

2014 Bound Volume 4 Issue 1-3: 1-198

ISSN 2219-2875 (online)

World Journal of *Immunology*

World J Immunol 2014 March 27; 4(1): 1-41



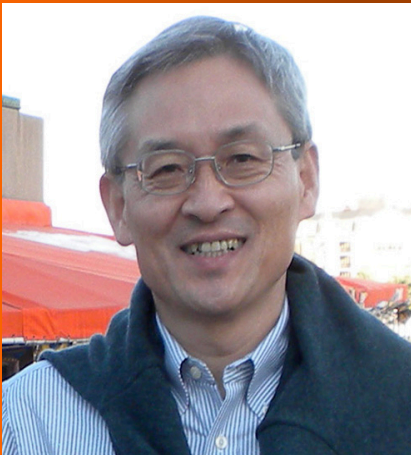
A portrait of a middle-aged man with dark hair and glasses, wearing a dark suit, white shirt, and a patterned tie. He is looking directly at the camera against a plain white background.

 *Immunology* Publishing Group
www.wjgnet.com

ISSN 2218-2624 (online)

World Journal of *Immunology*

World J Immunol 2014 July 27; 4(2): 42-129



 Published by Baishideng Publishing Group Inc

ISSN 2218-2824 (online)

World Journal of *Immunology*

World J Immunol 2014 November 27; 4(1): 130-138
Volume End



 Published by Baishideng Publishing Group Inc



Baishideng Publishing Group

-  Abstracting and Indexing
-  Alerts and Email
-  Journals
-  Submit a Manuscript

Welcome to Baishideng Publishing Group



Top 20 Articles of 20 World series journals in PubMed Central

LATEST NEWS [View More](#)

- Top 20 Articles of 20 World series journals in PubMed Central 2014-10-04
- 2014年世界20大期刊PubMed Central 文章排行 2014-10-04
- 2014年世界20大期刊PubMed Central 文章排行 2014-10-02
- Purpose of the Topics Highlight articles 2014-10-12
- 2014年世界20大期刊PubMed Central 文章排行 2014-10-12

BASHIDENG HOME

BASHIDENG MEMBERS

GENERAL INFORMATION

About the BPG

Abstracting and Indexing

Academic rules and norms

Awards

Advertising policies

Aims and scope

All issues

Annual rates and cost

Article formats

Articles in press

Establishment

BPG members

Board members

Clinical trial registration

Committee on publication ethics

Company registration

Copyright/ethical

Contact us

Copyright Assignment

Current issue

Data sharing

Declaration of interests

Direct marketing

E-books

Editorial board members



Baishideng Publishing Group Inc publishes 43 peer-reviewed, open access journals covering a broad range of topics in clinical medicine, as well as several topics in biochemistry and molecular biology; relevant to human health today.



Liang-Sheng Li, President and Company Editor-in-Chief, Baishideng Publishing Group Inc

Company Number: 3537345



Liang-Sheng Li, President and Company Editor-in-Chief, Baishideng Publishing Group Inc

Company Number: 3537345

About the BPG Journals

Format for

Clinicopathological

Conferences

Format for Bibliography

Format for Biochemistry

Guidelines for peer-reviewed

Publications process of the manuscript

Format for acid/cytogenetics of additional board members

Format for case control study

Format for clinical trials study

Format for diagnostic

Abstracts

[View More](#)

Featured Articles

1. Prospective rectal lymphatics: A potential cause of false positive T2D MRI 10
2. Can low-toxicity treatment be used alone in displaying apoptosis on CD44v3/CD117 25
3. VEGFR2 induces cellular apoptosis regulation in an in vitro prostate model of the transition after castration: drug delivery, drug 14

[View More](#)

World Journal of

Academic

Journal

Academy

Journal



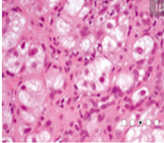
World Journal of Immunology

Submit a Manuscript

Submit a Reviewer

Submit a Manuscript

Welcome to World Journal of Immunology



Display of 1 cell culture in 3D.

Latest news View all

- 1. Heart activation of cluster of differentiation 74... 2014-11-15
- 1. Apoptosis signaling through redox signaling... 2014-11-15
- 1. Immunologic aspects of drug-induced liver injury 2014-11-15
- 1. Lipidosis and lipase in cancer for implication... 2014-11-15
- 1. CD226/CD134-EP and CD243/CD8, distribution and... 2014-11-15

JOURNAL HOME

- About the journal
- All article search
- Contact us
- Current issue
- Editorial board members
- Editorial board members
- Symptoms
- High impact papers
- Indexing/abstracting
- Permissions
- Previous issue
- Publication fee
- Submit a manuscript
- Subscribe

WJW EDITORIAL BOARD



Dr. J. J. Wang
Editor-in-Chief

The World Journal of Immunology Editorial Board covers all subjects. Agreeing with a list of worldwide experts in immunology. They are from 32 countries, including Argentina; 2, Australia (2), Austria (2), Belgium (2), Brazil (2), Canada (12), China (22), Czech Republic (2), Denmark (2), Russia(2), Germany (2), Greece (2), Hungary (2), India (2), Iceland (2), Israel (2), Italy (2), Japan (2), Lebanon (2), Mexico (2), Netherlands (2), Norway (2), Peru (2), Paraguay(2), Russia (2), Singapore (2), Slovenia (2), South Korea (2), Spain (2), Sweden (2), Switzerland (2), United Kingdom (2), and United States (76).

GENERAL INFORMATION

About the WJW

Editorial Board 2012-2015

Institutions for Authors

Journal for Editorial

Journal for Editorial

Journal for Editorial Highlight

Journal for Editorial

Journal for Editorial

Journal for Editorial for Basic Research

Journal for Editorial for Clinical Medicine

Journal for Editorial

more >>

Number of total issue: 7467

Number of issue today: 0

Number of issues online: 0

Number of downloads: 1107

BASHIDING JOURNALS

WJW + Immunology

Go

BASHIDING SUBMISSIONS

WJW + Immunology

Go

Express submission and peer-review system

Figure 1. Diffusion of oxygen and glucose across a cell membrane.

Express submission and peer-review system

Figure 1. Diffusion of oxygen and glucose across a cell membrane.

Express submission and peer-review system

Figure 1. Diffusion of oxygen and glucose across a cell membrane.

- EXPLOSIVE
- Added the E&P's
- All action research
- Articles in Press
- Author track manuscripts
- Conducts
- Current issue
- E-books
- E-exposed journals
- Editorial board members
- E-journals
- E&P's home
- Featured articles
- High impact articles
- Highly cited articles
- Interventions/strategies
- Interventions information
- Interventions in authors
- Journals
- Peer-reviewed track manuscripts
- Previous issue
- Publication fee
- Reviewer acknowledgement
- Submitted manuscript

- SUBMIT A MANUSCRIPT
- World Journal of Anaesthesiology
- World Journal of Biological Chemistry
- World Journal of Cardiology
- World Journal of Clinical Cases
- World Journal of Clinical Infectious Diseases
- World Journal of Clinical Oncology
- World Journal of Clinical Pediatrics
- World Journal of Clinical Urology
- World Journal of Critical Care Medicine
- World Journal of Dermatology
- World Journal of Diabetes
- World Journal of Experimental Medicine

TRACK MANUSCRIPTS

Author

Author
 Manuscript numbers
 Enter manuscript numbers, one per line. If you compare the manuscript numbers, in the respective list.
 Your email
 Password
 Submit

PUBLICATION FEE

PUBLICATION FEE

World Journal of Anaesthesiology
 World Journal of Biological Chemistry
 World Journal of Cardiology
 World Journal of Clinical Infectious Diseases
 World Journal of Clinical Oncology
 World Journal of Clinical Pediatrics
 World Journal of Clinical Urology
 World Journal of Critical Care Medicine
 World Journal of Dermatology
 World Journal of Diabetes
 World Journal of Experimental Medicine
 World Journal of Gastroenterology

CURRENT ISSUE

CURRENT ISSUE

World Journal of Anaesthesiology
 World Journal of Biological Chemistry
 World Journal of Cardiology
 World Journal of Clinical Infectious Diseases
 World Journal of Clinical Oncology
 World Journal of Clinical Pediatrics
 World Journal of Clinical Urology
 World Journal of Critical Care Medicine
 World Journal of Dermatology
 World Journal of Diabetes
 World Journal of Experimental Medicine
 World Journal of Gastroenterology



Editorial Board

2011-2015

The *World Journal of Immunology* Editorial Board consists of 247 members, representing a team of worldwide experts in immunology. They are from 33 countries, including Argentina (3), Australia (7), Austria (1), Belgium (1), Brazil (1), Canada (12), China (22), Czech Republic (1), Finland (1), France (14), Germany (8), Greece (2), Hungary (1), India (9), Ireland (1), Israel (6), Italy (16), Japan (9), Lebanon (1), Mexico (2), Netherlands (6), Norway (2), Peru (1), Portugal (2), Russia (3), Singapore (2), Slovenia (2), South Korea (6), Spain (7), Sweden (4), Switzerland (1), United Kingdom (14), and United States (79).

EDITORS-IN-CHIEF

Antonio La Cava, *Los Angeles*
Seung-Yong Seong, *Seoul*

GUEST EDITORIAL BOARD MEMBERS

Chen-Lung Steve Lin, *Kaohsiung*
Chien-Huang Lin, *Taipei*
Chih-Hsin Tang, *Taichung*
Chuen-Mao Yang, *Taoyuan*
Kuender D Yang, *Kaohsiung*
Wen-Chin Yang, *Taipei*

MEMBERS OF THE EDITORIAL BOARD



Argentina

Maria Silvia Di Genaro, *San Luis*
Rivero Virginia Elena, *Córdoba*
Marisa Vulcano, *Buenos Aires*



Australia

Antonio Ferrante, *Adelaide*
Leonard Charles Harrison, *Parkville*
Katherine Kedzierska, *Melbourne*
Mark J Kohler, *Adelaide*
Ian R Mackay, *Melbourne*
Mimi Lai-Kuan Tang, *Parkville*
Ban-Hock Toh, *Melbourne*



Austria

Doris Wilflingseder, *Innsbruck*



Belgium

Evelien Smits, *Antwerp*



Brazil

Marcelo Henrique Napimoga, *Uberaba*



Canada

Anshu Agrawal, *Irvine*
Zoulfia Allakhverdi, *Quebec*
Lbachir BenMohamed, *Irvine*
RM Gorczynski, *Toronto*
Subburaj Ilangumaran, *Sherbrooke*
Xiaoyan Jiang, *Vancouver*
Mladen Korbelik, *Vancouver*
François J M A Meurens, *Saskatoon*
Jean Sévigny, *Quebec*
Rajendra K Sharma, *Saskatoon*
Pingchang Yang, *Hamilton*
Zhu-Xu Zhang, *Ontario*



China

Wang-Sen Cao, *Nanjing*
Xin-Hua Chen, *Xiamen*
Ning Guo, *Beijing*
Xian-hui He, *Guangzhou*
Bo Huang, *Wuhan*
Bo Jin, *Beijing*
Ren Lai, *Kunming*
Zhan-Ju Liu, *Shanghai*
Chun-Feng Qu, *Beijing*
Fu-Dong Shi, *Tianjin*
Xiao Su, *Shanghai*

Jin-Xing Wang, *Jinan*
Yan-Jiang Wang, *Chongqing*
Li-Juan Zhang, *Beijing*
Shi-Cui Zhang, *Qingdao*
Zhi-Ren Zhang, *Chongqing*



Czech Republic

Josef Velisek, *Vodňany*



Finland

Yrjo Tapio Kontinen, *Helsinki*



France

Armand Bensussan, *Paris*
Christophe Borg, *Besançon*
Christophe Caux, *Lyon*
Mathias Chamaillard, *Lille*
Yves Denizot, *Limoges*
Philippe Marie Noel Georgel, *Strasbourg*
Sandra Kleinau, *Uppsala*
Guido Kroemer, *Villejuif*
Patrice N Marche, *Grenoble*
Jean-Louis Mege, *Marseille*
Julien Royet, *Marseille*
Bernhard Ryffel, *Orleans*
Guillaume Vogt, *Paris*
Renaudineau Yves, *Brest*



Germany

Nimmerjahn Falk, *Nuremberg*
Stephan Immenschuh, *Hannover*
Dieter Kabelitz, *Kiel*

Martin Leverkus, *Mannheim*
 Michael Linnebacher, *Rostock*
 Jan Hendrik Niess, *ULM/Donau*
 Enno Schmidt, *Luebeck*
 Robert Weissert, *Regensburg*



Greece

Giorgos T Bamias, *Athens*
 Clio P Mavragani, *Athens*



Hungary

Viktor Müller, *Budapest*



India

Arbind Acharya, *Varanasi*
 Atmaram Hari Bandivdekar, *Mumbai*
 Tapas Biswas, *Kolkata*
 Keya Chaudhuri, *Kolkata*
 Deepak Kaul, *Chandigarh*
 Debashis Mitra, *Pune*
 Praveen Rishi, *Chandigarh*
 Shyam Sundar, *Varanasi*
 Mohan R Wani, *Pune*



Ireland

Anne Fiona McGettrick, *Dublin*



Israel

Jacob George, *Rehovot*
 Noah Isakov, *Beer Sheva*
 Aaron Lerner, *Haifa*
 David Naor, *Jerusalem*
 Michal Schwartz, *Rehovot*
 Elias Toubi, *Haifa*



Italy

Roberto Biassoni, *Genoa*
 Francesco Indiveri, *Genoa*
 Pietro Invernizzi, *Rozzano*
 Lucia Lopalco, *Milan*
 Angelo Martino, *Rome*
 Ivano Mezzaroma, *Rome*
 Antonella d'Arminio Monforte, *Milan*
 Giulio Cesare Passali, *Siena*
 Carlo Perricone, *Rome*
 Alessandro Poggi, *Genoa*
 Antonella Prisco, *Naples*
 Francesco Recchia, *Avezzano*
 Carlo Riccardi, *Perugia*
 Domenico Sansonno, *Bari*
 Margherita Sisto, *Bari*
 Rosalinda Sorrentino, *Salerno-Fisciano*



Japan

Miyuki Azuma, *Tokyo*
 Kozo Fujisaki, *Kagoshima*

Shigetsugu Hatakeyama, *Sapporo*
 Kenji Kabashima, *Kyoto*
 Ryuji Kubota, *Kagoshima*
 Osam Mazda, *Kyoto*
 Toshi Nagata, *Hamamatsu*
 Toshimitsu Uede, *Sapporo*
 Hisanori Umehara, *Kahoku-gun*



Lebanon

Nayef E Saadé, *Beirut*



Mexico

Carlos Rosales, *Mexico City*
 Gilberto Vargas-Alarcón, *Mexico City*



Netherlands

Marianne Boes, *Utrecht*
 Niels Bovenschen, *Utrecht*
 Wouter J de Jonge, *Amsterdam*
 J Wouter Jukema, *Leiden*
 Frank A Redegeld, *Utrecht*
 Ruurd Torensma, *Nijmegen*



Norway

Guanglin Cui, *Tromso*
 Azzam A Maghazachi, *Oslo*



Peru

Salim Mohanna, *Lima*



Portugal

Alexandre M Carmo, *Porto*
 Nuno M de Oliveira Lages Alves, *Porto*



Russia

Alexander S Apt, *Moscow*
 Georgy A Nevinsky, *Novosibirsk*
 Alexander B Poletaev, *Moscow*



Singapore

Jeak Ling Ding, *Singapore*
 Alessandra Mortellaro, *Singapore*



Slovenia

Blaz Rozman, *Ljubljana*
 Snezna Sodin-Semrl, *Ljubljana*



South Korea

Sin-Hyeog Im, *Gwangju*

Mi-Yeon Kim, *Seoul*
 Hyung-Joo Kwon, *Chuncheon Gangwon-do*
 Won-Ha Lee, *Daegu*
 Cheol-Heui Yun, *Seoul*



Spain

Santos Mañes Brotón, *Madrid*
 Joan Claria, *Barcelona*
 Oscar J Cordero, *Santiago de Compostela*
 Victoriano Mulero, *Murcia*
 M^a Angeles Muñoz-Fernández, *Madrid*
 Yolanda Revilla Novella, *Madrid*
 Annabel F Valledor, *Barcelona*



Sweden

Francesco Dieli, *Stockholm*
 Levitskaya Jelena, *Stockholm*
 Stefan Karlsson, *Lund*
 Zou Xiang, *Gothenburg*



Switzerland

Silvia Monticelli, *Bellinzona*



United Kingdom

Peter Barnes, *London*
 Nicola Cirillo, *Bristol*
 Rossen Mintchev Donev, *Swansea*
 Eyad Elkord, *Manchester*
 Fang-Ping Huang, *London*
 John Maher, *London*
 Claudio Nicoletti, *Norwich*
 Dipak P Ramji, *Cardiff*
 Cordula Margaret Stover, *Leicester*
 Vadim V Sumbayev, *Chatham Maritime*
 Ying Sun, *London*
 Ping Wang, *London*
 Xiao-Qing Wei, *Cardiff*
 Heather M Wilson, *Aberdeen*



United States

Edward Abraham, *Birmingham*
 Jessie J Alexander, *Chicago*
 Robert J Amato, *Houston*
 Hossam M Ashour, *Detroit*
 Paul Ashwood, *Sacramento*
 Sami L Bahna, *Shreveport*
 Richard B Bankert, *Buffalo*
 Igor M Belyakov, *Frederick*
 Lauren Claire Berkow, *Baltimore*
 Michael Borchers, *Cincinnati*
 John J Bright, *Indianapolis*
 Stuart K Calderwood, *Boston*
 Christopher Chang, *Philadelphia*
 Arvind Chhabra, *Farmington*
 Lukasz K Chlewicki, *Chicago*
 Yingzi Cong, *Galveston*
 William Cruikshank, *Boston*
 Peter Demant, *Buffalo*
 Lauri J Diehl, *South San Francisco*

Nejat K Egilmez, *Buffalo*
D Mark Estes, *Athens*
Jie Fan, *Pittsburgh*
Angela Lee Foreman, *San Leandro*
Kenneth Adam Frauwirth, *College Park*
Mikhail A Gavrilin, *Columbus*
Alasdair M Gilfillan, *Bethesda*
Azizul Haque, *Charleston*
Jian Hong, *Houston*
Joseph Ugbodaga Igietseme, *Atlanta*
Rauno Joks, *Port Washington*
Janet Kalesnikoff, *Stanford*
Pravin TP Kaumaya, *Columbu*
Toshiaki Kawakami, *La Jolla*
Chang H Kim, *West Lafayette*
Hongmin Li, *New York*
Qiao Li, *Michigan*
Terry Lichtor, *Wilmette*
Tian Lin, *Boston*
Shu-Fang Liu, *Manhasset*

Yuan Liu, *Atlanta*
Binfeng Lu, *Pittsburgh*
Runqing Lu, *Omaha*
Yi Luo, *Iowa City*
Francesco M Marincola, *Potomac*
Kenneth R McLeish, *Louisville*
Song Qing Na, *Indianapolis*
SangKon Oh, *Dallas*
Kim Sung Ouk, *Ontario*
Kristen Page, *Cincinnati*
Kalipada Pahan, *Chicago*
Minggui Pan, *Santa Clara*
Manuel L Penichet, *Los Angeles*
Andras Perl, *Syracuse*
Edith Porter, *Los Angeles*
Hongwei Qin, *Birmingham*
Nguyen Cuong Quoc, *Gainesville*
Michael Karl Racke, *Columbus*
Mariusz Z Ratajczak, *Louisville*
Nicholas P Restifo, *Bethesda*

Prema Robinson, *Houston*
Rachel L Roper, *Greenville*
Kimberly S Schluns, *Houston*
Mohamed Tarek M Shata, *Cincinnati*
Haval Shirwan, *Louisville*
Judith Anne Smith, *Madison*
Zuoming Sun, *Duarte*
Dennis Daniel Taub, *Baltimore*
Georgios Christos Tsokos, *Boston*
Evros K Vassiliou, *Union*
Hongjun Wang, *Boston*
Min Wu, *Grand Forks*
Lihua Xiao, *Atlanta*
Dongxu Xie, *New York*
Baohui Xu, *Stanford*
Kejian Yang, *Worcester*
Xiao-Feng Yang, *Philadelphia*
Thomas Yankee, *Kansas City*
Song Guo Zheng, *Los Angeles*

World Journal of *Immunology*

World J Immunol 2014 March 27; 4(1): 1-41



**REVIEW**

- 1 Immune response after photodynamic therapy increases anti-cancer and anti-bacterial effects
Reginato E, Wolf P, Hamblin MR
- 12 Circulating immune cell activation and diet: A review on human trials
Chiva-Blanch G, Estruch R

MINIREVIEWS

- 20 Cigarette smoking and innate immune responses to influenza infection
Wu W, Metcalf JP
- 26 Role of myeloid-derived suppressor cells in autoimmune disease
Crook KR, Liu P
- 34 Immune thrombocytopenia in adults
Kurtoğlu E, Karakuş V

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Immunology*, Hyung-Joo Kwon, PhD, Professor, Department of Microbiology, Hallym University College of Medicine, 39 Hallymdaehak-gil, Chuncheon Gangwon-do 200-702, South Korea

AIM AND SCOPE *World Journal of Immunology* (*World J Immunol*, *WJI*, online ISSN 2219-2824, DOI: 10.5411) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJI covers a wide range of subjects including: (1) autoimmune diseases such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, myasthenia gravis, in both humans and animal models of disease, with an interest on aspects including the etiology, pathogenesis, mechanisms of disease induction, maintenance and progression; (2) tumor immunology including immunosurveillance, immunoediting and immunotherapies in animal models and in humans; (3) clinical immunology in humans and animal models including mechanisms of disease, regulation and therapy and immunodeficiencies; (4) innate immunity including cell subsets, receptors and soluble mediators, complement and inflammation; (5) adaptive immune mechanisms and cells including soluble mediators and antibodies; (6) immune cell development, differentiation, maturation; (7) control mechanisms for immune cells including immune tolerance and apoptosis; (8) immune cell interactions and immune cell receptors; (9) immunological methods and techniques; (10) immune cell activation including cell signaling pathways, biochemical and pharmacologic modulation studies; (11) infection; (12) different modalities of vaccination including gene therapy; (13) hypersensitivity and allergy; (14) transplantation.

We encourage authors to submit their manuscripts to *WJI*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING *World Journal of Immunology* is now indexed in Digital Object Identifier.

FLYLEAF I-III Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Su-Qing Liu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ling-Ling Wen*

NAME OF JOURNAL
World Journal of Immunology

ISSN
ISSN 2219-2824 (online)

LAUNCH DATE
December 27, 2011

FREQUENCY
Four-monthly

EDITORS-IN-CHIEF
Antonio La Cava, MD, PhD, Professor, Department of Medicine, University of California Los Angeles, Los Angeles, CA 90095-1670, United States

Seung-Yong Seong, MD, PhD, Professor, Department of Microbiology and Immunology, 103 Dachag-no, Jongno-gu, Seoul 110-799, South Korea

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Immunology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bjgoffice@wjgnet.com
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F, Lucky Plaza,
315-321 Lockhart Road, Wan Chai,
Hong Kong, China
Telephone: +852-6555-7188
Fax: +852-3177-9906
E-mail: bjgoffice@wjgnet.com
<http://www.wjgnet.com>

PUBLICATION DATE
March 27, 2014

COPYRIGHT
© 2014 Baishideng Publishing Group Co., Limited. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2219-2824/g_info_20100722180909.htm.

ONLINE SUBMISSION
<http://www.wjgnet.com/esp/>



Immune response after photodynamic therapy increases anti-cancer and anti-bacterial effects

Eleonora Reginato, Peter Wolf, Michael R Hamblin

Eleonora Reginato, Michael R Hamblin, Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA 02114, United States

Eleonora Reginato, Peter Wolf, Department of Dermatology, Medical University of Graz, Graz 8036, Austria

Michael R Hamblin, Department of Dermatology, Harvard Medical School, Boston, MA 02115, United States

Michael R Hamblin, Harvard-MIT Division of Health Science and Technology, Boston, MA 02139, United States

Author contributions: All authors contributed to this paper.

Supported by United States National Institute of Health grant AI050875; the PhD program of the Medical University of Graz, Austria, to Reginato E

Correspondence to: Michael R Hamblin, PhD, Wellman Center for Photomedicine, Massachusetts General Hospital, 55 Fruit St, Boston, MA 02114,

United States. hamblin@helix.mgh.harvard.edu

Telephone: +1-617-7266182 Fax: +1-617-7266566

Received: October 17, 2013 Revised: December 20, 2013

Accepted: February 16, 2014

Published online: March 27, 2014

Abstract

Photodynamic therapy (PDT) is a clinically approved procedure for treatment of cancer and infections. PDT involves systemic or topical administration of a photosensitizer (PS), followed by irradiation of the diseased area with light of a wavelength corresponding to an absorbance band of the PS. In the presence of oxygen, a photochemical reaction is initiated, leading to the generation of reactive oxygen species and cell death. Besides causing direct cytotoxic effects on illuminated tumor cells, PDT is known to cause damage to the tumor vasculature and induce the release of pro-inflammatory molecules. Pre-clinical and clinical studies have demonstrated that PDT is capable of affecting both the innate and adaptive arms of the immune system. Immune stimulatory properties of PDT may increase its beneficial effects giving the therapy wider potential to become more extensively used in clinical practice. Be-

sides stimulating tumor-specific cytotoxic T-cells capable to destroy distant untreated tumor cells, PDT leads to development of anti-tumor memory immunity that can potentially prevent the recurrence of cancer. The immunological effects of PDT make the therapy more effective also when used for treatment of bacterial infections, due to an augmented infiltration of neutrophils into the infected regions that seems to potentiate the outcome of the treatment.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Photodynamic therapy; Anti-tumor immunity; T-cell activation; Damage-associated molecular patterns; Inflammatory cells

Core tip: The immune stimulatory properties of photodynamic therapy (PDT) make this therapy one of the most promising therapeutic procedures for the management of cancer lesions and microbial infections. This review will focus on the current knowledge of the innate and adaptive immune responses induced by PDT against tumors and pathogens.

Reginato E, Wolf P, Hamblin MR. Immune response after photodynamic therapy increases anti-cancer and anti-bacterial effects. *World J Immunol* 2014; 4(1): 1-11 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i1/1.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i1.1>

INTRODUCTION

The ideal therapy for cancer should be able to selectively destroy the tumor cells at the primary site and at the same time trigger the immune system to recognize any remaining or recurring cancer cells. Compared to other unspecific and/or immunosuppressive cancer therapies

such as chemotherapy, ionizing radiation and surgery, photodynamic therapy (PDT) might have these desirable properties. PDT is a procedure that consists of three components: A photosensitizer (PS), light of appropriate wavelength to excite the PS and molecular oxygen^[1,2]. None of these three components is individually toxic, but when combined together they initiate a photochemical reaction that culminates in the generation of highly reactive oxygen species (ROS)^[3]. Most of the PSs used in PDT are based on a tetrapyrrole structure, similar to that of the protoporphyrin contained in hemoglobin^[4]. They have an absorption peak between 600 and 800 nm (red to deep red), since light at lower wavelengths would not penetrate efficiently through the tissue and light at longer wavelengths than 800 nm would not have sufficient energy to initiate a photochemical reaction and generate a substantial yield of ROS^[4].

The ROS produced during PDT can directly kill tumor cells by induction of necrosis and/or apoptosis^[5] and damage the tumor vasculature, leading to depletion of oxygen and nutrients in the tumor^[6,7]. As a result of this traumatic insult to the tumor and its microenvironment, a strong acute inflammatory reaction is provoked at the targeted site^[1]. The acute inflammatory response following PDT causes infiltration of host innate immune cells that carry out the removal of damaged cells. Acute inflammation also seems to be implicated in the development of adaptive anti-tumor immunity^[1]. In particular, the efficacy of PDT in some models has been shown to be dependent upon such induction of anti-tumor immunity. Early studies showed that while PDT of EMT6 tumors exhibited curative effects and long-term tumor control in Balb/c mice, the long-term protection from tumors was lost when PDT was performed in either *scid* (which lack T and B cells), or nude (which lack T cells) immune-compromised mice. However, when the *scid* mice were reconstituted with splenic T cells or bone marrow cells from Balb/c mice, the curative effect of PDT was restored^[8,9].

While the immune stimulatory effects of PDT have been widely studied, although not completely understood in cancer models, much effort still has to be done to understand these effects of PDT in microbial infections. Tanaka *et al.*^[10] discovered that the therapeutic effect of PDT in a mouse model of bacterial arthritis was dependent on the attraction and accumulation of neutrophils into the infected region and could also produce a protective effect if carried out before infection. This review will focus on the current knowledge of the beneficial immunological effects of PDT for cancer and bacterial infections. A list of notable publications that show that PDT can activate different constituents of the immune system is provided in Table 1.

DAMAGE-ASSOCIATED MOLECULAR PATTERNS

After the traumatic insult to the tumor induced by PDT,

one of the first events occurring at the treatment site is the generation of “danger” signals, so called damage-associated molecular patterns (DAMPs) or cell death-associated molecular patterns (CDAMPs) that serve as warning signals in innate immunity^[11-14]. DAMPs play a similar role to that of pathogen-associated molecular patterns, but instead of being associated with pathogenic microbes, they are associated with host tissue damage. DAMPs are endogenous intracellular molecules normally “hidden” within living cells, but upon exposure or secretion from dying and/or damaged cells, they acquire immune-stimulatory properties. DAMPs are thought to be the key mediators of the immunogenicity of tumor cells killed by PDT *via* necrosis or apoptosis. They constitute alarm signals warning that “self-altered” antigens were released from dying cells; the immune system recognizes them and triggers a vigorous immunological response. It is generally accepted that while necrotic cells are pro-inflammatory and immunogenic, some forms of apoptotic cells are efficiently engulfed and disposed of by macrophages and other phagocytic cells, therefore they should not induce inflammation and are unlikely to stimulate the immune system^[15,16]. However, it has been reported that under certain circumstances, other forms of apoptotic cells such as tumor cells undergoing apoptosis by some particular cancer therapies can effectively generate an immune response^[17,18]. In this case the process is defined as “immunogenic apoptosis” *vs* the conventional “non-immunogenic apoptosis”^[16,19,20].

It is conceivable that while the physiological programmed cell death is non-inflammatory and non-immunogenic, some cancer therapies (such as particular forms of chemotherapy and PDT) cause tumor damage, and produce an immunogenic form of apoptosis characterized by release of DAMPs and enhancement of inflammation.

The release of DAMPs after PDT has been investigated in some studies^[11,12,21]. Korbek *et al.*^[22] found that squamous cell carcinoma VII (SCCVII) cancer cells treated by *in vitro* photofrin-PDT expose on the surface heat shock proteins (HSPs) such as HSP60, HSP70 and glucose-regulated protein 94 (GRP94) and release HSP70 to the extracellular space. Interestingly, when PDT was applied in *in vivo* settings, they found a different spectrum of DAMPs exposed on the surface of treated SCCVII cells. While HSP70 was still exposed, HSP60 and GRP94 were no longer detected and replaced by GRP78 on the surface of PDT-treated SCCVII cancer cells. This indicated for the first time that the DAMPs associated with PDT can differ in the same cancer cells between *in vitro* and *in vivo* settings^[22].

It is worth mentioning also that the spectra of DAMPs exposed and/or released after PDT correlate with the sub-cellular localization patterns of the PS, where the ROS-based stress is originated. For instance, PSs targeting the endoplasmatic reticulum (*e.g.*, hypericin) are known to cause surface exposure of calreticulin (CRT); conversely, Photofrin (whose localization is mostly associated with lipid membranes)-PDT, has been linked primarily to surface exposure of HSP70^[23,24].

Table 1 Milestone studies on effects of photodynamic therapy affecting the immune system

Immune components	Immunomodulatory effect of PDT	Ref.
Pro-inflammatory cytokines	Production of pro-inflammatory cytokines after PDT <i>in vivo</i>	[26]
Macrophages	First evidence of cytokine production by PDT-treated macrophages <i>in vivo</i>	[107]
Dendritic cells	DCs can efficiently phagocytose PDT-treated tumor cells in <i>in vivo</i> experiments. Immature DCs administered in combination with PDT produce effective antitumor response <i>in vivo</i>	[38,108]
NKs	Role of NKs in immune response after PDT, control of distant untreated tumors	[43]
Neutrophils	Evidences that neutrophils have a crucial role in the PDT response <i>in vivo</i>	[30,109]
Memory immunity	First demonstration that a specific antitumor memory immunity is induced after PDT: resistance to tumor rechallenge in animals cured by PDT	[110]
T lymphocytes, memory immunity	Essential role of host T lymphocytes in immune response after PDT: curative effect of PDT in immune-competent Balb/c mice, but not in immune-suppressed <i>scid</i> mice. Adoptive transfer of splenocytes from PDT-cured mice to <i>scid</i> mice confers resistance to tumor rechallenge	[8,111]
Treg	Evidences for the role of Treg in inhibiting the immune response after PDT	[77]
Patient lymphocytes	First demonstration that an antigen-specific immune response can be observed after PDT	[65]

PDT: Photodynamic therapy; DCs: Dendritic cells; NKs: Natural killer cells; Treg: T regulatory cells.

Table 2 Damage-associated molecular pattern molecules that may be released or exposed on the outer leaflet of dying tumor cells after photodynamic therapy

DAMP	Function	Ref.
HSP60, HSP70, HSP90, gp96, GRP94, GRP78	Molecular chaperones that normally reside in intracellular regions/organelles, but under stress they are exposed on the damaged cell surface and prime immunomodulatory processes	[11,21,22,112]
Calreticulin	Calcium binding protein located in intracellular regions/organelles (mostly in ER), but under stress its presence on the PM is augmented. On the PM it acts as “danger signal” and increases the immunogenicity of the dying cells	[11,112]
ATP	High-energy molecule, normally intracellular, but can be released by necrotic and apoptotic cells under particular stresses. Extracellular ATP has the ability to help in chemoattraction of immune cells	[12,112]
Phosphatidylserine	When cells are damaged/dying, phosphatidylserine is transposed from the inner to the outer leaflet and acts as an “eat me” signal by interacting with multiple immune cells receptors, mediating efficient phagocytosis and anti-inflammatory responses	[112,113]
High mobility group box-1	Nuclear chromatin-binding protein; it has prominent cytokine-like properties and when released by dying cells tends to stimulate immune cells to produce various pro-inflammatory cytokines	[11,112]
Calgranulin family members (S100A8, S100A9, S100A12)	Calcium-binding proteins; when released by necrotic cells they act as “find me” signals attracting various immune cells and interacting with immune cell receptor (TLR4/RAGE) to induce the secretion of pro-inflammatory cytokines	[11,112,114]
Cross-linked dimer of ribosomal protein S19	Constituent of small ribosomal subunit; when released by necrotic cells it acts as a chemotactic factor for attracting various immune cells	[11,112]

DAMPs: Damage-associated molecular patterns; ER: Endoplasmic reticulum; PM: Plasma membrane; HSP: Heat shock protein; GRP: Glucose-regulated protein; ATP: Adenosine triphosphate; TLR4: Toll-like receptor 4; RAGE: Receptor for advanced glycation end-products.

Further investigations on cellular and molecular mechanisms are certainly required to establish in more detail the correlations between DAMPs and PDT. However, the most important examples of DAMPs which are produced after PDT reported so far are HSPs, CRT, adenosine triphosphate and other mediators^[21,22,25]. Table 2 lists the DAMPs that have been reported to be produced after PDT.

INFLAMMATION AND INNATE IMMUNE RESPONSES IN ANTI-CANCER PDT

The PDT-induced oxidative stress and traumatic insult to the tumor microenvironment are known to stimulate the release or expression of various proinflammatory mediators [tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-1, complement proteins, HSPs and arachidonic acid metabolites] from the treated site^[26]. Moreover, as men-

tioned above, immunogenic DAMPs are released after PDT and they can be detected by the innate immune cells that are programmed to detect microbial invasion^[27]. For these reasons, innate immune cells such as monocytes or macrophages, neutrophils and dendritic cells (DCs) are recruited to the treated site and infiltrate in large numbers to attack what is expected to be a microbial invasion but turns out to be damaged tumor cells^[28]. The primary function of the inflammatory cells is to neutralize the DAMPs by engulfing and eliminating the cellular debris as well as compromised tissue components. This promotes local healing with restoration of normal tissue function. At the onset of PDT-induced inflammation, the tumor vasculature undergoes significant changes and become permeable for blood proteins and pro-adhesive for inflammatory cells *via* over-expression of adhesion molecules (Intracellular Adhesion Molecule 1, Vascular Cell Adhesion Molecule 1, selectins)^[27], thus favoring the

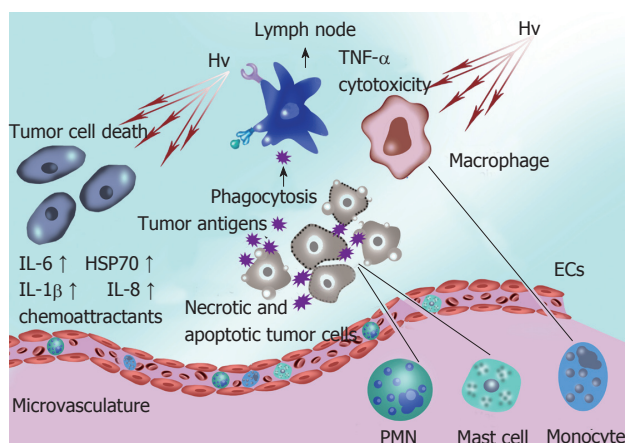


Figure 1 Innate immune responses in anti-cancer photodynamic therapy. Photodynamic therapy of tumors leads to the development of local inflammation mediated by the release of danger signals and cytokines. Various cells of the immune system infiltrate into the treated area. ECs: Endothelial cells; HSP70: Heat-shock protein; Hv: Light; PMNs: Polymorphonuclear neutrophils; TNF: Tumor necrosis factor; IL-6: Interleukin-6. Original figure based upon Ref. [115] and Ref. [116].

massive infiltration of the immune cells into the tumor.

The inflammatory cells are known to be necessary to achieve efficacious PDT, as several studies have shown that their depletion (or inhibition of their activity) diminishes the therapeutic effect of the treatment^[9,29,30]. Among all the cytokines involved in the PDT-induced inflammatory process, IL-1 β and IL-6 seem to play the most important role^[26,31] and conversely, IL-10 and transforming growth factor (TGF)- β seem to hamper PDT-effects as their blockade remarkably improves the cure rates after PDT^[27]. Also, blocking the function of various adhesion molecules can affect the efficacy of PDT^[26,32]. Figure 1 shows the important cells and mediators that are activated in the tumor environment after PDT of a tumor.

Although PDT is a local treatment, its effect is not limited to the local site, but it can induce a potent acute phase response with systemic consequences^[33]. Studies in mouse models have shown that PDT leads to drastic rise in serum levels of acute phase reactants such as serum amyloid P components (SAP), C-reactive protein (CRP) and mannose-binding lectin A (MBL-A)^[34]. SAP and CRP belong to the pentaxin family proteins and are involved in acute immunological responses^[35]. They are specialized in facilitating the phagocytosis and removal of dying cells such as those killed in PDT-treated tumors. SAP production and release is a hallmark acute phase reactant response in mice, but in humans CRP is a more important acute phase reactant than SAP and PDT dose-dependent up-regulation of CRP has been demonstrated in human lung tumor A549 cells^[35]. MBL-A is another important acute phase reactant with functional attributes similar to SAP^[36].

Furthermore, a rapid increase in peripheral neutrophils is observed immediately after PDT and it is still present 24 h later, that is correlated with the influx of neutrophils into the treated tumors^[37].

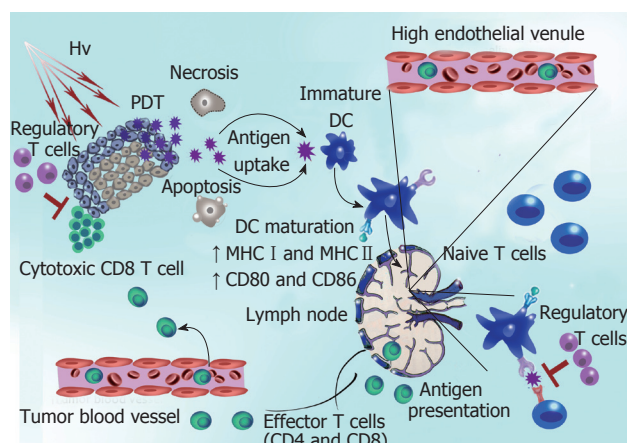


Figure 2 Stimulation of adaptive anti-tumor immunity by photodynamic therapy. PDT-treated tumor cells release the antigens, which are phagocytosed by DCs and presented to naive T cells in the tumor draining lymph node. Activated effector T cells return in circulation and migrate to the tumor. Regulatory T cells seem to inhibit the immune responses after PDT. DCs: Dendritic cells; Hv: Light; MHC I: Major histocompatibility class I; PDT: Photodynamic therapy. Original figure based upon Ref. [115] and Ref. [116].

ADAPTIVE IMMUNE RESPONSES IN ANTI-CANCER PDT

The PDT-induced local and systemic inflammatory responses can enhance the development of an adaptive immune response capable of protecting the host organism in an antigen-specific manner, owing to immunological memory. It can be asked what is mediating the crosstalk between the innate and adaptive arms of the immune system after PDT. It has been realized that PDT enhancement of adaptive anti-tumor immunity involves the activation of DCs. DC are stimulated by the recognition of DAMPs/CDAMPs released and/or exposed by dying tumor cells^[38]. One of the best characterized DAMPs induced by PDT is HSP70, which is released after PDT and forms stable chaperone complexes with cytoplasmic tumor antigens. Thereafter, the HSP-antigen complexes bind to the danger signal receptors, Toll-like receptors 2 and 4^[39] on the surface of DCs, which are most potent antigen presenting cells (APCs). In the absence of inflammation DCs remain in an immature state, but when tissue inflammation and release of DAMPs occur, they mature and migrate in large numbers to the draining lymph nodes. The transition to the mature state of DC involves the upregulation of surface major histocompatibility class I and II molecules (MHC I and MHC II) and of the costimulatory molecules CD80 and CD86. These changes allow the DCs to express peptide-MHC complexes at the cell surface and prime efficiently CD4⁺ T helper cells and CD8⁺ cytotoxic T lymphocytes (CTLs) and hence to initiate an adaptive immune response. Figure 2 shows the process by which DCs engulf tumor antigens, become activated, traffic to lymph nodes where antigen specific T-cells proliferate and then return to attack remaining tumor cells.

The generation of CD8⁺ effector and memory T cell

induction is generally, but not always dependent on CD4⁺ helper T cells^[40-42]. Kabingu *et al.*^[43] showed in fact that CD8⁺ T cell-mediated immunity is independent of CD4⁺ T cells and depends instead on natural killer cells. Also other studies suggest that CD8⁺ cells play the most critical role in PDT mediated anti-tumor immunity, as in the absence of their activation and/or tumor infiltration the efficacy of PDT is reduced^[9,43]. Furthermore, it has been shown that adoptive transfer of bare CD8⁺ T cells to immunocompromised *scid* mice can significantly restore PDT efficacy^[8].

The adaptive immunity is not provided only by antigen-specific T cells, but also by B cells. B cells produce antigen-specific immunoglobulins, mounting the so called humoral immune response. So far there is only one study showing that the activation of humoral immunity is implicated in the PDT-induced systemic antitumor protection, as seen by (1) increased serum IgG titers after PDT; (2) production of antibodies against existing antigens; and (3) marked B-cell infiltration in the tumor rim 24 h after PDT^[44]. Nonetheless, the importance of the humoral components to the tumor eradication process remains unclear and needs further investigations.

ROLE OF TUMOR ANTIGENS IN THE ANTI-TUMOR IMMUNE RESPONSE

Tumor antigens (TAs) represent a sort of “bait” for the immune system, since they activate DCs and allow the antigen-specific CTLs to recognize and destroy the tumor cells. Some TAs have been well defined in murine and human tumors^[45] and are generally classified in three distinct groups: (1) Antigens encoded by cancer-testis genes expressed in various tumors, but not in normal tissues, such as the mouse gene *P1A* and human genes of the melanoma antigen (MAGE)-type, B MAGE and G antigen families^[46-51]; (2) Differentiation antigens of the melanocytic lineage, which are present on most melanomas but also on normal melanocytes (*i.e.*, melanoma antigen recognized by T-cells 1, gp100)^[51-53]; and (3) Antigens that result from tumor-specific mutations in genes which are expressed in all tissues (*i.e.*, p53, p16) or come from viruses (*i.e.*, Epstein-Barr virus, Hepatitis B virus)^[54-58]. Successful immunotherapeutic strategies targeting the TAs have been developed in preclinical studies and early-phase clinical trials^[59,60], and our group was the first to realize the importance of TAs expression in PDT anti-tumor immunity.

We showed that a vascular PDT regimen was able to produce 100% of long term cures and rejection of rechallenge when tumors were induced in C3H mice with green fluorescent protein-expressing radiation-induced fibrosarcoma cells, but not with their wild-type counterpart^[61]. The same effect was observed when we used a pair of equally lethal Balb/c colon adenocarcinomas: The antigen negative CT26 wild-type and the CT26.CL25 transduced with *lacZ* gene, and thus expressing the tumor antigen β -galactosidase^[62]. We could further show that PDT of antigen positive tumors, but not of antigen neg-

ative tumors could trigger a highly potent antigen-specific systemic immune response capable to induce regression of distant untreated tumors. Recently we employed the P1A antigen positive mouse mastocytoma P815 wild-type and P1A antigen negative P1.204 (P815 derived) tumor models to study the antigen-specific PDT-induced antitumor immunity^[63]. This model is clinically more relevant than others as the P1A is a naturally occurring murine cancer antigen, homologue of the human MAGE-type antigen^[64]. We found that tumor cures, significantly higher survival and rejection of tumor rechallenged were obtained with P815, but not with P1.204 tumors that lack the antigen.

The role of the TAs in PDT anti-tumor immunity has been recently investigated also in the clinical setting. In a study published by Kabingu *et al.*^[65] in 2009, they demonstrated for the first time the enhancement of systemic immune reactivity to a basal cell carcinoma (BCC) associated TA (Hedgehog-interacting protein 1) following PDT in patients. These novel findings in patients are important as they are supporting the results in preclinical models, but more effort needs to be done in clinical trials to elucidate the PDT-induced systemic immune responses to tumor antigen.

IMPACT OF T REGULATORY CELLS IN THE ANTI-TUMOR IMMUNE RESPONSE

In addition to directly stimulating anti-tumor immunity by triggering DCs and T cells activation, PDT may also interfere with immune-suppressive T cells. The main class of T cells suppressing the immune response consists of CD4⁺CD25⁺FoxP3⁺ T regulatory cells (Treg)^[66]. The involvement of Treg in both autoimmune disease^[67] and cancer^[68] has been extensively described in mice and humans. Treg are thought to mediate their immunosuppressive effects by multiple mechanisms^[69]. Treg express the protein receptor cytotoxic T-lymphocyte antigen 4 (CTLA-4), which is similar to the T-cell costimulator protein CD28. CTLA-4 binds with much higher affinity to B7-1 and B7-2 costimulatory molecules on APCs compared to the equivalent molecule CD28 and transmits inhibitory signals, rather than stimulatory^[70].

Treg are generally classified into two main subpopulations: Natural Treg and induced Treg^[71]; the former are found in the thymus and thought to have T-cell receptors that recognize self-antigens, therefore important in the prevention of autoimmune disease, the latter can be induced and differentiate in the periphery, *i.e.*, upon influence by TGF- β in the tumor microenvironment^[72]. Several studies have shown that Treg inhibit the generation of immune responses against tumors^[71], but on the other hand, their depletion *in vivo* facilitates tumor eradication and enhances anti-tumor immunity^[73-75]. A summary of the features of Treg is provided in Table 3.

Our research group was the first to investigate the potential relationship between PDT and Treg and we realized that Treg play an important and negative role in PDT anti-tumor immunity. We observed that if Treg are

Table 3 Common features of T regulatory cells

Features of Treg	Ref.
Phenotypic and functional specialization	[66-68]
Treg are CD4 ⁺ CD25 ⁺ FoxP3 ⁺ immunosuppressive T cells. They are important for the maintenance of the immune homeostasis and involved in both autoimmune disease and cancer	[71,72]
Cells subpopulations	
Treg are generally classified into nTreg and iTreg. The former are found in the thymus and thought to have T-cell receptors that recognizes self-antigens, therefore important in the prevention of autoimmune disease, the latter can be induced and differentiate in the periphery, <i>i.e.</i> , upon influence by TGF-β in the tumor microenvironment	[69,70]
Immunosuppressive mechanisms	
Treg are thought to mediate their immunosuppressive effects by multiple mechanisms, among which	
Secretion of immunosuppressive cytokines	
High affinity binding of his CTLA-4 receptor to B7-1 and B7-2 costimulatory molecules on antigen presenting cells and transmission of inhibitory signals	
Role of Treg in anti-tumor immunity	[71,73-75]
Treg are known to inhibit the generation of immune responses against tumors. Treg depletion <i>in vivo</i> facilitates tumor eradication and enhances-anti-tumor immunity	

nTreg: Natural Treg; iTreg: Induced Treg; CTLA-4: Cytotoxic T-lymphocyte antigen 4; Treg: T regulatory cells; TGF: Transforming growth factor.

depleted by low-dose cyclophosphamide (CY) (a traditional cytotoxic cancer drug that at low doses selectively depletes Treg^[76]) prior to PDT, the anti-tumor immune responses are potentiated and a memory immunity is generated against metastatic J774 tumors^[77]. This effect was not seen when PDT and CY treatments were given separately or when PDT was combined with high-dose CY that destroyed all T-cells not just Treg. Another recently completed study involving the colon adenocarcinoma CT26 wild-type tumor model revealed that the combination of PDT with low-dose CY produced a dramatic improvement in long-term survival, compared with either treatment alone and led the development of immune response to the mouse cancer shared/auto-antigen gp70^[78]. Moreover, this combination treatment activated a long-lasting immune memory, that could however be uncovered only when Treg were depleted again by CY before rechallenge. These new findings are important, because they emphasizes that one of the most effective approaches for optimally improving anti-cancer PDT would be by restraining host's regulatory immune cell populations.

CLINICAL EVIDENCE FOR THE IMPACT OF THE IMMUNE SYSTEM IN ANTI-CANCER PDT EFFECTS

The first clinical use of PDT for cancer in modern times dates back to the beginning of the 20th century when Von Tappeiner *et al*^[79] used eosin as topical PS combined to sunlight to treat facial BCC. That first trial was successful as out of 6 patients, 4 showed complete tumor resolution. Many years later, in the 1970s, hematoporphyrin derivative (HPD) and light were administered to the tumor area of patients with bladder cancer^[80] and resulted in positive outcomes. In the same decade Dougherty *et al*^[81] tested for the first time HPD-PDT in a large series of patients with skin tumors reporting striking results: Complete or partial responses were observed in 111 out of 113 patients.

Since then, over 200 clinical trials for PDT as treatment for a large variety of tumors have been carried out. Some clinical studies have demonstrated that PDT efficacy seems to depend on antitumor immunity also in patients. Dragieva *et al*^[82] published a study comparing the efficacy of PDT for actinic keratosis and Bowen's disease in immune-competent patients *vs* immune-suppressed transplant patients. The two groups of patients showed comparable initial response, however the immune-suppressed patients had an increased propensity to develop new lesions after the treatment. It has also been shown that patients with vulval intraepithelial neoplasia (VIN) expressing MHC I molecules on the tumor cells were more likely to respond to aminolevulinic acid-PDT compared to patients whose tumors had down-regulated MHC I molecules^[83]. MHC I recognition is critical for activation of CD8⁺ T cells and the down-regulation of MHC I molecules is one of the mechanisms used by tumors to evade immune recognition in general and PDT-induced immunity in particular. VIN patients who did not respond to PDT had significantly lower CD8⁺ T cell infiltration into the treated tumors compared with responders, confirming the important role of CD8⁺ CTLs in PDT efficacy. The first clinical case of systemic PDT-immune response observed in patients has been published in 2007: PDT of multifocal angiosarcoma of the head and neck located on the right upper limb of a patient, resulted in a spontaneous regression of the untreated distant tumors on the contralateral left upper limb, accompanied by increased immune cell infiltration^[84]. Two years later Kabingu *et al*^[65] found that PDT treatment of BCC lesions enhanced the reactivity of patients lymphocytes against Hip1, a known BCC-associated TA, as seen by increased secretion of IFN-γ by patients lymphocytes following incubation with the TA derived peptide.

PDT FOR INFECTIONS

Although PDT was discovered in the field of microbiology over 100 years ago^[85], up to now PDT has been

studied and applied mainly as anticancer treatment. The discovery of antibiotics in 1940s revolutionized the treatment of infectious disease, limiting the development of other potential alternative anti-microbial treatments like PDT. However, the recent worldwide increase of resistance to antibiotics has strongly enhanced the interest in alternative therapeutic strategies for the treatment of infections. PDT is capable of killing a large variety of pathogens such as bacteria, parasitic protozoa, fungi, yeasts and viruses. Furthermore, PDT does not induce resistance itself and it is a non-invasive method. PDT is more effective in inactivating Gram (+) bacteria compared to Gram (-) due to the different structure of the cell walls^[86]. The membrane of Gram (+) bacteria is surrounded by a permeable layer of peptidoglycan and lipoteichoic acid that allows the PS to pass through it^[87]. Gram (-) species have an inner cytoplasmic membrane and an outer membrane, which are separated by a peptidoglycan-containing periplasm. The outer membrane constitutes a permeability barrier between the cell and its environment, limiting the PS penetration. Fungal cell walls have a moderately thick layer of chitin and β -glucan that result in a barrier with moderate permeability. Several *in vitro* and *in vivo* studies have been carried out to verify the efficacy of PDT for viral infections, soft tissues infections, oral and dental infections produced by different strains of bacteria. PDT has been shown to work efficiently against *Escherichia coli* and *Pseudomonas aeruginosa* in excisional wounds^[88,89] and against *Acinetobacter baumannii* and *Staphylococcus aureus* in burn infections^[90,91].

There are reports of PDT on its effects on certain species of fungus, including both filamentous fungi (*Trichophyton*^[92] and *Aspergillus*^[93]) and yeasts (*Saccharomyces*^[94] and *Candida albicans*^[95,96]). Also several types of virus have been tested for the affection by PDT, including herpes viruses HSV-1^[97] (PDT by methylene blue and light), enveloped RNA viruses from two different families, Semliki Forest Virus (*Togaviridae*) and vesicular stomatitis virus (*Rhabdoviridae*) (PDT by buckminsterfullerene and light)^[98] and others^[99].

Some clinical trials for PDT have been carried out for dental, gastric and dermatological infections such as acne as well as rosacea, a condition in which microbes may play a role in the pathogenesis^[100].

IMMUNE RESPONSES IN ANTI-BACTERIAL PDT

While the immune stimulating effects of PDT have been widely studied in cancer models, little is known about the immunological effects of PDT in bacterial infections. A recent study published by Tanaka *et al.*^[101] convincingly demonstrated for the first time that *in vivo* PDT can stimulate an innate immune response. They used a mouse model of bacterial arthritis (*Staphylococcus aureus* infection in the knee joint) and observed a strong infiltration of neutrophils in the PDT-treated area. In order to investigate the role of neutrophils in the PDT-mediated

bacteria inactivation, they administered anti-GR-1 (anti-neutrophil) antibody as well as antibodies to several pro-inflammatory mediators. The administration of such antibodies resulted in loss of the therapeutic effect of PDT. This suggests that not only killing of bacteria, but also attraction and accumulation of neutrophils into the infected regions were required mechanisms to achieve PDT-mediated clearance of bacterial infections. Additionally, PDT was tested also as a preventive therapeutic approach and delivered prior to the bacterial inoculation into the knee. PDT-mediated infiltration of neutrophils prevented the subsequent inoculation of bacteria from establishing the infection and again, such an effect was abrogated when antibodies against GR-1 and proinflammatory mediators were administered. To the best of our knowledge, this is the first demonstration of a protective innate immune response against a microbial pathogen being induced by PDT. It is well known that bacterial phagocytosis by innate immune cells such as neutrophils, plays a crucial role in the elimination of invading bacteria and, therefore, malfunction of the phagocytic immune system renders the host more susceptible to bacterial infections. Hence, it would be desirable to apply an antimicrobial PDT regimen that causes direct photoinactivation of bacteria, but at the same time that can minimize the damage to the host's neutrophils.

As described above, evidence indicated that PDT of cancer triggers the activation of both innate and adaptive arms of the immune system, while the early results from the bacterial infection models suggest that PDT is capable of stimulating (at least) the innate immune system. The biggest difference, however, could be in the stimulation of T- and B-cell-mediated adaptive immune responses. As antibodies produced by B cells are generally the most effective component of the immune response against bacterial infection, B cells are expected to be the main actors in the post-PDT immune response towards bacteria. However, to the best of our knowledge, nothing is known yet about humoral responses induced by PDT against bacterial infection.

On the other side, while the involvement of B cells in PDT-induced antitumor immunity still needs more investigation, it is widely accepted that the activation of T cell responses play a pivotal role in PDT-mediated immunity towards treated tumors.

CONCLUSION

Several studies in pre-clinical and clinical settings have demonstrated that PDT is capable of pronouncedly activating both the innate and adaptive arms of the immune system. Such effects on the immune system appear to be PDT regimen dependent and strictly linked to the degree of inflammation induced by PDT.

It has been speculated that PDT regimens causing a high degree of acute inflammation are better at immune activation compared to those in which the acute inflammation is lower. However, increase in inflammatory

mediators could promote tumor cell growth in certain circumstances^[101]. Moreover, PDT has been linked also to immunosuppressive effects. Such immunosuppressive effects have been established in model of suppression of induction of contact hypersensitivity (*i.e.*, afferent immune response), which involves the application of a hapten to the skin, followed by re-challenge^[102], and suppression of delayed-type hypersensitivity (Mantoux) reactions (*i.e.*, efferent immune response) for instance in healthy Mantoux-positive volunteers^[103,104]. In particular, such immunosuppressive responses seem to be dependent on the rate of light delivery^[105] and anatomic site of PDT^[106].

Further studies using a better targeted and dose-controlled PDT treatment would help to expand the knowledge on the activation/suppression of the immune system and the possibilities to improve it in clinical practice.

The proven ability of PDT to trigger inflammation and improve the anti-tumor immune response could be successfully employed in tandem with other treatment modalities, to combat cancer and to achieve long-term tumor control. Nevertheless, up to now PDT remains clinically underutilized. We must realize that with all probability it will take several years of further investigations and clinical trials before the use of PDT becomes a clinically accepted standard practice in cancer patients.

The innate immune responses seem to be of crucial importance also in the relatively new field of PDT as anti-microbial treatment. The activation of neutrophils after PDT, their mobilization from the bone marrow and their attraction to the site of inflammation appear to be important mechanisms, significantly potentiating the antibacterial effects, *e.g.*, in bacterial arthritis mouse models. However, it still remains to be elucidated whether the activation of the host neutrophils is applicable also to other infection models, with other classes of pathogens and/or using different PS. Many years of intense research will be required providing answers to these intriguing questions.

REFERENCES

- 1 **Dougherty TJ**, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbek M, Moan J, Peng Q. Photodynamic therapy. *J Natl Cancer Inst* 1998; **90**: 889-905 [PMID: 9637138]
- 2 **Dolmans DE**, Fukumura D, Jain RK. Photodynamic therapy for cancer. *Nat Rev Cancer* 2003; **3**: 380-387 [PMID: 12724736 DOI: 10.1038/nrc1071]
- 3 **Ochsner M**. Photophysical and photobiological processes in the photodynamic therapy of tumours. *J Photochem Photobiol B* 1997; **39**: 1-18 [PMID: 9210318]
- 4 **Agostinis P**, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, Hahn SM, Hamblin MR, Juzeniene A, Kessel D, Korbek M, Moan J, Mroz P, Nowis D, Piette J, Wilson BC, Golab J. Photodynamic therapy of cancer: an update. *CA Cancer J Clin* 2011; **61**: 250-281 [PMID: 21617154 DOI: 10.3322/caac.20114]
- 5 **Oleinick NL**, Morris RL, Belichenko I. The role of apoptosis in response to photodynamic therapy: what, where, why, and how. *Photochem Photobiol Sci* 2002; **1**: 1-21 [PMID: 12659143]
- 6 **Krammer B**. Vascular effects of photodynamic therapy. *Anticancer Res* 2001; **21**: 4271-4277 [PMID: 11908681]
- 7 **Dolmans DE**, Kadambi A, Hill JS, Waters CA, Robinson BC, Walker JP, Fukumura D, Jain RK. Vascular accumulation of a novel photosensitizer, MV6401, causes selective thrombosis in tumor vessels after photodynamic therapy. *Cancer Res* 2002; **62**: 2151-2156 [PMID: 11929837]
- 8 **Korbek M**, Kros J, Kros J, Dougherty GJ. The role of host lymphoid populations in the response of mouse EMT6 tumor to photodynamic therapy. *Cancer Res* 1996; **56**: 5647-5652 [PMID: 8971170]
- 9 **Korbek M**, Cecic I. Contribution of myeloid and lymphoid host cells to the curative outcome of mouse sarcoma treatment by photodynamic therapy. *Cancer Lett* 1999; **137**: 91-98 [PMID: 10376798]
- 10 **Tanaka M**, Mroz P, Dai T, Huang L, Morimoto Y, Kinoshita M, Yoshihara Y, Nemoto K, Shinomiya N, Seki S, Hamblin MR. Photodynamic therapy can induce a protective innate immune response against murine bacterial arthritis via neutrophil accumulation. *PLoS One* 2012; **7**: e39823 [PMID: 22761911 DOI: 10.1371/journal.pone.0039823]
- 11 **Garg AD**, Nowis D, Golab J, Vandenabeele P, Krysko DV, Agostinis P. Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation. *Biochim Biophys Acta* 2010; **1805**: 53-71 [PMID: 19720113 DOI: 10.1016/j.bbcan.2009.08.003]
- 12 **Garg AD**, Nowis D, Golab J, Agostinis P. Photodynamic therapy: illuminating the road from cell death towards anti-tumour immunity. *Apoptosis* 2010; **15**: 1050-1071 [PMID: 20221698 DOI: 10.1007/s10495-010-0479-7]
- 13 **Bianchi ME**. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007; **81**: 1-5 [PMID: 17032697 DOI: 10.1189/jlb.0306164]
- 14 **Manfredi AA**, Capobianco A, Bianchi ME, Rovere-Querini P. Regulation of dendritic- and T-cell fate by injury-associated endogenous signals. *Crit Rev Immunol* 2009; **29**: 69-86 [PMID: 19348611]
- 15 **Melcher A**, Todryk S, Hardwick N, Ford M, Jacobson M, Vile RG. Tumor immunogenicity is determined by the mechanism of cell death via induction of heat shock protein expression. *Nat Med* 1998; **4**: 581-587 [PMID: 9585232]
- 16 **Tesniere A**, Panaretakis T, Kepp O, Apetoh L, Ghiringhelli F, Zitvogel L, Kroemer G. Molecular characteristics of immunogenic cancer cell death. *Cell Death Differ* 2008; **15**: 3-12 [PMID: 18007663 DOI: 10.1038/sj.cdd.4402269]
- 17 **Scheffer SR**, Nave H, Korangy F, Schlote K, Pabst R, Jaffee EM, Manns MP, Greten TF. Apoptotic, but not necrotic, tumor cell vaccines induce a potent immune response in vivo. *Int J Cancer* 2003; **103**: 205-211 [PMID: 12455034 DOI: 10.1002/ijc.10777]
- 18 **Goldszmid RS**, Idoyaga J, Bravo AI, Steinman R, Mordoh J, Wainstok R. Dendritic cells charged with apoptotic tumor cells induce long-lived protective CD4+ and CD8+ T cell immunity against B16 melanoma. *J Immunol* 2003; **171**: 5940-5947 [PMID: 14634105]
- 19 **Kepp O**, Tesniere A, Schlemmer F, Michaud M, Senovilla L, Zitvogel L, Kroemer G. Immunogenic cell death modalities and their impact on cancer treatment. *Apoptosis* 2009; **14**: 364-375 [PMID: 19145485 DOI: 10.1007/s10495-008-0303-9]
- 20 **Zitvogel L**, Kroemer G. The immune response against dying tumor cells: avoid disaster, achieve cure. *Cell Death Differ* 2008; **15**: 1-2 [PMID: 18084310 DOI: 10.1038/sj.cdd.4402267]
- 21 **Garg AD**, Krysko DV, Vandenabeele P, Agostinis P. DAMPs and PDT-mediated photo-oxidative stress: exploring the unknown. *Photochem Photobiol Sci* 2011; **10**: 670-680 [PMID: 21258717 DOI: 10.1039/c0pp00294a]
- 22 **Korbek M**, Sun J, Cecic I. Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response. *Cancer Res* 2005; **65**: 1018-1026 [PMID: 15705903]
- 23 **Hsieh YJ**, Wu CC, Chang CJ, Yu JS. Subcellular localization of Photofrin determines the death phenotype of human epidermoid carcinoma A431 cells triggered by photodynamic therapy: when plasma membranes are the main targets. *J Cell*

- Physiol* 2003; **194**: 363-375 [PMID: 12548556 DOI: 10.1002/jcp.10273]
- 24 **Szokalska A**, Makowski M, Nowis D, Wilczynski GM, Kujawa M, Wójcik C, Mlynarczuk-Bialy I, Salwa P, Bil J, Janowska S, Agostinis P, Verfaillie T, Bugajski M, Gietka J, Issat T, Glodkowska E, Mrówka P, Stoklosa T, Hamblin MR, Mróz P, Jakóbsiak M, Gołab J. Proteasome inhibition potentiates antitumor effects of photodynamic therapy in mice through induction of endoplasmic reticulum stress and unfolded protein response. *Cancer Res* 2009; **69**: 4235-4243 [PMID: 19435917 DOI: 10.1158/0008-5472.CAN-08-3439]
 - 25 **Gomer CJ**, Ryter SW, Ferrario A, Rucker N, Wong S, Fisher AM. Photodynamic therapy-mediated oxidative stress can induce expression of heat shock proteins. *Cancer Res* 1996; **56**: 2355-2360 [PMID: 8625311]
 - 26 **Gollnick SO**, Evans SS, Baumann H, Owczarczak B, Maier P, Vaughan L, Wang WC, Unger E, Henderson BW. Role of cytokines in photodynamic therapy-induced local and systemic inflammation. *Br J Cancer* 2003; **88**: 1772-1779 [PMID: 12771994 DOI: 10.1038/sj.bjc.6600864]
 - 27 **Korbelik M**. PDT-associated host response and its role in the therapy outcome. *Lasers Surg Med* 2006; **38**: 500-508 [PMID: 16634073 DOI: 10.1002/lsm.20337]
 - 28 **Krosi G**, Korbelik M, Dougherty GJ. Induction of immune cell infiltration into murine SCCVII tumour by photofrin-based photodynamic therapy. *Br J Cancer* 1995; **71**: 549-555 [PMID: 7880738]
 - 29 **de Vree WJ**, Essers MC, Koster JF, Sluiter W. Role of interleukin 1 and granulocyte colony-stimulating factor in photofrin-based photodynamic therapy of rat rhabdomyosarcoma tumors. *Cancer Res* 1997; **57**: 2555-2558 [PMID: 9205052]
 - 30 **Kousis PC**, Henderson BW, Maier PG, Gollnick SO. Photodynamic therapy enhancement of antitumor immunity is regulated by neutrophils. *Cancer Res* 2007; **67**: 10501-10510 [PMID: 17974994]
 - 31 **Hunt DW**, Levy JG. Immunomodulatory aspects of photodynamic therapy. *Expert Opin Investig Drugs* 1998; **7**: 57-64 [PMID: 15991918 DOI: 10.1517/13543784.7.1.57]
 - 32 **Sun J**, Cecic I, Parkins CS, Korbelik M. Neutrophils as inflammatory and immune effectors in photodynamic therapy-treated mouse SCCVII tumours. *Photochem Photobiol Sci* 2002; **1**: 690-695 [PMID: 12665307]
 - 33 **Cecic I**, Stott B, Korbelik M. Acute phase response-associated systemic neutrophil mobilization in mice bearing tumors treated by photodynamic therapy. *Int Immunopharmacol* 2006; **6**: 1259-1266 [PMID: 16782538 DOI: 10.1016/j.intimp.2006.03.008]
 - 34 **Korbelik M**, Cecic I, Merchant S, Sun J. Acute phase response induction by cancer treatment with photodynamic therapy. *Int J Cancer* 2008; **122**: 1411-1417 [PMID: 18033689 DOI: 10.1002/ijc.23248]
 - 35 **Merchant S**, Korbelik M. Upregulation of genes for C-reactive protein and related pentraxin/complement proteins in photodynamic therapy-treated human tumor cells: enrolment of PI3K/Akt and AP-1. *Immunobiology* 2013; **218**: 869-874 [PMID: 23182717 DOI: 10.1016/j.imbio.2012.10.010]
 - 36 **Saevarsdottir S**, Vikingsdottir T, Valdimarsson H. The potential role of mannan-binding lectin in the clearance of self-components including immune complexes. *Scand J Immunol* 2004; **60**: 23-29 [PMID: 15238070 DOI: 10.1111/j.0300-9475.2004.01437.x]
 - 37 **Cecic I**, Korbelik M. Mediators of peripheral blood neutrophilia induced by photodynamic therapy of solid tumors. *Cancer Lett* 2002; **183**: 43-51 [PMID: 12049813]
 - 38 **Jalili A**, Makowski M, Switaj T, Nowis D, Wilczynski GM, Wilczek E, Chorazy-Massalska M, Radzikowska A, Maslinski W, Bialy L, Sienko J, Sieron A, Adamek M, Basak G, Mróz P, Krasnodebski IW, Jakóbsiak M, Gołab J. Effective photoimmunotherapy of murine colon carcinoma induced by the combination of photodynamic therapy and dendritic cells. *Clin Cancer Res* 2004; **10**: 4498-4508 [PMID: 15240542 DOI: 10.1158/1078-0432.CCR-04-0367]
 - 39 **Vabulas RM**, Wagner H, Schild H. Heat shock proteins as ligands of toll-like receptors. *Curr Top Microbiol Immunol* 2002; **270**: 169-184 [PMID: 12467251]
 - 40 **Marzo AL**, Vezys V, Klonowski KD, Lee SJ, Muralimohan G, Moore M, Tough DF, Lefrançois L. Fully functional memory CD8 T cells in the absence of CD4 T cells. *J Immunol* 2004; **173**: 969-975 [PMID: 15240684]
 - 41 **Wang J**, Santosuosso M, Ngai P, Zganiacz A, Xing Z. Activation of CD8 T cells by mycobacterial vaccination protects against pulmonary tuberculosis in the absence of CD4 T cells. *J Immunol* 2004; **173**: 4590-4597 [PMID: 15383593]
 - 42 **Castellino F**, Germain RN. Cooperation between CD4+ and CD8+ T cells: when, where, and how. *Annu Rev Immunol* 2006; **24**: 519-540 [PMID: 16551258 DOI: 10.1146/annurev.immunol.23.021704.115825]
 - 43 **Kabingu E**, Vaughan L, Owczarczak B, Ramsey KD, Gollnick SO. CD8+ T cell-mediated control of distant tumours following local photodynamic therapy is independent of CD4+ T cells and dependent on natural killer cells. *Br J Cancer* 2007; **96**: 1839-1848 [PMID: 17505510 DOI: 10.1038/sj.bjc.6603792]
 - 44 **Preise D**, Oren R, Glinert I, Kalchenko V, Jung S, Scherz A, Salomon Y. Systemic antitumor protection by vascular-targeted photodynamic therapy involves cellular and humoral immunity. *Cancer Immunol Immunother* 2009; **58**: 71-84 [PMID: 18488222 DOI: 10.1007/s00262-008-0527-0]
 - 45 **Van den Eynde BJ**, van der Bruggen P. T cell defined tumor antigens. *Curr Opin Immunol* 1997; **9**: 684-693 [PMID: 9368778]
 - 46 **Van den Eynde B**, Lethé B, Van Pel A, De Plaen E, Boon T. The gene coding for a major tumor rejection antigen of tumor P815 is identical to the normal gene of syngeneic DBA/2 mice. *J Exp Med* 1991; **173**: 1373-1384 [PMID: 1903428]
 - 47 **Van den Eynde B**, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. *J Exp Med* 1995; **182**: 689-698 [PMID: 7544395]
 - 48 **van der Bruggen P**, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; **254**: 1643-1647 [PMID: 1840703]
 - 49 **Gaugler B**, Van den Eynde B, van der Bruggen P, Romero P, Gafurio JJ, De Plaen E, Lethé B, Brasseur F, Boon T. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. *J Exp Med* 1994; **179**: 921-930 [PMID: 8113684]
 - 50 **Traversari C**, van der Bruggen P, Luescher IF, Lurquin C, Chomez P, Van Pel A, De Plaen E, Amar-Costesec A, Boon T. A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. *J Exp Med* 1992; **176**: 1453-1457 [PMID: 1402688]
 - 51 **Coulie PG**, Brichard V, Van Pel A, Wölfel T, Schneider J, Traversari C, Mattei S, De Plaen E, Lurquin C, Szikora JP, Renauld JC, Boon T. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med* 1994; **180**: 35-42 [PMID: 8006593]
 - 52 **Brichard V**, Van Pel A, Wölfel T, Wölfel C, De Plaen E, Lethé B, Coulie P, Boon T. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med* 1993; **178**: 489-495 [PMID: 8340755]
 - 53 **Bakker AB**, Schreurs MW, de Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. *J Exp Med* 1994; **179**: 1005-1009 [PMID: 8113668]
 - 54 **Coulie PG**, Lehmann F, Lethé B, Herman J, Lurquin C, Andrawiss M, Boon T. A mutated intron sequence codes for

- an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. *Proc Natl Acad Sci USA* 1995; **92**: 7976-7980 [PMID: 7644523]
- 55 **Mandelboim O**, Berke G, Fridkin M, Feldman M, Eisenstein M, Eisenbach L. CTL induction by a tumour-associated antigen octapeptide derived from a murine lung carcinoma. *Nature* 1994; **369**: 67-71 [PMID: 8164742 DOI: 10.1038/369067a0]
 - 56 **Monach PA**, Meredith SC, Siegel CT, Schreiber H. A unique tumor antigen produced by a single amino acid substitution. *Immunity* 1995; **2**: 45-59 [PMID: 7600302]
 - 57 **Robbins PF**, El-Gamil M, Li YF, Kawakami Y, Loftus D, Appella E, Rosenberg SA. A mutated beta-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J Exp Med* 1996; **183**: 1185-1192 [PMID: 8642260]
 - 58 **Dubey P**, Hendrickson RC, Meredith SC, Siegel CT, Shabanowitz J, Skipper JC, Engelhard VH, Hunt DF, Schreiber H. The immunodominant antigen of an ultraviolet-induced regressor tumor is generated by a somatic point mutation in the DEAD box helicase p68. *J Exp Med* 1997; **185**: 695-705 [PMID: 9034148]
 - 59 **Laheru D**, Jaffee EM. Immunotherapy for pancreatic cancer - science driving clinical progress. *Nat Rev Cancer* 2005; **5**: 459-467 [PMID: 15905855 DOI: 10.1038/nrc1630]
 - 60 **Marshall JL**, Gulley JL, Arlen PM, Beetham PK, Tsang KY, Slack R, Hodge JW, Doren S, Grosenbach DW, Hwang J, Fox E, Odogwu L, Park S, Panicali D, Schlom J. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen-expressing carcinomas. *J Clin Oncol* 2005; **23**: 720-731 [PMID: 15613691]
 - 61 **Castano AP**, Liu Q, Hamblin MR. A green fluorescent protein-expressing murine tumour but not its wild-type counterpart is cured by photodynamic therapy. *Br J Cancer* 2006; **94**: 391-397 [PMID: 16421588 DOI: 10.1038/sj.bjc.6602953]
 - 62 **Mroz P**, Szokalska A, Wu MX, Hamblin MR. Photodynamic therapy of tumors can lead to development of systemic antigen-specific immune response. *PLoS One* 2010; **5**: e15194 [PMID: 21179470 DOI: 10.1371/journal.pone.0015194]
 - 63 **Mroz P**, Vatansever F, Muchowicz A, Hamblin MR. Photodynamic therapy of murine mastocytoma induces specific immune responses against the cancer/testis antigen P1A. *Cancer Res* 2013; **73**: 6462-6470 [PMID: 24072749 DOI: 10.1158/0008-5472.CAN-11-2572]
 - 64 **Brändle D**, Bilsborough J, Rülcke T, Uyttenhove C, Boon T, Van den Eynde BJ. The shared tumor-specific antigen encoded by mouse gene P1A is a target not only for cytolytic T lymphocytes but also for tumor rejection. *Eur J Immunol* 1998; **28**: 4010-4019 [PMID: 9862337 DOI: 10.1002/(SICI)1521-4141(199812)28:12<4010::AID-IMMU4010>3.0.CO;2-5]
 - 65 **Kabingu E**, Oseroff AR, Wilding GE, Gollnick SO. Enhanced systemic immune reactivity to a Basal cell carcinoma associated antigen following photodynamic therapy. *Clin Cancer Res* 2009; **15**: 4460-4466 [PMID: 19549769 DOI: 10.1158/1078-0432.CCR-09-0400]
 - 66 **Campbell DJ**, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. *Nat Rev Immunol* 2011; **11**: 119-130 [PMID: 21267013 DOI: 10.1038/nri2916]
 - 67 **Dejaco C**, Duftner C, Grubeck-Loebenstien B, Schirmer M. Imbalance of regulatory T cells in human autoimmune diseases. *Immunology* 2006; **117**: 289-300 [PMID: 16476048 DOI: 10.1111/j.1365-2567.2005.02317.x]
 - 68 **Yamaguchi T**, Sakaguchi S. Regulatory T cells in immune surveillance and treatment of cancer. *Semin Cancer Biol* 2006; **16**: 115-123 [PMID: 16376102 DOI: 10.1016/j.semcancer.2005.11.005]
 - 69 **Bluestone JA**, Tang Q. How do CD4+CD25+ regulatory T cells control autoimmunity? *Curr Opin Immunol* 2005; **17**: 638-642 [PMID: 16209918 DOI: 10.1016/j.coi.2005.09.002]
 - 70 **Tang Q**, Boden EK, Henriksen KJ, Bour-Jordan H, Bi M, Bluestone JA. Distinct roles of CTLA-4 and TGF-beta in CD4+CD25+ regulatory T cell function. *Eur J Immunol* 2004; **34**: 2996-3005 [PMID: 15468055 DOI: 10.1002/eji.200425143]
 - 71 **Zou W**. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol* 2006; **6**: 295-307 [PMID: 16557261 DOI: 10.1038/nri1806]
 - 72 **Chen W**, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25-naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003; **198**: 1875-1886 [PMID: 14676299 DOI: 10.1084/jem.20030152]
 - 73 **Shimizu J**, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25+CD4+ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 1999; **163**: 5211-5218 [PMID: 10553041]
 - 74 **Golgher D**, Jones E, Powrie F, Elliott T, Gallimore A. Depletion of CD25+ regulatory cells uncovers immune responses to shared murine tumor rejection antigens. *Eur J Immunol* 2002; **32**: 3267-3275 [PMID: 12555672]
 - 75 **Tanaka H**, Tanaka J, Kjaergaard J, Shu S. Depletion of CD4+CD25+ regulatory cells augments the generation of specific immune T cells in tumor-draining lymph nodes. *J Immunother* 2002; **25**: 207-217 [PMID: 12000862]
 - 76 **Lutsiak ME**, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood* 2005; **105**: 2862-2868 [PMID: 15591121 DOI: 10.1182/blood-2004-06-2410]
 - 77 **Castano AP**, Mroz P, Wu MX, Hamblin MR. Photodynamic therapy plus low-dose cyclophosphamide generates antitumor immunity in a mouse model. *Proc Natl Acad Sci USA* 2008; **105**: 5495-5500 [PMID: 18378905 DOI: 10.1073/pnas.0709256105]
 - 78 **Reginato E**, Mroz P, Chung H, Kawakubo M, Wolf P, Hamblin MR. Photodynamic therapy plus regulatory T-cell depletion produces immunity against a mouse tumour that expresses a self-antigen. *Br J Cancer* 2013; **109**: 2167-2174 [PMID: 24064977 DOI: 10.1038/bjc.2013.580]
 - 79 **Von Tappeiner H**. Therapeutische Versuche mit fluoreszierenden Stoffen. *Muench Med Wochenschr* 1903; **47**: 2042-2044
 - 80 **Kelly JF**, Snell ME. Hematoporphyrin derivative: a possible aid in the diagnosis and therapy of carcinoma of the bladder. *J Urol* 1976; **115**: 150-151 [PMID: 1249866]
 - 81 **Dougherty TJ**, Kaufman JE, Goldfarb A, Weishaupt KR, Boyle D, Mittleman A. Photoradiation therapy for the treatment of malignant tumors. *Cancer Res* 1978; **38**: 2628-2635 [PMID: 667856]
 - 82 **Dragieva G**, Hafner J, Dummer R, Schmid-Grendelmeier P, Roos M, Prinz BM, Burg G, Binswanger U, Kempf W. Topical photodynamic therapy in the treatment of actinic keratoses and Bowen's disease in transplant recipients. *Transplantation* 2004; **77**: 115-121 [PMID: 14724445 DOI: 10.1097/01.TP.0000107284.04969.5C]
 - 83 **Abdel-Hady ES**, Martin-Hirsch P, Duggan-Keen M, Stern PL, Moore JV, Corbitt G, Kitchener HC, Hampson IN. Immunological and viral factors associated with the response of vulval intraepithelial neoplasia to photodynamic therapy. *Cancer Res* 2001; **61**: 192-196 [PMID: 11196160]
 - 84 **Thong PS**, Ong KW, Goh NS, Kho KW, Manivasager V, Bhuvaneshwari R, Olivo M, Soo KC. Photodynamic-therapy-activated immune response against distant untreated tumours in recurrent angiosarcoma. *Lancet Oncol* 2007; **8**: 950-952 [PMID: 17913664 DOI: 10.1016/S1470-2045(07)70318-2]
 - 85 **Hamblin MR**, Mroz P. History of PDT - the first hundred years, in *Advances in photodynamic therapy: basic, translational and clinical*. Hamblin M, Mroz P (eds). Artech House, Norwood, MA, 2008: 1-12
 - 86 **Nitzan Y**, Gutterman M, Malik Z, Ehrenberg B. Inactivation of gram-negative bacteria by photosensitized porphyrins. *Photochem Photobiol* 1992; **55**: 89-96 [PMID: 1534909]
 - 87 **Malik Z**, Ladan H, Nitzan Y. Photodynamic inactivation of

- Gram-negative bacteria: problems and possible solutions. *J Photochem Photobiol B* 1992; **14**: 262-266 [PMID: 1432395]
- 88 **Hamblin MR**, Zahra T, Contag CH, McManus AT, Hasan T. Optical monitoring and treatment of potentially lethal wound infections in vivo. *J Infect Dis* 2003; **187**: 1717-1725 [PMID: 12751029 DOI: 10.1086/375244]
 - 89 **Hamblin MR**, O'Donnell DA, Murthy N, Contag CH, Hasan T. Rapid control of wound infections by targeted photodynamic therapy monitored by in vivo bioluminescence imaging. *Photochem Photobiol* 2002; **75**: 51-57 [PMID: 11837327]
 - 90 **Dai T**, Tegos GP, Lu Z, Huang L, Zhiyentayev T, Franklin MJ, Baer DG, Hamblin MR. Photodynamic therapy for *Acinetobacter baumannii* burn infections in mice. *Antimicrob Agents Chemother* 2009; **53**: 3929-3934 [PMID: 19564369 DOI: 10.1128/AAC.00027-09]
 - 91 **Lambrechts SA**, Demidova TN, Aalders MC, Hasan T, Hamblin MR. Photodynamic therapy for *Staphylococcus aureus* infected burn wounds in mice. *Photochem Photobiol Sci* 2005; **4**: 503-509 [PMID: 15986057 DOI: 10.1039/b502125a]
 - 92 **Smijls TG**, van der Haas RN, Lugtenburg J, Liu Y, de Jong RL, Schuitmaker HJ. Photodynamic treatment of the dermatophyte *Trichophyton rubrum* and its microconidia with porphyrin photosensitizers. *Photochem Photobiol* 2004; **80**: 197-202 [PMID: 15244503 DOI: 10.1562/2004-04-22-RA-146]
 - 93 **Friedberg JS**, Skema C, Baum ED, Burdick J, Vinogradov SA, Wilson DF, Horan AD, Nachamkin I. In vitro effects of photodynamic therapy on *Aspergillus fumigatus*. *J Antimicrob Chemother* 2001; **48**: 105-107 [PMID: 11418518]
 - 94 **Paardekooper M**, De Bruijine AW, Van Steveninck J, Van den Broek PJ. Intracellular damage in yeast cells caused by photodynamic treatment with toluidine blue. *Photochem Photobiol* 1995; **61**: 84-89 [PMID: 7899497]
 - 95 **Chabrier-Roselló Y**, Foster TH, Pérez-Nazario N, Mitra S, Haidaris CG. Sensitivity of *Candida albicans* germ tubes and biofilms to photofrin-mediated phototoxicity. *Antimicrob Agents Chemother* 2005; **49**: 4288-4295 [PMID: 16189110 DOI: 10.1128/AAC.49.10.4288-4295.2005]
 - 96 **Lambrechts SA**, Aalders MC, Van Marle J. Mechanistic study of the photodynamic inactivation of *Candida albicans* by a cationic porphyrin. *Antimicrob Agents Chemother* 2005; **49**: 2026-2034 [PMID: 15855528 DOI: 10.1128/AAC.49.5.2026-2034.2005]
 - 97 **Müller-Breitkreutz K**, Mohr H. Infection cycle of herpes viruses after photodynamic treatment with methylene blue and light. *Beitr Infusionsther Transfusionsmed* 1997; **34**: 37-42 [PMID: 9356656]
 - 98 **Käsermann F**, Kempf C. Photodynamic inactivation of enveloped viruses by buckminsterfullerene. *Antiviral Res* 1997; **34**: 65-70 [PMID: 9107386]
 - 99 **Vatansever F**, Ferraresi C, de Sousa MV, Yin R, Rineh A, Sharma SK, Hamblin MR. Can biowarfare agents be defeated with light? *Virulence* 2013; **4**: 796-825 [PMID: 24067444]
 - 100 **Kharkwal GB**, Sharma SK, Huang YY, Dai T, Hamblin MR. Photodynamic therapy for infections: clinical applications. *Lasers Surg Med* 2011; **43**: 755-767 [PMID: 22057503 DOI: 10.1002/lsm.21080]
 - 101 **Sgambato A**, Cittadini A. Inflammation and cancer: a multifaceted link. *Eur Rev Med Pharmacol Sci* 2010; **14**: 263-268 [PMID: 20496533]
 - 102 **Mroz P**, Hamblin MR. The immunosuppressive side of PDT. *Photochem Photobiol Sci* 2011; **10**: 751-758 [PMID: 21437314 DOI: 10.1039/c0pp00345j]
 - 103 **Matthews YJ**, Damian DL. Topical photodynamic therapy is immunosuppressive in humans. *Br J Dermatol* 2010; **162**: 637-641 [PMID: 19863500 DOI: 10.1111/j.1365-2133.2009.09562.x]
 - 104 **Thanos SM**, Halliday GM, Damian DL. Nicotinamide reduces photodynamic therapy-induced immunosuppression in humans. *Br J Dermatol* 2012; **167**: 631-636 [PMID: 22709272 DOI: 10.1111/j.1365-2133.2012.11109.x]
 - 105 **Frost GA**, Halliday GM, Damian DL. Photodynamic therapy-induced immunosuppression in humans is prevented by reducing the rate of light delivery. *J Invest Dermatol* 2011; **131**: 962-968 [PMID: 21248771 DOI: 10.1038/jid.2010.429]
 - 106 **Musser DA**, Camacho SH, Manderscheid PA, Oseroff AR. The anatomic site of photodynamic therapy is a determinant for immunosuppression in a murine model. *Photochem Photobiol* 1999; **69**: 222-225 [PMID: 10048313]
 - 107 **Evans S**, Matthews W, Perry R, Fraker D, Norton J, Pass HI. Effect of photodynamic therapy on tumor necrosis factor production by murine macrophages. *J Natl Cancer Inst* 1990; **82**: 34-39 [PMID: 2293654]
 - 108 **Saji H**, Song W, Furumoto K, Kato H, Engleman EG. Systemic antitumor effect of intratumoral injection of dendritic cells in combination with local photodynamic therapy. *Clin Cancer Res* 2006; **12**: 2568-2574 [PMID: 16638867 DOI: 10.1158/1078-0432.CCR-05-1986]
 - 109 **de Vree WJ**, Essers MC, de Bruijn HS, Star WM, Koster JF, Sluiter W. Evidence for an important role of neutrophils in the efficacy of photodynamic therapy in vivo. *Cancer Res* 1996; **56**: 2908-2911 [PMID: 8674038]
 - 110 **Canti G**, Lattuada D, Nicolin A, Taroni P, Valentini G, Cubeddu R. Antitumor immunity induced by photodynamic therapy with aluminum disulfonated phthalocyanines and laser light. *Anticancer Drugs* 1994; **5**: 443-447 [PMID: 7949249]
 - 111 **Korbelik M**, Dougherty GJ. Photodynamic therapy-mediated immune response against subcutaneous mouse tumors. *Cancer Res* 1999; **59**: 1941-1946 [PMID: 10213504]
 - 112 **Zitvogel L**, Kepp O, Kroemer G. Decoding cell death signals in inflammation and immunity. *Cell* 2010; **140**: 798-804 [PMID: 20303871 DOI: 10.1016/j.cell.2010.02.015]
 - 113 **Subbarayan M**, Häfeli UO, Feyes DK, Unnithan J, Emancipator SN, Mukhtar H. A simplified method for preparation of ^{99m}Tc-annexin V and its biologic evaluation for in vivo imaging of apoptosis after photodynamic therapy. *J Nucl Med* 2003; **44**: 650-656 [PMID: 12679412]
 - 114 **Donato R**. RAGE: a single receptor for several ligands and different cellular responses: the case of certain S100 proteins. *Curr Mol Med* 2007; **7**: 711-724 [PMID: 18331229]
 - 115 **Mroz P**, Hashmi JT, Huang YY, Lange N, Hamblin MR. Stimulation of anti-tumor immunity by photodynamic therapy. *Expert Rev Clin Immunol* 2011; **7**: 75-91 [PMID: 21162652 DOI: 10.1586/eci.10.81]
 - 116 **Castano AP**, Mroz P, Hamblin MR. Photodynamic therapy and anti-tumour immunity. *Nat Rev Cancer* 2006; **6**: 535-545 [PMID: 16794636 DOI: 10.1038/nrc1894]

P- Reviewers: Kwon HJ, Nagata T, Yun CH **S- Editor:** Song XX

L- Editor: A **E- Editor:** Liu SQ



Circulating immune cell activation and diet: A review on human trials

Gemma Chiva-Blanch, Ramon Estruch

Gemma Chiva-Blanch, Ramon Estruch, Department of Internal Medicine, Hospital Clinic, 08036 Barcelona, Spain
 Gemma Chiva-Blanch, Ramon Estruch, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), University of Barcelona, 08041 Barcelona, Spain

Author contributions: Chiva-Blanch G substantially contributed to the conception and design and drafted the article; Estruch R revised it critically for important intellectual content; and Chiva-Blanch G and Estruch R finally approved the version to be published.

Correspondence to: Ramon Estruch, MD, PhD, Department of Internal Medicine, Hospital Clinic, Villarroel 170, 08036 Barcelona, Spain. restruch@clinic.ub.es

Telephone: +34-93-2275400 Fax: +34-93-2279365

Received: October 21, 2013 Revised: December 5, 2013

Accepted: February 16, 2014

Published online: March 27, 2014

tion; Leukocyte adhesion; Inflammation; Fatty acids; Fruits; Vegetables; Polyphenols; Cardiovascular disease; Cancer

Core tip: Immune cell activation is involved in several pathophysiological processes that play a crucial role in the appearance of cardiovascular disease or cancer. The aim of this review is to update the knowledge of the modulation of immune cell activation by different dietary patterns. A westernized high-saturated fat high-carbohydrate diet is positively associated with low-grade inflammation, but a Mediterranean diet, rich in vegetables and fruits decrease the levels of cellular and circulating inflammatory biomarkers thereby reducing the risk of related chronic diseases.

Chiva-Blanch G, Estruch R. Circulating immune cell activation and diet: A review on human trials. *World J Immunol* 2014; 4(1): 12-19 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i1/12.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i1.12>

Abstract

Protein energy malnutrition is the main cause of immunodeficiency and, secondarily, of several infections. However, immune cell activation is involved in several pathophysiological processes that play a crucial role in the appearance of cardiovascular disease (CVD) or cancer. The aim of this review is to update the knowledge of the modulation of immune cell activation by different dietary patterns and its components focusing on CVD or cancer. While a westernized high-saturated fat high-carbohydrate diet is positively associated with low-grade inflammation, vegetable- and fruit-based diets rich in monounsaturated fatty acids, polyunsaturated fatty acids and polyphenols, key nutrients of Mediterranean diet, decrease the levels of cellular and circulating inflammatory biomarkers thereby reducing the risk of related chronic diseases.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Mediterranean diet; Immune cell activa-

INTRODUCTION

Although some food compounds can originate several immune reactions such as allergies or celiac disease^[1], most of nutrients in the diet are essential for maintaining the function of immune cells. Thus, protein energy malnutrition is the main cause of immunodeficiency worldwide^[2] and deficits of nutrients commonly included in the diet such as vitamin E, vitamin C, β -carotene, selenium, copper, iron and zinc modify different immune functions related to infections caused by bacteria, viruses or parasites. A deficiency in vitamin E diminishes the ability of the immune system to respond to infectious microorganisms and under some conditions, a pharmacological level of vitamin E is needed to achieve an optimal immune response suggesting that the recommended dietary allowances for vitamin E might not be adequate for immuno-

logical vigor and health^[3]. Therefore, the knowledge of the effects of nutrition on immune function now extends beyond clinical nutrient deficiency. A growing body of literature demonstrates the immune benefits of increasing the intake of some specific nutrients. Compared with human infants fed formula without nucleotides, infants fed breast milk or formula supplemented with nucleotides had higher natural killer cell activity and interleukin (IL)-2 production^[4] and consequently, improved immune function. On the other hand, in an elderly population, zinc supplementation eliminated the effect of seasonal variations on the incidence of infections and also decreased their mean incidence compared to a placebo group (common cold, cold sores, and the flu)^[5].

In addition to protection against infections, immune cell activation is involved in several pathophysiological processes. Cell activation is a complex process, implying several plasma membrane-associated events in which chemokines and adhesion molecules play a pivotal role. These processes ultimately result in proliferation, target cell lysis, increased production of cytokines and the expression of immune cell activation markers.

Many chronic diseases, such as atherosclerosis, cancer, neurodegenerative disorders, rheumatoid arthritis, and even aging, are due to chronically increased pro-inflammatory cytokines and oxidative stress, and in consequence, due to immune cell activation. Therefore, dietetic strategies to decrease low-grade inflammation and immune cell activation may be useful tools for preventing or decreasing the progression of many chronic disorders.

Many studies have focused on the mechanisms by which one single nutrient or compound alters immune cell activation, but these studies have the limitation that the interactions between the different compounds of food are not considered. There is an increasing interest to consider a whole dietary pattern in addition to single compounds in order to have a holistic approach of the effects of diet on cell activation.

The aim of this review is to update the knowledge of the modulation of immune cell activation by diet and its components from a chronic disease point of view through human interventional studies, those which provide the greatest scientific evidence.

EFFECTS OF DIET ON INFLAMMATORY CHRONIC DISEASES

Chronic inflammatory diseases are defined by long-term inflammatory processes directed at a particular endogenous or exogenous antigen and considered as an underlying pathophysiological mechanism linking behavioral factors and obesity to risk of chronic disease. Inflammation is characterized by a complex biological cascade of molecular and cellular signals that alter physiological responses. At the site of the injury, cells release molecular signals such as cytokines that cause a number of changes in the affected area, such as dilation of blood vessels, increased blood flow, increased vascular permeability,

exudation of fluids containing antibodies and invasion by monocytes and macrophages and, to a lesser extent, lymphocytes, through the expression of integrins and other adhesion molecules. In addition, lesion progression is associated with the predominance of the proinflammatory M1 over the antiinflammatory M2 macrophage phenotype, which can be switched to M1 by several transcription factors, chemokines and lipid accumulation in macrophages^[6,7].

Elevated levels of inflammatory biomarkers such as C-reactive protein (CRP), tumor necrosis factor- α (TNF- α), IL-6 and 18, fibrinogen and adhesion molecules [E-selectin, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1)] have been shown to predict type 2 diabetes, cardiovascular disease (CVD) and cancer^[8-13]. In this setting, the activation of the inflammasome has been linked to the pathogenesis of obesity, type 2 diabetes and atherosclerosis^[14-19]. Several studies have suggested that the development of tolerance and control of inflammation are strongly correlated with specific immune mechanisms that may be altered by an inadequate supply of either macronutrients or micronutrients. Therefore, the intake of some nutrients or specific dietary patterns may influence the concentrations of inflammatory biomarkers and therefore, the risk and/or progression of inflammatory diseases.

A westernized high-fat high-carbohydrate diet is positively associated with low-grade inflammation, and therefore, contributes to disease development and progression. Likewise, these types of diets can have direct adverse effects on human physiology^[20] resulting in chronic immune and inflammatory imbalances. Overall, the intake of a high-fat, high-carbohydrate westernized diet has potent direct and indirect effects on local as well as systemic inflammation. This has led to a dramatic upswing in the incidence of inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, diabetes, and non-alcoholic fatty liver disease, among others^[21]. On the other hand, vegetable- and fruit-based diet and a priori healthy dietary patterns appeared to be inversely related to inflammatory biomarkers; this fact is particularly well supported by intervention studies investigating the effects of Mediterranean diet (MedDiet) on health.

EFFECTS OF DIET ON CVD AND METABOLIC SYNDROME

CVD is the main cause of mortality worldwide and is principally caused by the appearance and progression of atherosclerotic lesions. Although atherosclerosis has been historically considered an oxidative disease, nowadays it is considered a systemic disease characterized by low-grade arterial inflammation, in which the cell and endothelial expression of adhesion molecules and chemokines participate in the recruitment of circulating leukocytes to the vascular endothelium and further migration into sub-endothelial spaces. In addition, the metabolic syndrome is

a risk factor CVD, which also has an immunological and inflammatory component. Immune cell infiltration of adipose tissue giving rise to chronic low-grade inflammation is, in part, responsible for the pathogenesis of insulin resistance in obesity^[22] and lastly, CVD.

As explained before, the western diet enriched in total fat (and an imbalanced ratio of n-6:n-3^[23]), animal protein, n-6 polyunsaturated fatty acids (PUFA) and refined sugars, leads to an increased proinflammatory status^[24] and is, therefore, considered as a risk factor for the development of CVD^[25]. On the other hand, several studies have highlighted that a Mediterranean-like diet decreases cardiovascular risk^[26-29], by up to 30% in a high cardiovascular risk population^[30]. Several mechanisms have been proposed for the effects observed, and some of them, which will be discussed below, are related to immune cell activation.

While n-6 PUFA have been shown to exert an inflammatory effect^[31], it has been demonstrated that n-3 PUFAs have beneficial effects on cardiovascular and inflammatory diseases^[32,33], probably linked to the nuclear factor (NF)- κ B pathway^[34] and the inhibition of the inflammasome activation^[35]. Meta-analyses of randomized controlled trials studying the n-6 PUFA-specific effect on CVDs^[36] showed a direct effect of n-6 fatty acids on the risk of non fatal and fatal heart failure, although linoleic acid could not be linked to an increase in systemic inflammatory biomarkers^[37]. Hypercholesterolemic subjects receiving 2 diets low in saturated fat and cholesterol, and high in PUFA varying in α -linolenic acid (10.5% linoleic acid; 6.5% α -linolenic acid) and linoleic acid (12.6% linoleic acid; 3.6% α -linolenic acid) were compared with other who followed an average American diet (7.7% linoleic acid; 0.8% α -linolenic acid). The α -linolenic acid diet decreased circulating CRP, VCAM-1 and E-selectin plasma concentrations, and the 2 high-PUFA diets similarly decreased ICAM-1^[38], although other studies in hypercholesterolemic subjects observed no such results^[39]. In healthy women, a 2-wk intervention of a n-3 PUFA-enriched juice or a plain tomato juice decreased VCAM-1 levels but only the n-3 PUFA-enriched juice decreased ICAM-1 plasma concentrations^[40]. In addition, a high monounsaturated fatty acid (MUFA) intake has also been shown to exert anti-inflammatory effects. A crossover feeding trial observed that a breakfast rich in butter [saturated fatty acids (SFA)] increased leukocyte mRNA expression of TNF- α compared to an olive oil (rich in MUFA, and concretely oleic acid) or walnut breakfast (rich in PUFA n-6)^[41]. On the other hand, in overweight men, a low-fat and a very-low-carbohydrate diet resulted in significant decreases of TNF- α , IL-6, CRP and sICAM-1 but not P-Selectin^[42], although in another study with overweight or obese women with metabolic syndrome, the substitution of carbohydrates by PUFA resulted in no changes in CRP, TNF- α , IL-6, sICAM-1 and sVCAM-1 serum concentrations^[43]. It can be summarized that the overall quantity of fat intake, the sources and type of dietary fat, with special emphasis on

α -linolenic acid and oleic acid, and the ratio of n-6:n-3 fatty acids in the diet, collectively play a crucial role in modulating inflammation.

Other dietetic compounds influencing immune cell activation are polyphenols. These products are antioxidant phytochemicals that have been found in vegetables, fruits and derivatives such as cocoa, red wine or tea, shown to decrease TNF- α and CRP levels^[44]. In healthy volunteers cocoa consumption reduced NF- κ B activation in peripheral blood mononuclear cells^[45], and in men at high CVD risk, cocoa consumption decreased monocyte expression of very late antigen (VLA)-4, CD40 and CD36 and serum concentrations of P-selectin and ICAM-1^[46]. Grape polyphenols and specially resveratrol are among the polyphenols most frequently studied. In hemodialysis patients, red grape juice supplementation for 3-wk significantly reduced plasma monocyte chemoattractant protein 1 (MCP-1)^[47], and in overweight or obese subjects with metabolic syndrome, grapefruit supplementation for 6-wk decreased F2-isoprostane concentrations in those subjects with high baseline F2-isoprostane concentrations, but no changes in CRP and VCAM-1 were observed^[48]. These results suggest different responses to polyphenol intake depending on the pathophysiological conditions of the study subjects and probably the type of polyphenols administered in the intervention group. These differential effects were also observed after moderate red wine consumption, where in healthy male volunteers red wine consumption significantly reduced plasma concentrations of VCAM-1, ICAM-1 and IL-1 α and VLA-4 lymphocyte expression and lymphocyte function-associated antigen (LFA)-1, Mac-1, VLA-4 and MCP-1 monocyte expression^[49]. On the other hand, in high cardiovascular risk subjects, moderate red wine consumption and dealcoholized red wine consumption (therefore, the non alcoholic fraction of red wine, mainly polyphenols) decreased serum concentrations of CD40 antigen, CD40 Ligand, ICAM-1, E-Selectin, IL-16 and IL-6, MCP-1 and VCAM-1 and inhibited the expression of LFA-1 in T-lymphocytes and Mac-1, SLe^x and C-C chemokine receptor type 2 expression in monocytes^[50].

In the recent years, the effects of a MedDiet as a dietary pattern and not a sum of nutrients have been considered from a multidisciplinary point of view. The MedDiet is characterized by a high intake of cereals, fruit and vegetable products (and therefore, polyphenols), a moderate consumption of fish, olive oil, nuts and wine, and a low intake of meat and dairy and industrial bakery products^[51]. According to scientific evidence, the MedDiet is currently considered the more anti-inflammatory dietary pattern, and this is translated to a decreased risk in cardiovascular mortality^[50]. In patients with metabolic syndrome, a 2-year follow-up MedDiet reduced serum concentrations of CRP, IL-6, IL-7 and IL-18, accompanied with decreased insulin resistance and an improved endothelial function score^[52]. In older subjects with diabetes or ≥ 3 CVD risk factors randomly allocated to a 3-mo MedDiet with supplemented with extra-virgin

olive oil, a MedDiet supplemented with nuts or a low-fat diet, after both MedDiets CRP, IL-6, ICAM-1 and VCAM-1 plasma concentrations decreased as did CD40 and CD49d monocyte expression, whereas IL-6, ICAM-1 and VCAM-1 increased after the low-fat diet^[26]. In addition, after 1 year both MedDiet groups showed lower plasma concentrations of IL-6, tumor necrosis factor receptor 60 (TNFR60), and TNFR80, whereas ICAM-1, TNFR60, and TNFR80 concentrations increased in the low-fat diet group^[27]. The MedDiet has also shown anti-inflammatory effects in healthy subjects. Four weeks of a MedDiet compared to an ordinary Swedish diet decreased the number of platelets and leukocytes and serum concentrations of vascular endothelial growth factor (VEGF), although it did not change the CRP and IL-6 concentrations^[28], perhaps because of their low baseline concentration. Interestingly, in a middle-aged twin population, adherence to a MedDiet was highly associated with lower levels of IL-6 but not CRP^[29]. Overall, the MedDiet has an antiinflammatory and an inhibitory immune cell activation effect decreasing the onset and progression of CVD, while a low-fat diet or a westernized diet has the opposite effect.

EFFECTS OF DIET ON CANCER

Cancer is the second cause of mortality worldwide and is mediated by both the innate (nonspecific) and acquired (specific) immune systems^[53,54]. The molecular mechanisms by which chronic inflammation drives cancer initiation and promotion include increased production of pro-inflammatory mediators, such as cytokines, chemokines, reactive oxygen intermediates, increased expression of oncogenes, cyclooxygenases, lipoxygenases and matrix metalloproteinases, and pro-inflammatory transcription factors such as NF- κ B, that mediate tumor cell proliferation, transformation, metastasis, survival, invasion, angiogenesis, chemoresistance and radioresistance^[55]. Taking into account that adherence to ideal cardiovascular health, as proposed by the American Heart Association, is associated with a lower incidence of cancer^[56], one may suspect that dietary benefits on CVD may reduce the risk of cancer.

Although epidemiological studies have pinpointed that diet may influence more than one-third of human malignancies, probably through the high consumption of pesticides^[57], heavy metals^[58], heterocyclic amines from over-cooked meats and sex steroid hormones^[59], few interventional trials have focused on the modulation of angiogenesis and carcinogenesis through dietary patterns. A recent review^[60] of epidemiological studies concluded that there is no significant effect of n-3 PUFA on cancer risk. However these studies only accounted for absolute as opposed to relative levels of n-3 and n-6 PUFA. In fact, n-6 PUFA metabolites promote tumor angiogenesis through a variety of signaling pathways, encouraging epithelial cell proliferation and migration, and decreasing tumor apoptosis, while n-3 PUFA and their metabolites

can reverse the pro-angiogenic consequences of high n-6 fatty acids. On the other hand, a MedDiet supplemented with nuts and walnuts (rich in n-6 and polyphenols) also associated with a high intake of vegetables, fruit and fish, decreased the risk of cancer mortality^[61].

Intake of total catechin, epicatechin, kaempferol, and myricetin and consumption of black tea were associated with a decreased risk of stage III/IV or stage IV prostate cancer in the Netherlands Cohort study^[62], probably because of the anti-inflammatory and antiproliferative effects of flavonoids observed *in vitro*^[63]. In prostate cancer men, 30 d of low-fat diet decreased 19 cytokines and angiogenic factors including proangiogenic factors (stromal-cell derived-1 α) and myeloid factors [granulocyte-colony-stimulating factor, macrophage colony-stimulating factor (-M-CSF-)] and VEGF, probably through the NF- κ B pathway^[64]. Regarding breast cancer, diets high in n-6 PUFA have a clear stimulating influence on breast cancer development, whereas diets rich in extra virgin olive oil mainly have a negative modulatory effect^[65]. A recent meta-analysis^[66], showed a significant inverse association with the highest fiber intakes and the risk of esophageal cancer, probably through weight control and therefore, inflammation status control. The isothiocyanate sulforaphane [SF; 1-isothiocyanato-4(R)-methylsulfinylbutane] is abundant in broccoli sprouts in the form of its glucosinolate precursor (glucoraphanin). SF is powerful bactericidal against *Helicobacter pylori* (*H. pylori*) infections, which are strongly associated with the worldwide pandemic of gastric cancer. *H. pylori*-infected patients were randomly assigned to feeding of broccoli sprouts (70 g/d; containing 420 micromol of SF precursor) for 8 wk or to consumption of an equal weight of alfalfa sprouts (not containing SF) as placebo. Intervention with broccoli sprouts, but not with alfalfa, decreased the levels of urease measured by the urea breath test and *H. pylori* stool antigen (both biomarkers of *H. pylori* colonization) and serum pepsinogens I and II (biomarkers of gastric inflammation). Therefore, daily intake of SF-rich broccoli sprouts for 2 mo enhanced the chemoprotection of the gastric mucosa against *H. pylori*^[67]. In colorectal cancer (CRC) patients, drinking a slurry of black raspberry powder 3 times-a-day for 9 wk increased granulocyte -M-CSF- and decreased IL-8 plasma concentrations and CD105 colorectal tissue expression^[68], while in another large CRC cohort, red and processed meat intake before CRC diagnosis was associated with higher risks of death due to all causes and from CVD but not CRC. Although red and processed meat consumption after CRC diagnosis was not associated with mortality, survivors with consistently high (median or higher) intakes before and after diagnosis had a higher risk of CRC-specific mortality compared with those with consistently low intakes^[69]. Nonetheless, it should be taken into account that, in addition to the diet, colon cancer risk is influenced by the balance between microbial production of health-promoting metabolites and potentially carcinogenic metabolites^[70]. In summary, few interventional studies have been per-

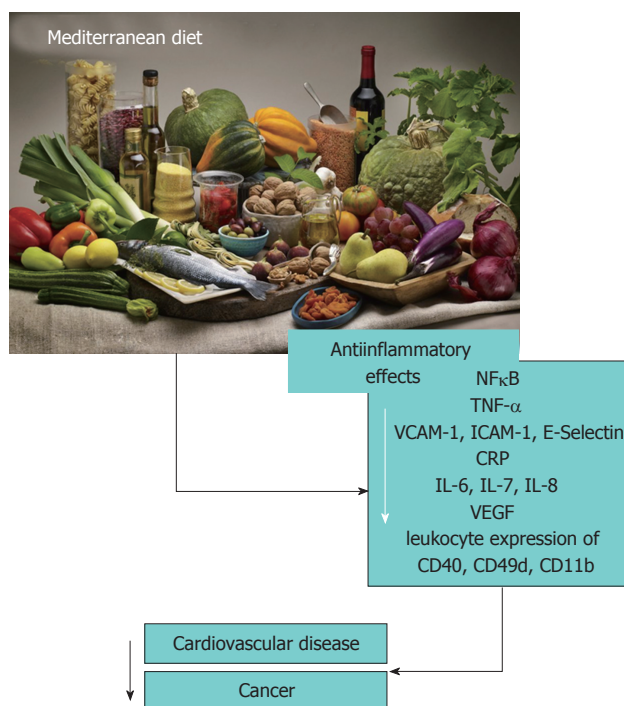


Figure 1 Summary of the anti-inflammatory effects of the Mediterranean Diet. CRP: C-reactive protein; TNF- α : tumor necrosis factor- α ; ICAM-1: Inter-cellular adhesion molecule 1; VCAM-1: Vascular cell adhesion protein 1; IL-6: Interleukin 6; VEGF: Vascular endothelial growth factor; NF: Nuclear factor.

formed investigating the link between diet, immune cell activation and cancer, but it can be postulated that a MedDiet brings together all the dietary protective nutrients related to cancer and specially cancers of the digestive system decreasing its risk of appearance^[71], although there are still not enough data to develop guidelines regarding specific foods and cancer risk.

FUTURE PERSPECTIVES

Dietary intake in relation to low-grade inflammation has been investigated in a number of studies exploring nutrients, foods or dietary patterns. Although there is increasing evidence that dietary patterns modulate immune cell activation and low-grade systemic inflammation, there is still a long way to understand the interactions between dietary compounds, dietary patterns, microbiota metabolites and individual polymorphisms and how these affect the body response to the intake of a determined food compound. The integration of dietary behaviors is warranted, given the fact that nutrients or foods are rarely eaten alone, and dietary patterns consider synergistic or antagonistic biochemical interactions among nutrients as well as different food sources of the same nutrient.

CONCLUSION

As summarized in Figure 1, there is compelling scientific evidence that a MedDiet rich in MUFA, PUFA (with an adequate ratio of n-3:n-6), polyphenols and with mild-to-

low carbohydrate, animal protein and SFA content is the most effective pattern to prevent immune cell activation and inflammation related to chronic diseases by decreasing the expression of leukocyte adhesion molecules and circulating inflammatory biomarkers.

ACKNOWLEDGMENTS

CIBERObn is an initiative of Instituto de Salud Carlos III, Spain.

REFERENCES

- 1 **Volta U**, Caio G, Tovoli F, De Giorgio R. Non-celiac gluten sensitivity: questions still to be answered despite increasing awareness. *Cell Mol Immunol* 2013; **10**: 383-392 [PMID: 23934026 DOI: 10.1038/cmi.2013.28]
- 2 **Delafuente JC**. Nutrients and immune responses. *Rheum Dis Clin North Am* 1991; **17**: 203-212 [PMID: 1907394]
- 3 **Beharka A**, Redican S, Leka L, Meydani SN. Vitamin E status and immune function. *Methods Enzymol* 1997; **282**: 247-263 [PMID: 9330293 DOI: 10.1016/S0076-6879(97)82112-X]
- 4 **Carver JD**. Dietary nucleotides: cellular immune, intestinal and hepatic system effects. *J Nutr* 1994; **124**: 144S-148S [PMID: 8283305]
- 5 **Prasad AS**, Beck FW, Bao B, Fitzgerald JT, Snell DC, Steinberg JD, Cardozo LJ. Zinc supplementation decreases incidence of infections in the elderly: effect of zinc on generation of cytokines and oxidative stress. *Am J Clin Nutr* 2007; **85**: 837-844 [PMID: 17344507]
- 6 **Chinetti-Gbaguidi G**, Staels B. Macrophage polarization in metabolic disorders: functions and regulation. *Curr Opin Lipidol* 2011; **22**: 365-372 [PMID: 21825981 DOI: 10.1097/MO.L.0b013e32834a77b4]
- 7 **Harford KA**, Reynolds CM, McGillicuddy FC, Roche HM. Fats, inflammation and insulin resistance: insights to the role of macrophage and T-cell accumulation in adipose tissue. *Proc Nutr Soc* 2011; **70**: 408-417 [PMID: 21835098 DOI: 10.1017/S0029665111000565]
- 8 **Panagiotakos DB**, Pitsavos C, Yannakoulia M, Chrysoshoou C, Stefanadis C. The implication of obesity and central fat on markers of chronic inflammation: The ATTICA study. *Atherosclerosis* 2005; **183**: 308-315 [PMID: 16285994 DOI: 10.1016/j.atherosclerosis.2005.03.010]
- 9 **Pradhan AD**, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001; **286**: 327-334 [PMID: 11466099 DOI: 10.1001/jama.286.3.327]
- 10 **Thorand B**, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, Illig T, Martin S, Herder C. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984-2002. *Diabetes* 2005; **54**: 2932-2938 [PMID: 16186395 DOI: 10.2337/diabetes.54.10.2932]
- 11 **Pearson TA**, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC, Taubert K, Tracy RP, Vinicor F. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for health-care professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003; **107**: 499-511 [PMID: 12551878 DOI: 10.1161/01.CIR.0000052939.59093.45]
- 12 **Il'yasova D**, Colbert LH, Harris TB, Newman AB, Bauer DC, Satterfield S, Kritchevsky SB. Circulating levels of inflammatory markers and cancer risk in the health aging and body composition cohort. *Cancer Epidemiol Biomarkers Prev* 2005;

- 14: 2413-2418 [PMID: 16214925 DOI: 10.1158/1055-9965.EPI-05-0316]
- 13 **Lindahl B**, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *N Engl J Med* 2000; **343**: 1139-1147 [PMID: 11036119 DOI: 10.1056/nejm200010193431602]
- 14 **Wen H**, Ting JP, O'Neill LA. A role for the NLRP3 inflammasome in metabolic diseases--did Warburg miss inflammation? *Nat Immunol* 2012; **13**: 352-357 [PMID: 22430788 DOI: 10.1038/ni.2228]
- 15 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; **482**: 179-185 [PMID: 22297845 DOI: 10.1038/nature10809]
- 16 **Strowig T**, Henao-Mejia J, Elinav E, Flavell R. Inflammasomes in health and disease. *Nature* 2012; **481**: 278-286 [PMID: 22258606 DOI: 10.1038/nature10759]
- 17 **Stienstra R**, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, Neale GA, Hooiveld GJ, Hijmans A, Vroegrijk I, van den Berg S, Romijn J, Rensen PC, Joosten LA, Netea MG, Kanneganti TD. Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc Natl Acad Sci USA* 2011; **108**: 15324-15329 [PMID: 21876127 DOI: 10.1073/pnas.1100255108]
- 18 **Freigang S**, Ampenberger F, Spohn G, Heer S, Shamshiev AT, Kisielow J, Hersberger M, Yamamoto M, Bachmann MF, Kopf M. Nrf2 is essential for cholesterol crystal-induced inflammasome activation and exacerbation of atherosclerosis. *Eur J Immunol* 2011; **41**: 2040-2051 [PMID: 21484785 DOI: 10.1002/eji.201041316]
- 19 **Duwell P**, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nuñez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010; **464**: 1357-1361 [PMID: 20428172 DOI: 10.1038/nature08938]
- 20 **Barbaresko J**, Koch M, Schulze MB, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. *Nutr Rev* 2013; **71**: 511-527 [PMID: 23865797 DOI: 10.1111/nure.12035]
- 21 **Huang EY**, Devkota S, Moscoso D, Chang EB, Leone VA. The role of diet in triggering human inflammatory disorders in the modern age. *Microbes Infect* 2013; **15**: 765-774 [PMID: 23876436 DOI: 10.1016/j.micinf.2013.07.004]
- 22 **Kalupahana NS**, Moustaid-Moussa N, Claycombe KJ. Immunity as a link between obesity and insulin resistance. *Mol Aspects Med* 2012; **33**: 26-34 [PMID: 22040698 DOI: 10.1016/j.mam.2011.10.011]
- 23 **Patterson E**, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutr Metab* 2012; **2012**: 539426 [PMID: 22570770 DOI: 10.1155/2012/539426]
- 24 **Giugliano D**, Ceriello A, Esposito K. The effects of diet on inflammation: emphasis on the metabolic syndrome. *J Am Coll Cardiol* 2006; **48**: 677-685 [PMID: 16904534 DOI: 10.1016/j.jacc.2006.03.052]
- 25 **Menotti A**, Kromhout D, Blackburn H, Fidanza F, Buzina R, Nissinen A. Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group. *Eur J Epidemiol* 1999; **15**: 507-515 [PMID: 10485342]
- 26 **Mena MP**, Sacanella E, Vazquez-Agell M, Morales M, Fitó M, Escoda R, Serrano-Martínez M, Salas-Salvadó J, Benages N, Casas R, Lamuela-Raventós RM, Masanes F, Ros E, Estruch R. Inhibition of circulating immune cell activation: a molecular antiinflammatory effect of the Mediterranean diet. *Am J Clin Nutr* 2009; **89**: 248-256 [PMID: 19056596 DOI: 10.3945/ajcn.2008.26094]
- 27 **Urpi-Sarda M**, Casas R, Chiva-Blanch G, Romero-Mamani ES, Valderas-Martínez P, Salas-Salvadó J, Covas MI, Toledo E, Andres-Lacueva C, Llorach R, García-Arellano A, Bulló M, Ruiz-Gutiérrez V, Lamuela-Raventós RM, Estruch R. The Mediterranean diet pattern and its main components are associated with lower plasma concentrations of tumor necrosis factor receptor 60 in patients at high risk for cardiovascular disease. *J Nutr* 2012; **142**: 1019-1025 [PMID: 22535754 DOI: 10.3945/jn.111.148726]
- 28 **Ambring A**, Johansson M, Axelsen M, Gan L, Strandvik B, Friberg P. Mediterranean-inspired diet lowers the ratio of serum phospholipid n-6 to n-3 fatty acids, the number of leukocytes and platelets, and vascular endothelial growth factor in healthy subjects. *Am J Clin Nutr* 2006; **83**: 575-581 [PMID: 16522903]
- 29 **Dai J**, Miller AH, Bremner JD, Goldberg J, Jones L, Shallenberger L, Buckham R, Murrain NV, Veledar E, Wilson PW, Vaccarino V. Adherence to the Mediterranean diet is inversely associated with circulating interleukin-6 among middle-aged men: a twin study. *Circulation* 2008; **117**: 169-175 [PMID: 18086924 DOI: 10.1161/CIRCULATIONAHA.107.710699]
- 30 **Estruch R**, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, Gómez-Gracia E, Ruiz-Gutiérrez V, Fiol M, Lapetra J, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Basora J, Muñoz MA, Sorlí JV, Martínez JA, Martínez-González MA. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013; **368**: 1279-1290 [PMID: 23432189 DOI: 10.1056/NEJMoa1200303]
- 31 **Ramsden CE**, Ringel A, Feldstein AE, Taha AY, MacIntosh BA, Hibbeln JR, Majchrzak-Hong SF, Faurot KR, Rapoport SI, Cheon Y, Chung YM, Berk M, Mann JD. Lowering dietary linoleic acid reduces bioactive oxidized linoleic acid metabolites in humans. *Prostaglandins Leukot Essent Fatty Acids* 2012; **87**: 135-141 [PMID: 22959954 DOI: 10.1016/j.plefa.2012.08.004]
- 32 **Wall R**, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr Rev* 2010; **68**: 280-289 [PMID: 20500789 DOI: 10.1111/j.1753-4887.2010.00287.x]
- 33 **Marchioli R**, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi MG, Geraci E, Levantesi G, Maggioni AP, Mantini L, Marfisi RM, Mastrogiuseppe G, Mininni N, Nicolosi GL, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C, Valagussa F. Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* 2002; **105**: 1897-1903 [PMID: 11997274 DOI: 10.1161/01.CIR.0000014682.14181.F2]
- 34 **Perkins ND**. Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nat Rev Mol Cell Biol* 2007; **8**: 49-62 [PMID: 17183360 DOI: 10.1038/nrm2083]
- 35 **Yan Y**, Jiang W, Spinetti T, Tardivel A, Castillo R, Bourquin C, Guarda G, Tian Z, Tschopp J, Zhou R. Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. *Immunity* 2013; **38**: 1154-1163 [PMID: 23809162 DOI: 10.1016/j.immuni.2013.05.015]
- 36 **Ramsden CE**, Hibbeln JR, Majchrzak SF, Davis JM. n-6 fatty acid-specific and mixed polyunsaturate dietary interventions have different effects on CHD risk: a meta-analysis of randomised controlled trials. *Br J Nutr* 2010; **104**: 1586-1600 [PMID: 21118617 DOI: 10.1017/S0007114510004010]
- 37 **Johnson GH**, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. *J Acad Nutr Diet* 2012; **112**: 1029-1041, 1041.e1-15 [PMID: 22889633 DOI: 10.1016/j.

- jand.2012.03.029]
- 38 **Zhao G**, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. *J Nutr* 2004; **134**: 2991-2997 [PMID: 15514264]
 - 39 **Bemelmans WJ**, Lefrandt JD, Feskens EJ, van Haelst PL, Broer J, Meyboom-de Jong B, May JF, Tervaert JW, Smit AJ. Increased alpha-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. *Eur J Clin Nutr* 2004; **58**: 1083-1089 [PMID: 15220952 DOI: 10.1038/sj.ejcn.1601938]
 - 40 **García-Alonso FJ**, Jorge-Vidal V, Ros G, Periago MJ. Effect of consumption of tomato juice enriched with n-3 polyunsaturated fatty acids on the lipid profile, antioxidant biomarker status, and cardiovascular disease risk in healthy women. *Eur J Nutr* 2012; **51**: 415-424 [PMID: 21755327 DOI: 10.1007/s00394-011-0225-0]
 - 41 **Jiménez-Gómez Y**, López-Miranda J, Blanco-Colio LM, Marín C, Pérez-Martínez P, Ruano J, Paniagua JA, Rodríguez F, Egido J, Pérez-Jiménez F. Olive oil and walnut breakfasts reduce the postprandial inflammatory response in mononuclear cells compared with a butter breakfast in healthy men. *Atherosclerosis* 2009; **204**: e70-e76 [PMID: 18952211 DOI: 10.1016/j.atherosclerosis.2008.09.011]
 - 42 **Sharmam MJ**, Volek JS. Weight loss leads to reductions in inflammatory biomarkers after a very-low-carbohydrate diet and a low-fat diet in overweight men. *Clin Sci (Lond)* 2004; **107**: 365-369 [PMID: 15265001 DOI: 10.1042/cs20040111]
 - 43 **Rajaie S**, Azadbakht L, Saneei P, Khazaei M, Esmailzadeh A. Comparative effects of carbohydrate versus fat restriction on serum levels of adipocytokines, markers of inflammation, and endothelial function among women with the metabolic syndrome: a randomized cross-over clinical trial. *Ann Nutr Metab* 2013; **63**: 159-167 [PMID: 24021709 DOI: 10.1159/000354868]
 - 44 **Bogdanski P**, Suliburska J, Szulinska M, Stepień M, Pupek-Musialik D, Jabłeczka A. Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. *Nutr Res* 2012; **32**: 421-427 [PMID: 22749178 DOI: 10.1016/j.nutres.2012.05.007]
 - 45 **Vázquez-Agell M**, Urpi-Sarda M, Sacanella E, Camino-López S, Chiva-Blanch G, Llorente-Cortés V, Tobias E, Roura E, Andres-Lacueva C, Lamuela-Raventós RM, Badimon L, Estruch R. Cocoa consumption reduces NF-κB activation in peripheral blood mononuclear cells in humans. *Nutr Metab Cardiovasc Dis* 2013; **23**: 257-263 [PMID: 21824756 DOI: 10.1016/j.numecd.2011]
 - 46 **Monagas M**, Khan N, Andres-Lacueva C, Casas R, Urpi-Sardà M, Llorach R, Lamuela-Raventós RM, Estruch R. Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. *Am J Clin Nutr* 2009; **90**: 1144-1150 [PMID: 19776136 DOI: 10.3945/ajcn.2009.27716]
 - 47 **Castilla P**, Echarri R, Dávalos A, Cerrato F, Ortega H, Teruel JL, Lucas MF, Gómez-Coronado D, Ortuño J, Lasunción MA. Concentrated red grape juice exerts antioxidant, hypolipidemic, and antiinflammatory effects in both hemodialysis patients and healthy subjects. *Am J Clin Nutr* 2006; **84**: 252-262 [PMID: 16825703]
 - 48 **Dow CA**, Wertheim BC, Patil BS, Thomson CA. Daily consumption of grapefruit for 6 weeks reduces urine F2-isoprostanes in overweight adults with high baseline values but has no effect on plasma high-sensitivity C-reactive protein or soluble vascular cellular adhesion molecule 1. *J Nutr* 2013; **143**: 1586-1592 [PMID: 23902962 DOI: 10.3945/jn.113.175166]
 - 49 **Estruch R**, Sacanella E, Badia E, Antúnez E, Nicolás JM, Fernández-Solá J, Rotilio D, de Gaetano G, Rubin E, Urbano-Marquez A. Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: a prospective randomized crossover trial. Effects of wine on inflammatory markers. *Atherosclerosis* 2004; **175**: 117-123 [PMID: 15186955 DOI: 10.1016/j.atherosclerosis.2004.03.006]
 - 50 **Chiva-Blanch G**, Urpi-Sarda M, Llorach R, Rotches-Ribalta M, Guillén M, Casas R, Arranz S, Valderas-Martínez P, Portoles O, Corella D, Tinahones F, Lamuela-Raventós RM, Andres-Lacueva C, Estruch R. Differential effects of polyphenols and alcohol of red wine on the expression of adhesion molecules and inflammatory cytokines related to atherosclerosis: a randomized clinical trial. *Am J Clin Nutr* 2012; **95**: 326-334 [PMID: 22205309 DOI: 10.3945/ajcn.111.022889]
 - 51 **Trichopoulou A**, Lagiou P. Healthy traditional Mediterranean diet: an expression of culture, history, and lifestyle. *Nutr Rev* 1997; **55**: 383-389 [PMID: 9420448]
 - 52 **Esposito K**, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004; **292**: 1440-1446 [PMID: 15383514 DOI: 10.1001/jama.292.12.1440]
 - 53 **Whiteside TL**, Herberman RB. The role of natural killer cells in immune surveillance of cancer. *Curr Opin Immunol* 1995; **7**: 704-710 [PMID: 8573315 DOI: 10.1016/0952-7915(95)80080-8]
 - 54 **Robins RA**. T-cell responses at the host: tumour interface. *Biochim Biophys Acta* 1986; **865**: 289-305 [PMID: 3539199]
 - 55 **Sethi G**, Shanmugam MK, Ramachandran L, Kumar AP, Tergaonkar V. Multifaceted link between cancer and inflammation. *Biosci Rep* 2012; **32**: 1-15 [PMID: 21981137 DOI: 10.1042/BSR20100136]
 - 56 **Rasmussen-Torvik LJ**, Shay CM, Abramson JG, Friedrich CA, Nettleton JA, Prizment AE, Folsom AR. Ideal cardiovascular health is inversely associated with incident cancer: the Atherosclerosis Risk In Communities study. *Circulation* 2013; **127**: 1270-1275 [PMID: 23509058 DOI: 10.1161/circulationaha.112.001183]
 - 57 **ACOG Committee Opinion No 575**. Exposure to toxic environmental agents. *Fertil Steril* 2013; **100**: 931-934 [PMID: 24070500 DOI: 10.1016/j.fertnstert.2013.08.043]
 - 58 **Mannello F**, Ligi D, Canale M. Aluminium, carbonyls and cytokines in human nipple aspirate fluids: Possible relationship between inflammation, oxidative stress and breast cancer microenvironment. *J Inorg Biochem* 2013; **128**: 250-256 [PMID: 23916117 DOI: 10.1016/j.jinorgbio.2013.07.003]
 - 59 **Lee HJ**, Wu K, Cox DG, Hunter D, Hankinson SE, Willett WC, Sinha R, Cho E. Polymorphisms in xenobiotic metabolizing genes, intakes of heterocyclic amines and red meat, and postmenopausal breast cancer. *Nutr Cancer* 2013; **65**: 1122-1131 [PMID: 24099317 DOI: 10.1080/01635581.2013.824991]
 - 60 **Kang JX**, Liu A. The role of the tissue omega-6/omega-3 fatty acid ratio in regulating tumor angiogenesis. *Cancer Metastasis Rev* 2013; **32**: 201-210 [PMID: 23090260 DOI: 10.1007/s10555-012-9401-9]
 - 61 **Guasch-Ferré M**, Bulló M, Martínez-González MÁ, Ros E, Corella D, Estruch R, Fitó M, Arós F, Wärnberg J, Fiol M, Lapetra J, Vinyoles E, Lamuela-Raventós RM, Serra-Majem L, Pintó X, Ruiz-Gutiérrez V, Basora J, Salas-Salvadó J. Frequency of nut consumption and mortality risk in the PREDIMED nutrition intervention trial. *BMC Med* 2013; **11**: 164 [PMID: 23866098 DOI: 10.1186/1741-7015-11-164]
 - 62 **Geybels MS**, Verhage BA, Arts IC, van Schooten FJ, Goldbohm RA, van den Brandt PA. Dietary flavonoid intake, black tea consumption, and risk of overall and advanced stage prostate cancer. *Am J Epidemiol* 2013; **177**: 1388-1398 [PMID: 23722011 DOI: 10.1093/aje/kws419]
 - 63 **García-Lafuente A**, Guillamón E, Villares A, Rostagno MA, Martínez JA. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm Res* 2009; **58**: 537-552 [PMID: 19381780 DOI: 10.1007/s00011-009-

- 0037-3]
- 64 **Heymach JV**, Shackelford TJ, Tran HT, Yoo SY, Do KA, Wergin M, Saintigny P, Vollmer RT, Polascik TJ, Snyder DC, Ruffin MT, Yan S, Dewhirst M, Kunnumakkara AB, Aggarwal BB, Demark-Wahnefried W. Effect of low-fat diets on plasma levels of NF- κ B-regulated inflammatory cytokines and angiogenic factors in men with prostate cancer. *Cancer Prev Res (Phila)* 2011; **4**: 1590-1598 [PMID: 21764858 DOI: 10.1158/1940-6207.CAPR-10-0136]
 - 65 **Escrib E**, Solanas M, Moral R. Olive oil and other dietary lipids in breast cancer. *Cancer Treat Res* 2014; **159**: 289-309 [PMID: 24114487 DOI: 10.1007/978-3-642-38007-5_17]
 - 66 **Coleman HG**, Murray LJ, Hicks B, Bhat SK, Kubo A, Corley DA, Cardwell CR, Cantwell MM. Dietary fiber and the risk of precancerous lesions and cancer of the esophagus: a systematic review and meta-analysis. *Nutr Rev* 2013; **71**: 474-482 [PMID: 23815145 DOI: 10.1111/nure.12032]
 - 67 **Yanaka A**, Fahey JW, Fukumoto A, Nakayama M, Inoue S, Zhang S, Tauchi M, Suzuki H, Hyodo I, Yamamoto M. Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in *Helicobacter pylori*-infected mice and humans. *Cancer Prev Res (Phila)* 2009; **2**: 353-360 [PMID: 19349290 DOI: 10.1158/1940-6207]
 - 68 **Mentor-Marcel RA**, Bobe G, Sardo C, Wang LS, Kuo CT, Stoner G, Colburn NH. Plasma cytokines as potential response indicators to dietary freeze-dried black raspberries in colorectal cancer patients. *Nutr Cancer* 2012; **64**: 820-825 [PMID: 22823889 DOI: 10.1080/01635581.2012.697597]
 - 69 **McCullough ML**, Gapstur SM, Shah R, Jacobs EJ, Campbell PT. Association between red and processed meat intake and mortality among colorectal cancer survivors. *J Clin Oncol* 2013; **31**: 2773-2782 [PMID: 23816965 DOI: 10.1200/JCO.2013.49.1126]
 - 70 **Ou J**, Carbonero F, Zoetendal EG, DeLany JP, Wang M, Newton K, Gaskins HR, O'Keefe SJ. Diet, microbiota, and microbial metabolites in colon cancer risk in rural Africans and African Americans. *Am J Clin Nutr* 2013; **98**: 111-120 [PMID: 23719549 DOI: 10.3945/ajcn.112.056689]
 - 71 **Bamia C**, Lagiou P, Buckland G, Grioni S, Agnoli C, Taylor AJ, Dahm CC, Overvad K, Olsen A, Tjønneland A, Cottet V, Boutron-Ruault MC, Morois S, Grote V, Teucher B, Boeing H, Buijsse B, Trichopoulos D, Adarakis G, Tumino R, Naccarati A, Panico S, Palli D, Bueno-de-Mesquita HB, van Duynhoven FJ, Peeters PH, Engeset D, Skeie G, Lund E, Sánchez MJ, Barricarte A, Huerta JM, Quirós JR, Dorronsoro M, Ljuslinder I, Palmqvist R, Drake I, Key TJ, Khaw KT, Wareham N, Romieu I, Fedirko V, Jenab M, Romaguera D, Norat T, Trichopoulou A. Mediterranean diet and colorectal cancer risk: results from a European cohort. *Eur J Epidemiol* 2013; **28**: 317-328 [PMID: 23579425 DOI: 10.1007/s10654-013-9795-x]

P- Reviewers: Kucuker A, Kindy MS, Movahed A
S- Editor: Song XX **L- Editor:** A **E- Editor:** Liu SQ





Cigarette smoking and innate immune responses to influenza infection

Wenxin Wu, Jordan P Metcalf

Wenxin Wu, Jordan P Metcalf, Department of Medicine, Pulmonary and Critical Care Division, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States

Jordan P Metcalf, Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, United States

Author contributions: Wu W and Metcalf JP wrote the review.

Supported by The Oklahoma Health Research Program from Oklahoma Center for the Advancement of Science and Technology, to Wu W; and by the National Institute of Allergy and Infectious Diseases, No. 1U19 AI62629, to Metcalf JP

Correspondence to: Wenxin Wu, PhD, Department of Medicine, Pulmonary and Critical Care Division, University of Oklahoma Health Sciences Center, 800 Research Pkwy, RP1, Rm 425, Oklahoma City, OK 73104, United States. wenxin-wu@ouhsc.edu

Telephone: +1-405-2711966 Fax: +1-405-2715440

Received: October 31, 2013 Revised: December 18, 2013

Accepted: February 16, 2014

Published online: March 27, 2014

Abstract

Cigarette smoking (CS) suppresses the immune system, and smoking is a well-known major risk factor for respiratory tract infections, including influenza infection. Both smoking cigarettes and passive smoking alter a wide range of immunological functions, including innate and adaptive immune responses. Past reviews on CS and innate immunity have been focused on the effects of CS on structural changes of the lung, as well as the effects on the function of alveolar macrophages, leukocytes, natural killer cells and dendritic cells. The study of innate immunity has developed rapidly in the last decade with the discovery of new receptors for virus recognition and interferon responses. This review aims to give a brief summary of recent findings on the suppressive effects of CS on the innate response to influenza virus, especially as it pertains to suppression of the function of pattern recognition receptors for influenza virus.

enza virus.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Smoking; Influenza; Innate immunity; Virus; Pattern recognition receptors; Immune response

Core tip: Cigarette smoking (CS) alters a wide range of immunological functions, including innate and adaptive immune responses to viral infection. This review aims to give a brief summary of recent findings on the suppressive effects of CS on the innate response to influenza virus, especially as it pertains to suppression of the function of pattern recognition receptors for influenza virus. Studies on CS inhibition to innate response will be important in designing strategies for the development of novel treatments to mitigate the adverse consequences of CS and Flu infection.

Wu W, Metcalf JP. Cigarette smoking and innate immune responses to influenza infection. *World J Immunol* 2014; 4(1): 20-25 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i1/20.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i1.20>

CIGARETTE SMOKING AND INFLUENZA INFECTION

Influenza virus is a major cause of infectious morbidity and mortality^[1]. Each year in the United States, 5% to 20% of the population are infected, 200000 are hospitalized, and 36000 die due to influenza virus infection, making it the leading infectious cause of death^[2,3]. There have been four pandemics (worldwide epidemics) in the last century, including the Spanish flu in 1918, the Asian flu in 1957, the Hong Kong flu in 1968, and the Swine flu in 2009. These were significant outbreaks. For example,

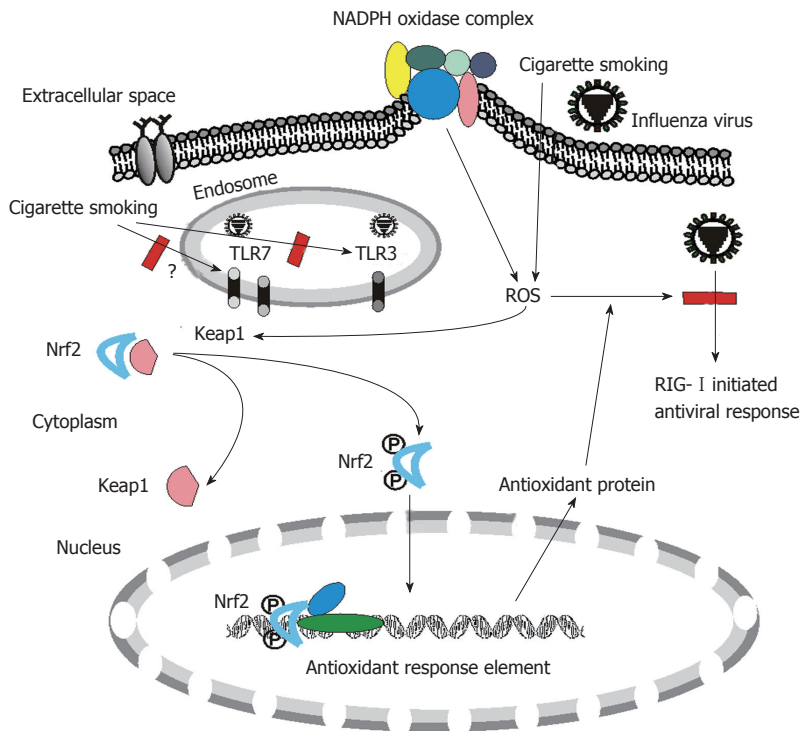


Figure 1 Cigarette smoking and its subsequent induced cellular oxidative stress suppress innate response to influenza virus. Influenza virus components are internalized from the cell surface in endosomes, and specific ligands are recognized by toll-like receptors (TLR)3/7 and cytosolic retinoic acid-inducible gene 1 (RIG- I). The PRRs then activate transcription factors that lead to the production of antiviral interferons and pro-inflammatory cytokines. Cigarette smoking (CS) inhibits RIG- I , TLR3 and possibly TLR7 recognition of influenza virus. Reactive oxygen species induced by CS are involved in the interference of PRR function. Reducing oxidative stress in cells, either by increasing Nrf2 or by Keap1 knockout, has potential therapeutic effect of restoring virus recognition by PRRs suppressed by CS. ROS: Reactive oxygen species; PRRs: Pattern recognition receptors.

the 1918 flu caused more deaths than those due to World War I. Influenza pandemics will continue as a threat to public health. The predisposition of cigarette smokers to have, and to have complications from, influenza infection is well recognized^[4]. Epidemiological studies show that influenza infection is seven times more common and is much more severe in smokers than nonsmokers^[5]. Influenza infections are more severe, with more cough, acute and chronic sputum production, breathlessness, and wheezing in smokers^[6]. Both active and passive cigarette smoke exposure increase the risk of infections^[7]. A cohort study of female military recruits showed that smoking was a risk factor for severe influenza-like illness during an outbreak of influenza A (H1N1) subtype infection^[8]. Thailand's National Avian Influenza Surveillance system reported that current or former smoking was among the several risk factors associated with a fatal outcome from human influenza infection^[9]. In the spring of 2013, the high mortality of avian influenza H7N9 in humans caused great concern in China and the world. Age along with a history of smoking are the most significant risk factors which predict a fatal outcome in human H7N9 infection^[10]. The mechanism of increased susceptibility to influenza infections in smokers is likely multifactorial, but clearly includes alteration of immunologic host defenses. Both smoking cigarettes and second-hand exposure to tobacco smoke alter a wide range of immunological functions, including innate and adaptive immune responses^[11].

CIGARETTE SMOKING SUPPRESSES INNATE RESPONSE RECEPTORS TO INFLUENZA VIRUS

Innate immunity is the first line of host defense against

invading microorganisms. Innate immune responses to viruses are triggered by recognition of specific structures of diversified pathogens called pathogen-associated molecular patterns (PAMPs). Host cells have multiple defensive mechanisms including pattern recognition receptors (PRRs) that can eliminate viruses through recognition of various viral PAMPs, such as ssRNA and dsRNA produced in virally infected cells. A recent triumph in research into immunity has been the discovery of three families of PRRs: Toll-like receptors (TLRs), Retinoic acid-inducible gene 1 (RIG- I) like helicases (RLRs) and nucleotide-binding domain and leucine-rich-repeat-containing proteins (NLRs). All three families are involved in influenza virus recognition and responses by the host^[12] (Figure 1).

RIG- I is a highly inducible cytoplasmic RNA helicase that activates antiviral responses to influenza virus by cell-signal mediated activation of interferon (IFN) production^[13,14]. Stimulation of RIG- I activates specific signaling pathways that lead to activation of nuclear factor- κ B (NF- κ B) which are crucial for inflammatory cytokine induction, and/or induction of interferon regulatory factor 3/7 (IRF3/7) which is important for the IFN-induced antiviral response. Many studies have confirmed that RIG- I regulation during influenza virus infection is important in the antiviral response and for modulation, either directly or indirectly, of proinflammatory cytokine responses^[15,16]. We have shown that RIG- I induction is inhibited by cigarette smoking (CS) in our human organ culture model^[17]. We demonstrated that 2%-20% cigarette smoke extract (CSE) inhibited influenza-induced RIG- I mRNA and protein expression as well as expression of the anti-viral cytokines interferon γ induced protein 10 and IFN- β in human lung.

Of the 13 mammalian TLRs, TLR3 and 7 are the most important PRRs for influenza virus recognition.

The influenza virus ssRNA genome is recognized by TLR7 in plasmacytoid dendritic cells (pDC) in humans^[18,19]. Others have shown that CS suppresses key pDC functions upon respiratory syncytial virus (RSV) infection by a mechanism that involves downregulation of TLR7 expression and decreased activation of IRF-7^[20]. The effect of CS on TLR7 in influenza virus infected pDC should be similar although it has not been evaluated.

Double-stranded RNA (dsRNA) is produced during viral replication and is recognized by endosomal TLR3^[21]. Surprisingly, TLR3-deficient mice appear to be even more resistant to influenza infections than wild type mice, in terms of mortality^[22]. Although high viral loads have been detected in the lung, viral load does not appear to underlie disease susceptibility in this model. In *in vitro* studies, CSE enhances rhinovirus-induced TLR3 expression and interleukin-8 secretion in A549 cells^[23]. In human bone marrow mononuclear cells, CSE induces TLR2, TLR3 and TLR4 expression^[24]. *In vivo*, CS augments the expression and responses of TLR3 in human macrophages^[25] and in murine lung tissue. However, CS exacerbated poly(I:C)-induced neutrophilia and airway hyperresponsiveness^[26]. Recently, Todt et al have reported that smoking decreased the response of human lung macrophages to dsRNA by reducing TLR3 expression. Alveolar macrophage of smokers show reduced C-X-C motif chemokine 10 production in response to poly(I:C) stimulation *in vitro*^[27]. Therefore, CS alone is likely to slightly induce TLR3 expression. However, CS may suppress additional induction of TLR3 by virus. TLR3 is highly expressed in mouse innate immune cells, but shows a low level of expression in human monocytes, macrophages and dendritic cells^[28]. This might lead to some conflicting results in studies of TLR3 expression in human and mouse models.

In the NLR family, Sabban *et al*^[29] found that nucleotide-binding oligomerization domain-containing protein 2 (NOD2) confers responsiveness to ssRNA in terms of IRF3 activation and IFN- β production. Furthermore, wild-type cells treated with NOD2-specific small interfering RNA or bone marrow-derived macrophages from NOD2-deficient mice failed to produce an antiviral response after transfection with ssRNA, as is contained in RSV and vesicular stomatitis virus. It has been reported that CSE delays NOD2 expression and affects NOD2/receptor-interacting serine-threonine kinase 2 interactions in intestinal epithelial cells^[30]. Thus, CS might interfere with the NLR-initiated innate response to influenza virus although further experiments are needed to examine this possibility.

In addition to inhibition of PRRs, CS could also affect the downstream signaling and transcription factors controlling the expression of IFN. For example, expression of IRF7 is critical for amplification of the type I interferon response. The expression of IRF7 was significantly decreased in influenza-infected nasal epithelium from smokers^[31]. Furthermore, the data indicated that

DNA methylation of the *IRF7* gene and expression of the DNA (cytosine-5)-methyltransferase1 was enhanced in cells from smokers. Previous studies demonstrated that hypermethylation of IRF7 results in decreased ability of type I IFNs to induce gene expression^[32]. In the above report, IRF7 induction after influenza was suppressed both *in vitro* in long-term differentiated cultures of nasal epithelium, and in freshly biopsied nasal epithelial cells obtained from smokers after inoculation with the live-attenuated influenza virus vaccine. Mechanistically, another group found that cigarette smoke-conditioned medium decreased the expression of IRF-7 transcripts and suppressed the nuclear translocation of the key transcription factors, NF- κ B and IRF-3, after poly(I:C) stimulation^[33].

CS INDUCED CELLULAR OXIDATIVE STRESS AND INFLUENZA INFECTION

CS may affect many physiologic conditions which further alter host defense and virus clearance of lung cells. One of the most important mechanisms of CS-induced alteration is by increasing cellular oxidant stress. CSE contains high concentrations of reactive oxygen species (ROS), nitric oxide, peroxynitrite, and free radicals of organic compounds^[34-36]. In addition to these short-lived, highly reactive substances, previous studies have shown that aqueous cigarette tar extracts also contain pro-oxidant substances that increase cellular production of ROS by NADPH oxidases^[37-39]. NADPH oxidase-mediated generation of ROS is part of the innate immune defense of phagocytic cells and a variety of non-phagocytic cells against foreign pathogens. Endogenous antioxidant systems cope with the oxidative burden and limit potential toxicity of ROS. However, excess ROS may overwhelm antioxidant capacity and perturb the balance in this reduction-oxidation equilibrium, and damage cells and tissues through oxidative stress. In this regard, ROS are involved in the tissue injury associated with a number of inflammatory diseases, including rheumatoid arthritis^[40], ischemia-reperfusion injury^[41] and the adult respiratory distress syndrome^[42]. Most importantly, mice lacking a functional NADPH oxidase exhibit increased viral clearance, reduced lung damage and improved lung function during influenza virus infection^[43]. Human and animal studies show that CS produces generalized endothelial dysfunction^[44-46], which is usually an indicator of increased oxidative stress which can be mediated by NADPH oxidases. Thus the increased NADPH oxidase activity induced by CS might play a major role in oxidative stress in human lung and inhibit the innate response to influenza virus (Figure 1).

CS increases the level of oxidants in the lungs, resulting in depletion of antioxidants. In response to CS, pulmonary epithelial cells counteract increased levels of oxidants by activating Nrf2-dependent pathways to augment the expression of detoxification and antioxidant enzymes. Nrf2 is a transcription factor and the Nrf2 antioxidant response pathway is the primary cellular defense

against the cytotoxic effects of oxidative stress. Among other effects, Nrf2 increases the expression of numerous antioxidant and pollutant-detoxifying genes and is essential to protect the lungs from oxidative injury and inflammation. Yageta *et al*^[47] have examined the role of Nrf2 in protection against influenza virus-induced pulmonary inflammation after CS exposure with both *in vitro* and *in vivo* approaches. Their data indicate that the antioxidant pathway controlled by Nrf2 is pivotal for protection against the development of influenza virus-induced pulmonary inflammation and injury under oxidative conditions^[47]. The results further proved that oxidant stress contributes to CS-mediated susceptibility to influenza infections.

Blake *et al*^[48] have developed a novel mouse model in which the cytosolic inhibitor of Nrf2, Keap1, is genetically deleted in Clara cells, which predominate in the upper airways in mice. Deletion of Keap1 in Clara cells resulted in increased expression of Nrf2-dependent genes. Deletion of Keap1 in airway epithelium also protected Clara cells against oxidative stress *ex vivo* and attenuated oxidative stress and CS-induced inflammation *in vivo*^[48]. Therefore, current reports suggest that reducing oxidative stress in cells has a potential therapeutic effect, not only restoring virus recognition by PRRs suppressed by CS, but also by decreasing oxidant-mediated inflammation and cellular injury.

Recent data from our laboratory also demonstrated that CS-mediated cellular oxidant stress is the major mechanism of suppression of viral-mediated induction of the major RNA virus sentinel, RIG- I, in human lung^[17]. We found that CSE treatment inhibited influenza-induced anti-viral cytokine expression in our human lung organ culture model. This is associated with CSE-inhibited mRNA and protein expression of RIG- I, which is important in the antiviral host response. However, inhibition of viral-mediated RIG- I induction by CSE was prevented and antiviral cytokine responses were restored by the antioxidant, N-acetyl cysteine (NAC). These findings show that CSE suppresses anti-viral responses in influenza virus infected human lung through oxidative inhibition of RIG- I. CS is the major cause of chronic obstructive pulmonary disease (COPD) and exacerbates the susceptibility of the host to respiratory infectious diseases and the attendant pathology^[49]. Restoration of these responses by NAC may be an important mechanism for the recent finding that treatment of COPD patients with high-dose NAC resulted in decreased exacerbations^[50].

In summary, epidemiological studies suggest that CS is a major risk factor for influenza caused morbidity and mortality. The innate immune system senses influenza virus invasion through recognition of specific ligands by TLR3/7, NLR and cytosolic RIG- I. CS inhibits RIG- I, TLR3 and possibly TLR7 recognition of influenza virus. ROS induced by CS are involved in the interference of PRR function. Reducing oxidative stress in cells, either by using antioxidants or by manipulating Nrf2 overexpression, has a potential therapeutic effect of restoring virus recognition by PRRs suppressed by CS.

More studies will be required to enhance our under-

standing of the mechanism whereby CS suppresses the human immune system and also of the process that controls influenza virus infection. This will be important in designing strategies for the development of novel treatments to mitigate the adverse consequences of CS and flu infection.

REFERENCES

- 1 **Davis MM**, Taubert K, Benin AL, Brown DW, Mensah GA, Baddour LM, Dunbar S, Krumholz HM. Influenza vaccination as secondary prevention for cardiovascular disease: a science advisory from the American Heart Association/American College of Cardiology. *J Am Coll Cardiol* 2006; **48**: 1498-1502 [PMID: 17010820 DOI: 10.1016/j.jacc.2006.09.004]
- 2 **Thompson WW**, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, Fukuda K. Influenza-associated hospitalizations in the United States. *JAMA* 2004; **292**: 1333-1340 [PMID: 15367555 DOI: 10.1001/jama.292.11.1333]
- 3 **Izurieta HS**, Thompson WW, Kramarz P, Shay DK, Davis RL, DeStefano F, Black S, Shinefield H, Fukuda K. Influenza and the rates of hospitalization for respiratory disease among infants and young children. *N Engl J Med* 2000; **342**: 232-239 [PMID: 10648764 DOI: 10.1056/NEJM200001273420402]
- 4 **Finklea JF**, Sandifer SH, Smith DD. Cigarette smoking and epidemic influenza. *Am J Epidemiol* 1969; **90**: 390-399 [PMID: 5356947]
- 5 **Arcavi L**, Benowitz NL. Cigarette smoking and infection. *Arch Intern Med* 2004; **164**: 2206-2216 [PMID: 15534156 DOI: 10.1001/archinte.164.20.2206]
- 6 **Kark JD**, Lebiush M, Rannon L. Cigarette smoking as a risk factor for epidemic a(h1n1) influenza in young men. *N Engl J Med* 1982; **307**: 1042-1046 [PMID: 7121513 DOI: 10.1056/NEJM198210213071702]
- 7 **Peat JK**, Keena V, Harakeh Z, Marks G. Parental smoking and respiratory tract infections in children. *Paediatr Respir Rev* 2001; **2**: 207-213 [PMID: 12052321 DOI: 10.1053/prrv.2001.0142]
- 8 **Kark JD**, Lebiush M. Smoking and epidemic influenza-like illness in female military recruits: a brief survey. *Am J Public Health* 1981; **71**: 530-532 [PMID: 7212144 DOI: 10.2105/AJPH.71.5.530]
- 9 **Hanshaoworakul W**, Simmerman JM, Narueponjirakul U, Sanasuttipun W, Shinde V, Kaewchana S, Areechokechai D, Levy J, Ungchusak K. Severe human influenza infections in Thailand: oseltamivir treatment and risk factors for fatal outcome. *PLoS One* 2009; **4**: e6051 [PMID: 19557130 DOI: 10.1371/journal.pone.0006051]
- 10 **Liu S**, Sun J, Cai J, Miao Z, Lu M, Qin S, Wang X, Lv H, Yu Z, Amer S, Chai C. Epidemiological, clinical and viral characteristics of fatal cases of human avian influenza A (H7N9) virus in Zhejiang Province, China. *J Infect* 2013; **67**: 595-605 [PMID: 23958687 DOI: 10.1016/j.jinf.2013.08.007]
- 11 **Sopori M**. Effects of cigarette smoke on the immune system. *Nat Rev Immunol* 2002; **2**: 372-377 [PMID: 12033743 DOI: 10.1038/nri803]
- 12 **Wu S**, Metcalf JP, Wu W. Innate immune response to influenza virus. *Curr Opin Infect Dis* 2011; **24**: 235-240 [PMID: 21330918 DOI: 10.1097/QCO.0b013e328344c0e3]
- 13 **Kato H**, Sato S, Yoneyama M, Yamamoto M, Uematsu S, Matsui K, Tsujimura T, Takeda K, Fujita T, Takeuchi O, Akira S. Cell type-specific involvement of RIG-I in antiviral response. *Immunity* 2005; **23**: 19-28 [PMID: 16039576 DOI: 10.1016/j.immuni.2005.04.010]
- 14 **Sumpter R**, Loo YM, Foy E, Li K, Yoneyama M, Fujita T, Lemon SM, Gale M. Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. *J Virol* 2005; **79**: 2689-2699 [PMID: 15708988 DOI: 10.1128/JVI.79.5.2689-2699.2005]
- 15 **Kobasa D**, Jones SM, Shinya K, Kash JC, Copps J, Ebihara

- H, Hatta Y, Kim JH, Halfmann P, Hatta M, Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawakawa Y. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature* 2007; **445**: 319-323 [PMID: 17230189 DOI: 10.1038/nature05495]
- 16 Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imai-zumi T, Miyagishi M, Taira K, Akira S, Fujita T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004; **5**: 730-737 [PMID: 15208624 DOI: 10.1038/ni1087]
- 17 Wu W, Patel KB, Booth JL, Zhang W, Metcalf JP. Cigarette smoke extract suppresses the RIG-I-initiated innate immune response to influenza virus in the human lung. *Am J Physiol Lung Cell Mol Physiol* 2011; **300**: L821-L830 [PMID: 21335520 DOI: 10.1152/ajplung.00267.2010]
- 18 Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 2004; **303**: 1529-1531 [PMID: 14976261 DOI: 10.1126/science.1093616]
- 19 Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW, Iwasaki A, Flavell RA. Recognition of single-stranded RNA viruses by Toll-like receptor 7. *Proc Natl Acad Sci USA* 2004; **101**: 5598-5603 [PMID: 15034168 DOI: 10.1073/pnas.0400937101]
- 20 Castro SM, Chakraborty K, Guerrero-Plata A. Cigarette smoke suppresses TLR-7 stimulation in response to virus infection in plasmacytoid dendritic cells. *Toxicol In Vitro* 2011; **25**: 1106-1113 [PMID: 21435390 DOI: 10.1016/j.tiv.2011.03.011]
- 21 Guillot L, Le Goffic R, Bloch S, Esciou N, Akira S, Chignard M, Si-Tahar M. Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and influenza A virus. *J Biol Chem* 2005; **280**: 5571-5580 [PMID: 15579900 DOI: 10.1074/jbc.M410592200]
- 22 Le Goffic R, Balloy V, Lagranderie M, Alexopoulou L, Esciou N, Flavell R, Chignard M, Si-Tahar M. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. *PLoS Pathog* 2006; **2**: e53 [PMID: 16789835 DOI: 10.1371/journal.ppat.0020053]
- 23 Wang JH, Kim H, Jang YJ. Cigarette smoke extract enhances rhinovirus-induced toll-like receptor 3 expression and interleukin-8 secretion in A549 cells. *Am J Rhinol Allergy* 2009; **23**: e5-e9 [PMID: 19769802 DOI: 10.2500/ajra.2009.23.3407]
- 24 Zhou J, Eksioglu EA, Fortenberry NR, Chen X, Wang H, Epling-Burnette PK, Djeu JY, Wei S. Bone marrow mononuclear cells up-regulate toll-like receptor expression and produce inflammatory mediators in response to cigarette smoke extract. *PLoS One* 2011; **6**: e21173 [PMID: 21738617 DOI: 10.1371/journal.pone.0021173]
- 25 Koarai A, Yanagisawa S, Sugiura H, Ichikawa T, Akamatsu K, Hirano T, Nakanishi M, Matsunaga K, Minakata Y, Ichinose M. Cigarette smoke augments the expression and responses of toll-like receptor 3 in human macrophages. *Respirology* 2012; **17**: 1018-1025 [PMID: 22591330 DOI: 10.1111/j.1440-1843.2012.02198.x]
- 26 Kimura G, Ueda K, Eto S, Watanabe Y, Masuko T, Kusama T, Barnes PJ, Ito K, Kizawa Y. Toll-like receptor 3 stimulation causes corticosteroid-refractory airway neutrophilia and hyperresponsiveness in mice. *Chest* 2013; **144**: 99-105 [PMID: 23348232 DOI: 10.1378/chest.12-2610]
- 27 Todd JC, Freeman CM, Brown JP, Sonstein J, Ames TM, McCubrey AL, Martinez FJ, Chensue SW, Beck JM, Curtis JL. Smoking decreases the response of human lung macrophages to double-stranded RNA by reducing TLR3 expression. *Respir Res* 2013; **14**: 33 [PMID: 23497334 DOI: 10.1186/1465-9921-14-33]
- 28 Heinz S, Haehnel V, Karaghiosoff M, Schwarzfischer L, Müller M, Krause SW, Rehli M. Species-specific regulation of Toll-like receptor 3 genes in men and mice. *J Biol Chem* 2003; **278**: 21502-21509 [PMID: 12672806 DOI: 10.1074/jbc.M301476200]
- 29 Sabbah A, Chang TH, Harnack R, Frohlich V, Tominaga K, Dube PH, Xiang Y, Bose S. Activation of innate immune antiviral responses by Nod2. *Nat Immunol* 2009; **10**: 1073-1080 [PMID: 19701189 DOI: 10.1038/ni.1782]
- 30 Aldhous MC, Soo K, Stark LA, Ulanicka AA, Easterbrook JE, Dunlop MG, Satsangi J. Cigarette smoke extract (CSE) delays NOD2 expression and affects NOD2/RIPK2 interactions in intestinal epithelial cells. *PLoS One* 2011; **6**: e24715 [PMID: 21931826 DOI: 10.1371/journal.pone.0024715]
- 31 Jaspers I, Horvath KM, Zhang W, Brighton LE, Carson JL, Noah TL. Reduced expression of IRF7 in nasal epithelial cells from smokers after infection with influenza. *Am J Respir Cell Mol Biol* 2010; **43**: 368-375 [PMID: 19880818 DOI: 10.1165/rcmb.2009-0254OC]
- 32 Li Q, Tang L, Roberts PC, Kraniak JM, Fridman AL, Kulaeva OI, Tehrani OS, Tainsky MA. Interferon regulatory factors IRF5 and IRF7 inhibit growth and induce senescence in immortal Li-Fraumeni fibroblasts. *Mol Cancer Res* 2008; **6**: 770-784 [PMID: 18505922 DOI: 10.1158/1541-7786.MCR-07-0114]
- 33 Bauer CM, Dewitte-Orr SJ, Hornby KR, Zavitz CC, Lichty BD, Stämpfli MR, Mossman KL. Cigarette smoke suppresses type I interferon-mediated antiviral immunity in lung fibroblast and epithelial cells. *J Interferon Cytokine Res* 2008; **28**: 167-179 [PMID: 18338949 DOI: 10.1089/jir.2007.0054]
- 34 Nakayama T, Church DF, Pryor WA. Quantitative analysis of the hydrogen peroxide formed in aqueous cigarette tar extracts. *Free Radic Biol Med* 1989; **7**: 9-15 [PMID: 2753397 DOI: 10.1016/0891-5849(89)90094-4]
- 35 Pryor WA, Prier DG, Church DF. Electron-spin resonance study of mainstream and sidestream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. *Environ Health Perspect* 1983; **47**: 345-355 [PMID: 6297881 DOI: 10.1289/ehp.8347345]
- 36 Zang LY, Stone K, Pryor WA. Detection of free radicals in aqueous extracts of cigarette tar by electron spin resonance. *Free Radic Biol Med* 1995; **19**: 161-167 [PMID: 7649487 DOI: 10.1016/0891-5849(94)00236-D]
- 37 Pryor WA, Stone K, Zang LY, Bermúdez E. Fractionation of aqueous cigarette tar extracts: fractions that contain the tar radical cause DNA damage. *Chem Res Toxicol* 1998; **11**: 441-448 [PMID: 9585474 DOI: 10.1021/tx970159y]
- 38 Squadrito GL, Cueto R, Dellinger B, Pryor WA. Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter. *Free Radic Biol Med* 2001; **31**: 1132-1138 [PMID: 11677046 DOI: 10.1016/S0891-5849(01)00703-1]
- 39 Bock FG, Swain AP, Stedman RL. Carcinogenesis assay of subfractions of cigarette smoke condensate prepared by solvent-solvent separation of the neutral fraction. *J Natl Cancer Inst* 1972; **49**: 477-483 [PMID: 5076828]
- 40 Kitsis E, Weissmann G. The role of the neutrophil in rheumatoid arthritis. *Clin Orthop Relat Res* 1991; **(265)**: 63-72 [PMID: 2009678]
- 41 Zimmerman BJ, Granger DN. Mechanisms of reperfusion injury. *Am J Med Sci* 1994; **307**: 284-292 [PMID: 8160724 DOI: 10.1097/0000441-199404000-00009]
- 42 Boxer LA, Axtell R, Suchard S. The role of the neutrophil in inflammatory diseases of the lung. *Blood Cells* 1990; **16**: 25-40; discussion 41-2 [PMID: 2190649]
- 43 Snelgrove RJ, Edwards L, Rae AJ, Hussell T. An absence of reactive oxygen species improves the resolution of lung influenza infection. *Eur J Immunol* 2006; **36**: 1364-1373 [PMID: 16703568 DOI: 10.1002/eji.200635977]
- 44 Adams MR, Jessup W, Celermajer DS. Cigarette smoking is associated with increased human monocyte adhesion to endothelial cells: reversibility with oral L-arginine but not vitamin C. *J Am Coll Cardiol* 1997; **29**: 491-497 [PMID: 9060883 DOI: 10.1016/S0735-1097(96)00537-2]
- 45 Czernin J, Waldherr C. Cigarette smoking and coronary

- blood flow. *Prog Cardiovasc Dis* 2003; **45**: 395-404 [PMID: 12704596]
- 46 **Raij L**, DeMaster EG, Jaimes EA. Cigarette smoke-induced endothelium dysfunction: role of superoxide anion. *J Hypertens* 2001; **19**: 891-897 [PMID: 11393672 DOI: 10.1097/00004872-200105000-00009]
- 47 **Yageta Y**, Ishii Y, Morishima Y, Masuko H, Ano S, Yamadori T, Itoh K, Takeuchi K, Yamamoto M, Hizawa N. Role of Nrf2 in host defense against influenza virus in cigarette smoke-exposed mice. *J Virol* 2011; **85**: 4679-4690 [PMID: 21367886 DOI: 10.1128/JVI.02456-10]
- 48 **Blake DJ**, Singh A, Kombairaju P, Malhotra D, Mariani TJ, Tudor RM, Gabrielson E, Biswal S. Deletion of Keap1 in the lung attenuates acute cigarette smoke-induced oxidative stress and inflammation. *Am J Respir Cell Mol Biol* 2010; **42**: 524-536 [PMID: 19520915 DOI: 10.1165/rcmb.2009-0054OC]
- 49 **Sutherland ER**, Cherniack RM. Management of chronic obstructive pulmonary disease. *N Engl J Med* 2004; **350**: 2689-2697 [PMID: 15215485 DOI: 10.1056/NEJMra030415]
- 50 **Tse HN**, Raiteri L, Wong KY, Yee KS, Ng LY, Wai KY, Loo CK, Chan MH. High-dose N-acetylcysteine in stable COPD: the 1-year, double-blind, randomized, placebo-controlled HIACE study. *Chest* 2013; **144**: 106-118 [PMID: 23348146 DOI: 10.1378/chest.12-2357]

P- Reviewers: Fujita J, Julio RL, Ryffel B, Yokota S
S- Editor: Song XX **L- Editor:** A **E- Editor:** Liu SQ





Role of myeloid-derived suppressor cells in autoimmune disease

Kristen R Crook, Peng Liu

Kristen R Crook, Peng Liu, Department of Medicine, Thurston Arthritis Research Center, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, United States

Author contributions: Crook KR and Liu P designed the review, wrote the paper, and approved for publication.

Correspondence to: Peng Liu, MD, PhD, Associate Professor, Department of Medicine, Thurston Arthritis Research Center, School of Medicine, University of North Carolina at Chapel Hill, 3300 Thurston Building, CB# 7280, 104 Manning Drive, Chapel Hill, NC 27599, United States. liupz@med.unc.edu

Telephone: +1-919-9660570 Fax: +1-919-9669269

Received: October 25, 2013 Revised: December 12, 2013

Accepted: January 17, 2014

Published online: March 27, 2014

Abstract

Myeloid-derived suppressor cells (MDSCs) represent an important class of immunoregulatory cells that can be activated to suppress T cell functions. These MDSCs can inhibit T cell functions through cell surface interactions and the release of soluble mediators. MDSCs accumulate in the inflamed tissues and lymphoid organs of patients with autoimmune diseases. Much of our knowledge of MDSC function has come from studies involving cancer models, however many recent studies have helped to characterize MDSC involvement in autoimmune diseases. MDSCs are a heterogeneous group of immature myeloid cells with a number of different functions for the suppression of T cell responses. However, we have yet to fully understand their contributions to the development and regulation of autoimmune diseases. A number of studies have described beneficial functions of MDSCs during autoimmune diseases, and thus there appears to be a potential role for MDSCs in the treatment of these diseases. Nevertheless, many questions remain as to the activation, differentiation, and inhibitory functions of MDSCs. This review aims to summarize our current knowledge of MDSC subsets and suppressive functions in tissue-specific autoimmune disorders. We also describe the potential of MDSC-based

cell therapy for the treatment of autoimmune diseases and note some of hurdles facing the implementation of this therapy.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Myeloid-derived suppressor cells; Autoimmune disease; Autoimmunity; T cells; Chronic inflammation; Immune regulation

Core tip: Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of cells with immunosuppressive abilities. MDSCs inhibit T cell function and regulate immune responses in cancer and autoimmune diseases. Therapeutic administration of MDSCs in the mouse models of multiple sclerosis, rheumatoid arthritis, and diabetes has shown promising results. Thus, MDSCs have potential in cell-based treatments of autoimmune disorders. However, the role of MDSCs in autoimmunity is complex and not fully understood. Further studies are needed before new therapies can be implemented.

Crook KR, Liu P. Role of myeloid-derived suppressor cells in autoimmune disease. *World J Immunol* 2014; 4(1): 26-33 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i1/26.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i1.26>

INTRODUCTION

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of immature myeloid cells that have the ability to suppress T cell functions^[1]. MDSCs are derived from the bone marrow and arise from a delay in maturation during pathologic conditions, such as cancer, chronic inflammation, infection, and traumatic stress^[2]. Most studies focus on the pathogenic nature of MDSCs in cancer, where suppression of T cell-mediated immune

responses prevents immune surveillance and clearance of developing tumors^[3-5]. Recently, MDSCs have been reported to regulate autoimmunity and control the generation and perpetuation of autoimmune diseases^[6]. In this review, we will summarize the current knowledge of MDSC subsets and suppressive functions in tissue-specific autoimmune disorders. We also describe the potential of MDSC-based cell therapy for the treatment of these autoimmune diseases, while noting some of the obstacles that may hinder the implementation of this therapy.

MDSC INVOLVEMENT IN AUTOIMMUNE DISEASES

Our knowledge of the origination and functions of MDSCs has come mainly from studies in tumor models and from cancer patients^[1,5,7]. The role of MDSCs in autoimmune diseases is only starting to be elucidated. We now know that MDSCs are involved in a number of different autoimmune disorders, including multiple sclerosis (MS), type 1 diabetes, rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and autoimmune hepatitis. In steady state conditions, MDSCs reside primarily in the bone marrow. Under pathological conditions, MDSC populations expand and can be detected in the spleen, lymph nodes, cancerous tumors, and bloodstream. An early study using a mouse model of autoimmune uveoretinitis showed that the accumulation of nitric oxide-producing monocytes in the choroid and retina of the eye correlated with the severity of disease^[8]. A later study showed similar results and confirmed the identity of these cells to be MDSCs^[9]. Studies using the mouse model of MS, experimental autoimmune encephalomyelitis (EAE), showed that MDSCs were present in the demyelinated areas of the spinal cord tissue of mice. Another EAE model showed that MDSC accumulation in the spleen correlated with disease progression^[10]. Here, they showed that the start of MDSC accumulation occurred during the asymptomatic phase and increased throughout the onset phase. At the peak of the disease, MDSC accumulation reached its highest level, and then began to decrease during the recovery phase and returned to steady state levels by disease resolution. Similar results were found using collagen-induced arthritis (CIA), a mouse model of RA, where MDSC accumulation in the spleen correlated with the course of disease^[11]. In humans, MDSCs were found to be enriched in the bloodstream of patients with active MS, but were only slightly elevated in the blood of patients in recovery^[12].

MDSCs require certain signals for their expansion and activation. The factors responsible for driving the expansion of MDSCs include cyclooxygenase-2, prostaglandins, interleukin 6 (IL-6), macrophage colony-stimulating factor (M-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF)^[9,13-18]. Most of these factors trigger signaling pathways that stimulate the proliferation of myeloid cells in the bone marrow and inhibit their differentiation into mature cells^[3]. MDSCs

can be activated to suppress T cell functions *via* interferon gamma (IFN γ) and transforming growth factor beta (TGF- β)^[13]. Blocking IFN γ production by activated T cells abolishes MDSC-mediated T cell suppression^[1,11,19]. Cancer models have identified IL-6, IL-1 β , prostaglandin E₂, and the calcium binding proteins S100A8 and S100A9, as factors important for the accumulation of MDSCs at sites of inflammation^[17,20,21]. Tumor necrosis factor (TNF) signaling drives MDSC accumulation in the periphery by promoting MDSC survival and inhibiting apoptosis^[22]. Treatment with a TNF- α antagonist showed decreased MDSC accumulation in the spleen in response to chronic inflammation^[23].

MDSC SUBSETS IN AUTOIMMUNITY

Early classification of MDSCs was based on cell surface expression of CD11b and Gr-1. The CD11b⁺Gr-1⁺ subgroup is now divided into two separate groups, exhibiting either a monocytic morphology or a granulocytic morphology^[24]. Granulocytic MDSCs (G-MDSCs) display a CD11b⁺Ly6C^{low}Ly6G⁺ phenotype, whereas monocytic MDSCs (M-MDSCs) are CD11b⁺Ly6C⁺Ly6G⁻^[18,24-26]. The two groups also differ in functionality^[18,25,27]. MDSCs suppress T cell functions *via* a number of different mechanisms involving the production of soluble mediators or through cell-cell contact^[28-31]. G-MDSCs frequently inhibit T cell function through arginase-1 enzyme activity. M-MDSCs more commonly inhibit T cell functions *via* nitric oxide production. IFN γ -mediated activation of MDSCs results in the upregulation of arginase-1 and nitric oxide production. In the CIA model, MDSCs were found to inhibit both T cell proliferation and CD4⁺ T cell differentiation into Th17 cells^[11]. Here, the researchers used the total CD11b⁺Gr-1⁺ population from the spleen and found both arginase-1 and nitric oxide to be mechanisms of inhibition. The Gr-1 antibody recognizes both Ly6G and Ly6C surface antigens, therefore the population of cells used for their studies contained both G-MDSCs and M-MDSCs. In a mouse model of diabetes, CD11b⁺Gr-1⁺ cells were found to inhibit CD8⁺ and CD4⁺ T cell responses *via* nitric oxide- and IL-10-dependent mechanisms^[32]. In the EAE model, G-MDSCs from myelin oligodendrocyte glycoprotein-immunized mice were found to express high levels of programmed cell death 1 ligand 1 (PD-L1), a costimulatory molecule that negatively regulates T cell proliferation. G-MDSCs were found to inhibit autoantigen-priming of Th1 and Th17 cells in a PD-L1-dependent manner^[12]. Interestingly, one report showed that CD11b⁺Gr-1⁺ cells isolated from mice with EAE inhibited T cell proliferation in co-culture but promoted Th17 cell differentiation under Th17-polarizing conditions^[33].

M-MDSCs also display immunosuppressive effects during autoimmune diseases. Recent data showed that M-MDSCs induced during the priming phase of EAE were potent suppressors of activated T cells and mediated T cell inhibition through the production of nitric

oxide^[18]. Nitric oxide production by MDSCs results in the nitrosylation of cysteine residues, leading to a significant decrease in mRNA stability, and thereby preventing the production of cytokines required for T cell proliferation^[28]. Another study demonstrated that activation of M-MDSC suppressive function occurred at the peak of EAE disease^[34]. This study determined that the suppression of T cell responses was due to M-MDSC-mediated nitric oxide production. Furthermore, transfer of activated M-MDSCs led to apoptosis of T cells in the central nervous system and decreased EAE severity. In autoimmune arthritis, clinical trials against C-C chemokine receptor 2 (CCR2), the major chemokine receptor mediating monocyte recruitment, were surprisingly unsuccessful as monocytes/macrophages were thought to be pathogenic in RA^[35-37]. Interestingly, CCR2-deficient mice are now known to develop exacerbated CIA^[38,39]. The underlying mechanisms contributing to the aggravated disease are not clear. However, our data showed that M-MDSCs were absent from the periphery of collagen-immunized CCR2-deficient mice, as CCR2 is required for the emigration of M-MDSCs from the bone marrow^[38,40]. Further, M-MDSCs isolated from the bone marrow of CCR2-deficient mice with CIA inhibited CD4⁺ T cell proliferation and mitigated CIA severity, suggesting M-MDSCs are required for the regulation of autoimmune arthritis^[41].

Human MDSCs are identified as CD14⁺CD16⁺ and CD14⁺CD16⁻ cells. These CD14⁺ cells were found to be abundant in the blood and synovial fluid of RA patients^[42,43]. Recently, MDSCs were shown to mediate enhancement of regulatory T cell (Treg) suppressive functions^[43]. Here, Tregs were isolated from healthy subjects and their suppressive activity and cytokine expression were analyzed after co-culture with CD14⁺ cells. Results showed an increase in the expression of IFN γ , TNF- α , IL-17, and IL-10 by Tregs, a sustained Treg phenotype, and an enhanced capacity to suppress T cell-mediated proinflammatory cytokine production and T cell proliferation.

Taken together, these studies demonstrate that MDSCs can use various functions to suppress T cell responses and suggest that MDSC differentiation and function may be influenced by the distinct environment associated with each type of disease. Although both G-MDSCs and M-MDSCs can suppress T cell functions, further research is needed to confirm whether the two subsets have different outcomes in different diseases (Table 1).

MDSC-MEDIATED SUPPRESSION OF ANTIGEN-SPECIFIC IMMUNE RESPONSES

Loss of immunological tolerance is the basis for the development of autoimmune diseases. Recognition of self-antigens leads to autoimmune-driven tissue inflammation. However, regulation of the responses to self-antigens must be highly specific in order for the host immune recognition of pathogens to remain intact. MDSCs may play

a crucial role in maintaining this balance as they are capable of suppressing antigen-specific immune responses. It is believed that MDSCs internalize antigens and present them to T cells, bringing the two cells into close contact. Peroxynitrite, a derivative of nitric oxide, causes nitration of tyrosine residues on the T cell receptor (TCR), thereby preventing binding between the major histocompatibility complex (MHC) and peptide^[44]. Increased levels of nitrotyrosine have been documented for patients suffering from MS, RA, autoimmune myocarditis, and diabetes^[45-48]. In a cancer model, increased production of peroxynitrite and hydrogen peroxide resulted from the interaction between immature myeloid cells and antigen-specific CD8⁺ T cells in the presence of the specific antigen, but not in the presence of the control antigen^[29]. In some cancer models, arginase-1 production is the mechanism of MDSC-mediated suppression^[31,49]. The arginase-1 enzyme hydrolyzes arginine, depleting the pool of arginine available to the cell^[50-52]. A deficiency in arginine prevents the formation of CD3 molecules^[53]. The absence of CD3 prevents signaling through the TCR upon recognition of a specific antigen-MHC complex.

In one study of autoimmune diabetes, MDSCs induced the antigen-specific expansion of Tregs, which resulted in the suppression of T cell proliferation and prevented the onset of disease^[54]. The authors described that MDSC-mediated expansion of Tregs was dependent on antigen presentation by MHC class II molecules. For these experiments, hemagglutinin (HA)-specific CD4⁺ T cells were adoptively transferred to mice, followed by the administration of MDSCs and HA antigen. The results showed a significant reduction in disease upon administration of MDSCs and HA, but no decrease in disease when MDSCs were administered with the ovalbumin peptide, confirming that the MDSC-mediated suppression was antigen-specific.

MDSCs also mediate suppression of non-specific T cell responses, *i.e.*, mitogen-activated T cell responses, suggesting MDSCs may be involved in the late phase of tissue inflammation during autoimmune diseases. Others have hypothesized that MDSCs function in both antigen-specific and non-specific manners depending on the signals they are exposed to in a particular microenvironment^[55]. Indeed, comparison of MDSCs isolated from the spleen to those isolated from a tumor showed that splenic MDSCs were able to inhibit antigen-specific T cell responses *via* the production of reactive oxygen species, whereas MDSCs isolated from the tumor inhibited T cells nonspecifically and more potently than those from the spleen^[56]. T cells isolated from the peripheral lymphoid organs of human cancer patients, or from a mouse tumor model, are still responsive to non-cancer related stimuli, including viruses, IL-2, and anti-CD3/CD28 antibodies^[1,57]. This suggests that the expansion of MDSCs does not induce systemic immune suppression. Taken together, these data suggest that MDSCs from the site of inflammation may be more potent and far-reaching

Table 1 Myeloid-derived suppressor cells in autoimmune disease models

Human disease	Mouse model	Phenotype	T cell suppression	Suppressive mechanism	Suppressive role <i>in vivo</i>
Multiple sclerosis	EAE	CD11b ⁺ Ly6C ^{high} (M-MDSCs)	CD4 ⁺ T cells	NO-apoptosis	Not determined ^[18]
	EAE	CD11b ⁺ Ly6G ⁺ (M-MDSCs)	CD4 ⁺ , CD8 ⁺ , Ag-specific CD4 ⁺ T cells	NOS	No effect by naïve MDSCs ^[77]
	EAE	CD11b ⁺ Ly6C ^{high} (M-MDSCs)	Not determined	Not determined	Increase severity ^[73]
	EAE	Arg-1 ⁺ CD11b ⁺ Gr-1 ^{low} (M-MDSCs)	CD3 ⁺ T cells	Apoptosis	Not determined ^[10]
	EAE	CD11b ⁺ Ly6C ⁺ (M-MDSCs)	CD4 ⁺ T cells	NO	Reduce severity by late phase MDSCs ^[34]
	EAE	CD11b ^{high} Ly6G ⁺ Ly6C ⁻ (G-MDSCs)	Th1 and Th17 cells	PD-L1	Reduce severity ^[12]
	EAE	CD11b ⁺ Gr-1 ⁺	Promote Th17 cells	IL-1β	Increase severity ^[33]
	EAE	CD11b ⁺ Gr-1 ⁺	Ag-specific Th17 cells	iNOS, arginase-1 and IL-10	Ablated iNKT-induced disease mitigation ^[78]
Rheumatoid arthritis	CIA	CD11b ⁺ Ly6C ⁺ Ly6G ⁻ (M-MDSC)	CD4 ⁺ T cells	NO	Reduce severity ^[41]
	CIA	CD11b ⁺ Gr-1 ⁺	Th17 cells	Arginase and iNOS	Reduce severity ^[11]
	Proteoglycan-induced arthritis	CD11b ⁺ Gr-1 ⁺	Ag-specific T cells	NO and ROS	Not determined ^[79]
Systemic lupus erythematosus	MRL-fas ^{lpr}	CD11b ⁺ Gr-1 ^{low} (M-MDSCs)	CD4 ⁺ T cells	Arginase-1	Not determined ^[80]
Inflammatory bowel disease	HA-transgenic mice	CD11b ⁺ Gr-1 ⁺	Ag-specific CD8 ⁺ T cells	NO-apoptosis	Reduce severity ^[14]
	DDS-induced colitis	CD11b ⁺ Gr-1 ⁺	Not determined	Not determined	Reduce severity ^[75]
	IL-10 ^{-/-}	CD11b ⁺ Gr-1 ⁺	MLN T cells	Not determined	Not determined ^[81]
	TNBS-induced colitis	CD11b ⁺ Gr-1 ⁺	Splenocytes	Not determined	Reduce severity ^[74]
T1D	INS-HA/RAG ^{-/-}	Gr-1 ⁺ CD115 ⁺ (M-MDSCs)	Induce Tregs and inhibit Teff cells	TGF-β and IL-10	Reduce severity ^[54]
	h-CD20/NOD	CD11b ⁺ Gr-1 ⁺	CD4 ⁺ and CD8 ⁺ T cells	NO and IL-10	Not determined ^[32]
			induce Tregs		
Autoimmune hepatitis	Tgfb ^{-/-}	CD11b ⁺ Ly6C ^{high} Ly6G ⁻ (M-MDSCs)	CD4 ⁺ T cells	NO	Not determined ^[82]
Inflammatory eye disease	EAU	CD11b ⁺ Gr-1 ⁺ Ly6G ⁺ (M-MDSCs)	CD4 ⁺ T cells	TNFR-dependent, Arginase	Not Determined ^[9]
	EAU	REP-induced CD11b ⁺ Gr-1 ⁺	CD4 ⁺ T cells	Not determined	Reduce severity ^[76]
Alopecia areata	Alopecia areata-eczema	CD11b ⁺ Gr-1 ⁺	CD4 ⁺ and CD8 ⁺ T cells	CD3-zeta down-regulation	Local MDSC administration reduces severity ^[83]

T1D: Type 1 diabetes; EAE: Experimental autoimmune encephalomyelitis; CIA: Collagen-induced arthritis; MLR: Murphy roths large; HA: Hemagglutinin; DDS: Dextran sulphate sodium; INS: Insulin; RAG: Recombination-activating gene; NOD: Non-obese diabetic; EAU: Experimental autoimmune uveitis; M-MDSCs: Monocytic myeloid-derived suppressor cells; G-MDSCs: Granulocytic myeloid-derived suppressor cells; MLN: Mesenteric lymph node; NO: Nitric oxide; NOS: Nitric oxide synthase; PD-L1: Programmed cell death 1 ligand 1; iNOS: Inducible nitric oxide synthase; ROS: Reactive oxygen species; IL-10: Interleukin 10; TGF-β: Transforming growth factor beta; IL-1β: Interleukin 1 beta; TNFR: Tumor necrosis factor receptor.

in their suppressive effects than those MDSCs in the peripheral organs. The MDSCs in circulation may function to prevent the spread of inflammation to other areas of the body, without compromising immune recognition of pathogens.

THERAPEUTIC POTENTIAL OF MDSC-BASED TREATMENTS

Therapeutic approaches involving MDSCs require their purification and/or proliferation *in vitro*. MDSCs migrate to peripheral lymphoid organs where they differentiate into granulocytes, monocytes/macrophages, and dendritic cells (DCs). GM-CSF has been shown to drive MDSC accumulation at sites of inflammation^[58,59] and has been used to generate MDSCs from bone marrow cells *in vi-*

tro^[60]. However, the concentration of GM-CSF in the media must be tightly regulated as different concentrations of GM-CSF may lead to the generation of neutrophils or DCs^[60,61]. Vascular endothelial growth factor (VEGF) is important in the differentiation of hematopoietic progenitor cells^[62], and studies have shown that blocking VEGF binding leads to increased differentiation of MDSCs into DCs^[63]. Similar results were shown for stem cell factor, where blocking its function led to reduced MDSC expansion^[64]. Factors such as granulocyte colony-stimulating factor (G-CSF) and M-CSF are also known to induce MDSC expansion. G-CSF induces the proliferation of G-MDSCs *via* the Janus kinase/signal transducers and activators of transcription pathway (Jak/STAT)^[65]. In the presence of IL-6, M-CSF was shown to inhibit DC generation from hematopoietic stem cells (HSCs), thereby redirecting HSC differentiation towards MDSCs^[66].

The calcium binding proteins, S100A8 and S100A9, are upregulated in some autoimmune conditions, including RA, MS, and IBD^[66-68]. These proteins are secreted by MDSCs^[69] and may work in an autocrine fashion to promote the accumulation of MDSCs while simultaneously preventing their differentiation into DCs^[70]. MDSC generation, expansion, and gain of specific suppressive abilities occur primarily under inflammatory conditions such as infection, cancer, trauma, and autoimmune diseases. It is important to note that MDSCs are not terminally differentiated, and thus may mature into antigen-presenting cells, such as macrophages or DCs, highlighting a potential complication for therapeutic attempts. Therefore, in order to develop effective MDSC-based therapies, we must first understand how different cell types respond to different inflammatory mediators and determine how these inflammatory mediators affect the potency and/or suppressive mechanisms of MDSCs.

A number of studies have provided insight into the use of MDSCs for treatment of autoimmune diseases. In a murine model of diabetes, MDSCs were generated *in vitro* by culturing hepatic stellate cells with DCs^[71]. This method of MDSC generation was previously shown to produce highly suppressive cells in an IFN γ -dependent manner^[72]. In the diabetes study, these *in vitro*-generated MDSCs were mixed with pancreatic islet cells and transplanted into diabetic mice. The MDSCs induced Treg expansion in the allograft site, resulting in the inhibition of CD8⁺ T cell responses^[71]. In a mouse model of IBD, MDSCs were found to be upregulated in the spleen and intestine of IBD mice^[14]. Further data showed that these MDSCs effectively prevented T cell proliferation and induced T cell apoptosis after transfer of CD8⁺ T cells^[14]. One report showed that the *in vivo* transfer of G-MDSCs in the EAE model resulted in the delayed onset of disease and a significant reduction in demyelination^[12], however other studies were not as successful^[33,73]. Adoptive transfer of MDSCs also led to reduced disease severity in models of RA^[11,41], IBD^[74,75], and inflammatory eye disease^[76].

CONCLUSION

MDSCs represent an important class of immunoregulatory cells. MDSCs display particular heterogeneity and plasticity, and for these reasons they have become an attractive candidate for the treatment of autoimmune diseases. On the other hand, MDSCs are very difficult to work with because of their diverse nature. MDSCs have multiple phenotypes which inhibit T cell responses by multiple mechanisms, and their environment dictates the development of suppressive properties and activation pathways. Additionally, the maturation/differentiation of these cells may depend on the particular inflammatory signals received from their microenvironment. Though MDSCs hold promise in the treatment of autoimmune diseases, their full utilization is stalled by our limited understanding of their phenotype, differentiation, cellular

functions, and influence on the microenvironment.

REFERENCES

1. **Gabrilovich DI**, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009; **9**: 162-174 [PMID: 19197294]
2. **Nagaraj S**, Collazo M, Corzo CA, Youn JI, Ortiz M, Quiceno D, Gabrilovich DI. Regulatory myeloid suppressor cells in health and disease. *Cancer Res* 2009; **69**: 7503-7506 [PMID: 19752086]
3. **Condamine T**, Gabrilovich DI. Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. *Trends Immunol* 2011; **32**: 19-25 [PMID: 21067974 DOI: 10.1016/j.it.2010.10.002]
4. **Gabrilovich DI**, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012; **12**: 253-268 [PMID: 22437938 DOI: 10.1038/nri3175]
5. **Khaled YS**, Ammori BJ, Elkord E. Myeloid-derived suppressor cells in cancer: recent progress and prospects. *Immunol Cell Biol* 2013; **91**: 493-502 [PMID: 23797066 DOI: 10.1038/icb.2013.29]
6. **Cripps JG**, Gorham JD. MDSC in autoimmunity. *Int Immunopharmacol* 2011; **11**: 789-793 [PMID: 21310255 DOI: 10.1016/j.intimp.2011.01.026]
7. **Greten TF**, Manns MP, Korangy F. Myeloid derived suppressor cells in human diseases. *Int Immunopharmacol* 2011; **11**: 802-807 [PMID: 21237299 DOI: 10.1016/j.intimp.2011.01.003]
8. **Liversidge J**, Dick A, Gordon S. Nitric oxide mediates apoptosis through formation of peroxynitrite and Fas/Fas-ligand interactions in experimental autoimmune uveitis. *Am J Pathol* 2002; **160**: 905-916 [PMID: 11891189 DOI: 10.1016/S0002-9440(10)64913-9]
9. **Kerr EC**, Raveney BJ, Copland DA, Dick AD, Nicholson LB. Analysis of retinal cellular infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations. *J Autoimmun* 2008; **31**: 354-361 [PMID: 18838247 DOI: 10.1016/j.jaut.2008.08.006]
10. **Moliné-Velázquez V**, Cuervo H, Vila-Del Sol V, Ortega MC, Clemente D, de Castro F. Myeloid-derived suppressor cells limit the inflammation by promoting T lymphocyte apoptosis in the spinal cord of a murine model of multiple sclerosis. *Brain Pathol* 2011; **21**: 678-691 [PMID: 21507122 DOI: 10.1111/j.1750-3639.2011.00495.x]
11. **Fujii W**, Ashihara E, Hirai H, Nagahara H, Kajitani N, Fujioka K, Murakami K, Seno T, Yamamoto A, Ishino H, Kohno M, Maekawa T, Kawahito Y. Myeloid-derived suppressor cells play crucial roles in the regulation of mouse collagen-induced arthritis. *J Immunol* 2013; **191**: 1073-1081 [PMID: 23804709 DOI: 10.4049/jimmunol.1203535]
12. **Ioannou M**, Alissafi T, Lazaridis I, Deraos G, Matsoukas J, Gravanis A, Mastorodemos V, Plaitakis A, Sharpe A, Boumpas D, Verginis P. Crucial role of granulocytic myeloid-derived suppressor cells in the regulation of central nervous system autoimmune disease. *J Immunol* 2012; **188**: 1136-1146 [PMID: 22210912 DOI: 10.4049/jimmunol.1101816]
13. **Fujio K**, Okamura T, Sumitomo S, Yamamoto K. Regulatory T cell-mediated control of autoantibody-induced inflammation. *Front Immunol* 2012; **3**: 28 [PMID: 22566912 DOI: 10.3389/fimmu.2012.00028]
14. **Haile LA**, von Wasielowski R, Gamrekashvili J, Krüger C, Bachmann O, Westendorf AM, Buer J, Liblau R, Manns MP, Korangy F, Greten TF. Myeloid-derived suppressor cells in inflammatory bowel disease: a new immunoregulatory pathway. *Gastroenterology* 2008; **135**: 871-881, 881.e1-5 [PMID: 18674538 DOI: 10.1053/j.gastro.2008.06.032]
15. **Makarenkova VP**, Bansal V, Matta BM, Perez LA, Ochoa JB. CD11b+/Gr-1+ myeloid suppressor cells cause T cell dysfunction after traumatic stress. *J Immunol* 2006; **176**: 2085-2094

- [PMID: 16455964]
- 16 **Serafini P**, Carbley R, Noonan KA, Tan G, Bronte V, Borrello L. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res* 2004; **64**: 6337-6343 [PMID: 15342423 DOI: 10.1158/0008-5472.CAN-04-0757]
 - 17 **Sinha P**, Clements VK, Fulton AM, Ostrand-Rosenberg S. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. *Cancer Res* 2007; **67**: 4507-4513 [PMID: 17483367 DOI: 10.1158/0008-5472.CAN-06-4174]
 - 18 **Zhu B**, Bando Y, Xiao S, Yang K, Anderson AC, Kuchroo VK, Khoury SJ. CD11b+Ly-6C(hi) suppressive monocytes in experimental autoimmune encephalomyelitis. *J Immunol* 2007; **179**: 5228-5237 [PMID: 17911608]
 - 19 **Corzo CA**, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, Padhya T, McCaffrey TV, McCaffrey JC, Gabrilovich DI. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol* 2009; **182**: 5693-5701 [PMID: 19380816 DOI: 10.4049/jimmunol.0900092]
 - 20 **Bunt SK**, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression. *J Immunol* 2006; **176**: 284-290 [PMID: 16365420]
 - 21 **Sinha P**, Okoro C, Foell D, Freeze HH, Ostrand-Rosenberg S, Srikrishna G. Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. *J Immunol* 2008; **181**: 4666-4675 [PMID: 18802069]
 - 22 **Zhao X**, Rong L, Zhao X, Li X, Liu X, Deng J, Wu H, Xu X, Erben U, Wu P, Syrbe U, Sieper J, Qin Z. TNF signaling drives myeloid-derived suppressor cell accumulation. *J Clin Invest* 2012; **122**: 4094-4104 [PMID: 23064360 DOI: 10.1172/JCI64115]
 - 23 **Sade-Feldman M**, Kanterman J, Ish-Shalom E, Elnekave M, Horwitz E, Baniyash M. Tumor necrosis factor- α blocks differentiation and enhances suppressive activity of immature myeloid cells during chronic inflammation. *Immunity* 2013; **38**: 541-554 [PMID: 23477736 DOI: 10.1016/j.immuni.2013.02.007]
 - 24 **Youn JI**, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol* 2008; **181**: 5791-5802 [PMID: 18832739]
 - 25 **Dietlin TA**, Hofman FM, Lund BT, Gilmore W, Stohlman SA, van der Veen RC. Mycobacteria-induced Gr-1+ subsets from distinct myeloid lineages have opposite effects on T cell expansion. *J Leukoc Biol* 2007; **81**: 1205-1212 [PMID: 17307863 DOI: 10.1189/jlb.1006640]
 - 26 **Hettinger J**, Richards DM, Hansson J, Barra MM, Joschko AC, Krijgsvelde J, Feuerer M. Origin of monocytes and macrophages in a committed progenitor. *Nat Immunol* 2013; **14**: 821-830 [PMID: 23812096 DOI: 10.1038/ni.2638]
 - 27 **Movahedi K**, Williams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, De Baetselier P, Van Ginnecht JA. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood* 2008; **111**: 4233-4244 [PMID: 18272812 DOI: 10.1182/blood-2007-07-099226]
 - 28 **Bronte V**, Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* 2005; **5**: 641-654 [PMID: 16056256 DOI: 10.1038/nri1668]
 - 29 **Kusmartsev S**, Nefedova Y, Yoder D, Gabrilovich DI. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J Immunol* 2004; **172**: 989-999 [PMID: 14707072]
 - 30 **Nagaraj S**, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, Herber DL, Schneck J, Gabrilovich DI. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat Med* 2007; **13**: 828-835 [PMID: 17603493 DOI: 10.1038/nm1609]
 - 31 **Rodríguez PC**, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. *Immunol Rev* 2008; **222**: 180-191 [PMID: 18364002 DOI: 10.1111/j.1600-065X.2008.00608.x]
 - 32 **Hu C**, Du W, Zhang X, Wong FS, Wen L. The role of Gr1+ cells after anti-CD20 treatment in type 1 diabetes in nonobese diabetic mice. *J Immunol* 2012; **188**: 294-301 [PMID: 22140261 DOI: 10.4049/jimmunol.1101590]
 - 33 **Yi H**, Guo C, Yu X, Zuo D, Wang XY. Mouse CD11b+Gr-1+ myeloid cells can promote Th17 cell differentiation and experimental autoimmune encephalomyelitis. *J Immunol* 2012; **189**: 4295-4304 [PMID: 23034169 DOI: 10.4049/jimmunol.1200086]
 - 34 **Zhu B**, Kennedy JK, Wang Y, Sandoval-Garcia C, Cao L, Xiao S, Wu C, Elyaman W, Khoury SJ. Plasticity of Ly-6C(hi) myeloid cells in T cell regulation. *J Immunol* 2011; **187**: 2418-2432 [PMID: 21824867 DOI: 10.4049/jimmunol.1100403]
 - 35 **Horuk R**. Chemokine receptor antagonists: overcoming developmental hurdles. *Nat Rev Drug Discov* 2009; **8**: 23-33 [PMID: 19079127 DOI: 10.1038/nrd2734]
 - 36 **Vergunst CE**, Gerlag DM, Lopatinskaya L, Klareskog L, Smith MD, van den Bosch F, Dinant HJ, Lee Y, Wyant T, Jacobson EW, Baeten D, Tak PP. Modulation of CCR2 in rheumatoid arthritis: a double-blind, randomized, placebo-controlled clinical trial. *Arthritis Rheum* 2008; **58**: 1931-1939 [PMID: 18576354]
 - 37 **Kalinowska A**, Losy J. Investigational C-C chemokine receptor 2 antagonists for the treatment of autoimmune diseases. *Expert Opin Investig Drugs* 2008; **17**: 1267-1279 [PMID: 18694362]
 - 38 **Rampersad RR**, Tarrant TK, Vallanat CT, Quintero-Matthews T, Weeks MF, Esserman DA, Clark J, Di Padova F, Patel DD, Fong AM, Liu P. Enhanced Th17-cell responses render CCR2-deficient mice more susceptible for autoimmune arthritis. *PLoS One* 2011; **6**: e25833 [PMID: 21991368 DOI: 10.1371/journal.pone.0025833]
 - 39 **Quinones MP**, Estrada CA, Kalkonde Y, Ahuja SK, Kuziel WA, Mack M, Ahuja SS. The complex role of the chemokine receptor CCR2 in collagen-induced arthritis: implications for therapeutic targeting of CCR2 in rheumatoid arthritis. *J Mol Med (Berl)* 2005; **83**: 672-681 [PMID: 15827759]
 - 40 **Serbina NV**, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 2006; **7**: 311-317 [PMID: 16462739]
 - 41 **Crook K**, Weeks M, Rampersad R, Baldi R, Liu P. Myeloid-derived suppressor cells ameliorate the pathogenesis of autoimmune arthritis (P1058). *J Immunol* 2013; **190**: 185.3
 - 42 **Evans HG**, Gullick NJ, Kelly S, Pitzalis C, Lord GM, Kirkham BW, Taams LS. In vivo activated monocytes from the site of inflammation in humans specifically promote Th17 responses. *Proc Natl Acad Sci USA* 2009; **106**: 6232-6237 [PMID: 19325128 DOI: 10.1073/pnas.0808144106]
 - 43 **Walter GJ**, Evans HG, Menon B, Gullick NJ, Kirkham BW, Cope AP, Geissmann F, Taams LS. Interaction with activated monocytes enhances cytokine expression and suppressive activity of human CD4+CD45ro+CD25+CD127(low) regulatory T cells. *Arthritis Rheum* 2013; **65**: 627-638 [PMID: 23280063 DOI: 10.1002/art.37832]
 - 44 **Birnboim HC**, Lemay AM, Lam DK, Goldstein R, Webb JR. Cutting edge: MHC class II-restricted peptides containing the inflammation-associated marker 3-nitrotyrosine evade central tolerance and elicit a robust cell-mediated immune response. *J Immunol* 2003; **171**: 528-532 [PMID: 12847213]
 - 45 **Bachmaier K**, Neu N, Pummerer C, Duncan GS, Mak TW, Matsuyama T, Penninger JM. iNOS expression and nitrotyrosine formation in the myocardium in response to inflammation is controlled by the interferon regulatory transcription factor 1. *Circulation* 1997; **96**: 585-591 [PMID: 9244230]
 - 46 **Bagasra O**, Michaels FH, Zheng YM, Bobroski LE, Spitsin SV, Fu ZF, Tawadros R, Koprowski H. Activation of the in-

- ducible form of nitric oxide synthase in the brains of patients with multiple sclerosis. *Proc Natl Acad Sci USA* 1995; **92**: 12041-12045 [PMID: 8618840]
- 47 **Ceriello A**, Mercuri F, Quagliaro L, Assaloni R, Motz E, Tonutti L, Taboga C. Detection of nitrotyrosine in the diabetic plasma: evidence of oxidative stress. *Diabetologia* 2001; **44**: 834-838 [PMID: 11508267 DOI: 10.1007/s001250100529]
 - 48 **Kaur H**, Halliwell B. Evidence for nitric oxide-mediated oxidative damage in chronic inflammation. Nitrotyrosine in serum and synovial fluid from rheumatoid patients. *FEBS Lett* 1994; **350**: 9-12 [PMID: 8062931]
 - 49 **Bronte V**, Serafini P, De Santo C, Marigo I, Tosello V, Mazoni A, Segal DM, Staib C, Lowel M, Sutter G, Colombo MP, Zanovello P. IL-4-induced arginase 1 suppresses alloreactive T cells in tumor-bearing mice. *J Immunol* 2003; **170**: 270-278 [PMID: 12496409]
 - 50 **Cederbaum SD**, Yu H, Grody WW, Kern RM, Yoo P, Iyer RK. Arginases I and II: do their functions overlap? *Mol Genet Metab* 2004; **81** Suppl 1: S38-S44 [PMID: 15050972 DOI: 10.1016/j.ymgme.2003.10.012]
 - 51 **Hesse M**, Modolell M, La Flamme AC, Schito M, Fuentes JM, Cheever AW, Pearce EJ, Wynn TA. Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. *J Immunol* 2001; **167**: 6533-6544 [PMID: 11714822]
 - 52 **Jenkinson CP**, Grody WW, Cederbaum SD. Comparative properties of arginases. *Comp Biochem Physiol B Biochem Mol Biol* 1996; **114**: 107-132 [PMID: 8759304]
 - 53 **Rodriguez PC**, Zea AH, Culotta KS, Zabaleta J, Ochoa JB, Ochoa AC. Regulation of T cell receptor CD3zeta chain expression by L-arginine. *J Biol Chem* 2002; **277**: 21123-21129 [PMID: 11950832 DOI: 10.1074/jbc.M110675200]
 - 54 **Yin B**, Ma G, Yen CY, Zhou Z, Wang GX, Divino CM, Casares S, Chen SH, Yang WC, Pan PY. Myeloid-derived suppressor cells prevent type 1 diabetes in murine models. *J Immunol* 2010; **185**: 5828-5834 [PMID: 20956337 DOI: 10.4049/jimmunol.0903636]
 - 55 **Nagaraj S**, Gabrilovich DI. Tumor escape mechanism governed by myeloid-derived suppressor cells. *Cancer Res* 2008; **68**: 2561-2563 [PMID: 18413722 DOI: 10.1158/0008-5472.CAN-07-6229]
 - 56 **Corzo CA**, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T, McCaffrey TV, McCaffrey JC, Gabrilovich DI. HIF-1 α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med* 2010; **207**: 2439-2453 [PMID: 20876310 DOI: 10.1084/jem.20100587]
 - 57 **Solito S**, Bronte V, Mandruzzato S. Antigen specificity of immune suppression by myeloid-derived suppressor cells. *J Leukoc Biol* 2011; **90**: 31-36 [PMID: 21486906 DOI: 10.1189/jlb.0111021]
 - 58 **Fu YX**, Watson G, Jimenez JJ, Wang Y, Lopez DM. Expansion of immunoregulatory macrophages by granulocyte-macrophage colony-stimulating factor derived from a murine mammary tumor. *Cancer Res* 1990; **50**: 227-234 [PMID: 2136804]
 - 59 **Ilkoviitch D**, Lopez DM. Urokinase-mediated recruitment of myeloid-derived suppressor cells and their suppressive mechanisms are blocked by MUC1/sec. *Blood* 2009; **113**: 4729-4739 [PMID: 19196663 DOI: 10.1182/blood-2008-08-176438]
 - 60 **Rössner S**, Voigtländer C, Wiethe C, Hänig J, Seifarth C, Lutz MB. Myeloid dendritic cell precursors generated from bone marrow suppress T cell responses via cell contact and nitric oxide production in vitro. *Eur J Immunol* 2005; **35**: 3533-3544 [PMID: 16331707 DOI: 10.1002/eji.200526172]
 - 61 **Lutz MB**, Suri RM, Niimi M, Ogilvie AL, Kukutsch NA, Rössner S, Schuler G, Austyn JM. Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival in vivo. *Eur J Immunol* 2000; **30**: 1813-1822 [PMID: 10940870 DOI: 10.1002/1521-4141(200007)30:7<1813::AID-IMMU1813>3.0.CO;2-8]
 - 62 **Gabrilovich D**, Ishida T, Oyama T, Ran S, Kravtsov V, Nadaf S, Carbone DP. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. *Blood* 1998; **92**: 4150-4166 [PMID: 9834220]
 - 63 **Roland CL**, Lynn KD, Toombs JE, Dineen SP, Udugamasooriya DG, Brekken RA. Cytokine levels correlate with immune cell infiltration after anti-VEGF therapy in preclinical mouse models of breast cancer. *PLoS One* 2009; **4**: e7669 [PMID: 19888452 DOI: 10.1371/journal.pone.0007669]
 - 64 **Pan PY**, Wang GX, Yin B, Ozao J, Ku T, Divino CM, Chen SH. Reversion of immune tolerance in advanced malignancy: modulation of myeloid-derived suppressor cell development by blockade of stem-cell factor function. *Blood* 2008; **111**: 219-228 [PMID: 17885078 DOI: 10.1182/blood-2007-04-086835]
 - 65 **Nefedova Y**, Nagaraj S, Rosenbauer A, Muro-Cacho C, Sebt SM, Gabrilovich DI. Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. *Cancer Res* 2005; **65**: 9525-9535 [PMID: 16230418 DOI: 10.1158/0008-5472.CAN-05-0529]
 - 66 **Leach ST**, Yang Z, Messina I, Song C, Geczy CL, Cunningham AM, Day AS. Serum and mucosal S100 proteins, calprotectin (S100A8/S100A9) and S100A12, are elevated at diagnosis in children with inflammatory bowel disease. *Scand J Gastroenterol* 2007; **42**: 1321-1331 [PMID: 17852869 DOI: 10.1080/00365520701416709]
 - 67 **Nukui T**, Ehama R, Sakaguchi M, Sonogawa H, Katagiri C, Hibino T, Huh NH. S100A8/A9, a key mediator for positive feedback growth stimulation of normal human keratinocytes. *J Cell Biochem* 2008; **104**: 453-464 [PMID: 18044712 DOI: 10.1002/jcb.21639]
 - 68 **Roth J**, Goebeler M, Sorg C. S100A8 and S100A9 in inflammatory diseases. *Lancet* 2001; **357**: 1041 [PMID: 11293617 DOI: 10.1016/S0140-6736(05)71610-X]
 - 69 **Zhao F**, Hoechst B, Duffy A, Gamrekashvili J, Fioravanti S, Manns MP, Greten TF, Korangy F. S100A9 a new marker for monocytic human myeloid-derived suppressor cells. *Immunology* 2012; **136**: 176-183 [PMID: 22304731 DOI: 10.1111/j.1365-2567.2012.03566.x]
 - 70 **Cheng P**, Corzo CA, Luetke N, Yu B, Nagaraj S, Bui MM, Ortiz M, Nacken W, Sorg C, Vogl T, Roth J, Gabrilovich DI. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *J Exp Med* 2008; **205**: 2235-2249 [PMID: 18809714 DOI: 10.1084/jem.20080132]
 - 71 **Chou HS**, Hsieh CC, Charles R, Wang L, Wagner T, Fung JJ, Qian S, Lu LL. Myeloid-derived suppressor cells protect islet transplants by B7-H1 mediated enhancement of T regulatory cells. *Transplantation* 2012; **93**: 272-282 [PMID: 22179405 DOI: 10.1097/TP.0b013e31823ff39]
 - 72 **Chou HS**, Hsieh CC, Yang HR, Wang L, Arakawa Y, Brown K, Wu Q, Lin F, Peters M, Fung JJ, Lu L, Qian S. Hepatic stellate cells regulate immune response by way of induction of myeloid suppressor cells in mice. *Hepatology* 2011; **53**: 1007-1019 [PMID: 21374665 DOI: 10.1002/hep.24162]
 - 73 **King IL**, Dickendersher TL, Segal BM. Circulating Ly-6C⁺ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. *Blood* 2009; **113**: 3190-3197 [PMID: 19196868 DOI: 10.1182/blood-2008-07-168575]
 - 74 **Guan Q**, Moreno S, Qing G, Weiss CR, Lu L, Bernstein CN, Warrington RJ, Ma Y, Peng Z. The role and potential therapeutic application of myeloid-derived suppressor cells in TNBS-induced colitis. *J Leukoc Biol* 2013; **94**: 803-811 [PMID: 23901119 DOI: 10.1189/jlb.0113050]
 - 75 **Zhang R**, Ito S, Nishio N, Cheng Z, Suzuki H, Isobe KI. Dex-

- tran sulphate sodium increases splenic Gr1(+)CD11b(+) cells which accelerate recovery from colitis following intravenous transplantation. *Clin Exp Immunol* 2011; **164**: 417-427 [PMID: 21413942 DOI: 10.1111/j.1365-2249.2011.04374.x]
- 76 **Tu Z**, Li Y, Smith D, Doller C, Sugita S, Chan CC, Qian S, Fung J, Caspi RR, Lu L, Lin F. Myeloid suppressor cells induced by retinal pigment epithelial cells inhibit autoreactive T-cell responses that lead to experimental autoimmune uveitis. *Invest Ophthalmol Vis Sci* 2012; **53**: 959-966 [PMID: 22247470 DOI: 10.1167/iovs.11-8377]
- 77 **Slaney CY**, Toker A, La Flamme A, Bäckström BT, Harper JL. Naïve blood monocytes suppress T-cell function. A possible mechanism for protection from autoimmunity. *Immunol Cell Biol* 2011; **89**: 7-13 [PMID: 21060323 DOI: 10.1038/icb.2010.110]
- 78 **Parekh VV**, Wu L, Olivares-Villagómez D, Wilson KT, Van Kaer L. Activated invariant NKT cells control central nervous system autoimmunity in a mechanism that involves myeloid-derived suppressor cells. *J Immunol* 2013; **190**: 1948-1960 [PMID: 23345328 DOI: 10.4049/jimmunol.1201718]
- 79 **Egelston C**, Kurkó J, Besenyei T, Tryniszewska B, Rauch TA, Glant TT, Mikecz K. Suppression of dendritic cell maturation and T cell proliferation by synovial fluid myeloid cells from mice with autoimmune arthritis. *Arthritis Rheum* 2012; **64**: 3179-3188 [PMID: 22492217 DOI: 10.1002/art.34494]
- 80 **Iwata Y**, Furuichi K, Kitagawa K, Hara A, Okumura T, Kokubo S, Shimizu K, Sakai N, Sagara A, Kurokawa Y, Ueha S, Matsushima K, Kaneko S, Wada T. Involvement of CD11b+ GR-1 low cells in autoimmune disorder in MRL-Fas lpr mouse. *Clin Exp Nephrol* 2010; **14**: 411-417 [PMID: 20652350 DOI: 10.1007/s10157-010-0309-9]
- 81 **Singh UP**, Singh NP, Singh B, Hofseth LJ, Taub DD, Price RL, Nagarkatti M, Nagarkatti PS. Role of resveratrol-induced CD11b(+) Gr-1(+) myeloid derived suppressor cells (MDSCs) in the reduction of CXCR3(+) T cells and amelioration of chronic colitis in IL-10(-/-) mice. *Brain Behav Immun* 2012; **26**: 72-82 [PMID: 21807089 DOI: 10.1016/j.bbi.2011.07.236]
- 82 **Cripps JG**, Wang J, Maria A, Blumenthal I, Gorham JD. Type 1 T helper cells induce the accumulation of myeloid-derived suppressor cells in the inflamed Tgfb1 knockout mouse liver. *Hepatology* 2010; **52**: 1350-1359 [PMID: 20803559 DOI: 10.1002/hep.23841]
- 83 **Marhaba R**, Vitacolonna M, Hildebrand D, Baniyash M, Freyschmidt-Paul P, Zöller M. The importance of myeloid-derived suppressor cells in the regulation of autoimmune effector cells by a chronic contact eczema. *J Immunol* 2007; **179**: 5071-5081 [PMID: 17911592]

P- Reviewers: Sotelo J, Yves R **S- Editor:** Song XX

L- Editor: A **E- Editor:** Liu SQ





Immune thrombocytopenia in adults

Erdal Kurtoğlu, Volkan Karakuş

Erdal Kurtoğlu, Volkan Karakuş, Department of Hematology, Antalya Training and Research Hospital, 07070 Antalya, Turkey
Author contributions: Kurtoğlu E and Karakuş V contributed equally to this work, wrote the manuscript and generated the figures; Kurtoğlu E additionally designed the aim of the manuscript.
Correspondence to: Erdal Kurtoğlu, MD, Associated Professor, Department of Hematology, Antalya Training and Research Hospital, Varlık Mah., 07070 Antalya, Turkey. erdalkurtoglu@yahoo.com
Telephone: +90-242-2494400 Fax: +90-242-2494462
Received: September 27, 2013 Revised: November 21, 2013
Accepted: December 13, 2013
Published online: March 27, 2014

Core tip: In this manuscript we evaluate all aspects of immune thrombocytopenia (ITP). We outline the etiology, pathogenesis, diagnosis and treatment of ITP. We describe the first and second-line therapies in detail. Also, the mechanism of the actions of drugs is described.

Kurtoğlu E, Karakuş V. Immune thrombocytopenia in adults. *World J Immunol* 2014; 4(1): 34-41 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i1/34.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i1.34>

Abstract

Immune thrombocytopenia is an autoimmune disease resulting in the destruction of platelets. It is classified as acute, thrombocytopenia occurring for < 6 mo and usually resolving spontaneously, and chronic, lasting > 6 mo and requiring therapy to improve the thrombocytopenia. The underlying defects leading to autoantibody production are unknown. Molecular mimicry appears to play a role in the development of self-reactive platelet antibodies after vaccination and certain viral infections. Platelet life span is reduced as a consequence of antibody-mediated clearance by tissue macrophages in essentially all patients. Diagnosis is based on the exclusion of the other causes of thrombocytopenia. Steroid is the first choice of the treatment, often followed by splenectomy in unresponsive cases. Intravenous immunoglobulin, anti-Rho(D) immune globulin, azathioprine, cyclosporine A, cyclophosphamide, danazol, dapsone, mycophenolate mofetil, rituximab, thrombopoietin receptor agonists and vinca alkaloids are other choices of treatment.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Immune thrombocytopenia; Splenectomy; Intravenous immunoglobulin; Autoimmune thrombocytopenia

INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune disease involving antibody and cell-mediated destruction of platelets and suppression of platelet production that may predispose to bleeding which may be even fatal. Recent recommendations from an international working group suggest that ITP be used to designate all cases of immune-mediated thrombocytopenia, whether occurring as a component of another clinically evident disorder or drug exposure, secondary ITP or, in the absence of a clear predisposing etiology, primary ITP^[1,2].

The international working group also recommends that a platelet count below $100 \times 10^9/L$, rather than $150 \times 10^9/L$, be required for diagnosis. This threshold is based on observational evidence that fewer than 10% of otherwise healthy individuals with a stable platelet count between 100 and $150 \times 10^9/L$ develop more severe unexplained ITP over the ensuing 10 years. This review focuses on primary ITP in the adult population but includes certain aspects of secondary forms and pediatric ITP where pertinent^[3].

INCIDENCE AND PREVALENCE

The annual incidence of ITP in the United States is estimated to be 1.6/100000. Acute ITP, defined as thrombocytopenia occurring for < 6 mo and usually resolving

spontaneously, most often affects children and young adults. The incidence peaks in the winter and spring, following viral infections. Acute ITP is most common between 2 and 6 years of age. Approximately 7% to 28% of children with acute ITP develop the chronic form. Chronic ITP, lasting > 6 mo and requiring therapy to improve the thrombocytopenia, occurs most commonly in adults, as emphasized in the oldest reported series in the literature. In the reported series, both acute and chronic ITP cases were reviewed, 67% of 271 patients and 45% of 737 patients were below 21 and 15 years of age, respectively. In chronic ITP in adults, the median age is usually 40 to 45 years, although in one large series, 74% of 934 cases were younger than the of age 40. The ratio of female to male is nearly 1:1 in acute ITP and 2 to 3:1 in chronic ITP^[4].

Estimates of the incidence of adult-onset ITP range from approximately 1.6 to 3.9 per 100000 persons per year, with a prevalence ranging from 9.5 to 23.6 per 100000 persons, based on diagnostic codes in the United Kingdom health registry^[5,6]. Estimates based on the International Classification of Diseases, 9th revision codes at hospital discharge in the United States are somewhat lower^[7]. However, in light of the vagaries of diagnosis and diagnostic coding, as well as the likelihood that some affected patients may not seek medical attention, the actual frequency of ITP and the number of individuals requiring therapy is uncertain.

ETIOLOGY

The underlying defects leading to autoantibody production are unknown. Heritability is uncommon, although predisposing polymorphisms in cytokines and Fcγ receptors have been described. A Th1/Th0 cytokine profile, a reduction in suppressor T-regulatory cells, and an increase in B-cell-activating factor may predispose to emergence of autoantibodies in response to exogenous antigens. Molecular mimicry appears to play a role in the development of self-reactive platelet antibodies after vaccination and certain viral infections^[8-12]. Thrombocytopenia can be caused by a myriad of conditions, including systemic disease, infection, drugs and primary hematological disorders (Table 1)^[2].

PATHOGENESIS

Platelet life span is reduced as a consequence of antibody-mediated clearance by tissue macrophages in essentially all patients. Accumulating evidence from studies of platelet kinetics also points to the contribution of immune-mediated suppression of megakaryocyte and platelet development in many patients; megakaryocyte apoptosis and suppression of megakaryopoiesis *in vitro* by ITP plasma/immunoglobulin G (IgG) or T-cells, and responsiveness to thrombopoietin receptor agonists (TRAs)^[13-19]. Platelet-reactive antibodies are not detected in all individuals with ITP and a subset of patients do not respond to pharmacological or surgical inhibition of an-

Table 1 Frequent examples of differential diagnosis of immune thrombocytopenia and potential alternative causes of thrombocytopenia identified by patient history

Previously diagnosed or possible high risk of conditions that may be associated with autoimmune thrombocytopenia, for example, HIV, HCV or other infection; other autoimmune/immunodeficiency disorders (including systemic lupus erythematosus; malignancy (e.g., lymphoproliferative disorders); recent vaccination
Liver disease (including alcoholic liver cirrhosis)
Drugs (prescription or non-prescription), alcohol abuse, consumption of quinine, tonic water, exposure to environmental toxins
Bone marrow diseases including myelodysplastic syndromes, leukemias, other malignancies, fibrosis, aplastic anemia and megaloblastic anemia
Recent transfusions (possibility of post-transfusion purpura) and recent immunizations
Inherited thrombocytopenia: thrombocytopenia-absent radius syndrome, radioulnar synostosis, congenital amegakaryocytic thrombocytopenia, Wiskott-Aldrich syndrome, MYH9-related disease, Bernard-Soulier syndrome, type II B von Willebrand disease

HIV: Human immunodeficiency virus; HCV: Hepatitis C virus; MYH9: Myosin heavy chain 9.

tibody-mediated platelet clearance or B-cell suppression, suggesting the possible involvement of other pathogenic mechanisms such as antibody-mediated apoptosis, antigen shedding and T-cell mediated platelet destruction or marrow suppression^[20].

Although the initial inciting event resulting in provocation of antiplatelet antibodies remains unknown, platelet autoantibodies are often present by the time of diagnosis. Macrophages and dendritic cells of the reticuloendothelial system function to phagocytose circulating antibody-bound antigens, including antibody-targeted platelets. Opsonization of antibody-platelet complexes facilitates intracellular processing of platelets and can lead to presentation by T cells *via* major histocompatibility complex (MHC) II as an array of “foreign” platelet peptides. Presentation of platelet peptides by MHC II in a stimulatory context activates T cells, leading to enhancement of the antiplatelet immune response and the possibility of epitope spread to additional platelet antigens^[21].

In patients with ITP, autoantibodies frequently appear to be directed against Gp I b/IX and GP II b/IIIa, although specificity for other platelet antigens can occur. Although antiplatelet autoantibodies appear to play a central role in the pathogenesis of ITP, some patients have no detectable antibodies at the time of diagnosis. This may be explained by limitations inherent to laboratory testing methods and the biology of ITP: Brisk clearance of some types of antibody-platelet complexes may reduce circulating antiplatelet antibody titers to below the threshold of detection; tightly bound antiplatelet antibodies may be difficult to dissociate for study; antibodies with specificity to minor or cryptic antigens on platelets or antigens that reside primarily on megakaryocytes may be missed; and there may simply be a subset of patients in which antiplatelet antibodies are not present. Therefore, although the majority of ITP patients present with features consistent with antibody-mediated autoimmunity

as a central feature of their disease, there exists considerable heterogeneity in the types, titers and likely biology of antiplatelet antibodies in ITP^[22,23].

As discussed in more detail in a recent review, the presentation of secondary ITP is often more complex than primary ITP. Similar to the antiplatelet antibodies provoked during *Helicobacter pylori* (*H. pylori*) infection, human immunodeficiency virus (HIV) can provoke anti-HIV antibodies that cross-react with platelet glycoproteins and form immune complexes, as hepatitis C virus (HCV) does. Additional mechanisms of platelet destruction also become apparent from studies in virus-associated ITP^[24]. In both HIV and HCV, suppression of viral replication can result in improvement in thrombocytopenia. Interestingly, HIV-associated ITP tends to occur early in HIV infection, whereas non-ITP tends to predominate in more advanced HIV when the immune system has suffered from greater effects of the infection. One possible explanation is that the immune system of HIV patients is more capable of developing autoimmunity in the earlier phases of the disease.

An acute infectious event has long been suspected to be a trigger in the initiation of primary ITP. Acute infection remains a plausible candidate to induce ITP either by providing an opportunity for molecular mimicry or similar targeting of the immune system to platelets or by the mere presence of an acute inflammatory response tipping the balance in a predisposed patient to break tolerance^[21].

Patients with systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), antiphospholipid antibody syndrome and rheumatoid arthritis, are prone to developing ITP. A diagnosis of secondary ITP in these patients is complex because non-ITP due to underlying disease or related therapies is also common. These observations are consistent with the notion that a patient with one autoimmune disease is at high risk of developing a second. The mechanisms underlying the development of many autoimmune disorders, including ITP, is unknown. It may also be that during the immune dysregulation leading to autoimmunity to one self-antigen, there is a risk of immune presentation of other self-antigens. Interestingly, many of the features of immune dysregulation described in ITP, such as the shift in Th1/Th2 balance, increased Th17 and altered Treg profiles described above, are also common to other autoimmune diseases^[25].

DIAGNOSIS

Personal history, with special attention to drugs and medical conditions that could cause thrombocytopenia, is very important. With a family history, ITP may occasionally be mistaken for an inherited cause of thrombocytopenia. The presence of the latter can often be confirmed by review of the peripheral blood film of the patient as well as other family members with thrombocytopenia. ITP is generally not considered to be an inherited disorder, although some HLA alleles may be more prevalent in ITP patients^[26].

Physical examination should be normal aside from

bleeding manifestations. Mild splenomegaly may be found in younger patients, but moderate or massive splenomegaly suggests an alternative cause. Constitutional symptoms, such as fever or weight loss, hepatomegaly or lymphadenopathy, might indicate an underlying disorder such as HIV, SLE or a lymphoproliferative disease^[27].

ITP is characterized by isolated thrombocytopenia with an otherwise normal complete blood count. Anemia from blood loss may be present but it should be proportional to the amount and duration of bleeding and may result in iron deficiency^[2].

In ITP, the peripheral blood smear should appear normal except for the presence of thrombocytopenia, although platelets may be mildly enlarged in some individuals. Both red cell and leukocyte morphologies are normal^[26].

Bone marrow examination may be informative in patients older than 60 years of age, in those with systemic symptoms or abnormal signs, or in some cases in which splenectomy is considered. Both bone marrow aspirate and biopsy should be performed. In addition to the morphological assessment, flow cytometry and cytogenetic testing should be considered (evidence level II b-IV). Flow cytometry may be particularly helpful in identifying patients with ITP secondary to chronic lymphocytic leukemia^[2,27].

International guidelines suggest that testing for reduced immunoglobulin levels and HIV, HCV and *H. pylori* infections should also be considered. Testing for antiphospholipid antibodies, antinuclear antibodies, parvovirus and cytomegalovirus may also be indicated in specific individuals. Testing for antiplatelet antibodies is not commonly performed in the current era because of its relatively low sensitivity and specificity^[26].

MANAGEMENT OF ADULT ITP

Relevant factors that contribute to management decisions include the extent of bleeding, comorbidities predisposition to bleeding, complications of specific therapies, activity and lifestyle, tolerance of side effects, potential interventions that may cause bleeding, accessibility of care, patient expectations, patient's worry or anxiety about disease burden, and patient's need for non-ITP medications that may create a bleeding risk^[28,29].

Treatment is rarely indicated in patients with platelet counts above $50 \times 10^9/L$ in the absence of the following: Bleeding due to platelet dysfunction or another hemostatic defect, trauma, surgery, clearly identified comorbidities for bleeding, mandated anticoagulation therapy, or in persons whose profession or lifestyle predisposes them to trauma. Patient's preference must also be considered when discussing treatment options^[30].

FIRST-LINE TREATMENT

First-line therapies for ITP include corticosteroids, intravenous immunoglobulin (IVIg) and anti-Rho(D) immune globulin^[2].

Table 2 Summary of dosage and toxicity of drugs

Agent	Typical dosing	Time to response	Selected toxicities
Prednis(ol)one	0.5-2 mg/kg per day 2-4 wk followed by slow taper	Several days to several weeks	Mood swings, insomnia, anxiety, psychosis, weight gain, cushingoid facies, hyperglycemia, decreased bone density, hypertension, skin changes, gastrointestinal distress and ulceration, avascular necrosis, increased susceptibility to infections, cataracts, adrenal insufficiency
Methylprednisolone	30 mg/kg per day 7 d	2-7 d	
Dexamethasone	40 mg/d for 4 d every 2-4 wk for 1-4 cycles	Several days to several weeks	
IVIg	0.4 g/kg per day 5 d or 1 g/kg per day 1-2 d	1-4 d	Headache, aseptic meningitis, renal insufficiency, fever, chills, nausea, thromboembolism, anaphylactoid reactions in patients with IgA-deficiency
Anti-Rh(D)	50-75 mcg/kg	1-5 d	Hemolytic anemia, fever, chills. Rarely, intravascular hemolysis, DIC, and renal failure
Splenectomy	N/A	0-24 d	Adverse effects of surgery and anesthesia, increased risk of infection, long-term vascular complications
Rituximab	375 mg/m ² weekly 4 wk (lower doses may be effective)	1-8 wk	Infusion reactions, reactivation of hepatitis B infection, rare cases of progressive multifocal leukoencephalopathy
Eltrombopag	12.5-75 mg PO daily	1-4 wk	Increased bone marrow reticulin, rebound thrombocytopenia, thrombosis,
Romiplostim	(1-10 mcg/kg) SC weekly	1-4 wk	eltrombopag also associated with liver function test abnormalities
Azathioprine	1-2 mg/kg per day (maximum 150 mg day)	1-4 wk	Liver function abnormalities, neutropenia, anemia, infection
Cyclosporine	5 mg/kg per day 6 d, then 2.5-3 mg/kg per day (titrated to blood levels of 100-200 ng/mL)	1-4 wk	Renal failure, hypertension, tremor, infection
Cyclophosphamide	1-2 mg/kg PO daily or 0.3-1 g/m ² <i>iv</i> every 2-4 wk 1-3 doses	1-4 wk	Myelosuppression, infection, secondary malignancy
Danazol	200 mg 2-4 times per day	1-4 wk	Acne, hirsutism, dyslipidemia, amenorrhea, liver function abnormalities
Dapsone	75-100 mg daily	1-4 wk	Hemolytic anemia in patients with G6PD deficiency, rash, methemoglobinemia
Mycophenolate mofetil	1000 mg twice daily	1-4 wk	Headache, back pain, infection
Vincristine	1-2 mg <i>iv</i> weekly (total dose 6 mg)	1-4 wk	Neuropathy, constipation, cytopenias, thrombophlebitis at the infusion site

IVIg: Intravenous immunoglobulin; DIC: Disseminated intravascular coagulation; PO: Per oral.

Corticosteroids

Standard prednisone therapy, 1 to 2 mg/kg per day, is given until a response is seen and then tapered. Some maintain therapy for an additional week before tapering. There are no guidelines about how to taper: Some decrease the dosage by 50% per week, although many recommend going more slowly, particularly at the lower range of dosing. Up to 85% of patients achieve a clinical response, usually within 7 to 10 d, with platelet counts peaking in 2 to 4 wk. Unfortunately, only about 15% of patients maintain the response over the subsequent 6 to 12 mo. Restarting prednisone often initiates a vicious circle and makes patients vulnerable to steroid toxicities (Table 2)^[26].

Pulse dexamethasone therapy consists of 40 mg/d for 4 d for one to three cycles (Dexamethasone 1 mg is equivalent to about 10 mg of prednisone). Pulse dexamethasone therapy as an initial approach to ITP has been developed during the past decade and has been used primarily in research studies. This regimen evolved from studies of patients with multiple myelomas and has the potential to induce more durable remissions in some patients with newly diagnosed ITP. However, high-dose corticosteroids may be associated with increased toxicity, at least in the short term, and should be used cautiously^[2].

IVIg

Another primary therapy for ITP is IVIg 0.4 g/kg per day for 5 d or infusions of 1 g/kg per day for 1-2 d^[2]. IVIg

is associated with numerous adverse effects, including thrombosis, renal insufficiency, headache and anaphylaxis in IgA-deficient patients. It also converts the direct anti-globulin test to positive. IVIg is expensive, inconvenient to administer, and may require lengthy infusions depending on the formulation.

Although IVIg is not a good long-term therapy, it can help raise the platelet count relatively quickly in patients who present with severe thrombocytopenia accompanied by bleeding. Such patients should be treated with high-dose steroids, IVIg and platelet transfusions. IVIg may also be useful to increase platelet counts prior to interventional procedures^[26].

Platelet clearance in ITP mediated by most anti-GPIIb antibodies may occur through an Fc-independent process, likely *via* a system that evolved for our innate immunity and for clearance of senescent cells. This type of ITP may not be sensitive to IVIg and other therapies designed based on Fc receptor blockage^[31].

Antibody Fc-independent phagocytosis has also been well described in mammals, including Fc-independent opsonization by antibodies^[32], as well as antibody and Fc receptor-independent phagocytosis of microbes and other senescent cells^[33-36]. In the absence of antibody, specific ligands from bacteria, other foreign microorganisms or the host's senescent cells, may engage receptors directly on phagocytes, such as scavenger receptors, phosphatidylserine counter-receptors, V integrins, com-

plement receptors or C-type lectins^[33-38]. In some cases, this engagement can be enhanced by F(ab')₂ fragments of antibodies or non-antibody opsonins. These Fc-absent antibodies may bind to receptors on phagocytic cells (*e.g.*, scavenger receptors) or their ligands and induce changes in conformation and affinity of these molecules, which facilitate phagocytosis^[32]. Thus, by directly engaging the target, phagocytosis without the need for antibody is an effective mechanism for clearance of microorganisms and senescent cells.

It was demonstrated that the removal of the Fc region of anti-GPIIb monoclonal antibodies did not affect the ability of these antibodies to induce thrombocytopenia [*i.e.*, the F(ab')₂ portions were as effective as intact antibodies in inducing platelet clearance]. However, when the Fc region of anti-GP IIb/IIIa antibodies was removed, thrombocytopenia was not induced in the same animal model^[39].

Intravenous anti-D

An alternative to IVIg for Rh(D)-positive patients before splenectomy is anti-D Ig. At doses of 75 µg/kg, anti-D may increase the platelet count more rapidly compared with the standard dose of 50 µg/kg. Subcutaneous anti-D has been administered to a few patients suffering from chronic ITP who appeared to have the same response rate as those treated with intravenous delivery without relevant side effects. Evidence of hemolysis is present in most patients treated with anti-D. While the decline in hemoglobin concentration rarely exceeds 2 g/dL, several cases of massive intravascular hemolysis and disseminated intravascular coagulation have been reported. Elderly patients, above 65 years of age, with a coexisting infection, autoimmune hemolytic anemia (Evans syndrome), autoimmune disorders or lymphoproliferative disorders appear to be more susceptible to these complications^[40].

Platelet transfusions with or without IVIg

Platelet transfusion increases the post-transfusion platelet count by more than $20 \times 10^9/L$ in 42% of bleeding ITP patients and may reduce bleeding. In a retrospective study of 40 patients, concurrent administration of platelet transfusions and IVIg was associated with resolution of bleeding, rapid restoration of adequate platelet counts and minimal side effects^[40,41].

Antifibrinolytics

Antifibrinolytic agents, such as oral or IV tranexamic acid and epsilon-aminocaproic acid, may be useful in preventing recurrent bleeding in patients with severe thrombocytopenia; however, the efficacy has not been evaluated by randomized trials in ITP patients. Tranexamic acid (1 g, 3 times daily orally) and epsilon-aminocaproic acid (1-4 g every 4-6 h maximum dose, 24 g/d) may be of special value in certain dental or surgical procedures^[40-42].

SECOND-LINE TREATMENT

Second-line therapies, as designated by the international

working group, include azathioprine, cyclosporine A, cyclophosphamide, danazol, dapsone, mycophenolate mofetil, rituximab, splenectomy, TRAs and vinca alkaloids. The evidence for efficacy of the cytotoxic agents, *i.e.*, cyclophosphamide, the vinca alkaloids and azathioprine, comes from small, non-randomized studies^[30]. Although these agents are useful in some patients, they may be associated with significant toxicities and are used less commonly than in the past (Table 2)^[26,42].

Splenectomy

Splenectomy probably offers the best response of any treatment for ITP. About 80% of patients with ITP respond rapidly, often within 1 wk. Of those, 15% relapse within the first year and after 10 years, two-thirds remain in remission^[43,44].

Splenectomy increases the risk of subsequent infection by encapsulated organisms and patients should be immunized with pneumococcal, *Haemophilus influenzae type B* and meningococcal vaccines, preferably at least 3 wk before the spleen is removed. Splenectomy is associated with pulmonary hypertension and thrombosis, primarily in patients who have had their spleens removed because of accelerated red cell destruction. Whether these risks are applicable to patients with ITP is unknown but if so, they are probably much lower than in patients with red cell disorders^[26].

Rituximab

Rituximab, an anti-CD20 monoclonal antibody, has produced variable objective responses. Rituximab causes selective B-cell lysis *in vitro* and B-cell depletion *in vivo*. Involved mechanisms of action include apoptosis, antibody-dependent cytotoxicity. Recovery of B-cell counts usually occurs by 6 to 12 mo after completion of treatment^[45].

Several publications have reported the use of rituximab in ITP patients since previous consensus documents were issued and suggest that about 60% of patients respond, with approximately 40% achieving complete response. Responses generally occur after 1 to 2 wk to 6 to 8 wk and last from 2 mo in partial responders to 5 years or longer in 15% to 20% of initially treated patients. Most patients with a durable (> 1 year) complete response will respond to repeat treatment if they relapse^[46-48].

Romiplostim

Romiplostim is a peptibody (comprising of an IgG Fc region and four peptidomimetics regions that interact with the thrombopoietin receptor, c-mpl) that is given subcutaneously once a week. Romiplostim performed well in several phase I clinical trials. In a 24 wk phase III trial that compared romiplostim against placebo in patients with ITP that had been refractory to other primary treatments, 79% of splenectomized patients and 88% of non-splenectomized patients had an overall response (defined as a platelet count $> 50 \times 10^9/L$ for 4 wk during the study period) and 38% of splenectomized patients and 61% of non-splenectomized patients had a durable response (platelet count $> 50 \times 10^9/L$ for 6 of the last 8 wk of

the study). In an ongoing long-term extension study of romiplostim that allows dose adjustments to maintain a platelet count between $50\text{--}200 \times 10^9/\text{L}$, romiplostim dosage and efficacy have remained stable over 5 years^[18,49,50].

Dapsone

Dapsone is a moderate corticosteroid-sparing agent that is usually administered orally at a dose of 75 to 100 mg/d. Dapsone may delay splenectomy for up to 32 mo in patients who have not responded to first-line corticosteroid therapy. However, splenectomized patients have a low response rate^[2].

Eltrombopag

Eltrombopag is a nonpeptide small-molecule c-mpl agonist that is taken orally once daily. A recent randomized, placebo-controlled study in patients with ITP refractory to other primary treatments found that eltrombopag was highly effective in raising platelet counts over the 6 mo of the study. Like romiplostim, it was effective in both splenectomized and non-splenectomized patients.

Although eltrombopag has not been studied for as long as romiplostim, data over 3 years indicate that increased platelet counts are maintained without the emergence of drug resistance or cumulative toxicity. Several other drugs in this class are currently in development^[51,52].

CONCLUSION

The pathophysiology of ITP is complex and abnormalities of both the B and the T-cell compartments have been identified. The mechanisms of thrombocytopenia involve both increased platelet destruction and, in a significant proportion of cases, impaired platelet production.

Splenectomy has historically been the second-line therapy for adults with ITP in whom achieving a safe platelet count with initial corticosteroid and/or immunoglobulin therapy has failed. Although it still remains the therapeutic modality that offers the highest chance of cure, its position in the therapeutic algorithm of ITP is currently challenged. Rituximab has been shown to have a limited but valuable activity as a splenectomy sparing agent and is generally tolerated very well. The Thrombopoietin-receptor agonists have undergone a formal, systematic investigation and have been licensed for use in adult patients with ITP. These agents appear to be very effective in a high percentage of patients with chronic and refractory disease and appear to have a favorable side-effect profile in the short and medium term. Potential long-term side effects of TPO-receptor agonists remain a concern and suggest their prudent use in young, non-splenectomized patients.

REFERENCES

- 1 Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, Bussel JB, Cines DB, Chong BH, Cooper N, Godeau B, Lechner K, Mazzucconi MG, McMillan R, Sanz MA, Imbach P, Blanchette V, Kühne T, Ruggeri M, George JN. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood* 2009; **113**: 2386-2393 [PMID: 19005182 DOI: 10.1182/blood-2008-07-162503]
- 2 Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, Chong BH, Cines DB, Gernsheimer TB, Godeau B, Grainger J, Greer I, Hunt BJ, Imbach PA, Lyons G, McMillan R, Rodeghiero F, Sanz MA, Tarantino M, Watson S, Young J, Kuter DJ. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood* 2010; **115**: 168-186 [PMID: 19846889 DOI: 10.1182/blood-2009-06-225565]
- 3 Stasi R, Amadori S, Osborn J, Newland AC, Provan D. Long-term outcome of otherwise healthy individuals with incidentally discovered borderline thrombocytopenia. *PLoS Med* 2006; **3**: e24 [PMID: 16401142 DOI: 10.1371/journal.pmed.0030024]
- 4 Thienelt CD, Calverley DC. Thrombocytopenia Caused by Immunologic Platelet Destruction. Greer JP, Foerster J, Rodgers GM at all. Wintrobe's Clinical Hematology 12th ed. Lippincott Williams & Wilkins, Philadelphia, USA, 2009: 1292-313
- 5 Schoonen WM, Kucera G, Coalson J, Li L, Rutstein M, Mowat F, Fryzek J, Kaye JA. Epidemiology of immune thrombocytopenic purpura in the General Practice Research Database. *Br J Haematol* 2009; **145**: 235-244 [PMID: 19245432 DOI: 10.1111/j.1365-2141.2009.07615]
- 6 Abrahamson PE, Hall SA, Feudjo-Tepie M, Mitrani-Gold FS, Logie J. The incidence of idiopathic thrombocytopenic purpura among adults: a population-based study and literature review. *Eur J Haematol* 2009; **83**: 83-89 [PMID: 19245532 DOI: 10.1111/j.1600-0609.2009.01247]
- 7 Segal JB, Powe NR. Prevalence of immune thrombocytopenia: analyses of administrative data. *J Thromb Haemost* 2006; **4**: 2377-2383 [PMID: 16869934 DOI: 10.1111/j.1538-7836.2006.02147.x]
- 8 Rischewski JR, Imbach P, Paulussen M, Kühne T. Idiopathic thrombocytopenic purpura (ITP): is there a genetic predisposition? *Pediatr Blood Cancer* 2006; **47**: 678-680 [PMID: 16933243 DOI: 10.1002/pbc.21005]
- 9 Coopamah MD, Garvey MB, Freedman J, Semple JW. Cellular immune mechanisms in autoimmune thrombocytopenic purpura: An update. *Transfus Med Rev* 2003; **17**: 69-80 [PMID: 12522773 DOI: 10.1053/tmrv.2003.50004]
- 10 Yu J, Heck S, Patel V, Levan J, Yu Y, Bussel JB, Yazdankhsh K. Defective circulating CD25 regulatory T cells in patients with chronic immune thrombocytopenic purpura. *Blood* 2008; **112**: 1325-1328 [PMID: 18420827 DOI: 10.1182/blood-2008-01-135335]
- 11 Emmerich F, Bal G, Barakat A, Milz J, Mühle C, Martinez-Gamboa L, Dörner T, Salama A. High-level serum B-cell activating factor and promoter polymorphisms in patients with idiopathic thrombocytopenic purpura. *Br J Haematol* 2007; **136**: 309-314 [PMID: 17156395 DOI: 10.1111/j.1365-2141.2006.06431.x]
- 12 Craig VJ, Arnold I, Gerke C, Huynh MQ, Wündisch T, Neubauer A, Renner C, Falkow S, Müller A. Gastric MALT lymphoma B cells express polyreactive, somatically mutated immunoglobulins. *Blood* 2010; **115**: 581-591 [PMID: 19965661 DOI: 10.1182/blood-2009-06-228015]
- 13 Ballem PJ, Belzberg A, Devine DV, Lyster D, Spruston B, Chambers H, Doubroff P, Mikulash K. Kinetic studies of the mechanism of thrombocytopenia in patients with human immunodeficiency virus infection. *N Engl J Med* 1992; **327**: 1779-1784 [PMID: 1435932 DOI: 10.1056/NEJM199212173272503]
- 14 Houwerzijl EJ, Blom NR, van der Want JJ, Esselink MT, Koornstra JJ, Smit JW, Louwes H, Vellenga E, de Wolf JT. Ultrastructural study shows morphologic features of apop-

- tosis and para-apoptosis in megakaryocytes from patients with idiopathic thrombocytopenic purpura. *Blood* 2004; **103**: 500-506 [PMID: 12969975 DOI: 10.1182/blood-2003-01-0275]
- 15 **McMillan R**, Wang L, Tomer A, Nichol J, Pistillo J. Suppression of in vitro megakaryocyte production by antiplatelet autoantibodies from adult patients with chronic ITP. *Blood* 2004; **103**: 1364-1369 [PMID: 14576051 DOI: 10.1182/blood-2003-08-2672]
 - 16 **Olsson B**, Ridell B, Carlsson L, Jacobsson S, Wadenvik H. Recruitment of T cells into bone marrow of ITP patients possibly due to elevated expression of VLA-4 and CX3CR1. *Blood* 2008; **112**: 1078-1084 [PMID: 18519809 DOI: 10.1182/blood-2008-02-139402]
 - 17 **Bussell JB**, Provan D, Shamsi T, Cheng G, Psaila B, Kovaleva L, Salama A, Jenkins JM, Roychowdhury D, Mayer B, Stone N, Arning M. Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: a randomised, double-blind, placebo-controlled trial. *Lancet* 2009; **373**: 641-648 [PMID: 19231632 DOI: 10.1016/S0140-6736(09)60402-5]
 - 18 **Kuter DJ**, Bussell JB, Lyons RM, Pullarkat V, Gernsheimer TB, Senecal FM, Aledort LM, George JN, Kessler CM, Sanz MA, Liebman HA, Slovick FT, de Wolf JT, Bourgeois E, Guthrie TH, Newland A, Wasser JS, Hamburg SI, Grande C, Lefrère F, Lichtin AE, Tarantino MD, Terebello HR, Viallard JF, Cuevas FJ, Go RS, Henry DH, Redner RL, Rice L, Schipperus MR, Guo DM, Nichol JL. Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. *Lancet* 2008; **371**: 395-403 [PMID: 18242413 DOI: 10.1016/S0140-6736(08)60203-2]
 - 19 **Bussell JB**, Kuter DJ, Pullarkat V, Lyons RM, Guo M, Nichol JL. Safety and efficacy of long-term treatment with romiplostim in thrombocytopenic patients with chronic ITP. *Blood* 2009; **113**: 2161-2171 [PMID: 18981291 DOI: 10.1182/blood-2008-04-150078]
 - 20 **Olsson B**, Andersson PO, Jernäs M, Jacobsson S, Carlsson B, Carlsson LM, Wadenvik H. T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. *Nat Med* 2003; **9**: 1123-1124 [PMID: 12937414 DOI: 10.1038/nm921]
 - 21 **Cines DB**, Bussell JB, Liebman HA, Luning Prak ET. The ITP syndrome: pathogenic and clinical diversity. *Blood* 2009; **113**: 6511-6521 [PMID: 19395674 DOI: 10.1182/blood-2009-01-129155]
 - 22 **Sample JW**, Provan D, Garvey MB, Freedman J. Recent progress in understanding the pathogenesis of immune thrombocytopenia. *Curr Opin Hematol* 2010; **17**: 590-595 [PMID: 20739879 DOI: 10.1097/MOH.0b013e32833eae3f]
 - 23 **Najaoui A**, Bakchoul T, Stoy J, Bein G, Rummel MJ, Santoso S, Sachs UJ. Autoantibody-mediated complement activation on platelets is a common finding in patients with immune thrombocytopenic purpura (ITP). *Eur J Haematol* 2012; **88**: 167-174 [PMID: 21985182 DOI: 10.1111/j.1600-0609.2011.01718.x]
 - 24 **Stasi R**, Willis F, Shannon MS, Gordon-Smith EC. Infectious causes of chronic immune thrombocytopenia. *Hematol Oncol Clin North Am* 2009; **23**: 1275-1297 [PMID: 19932434 DOI: 10.1016/j.hoc.2009.08.009]
 - 25 **Arkfeld DG**, Weitz IC. Immune thrombocytopenia in patients with connective tissue disorders and the antiphospholipid antibody syndrome. *Hematol Oncol Clin North Am* 2009; **23**: 1239-1249 [PMID: 19932431 DOI: 10.1016/j.hoc.2009.08.010]
 - 26 **McCrae K**. Immune thrombocytopenia: no longer 'idiopathic'. *Cleve Clin J Med* 2011; **78**: 358-373 [PMID: 21632906 DOI: 10.3949/ccjm.78gr.10005]
 - 27 **Mittal S**, Blaylock MG, Culligan DJ, Barker RN, Vickers MA. A high rate of CLL phenotype lymphocytes in autoimmune hemolytic anemia and immune thrombocytopenic purpura. *Haematologica* 2008; **93**: 151-152 [PMID: 18166805 DOI: 10.3324/haematol.11822]
 - 28 **Cortelazzo S**, Finazzi G, Buelli M, Molteni A, Viero P, Barbui T. High risk of severe bleeding in aged patients with chronic idiopathic thrombocytopenic purpura. *Blood* 1991; **77**: 31-33 [PMID: 1984800]
 - 29 **Portielje JE**, Westendorp RG, Kluin-Nelemans HC, Brand A. Morbidity and mortality in adults with idiopathic thrombocytopenic purpura. *Blood* 2001; **97**: 2549-2554 [PMID: 11313240 DOI: 10.1182/blood.V97.9.2549]
 - 30 **Yang R**, Han ZC. Pathogenesis and management of chronic idiopathic thrombocytopenic purpura: an update. *Int J Hematol* 2000; **71**: 18-24 [PMID: 10729989]
 - 31 **Li J**, van der Wal DE, Zhu L, Vadasz B, Simpson EK, Li C, Webster ML, Zhu G, Lang S, Chen P, Zeng Q, Ni H. Fc-independent phagocytosis: implications for IVIG and other therapies in immune-mediated thrombocytopenia. *Cardiovasc Hematol Disord Drug Targets* 2013; **13**: 50-58 [PMID: 23082940 DOI: 10.2174/1871529X11313010006]
 - 32 **Netski D**, Kozel TR. Fc-dependent and Fc-independent opsonization of *Cryptococcus neoformans* by anticapsular monoclonal antibodies: importance of epitope specificity. *Infect Immun* 2002; **70**: 2812-2819 [PMID: 12010967 DOI: 10.1128/IAI.70.6.2812-2819.2002]
 - 33 **Fujiwara N**, Kobayashi K. Macrophages in inflammation. *Curr Drug Targets Inflamm Allergy* 2005; **4**: 281-286 [PMID: 16101534]
 - 34 **Plüddemann A**, Mukhopadhyay S, Gordon S. The interaction of macrophage receptors with bacterial ligands. *Expert Rev Mol Med* 2006; **8**: 1-25 [PMID: 17118220 DOI: 10.1017/S1462399406000159]
 - 35 **Plüddemann A**, Neyen C, Gordon S. Macrophage scavenger receptors and host-derived ligands. *Methods* 2007; **43**: 207-217 [PMID: 17920517 DOI: 10.1016/j.ymeth.2007.06.004]
 - 36 **Lauber K**, Blumenthal SG, Waibel M, Wesselborg S. Clearance of apoptotic cells: getting rid of the corpses. *Mol Cell* 2004; **14**: 277-287 [PMID: 15125832 DOI: 10.1016/S1097-2765(04)00237-0]
 - 37 **East L**, Isacke CM. The mannose receptor family. *Biochim Biophys Acta* 2002; **1572**: 364-386 [PMID: 12223280]
 - 38 **Carroll MC**. The role of complement and complement receptors in induction and regulation of immunity. *Annu Rev Immunol* 1998; **16**: 545-568 [PMID: 9597141 DOI: 10.1146/annurev.immunol.16.1.545]
 - 39 **Nieswandt B**, Bergmeier W, Rackebrandt K, Gessner JE, Zirngibl H. Identification of critical antigen-specific mechanisms in the development of immune thrombocytopenic purpura in mice. *Blood* 2000; **96**: 2520-2527 [PMID: 11001906]
 - 40 **Spahr JE**, Rodgers GM. Treatment of immune-mediated thrombocytopenia purpura with concurrent intravenous immunoglobulin and platelet transfusion: a retrospective review of 40 patients. *Am J Hematol* 2008; **83**: 122-125 [PMID: 17874448 DOI: 10.1002/ajh.21060]
 - 41 **Carr JM**, Kruskall MS, Kaye JA, Robinson SH. Efficacy of platelet transfusions in immune thrombocytopenia. *Am J Med* 1986; **80**: 1051-1054 [PMID: 3728504]
 - 42 **George JN**, Kojouri K, Perdue JJ, Vesely SK. Management of patients with chronic, refractory idiopathic thrombocytopenic purpura. *Semin Hematol* 2000; **37**: 290-298 [PMID: 10942223]
 - 43 **Schwartz J**, Leber MD, Gillis S, Giunta A, Eldor A, Bussell JB. Long term follow-up after splenectomy performed for immune thrombocytopenic purpura (ITP). *Am J Hematol* 2003; **72**: 94-98 [PMID: 12555211 DOI: 10.1002/ajh.10253]
 - 44 **Kojouri K**, Vesely SK, Terrell DR, George JN. Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. *Blood* 2004; **104**: 2623-2634 [PMID: 15217831]
 - 45 **Arnold DM**, Dentali F, Crowther MA, Meyer RM, Cook RJ, Sigouin C, Fraser GA, Lim W, Kelton JG. Systematic review:

- efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. *Ann Intern Med* 2007; **146**: 25-33 [PMID: 17200219 DOI: 10.7326/0003-4819-146-1-200701020-00006]
- 46 **Thienelt CD**, Calverley DC. Thrombocytopenia Caused by Immunologic Platelet Destruction. Wintrobe's Clinical Hematoloji. 12th Ed. Lippincott Williams&Wilkins. Philadelphia. 2009: 1292-1313
 - 47 **Stasi R**, Stipa E, Forte V, Meo P, Amadori S. Variable patterns of response to rituximab treatment in adults with chronic idiopathic thrombocytopenic purpura. *Blood* 2002; **99**: 3872-3873 [PMID: 12014370 DOI: 10.1182/blood-2002-02-0392]
 - 48 **Cooper N**, Stasi R, Cunningham-Rundles S, Feuerstein MA, Leonard JP, Amadori S, Bussel JB. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. *Br J Haematol* 2004; **125**: 232-239 [PMID: 15059147 DOI: 10.1111/j.1365-2141.]
 - 49 **Bussel JB**, Kuter DJ, George JN, McMillan R, Aledort LM, Conklin GT, Lichtin AE, Lyons RM, Nieva J, Wasser JS, Wiznitzer I, Kelly R, Chen CF, Nichol JL. AMG 531, a thrombopoiesis-stimulating protein, for chronic ITP. *N Engl J Med* 2006; **355**: 1672-1681 [PMID: 17050891 DOI: 10.1056/NEJMoa054626]
 - 50 **Gernsheimer TB**, George JN, Aledort LM, Tarantino MD, Sunkara U, Matthew Guo D, Nichol JL. Evaluation of bleeding and thrombotic events during long-term use of romiplostim in patients with chronic immune thrombocytopenia (ITP). *J Thromb Haemost* 2010; **8**: 1372-1382 [PMID: 20230419 DOI: 10.1111/j.1538-7836.2010.03830.x]
 - 51 **Cheng G**, Saleh MN, Marcher C, Vasey S, Mayer B, Aivado M, Arning M, Stone NL, Bussel JB. Eltrombopag for management of chronic immune thrombocytopenia (RAISE): a 6-month, randomised, phase 3 study. *Lancet* 2011; **377**: 393-402 [PMID: 20739054 DOI: 10.1016/S0140-6736(10)60959-2]
 - 52 **Saleh MN**, Bussel JB, Cheng G, Meddeb B, Mayer B, Bailey C, Aivado M. Long-term treatment of chronic immune thrombocytopenic purpura with oral eltrombopag. Abstract #682 presented at the 51st American Society of Hematology Annual Meeting and Exposition; New Orleans, LA. December 5-8, 2009. Available from: URL: <http://ash.confex.com/ash/2009/webprogram/Paper24081.html>

P- Reviewers: Kim K, Yazdanbakhsh K, Zimmer J
S- Editor: Song XX **L- Editor:** Roemmele A **E- Editor:** Liu SQ





INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Immunology (*World J Immunol*, *WJI*, online ISSN 2219-2824, DOI: 10.5411) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aims and scope

WJI covers a wide range of subjects including: (1) autoimmune diseases such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, myasthenia gravis, in both humans and animal models of disease, with an interest on aspects including the etiology, pathogenesis, mechanisms of disease induction, maintenance and progression; (2) tumor immunology including immunosurveillance, immunoediting and immunotherapies in animal models and in humans; (3) clinical immunology in humans and animal models including mechanisms of disease, regulation and therapy and immunodeficiencies; (4) innate immunity including cell subsets, receptors and soluble mediators, complement and inflammation; (5) adaptive immune mechanisms and cells including soluble mediators and antibodies; (6) immune cell development, differentiation, maturation; (7) control mechanisms for immune cells including immune tolerance and apoptosis; (8) immune cell interactions and immune cell receptors; (9) immunological methods and techniques; (10) immune cell activation including cell signaling pathways, biochemical and pharmacologic modulation studies; (11) infection; (12) different modalities of vaccination including gene therapy; (13) hypersensitivity and allergy; (14) transplantation.

We encourage authors to submit their manuscripts to *WJI*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

WJI is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15471 editorial board members or peer reviewers, and is a world first-class publisher

Columns

The columns in the issues of *WJI* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, phys-

ical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers); (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, etc.; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in immunology; (12) Brief Articles: To briefly report the novel and innovative findings in immunology; (13) Meta-Analysis: To summarize a given quantitative effect, e.g., the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJI*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of immunology; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Immunology

Instructions to authors

ISSN

ISSN 2219-2824 (online)

Launch date

December 27, 2011

Frequency

Four-monthly

Editors-in-Chief

Antonio La Cava, MD, PhD, Professor, Department of Medicine, University of California Los Angeles, Los Angeles, CA 90095-1670, United States

Seung-Yong Seong, MD, PhD, Professor, Department of Microbiology and Immunology, 103 Daehag-ro, Jongno-gu, Seoul 110-799, South Korea

Editorial office

Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director

World Journal of Immunology

Room 903, Building D, Ocean International Center,

No. 62 Dongsihuan Zhonglu, Chaoyang District,

Beijing 100025, China

E-mail: bpgoffice@wjgnet.com

<http://www.wjgnet.com>

Telephone: +86-10-85381891

Fax: +86-10-85381893

Publisher

Baishideng Publishing Group Co., Limited

Flat C, 23/F, Lucky Plaza,

315-321 Lockhart Road, Wan Chai,

Hong Kong, China

Telephone: +852-6555-7188

Fax: +852-3177-9906

E-mail: bpgoffice@wjgnet.com

<http://www.wjgnet.com>

Production center

Beijing Baishideng BioMed Scientific Co., Limited

Room 903, Building D, Ocean International Center,

No. 62 Dongsihuan Zhonglu, Chaoyang District,

Beijing 100025, China

Telephone: +86-10-85381892

Fax: +86-10-85381893

Representative office

USA Office

8226 Regency Drive,

Pleasanton, CA 94588-3144, United States

Instructions to authors

Full instructions are available online at http://www.wjgnet.com/2219-2824/g_info_20100316161927.htm.

Indexed and Abstracted in

Digital Object Identifier.

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing

Statistical review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Redit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance,

etc. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement

In the interests of transparency and to help reviewers assess any potential bias, *WJI* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent

Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights

When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Leg-

ends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is <http://www.clinicaltrials.gov> sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: <http://www.wjgnet.com/esps/>. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2219-2824/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to bpgoffice@wjgnet.com, or by telephone: +86-10-85381891. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally

to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomerybissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/..."), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g., 6.92 ± 3.86 vs 3.61 ± 1.67 , $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, etc., and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names

Instructions to authors

should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...*etc.* It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. ^a*P* < 0.05, ^b*P* < 0.01 should be noted (*P* > 0.05 should not be noted). If there are other series of *P* values, ^c*P* < 0.05 and ^d*P* < 0.01 are used. A third series of *P* values can be expressed as ^e*P* < 0.05 and ^f*P* < 0.01. Other notes in tables or under illustrations should be expressed as ¹F, ²F, ³F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability^[12]". If references are cited directly in the text, they should be put together within the text, for example, "From references^[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Please provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed> and <http://www.crossref.org/SimpleTextQuery/>, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-

Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

- 1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

- 2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarhoea. *Shijie Huaren Xiaobua Zazhi* 1999; **7**: 285-287

In press

- 3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

- 4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

- 5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

- 6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

- 7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

- 8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; **(401)**: 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

- 9 Outreach: Bringing HIV-positive individuals into care. *HRS A Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

- 11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

- 12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wiczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

- 13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

- 14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA,

Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

- 15 Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: <http://www.cdc.gov/ncidod/eid/index.htm>

Patent (list all authors)

- 16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean $\pm$ SD or mean $\pm$ SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as *v* (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, *m* (B) = 78 kg; blood pressure, *p* (B) = 16.2/12.3 kPa; incubation time, *t* (incubation) = 96 h, blood glucose concentration, *c* (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, *p* (CEA) = 8.6 $24.5 \mu\text{g/L}$; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23243641.

The format for how to accurately write common units and quantum numbers can be found at: http://www.wjgnet.com/2219-2824/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg 1*, *c myc*, *c fos*, *etc.*

Restriction enzymes: *EcoRI*, *HindIII*, *BamHI*, *Kho I*, *Kpn I*, *etc.*

Biology: *H. pylori*, *E. coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the

link: <http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15>

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2219-2824/g_info_20100725073806.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2219-2824/g_info_20100725073806.htm.

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

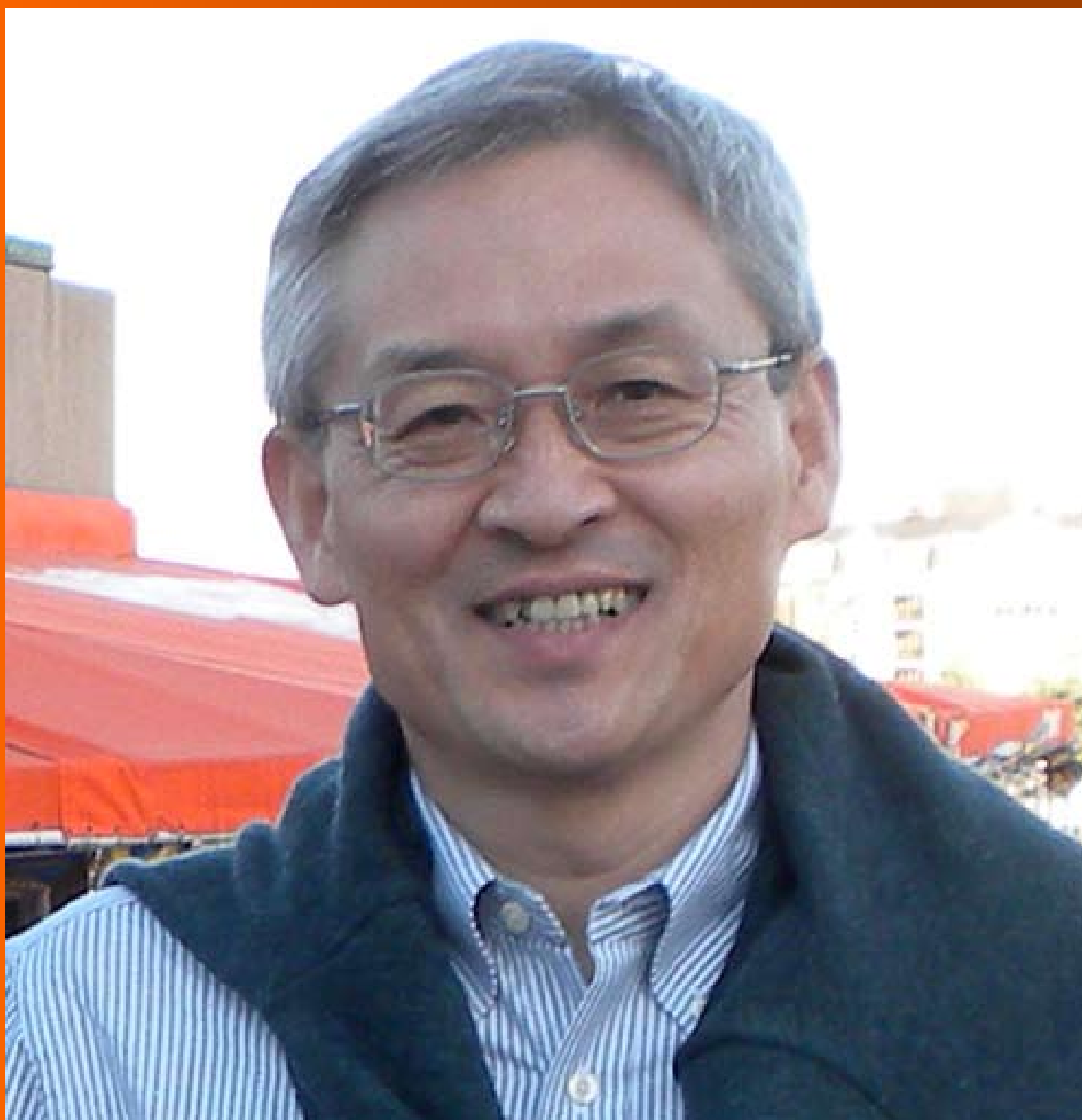
In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJI is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. All invited articles are published free of charge.

World Journal of *Immunology*

World J Immunol 2014 July 27; 4(2): 42-129



**REVIEW**

- 42 Lysosomal acid lipase is critical for myeloid-derived suppressive cell differentiation, development, and homeostasis
Yan C, Du H
- 52 Gut immune response in the presence of hepatitis C virus infection
Hetta HF, Mehta MJ, Shata MTM
- 63 CD28/CTLA-4/B7 and CD40/CD40L costimulation and activation of regulatory T cells
Vogel IT, Van Gool SW, Ceuppens JL
- 78 Immunopathogenesis of reactive arthritis: Role of the cytokines
Eliçabe RJ, Di Genaro MS
- 88 Update on pythiosis immunobiology and immunotherapy
Loreto ÉS, Tondolo JSM, Zanette RA, Alves SH, Santurio JM
- 98 GM3-containing nanoparticles in immunosuppressed hosts: Effect on myeloid-derived suppressor cells
Fernández A, Oliver L, Alvarez R, Fernández LE, Mesa C
- 107 Role of host immune responses in sequence variability of HIV-1 Vpu
Hasan Z, Kamori D, Ueno T
- 116 Targeting TLR4/MAPKs signaling pathway: A better option for therapeutic inhibition of atherosclerosis
Plakkal Ayyappan J, Abraham A

MINIREVIEWS

- 122 Biologic response modifiers in retinal vasculitis
Saxena S, Srivastav K

Contents

World Journal of Immunology
Volume 4 Number 2 July 27, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Immunology*, Hyung-Joo Kwon, PhD, Professor, Department of Microbiology, Hallym University College of Medicine, 39 Hallymdaehak-gil, Chuncheon Gangwon-do 200-702, South Korea

AIM AND SCOPE *World Journal of Immunology* (*World J Immunol*, *WJI*, online ISSN 2219-2824, DOI: 10.5411) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJI covers a wide range of subjects including: (1) autoimmune diseases such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, myasthenia gravis, in both humans and animal models of disease, with an interest on aspects including the etiology, pathogenesis, mechanisms of disease induction, maintenance and progression; (2) tumor immunology including immunosurveillance, immunoediting and immunotherapies in animal models and in humans; (3) clinical immunology in humans and animal models including mechanisms of disease, regulation and therapy and immunodeficiencies; (4) innate immunity including cell subsets, receptors and soluble mediators, complement and inflammation; (5) adaptive immune mechanisms and cells including soluble mediators and antibodies; (6) immune cell development, differentiation, maturation; (7) control mechanisms for immune cells including immune tolerance and apoptosis; (8) immune cell interactions and immune cell receptors; (9) immunological methods and techniques; (10) immune cell activation including cell signaling pathways, biochemical and pharmacologic modulation studies; (11) infection; (12) different modalities of vaccination including gene therapy; (13) hypersensitivity and allergy; (14) transplantation.

We encourage authors to submit their manuscripts to *WJI*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING *World Journal of Immunology* is now indexed in Digital Object Identifier.

FLYLEAF I-III Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Cui-Hong Wang*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Ling-Ling Wen*
Proofing Editorial Office Director: *Xiu-Xia Song*

NAME OF JOURNAL
World Journal of Immunology

ISSN
ISSN 2219-2824 (online)

LAUNCH DATE
December 27, 2011

FREQUENCY
Four-monthly

EDITORS-IN-CHIEF
Antonio La Cava, MD, PhD, Professor, Department of Medicine, University of California Los Angeles, Los Angeles, CA 90095-1670, United States

Seung-Yong Seong, MD, PhD, Professor, Department of Microbiology and Immunology, 103 Daehag-ro, Jongno-gu, Seoul 110-799, South Korea

EDITORIAL OFFICE
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director
World Journal of Immunology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>

PUBLICATION DATE
July 27, 2014

COPYRIGHT

© 2014 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS

Full instructions are available online at http://www.wjgnet.com/2219-2824/g_info_20100722180909.htm.

ONLINE SUBMISSION
<http://www.wjgnet.com/esps/>

Lysosomal acid lipase is critical for myeloid-derived suppressive cell differentiation, development, and homeostasis

Cong Yan, Hong Du

Cong Yan, Center for Immunobiology, Indiana University School of Medicine, Indianapolis, IN 46202, United States

Cong Yan, Hong Du, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, United States

Cong Yan, Hong Du, IU Simon Cancer Center, Indiana University School of Medicine, Indianapolis, IN 46202, United States

Author contributions: Yan C and Du H wrote and edited the review.

Supported by National Institutes of Health, No.CA138759, CA152099, to Yan C; HL087001, to Du H, and HL-061803 and HL-067862 to Yan C and Du H

Correspondence to: Dr. Cong Yan, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, 975 W Walnut Street, IB424G, Indianapolis, IN 46202, United States. coyan@iupui.edu

Telephone: +1-317-2786005 Fax: +1-317-2788198

Received: February 26, 2014 Revised: April 2, 2014

Accepted: June 18, 2014

Published online: July 27, 2014

Abstract

Lysosomal acid lipase (LAL) cleaves cholesteryl esters (CE) and triglycerides (TG) to generate cholesterol and free fatty acid in lysosomes of cells. The downstream metabolic products of fatty acids are ligands for activation of peroxisome proliferator-activated receptor gamma (PPAR γ). Accumulation of CEs and TGs is resulted from lack of functional LAL in lysosomes of cells, especially in myeloid cells. One characteristic phenotype in LAL knock-out (*lal*^{-/-}) mice is systemic elevation of myeloid-derived suppressive cells (MDSCs). MDSCs infiltrate into multiple distal organs, alter T cell development, and suppress T cell proliferation and lymphokine production in *lal*^{-/-} mice, which lead to severe pathogenesis in multiple organs. The gene transcriptional profile analysis in MDSCs from the bone marrow has identified multiple defects responsible for MDSCs malformation and malfunction in *lal*^{-/-} mice, including G protein signaling, cell cycles, glycolysis metabolism, mi-

tochondrial bioenergetics, mTOR pathway etc. In a separate gene transcriptional profile analysis in the lung of *lal*^{-/-} mice, matrix metalloproteinase 12 (MMP12) and apoptosis inhibitor 6 (Api6) are highly overexpressed due to lack of ligand synthesis for PPAR γ . PPAR γ negatively regulates MMP12 and Api6. Blocking the PPAR signaling by overexpression of a dominant negative PPAR γ (dnPPAR γ) form, or overexpressing MMP12 or Api6 in myeloid or lung epithelial cells in inducible transgenic mouse models results in elevated MDSCs and inflammation-induced tumorigenesis. These studies demonstrate that LAL and its downstream effectors are critical for MDSCs development, differentiation and malfunction.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Lysosomal acid lipase; Myeloid-derived suppressor cells; Immunosuppression; Myeloid-derived suppressive cell development; Hematopoiesis

Core tip: Neutral lipid metabolism is essential for myeloid cell proliferation and differentiation. This review summarizes the most recent discoveries that lysosomal acid lipase (LAL), an enzyme hydrolysing cholesteryl esters and triglycerides in lysosomes, plays a critical role in myeloid-derived suppressive cells (MDSCs) development, differentiation, and immune suppressive function. Both LAL knock-out and myeloid specific rescue of LAL knock-out mice are used in the studies. Doxycycline-inducible bitransgenic mouse models of LAL downstream genes are also generated to study MDSCs malformation and malfunction. The molecular pathways/mechanisms to connect LAL and MDSCs are characterized by microarray analyses of gene transcriptional profiles.

Yan C, Du H. Lysosomal acid lipase is critical for myeloid-derived suppressive cell differentiation, development, and homeostasis. *World J Immunol* 2014; 4(2): 42-51 Available from: <http://www.wjgnet.com/2219-2824/full/v4/i2/42.htm> DOI:

<http://dx.doi.org/10.5411/wji.v4.i2.42>

HISTORY OF LYSOSOMAL ACID LIPASE

Lysosomal acid lipase (LAL) cleaves cholesteryl esters (CE) and triglycerides (TG) in cell lysosomes. Mutation in the LAL gene results in Wolman disease (WD) of early infantile onset, and cholesteryl ester storage disease (CESD) of late onset. WD was first described by Dr. Wolman^[1] in 1956 as severe malnutrition, hepatosplenomegaly, calcified adrenal glands, and death of children within the first few months of life. Affected WD infants display massive accumulations of CE and TG in the lysosomes of hepatocytes and Kupffer cells, as well as in macrophages throughout the viscera, which lead to liver failure, severe hepatosplenomegaly, steatorrhea, pulmonary fibrosis^[2,3], and adrenal calcification and insufficiency^[4,5]. Lipid engorged macrophages in intestinal villi lead to severe malabsorption and cachexia^[2,4]. The average life span of WD is 3.5 mo^[6]. CESD was initially described by Fredrickson, Schiff, Langeron, and Infante and their colleagues in 1967^[7-10] and named by Partin and Schubert based on phenotype that exhibited hepatomegaly with increased hepatic levels of cholesteryl esters in 1969. CESD can be a more indolent progressive disease, which shows microvesicular steatosis leading to fibrosis and cirrhosis in the liver, increases atherosclerosis and premature demise^[11-13]. Wolman disease and CESD result from allelic mutations at the LAL locus on human chromosome 10q23.2-q23.3 and are autosomal recessive traits. The gene spans 45 kb, has 10 exons, and contains no unusual structures, except for a large intron 3. The *LIPA* mutations found in Wolman disease include deletions and insertions that lead to premature stop codons and the consequent loss of LAL protein and activity^[14]. The mutations found in CESD are usually missense mutations, either heteroallelic or homoallelic with another mutant *LIPA* gene^[14].

Recently, some evidence started to emerge, showing altered mononuclear phagocyte differentiation [increased CD14⁺CD16⁺ and CD14⁺CD33⁺ cells, subsets of human myeloid-derived suppressive cells, or myeloid-derived suppressive cells (MDSCs)] in humans that were heterozygote carriers of LAL mutations^[15]. Furthermore, patients with mutations in the *LAL* gene have been reported to be associated with carcinogenesis^[16]. These clinical observations support the extensive characterization in animal models as described below.

LAL PROPERTIES

LAL is a key player in the modulation of cholesterol metabolism in all cells. On the surface membranes of various cells, there are multiple receptors that can deliver LDL-bound cholesteryl esters/triglycerides to lysosomes, but LAL is the only lipase in the lysosomes that hydrolyzes cholesteryl esters and triglycerides. Once cleaved by

LAL, the free cholesterol and fatty acids enter the cytosol from lysosome. In LAL deficiency, cholesteryl esters and triglycerides cannot be cleaved; therefore, free cholesterol and fatty acids cannot leave the lysosome^[17,18]. Cells sense this as an intracellular (cytosolic) cholesterol deficiency, and the cholesterol biosynthetic pathway is up-regulated to compensate.

Synthesized in the rough endoplasmic reticulum, LAL is a typical soluble lysosomal hydrolase, which is co-translationally glycosylated when it emerges into the endoplasmic reticulum lumen^[18,19]. Following the removal of the leader sequence (21 amino acids), LAL is decorated with oligosaccharides that are remodeled during transit through the Golgi apparatus. The N-linked oligosaccharides are remodeled from high mannosyl to complex forms, with a mannose 6-phosphate being added, which serves as the lysosomal sorting targeting signal. The mannose 6-phosphate receptor system is used to deliver the newly synthesized LAL to the lysosome. LAL is not known to require cofactors for optimal hydrolysis, and it functions as a monomer. Unmodified mature protein (378 amino acids) has a predicted molecular weight approximately 42.5 kDa. Different molecular weights have been reported for purified human LAL^[20-24]. Occupancy of the LAL N-glycosylation is essential for enzyme stability, *i.e.*, protection from rapid degradation^[25].

LAL has significant similarity to other acidic lipases, for example, lingual lipase and gastric lipases that cleave similar substrates in the stomach. However, LAL is distinct from other lipases, including hormone-sensitive lipase, pancreatic lysophospholipid lipase, lecithin cholesterol acyl transferase, lipoprotein lipase, hepatic lipase, and pancreatic lipase^[26]. All such lipases share a motif, Gly-X-Ser-X-Gly, that is an essential pentapeptide in the active site^[27,28]. This pentapeptide occurs twice in LAL at serine 99 and serine 153, and specific mutation of serine 153 identified this residue as important to catalytic activity^[23]. Like other lipases, LAL also has a catalytic triad of Ser₁₅₃, Asp₄₂₃ and His₃₅₃^[27].

GENE KNOCK-OUT PHENOTYPES AND MDSCS IN MICE

A *Lipa* knock-out mouse (*lal*^{-/-}) has been created to understand the functional roles of LAL in disease pathophysiology, lipid metabolism, and therapeutic approaches^[29,30]. The *lal*^{-/-} phenotype resembles human CESD. Its histopathologic and biochemical phenotypes are similar to human WD. The *lal*^{-/-} mice are normal appearing at birth, but develop liver enlargement by 4 wk and have a grossly enlarged abdomen with hepatosplenomegaly, lymph node enlargement, and intestinal villus infiltration by foamy macrophages by 16 wk. Massive accumulation of CE and TG and macrophage storage develops in these and other organs^[29,31-34]. Enzyme therapy has been studied in this model using human recombinant LAL (rhLAL) produced in several different eukaryotic systems^[24,35,36]. These studies clearly show the potential for correction of

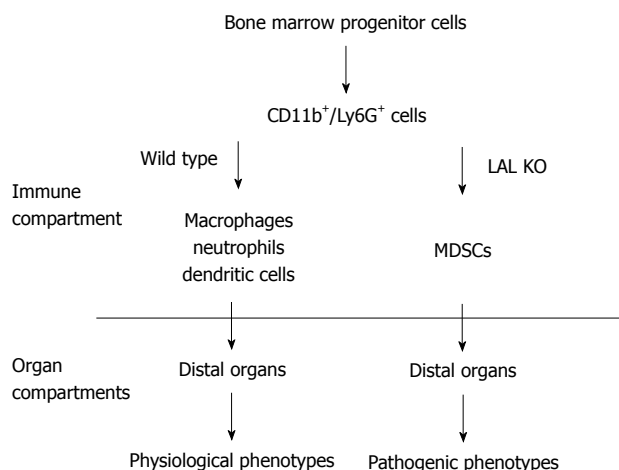


Figure 1 The functional role of Lysosomal acid lipase in myeloid lineage cells. In the wild type mice, the CD11b⁺Ly6G⁺ cells are myeloid lineage precursors for monocytes/macrophages, neutrophils, and dendritic cells, which participate in the normal physiological functions of the distal organs (e.g., lung, liver, etc.), such as clearance of invading pathogens. The lysosomal acid lipase (LAL) activity is essential for normal myeloid lineage cell development, differentiation and function. LAL deficiency leads to neutral lipid accumulation in myeloid cells and blocks CD11b⁺Ly6G⁺ cells from further differentiation into mature myeloid lineage cells. The accumulated CD11b⁺Ly6G⁺ cells possess various malfunctions that participate in the pathogenic conditions in the residing organs. MDSCs: Myeloid-derived suppressive cells.

the manifestations if enzyme therapy is begun early in the course of the disease^[36,37].

Many phenotypes of seemingly unrelated diseases in various organs co-exist in *lal*^{-/-} mice. Therefore, these diseases must share common cellular and molecular mechanisms that link these pathological processes. Extensive characterization of *lal*^{-/-} mice shows that elevation of systemic MDSCs is a major manifestation in association with most of the pathogenic conditions (e.g., > 70% in the bone marrow and > 40% in the blood), suggesting that MDSCs play a central role in mediating LAL deficiency-induced pathogenic progression^[29,31-34,36,38-41]. MDSCs was originally identified in tumor pathogenesis^[42]. Recent studies have linked this cell population to many other chronic inflammatory diseases^[43-50]. MDSCs are a mixture of myeloid cells that express CD11b and Gr-1 antigens in mice. In certain disease conditions (cancer), MDSCs are categorized into granulocytic (CD11b⁺, Ly6G⁺) and monocytic (CD11b⁺Ly6C⁺) MDSC^[51]. Interestingly, most gated *lal*^{-/-} CD11b⁺ cells show Ly6C⁺ and Ly6G⁺ double positive, making them CD11b⁺Ly6C⁺Ly6G⁺ cells^[34]. Normally, healthy immature myeloid lineage cells differentiate into dendritic cells (DCs), macrophages, or granulocytes in response to environmental changes. However, this process is blocked by LAL deficiency, leading to accumulation and expansion of MDSCs with immune suppressive function^[51-53]. This is similar to what has been observed in the tumor environment^[54]. It is conceivable that through paracrine and autocrine mechanisms, abnormally elevated MDSCs generate and secrete growth factors, chemokines and cytokines to influence cell differentiation, cell proliferation, cell apoptosis and gene expression in residing or-

gan tissues, contributing to the physiological progression of various diseases. Direct cell-cell contact by MDSCs and other cells through the juxtacrine mechanism also contributes to this pathogenic process.

The functional roles of LAL in myeloid cells have been specifically evaluated by creating a myeloid-specific doxycycline-inducible c-fms-rtTA/(tetO)⁷-CMV-hLAL; *lal*^{-/-} triple mouse model, in which human LAL is expressed in myeloid cells under the control of the 7.2 kb c-fms promoter/intron2 regulatory sequence in *lal*^{-/-} mice^[32,34,55]. The hLAL expression in myeloid lineage cells in this triple mouse model significantly reduced systemic MDSCs accumulation^[34], reversed aberrant gene expression, and ameliorated pathogenic phenotypes^[32]. Therefore, the normal biological function of myeloid cells requires normal neutral lipid metabolism (Figure 1).

MDSCS DIFFERENTIATION AND DEVELOPMENT

The myeloid lineage cells undergo the sequentially differentiated and proliferated from hematopoietic stem cells through an increasingly lineage-restricted intermediate progenitors including common myeloid progenitors (CMPs) and granulocyte-macrophage progenitors (GMPs) in the bone marrow^[56,57]. The number and frequency of primitive LSK (Lin⁻/Sca-1⁺/c-kit⁺), CMP, and GMP populations in the bone marrow, systemic myeloid cell distribution are changed in *lal*^{-/-} mice, leading to an expansion in CD11b⁺/Gr-1⁺ MDSCs^[41]. Both increased proliferation and decreased apoptosis contribute to the expansion of MDSCs in *lal*^{-/-} mice. *Lal*^{-/-} mice also display increased numbers of high proliferative potential colony-forming cells (HPP-CFC), colony-forming unit of granulocyte and macrophage progenitor cells (CFU-GM), colony-forming unit of granulocytes (CFU-G) and colony-forming unit of macrophages (CFU-M) colonies from cultured bone marrow cells. When *lal*^{-/-} bone marrow cells are transplanted into wild type mice, the donor CD11b⁺/Gr-1⁺ myeloid cells in the blood, spleen, lung and bone marrow of recipient mice are increased, confirming that the MDSCs increase is primarily due to the intrinsic defect in myeloid lineage progenitor cells. In addition to the intrinsic progenitor problem, the environment in *lal*^{-/-} mice also contributes to myeloid cell hyper-expansion, since the donor CD11b⁺/Gr-1⁺ myeloid cell population in *lal*^{-/-} recipient mice that are transplanted with wild type bone marrow cells is expanded. Therefore, the *lal*^{-/-} environment does not normally support hematopoiesis. Deregulated bone marrow progenitor cell differentiation is a primary cause for expansion of *lal*^{-/-} MDSCs, which is attributed to both cell-autonomous and environmental factors. Taken together, LAL expression in myeloid lineage cells is critical to maintain hematopoiesis and myelopoiesis. After MDSCs infiltration into distal organs, at least two mechanisms can explain how the cell-autonomous defect and environmental factors influence each other. Firstly, MDSCs and other regional

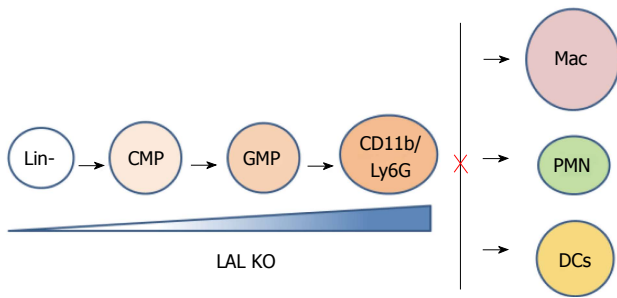


Figure 2 Lysosomal acid lipase is required for normal myeloid lineage cell development and differentiation. Lysosomal acid lipase (LAL) deficiency leads to increased myeloid-derived suppressive cells differentiation from Lin⁻ progenitor cells in the bone marrow, and decreased differentiation to mature macrophages, neutrophils, and dendritic cells in other compartments. Lin⁻: Lineage negative progenitor; CMP: Common myeloid progenitor; GMP: Granulocyte-macrophage progenitor; Mac: Macrophage; PMN: Polymorphonuclear cell, or neutrophil; DC: Dendritic cell.

cells in distal organs influence each other by the paracrine mechanism as both sides secrete cytokines and chemokines. Secondly, MDSCs and other cells can influence each other by direct contact (juxtacrine mechanism). Starting at the GMP stage, hLAL expression in myeloid cells reverses abnormal myeloid development in the bone marrow, and reduces systemic expansion of MDSCs in c-fms-rtTA/(tetO)⁷-CMV-hLAL; *lal*^{-/-} triple mice. In addition, differentiation from Lin⁻ progenitor cells to CD11b⁺GR-1⁺ cells is abnormally increased in *lal*^{-/-} mice (Figure 2). This further supports that the cell-autonomous effect of MDSCs expansion in *lal*^{-/-} mice. Myeloid hLAL expression in c-fms-rtTA/(tetO)⁷-CMV-hLAL; *lal*^{-/-} triple mice successfully reverses this abnormality^[32]. The environmental effects on MDSCs malformation are further supported by an observation that when the Stat3 pathway is overly activated in lung epithelial cells^[58], secretion of Stat3-induced pro-inflammatory cytokines in epithelial cells reversed mature myeloid lineage cells to MDSCs^[59].

MDSCS IMMUNOSUPPRESSION

In contrast to myeloid lineage cells, T cells are systemically decreased in *lal*^{-/-} mice. *Lal*^{-/-} T cells behave abnormally. In response to stimulation of anti-CD3 plus anti-CD28 antibodies, or phorbol-12-myristate-13-acetate (agonist to activate PKC) and ionomycin (calcium ionophore), there is severely diminished T cell proliferation, decreased CD69 expression, and decreased expression of T cell lymphokines. LAL deficiency does not drive effector T cells into either Th1 or Th2 status^[33]. The thymus is the most important organ for T cell development, which is divided into different developmental stages that are marked by CD4⁻CD8⁻ double negative (DN) 1 to 4 stages, CD4⁺CD8⁺ double positive (DP) stage and CD4⁺ or CD8⁺ single positive (SP) stage. The earliest stage for thymocyte paucity appears at the DN4 (CD25⁻CD44⁻) stage in the *lal*^{-/-} thymus. After this developmental point, thymocytes are declining at all stages, suggesting that

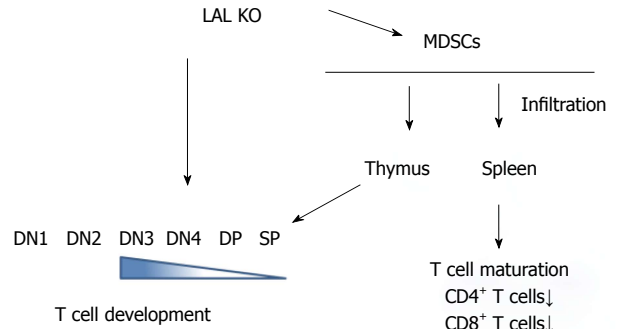


Figure 3 Lysosomal acid lipase is required for normal T cell development and differentiation. Lysosomal acid lipase (LAL) deficiency can cause the intrinsic defect in T cell development, starting at the double negative 3 (DN3) stage. In addition, myeloid-derived suppressive cells infiltrate into the thymus and spleen, resulting in blockage of normal T cell development, differentiation, and maturation. DN: CD4 and CD8 double negative; DP: CD4 and CD8 double positive; SP: CD4 or CD8 single positive.

the blockage of T cell development initially occurs at the DN3 to DN4 transition (Figure 3)^[33]. Decrease of T cell development and maturation was also observed in *lal*^{-/-} mice due to the defects in lymphoid progenitors in the bone marrow chimeras study. This notion has been supported by the bone marrow profile analysis, in which common lymphoid progenitor development is blocked in the bone marrow of *lal*^{-/-} mice^[33,41].

In addition to the above intrinsic defect, extensive analyses have revealed a second mechanism that contributes to systemic reduction of T cell populations. Strikingly, LAL deficiency dramatically increases MDSCs expansion and infiltration in the thymus and the spleen of *lal*^{-/-} mice, leading to neutral lipid accumulation and abnormal organization of the thymus and spleen^[33]. Infiltration of MDSCs in these important T cell organs affects T cell development, differentiation and maturation. Functional analyses have shown that MDSCs from *lal*^{-/-} mice strongly inhibit proliferation and function of T cells (Figure 3)^[34,40,41].

Direct connection between LAL in MDSCs and T cell abnormalities comes from the c-fms-rtTA/(tetO)⁷-CMV-hLAL;*lal*^{-/-} triple mouse study. MDSCs expansion and infiltration into the thymus and spleen are reduced in this mouse model. This leads to restoration of T cell proliferation in the spleen and normal T cell development in the thymus^[34]. Stat3 and NFκB p65 signaling play a critical role in *lal*^{-/-} MDSCs immune suppressive function^[34]. The above observations are further proved by an MDSCs depletion study, in which anti-Gr-1 antibody treatment recovers T cell numbers in *lal*^{-/-} mice^[34]. *lal*^{-/-} MDSCs also inhibits T cell lymphokine production, which is resulted from inactivation of the pZAP-70/Syk intracellular signaling, loss of expression of TCR ξ chain and CD69, a failure to respond to TCR stimulation^[33]. These defects can also be reversed by myeloid hLAL expression^[34]. Lastly, Treg cells inhibit CD4⁺ T cell lymphokine production and proliferation^[60]. LAL deficiency substantially increases CD4⁺FoxP3⁺ Treg cells in *lal*^{-/-} mice^[33].

GENE PROFILES IN LAL DEFICIENCY-INDUCED MDSCS

Since LAL controls homeostasis and development of MDSCs, which have profound pathogenic impact on various disease development, it is essential to identify the intrinsic defects that are involved in the MDSCs homeostasis and function for future targeting. In a comprehensive gene transcriptional profile study by Affymetrix GeneChip microarray analysis, multiple pathways have been revealed in *lal*^{-/-} bone marrow MDSCs. Below are lists of some major (but not limited) changed pathways in *lal*^{-/-} MDSCs.

Genes of G-protein superfamily

Expression changes of both large and small GTPases have been detected in *lal*^{-/-} MDSCs, which have diverse functions in cells^[61,62]. They include: (1) Rab GTPases, which control vesicle formation, receptor internalization, and trafficking to the nucleus, lysosome and plasma membrane. Rab GTPases regulate cellular proliferation, apoptosis and migration by integrating signaling pathways; (2) Rho GTPases, which organize actin cytoskeleton, cell adhesion and cell motility^[63]; and (3) Ras GTPases mediate cell-cycle entry, cell growth, cell survival, cell growth and cellular metabolism by phosphorylating transcription factors through activation of the Raf/Mek/Erk pathway. Activation of Erk and p38 phosphorylation has been observed in *lal*^{-/-} MDSCs^[41].

Histone cluster genes and cell cycle genes

Cell cycle regulating genes are upregulated in *lal*^{-/-} MDSCs. They include: (1) Histone-variants cluster genes, which favor the epigenetic microenvironment change to promote MDSCs expansion. Histone-variants exchange also contributes to formation of centromeric and telomeric chromatin during cell cycles. Indeed, G1/M phases of *lal*^{-/-} MDSCs are increased in a cell cycle analysis^[64]; (2) Cell cycle related genes^[65], including Cdk1, Cdk2, Cdk5, Cdk9, and all Cdk regulatory cyclins (A, B, D, E-type), suggesting constitutive mitogenic signaling and defective responses to anti-mitogenic signals; and (3) Ubiquitination and proteasome enzymes/protein factors, which direct proteins to proteolysis within proteasome for recycling^[66].

Metabolism and bioenergetics

Bioenergetic and metabolic genes are abnormally upregulated in *lal*^{-/-} MDSCs, which control mitochondrial oxidative phosphorylation and energy (ATP production) for cellular activities. These include: (1) lactate dehydrogenase A and B, which produce large quantities of secreted lactate, suggesting that *lal*^{-/-} MDSCs use an aerobic glycolysis; (2) nitric oxide/reactive oxygen species (ROS) production genes, glutathione peroxidase/glutathione reductase genes, and glucose 6-phosphate dehydrogenase gene, which are involved in production of ROS. The concentration of ROS is significantly increased in *lal*^{-/-}

MDSCs; (3) enzymes and proteins in glycolysis and citric acid cycles; and (4) respiratory chain proteins (NADH dehydrogenases, cytochrome proteins, ATPases and mitochondrial ribosomal proteins).

The mTOR pathway in LAL deficiency induced MDSCs

PI3K/thymoma viral proto-oncogene (AKT)/mammalian target of rapamycin (mTOR) is activated in *lal*^{-/-} MDSCs^[64]. mTOR is a lysosomal membrane-bound protein, which controls apoptosis, promotes influx of glucose and amino acids into the cells, stimulates ATP production^[67], contributes to cell growth, cell cycle entry, cell survival, and cell motility^[68,69]. Lack of the LAL activity changes lipid composition and dynamics on the lysosomal membrane that potentially influence endomembrane trafficking and stimulate the mTOR activity, which in turn coordinates the cellular metabolism^[64,69,70]. It has been demonstrated that mTOR plays a critical role in modulating cellular immune functions^[71,72], activation of the mTOR pathway contributes to *lal*^{-/-} MDSCs production and function^[40]. mTOR is the catalytic subunit of two distinctive complexes; mTOR complex 1 (mTORC1) and mTOR complex (mTORC2). mTORC1 contains unique regulatory associated proteins of mTOR (RAPTOR) while mTORC2 contains rapamycin-insensitive companion of mTOR (RICTOR)^[67,72-75]. Inhibition of mTOR and associated proteins (Raptor, Rictor, and Akt1) corrects *lal*^{-/-} MDSCs development, increased cell proliferation, decreased cellular apoptosis, and immune suppression in association with decreased ROS production, recovery from impairment of the mitochondrial membrane potential, increased ATP synthesis, and increased cell cycling. Potentially, the mTOR pathway can serve as a target to modulate the emergence of MDSCs in various pathophysiologic states where these cells play an immunosuppressive role (Figure 4).

The Stat3 and NFκB pathways

Although upregulation of Signal Transducer and Activator of Transcription (Stat) family members and NFκB family members are not detected by microarray analysis, phosphorylation of Stat3 and NFκB has been detected in expanded *lal*^{-/-} MDSCs^[34,41]. Activation of Stat3 directly leads to MDSCs expansion *in vivo*^[58,59].

STUDY OF LAL DOWNSTREAM GENES

The gene profile study in the lung of *lal*^{-/-} mice by Affymetrix GeneChip microarray analysis has also been performed. This is because the lung is a lipid rich organ and highly responsive to inflammation. Neutral lipids account for 10% of the composition of pulmonary surfactant that protects alveoli from collapse during respiratory cycles^[76]. LAL deficiency results in massive myeloid cell infiltration, hyperplasia and emphysema in the *lal*^{-/-} lung^[32,39]. Comparison between the changed gene lists of bone marrow MDSCs and the whole lung by Affymetrix GeneChip microarray analyses reveals a few overlapping

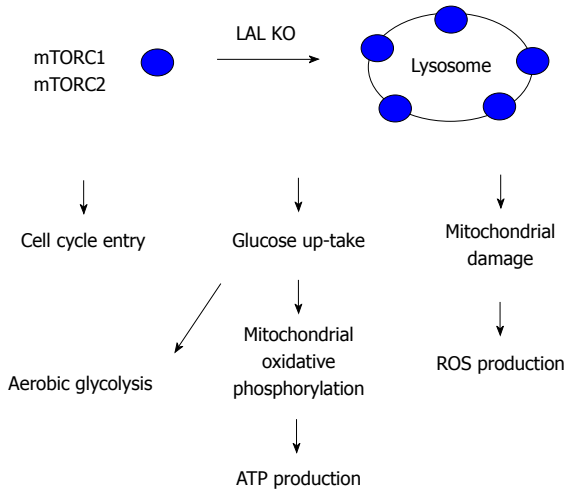
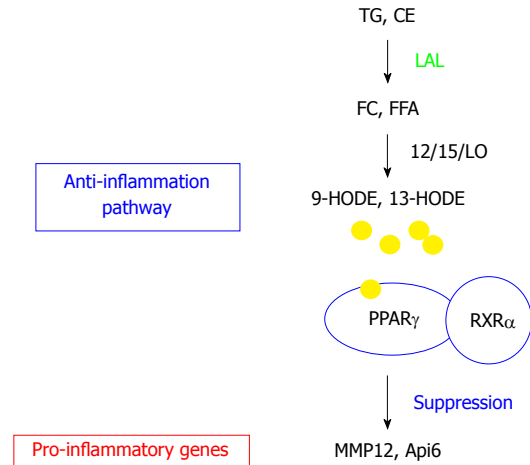


Figure 4 Lysosomal acid lipase deficiency induces overactivation of the mTOR pathway in myeloid-derived suppressive cells. Lysosomal acid lipase (LAL) is a lysosome-associated enzyme. LAL deficiency increases mTOR complexes anchoring on lysosomes and stimulates the mTOR1 activity to influence the cellular metabolism and proliferation of *lal*^{-/-} myeloid-derived suppressive cells (MDSCs). These include an increased influx of glucose through aerobic glycolysis, an increased mitochondrial oxidative phosphorylation and ATP production, an impairment of the mitochondrial membrane potential in association with increased reactive oxygen species (ROS) production, and an increased cell cycle entry in *lal*^{-/-} MDSCs.

genes. Therefore, LAL performs differential roles in different compartments. LAL exerts its biological effects through its downstream genes. In order to fully understand the LAL functions, it is necessary and essential to characterize its downstream genes. From the whole lung gene list, the two most up-regulated genes matrix metal proteinase 12 (MMP12) and apoptosis inhibitor 6 (Api6) are characterized extensively. The functional role of LAL downstream effector peroxisome proliferator-activated receptor gamma (PPAR γ) has also been studied in depth. Figure 5 shows the relationship between LAL and its downstream effectors.

PPAR γ

Involvement of the receptor network in the metabolic programming of myeloid lineage cells is essential to the innate immune system^[77,78]. PPAR γ is of high interest for several reasons. Firstly, the metabolites of LAL hydrolysis, 9-hydroxyoctadecanoic acids (9-HODE) or 13-HODE from linoleic acid, serve as ligands for PPAR γ . Upon binding to the ligands, PPAR interacts with the retinoid X receptor (RXR) to form the PPAR γ /RXR dimer on target genes. Secondly, PPAR γ plays an important role in anti-inflammation of various tissues^[77,79,80]. It has been shown that PPAR γ agonists suppress gene expression of inflammatory cytokines^[79]. In the *lal*^{-/-} lung, these pro-inflammatory cytokines are up-regulated (Figure 5)^[39]. Therefore, LAL deficiency causes inactivation of PPAR γ by depleting ligand production. Using the lung as a model system, reintroduction of LAL downstream metabolic derivative 9-HODE (a natural occurring ligand for PPAR γ) and a synthetic ligand compound ciglitazone for PPAR γ improves the inflammatory status and pathogen-



TGF- β , IL-1 β , MCP-1, IL-6, TNF- α , G-CSF, GM-CSF, NOS, NF- κ B, KC (CXCL1), EP2, MMP9, PEG2

Figure 5 Lysosomal acid lipase and its downstream effector genes. Lysosomal acid lipase (LAL) cleaves cholesteryl esters (CE) and triglycerides (TG) to produce free cholesterol (FC) and fatty acids (FFA) in lysosomes of cells. The lipid derivatives (9-HODE, 13-HODE) of FFA serve as ligands for PPAR γ in coupling with retinoid X receptor α (RXR α), which suppresses gene expression of a variety of pro-inflammatory cytokines. The LAL/PPAR γ axis serves as an anti-inflammatory pathway. LAL deficiency blocks this metabolic pathway to provoke up-regulation of pro-inflammatory cytokines (e.g., Api6, MMP12). TGF: Transforming growth factor beta; IL: Interleukin; MCP: Monocyte chemotactic protein; TNF: Tumor necrosis factor; NF: Nuclear factor.

esis in the *lal*^{-/-} lung. Therefore, the ligands/PPAR γ axis controls inflammation-triggered elevated gene expression and pathogenesis in the *lal*^{-/-} mice^[31].

To directly evaluate functional role of LAL downstream effector PPAR γ in myeloid cells, dominant negative PPAR γ (dnPPAR γ) is overexpressed in a myeloid-specific c-fms-rtTA/(TetO) γ -CMV-dnPPAR γ bitransgenic mouse model^[81]. In this bitransgenic system, total numbers and frequencies of LK, LSK, CMP and GMP progenitor cells in the bone marrow are abnormally elevated. DnPPAR γ overexpression leads to up-regulation of IL-1 β , IL-6 and TNF α in the blood plasma. MDSCs from this bitransgenic mouse model inhibit the proliferation and lymphokine production of wild type CD4⁺ T cells *in vitro*. Both CD4⁺ and CD8⁺ T cell populations are decreased in doxycycline-induced dnPPAR γ expressed mice. Bone marrow transplantation reveals that a myeloid autonomous defect is responsible for MDSC expansion, immunosuppression and tumorigenesis in this myeloid-specifically expressed dnPPAR γ bitransgenic mice. Multiple forms of carcinoma and sarcoma in various organs (the lung, liver, spleen and lymph nodes) are observed in this mouse model. Therefore, the LAL/hormonal ligands/PPAR γ axis is critical to control inflammation and the induction of various tumors. Disruption of this pathway in myeloid cells, either by blocking ligand synthesis (as in *lal*^{-/-} mice), or inhibition of PPAR γ (as in c-fms-rtTA/(TetO) γ -CMV-dnPPAR γ bitransgenic mice) can initiate up-regulation of inflammatory molecules which cause hematopoietic progenitors skewing towards myeloid lineage expansion to form MDSCs.

Matrix metalloproteinases12

Zinc-dependent MMPs act as modulators for inflammation and innate immunity by activating, deactivating or modifying the activities of signaling cytokines, chemokines and receptors through proteolytic and nonproteolytic functions^[82-84]. Among MMPs, MMP12 is a 22-kDa secretory proteinase that is predominantly expressed in macrophages as previously reported^[85]. MMP12 degrades extracellular matrix components, such as type IV collagen, fibronectin, laminin, gelatin, vitronectin, entactin, heparin, and chondroitin sulphates, to facilitate tissue remodeling^[86]. The expression of MMP12 in macrophages is induced in the lung of cigarette smokers^[87]. Inactivation of the MMP12 gene in knock-out mice demonstrates a critical role of MMP12 in smoking-induced chronic obstructive pulmonary disease (COPD)^[88], a disease highly related to lung cancer. From clinical studies, MMP12 correlates with early cancer-related deaths in non-small cell lung cancer (NSCLC), especially with those associated with tobacco cigarette smoke exposure^[89,90]. In the *lal*^{-/-} lung, MMP-12 is the highest upregulated gene^[31]. In the *lal*^{-/-} lung, both macrophages and lung epithelial alveolar type II (AT II) cells are responsible for MMP-12 increase^[31,91,92]. Both myeloid-specific and lung epithelial-specific MMP12 bitransgenic mouse models have been created to study the functional roles of this LAL/PPAR γ downstream molecule.

In the myeloid-specific c-fms-rtTA/(TetO) γ -CMV-MMP12 bitransgenic mouse model, induction of MMP12 abnormally elevates numbers and frequencies of CMP and GMP populations in the bone marrow, similar to that observed in *lal*^{-/-} mice. Addition of activated MMP12 is able to stimulate wild type Lin⁻ progenitor cells to differentiate into the MDSC population, suggesting that MMP12 directly exerts its effect on hematopoietic progenitor cells. The MDSCs are systemically increased in multiple organs of MMP12 bitransgenic mice. MDSCs from MMP12-overexpressed bitransgenic mice suppress T cell proliferation and function. MMP12 directly stimulates differentiation of CD11b⁺Gr-1⁺ cells from Lin⁻ progenitor cells. In the lung, the concentration of IL-6 is increased, which aberrantly activates oncogenic Stat3 and increases expression of Stat3 downstream genes in epithelial tumor progenitor cells. As a result, spontaneous emphysema and lung adenocarcinoma are sequentially developed in MMP12-overexpressive bitransgenic mice, suggesting a critical role of MMP12 in the transition from emphysema to lung cancer.

In epithelial-specific CCSP-rtTA/(TetO) γ -CMV-MMP12 bitransgenic mice, MMP12 overexpression induces regional MDSCs infiltration and increases epithelial growth. Again, spontaneous emphysema and bronchioalveolar adenocarcinoma are developed sequentially. Importantly, MMP12 upregulation is highly associated with COPD and lung cancer in human patients. Together, these studies support that LAL/PPAR γ downstream MMP12 plays a critical role in emphysema to lung cancer transition that is facilitated by inflammation.

Clinically, it has been reported that there is a pathophysiological connection between emphysema/COPD and lung cancers^[93,94].

Apoptosis inhibitor 6

Apoptosis inhibitor 6 (Api6) belongs to the macrophage scavenger receptor cysteine-rich domain superfamily (SRCR-SF)^[95,96]. Api6 expression is the second highest induced gene in the *lal*^{-/-} lung. Api6 is regulated by LAL metabolic derivatives (*e.g.*, 9-HODE) and PPAR γ ^[31]. In a myeloid-specific c-fms-rtTA/(TetO) γ -CMV-Api6 bitransgenic mouse model, many phenotypes are similar to those observed in *lal*^{-/-} mice. Overexpression of Api6 abnormally elevates MDSCs in the bone marrow, blood and lung with increased cell proliferation and decreased apoptotic activities. Api6 overexpression activates Stat3, Erk1/2 and p38 in myeloid lineage cells. Persistent inflammation in myeloid-specific Api6 bitransgenic mice causes lung adenocarcinoma^[97].

Pathogenic overexpression of Api6 is also observed in *lal*^{-/-} AT II cells. In an epithelial-specific CCSP-rtTA/(TetO) γ -CMV-Api6 bitransgenic mice, Api6 overexpression in AT II cells increases pro-inflammatory cytokine/chemokine levels in bronchoalveolar lavage fluid and serum, activates oncogenic signaling and inhibits apoptosis, promotes expansion of MDSCs in lung and blood but not in the bone marrow or spleen. Lung MDSCs from this bitransgenic mouse model suppress T cell proliferation and function, which results in occurrence of emphysema and adenocarcinoma.

CONCLUSION

MDSCs play vital roles in various inflammation-induced chronic diseases. Elimination or reduction of MDSCs populations can slow down disease formation and progression. It is important to identify the molecular pathways in order to effectively block MDSCs homeostasis and function. Extensive studies outlined in this review have shown that the role of LAL in controlling neutral lipid metabolism is a key player in MDSCs development, homeostasis and function, therefore, providing a new avenue to develop therapeutic or immunologic approaches for clinical application. Through studies of the LAL function, defective gene expression patterns have been mapped in *lal*^{-/-} MDSCs. These provide novel targets for controlling MDSCs and associated diseases by designing small molecule inhibitors. Clinically, small molecule inhibitors for c-kit have been tested to target MDSCs^[98]. Using the gene profile list from LAL deficiency-induced MDSCs, more small molecule inhibitors can and will be identified to inhibit MDSCs pathogenic functions in various disease conditions.

ACKNOWLEDGMENTS

The authors thank Miss Katlin Walls for proof-reading the manuscript.

REFERENCES

- Abramov A, Schorr S, Wolman M. Generalized xanthomatosis with calcified adrenals. *AMA J Dis Child* 1956; **91**: 282-286 [PMID: 13301142]
- Boldrini R, Devito R, Biselli R, Filocamo M, Bosman C. Wolman disease and cholesteryl ester storage disease diagnosed by histological and ultrastructural examination of intestinal and liver biopsy. *Pathol Res Pract* 2004; **200**: 231-240 [PMID: 15200275 DOI: 10.1016/j.prp.2003.11.001]
- Krivit W, Peters C, Dusenbery K, Ben-Yoseph Y, Ramsay NK, Wagner JE, Anderson R. Wolman disease successfully treated by bone marrow transplantation. *Bone Marrow Transplant* 2000; **26**: 567-570 [PMID: 11019848 DOI: 10.1038/sj.bmt.1702557]
- Assmann G, Seedorf U. Acid lipase deficiency: Wolman disease and cholesteryl ester storage disease. The Metabolic and Molecular Bases of Inherited Disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. 6 ed. New York: McGraw-Hill, 1995: 2563-2587
- Stein J, Garty BZ, Dror Y, Fenig E, Zeigler M, Yaniv I. Successful treatment of Wolman disease by unrelated umbilical cord blood transplantation. *Eur J Pediatr* 2007; **166**: 663-666 [PMID: 17033804 DOI: 10.1007/s00431-006-0298-6]
- Reynolds T. Cholesteryl ester storage disease: a rare and possibly treatable cause of premature vascular disease and cirrhosis. *J Clin Pathol* 2013; **66**: 918-923 [PMID: 23999269 DOI: 10.1136/jclinpath-2012-201302]
- Fredrickson DS, Sloan HR, Ferrans VJ, Demosky SJ. Cholesteryl ester storage disease: a most unusual manifestation of deficiency of two lysosomal enzyme activities. *Trans Assoc Am Physicians* 1972; **85**: 109-119 [PMID: 4660005]
- Schiff L, Schubert WK, McAdams AJ, Spiegel EL, O'Donnell JF. Hepatic cholesterol ester storage disease, a familial disorder. I. Clinical aspects. *Am J Med* 1968; **44**: 538-546 [PMID: 5642714 DOI: 10.1016/0002-9343(68)90054-5]
- Lageron A, Caroli J, Stralin H, Barbier P. [Cholesterolic polycoria in adults. I. Clinical and histochemical study]. *Presse Med* 1967; **75**: 2785 [PMID: 5583022]
- Infante R, Polonovski J, Caroli J. [Cholesterolic polycoria in adults. II. Biochemical study]. *Presse Med* 1967; **75**: 2829-2832 [PMID: 5583895]
- Beaudet AL, Ferry GD, Nichols BL, Rosenberg HS. Cholesterol ester storage disease: clinical, biochemical, and pathological studies. *J Pediatr* 1977; **90**: 910-914 [PMID: 859064 DOI: 10.1016/S0022-3476(77)80557-X]
- Bernstein DL, Hůlková H, Bialer MG, Desnick RJ. Cholesteryl ester storage disease: review of the findings in 135 reported patients with an underdiagnosed disease. *J Hepatol* 2013; **58**: 1230-1243 [PMID: 23485521 DOI: 10.1016/j.jhep.2013.02.014]
- Fouchier SW, Defesche JC. Lysosomal acid lipase A and the hypercholesterolaemic phenotype. *Curr Opin Lipidol* 2013; **24**: 332-338 [PMID: 23652569 DOI: 10.1097/MOL.0b013e328361f6c6]
- Grabowski GA, Du H. Lysosomal Acid Lipase Deficiencies: The Wolman Disease/Cholesteryl ester storage disease Spectrum. The Online Metabolic and Molecular Bases of inherited Disease (OMMBID). In: Valle D BA, Voglstein B, Kinzler KW, Antonarakis SE, editors. 9th ed. New York: McGraw-Hill, 2012
- Rothe G, Stöhr J, Fehringer P, Gasche C, Schmitz G. Altered mononuclear phagocyte differentiation associated with genetic defects of the lysosomal acid lipase. *Atherosclerosis* 1997; **130**: 215-221 [PMID: 9126667 DOI: 10.1016/S0021-9150(97)06065-6]
- Elleder M, Chlumská A, Hyánek J, Poupětová H, Ledvinová J, Maas S, Lohse P. Subclinical course of cholesteryl ester storage disease in an adult with hypercholesterolemia, accelerated atherosclerosis, and liver cancer. *J Hepatol* 2000; **32**: 528-534 [PMID: 10735626 DOI: 10.1016/S0168-8278(00)80407-9]
- Brown MS, Sobhani MK, Brunschede GY, Goldstein JL. Retention of a regulatory response to low density lipoprotein in acid lipase-deficient human fibroblasts. *J Biol Chem* 1976; **251**: 3277-3286 [PMID: 179993]
- Sando GN, Ma GP, Lindsley KA, Wei YP. Intercellular transport of lysosomal acid lipase mediates lipoprotein cholesteryl ester metabolism in a human vascular endothelial cell-fibroblast coculture system. *Cell Regul* 1990; **1**: 661-674 [PMID: 2150334]
- Sando GN, Henke VL. Recognition and receptor-mediated endocytosis of the lysosomal acid lipase secreted by cultured human fibroblasts. *J Lipid Res* 1982; **23**: 114-123 [PMID: 7057100]
- Sando GN, Rosenbaum LM. Human lysosomal acid lipase/cholesteryl ester hydrolase. Purification and properties of the form secreted by fibroblasts in microcarrier culture. *J Biol Chem* 1985; **260**: 15186-15193 [PMID: 4066668]
- Ameis D, Merkel M, Eckerskorn C, Greten H. Purification, characterization and molecular cloning of human hepatic lysosomal acid lipase. *Eur J Biochem* 1994; **219**: 905-914 [PMID: 8112342 DOI: 10.1111/j.1432-1033.1994.tb18572.x]
- Du H, Sheriff S, Bezerra J, Leonova T, Grabowski GA. Molecular and enzymatic analyses of lysosomal acid lipase in cholesteryl ester storage disease. *Mol Genet Metab* 1998; **64**: 126-134 [PMID: 9705237 DOI: 10.1006/mgme.1998.2707]
- Sheriff S, Du H, Grabowski GA. Characterization of lysosomal acid lipase by site-directed mutagenesis and heterologous expression. *J Biol Chem* 1995; **270**: 27766-27772 [PMID: 7499245 DOI: 10.1074/jbc.270.46.27766]
- Du H, Cameron TL, Garger SJ, Pogue GP, Hamm LA, White E, Hanley KM, Grabowski GA. Wolman disease/cholesteryl ester storage disease: efficacy of plant-produced human lysosomal acid lipase in mice. *J Lipid Res* 2008; **49**: 1646-1657 [PMID: 18413899 DOI: 10.1194/jlr.M700482-JLR200]
- Zschenker O, Bähr C, Hess UF, Ameis D. Systematic mutagenesis of potential glycosylation sites of lysosomal acid lipase. *J Biochem* 2005; **137**: 387-394 [PMID: 15809341 DOI: 10.1093/jb/mvi043]
- Komaromy MC, Schotz MC. Cloning of rat hepatic lipase cDNA: evidence for a lipase gene family. *Proc Natl Acad Sci USA* 1987; **84**: 1526-1530 [PMID: 3470738 DOI: 10.1073/pnas.84.6.1526]
- Lohse P, Lohse P, Chahrokh-Zadeh S, Seidel D. Human lysosomal acid lipase/cholesteryl ester hydrolase and human gastric lipase: site-directed mutagenesis of Cys227 and Cys236 results in substrate-dependent reduction of enzymatic activity. *J Lipid Res* 1997; **38**: 1896-1905 [PMID: 9323599]
- Roussel A, Canaan S, Egloff MP, Rivière M, Dupuis L, Vergier R, Cambillau C. Crystal structure of human gastric lipase and model of lysosomal acid lipase, two lipolytic enzymes of medical interest. *J Biol Chem* 1999; **274**: 16995-17002 [PMID: 10358049 DOI: 10.1074/jbc.274.24.16995]
- Du H, Duanmu M, Witte D, Grabowski GA. Targeted disruption of the mouse lysosomal acid lipase gene: long-term survival with massive cholesteryl ester and triglyceride storage. *Hum Mol Genet* 1998; **7**: 1347-1354 [PMID: 9700186 DOI: 10.1093/hmg/7.9.1347]
- Yan C, Lian X, Dai Y, Wang X, Qu P, White A, Qin Y, Du H. Gene delivery by the hSP-B promoter to lung alveolar type II epithelial cells in LAL-knockout mice through bone marrow mesenchymal stem cells. *Gene Ther* 2007; **14**: 1461-1470 [PMID: 17700706 DOI: 10.1038/sj.gt.3303006]
- Lian X, Yan C, Qin Y, Knox L, Li T, Du H. Neutral lipids and peroxisome proliferator-activated receptor- γ control pulmonary gene expression and inflammation-triggered pathogenesis in lysosomal acid lipase knockout mice. *Am J Pathol* 2005; **167**: 813-821 [PMID: 16127159 DOI: 10.1016/S0002-9440(10)62053-6]
- Yan C, Lian X, Li Y, Dai Y, White A, Qin Y, Li H, Hume DA, Du H. Macrophage-specific expression of human lysosomal acid lipase corrects inflammation and pathogenic phenotypes in *lal*^{-/-} mice. *Am J Pathol* 2006; **169**: 916-926 [PMID: 16800000 DOI: 10.1016/j.ajpath.2006.05.010]

- 16936266 DOI: 10.2353/ajpath.2006.051327]
- 33 **Qu P**, Du H, Wilkes DS, Yan C. Critical roles of lysosomal acid lipase in T cell development and function. *Am J Pathol* 2009; **174**: 944-956 [PMID: 19179613 DOI: 10.2353/ajpath.2009.080562]
- 34 **Qu P**, Yan C, Blum JS, Kapur R, Du H. Myeloid-specific expression of human lysosomal acid lipase corrects malformation and malfunction of myeloid-derived suppressor cells in *lal*^{-/-} mice. *J Immunol* 2011; **187**: 3854-3866 [PMID: 21900179 DOI: 10.4049/jimmunol.1003358]
- 35 **Du H**, Levine M, Ganesa C, Witte DP, Cole ES, Grabowski GA. The role of mannosylated enzyme and the mannose receptor in enzyme replacement therapy. *Am J Hum Genet* 2005; **77**: 1061-1074 [PMID: 16380916]
- 36 **Du H**, Schiavi S, Levine M, Mishra J, Heur M, Grabowski GA. Enzyme therapy for lysosomal acid lipase deficiency in the mouse. *Hum Mol Genet* 2001; **10**: 1639-1648 [PMID: 11487567]
- 37 **Balwani M**, Breen C, Enns GM, Deegan PB, Honzik T, Jones S, Kane JP, Malinova V, Sharma R, Stock EO, Valayannopoulos V, Wraith JE, Burg J, Eckert S, Schneider E, Quinn AG. Clinical effect and safety profile of recombinant human lysosomal acid lipase in patients with cholesteryl ester storage disease. *Hepatology* 2013; **58**: 950-957 [PMID: 23348766 DOI: 10.1002/hep.26289]
- 38 **Du H**, Heur M, Duanmu M, Grabowski GA, Hui DY, Witte DP, Mishra J. Lysosomal acid lipase-deficient mice: depletion of white and brown fat, severe hepatosplenomegaly, and shortened life span. *J Lipid Res* 2001; **42**: 489-500 [PMID: 11290820]
- 39 **Lian X**, Yan C, Yang L, Xu Y, Du H. Lysosomal acid lipase deficiency causes respiratory inflammation and destruction in the lung. *Am J Physiol Lung Cell Mol Physiol* 2004; **286**: L801-L807 [PMID: 14644759]
- 40 **Ding X**, Du H, Yoder MC, Yan C. Critical role of the mTOR pathway in development and function of myeloid-derived suppressor cells in *lal*^{-/-} mice. *Am J Pathol* 2014; **184**: 397-408 [PMID: 24287405 DOI: 10.1016/j.ajpath.2013.10.015]
- 41 **Qu P**, Shelley WC, Yoder MC, Wu L, Du H, Yan C. Critical roles of lysosomal acid lipase in myelopoiesis. *Am J Pathol* 2010; **176**: 2394-2404 [PMID: 20348241 DOI: 10.2353/ajpath.2010.091063]
- 42 **Talmadge JE**, Gabrilovich DI. History of myeloid-derived suppressor cells. *Nat Rev Cancer* 2013; **13**: 739-752 [PMID: 24060865 DOI: 10.1038/nrc3581]
- 43 **Okwan-Duodu D**, Umpierrez GE, Brawley OW, Diaz R. Obesity-driven inflammation and cancer risk: role of myeloid derived suppressor cells and alternately activated macrophages. *Am J Cancer Res* 2013; **3**: 21-33 [PMID: 23359288]
- 44 **Cuenca AG**, Delano MJ, Kelly-Scumpia KM, Moreno C, Scumpia PO, Laface DM, Heyworth PG, Efron PA, Moldawer LL. A paradoxical role for myeloid-derived suppressor cells in sepsis and trauma. *Mol Med* 2011; **17**: 281-292 [PMID: 21085745 DOI: 10.2119/molmed.2010.00178]
- 45 **Goh C**, Narayanan S, Hahn YS. Myeloid-derived suppressor cells: the dark knight or the joker in viral infections? *Immunol Rev* 2013; **255**: 210-221 [PMID: 23947357 DOI: 10.1111/immr.12084]
- 46 **Natarajan S**, Thomson AW. Tolerogenic dendritic cells and myeloid-derived suppressor cells: potential for regulation and therapy of liver auto- and alloimmunity. *Immunobiology* 2010; **215**: 698-703 [PMID: 20605054 DOI: 10.1016/j.imbio.2010.05.024]
- 47 **Dilek N**, Vuillefroy de Silly R, Blanche G, Vanhove B. Myeloid-derived suppressor cells: mechanisms of action and recent advances in their role in transplant tolerance. *Front Immunol* 2012; **3**: 208 [PMID: 22822406 DOI: 10.3389/fimmu.2012.00208]
- 48 **Yin B**, Ma G, Yen CY, Zhou Z, Wang GX, Divino CM, Casares S, Chen SH, Yang WC, Pan PY. Myeloid-derived suppressor cells prevent type 1 diabetes in murine models. *J Immunol* 2010; **185**: 5828-5834 [PMID: 20956337 DOI: 10.4049/jimmunol.0903636]
- 49 **Morales JK**, Saleem SJ, Martin RK, Saunders BL, Barnstein BO, Faber TW, Pullen NA, Kolawole EM, Brooks KB, Norton SK, Sturgill J, Graham L, Bear HD, Urban JF, Lantz CS, Conrad DH, Ryan JJ. Myeloid-derived suppressor cells enhance IgE-mediated mast cell responses. *J Leukoc Biol* 2014; **95**: 643-650 [PMID: 24338630 DOI: 10.1189/jlb.0913510]
- 50 **Ostanin DV**, Bhattacharya D. Myeloid-derived suppressor cells in the inflammatory bowel diseases. *Inflamm Bowel Dis* 2013; **19**: 2468-2477 [PMID: 23811636 DOI: 10.1097/MIB.0b013e3182902b11]
- 51 **Gabrilovich DI**, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009; **9**: 162-174 [PMID: 19197294 DOI: 10.1038/nri2506]
- 52 **Ostrand-Rosenberg S**, Sinha P. Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol* 2009; **182**: 4499-4506 [PMID: 19342621 DOI: 10.4049/jimmunol.0802740]
- 53 **Sica A**, Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest* 2007; **117**: 1155-1166 [PMID: 17476345]
- 54 **Lindau D**, Gielen P, Kroesen M, Wesseling P, Adema GJ. The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. *Immunology* 2013; **138**: 105-115 [PMID: 23216602 DOI: 10.1111/imm.12036]
- 55 **Sasmono RT**, Oceandy D, Pollard JW, Tong W, Pavli P, Wainwright BJ, Ostrowski MC, Himes SR, Hume DA. A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. *Blood* 2003; **101**: 1155-1163 [PMID: 12393599 DOI: 10.1182/blood-2002-02-0569]
- 56 **Weissman IL**, Shizuru JA. The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. *Blood* 2008; **112**: 3543-3553 [PMID: 18948588 DOI: 10.1182/blood-2008-08-078220]
- 57 **Blank U**, Karlsson G, Karlsson S. Signaling pathways governing stem-cell fate. *Blood* 2008; **111**: 492-503 [PMID: 17914027 DOI: 10.1182/blood-2007-07-075168]
- 58 **Li Y**, Du H, Qin Y, Roberts J, Cummings OW, Yan C. Activation of the signal transducers and activators of the transcription 3 pathway in alveolar epithelial cells induces inflammation and adenocarcinomas in mouse lung. *Cancer Res* 2007; **67**: 8494-8503 [PMID: 17875688 DOI: 10.1158/0008-5472.CAN-07-0647]
- 59 **Wu L**, Du H, Li Y, Qu P, Yan C. Signal transducer and activator of transcription 3 (Stat3C) promotes myeloid-derived suppressor cell expansion and immune suppression during lung tumorigenesis. *Am J Pathol* 2011; **179**: 2131-2141 [PMID: 21864492 DOI: 10.1016/j.ajpath.2011.06.028]
- 60 **Wang HY**, Wang RF. Regulatory T cells and cancer. *Curr Opin Immunol* 2007; **19**: 217-223 [PMID: 17306521 DOI: 10.1016/j.coi.2007.02.004]
- 61 **Neves SR**, Ram PT, Iyengar R. G protein pathways. *Science* 2002; **296**: 1636-1639 [PMID: 12040175 DOI: 10.1126/science.1071550]
- 62 **Gavi S**, Shumay E, Wang HY, Malbon CC. G-protein-coupled receptors and tyrosine kinases: crossroads in cell signaling and regulation. *Trends Endocrinol Metab* 2006; **17**: 48-54 [PMID: 16460957 DOI: 10.1016/j.tem.2006.01.006]
- 63 **Konstantinopoulos PA**, Karamouzis MV, Papavassiliou AG. Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nat Rev Drug Discov* 2007; **6**: 541-555 [PMID: 17585331 DOI: 10.1038/nrd2221]
- 64 **Yan C**, Ding X, Dasgupta N, Wu L, Du H. Gene profile of myeloid-derived suppressive cells from the bone marrow of lysosomal acid lipase knock-out mice. *PLoS One* 2012; **7**: e30701 [PMID: 22383970 DOI: 10.1371/journal.pone.0030701]
- 65 **Malumbres M**, Barbacid M. Cell cycle, CDKs and cancer: a

- changing paradigm. *Nat Rev Cancer* 2009; **9**: 153-166 [PMID: 19238148 DOI: 10.1038/nrc2602]
- 66 **Ovaa H.** Active-site directed probes to report enzymatic action in the ubiquitin proteasome system. *Nat Rev Cancer* 2007; **7**: 613-620 [PMID: 17646866 DOI: 10.1038/nrc2128]
- 67 **Guertin DA, Sabatini DM.** Defining the role of mTOR in cancer. *Cancer Cell* 2007; **12**: 9-22 [PMID: 17613433]
- 68 **Bousquet M, Recher C, Queleen C, Demur C, Payrastre B, Brousset P.** Assessment of somatic mutations in phosphatidylinositol 3-kinase gene in human lymphoma and acute leukaemia. *Br J Haematol* 2005; **131**: 411-413 [PMID: 16225664]
- 69 **Zoncu R, Efeyan A, Sabatini DM.** mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol* 2011; **12**: 21-35 [PMID: 21157483 DOI: 10.1038/nrm3025]
- 70 **Korolchuk VI, Saiki S, Lichtenberg M, Siddiqi FH, Roberts EA, Imarisio S, Jahreiss L, Sarkar S, Futter M, Menzies FM, O' Kane CJ, Deretic V, Rubinsztein DC.** Lysosomal positioning coordinates cellular nutrient responses. *Nat Cell Biol* 2011; **13**: 453-460 [PMID: 21394080 DOI: 10.1038/ncb2204]
- 71 **Thomson AW, Turnquist HR, Raimondi G.** Immunoregulatory functions of mTOR inhibition. *Nat Rev Immunol* 2009; **9**: 324-337 [PMID: 19390566 DOI: 10.1038/nri2546]
- 72 **Weichhart T, Säemann MD.** The multiple facets of mTOR in immunity. *Trends Immunol* 2009; **30**: 218-226 [PMID: 19362054 DOI: 10.1016/j.it.2009.02.002]
- 73 **Heitman J, Movva NR, Hall MN.** Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 1991; **253**: 905-909 [PMID: 1715094]
- 74 **Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH.** RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell* 1994; **78**: 35-43 [PMID: 7518356]
- 75 **Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL.** A mammalian protein targeted by G1-arresting rapamycin-receptor complex. *Nature* 1994; **369**: 756-758 [PMID: 8008069]
- 76 **Yan C, Du H.** Alveolus formation: what have we learned from genetic studies? *J Appl Physiol* 2004; **97**: 1543-1548 [PMID: 15358757]
- 77 **Kiss M, Czimmerer Z, Nagy L.** The role of lipid-activated nuclear receptors in shaping macrophage and dendritic cell function: From physiology to pathology. *J Allergy Clin Immunol* 2013; **132**: 264-286 [PMID: 23905916 DOI: 10.1016/j.jaci.2013.05.044]
- 78 **Nagy L, Szanto A, Szatmari I, Széles L.** Nuclear hormone receptors enable macrophages and dendritic cells to sense their lipid environment and shape their immune response. *Physiol Rev* 2012; **92**: 739-789 [PMID: 22535896 DOI: 10.1152/physrev.00004.2011]
- 79 **Jiang C, Ting AT, Seed B.** PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998; **391**: 82-86 [PMID: 9422509]
- 80 **Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK.** The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature* 1998; **391**: 79-82 [PMID: 9422508]
- 81 **Wu L, Yan C, Czader M, Foreman O, Blum JS, Kapur R, Du H.** Inhibition of PPAR γ in myeloid-lineage cells induces systemic inflammation, immunosuppression, and tumorigenesis. *Blood* 2012; **119**: 115-126 [PMID: 22053106 DOI: 10.1182/blood-2011-06-363093]
- 82 **Page-McCaw A, Ewald AJ, Werb Z.** Matrix metalloproteinases and the regulation of tissue remodelling. *Nat Rev Mol Cell Biol* 2007; **8**: 221-233 [PMID: 17318226]
- 83 **Kessenbrock K, Plaks V, Werb Z.** Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010; **141**: 52-67 [PMID: 20371345 DOI: 10.1016/j.cell.2010.03.015]
- 84 **Marco M, Fortin C, Fulop T.** Membrane-type matrix metalloproteinases: key mediators of leukocyte function. *J Leukoc Biol* 2013; **94**: 237-246 [PMID: 23695309 DOI: 10.1189/jlb.0612267]
- 85 **Werb Z, Gordon S.** Elastase secretion by stimulated macrophages. Characterization and regulation. *J Exp Med* 1975; **142**: 361-377 [PMID: 167096]
- 86 **Gronski TJ, Martin RL, Kobayashi DK, Walsh BC, Holman MC, Huber M, Van Wart HE, Shapiro SD.** Hydrolysis of a broad spectrum of extracellular matrix proteins by human macrophage elastase. *J Biol Chem* 1997; **272**: 12189-12194 [PMID: 9115292]
- 87 **Shapiro SD, Kobayashi DK, Ley TJ.** Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages. *J Biol Chem* 1993; **268**: 23824-23829 [PMID: 8226919]
- 88 **Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD.** Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice. *Science* 1997; **277**: 2002-2004 [PMID: 9302297]
- 89 **Hofmann HS, Hansen G, Richter G, Taege C, Simm A, Silber RE, Burdach S.** Matrix metalloproteinase-12 expression correlates with local recurrence and metastatic disease in non-small cell lung cancer patients. *Clin Cancer Res* 2005; **11**: 1086-1092 [PMID: 15709175]
- 90 **Qu P, Du H, Wang X, Yan C.** Matrix metalloproteinase 12 overexpression in lung epithelial cells plays a key role in emphysema to lung bronchioalveolar adenocarcinoma transition. *Cancer Res* 2009; **69**: 7252-7261 [PMID: 19706765 DOI: 10.1158/0008-5472.CAN-09-0577]
- 91 **MacIvor DM, Shapiro SD, Pham CT, Belaaouaj A, Abraham SN, Ley TJ.** Normal neutrophil function in cathepsin G-deficient mice. *Blood* 1999; **94**: 4282-4293 [PMID: 10590073]
- 92 **Shapiro SD, Senior RM.** Matrix metalloproteinases. Matrix degradation and more. *Am J Respir Cell Mol Biol* 1999; **20**: 1100-1102 [PMID: 10340927]
- 93 **Lee G, Walser TC, Dubinett SM.** Chronic inflammation, chronic obstructive pulmonary disease, and lung cancer. *Curr Opin Pulm Med* 2009; **15**: 303-307 [PMID: 19417670 DOI: 10.1097/MCP.0b013e32832c975a]
- 94 **Sohal SS, Ward C, Danial W, Wood-Baker R, Walters EH.** Recent advances in understanding inflammation and remodeling in the airways in chronic obstructive pulmonary disease. *Expert Rev Respir Med* 2013; **7**: 275-288 [PMID: 23734649 DOI: 10.1586/ers.13.26]
- 95 **Gebe JA, Llewellyn M, Hoggatt H, Aruffo A.** Molecular cloning, genomic organization and cell-binding characteristics of mouse Spalpa. *Immunology* 2000; **99**: 78-86 [PMID: 10651944]
- 96 **Miyazaki T, Hirokami Y, Matsushashi N, Takatsuka H, Naito M.** Increased susceptibility of thymocytes to apoptosis in mice lacking AIM, a novel murine macrophage-derived soluble factor belonging to the scavenger receptor cysteine-rich domain superfamily. *J Exp Med* 1999; **189**: 413-422 [PMID: 9892623]
- 97 **Qu P, Du H, Li Y, Yan C.** Myeloid-specific expression of Api6/AIM/Sp alpha induces systemic inflammation and adenocarcinoma in the lung. *J Immunol* 2009; **182**: 1648-1659 [PMID: 19155514]
- 98 **Kao J, Ko EC, Eisenstein S, Sikora AG, Fu S, Chen SH.** Targeting immune suppressing myeloid-derived suppressor cells in oncology. *Crit Rev Oncol Hematol* 2011; **77**: 12-19 [PMID: 20304669 DOI: 10.1016/j.critrevonc.2010.02.004]

P- Reviewer: Gopinath SCB, Schuurman HJ, Saeki K
S- Editor: Ji FF L- Editor: A E- Editor: Wang CH



Gut immune response in the presence of hepatitis C virus infection

Helal F Hetta, Minesh J Mehta, Mohamed Tarek M Shata

Helal F Hetta, Minesh J Mehta, Mohamed Tarek M Shata, Department of Internal Medicine, Division of Digestive Diseases, University of Cincinnati, Cincinnati, OH 45267, United States
 Helal F Hetta, Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut 71515, Egypt

Author contributions: All the authors contributed to this paper. Supported by Egyptian Government Scholarship for Helal Hetta; and Merck Investigator Initiated Studies (IIS) IISP, No. 40458 (Shata)

Correspondence to: Mohamed Tarek M Shata, MD, PhD, Department of Internal Medicine, Division of Digestive Diseases, University of Cincinnati, 231 Albert B. Sabin Way, Cincinnati, OH 45267, United States. mohamed.shata@uc.edu

Telephone: +1-513-5586110 Fax: +1-513-5581744

Received: April 2, 2014 Revised: May 22, 2014

Accepted: June 20, 2014

Published online: July 27, 2014

Abstract

Hepatitis C virus (HCV) is an important etiologic agent of hepatitis and a major cause of chronic liver infection that often leads to cirrhosis, fibrosis and hepatocellular carcinoma. Although, HCV is a hepatotropic virus, there is strong evidence that HCV could replicate extra-hepatic in the gastrointestinal tissue which could serve as a reservoir for HCV. The outcome of HCV infection depends mainly on the host innate and adaptive immune responses. Innate immunity against HCV includes mainly nuclear factor cells and activation of IFN-related genes. There is an immunologic link between the gut and the liver through a population of T-cells that are capable of homing to both the liver and gut *via* the portal circulation. However, little is known on the role of Gut immune response in HCV. In this review we discussed the immune regulation of Gut immune cells and its association with HCV pathogenesis, various outcomes of anti-HCV therapy, viral persistence and degree of liver inflammation. Additionally, we investigated the relationship between Gut immune responses to HCV and IL28B

genotypes, which were identified as a strong predictor for HCV pathogenesis and treatment outcome after acute infection.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Hepatitis C virus; Colonic T_{reg}; Mucosal; Immune regulation; Liver inflammation; Interleukin-28B

Core tip: Chronic hepatitis C (CHC) is a global worldwide health problem with approximately 200 million people worldwide infected with hepatitis C virus (HCV). It is also a major cause of chronic liver infection that often leads to chronic hepatitis which may progress to cirrhosis, fibrosis and finally hepatocellular carcinoma. In CHC, immune responses play an important role in HCV pathogenesis and responses to therapy. Intra-hepatic immune responses to HCV are highly regulated. There is a clear relationship between hepatic immune responses and mucosal immune response in the gut. Additionally, genetic immunological markers have been proposed to predict response to HCV treatment, and outcome of infection.

Hetta HF, Mehta MJ, Shata MTM. Gut immune response in the presence of hepatitis C virus infection. *World J Immunol* 2014; 4(2): 52-62 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/52.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.52>

INTRODUCTION

Hepatitis C virus (HCV) was first identified by Harvey Alter in 1978 and named non-A, non-B hepatitis^[1], and cloned by Houghton in 1986^[2]. HCV is a single-stranded, positive sense RNA virus belonging to *Hepacivirus* group in the family *Flaviviridae*^[3]. There are 6 HCV genotypes. Due to the low fidelity and lack of proofreading of HCV

polymerase enzymes used for viral genome amplification, multiple mutations occur within a genotype to produce quasi-species^[4,5].

Chronic hepatitis C infection (CHC) is a global worldwide health care problem with an increasing burden year-by-year^[6-8]. The World Health Organization estimates that approximately 200 million people worldwide are infected with HCV^[9]. It is also a major cause of chronic liver infection that often leads to chronic hepatitis which may progress to cirrhosis, fibrosis and finally hepatocellular carcinoma^[3,10].

HCV is one of the most important etiologic agents of post transfusion hepatitis. HCV is usually spread by sharing infected needles with a carrier, from receiving infected blood, and from accidental exposure to infected blood. Some people acquire the infection through non parenteral means that have not been fully defined, but include sexual transmission in persons with high risk behaviors^[11]. It is not reported that HCV can spread orally by food, water, breast feeding, or by normal social contact as sneezing, coughing, hugging, sharing eating utensils or drinking glasses^[12]. Mother-to-baby transmission is rare and needs a high viremia as found in HIV co-infection^[13].

HCV VIROLOGY

HCV is a single stranded RNA virus which produces negative strand RNA as a replicative intermediate. The HCV genome is about 9.6 kb in length. During HCV replication cycle, one large precursor protein is synthesized from an open reading frame then cleaved to produce 10 proteins including three structural proteins which are Core, two envelope proteins (E1 and E2)^[3], and P7 which results from cleavage of E2 protein^[14]. The other six proteins that are not in the viral particle called non-structural proteins (NS) including NS2, NS3, NS4A, NS4B, NS5A, and NS5B^[3]. Non-structural (NS) proteins are not found in the virion, therefore, presence of NS proteins inside cells suggests that HCV replication occurred in those cells^[3]. Replication of HCV involves converting the viral genomic positive strand into a negative strand, and then back to the genomic strand. Thus, the presence of the negative strand strongly suggests that replication^[15].

HCV REPLICATION

HCV is primarily a hepatotropic virus^[15]. However, a broad spectrum of extra-hepatic manifestations may be associated with HCV infection, including mixed cryoglobulinemia, non-Hodgkin's lymphoma, arthralgia, paresthesia, myalgia, pruritis, cutaneous vasculitis, glomerulonephritis, neuropathy and lymphoproliferative disorders^[16,17].

HCV was believed to infect only hepatocytes^[3]. However, recent studies have reported HCV infection of other cell types^[15,18-21]. In fact, viral replication has been reported in B cells, T cells, monocytes, macrophages, and

macrophage-like cells such as Kupffer cells, dendritic cells (DCs), renal cells, thyroid cells, and gastric cells. There is mounting evidence that these cells could represent replicative compartments for the virus^[3,22,23]. In addition, it has been proposed that peripheral blood monocytes (PBMC) could be the source of recurrent HCV infection after liver transplantation^[24]. Despite these reports, extra-hepatic replication of HCV is still controversial by some investigators. However, the importance of extra-hepatic HCV replication in HCV pathogenesis is clear. Extra-hepatic compartments might serve as reservoirs for HCV, and hence HCV persistence, reactivation after antiviral therapy and also may contribute to the HCV extra-hepatic manifestations^[24].

HCV IN THE GUT

There is a molecular evidence that HCV may infect and replicate in oral mucosa and gastric cells^[23]. Moreover, HCV seems to be involved in development of B-cell non-Hodgkin's lymphoma of the gastric mucosa^[25]. Miglioresi *et al*^[26], reported that Gut mucosa may serve as possible reservoir for HCV relapse after viral clearance. They analyzed HCV gastric localization in 15 patients and compared their levels of viremia with the status of HCV in gastric biopsy specimens and PBMCs. In that study, all 15 patients with positive viremia were positive for HCV RNA on Gut tissue and PBMCs. In 2 patients, HCV RNA was positive on serum, negative at Gut biopsy but their PBMCs were positive. Two patients with negative viremia and PBMCs after antiviral treatment were positive for HCV RNA on gastric sample and eventually relapsed (after 6 and 18 wk). The finding of a positive hidden compartment for HCV and simultaneous negative viremia had previously reported in HCV infected liver without detectable viremia^[27]. Replication of HCV in gastrointestinal tissue represents a continuous new source as an extra-hepatic reservoir of viral particles for re-infection of hepatocytes^[26].

IMMUNE RESPONSE TO HCV

Systemic immune responses

The immune response against HCV involves innate and adaptive immunity^[9]. Innate immunity against HCV is mediated by several innate immune effector cells such as NK cells, and activation of the interferons-stimulated genes (ISGs) response^[28]. Recent studies have revealed that the *IL28B* gene locus, which codes for a type III interferon is a critical locus for outcome after acute infection^[29], and response to therapy^[29,30]. However, HCV may develop several strategies to overcome these responses. For example, viral NS3 and NS4a protease can cause disruption of important components of type I interferon activation cascade through inactivation of several ISGs^[31,32].

Adaptive immunity against HCV is mediated by both humoral and cellular immune responses. Most HCV-

infected individuals develop antibodies against HCV, regardless of the outcome of infection. Few of these antibodies can neutralize viral particles and may limit viral spread^[33]. However, neutralizing antibodies have a limited role in most of the infected patients due to the high replication and mutation rate of HCV^[34]. In fact, HCV clearance had been observed in some patients in the absence of neutralising antibodies^[35]. Therefore, despite the potential protective role of innate and humoral immunity in the outcome of infection, it is clear that protection and viral clearance depend primarily on cellular adaptive immune responses through a complex interplay between CD4⁺ and CD8⁺ T-cell responses^[9]. Unfortunately, in some patients, cellular immune responses are inadequate and fail to clear the infection with a subsequent viral persistence^[9]. Fully functional virus-specific CD4⁺T-cell responses are detectable in patients who cleared infection^[9,36-38]. The role of HCV-specific CD4⁺T-cell was further supported by the finding of *in vivo* depletion of CD4⁺T cells from HCV-recovered chimpanzees was associated with viral persistence^[38]. Moreover, several studies have shown that HCV-specific CD8⁺T-cells derived from the peripheral blood or liver are functionally impaired and display a reduced ability to proliferate or secrete anti-viral cytokines such as IFN- γ ^[39-41]. The mechanisms contributing to CD8⁺T cell exhaustion in HCV are not fully understood, however, it may be partially explained by the intrinsic regulatory pathways such as signals mediated by the inhibitory receptor PD-1^[40,42-45] and extrinsic regulatory pathways as regulatory T cells (T_{reg}) or secretion of immunoregulatory cytokines such as IL-10^[46-51]. Ultimately, the outcome of HCV infection, viral persistence or clearance, is determined by the host immune response^[9,52,53]. Additionally, sustained HCV-specific cytotoxic T cell responses in the liver have been associated with the development of hepatic immunopathology and liver necrosis which may lead to liver cirrhosis^[52,53]. The mechanisms that mediate liver inflammation and damage in CHC are not yet fully elucidated^[9,54]. One of the potential mechanisms that might modulate HCV-specific immune responses is T_{reg} cells which are a subtype of T cells that play a fundamental role in maintaining immune homeostasis and the balance between the tissue-damaging and protective effects of the immune response^[54-56]. It is characterized by the expression of a unique transcription factor Forkhead box protein P3 (FoxP3), which is highly expressed in the nucleus of T_{reg} cells and is generally accepted as the single best marker to quantify T_{reg} cells^[53,56-58]. In cases with CHC, it was reported that the frequency of T_{reg} cells were negatively correlated with the degree of necro-inflammatory scores and their frequency is higher than that in healthy individuals^[47,59,60]. Thus, T_{reg} cells appear to assist in the maintenance of chronicity by inhibition of anti-HCV immune responses and consequently attenuate the intrahepatic tissue-damaging response to infection^[49,53].

MUCOSAL (GUT) IMMUNE RESPONSE IN HCV

The mucosal immune system is considered the first line of defense that reduces the need for elimination of exogenous invading antigens by pro-inflammatory immune response^[61]. The mucosal immune system maintains homeostasis through evolution of two layers of adaptive non-inflammatory defense; the first strategy is immune exclusion by secretory IgA (and IgM) antibodies to limit epithelial contact and penetration of invading microorganisms and other potentially dangerous antigens^[61], and the second strategy is oral tolerance by development of immunosuppressive mechanisms to inhibit over-reaction against food antigens and commensal bacteria^[62]. Oral tolerance depends mainly on the induction of T_{reg} cells in mesenteric lymph nodes to which mucosal DCs carry and present food and commensal microbial antigens^[63]. Gut induced tolerance include other suppressive mechanisms to ensure that persistent food allergy is relatively rare^[64].

Some pathogens and food antigens could enter the liver via the portal circulation^[65] within 2 h of ingestion^[66] and presented on liver endothelial cells. The liver is critical in the regulation of immune responses to pathogens entering *via* portal circulation^[67]. It receives 75% of its blood supply from the portal vein, which drains the gut. Oral tolerance is usually lost in case of a portal-systemic shunt, which allows portal blood to bypass the liver and goes directly from the gut to the systemic circulation^[67,68].

To understand the interactions between the immune responses in the Gut and the liver during HCV infection, we have to dissect the immune responses in each organ. The intestinal immune system can be divided into inductive and effector sites based upon their anatomical and functional properties^[61,63]. Inductive sites include the gut-associated lymphoid tissues (GALT) such as Peyer's patches (PP) and isolated lymphoid follicles and the mesenteric lymph nodes (mLNs). The GALT contains a wide variety of cells, such as Microfold (M) cells, DCs, intraepithelial lymphocytes (IEL), macrophages and T_{reg} cells^[61]. The main effector sites of the intestinal immune system are the lamina propria (LP) and epithelium, which harbor large populations of activated T cells and antibody-secreting plasma cells. The LP may also contribute to the induction of tolerance. It is a site of antigen uptake and loading of the migratory DCs that encounter naïve T cells in the mLNs^[61]. Antigen are up-taken by absorptive epithelial and M cells in the mucosal inductive sites or directly captured by professional APCs (including DCs, Macrophage and B lymphocytes)^[69]. M cells take up molecules and particles from the gut lumen by endocytosis or phagocytosis then sample them to the immune cells. Antigens are transported through M cells by the process of transcytosis. The cell membrane at the base of M cells is folded around lymphocytes and dendritic cells within the Peyer's patches^[69]. M cells present the antigen to conventional CD4⁺ and CD8⁺ $\alpha\beta$ T cells at the inductive site.

At the same time, epithelial cells may process and present certain antigens directly to neighboring intraepithelial T cells such as NKT cells and $\gamma\delta$ T cells which are T cells with limited repertoire diversity^[69]. Naive B and T cells enter GALT and are primed to become memory/effector B and T cells, then migrate from GALT to mesenteric blood and the liver or to the lymph nodes *via* lymph and then *via* thoracic duct to peripheral blood for subsequent extravasation at mucosal effector sites. A system of Gut-specific lymphocyte trafficking has been evolved to target lymphocyte to the area of injury or infection through vascular adhesion molecules and chemokines. Thus, the endothelial cells act as a local gatekeeper for mucosal immunity^[61]. Under normal physiological conditions enteric antigens are presented to naïve lymphocytes in the draining mesenteric lymph nodes. Lymphocytes activated by gut dendritic cells express a gut-homing phenotype characterized by expression of the chemokine receptor CCR9 and the integrin $\alpha 4\beta 7$ which direct the migration of the activated lymphocytes back to gut tissue where their respective ligands CCL25 and MAdCAM-1 are expressed^[67,70]. Lymphocytes that are primed to hepatic antigens acquire expression of adhesion molecules that direct them to traffic to the liver by interacting with molecules expressed on hepatic endothelium such as VAP-1.

EFFECTOR MECHANISMS OF THE GUT IMMUNE RESPONSES

Innate immune system in the gut includes the lining epithelium which provides barrier function, mechanical cleaning and defensins which act as chemical antimicrobial factors^[71]. The gut mucosa contains a number of other cells as part of the innate immune system, including phagocytic neutrophils and macrophages, DCs, NK cells and mast cells. These cells contribute significantly to host defense against pathogens^[22] and also initiate adaptive mucosal immune responses^[69,72].

The adaptive humoral immune defense at the gut mucosal surfaces is mainly mediated by secretory IgA (sIgA) antibody, which is the ideal antibody for functioning in mucosal secretions due to its resistance to proteases^[61]. sIgA plays a protective role against a variety of foreign antigens such as food antigens, toxins, bacteria and viruses^[72]. sIgA blocks the access of potentially allergenic molecules derived from food or drugs^[73]. Because some dietary antigen is clearly absorbed by normal subjects, the importance of sIgA antibody may lie in reducing the amount of antigen that gains access to the lamina propria^[73,74]. sIgA can neutralize biologically active antigens as bacteria, toxins, enzymes and viruses. The effectiveness of sIgA as a neutralizing antibody against viruses is shown for example in the responses to oral live-attenuated poliovirus vaccine where protection correlates with levels of secretory antibody^[75]. Additionally, sIgA is an efficient agglutinin that can prevent adherence of pathogenic bacteria to the epithelial surfaces and enhance the antibacterial efficiency of other effector immune system; sIgA has bactericidal

potential by cooperation with complement and lysozyme and also can act as opsonin. However, the role of sIgA during HCV infection is limited.

The development of IgA immune response against mucosal pathogens and soluble protein antigens is dependent on T helper cells^[76]. Mucosal T cells produce large amounts of transforming growth factor (TGF)- β , interleukin (IL)-10 and IL-4 to promote B-cell isotype class switching to IgA^[77,78]. Additionally, muco-epithelial cells, and T_{reg} cells are the major sources of TGF- β and IL-10, suggesting that cooperation between neighboring lymphocytes and epithelial cells in the mucosal microenvironment is pivotal to promote B-cell switch to IgA and differentiation into IgA-committed B cells^[69].

One of the important cellular immune defense at the gut mucosal surfaces is mainly mediated by cytotoxic T lymphocyte (CTL) responses^[69]. It is reported that mucosal CTLs are crucial for the immune clearance of pathogens in several animal models of infection with enteric viruses like Rota virus^[79] and intracellular parasites^[80]. Besides CTLs, induced IFN- γ producing CD4⁺ T cells, have been found to be important for mucosal immune defense to both viral and bacterial infections^[69].

REGULATORY MECHANISMS OF GUT IMMUNE RESPONSE AND ORAL TOLERANCE

Gut immune response is controlled by the local microenvironment, the nature of the antigen and the type of APCs. In case of foreign food proteins and non-pathogen antigens, the default pathway for mucosal DCs and other APCs is to generate Th2 and various regulatory T cell types of responses mainly T_{reg}^[81], and Th17 cells^[82] which usually leads to down-regulatory or active suppression of systemic immunity (oral tolerance). On the other hand, antigens, most pathogens harboring motifs which could bind to Toll-like receptor (TLR), and be sensed by mucosal APCs as 'danger signals' and pro-inflammatory conditions in general favor the development of stronger and broader immune responses but do not lead to oral tolerance^[81,83,84]. Oral tolerance can be achieved through different mechanisms, including anergy, activation-induced cell death and most important, the induction of regulatory T cells^[69,85]. Anergy of antigen-specific T cells has been reported after ingestion of large quantities of soluble proteins^[86], and deletion of specific T cells only after mucosal administration of massive, non-physiological antigen doses^[87]. Induction of regulatory T cells after mucosal delivery of antigens has been reported and received major attention given the potential of manipulating these regulatory cells as therapeutic agents in immune-mediated diseases^[69].

Regulatory T cells includes: (1) CD4⁺CD45RB^{low} Tr1 cells that function through the production of IL-10 to suppresses antigen-specific T cell responses and actively down-regulates a pathological immune response^[88]; (2)

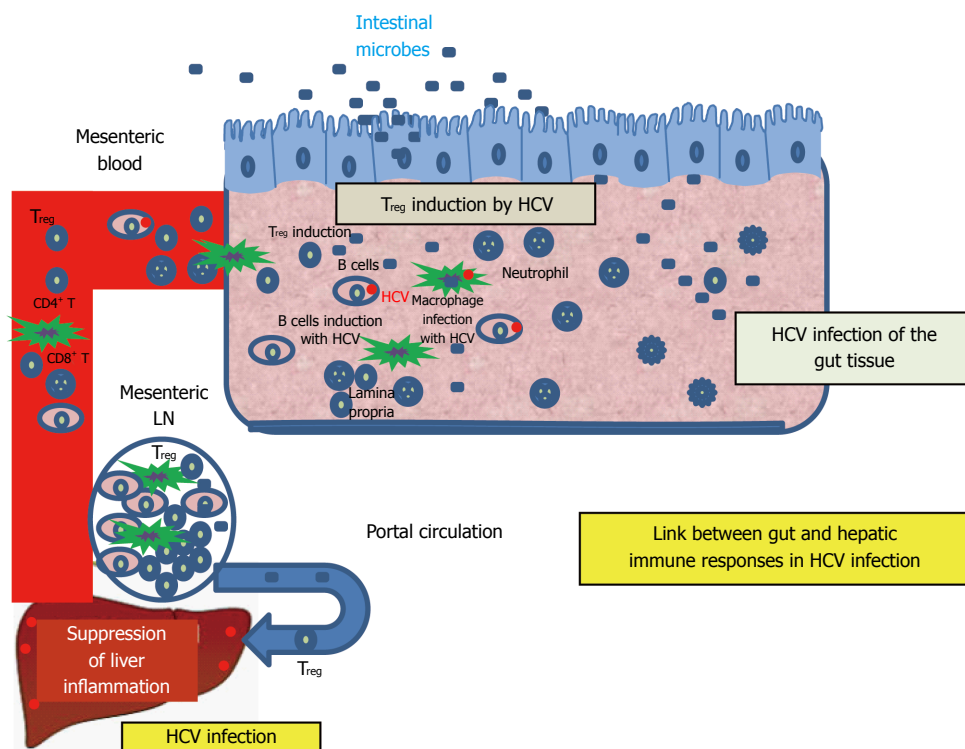


Figure 1 Link between Gut and hepatic immune responses in hepatitis C virus infection. Hepatitis C virus (HCV) replicates in the Gut B cells and macrophages and stimulates Treg cells. Colonic Treg cells migrate to the liver and inhibit immune responses to HCV infection, and inhibit liver inflammation and fibrosis.

T_H3 cell which are $CD4^+$ or $CD8^+$ T cells producing TGF- β with various amounts of interleukin-4 and interleukin-10^[89]; and (3) T_{reg} cells, a population of naturally occurring $CD4^+CD25^+$ regulatory T cells that suppress proliferation through a cell contact-dependent mechanism^[90] followed by cell-contact-independent mechanism mediated by soluble factors such as IL-10 and TGF- β ^[91]. Induction of tolerance is a contact-dependent mechanism used by naturally occurring $CD4^+CD25^+$ T_{reg} to confer suppressive activity upon conventional antigen-specific $CD4^+$ T cells through the expression of the transcription factor Foxp3 and/or the major histocompatibility complex (MHC) class II-binding molecule LAG-3 in such cells^[69,91], and inhibit T cell activation *via* soluble mediators. $CD4^+CD25^+$ T_{reg} cells expressing the mucosal $\alpha 4\beta 7$ integrin, when co-cultured with conventional $CD4^+$ T cells, induced Tr1-like IL-10-secreting T cells with strong suppressor activity on effector T cells. While $\alpha 4\beta 1$ -positive T_{reg} induced T_H3 -like TGF- β secreting suppressor T cells^[91]. Moreover, intraepithelial $CD8^+\gamma\delta$ T cells in the small intestine have been involved in mucosal tolerance and are the first T cells to encounter pathogens that have invaded an epithelial surface^[92].

ROLE OF LIVER IN ORAL TOLERANCE

Although the liver is capable of generating vigorous immune responses to infections such as hepatitis A and hepatitis E viruses, both of which enter *via* the gut, it is also characterized by immune tolerance in several settings^[93,94]. A vigorous intrahepatic immune response

depends on activation of T cells by fully activated DCs within secondary lymphoid tissues whereas direct activation within the liver by resident APCs including endothelial cells and hepatocytes usually results in tolerance^[95]. This is logic, as it allows the liver to tolerate soluble food antigens captured by liver endothelial cells and self-antigens on hepatocytes that fail to cause damage whilst responding appropriately to infections that cause injury, inflammation and full activation of DCs^[67].

Regulatory T cells as well as NK and CD1-restricted NKT cells seem to contribute to the overall bias of hepatic immune responses toward tolerance. The tolerance microenvironment of the liver may account for the survival of liver allografts and the persistence of certain liver pathogens such as hepatitis viruses^[94].

LINK BETWEEN THE GUT AND LIVER IMMUNE RESPONSES DURING HCV INFECTION

The Gut and the liver share common embryological origins; the liver develops from the ventral floor of the foregut as the liver diverticulum from the undifferentiated gut endoderm^[96]. Subsequently, the gut is populated by lymphocyte precursors derived from the developing liver^[97] (Figure 1).

There is an immunologic link between the gut and the liver through a population of T-cells that are capable of homing to both the liver and gut *via* portal circulation^[96]. Additionally, the liver is considered an important

toleragenic organ for all of foreign proteins we are eating that are probably mediated through the T_{reg} cells, which in turn act as a link between the gut and the liver^[67,96]. Most of the infiltrating T-cells in the liver are primed cells suggesting that trafficking of memory T-cells through the liver might contribute to immune surveillance^[98]. Evidence, that supports such findings, comes from observations that the gut adhesion molecules and chemokine (such as CCL25) are also detected on liver endothelium^[99] providing a mechanism for the recruitment of mucosal lymphocytes to the liver^[100].

Evaluation of the gut immune cells for the intrinsic gut-liver immune axis of the shared lymphocytes that recirculate between the gut and liver through the portal circulation may be considered a useful image of the intrahepatic micro-environment during HCV infection. Based on this relationship, the frequency of T_{reg} cells in colonic tissue and its association with the various outcomes of anti-HCV therapy, viral persistence and degree of liver inflammation were examined in our laboratory. Our data indicated that the frequency of colonic T_{reg} in CHC patients is higher than control and our findings are in concordance with previous reports that demonstrated a higher number of FoxP3⁺ T_{reg} cells in the liver of HCV-infected patients compared to healthy control^[47,59,60]. These findings support that T_{reg} plays a prominent role in maintaining the balance between tissue damaging and protective effects of immune responses to HCV.

While attempting to limit viral replication, T-cells inadvertently play a pivotal role in limiting hepatic necro-inflammation and subsequent fibrosis^[28,101-103] by suppressing HCV-specific immune responses^[48]. In our study, we found a significant inverse correlation between the frequency of colonic T_{reg} and liver pathology indicating a role of colonic T_{reg} in controlling the chronic inflammatory response and limit liver damage in CHC infection.

There is still an open question whether T_{reg} cells are protective or harmful in CHC. The effective host anti-HCV immune response may be associated with strong inflammatory reactions and liver damage. To minimize the damage to self, the activation of the immune system also triggers anti-inflammatory pathways through T_{reg} responses. Both inflammatory and anti-inflammatory reactions are normal components of the immune response, which together, fight infections while preventing immunopathology.

TREATMENT OF HCV AND RELATIONSHIP TO IMMUNE RESPONSES

Until 2011, the standard of care for chronic hepatitis C patients was combined treatment with Peginterferon (Peg-IFN) and ribavirin (RBV). The combination of Peg-IFN and RBV induced sustained virologic response (SVR) in 40%-50% of genotype 1 and 80% or more in genotype 2 and 3 infections^[104-106]. The lack of effective regimens across all genotypes and alternative therapeutics for patients who suffered serious side effects prompted basic

science research and numerous clinical trials leading to the development of direct-acting antiviral (DAA) agents. The US Food and Drug Administration approved Telaprevir (TVR) and Boceprevir (BOC) for HCV genotype 1. They inhibit HCV nonstructural protein 3/4A (NS3/4A) serine protease, which is critical for HCV replication. TVR and BOC are approved for use in combination therapies with Peg-IFN-alpha and RBV as they improved SVR rates to 75% and 66% respectively for adult HCV genotype 1 patients with compensated liver cirrhosis^[107]. However, these DAAs incur their own set of severe side effects including anemia, rash, and hyperbilirubinemia. New drugs classified as second-wave protease inhibitors, second-generation protease inhibitors, and polymerase inhibitors are being developed and currently undergoing clinical trials^[108]. The NS5B polymerase inhibitor, sofosbuvir has been recently approved by the FDA for treatment of hepatitis C genotype 1, 2, and 3 patients^[109].

Identifying patients that are likely to achieve SVR versus those that are likely to be non-responders is crucial for disease prognosis, providing optimal therapy, avoiding side effects, and reducing costs associated with Hepatitis C therapy. Since sequencing of the human genome in 2001, advancements along with decrease costs in genotyping technologies have led to investigation of genomic markers associated with a response to Peg-IFN and RBV in patients with chronic hepatitis C. The rs12979860 SNP located on chromosome 19 upstream of the *IL-28B* gene has been identified as a significant predictor of SVR in HCV Genotype 1 chronically infected patients that underwent standard therapy^[110]. The same rs12979860 SNP has the ability to predict natural clearance of the hepatitis C virus^[30]. Genotype C/C at the rs12979860 SNP was associated with a higher likelihood of natural clearance and therapy induced clearance of hepatitis C genotype 1, while T/T genotype was the most unfavorable^[111]. Studies have confirmed that rs12979860 is the strongest predictor of SVR and can effectively predict response to IFN/RBV based therapy^[112]. The mechanisms by which the rs12979860 affects HCV pathogenesis are still unclear. However, it is well-known that the *IL-28B* gene codes for cytokine IL-28B also known as interferon (IFN) λ -3, which belongs to the type III IFN family. IFN- λ is mainly produced by macrophages and DCs in response to viral proteins and plays an important role in antiviral responses to hepatitis C^[30,113]. IFN λ receptors are predominantly expressed on hepatocytes, which may explain its ability to counteract hepatotropic viruses^[114]. Therefore, stimulation of IFN λ receptors on hepatocytes by IFN- λ secreted by DCs induces ISGs^[115] which have the ability to suppress viral replication and protein synthesis of HCV^[116]. Additionally, IFN- λ promotes differentiation of monocyte-derived dendritic cells (DCs) with high PD-L1 expression and further promoted expansion of T_{reg} cells^[117] locally and suppressed the inflammatory responses in the liver. Recent data by our laboratory (Hetta *et al*, 2014 submitted) as well as others^[118] identified a correlation between IL28B SNP rs12979860 genotype TT

s and T_{reg} frequencies. The mechanism responsible for elevated T_{reg} in patients with TT genotype may be related to the precise location of rs12979860 in the promoter region of the *IL-28B* gene. The promoter region plays an important role in gene expression, and the TT genotype might favor increased IL-28B expression in turn resulting in higher T_{reg} frequencies. In support of the relationship between IL-28B phenotypes, T_{reg} frequency, and HCV pathogenesis, recent reports found elevated T_{reg} in acute HCV as a predictor for viral persistence and CHC as well as increased levels of IFN- λ , IL-28, and IL-29 in serum in chronic HCV patients^[117].

The association between IL-28B polymorphism and SVR in genotype 2 and 3 infected patients has produced mixed results making its clinical utility less clear. For instance, one study found IL-28B polymorphism to be associated with SVR in patients infected by genotype 2/3 HCV in whom RVR was not achieved^[119]. On the other hand, in a study of hepatitis C Genotype 3 infected patients, rs12979860 SNP genotype C/C did not correlate with SVR to PEG-IFN/ribavirin therapy^[120]. The majority of studies to this point have focused on IL-28B SNPs in HCV Genotype 1, 2, and 3. The clinical utility of IL-28B testing is probably best served in HCV genotype 1 infected-patients for prediction of outcomes and to limit expenses and side effects associated with IFN-based therapy^[110].

REFERENCES

- 1 Alter HJ, Purcell RH, Holland PV, Popper H. Transmissible agent in non-A, non-B hepatitis. *Lancet* 1978; **1**: 459-463 [PMID: 76017 DOI: 10.1016/S0140-6736(78)90131-9]
- 2 Wang KS, Choo QL, Weiner AJ, Ou JH, Najarian RC, Thayer RM, Mullenbach GT, Denniston KJ, Gerin JL, Houghton M. Structure, sequence and expression of the hepatitis delta (delta) viral genome. *Nature* 1986; **323**: 508-514 [PMID: 3762705]
- 3 Revie D, Salahuddin SZ. Human cell types important for hepatitis C virus replication in vivo and in vitro: old assertions and current evidence. *Viral J* 2011; **8**: 346 [PMID: 21745397 DOI: 10.1186/1743-422x-8-346]
- 4 Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, Halfon P, Inchauspé G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology* 2005; **42**: 962-973 [PMID: 16149085 DOI: 10.1002/hep.20819]
- 5 Farci P, Purcell RH. Clinical significance of hepatitis C virus genotypes and quasispecies. *Semin Liver Dis* 2000; **20**: 103-126 [PMID: 10895435]
- 6 Lauer GM, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52 [PMID: 11439948 DOI: 10.1056/nejm200107053450107]
- 7 Gouda I, Nada O, Ezzat S, Eldaly M, Loffredo C, Taylor C, Abdel-Hamid M. Immunohistochemical detection of hepatitis C virus (genotype 4) in B-cell NHL in an Egyptian population: correlation with serum HCV-RNA. *Appl Immunohistochem Mol Morphol* 2010; **18**: 29-34 [PMID: 19644357 DOI: 10.1097/PAI.0b013e3181ae9e82]
- 8 Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet* 2000; **355**: 887-891 [PMID: 10752705 DOI: 10.1016/S0140-6736(99)06527-7]
- 9 Klennerman P, Thimme R. T cell responses in hepatitis C: the good, the bad and the unconventional. *Gut* 2012; **61**: 1226-1234 [PMID: 21873736 DOI: 10.1136/gutjnl-2011-30062]
- 10 Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989; **244**: 362-364 [PMID: 2496467 DOI: 10.1126/science.2496467]
- 11 Alipour A, Rezaianzadeh A, Hasanazadeh J, Rajaefard A, Davarpanah MA. Sexual Transmission of Hepatitis C Virus Between HIV Infected Subjects and Their Main Heterosexual Partners. *Hepat Mon* 2013; **13**: e13593 [PMID: 24348647 DOI: 10.5812/hepatmon.13593]
- 12 Mast EE, Alter MJ, Margolis HS. Strategies to prevent and control hepatitis B and C virus infections: a global perspective. *Vaccine* 1999; **17**: 1730-1733 [PMID: 10194830 DOI: 10.1016/S0264-410X(98)00415-0]
- 13 EASL International Consensus Conference on hepatitis C. Paris, 26-27 February 1999. Consensus statement. *J Hepatol* 1999; **31** Suppl 1: 3-8 [PMID: 10622553]
- 14 Griffin SD, Beales LP, Clarke DS, Worsfold O, Evans SD, Jaeger J, Harris MP, Rowlands DJ. The p7 protein of hepatitis C virus forms an ion channel that is blocked by the antiviral drug, Amantadine. *FEBS Lett* 2003; **535**: 34-38 [PMID: 12560074 DOI: 10.1016/S0014-5793(02)03851-6]
- 15 Castillo I, Rodríguez-Iñigo E, Bartolomé J, de Lucas S, Ortíz-Movilla N, López-Alcorocho JM, Pardo M, Carreño V. Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. *Gut* 2005; **54**: 682-685 [PMID: 15831916 DOI: 10.1136/gut.2004.057281]
- 16 Blackard JT, Kemmer N, Sherman KE. Extrahepatic replication of HCV: insights into clinical manifestations and biological consequences. *Hepatology* 2006; **44**: 15-22 [PMID: 16799966 DOI: 10.1002/hep.21283]
- 17 Agnello V, De Rosa FG. Extrahepatic disease manifestations of HCV infection: some current issues. *J Hepatol* 2004; **40**: 341-352 [PMID: 14739110 DOI: 10.1016/j.jhep.2003.10.009]
- 18 Manzin A, Candela M, Paolucci S, Caniglia ML, Gabrielli A, Clementi M. Presence of hepatitis C virus (HCV) genomic RNA and viral replicative intermediates in bone marrow and peripheral blood mononuclear cells from HCV-infected patients. *Clin Diagn Lab Immunol* 1994; **1**: 160-163 [PMID: 7496938]
- 19 Wang JT, Sheu JC, Lin JT, Wang TH, Chen DS. Detection of replicative form of hepatitis C virus RNA in peripheral blood mononuclear cells. *J Infect Dis* 1992; **166**: 1167-1169 [PMID: 1328405 DOI: 10.1093/infdis/166.5.1167]
- 20 Chang TT, Young KC, Yang YJ, Lei HY, Wu HL. Hepatitis C virus RNA in peripheral blood mononuclear cells: comparing acute and chronic hepatitis C virus infection. *Hepatology* 1996; **23**: 977-981 [PMID: 8621178 DOI: 10.1002/hep.510230506]
- 21 Saleh MG, Tibbs CJ, Koskinas J, Pereira LM, Bomford AB, Portmann BC, McFarlane IG, Williams R. Hepatic and extrahepatic hepatitis C virus replication in relation to response to interferon therapy. *Hepatology* 1994; **20**: 1399-1404 [PMID: 7982638 DOI: 10.1002/hep.1840200604]
- 22 Yan FM, Chen AS, Hao F, Zhao XP, Gu CH, Zhao LB, Yang DL, Hao LJ. Hepatitis C virus may infect extrahepatic tissues in patients with hepatitis C. *World J Gastroenterol* 2000; **6**: 805-811 [PMID: 11819700]
- 23 Carrozzo M, Quadri R, Latorre P, Pentenero M, Paganin S, Bertolusso G, Gandolfo S, Negro F. Molecular evidence that the hepatitis C virus replicates in the oral mucosa. *J Hepatol* 2002; **37**: 364-369 [PMID: 12175632 DOI: 10.1016/S0168-8278(02)00183-6]

- 24 **Féray C**, Samuel D, Thiers V, Gigou M, Pichon F, Bismuth A, Reynes M, Maisonneuve P, Bismuth H, Bréchet C. Reinfection of liver graft by hepatitis C virus after liver transplantation. *J Clin Invest* 1992; **89**: 1361-1365 [PMID: 1313453 DOI: 10.1172/jci115723]
- 25 **Tursi A**, Brandimante G, Chiarelli F, Spagnoli A, Torello M. Detection of HCV RNA in gastric mucosa-associated lymphoid tissue by in situ hybridization: evidence of a new extrahepatic localization of HCV with increased risk of gastric malt lymphoma. *Am J Gastroenterol* 2002; **97**: 1802-1806 [PMID: 12135039 DOI: 10.1111/j.1572-0241.2002.05848.x]
- 26 **Miglioresi L**, Riva E, Antonelli G, Russo F, Ricci GL. Localization of hepatitis C virus in gastrointestinal mucosa: a possible reservoir for relapse. *Hepatology* 2003; **38**: 775 [PMID: 12939605 DOI: 10.1053/jhep.2003.50322]
- 27 **McHutchison JG**, Poynard T, Esteban-Mur R, Davis GL, Goodman ZD, Harvey J, Ling MH, Garaud JJ, Albrecht JK, Patel K, Dienstag JL, Morgan T. Hepatic HCV RNA before and after treatment with interferon alone or combined with ribavirin. *Hepatology* 2002; **35**: 688-693 [PMID: 11870385 DOI: 10.1053/jhep.2002.31870]
- 28 **Rehermann B**. Interaction between the hepatitis C virus and the immune system. *Semin Liver Dis* 2000; **20**: 127-141 [PMID: 10946419 DOI: 10.1055/s-2000-9946]
- 29 **Kelly C**, Klenerman P, Barnes E. Interferon lambdas: the next cytokine storm. *Gut* 2011; **60**: 1284-1293 [PMID: 21303914 DOI: 10.1136/gut.2010.222976]
- 30 **Thomas DL**, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798-801 [PMID: 19759533 DOI: 10.1038/nature08463]
- 31 **Rehermann B**. Hepatitis C virus versus innate and adaptive immune responses: a tale of coevolution and coexistence. *J Clin Invest* 2009; **119**: 1745-1754 [PMID: 19587449 DOI: 10.1172/jci39133]
- 32 **Walker CM**. Adaptive immunity to the hepatitis C virus. *Adv Virus Res* 2010; **78**: 43-86 [PMID: 21040831 DOI: 10.1016/b978-0-12-385032-4.00002-1]
- 33 **Pestka JM**, Zeisel MB, Bläser E, Schürmann P, Bartosch B, Cosset FL, Patel AH, Meisel H, Baumert J, Viazov S, Rispeter K, Blum HE, Roggendorf M, Baumert TF. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc Natl Acad Sci USA* 2007; **104**: 6025-6030 [PMID: 17392433 DOI: 10.1073/pnas.0607026104]
- 34 **Spengler U**, Nattermann J. Immunopathogenesis in hepatitis C virus cirrhosis. *Clin Sci (Lond)* 2007; **112**: 141-155 [PMID: 17199558 DOI: 10.1042/cs20060171]
- 35 **Post JJ**, Pan Y, Freeman AJ, Harvey CE, White PA, Palladinetti P, Haber PS, Marinos G, Levy MH, Kaldor JM, Dolan KA, Ffrench RA, Lloyd AR, Rawlinson WD. Clearance of hepatitis C viremia associated with cellular immunity in the absence of seroconversion in the hepatitis C incidence and transmission in prisons study cohort. *J Infect Dis* 2004; **189**: 1846-1855 [PMID: 15122521 DOI: 10.1086/383279]
- 36 **Lucas M**, Ulsenheimer A, Pfafferot K, Heeg MH, Gaudieri S, Grüner N, Rauch A, Gerlach JT, Jung MC, Zachoval R, Pape GR, Schraut W, Santantonio T, Nitschko H, Obermeier M, Phillips R, Scriba TJ, Semmo N, Day C, Weber JN, Fidler S, Thimme R, Haberstroh A, Baumert TF, Klenerman P, Diepolder HM. Tracking virus-specific CD4+ T cells during and after acute hepatitis C virus infection. *PLoS One* 2007; **2**: e649 [PMID: 17653276 DOI: 10.1371/journal.pone.0000649]
- 37 **Missale G**, Bertoni R, Lamona V, Valli A, Massari M, Mori C, Rumi MG, Houghton M, Fiaccadori F, Ferrari C. Different clinical behaviors of acute hepatitis C virus infection are associated with different vigor of the anti-viral cell-mediated immune response. *J Clin Invest* 1996; **98**: 706-714 [PMID: 8698862 DOI: 10.1172/jci118842]
- 38 **Grakoui A**, Shoukry NH, Woollard DJ, Han JH, Hanson HL, Ghayeb J, Murthy KK, Rice CM, Walker CM. HCV persistence and immune evasion in the absence of memory T cell help. *Science* 2003; **302**: 659-662 [PMID: 14576438 DOI: 10.1126/science.1088774]
- 39 **Gruener NH**, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, Walker B, Sullivan J, Phillips R, Pape GR, Klenerman P. Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus. *J Virol* 2001; **75**: 5550-5558 [PMID: 11356962 DOI: 10.1128/jvi.75.12.5550-5558.2001]
- 40 **Nakamoto N**, Kaplan DE, Coleclough J, Li Y, Valiga ME, Kaminski M, Shaked A, Olthoff K, Gostick E, Price DA, Freeman GJ, Wherry EJ, Chang KM. Functional restoration of HCV-specific CD8 T cells by PD-1 blockade is defined by PD-1 expression and compartmentalization. *Gastroenterology* 2008; **134**: 1927-1937, 1937.e1-2 [PMID: 18549878 DOI: 10.1053/j.gastro.2008.02.033]
- 41 **Spangenberg HC**, Viazov S, Kersting N, Neumann-Haefelin C, McKinney D, Roggendorf M, von Weizsäcker F, Blum HE, Thimme R. Intrahepatic CD8+ T-cell failure during chronic hepatitis C virus infection. *Hepatology* 2005; **42**: 828-837 [PMID: 16175596 DOI: 10.1002/hep.20856]
- 42 **McMahon RH**, Golden-Mason L, Nishimura MI, McMahon BJ, Kemper M, Allen TM, Gretch DR, Rosen HR. Tim-3 expression on PD-1+ HCV-specific human CTLs is associated with viral persistence, and its blockade restores hepatocyte-directed in vitro cytotoxicity. *J Clin Invest* 2010; **120**: 4546-4557 [PMID: 21084749 DOI: 10.1172/jci43127]
- 43 **Radziejewicz H**, Ibegbu CC, Fernandez ML, Workowski KA, Obideen K, Wehbi M, Hanson HL, Steinberg JP, Masopust D, Wherry EJ, Altman JD, Rouse BT, Freeman GJ, Ahmed R, Grakoui A. Liver-infiltrating lymphocytes in chronic human hepatitis C virus infection display an exhausted phenotype with high levels of PD-1 and low levels of CD127 expression. *J Virol* 2007; **81**: 2545-2553 [PMID: 17182670 DOI: 10.1128/jvi.02021-06]
- 44 **Bengsch B**, Seigel B, Ruhl M, Timm J, Kuntz M, Blum HE, Pircher H, Thimme R. Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8+ T cells is linked to antigen recognition and T cell differentiation. *PLoS Pathog* 2010; **6**: e1000947 [PMID: 20548953 DOI: 10.1371/journal.ppat.1000947]
- 45 **Rutebemberwa A**, Ray SC, Astemborski J, Levine J, Liu L, Dowd KA, Clute S, Wang C, Korman A, Sette A, Sidney J, Pardoll DM, Cox AL. High-programmed death-1 levels on hepatitis C virus-specific T cells during acute infection are associated with viral persistence and require preservation of cognate antigen during chronic infection. *J Immunol* 2008; **181**: 8215-8225 [PMID: 19050238 DOI: 10.4049/jimmunol.181.12.8215]
- 46 **Thimme R**, Opitz OG. Interleukin-10 and viral clearance: translation to viral hepatitis. *Gastroenterology* 2007; **132**: 2611-2613 [PMID: 17570238 DOI: 10.1053/j.gastro.2007.04.049]
- 47 **Boettler T**, Spangenberg HC, Neumann-Haefelin C, Panther E, Urbani S, Ferrari C, Blum HE, von Weizsäcker F, Thimme R. T cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. *J Virol* 2005; **79**: 7860-7867 [PMID: 15919940 DOI: 10.1128/jvi.79.12.7860-7867.2005]
- 48 **Cabrera R**, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, Nelson DR. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. *Hepatology* 2004; **40**: 1062-1071 [PMID: 15486925 DOI: 10.1002/hep.20454]
- 49 **Amoroso A**, D'Amico F, Consolo M, Skarmoutsou E, Neri S, Dianzani U, Spandidos DA, Mazzarino MC. Evaluation of circulating CD4+CD25+ and liver-infiltrating Foxp3+ cells in HCV-associated liver disease. *Int J Mol Med* 2012; **29**: 983-988

- [PMID: 22446965 DOI: 10.3892/ijmm.2012.947]
- 50 Sugimoto K, Ikeda F, Stadanlick J, Nunes FA, Alter HJ, Chang KM. Suppression of HCV-specific T cells without differential hierarchy demonstrated ex vivo in persistent HCV infection. *Hepatology* 2003; **38**: 1437-1448 [PMID: 14647055 DOI: 10.1016/j.hep.2003.09.026]
 - 51 Ward SM, Fox BC, Brown PJ, Worthington J, Fox SB, Chapman RW, Fleming KA, Banham AH, Klenerman P. Quantification and localisation of FOXP3+ T lymphocytes and relation to hepatic inflammation during chronic HCV infection. *J Hepatol* 2007; **47**: 316-324 [PMID: 17475362 DOI: 10.1016/j.jhep.2007.03.023]
 - 52 Bowen DG, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. *Nature* 2005; **436**: 946-952 [PMID: 16107834 DOI: 10.1038/nature04079]
 - 53 Keynan Y, Card CM, McLaren PJ, Dawood MR, Kasper K, Fowke KR. The role of regulatory T cells in chronic and acute viral infections. *Clin Infect Dis* 2008; **46**: 1046-1052 [PMID: 18444822 DOI: 10.1086/529379]
 - 54 Hartling HJ, Gaardbo JC, Ronit A, Knudsen LS, Ullum H, Vainer B, Clausen MR, Skogstrand K, Gerstoft J, Nielsen SD. CD4⁺ and CD8⁺ regulatory T cells (Tregs) are elevated and display an active phenotype in patients with chronic HCV mono-infection and HIV/HCV co-infection. *Scand J Immunol* 2012; **76**: 294-305 [PMID: 22671952 DOI: 10.1111/j.1365-3083.2012.02725.x]
 - 55 Bolacchi F, Sinistro A, Ciaprini C, Demin F, Capozzi M, Carducci FC, Drapeau CM, Rocchi G, Bergamini A. Increased hepatitis C virus (HCV)-specific CD4⁺CD25⁺ regulatory T lymphocytes and reduced HCV-specific CD4⁺ T cell response in HCV-infected patients with normal versus abnormal alanine aminotransferase levels. *Clin Exp Immunol* 2006; **144**: 188-196 [PMID: 16634790]
 - 56 Sturm N, Thélou MA, Camous X, Dimitrov G, Ramzan M, Dufeu-Duchesne T, Bonorino P, Guillermet C, Brambilla E, Arvers P, Pernollet M, Leroy V, Zarski JP, Marche PN, Jouvin-Marche E. Characterization and role of intra-hepatic regulatory T cells in chronic hepatitis C pathogenesis. *J Hepatol* 2010; **53**: 25-35 [PMID: 20452085 DOI: 10.1016/j.jhep.2010.02.024]
 - 57 Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008; **133**: 775-787 [PMID: 18510923 DOI: 10.1016/j.cell.2008.05.009]
 - 58 Magg T, Mannert J, Ellwart JW, Schmid I, Albert MH. Subcellular localization of FOXP3 in human regulatory and non-regulatory T cells. *Eur J Immunol* 2012; **42**: 1627-1638 [PMID: 22678915 DOI: 10.1002/eji.201141838]
 - 59 Rushbrook SM, Ward SM, Unitt E, Vowler SL, Lucas M, Klenerman P, Alexander GJ. Regulatory T cells suppress in vitro proliferation of virus-specific CD8⁺ T cells during persistent hepatitis C virus infection. *J Virol* 2005; **79**: 7852-7859 [PMID: 15919939 DOI: 10.1128/jvi.79.12.7852-7859.2005]
 - 60 Itose I, Kanto T, Kakita N, Takebe S, Inoue M, Higashitani K, Miyazaki M, Miyatake H, Sakakibara M, Hiramatsu N, Takehara T, Kasahara A, Hayashi N. Enhanced ability of regulatory T cells in chronic hepatitis C patients with persistently normal alanine aminotransferase levels than those with active hepatitis. *J Viral Hepat* 2009; **16**: 844-852 [PMID: 19486278 DOI: 10.1111/j.1365-2893.2009.01131.x]
 - 61 Brandtzaeg P. Mucosal immunity: induction, dissemination, and effector functions. *Scand J Immunol* 2009; **70**: 505-515 [PMID: 19906191 DOI: 10.1111/j.1365-3083.2009.02319.x]
 - 62 Brandtzaeg P. History of oral tolerance and mucosal immunity. *Ann N Y Acad Sci* 1996; **778**: 1-27 [PMID: 8610963 DOI: 10.1111/j.1749-6632.1996.tb21110.x]
 - 63 Brandtzaeg P. 'ABC' of mucosal immunology. *Nestle Nutr Workshop Ser Pediatr Program* 2009; **64**: 23-38; discussion 38-43, 251-257 [PMID: 19710513 DOI: 10.1159/000235781]
 - 64 Artis D. Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. *Nat Rev Immunol* 2008; **8**: 411-420 [PMID: 18469830 DOI: 10.1038/nri2316]
 - 65 Husby S, Jensenius JC, Svehaug SE. Passage of undegraded dietary antigen into the blood of healthy adults. Quantification, estimation of size distribution, and relation of uptake to levels of specific antibodies. *Scand J Immunol* 1985; **22**: 83-92 [PMID: 4023632 DOI: 10.1111/j.1365-3083.1985.tb01862.x]
 - 66 Limmer A, Ohl J, Wingender G, Berg M, Jüngerkes F, Schumak B, Djandji D, Scholz K, Klevenz A, Hegenbarth S, Momburg F, Hämmerling GJ, Arnold B, Knolle PA. Cross-presentation of oral antigens by liver sinusoidal endothelial cells leads to CD8 T cell tolerance. *Eur J Immunol* 2005; **35**: 2970-2981 [PMID: 16163670 DOI: 10.1002/eji.200526034]
 - 67 Adams DH, Eksteen B, Curbishley SM. Immunology of the gut and liver: a love/hate relationship. *Gut* 2008; **57**: 838-848 [PMID: 18203807 DOI: 10.1136/gut.2007.122168]
 - 68 Yang R, Liu Q, Grosfeld JL, Pescovitz MD. Intestinal venous drainage through the liver is a prerequisite for oral tolerance induction. *J Pediatr Surg* 1994; **29**: 1145-1148 [PMID: 7965523 DOI: 10.1016/0022-3468(94)90297-6]
 - 69 Holmgren J, Czerkinsky C. Mucosal immunity and vaccines. *Nat Med* 2005; **11**: S45-S53 [PMID: 15812489 DOI: 10.1038/nm1213]
 - 70 Agace WW. Tissue-tropic effector T cells: generation and targeting opportunities. *Nat Rev Immunol* 2006; **6**: 682-692 [PMID: 16932753 DOI: 10.1038/nri1869]
 - 71 Shata MT, Abdel-Hameed EA, Hetta HF, Sherman KE. Immune activation in HIV/HCV-infected patients is associated with low-level expression of liver expressed antimicrobial peptide-2 (LEAP-2). *J Clin Pathol* 2013; **66**: 967-975 [PMID: 23940131 DOI: 10.1136/jclinpath-2013-201581]
 - 72 Yuan Q, Walker WA. Innate immunity of the gut: mucosal defense in health and disease. *J Pediatr Gastroenterol Nutr* 2004; **38**: 463-473 [PMID: 15097431 DOI: 10.1097/00005176-200405000-00001]
 - 73 Heremans JF, Bazin H. Antibodies induced by local antigenic stimulation of mucosal surfaces. *Ann N Y Acad Sci* 1971; **190**: 268-275 [PMID: 5290019 DOI: 10.1111/j.1749-6632.1971.tb13540.x]
 - 74 Doe WF. The intestinal immune system. *Gut* 1989; **30**: 1679-1685 [PMID: 2693229 DOI: 10.1136/gut.30.12.1679]
 - 75 Ogra PL, Karzon DT. Poliovirus antibody response in serum and nasal secretions following intranasal inoculation with inactivated poliovaccine. *J Immunol* 1969; **102**: 15-23 [PMID: 4303877]
 - 76 Lycke N, Eriksen L, Holmgren J. Protection against cholera toxin after oral immunization is thymus-dependent and associated with intestinal production of neutralizing IgA antitoxin. *Scand J Immunol* 1987; **25**: 413-419 [PMID: 3576135 DOI: 10.1111/j.1365-3083.1987.tb02208.x]
 - 77 Goodrich ME, McGee DW. Regulation of mucosal B cell immunoglobulin secretion by intestinal epithelial cell-derived cytokines. *Cytokine* 1998; **10**: 948-955 [PMID: 10049518 DOI: 10.1006/cyto.1998.0385]
 - 78 Asano T, Kaneko H, Terada T, Kasahara Y, Fukao T, Kasahara K, Kondo N. Molecular analysis of B-cell differentiation in selective or partial IgA deficiency. *Clin Exp Immunol* 2004; **136**: 284-290 [PMID: 15086392 DOI: 10.1111/j.1365-2249.2004.02440.x]
 - 79 Franco MA, Greenberg HB. Role of B cells and cytotoxic T lymphocytes in clearance of and immunity to rotavirus infection in mice. *J Virol* 1995; **69**: 7800-7806 [PMID: 7494291]
 - 80 Buzoni-Gatel D, Lepage AC, Dimier-Poisson IH, Bout DT, Kasper LH. Adoptive transfer of gut intraepithelial lymphocytes protects against murine infection with *Toxoplasma gondii*. *J Immunol* 1997; **158**: 5883-5889 [PMID: 9190941]
 - 81 Iwasaki A, Kelsall BL. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J Exp Med* 1999; **190**: 229-239 [PMID: 10432286 DOI: 10.1084/jem.190.2.229]

- 82 **Rossi M**, Bot A. The Th17 cell population and the immune homeostasis of the gastrointestinal tract. *Int Rev Immunol* 2013; **32**: 471-474 [PMID: 24164337 DOI: 10.3109/08830185.2013.843983]
- 83 **Bilsborough J**, Viney JL. Gastrointestinal dendritic cells play a role in immunity, tolerance, and disease. *Gastroenterology* 2004; **127**: 300-309 [PMID: 15236195 DOI: 10.1053/j.gastro.2004.01.028]
- 84 **Mowat AM**. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol* 2003; **3**: 331-341 [PMID: 12669023 DOI: 10.1038/nri1057]
- 85 **Faria AM**, Weiner HL. Oral tolerance. *Immunol Rev* 2005; **206**: 232-259 [PMID: 16048553 DOI: 10.1111/j.0105-2896.2005.00280.x]
- 86 **Whitacre CC**, Gienapp IE, Orosz CG, Bitar DM. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. *J Immunol* 1991; **147**: 2155-2163 [PMID: 1717550]
- 87 **Chen Y**, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995; **376**: 177-180 [PMID: 7603570 DOI: 10.1038/376177a0]
- 88 **Groux H**, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; **389**: 737-742 [PMID: 9338786 DOI: 10.1038/39614]
- 89 **Chen Y**, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994; **265**: 1237-1240 [PMID: 7520605 DOI: 10.1126/science.7520605]
- 90 **Thornton AM**, Shevach EM. CD4⁺CD25⁺ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998; **188**: 287-296 [PMID: 9670041 DOI: 10.1084/jem.188.2.287]
- 91 **Stassen M**, Fondel S, Bopp T, Richter C, Müller C, Kubach J, Becker C, Knop J, Enk AH, Schmitt S, Schmitt E, Jonuleit H. Human CD25⁺ regulatory T cells: two subsets defined by the integrins alpha 4 beta 7 or alpha 4 beta 1 confer distinct suppressive properties upon CD4⁺ T helper cells. *Eur J Immunol* 2004; **34**: 1303-1311 [PMID: 15114663 DOI: 10.1002/eji.200324656]
- 92 **McMenamin C**, Pimm C, McKersey M, Holt PG. Regulation of IgE responses to inhaled antigen in mice by antigen-specific gamma delta T cells. *Science* 1994; **265**: 1869-1871 [PMID: 7916481 DOI: 10.1126/science.7916481]
- 93 **Knolle PA**, Limmer A. Neighborhood politics: the immunoregulatory function of organ-resident liver endothelial cells. *Trends Immunol* 2001; **22**: 432-437 [PMID: 11473832 DOI: 10.1016/S1471-4906(01)01957-3]
- 94 **Crispe IN**. Hepatic T cells and liver tolerance. *Nat Rev Immunol* 2003; **3**: 51-62 [PMID: 12511875 DOI: 10.1038/nri981]
- 95 **Bowen DG**, McCaughan GW, Bertolino P. Intrahepatic immunity: a tale of two sites? *Trends Immunol* 2005; **26**: 512-517 [PMID: 16109501 DOI: 10.1016/j.it.2005.08.005]
- 96 **Gualdi R**, Bossard P, Zheng M, Hamada Y, Coleman JR, Zaret KS. Hepatic specification of the gut endoderm in vitro: cell signaling and transcriptional control. *Genes Dev* 1996; **10**: 1670-1682 [PMID: 8682297 DOI: 10.1101/gad.10.13.1670]
- 97 **Yoshida H**, Kawamoto H, Santee SM, Hashi H, Honda K, Nishikawa S, Ware CF, Katsura Y, Nishikawa SI. Expression of alpha(4)beta(7) integrin defines a distinct pathway of lymphoid progenitors committed to T cells, fetal intestinal lymphotoxin producer, NK, and dendritic cells. *J Immunol* 2001; **167**: 2511-2521 [PMID: 11509590 DOI: 10.4049/jimmunol.167.5.2511]
- 98 **Ward SM**, Jonsson JR, Sierro S, Clouston AD, Lucas M, Vargas AL, Powell EE, Klenerman P. Virus-specific CD8⁺ T lymphocytes within the normal human liver. *Eur J Immunol* 2004; **34**: 1526-1531 [PMID: 15162421 DOI: 10.1002/eji.200324275]
- 99 **Grant AJ**, Lalor PF, Hübscher SG, Briskin M, Adams DH. MAdCAM-1 expressed in chronic inflammatory liver disease supports mucosal lymphocyte adhesion to hepatic endothelium (MAdCAM-1 in chronic inflammatory liver disease). *Hepatology* 2001; **33**: 1065-1072 [PMID: 11343233 DOI: 10.1053/jhep.2001.24231]
- 100 **Eksteen B**, Grant AJ, Miles A, Curbishley SM, Lalor PF, Hübscher SG, Briskin M, Salmon M, Adams DH. Hepatic endothelial CCL25 mediates the recruitment of CCR9⁺ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. *J Exp Med* 2004; **200**: 1511-1517 [PMID: 15557349 DOI: 10.1084/jem.20041035]
- 101 **Napoli J**, Bishop GA, McGuinness PH, Painter DM, McCaughan GW. Progressive liver injury in chronic hepatitis C infection correlates with increased intrahepatic expression of Th1-associated cytokines. *Hepatology* 1996; **24**: 759-765 [PMID: 8855173 DOI: 10.1002/hep.510240402]
- 102 **Quiroga JA**, Martín J, Navas S, Carreño V. Induction of interleukin-12 production in chronic hepatitis C virus infection correlates with the hepatocellular damage. *J Infect Dis* 1998; **178**: 247-251 [PMID: 9652448 DOI: 10.1086/517446]
- 103 **Abregnani S**. Immune responses throughout hepatitis C virus (HCV) infection: HCV from the immune system point of view. *Springer Semin Immunopathol* 1997; **19**: 47-55 [PMID: 9266630 DOI: 10.1007/BF00945024]
- 104 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçalves FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]
- 105 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749 DOI: 10.1016/S0140-6736(01)06102-5]
- 106 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676 DOI: 10.7326/0003-4819-140-5-200403020-00010]
- 107 **Ghany MG**, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 1433-1444 [PMID: 21898493 DOI: 10.1002/hep.24641]
- 108 **Chae HB**, Park SM, Youn SJ. Direct-acting antivirals for the treatment of chronic hepatitis C: open issues and future perspectives. *ScientificWorldJournal* 2013; **2013**: 704912 [PMID: 23844410 DOI: 10.1155/2013/704912]
- 109 **Koff RS**. Review article: the efficacy and safety of sofosbuvir, a novel, oral nucleotide NS5B polymerase inhibitor, in the treatment of chronic hepatitis C virus infection. *Aliment Pharmacol Ther* 2014; **39**: 478-487 [PMID: 24387618 DOI: 10.1111/apt.12601]
- 110 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]
- 111 **Pearlman BL**. The IL-28 genotype: how it will affect the care of patients with hepatitis C virus infection. *Curr Gastroenterol Rep* 2011; **13**: 78-86 [PMID: 21080244 DOI: 10.1007/s11894-010-0161-9]
- 112 **Thompson AJ**, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW,

- Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* 2010; **139**: 120-129.e18 [PMID: 20399780 DOI: 10.1053/j.gastro.2010.04.013]
- 113 **Ank N**, Iversen MB, Bartholdy C, Staeheli P, Hartmann R, Jensen UB, Dagnaes-Hansen F, Thomsen AR, Chen Z, Haugen H, Klucher K, Paludan SR. An important role for type III interferon (IFN- λ 28) in TLR-induced antiviral activity. *J Immunol* 2008; **180**: 2474-2485 [PMID: 18250457 DOI: 10.4049/jimmunol.180.4.2474]
- 114 **Sheppard P**, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, Kuestner R, Garrigues U, Birks C, Roraback J, Ostrander C, Dong D, Shin J, Presnell S, Fox B, Haldeman B, Cooper E, Taft D, Gilbert T, Grant FJ, Tackett M, Krivan W, McKnight G, Clegg C, Foster D, Klucher KM. IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nat Immunol* 2003; **4**: 63-68 [PMID: 12469119 DOI: 10.1038/ni873]
- 115 **Stark GR**, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. *Annu Rev Biochem* 1998; **67**: 227-264 [PMID: 9759489 DOI: 10.1146/annurev.biochem.67.1.227]
- 116 **Samuel CE**. Antiviral actions of interferons. *Clin Microbiol Rev* 2001; **14**: 778-809, table of contents [PMID: 11585785 DOI: 10.1128/CMR.14.4.778-809.2001]
- 117 **Dolganiuc A**, Kodys K, Marshall C, Saha B, Zhang S, Bala S, Szabo G. Type III interferons, IL-28 and IL-29, are increased in chronic HCV infection and induce myeloid dendritic cell-mediated FoxP3+ regulatory T cells. *PLoS One* 2012; **7**: e44915 [PMID: 23071503 DOI: 10.1371/journal.pone.0044915]
- 118 **Perrella A**, Vitiello L, Atripaldi L, Conti P, Sbriglia C, Altamura S, Patarino T, Vela R, Morelli G, Bellopede P, Alone C, Racioppi L, Perrella O. Elevated CD4+/CD25+ T cell frequency and function during acute hepatitis C presage chronic evolution. *Gut* 2006; **55**: 1370-1371 [PMID: 16905711 DOI: 10.1136/gut.2006.099887]
- 119 **Mangia A**, Thompson AJ, Santoro R, Piazzolla V, Tillmann HL, Patel K, Shianna KV, Mottola L, Petruzzellis D, Bacca D, Carretta V, Minerva N, Goldstein DB, McHutchison JG. An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010; **139**: 821-827, 827.e1 [PMID: 20621700 DOI: 10.1053/j.gastro.2010.05.079]
- 120 **Moghaddam A**, Melum E, Reinton N, Ring-Larsen H, Verbaan H, Bjørø K, Dalgard O. IL28B genetic variation and treatment response in patients with hepatitis C virus genotype 3 infection. *Hepatology* 2011; **53**: 746-754 [PMID: 21374656 DOI: 10.1002/hep.24154]

P- Reviewer: Dirchwolf M, Narciso-Schiavon JL, Puoti C, Sagnelli E **S- Editor:** Song XX **L- Editor:** A **E- Editor:** Wang CH





CD28/CTLA-4/B7 and CD40/CD40L costimulation and activation of regulatory T cells

Isabel T Vogel, Stefaan W Van Gool, Jan L Ceuppens

Isabel T Vogel, Stefaan W Van Gool, Jan L Ceuppens, Laboratory of Clinical Immunology, Department of Microbiology and Immunology, KU Leuven, University Hospital Gasthuisberg, 3000 Leuven, Belgium

Stefaan W Van Gool, Laboratory of Pediatric Immunology, KU Leuven, University Hospital Gasthuisberg, 3000 Leuven, Belgium

Author contributions: All authors contributed to this manuscript.
Correspondence to: Jan L Ceuppens, MD, Laboratory of Clinical Immunology, Department of Microbiology and Immunology, KU Leuven, University Hospital Gasthuisberg, Herestraat 49, 3000 Leuven, Belgium. jan.ceuppens@uzleuven.be

Telephone: +32-16-346137 Fax: +32-16-346035

Received: March 27, 2014 Revised: May 12, 2014

Accepted: June 10, 2014

Published online: July 27, 2014

Abstract

Costimulatory signals are crucial for T cell activation. Attempts to block costimulatory pathways have been effective in preventing unwanted immune reactions. In particular, blocking the CD28/cytotoxic T lymphocyte antigen (CTLA)-4/B7 interaction (using CTLA-4Ig) and the CD40/CD40L interaction (using anti-CD40L antibodies) prevents T cell mediated autoimmune diseases, transplant rejection and graft vs host disease in experimental models. Moreover, CTLA-4Ig is in clinical use to treat rheumatoid arthritis (abatacept) and to prevent rejection of renal transplants (belatacept). Under certain experimental conditions, this treatment can even result in tolerance. Surprisingly, the underlying mechanisms of immune modulation are still not completely understood. We here discuss the evidence that costimulation blockade differentially affects effector T cells (Teff) and regulatory T cells (Treg). The latter are required to control inappropriate and unwanted immune responses, and their activity often contributes to tolerance induction and maintenance. Unfortunately, our knowledge on the costimulatory requirements of Treg cells is very limited. We therefore summarize the current understanding of

the costimulatory requirements of Treg cells, and elaborate on the effect of anti-CD40L antibody and CTLA-4Ig treatment on Treg cell activity. In this context, we point out that the outcome of a treatment aiming at blocking the CD28/CTLA-4/B7 costimulatory interaction can vary with dosing, timing and underlying immunopathology.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Regulatory T cells; Tolerance; Cytotoxic T lymphocyte antigen-4Ig; Anti-CD40L; Costimulation

Core tip: Costimulation blockade (*e.g.*, CD28/B7 and CD40/CD40L blockade) has been successfully used experimentally to induce tolerance to allo- or auto-antigens. Several studies suggest that effector T cells (Teff) and regulatory T cells (Treg) have different requirements regarding costimulation. While blockade of the CD40L receptor does not affect Treg cells and targets Teff cells, the effect of blocking the CD28/cytotoxic T lymphocyte antigen (CTLA)-4/B7 interaction (with CTLA-4Ig) is more difficult to predict and depends on the type, the strength and the stage of an immune process. Importantly, manipulating these costimulatory signals can therefore shift the Treg/Teff cell balance towards dominant Treg cell activity.

Vogel IT, Van Gool SW, Ceuppens JL. CD28/CTLA-4/B7 and CD40/CD40L costimulation and activation of regulatory T cells. *World J Immunol* 2014; 4(2): 63-77 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/63.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.63>

INTRODUCTION

Costimulatory interactions between T cells and antigen presenting cells (APCs), such as the CD28/B7 pathway

and the CD40/CD40L pathway, are essential for T cell activation. As a consequence, reagents that deliberately block those costimulatory signals (*e.g.*, the CTLA-4Ig fusion protein or antagonistic anti-CD40L antibodies) can be used to prevent unwanted or inappropriate T cell activation. Blocking costimulation, therefore, has been used to treat T cell mediated autoimmune diseases, transplant rejection or graft *vs* host disease (GvHD). Although anti-CD40L antibodies showed great potential in pre-clinical animal models and cytotoxic T lymphocyte antigen (CTLA)-4Ig is successfully used in clinical practice to treat rheumatoid arthritis and to prevent rejection of renal transplants, the precise mechanisms underlying their efficacy are still not fully understood. While effector T cells (Teff) clearly depend on costimulation for their activation, the costimulatory requirements of a suppressive T cell population, the regulatory T cells (Treg), are not completely clear. Several studies suggest that Treg and Teff cells have different requirements regarding costimulation. Furthermore, it has been suggested that Treg cells play an important role in the process of tolerance induction by costimulation blockade. In this review we discuss some possibilities to modulate costimulation in such a way that Teff cells are blocked but Treg cells remain active and functional. In this context, we summarize the current understanding of the costimulatory requirements of Treg cells, and elaborate on the effect of anti-CD40L antibody and CTLA-4Ig treatment on Treg cells. We point out that CTLA-4Ig has a quite complex effect on Treg cells, which should be taken into account when interfering with the CD28/CTLA-4/B7 interaction.

MECHANISMS OF PERIPHERAL TOLERANCE

Immune tolerance refers to a state of specific immune non-responsiveness of the immune system to a particular antigen or a group of antigens. Tolerance to self-antigens is a hallmark of an effectively functioning immune system and disabling tolerance to self-antigens can lead to autoimmune diseases. In a similar way, an inappropriate response to a harmless environmental antigen can result in allergies. To avoid such harmful reactions, the immune system has developed several sophisticated mechanisms to induce and maintain tolerance.

During the maturation in the thymus, T cells undergo positive and negative selection. T cells which recognize a self-antigen presented by major histocompatibility complex (MHC) molecules, can be eliminated (negative selection)^[1]. In this process, the signal strength with which the T cell receptor (TCR) recognizes its antigen determines the fate of the T cell. A strong signal and definite recognition of the auto-antigen leads to immediate deletion of the responding cell. A weak signal often leads to ignorance and migration to the periphery^[2]. This is reasonable in order to maintain a pool of variable TCRs in the periphery. However, these cells might regain self-reactivity later on. Furthermore, some T cells escape thymic selec-

tion. Under these circumstances, peripheral tolerance induction should come into action.

Peripheral tolerance is maintained by mechanisms such as anergy (which results from a lack of sufficient activation signals)^[3], deletion by apoptosis^[4,5] and control by regulatory T (Treg) cells. The role of regulatory T cells, as well as the importance of costimulation for the induction and maintenance of peripheral tolerance, will be discussed in the following section.

Costimulatory signals

Naïve T cells need two distinct signals in order to get fully activated^[6]. The first signal is transmitted through the TCR, which recognizes an antigen presented by specialized antigen-presenting cells (APCs) on MHC molecules. This signal determines the specificity of the T cell response. The second (or accessory) signal is provided by the ligation of costimulatory receptors on the cell surface^[7]. Without proper costimulation, T cells fail to become fully activated and enter a state of hypo-responsiveness (anergy)^[8]. Up to now, many costimulatory signals and pathways have been identified, among which the best characterized are the CD28/CTLA-4/B7 pathway and the CD40/CD40L pathway.

The CD28/CTLA-4/B7 interaction: Mice deficient in CD28 are unable to mount an effective immune response to foreign antigens, pathogens or allografts. The CD28 receptor is a disulfide-linked homodimer, which is constitutively expressed on T cells and is engaged by both the CD80 (B7-1) and CD86 (B7-2) molecule on activated APC^[9]. The monomeric CD86 ligand is constitutively expressed in low amounts on professional APC and up-regulated upon activation, while CD80 is expressed as a dimer on activated APC. The up-regulation of CD86 occurs rapidly after activation and reaches its maximum 18 to 24 h after stimulation, while the up-regulation of CD80 is delayed and reaches a maximum after 48 to 72 h^[10,11]. Studies with knock-out (KO) mice have shown that CD86 is more important for initiating an immune response than CD80. Otherwise the functions of the two B7 molecules are largely overlapping^[12]. Signalling *via* CD28 is mediated through the phosphatidylinositol 3-kinase-protein kinase B (PKB/Akt) and the growth factor-receptor-bound protein 2 (Grb2) pathways and promotes IL-2 production^[13] and T cell proliferation^[14] by decreasing the threshold for activation *via* the TCR^[15]. In addition, T cell survival is strengthened by up-regulation of the anti-apoptotic factor Bcl-xL^[16]. CD28 engagement also up-regulates or induces the expression of additional costimulatory receptors such as ICOS and CTLA-4^[17]. While CD28/B7 signalling is crucial for the activation of naïve T cells, previously activated cells are less dependent on costimulation. After priming and differentiation are completed, the production of effector cytokines (*e.g.*, IL-4 or IFN γ) does not require further costimulation. Only IL-2 production depends on continuous costimulatory signalling^[12].

Another receptor molecule, which binds to both B7 molecules and is structurally homologous to CD28, is the “cytotoxic T lymphocyte antigen 4” (CTLA-4) or CD152. It is up-regulated on T cells upon activation with a peak at 24-48 h after initial priming^[18]. However, its expression on the surface is not stable and the CTLA-4 molecule is continuously internalized in a clathrin dependent way, degraded in lysosomes and recycled to the cell surface^[19]. CTLA-4 binds CD80 and CD86 with a 10-20 fold higher affinity compared to CD28^[20] and consequently outcompetes CD28 mediated activation^[21]. Furthermore, CTLA-4 has an advantage in engaging to B7 molecules as it binds divalently, while CD28 binds monovalently^[22]. In contrast to CD28 signalling, the CTLA-4 pathway has a suppressive character, and CTLA-4 deficient mice develop severe lymphoproliferative disease and die 3 to 4 wk after birth^[23]. Of note, CTLA-4 KO mice deficient in B7-1 and B7-2, as well as CTLA-4 KO mice with a defective CD28 receptor are protected from this fatal disease^[24,25]. This suggests that CTLA-4 selectively regulates CD28 mediated activation. Binding of CTLA-4 to its ligands recruits phosphatases (SHP-1, SHP-2 and PP2A), which inhibit TCR phosphorylation and several other pathways such as the PKB/Akt activation as well as the phosphorylation of extracellular-signal-regulated kinases (ERK) and c-Jun N-terminal kinases (JNK)^[26]. This reduces the production of IL-2 and its receptor, inhibits T cell proliferation, and consequently results in termination of the immune response^[18,27].

Other members of the B7 and CD28 superfamilies:

Other members of the B7 superfamily, which have been studied extensively, are the inducible costimulator ligand (ICOSL, CD275, B7h or B7-PR-1), which binds to ICOS (CD278) and the programmed death ligands 1 and 2 (PD-L1 and PD-L2) which binds to programmed death 1 (PD 1). ICOS is structurally and genetically related to CD28 and up-regulated in the course of activation^[28]. ICOSL is expressed on APCs and some non-hematopoietic cells (*e.g.*, endothelial cells). Different from CD28/B7 signalling, ICOS/ICOSL interaction is not essential for T cell activation, but rather acts by fine-tuning effector T cell differentiation and cytokine production^[29]. Furthermore, ICOS is crucial for germinal centre formation and class switching in B cells^[30,31].

PD-1 is a suppressive member of the CD28 superfamily. Different from CD28 and CTLA-4, PD-1 is not expressed as a dimer and its expression is not limited to T cells. It can be found on activated T cells, but also on B cells and myeloid cells, which suggests a broader spectrum of regulation compared to CTLA-4^[32]. Ligation to PD-L1 and PD-L2, which are expressed on activated APCs, inhibits cytokine production and leads to cell cycle arrest^[33,34]. Furthermore, PD-1 signalling was found to be involved in CD8⁺ T cell differentiation and regulation^[35].

The CD40/CD40L interaction: CD40 (TNFRSF5) is a type I trans-membrane protein, which clusters upon en-

gagement to its ligand CD40L (CD154, TNFSF5, gp39, T-BAM, or TRAP)^[36]. CD40 ligation further induces the recruitment of adaptor proteins (TNF-associated factors), which then in turn trigger several possible pathways including the canonical and non-canonical nuclear factor κ B (NF κ B) signalling pathway, the mitogen activated protein kinases (MAPK), the phosphoinositide 3-kinase (PI3K) and the phospholipase γ (PLC γ) pathways^[37]. CD40 is constitutively expressed on APC and on many other cell types including non-hematopoietic cells (*e.g.*, fibroblasts and epithelial cells)^[38]. CD40L forms a sandwich structure composed of a β -sheet, an α -helix loop and another β -sheet and is expressed as a trimeric complex on activated T cells and platelets^[39]. Under inflammatory conditions it can also be found on natural killer (NK) cells, mastocytes and eosinophils^[38]. Its expression on T cells is mainly restricted to CD4⁺ T helper (Th) cells, but there is also a small population of CD8⁺ T cells and $\gamma\delta$ T cells which can express CD40L^[36]. Furthermore, it has been shown that CD40L is expressed on CD8⁺ T cells in the presence of IL-12 and that these cells potentially represent a CD8⁺ T helper cell subset^[40,41].

Upon activation, CD40L is up-regulated as early as 5 to 15 min after stimulation and reaches a maximum after 6 to 8 h^[36]. This fast up-regulation is made possible *via* preformed CD40L (pCD40L), which is stored in lysosomal compartments and can be mobilised in response to an activation signal^[42].

The broad expression of CD40 suggests involvement in many different immune modulatory mechanisms. In this context, CD40L engagement to CD40 results in increased survival of APC^[43], production of cytokines^[44], up-regulation of B7 molecules and nitric oxide (NO) production^[45] and is critical for full maturation of dendritic cells (DC)^[46]. Furthermore, CD40 signalling is crucial for B cell activation and differentiation, antibody production, immunoglobulin-class switching and germinal centre formation^[47,48]. CD40/CD40L KO mice do not only show hyper-IgM syndrome, but also exhibit deficiency in priming of T cells^[36]. Signalling *via* CD40/CD40L results in enforcement of the CD28-B7 interaction and antigen presentation and is crucial for expansion and maturation of effector T (Teff) cells^[38,49]. Furthermore, CD40/CD40L mediated contact between CD4⁺ T helper cells and professional APC (DC) is important to enable DC to subsequently prime CD8⁺ cytotoxic T lymphocytes (CTL)^[50].

Other members of the TNF and TNFR superfamilies:

Other members of the TNF/TNFR superfamily have gained importance during the last years. Among those are the interactions between the glucocorticoid-induced tumour necrosis factor related receptor (GITR) and its ligand GITR-L, between OX40 (CD134 or TNFRSF4) and OX40 ligand (OX40L, CD252 or TNFSF4), between 4-1BB (CD137 or TNFRSF9) and 4-1BB ligand (4-1BBL or TNFSF9) and CD27 (TNFRSF7) and CD70 (TNFSF7). In general, these

TNF/TNFR superfamily members are up-regulated or induced upon activation on T cells and their ligands on APCs. Signalling *via* these pathways regulates the frequency of effector or memory cells, provides proliferation and survival signals and promotes cytokine production^[51]. The expression of OX40L, 4-1BBL and CD70 on non-immune cells (*e.g.*, endothelial cells or smooth muscle cells) further suggests a role in tissue inflammation in different disease settings^[52,53]. In addition, TNF/TNFR superfamily members are expressed on natural killer (NK) and natural killer T (NKT) cells and signalling increases their effector function^[51].

Regulatory T cells

A subset of CD4⁺ T cells has regulatory capacity. In a healthy individual they constitute about 10% of circulating CD4⁺ T cells. Treg cells play a key role in dampening of immune responses, prevention of autoimmune and allergic diseases, as well as in tolerance after transplantation^[54]. They are characterized by constitutive expression of the IL-2 receptor α -chain CD25, CTLA-4 and the forkhead transcription factor Foxp3^[55,56]. The latter one is crucial for the suppressive function of Treg cells, as ectopic expression of Foxp3 can induce regulatory function in naïve T cells^[57]. Loss of Foxp3 results in impairment of Treg cells and in autoimmune disorders in mice (Scurfy)^[58] and humans (IPEX-syndrome)^[59].

Two subgroups of Foxp3 expressing Treg cells have been identified: the so called thymus derived Treg cells (tTreg) and induced Treg cells (iTreg), which are generated in the periphery from naïve CD4⁺ T cells. *In vitro*, iTreg cells can be induced by antigenic stimulation in the presence of IL-2 and TGF- β ^[60,61]. Although the situation *in vivo* is less clear, iTreg cells are thought to be generated under non-inflammatory conditions in the presence of IL-2 and TGF- β by chronic sub-optimal antigen exposure^[62-64], *e.g.*, by recognition of an antigen on immature DC which do not provide costimulation^[65]. Furthermore, a role for retinoic acid (RA), which increases TGF- β production and favors Foxp3 polarization, has been unraveled^[66,67]. During an acute inflammation (*e.g.*, in allergic or autoimmune diseases or during the course of an infection), in the presence of high amounts of inflammatory cytokines, the generation of Teff cells is favored over Treg cell induction^[68].

Unfortunately it is not yet possible to distinguish tTreg and iTreg cells since both of them express CTLA-4, CD25 and Foxp3. Helios (a member of the Ikaros transcription factor family) and Neuropilin-1 (Nrp1) have been suggested as specific markers for tTreg cells, but controversial findings regarding their expression on tTreg *vs* iTreg cells limit their use as reliable markers^[69-72].

There are also CD4⁺ Treg cell subtypes induced in the periphery which do not express Foxp3. Among those are T regulatory cells 1 (Tr1), which can be induced from naïve CD4⁺ T cells in the presence of IL-10^[73] and T helper cells type 3 (Th3), which require TGF- β ^[74]. Up to now, it is difficult to identify those Treg cell subsets by

means of a specific surface marker. Therefore, they are predominantly defined by their cytokine profile. Tr1 cells are characterized by a high IL-10 and TGF- β production, low levels of IL-2, variable levels of IL-5 and IFN- γ and no IL-4^[73]. Th3 cells produce mainly TGF- β and variable levels of IL-10 and IL-4^[75].

Activation and expansion of Treg cells requires a TCR signal *in vitro*^[76,77] and *in vivo*^[78,79] and is consequently antigen specific. Whether or not they suppress in an antigen-specific way is still a matter of debate. A key molecule in suppression by Treg cells is CTLA-4. Mice which display a Treg-specific deficiency in CTLA-4 develop severe autoimmune diseases, and Treg cells from these mice show reduced suppressive capacity *in vitro*^[80]. In contrast to conventional T cells, Treg cells express CTLA-4 constitutively^[81] and therefore have a natural advantage over naïve T cells in terms of CD80/CD86 engagement. In addition, CTLA-4 expressed by Treg cells also has a cell-extrinsic mechanism of action. It has been demonstrated by Qureshi and coworkers that CTLA-4 engagement to the B7 molecules leads to trans-endocytosis and degradation of CD80 and CD86 on the surface of APCs^[82]. This effect can only be mediated by CTLA-4 expressed on the cell surface, but not by soluble CTLA-4. As a result, the availability of B7 receptors and consequently the CD28 mediated activation of T cells are reduced. Moreover, CTLA-4/B7 interaction might lead to “reverse signalling” in APC. In the course of CTLA-4 engagement, APC start to produce indoleamine 2,3-dioxygenase (IDO), which catalyses the degradation of tryptophan and thus creates a local inhibitory environment for T cells^[83]. This also induces the nuclear translocation of the transcription factor Foxo3^[84], which inhibits the production of IL-6 and of tumor necrosis factor alpha (TNF α) but increases the secretion of suppressive cytokines such as IL-10^[85]. Apart from mechanisms mediated by direct cell contact to APCs, Treg cells also secrete suppressive molecules such as IL-10^[86], TGF β ^[87] and IL-35^[88] and molecules which can directly kill Teff cells, such as granzyme B and perforin^[89]. Membrane-bound TGF β ^[90] or production of cyclic adenosine monophosphate (cAMP), which can be transferred to Teff cells *via* gap junctions, can suppress Teff cells *via* direct cell-cell contact^[91]. Other suppressive mechanisms involve CD39 and CD72 mediated degradation of adenosine monophosphate (AMP) and adenosine triphosphate (ATP) to adenosine^[92] or suppression by Galectin-1^[93]. Finally, Treg cells are thought to suppress Teff cells by IL-2 deprivation and subsequent apoptosis^[94]. IL-2 is crucial for Treg cell generation, induction and maintenance^[95], but, in contrast to Teff cells, Treg cells lack the ability to produce IL-2 and are consequently dependent on an external source^[96]. Since Treg cells constitutively express the high affinity receptor for IL-2 (CD25)^[55], they have an advantage over Teff cells in terms of binding IL-2. In an inflammatory setting, however, when Teff cells also up-regulate CD25, this advantage is lost. Therefore, it was suggested that suppression by IL-2 consumption is predominantly important

in steady-state conditions as a feed-back mechanism to prevent Treg cell overgrowth and not in an inflammatory setting^[97].

Since none of the above described mechanisms results in a complete absence of regulatory activity when deleted, there is most likely not one core-mechanism of suppression. In this context, Treg-specific CTLA-4 deficiency resulted in systemic autoimmune diseases^[80], but transfer of CTLA-4 deficient Treg cells could prevent experimental colitis *in vivo*^[98] and IL-10 deficient Treg cells are able to suppress auto-immunity, but cannot prevent experimental colitis^[86,99]. Thus, Treg cells can compensate for defects and adapt to environmental circumstances.

THE EFFECTS OF BLOCKING COSTIMULATORY SIGNALS

Since the “second” or “costimulatory” signal is of great importance for the activation and successful differentiation of naive T cells into fully functional Teff cells^[6], blocking these pathways presents a promising approach to treat T cell mediated autoimmune diseases (*e.g.*, rheumatoid arthritis or multiple sclerosis), transplant rejections or graft *vs* host disease (GvHD). Compared to conventional immunosuppressive drugs, costimulation blockade provides the advantage of selective inhibition of T cell responses and has the potential of inducing long-lasting antigen-specific tolerance^[100]. The most promising and best studied candidates for such manipulations are the CD28/B7 and CD40/CD40L pathways as they are both critical for T cell activation.

Blocking the CD28/B7 pathway using CTLA-4Ig

Up to now, the most promising candidate to achieve CD28/B7 costimulation blockade is the CTLA-4Ig fusion protein. It consists of the extracellular domain of the CTLA-4 molecule fused to the Fc-region of IgG. CTLA-4Ig binds both B7 molecules with the same high binding affinity as CTLA-4. The effect of CTLA-4Ig has first been demonstrated in an animal model of islet transplantation, where CTLA-4Ig treatment led to long-term acceptance of xenografts^[101]. Also in systems of allogeneic islet or cardiac transplantation or graft *vs* host disease (GvHD), CTLA-4Ig could prolong survival and reduce rejection^[102-104]. Furthermore, CTLA-4Ig is a potent immunosuppressor in animal models of autoimmunity such as experimental autoimmune encephalomyelitis (EAE)^[105], diabetes^[106] and systemic lupus erythematosus (SLE)^[107].

CTLA-4Ig has also been used effectively in clinical trials. Davies and co-worker showed that tolerizing bone marrow cells *ex vivo* in the presence of CTLA-4Ig prior to transplantation to a MHC-matched recipient reduces the incidence of acute and chronic GvHD^[108]. Furthermore, CTLA-4Ig (abatacept) treatment in combination with cyclosporin and methotrexate prevents acute GvHD after hematopoietic cell transplantation from an unrelated donor^[109]. Since 2005, CTLA-4Ig (abatacept) is approved by the FDA for the treatment of rheumatoid arthritis (RA)^[110]

and a second-generation molecule (belatacept) with higher binding affinity for B7-1 and B7-2 was approved in 2011 to prevent rejection after renal transplantation^[111].

Blocking the CD40/CD40L pathway

Antagonistic anti-CD40L monoclonal antibodies (mAb) have shown impressive effects in many animal models. Blocking CD40L prevents acute and chronic GvHD^[112]. If given at the time of transplantation, anti-CD40L treatment prolongs graft survival in a model of heart, islet, liver and limb transplantation^[113-116]. Targeting the CD40L receptor proved to be efficient in animal models of autoimmune diseases such as EAE, arthritis, SLE, colitis and arteriosclerosis^[117]. However, clinical trials with an anti-CD40L mAb (Ruplizumab) in SLE patients have led to thromboembolic side-effects and had to be halted^[118]. This effect was caused by the Fc-fragment of the antibody bound to a receptor on platelets which also express CD40L. Nonetheless, the findings in animal systems are extremely promising and, consequently, it is attempted to find alternative ways to achieve CD40L blockade. mAb with an engineered, aglycosylated or mutated Fc-part were created^[119-121]. The modifications alter the antibody in a way that Fc-receptor or complement mediated platelet aggregation and subsequent thromboembolic events are prevented. Furthermore, alternative blocking reagents such as small molecules or peptides are currently explored^[122,123].

The CD40/CD40L interaction can also be interrupted by targeting the CD40 receptor. A human antagonistic anti-CD40 antibody showed some effect in *ex vivo* studies^[124,125] and proved to be safe in a Phase I clinical trial on lymphocytic leukaemia patients^[126]. Another antagonistic anti-CD40 antibody, chimeric 5D12, was tested successfully in an EAE model in marmoset monkeys^[127]. Furthermore, we showed that 5D12 was well tolerated in a phase I clinical trial in patients with Crohn's disease^[128]. However, CD40 is expressed on many different cell types and consequently targeting this molecule might have broad and undesired effects. Additionally, most antibodies directed against CD40 are stimulatory for APC and B cells by cross-linking the trimeric receptor.

Combined blockade of the CD28/B7 and the CD40/CD40L pathway

Although CTLA-4Ig and anti-CD40L antibodies show great potential in various disease models, the combination of both is often superior. It is indeed possible that in the absence of CD40L or CD28 triggering, the T cell can still receive sufficient activation signals from other costimulatory pathways^[129,130]. Especially in animal models of solid organ transplantation, combined blockade of CD28/B7 and CD40/CD40L is required for permanent tolerance induction in mice^[131] and non-human primates^[132]. Also, in animal models of leukaemia^[133] or autoimmune diseases such as EAE^[134] and SLE^[135], the combination of CTLA-4Ig and MR1 (an anti-CD40L mAb) could more effectively reduce disease symptoms than both alone.

We made similar observations in a fully MHC mismatch model of GvHD with allogeneic bone marrow transfer. In our study, only the combined blockade of the CD28/B7 pathway (using CTLA-4Ig) and the CD40/CD40L pathway (using MR1) prevented lethal GvHD and resulted in long-lasting tolerance and the induction of stable mixed chimerism^[136].

Mechanisms of suppression by CD28/CTLA-4/B7 and CD40/CD40L blockade

The mechanisms of tolerance induction by costimulation blockade, in particular of the CD28/CTLA-4/B7 and the CD40/CD40L interaction, have extensively been studied in allo-responses such as GvHD or transplant rejection. In these settings, deprivation of necessary activation signals (CD28 and/or CD40 triggering) leads to T cell hypo-responsiveness^[8], which is followed by peripheral clonal deletion^[136,137]. Elimination of the hypo-responsive T cells is predominantly mediated by apoptosis^[138-140]. In a fully miss-matched transplantation model, the tolerising effect of combined CD28/B7 (using CTLA-4Ig) and CD40/CD40L (using MR1) blockade can be reversed by the calcineurin inhibitor cyclosporine A (CsA), which prevents apoptosis^[138]. In contrast, rapamycin (which favours apoptosis) acts synergistically with costimulation blockade. While activation induced cell death (AICD) seems not to be essential, passive cells death is crucial for the induction of tolerance under the cover of CTLA-4Ig and MR1. Heart allografts were rejected in Bcl-xL deficient mice despite costimulation blockade^[139], but Fas-deficiency was not able to break tolerance^[140]. Additionally, CTLA-4Ig has been suggested to act *via* reverse signalling to APCs and to induce IDO production, which contributes to creating a suppressive environment^[141].

THE ROLE OF TREG CELLS IN IMMUNE SUPPRESSION BY COSTIMULATION BLOCKADE

Although apoptosis of Teff cells after activation in the absence of costimulatory has been demonstrated by many research groups, complete deletion of responsive T cells takes several weeks^[137] while tolerance can already be observed shortly after treatment^[142]. In this context, it has been demonstrated by the group of Waldmann that CD4⁺ cells, which have been tolerized to allo-antigens by CD40L blockade, are not only hypo-responsive but moreover display a suppressive function^[143,144]. Therefore, it has been suggested that Treg cells, at least partially, mediate tolerance until Teff cells have been eliminated. In line with this, it has been demonstrated that tolerance induction by CD40L or B7 blockade is abrogated when Treg cells are depleted. In a study performed by Taylor and co-workers, CD4⁺ cells were tolerized to allo-antigens *ex vivo* in the presence of antagonistic anti-CD40L or anti-B7 antibodies. Transfer of these cells to animals suffering from GvHD did abrogate the disease. However,

if Treg cells were depleted prior to the transfer, GvHD was not suppressed^[145]. Also, long-term acceptance of a skin or a heart allograft under the cover of CD40L blockade could be abrogated if recipient Treg cells were depleted^[146,147]. However, Kurtz *et al.*^[148] showed that it is possible to induce mixed chimerism after allogeneic bone marrow transplantation under the cover of CD40L blockade, but they did not find evidence for an involvement of Treg in this system. In line with this, we have previously shown in a model of GvHD with allogeneic bone marrow transplantation that tolerance induction by combined CD40/CD40L and CD28/B7 blockade and the development of mixed chimerism are still possible despite the absence of donor Treg cells^[136]. In both studies T cell hypo-responsiveness and deletion were the main mechanisms by which tolerance was achieved. The importance of Treg cells for tolerance induction by costimulation blockade thus might depend on the disease model. The recipient Treg cells might be important in the setting of a solid organ transplant, while in GvHD the presence of Treg cells within the donor cell transplant might not be crucial for the outcome of the disease.

Costimulatory requirements of Treg cells

Involvement of Treg cells in tolerance induction by costimulation blockade implies that Teff cells and Treg cells have different requirements regarding costimulation. Such different requirements could result in differential modulation of Teff cells and Treg cells by costimulation blockade. Both cell types share the TCR-mediated recognition of an antigen as the first signal for activation. However, the costimulatory requirements for Treg cells are less clear than those for Teff cells (Figure 1). CD28/B7 signalling is crucial for thymic Treg cell generation and homeostasis since mice deficient in CD28 or B7 molecules have a significantly reduced number of Treg cells in the thymus as well as in the periphery^[149,150]. CD40L and glucocorticoid-induced tumour necrosis factor related receptor (GITR) signalling also play an important role during thymic development of Treg cells^[151-153]. Whether CD28 and/or CD40L costimulation is equally important for the activation or the induction of Treg cells in peripheral lymphoid organs as it is for Teff cells, however, is still a matter of debate. We have shown that blocking the B7 molecules using anti-B7-1 and anti-B7-2 antibodies in combination with an antagonistic anti-CD40 antibody resulted in human T cell hypo-responsiveness *in vitro*. This effect was associated with the induction of a T cell subset with suppressive activity, which expressed high levels of ICOS and produced IL-10^[154]. Furthermore, we have shown that the beneficial effect of combined CTLA-4Ig and MR1 treatment in a mouse model of GvHD is associated with an increase in the frequency of Foxp3⁺ Treg cells between day 6 and 30 after T cell transfer^[136]. Both findings argue for costimulation independent Treg induction and expansion. We further conducted a more detailed examination of the effect of CTLA-4Ig and MR1 on murine Treg cells *in vitro*. Here, we showed that Treg cells can proliferate

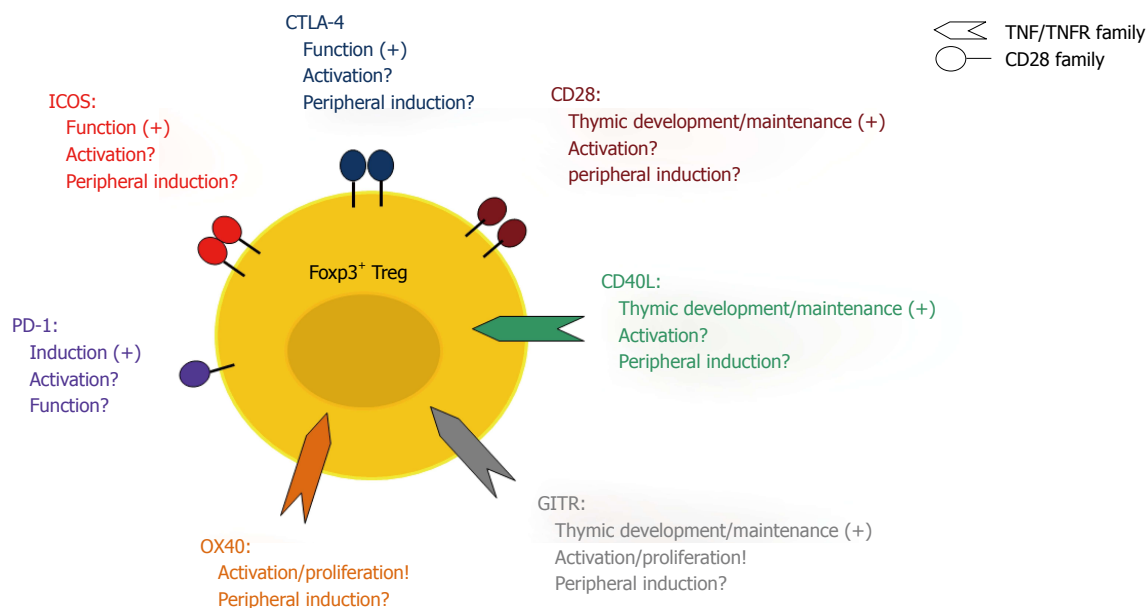


Figure 1 Costimulatory requirements of Foxp3⁺ Treg cells. Treg cells, similar to Teff cells, depend on T cell receptor (TCR)-mediated recognition of an antigen for activation (signal 1). The requirements of Treg cells regarding the second, costimulatory signal are less clear. The exact pathways and necessary signals are still a matter of debate. So far, it is well established that Treg cells depend on CD28 and CD40L for their thymic development. Also, glucocorticoid-induced tumour necrosis factor related receptor (GITR) is stabilizing Foxp3 expression during maturation in the thymus. In order to get properly activated and to proliferate, triggering of OX40 and GITR, in concert with IL-2, was reported to be crucial. The induction of iTreg cells in the periphery is promoted by PD-1 signalling. The function of Treg cells depends on cytotoxic T lymphocyte antigen (CTLA)-4 and ICOS. See text for more details and references.

and be activated if CTLA-4Ig and MR1 were added to the cultures at a dose where Teff cells are inhibited^[155]. Also other laboratories, in which the blockade of the CD28/B7 and/or the CD40/CD40L interaction was studied, have observed an increase of functional Treg cells *in vitro*^[145,156]. Furthermore, a selective non-cross-linking CD28 antagonist induced tolerance to renal and cardiac allografts in non-human primates and this was associated with an increased frequency of Foxp3⁺ Treg cells^[157]. In a mouse model of heart transplantation under the cover of an anti-CD40L mAb, Treg cell were functional and crucial to prevent rejection^[147]. Altogether, these findings suggests that Treg cells are less dependent on CD28/B7 and CD40/CD40L costimulation compared to Teff cells and can therefore still be activated and expand in the presence of CTLA-4Ig and MR1.

However, Treg cells are probably not completely independent of CD28/B7 and CD40/CD40L costimulation. In this context, we showed that the increase in the Treg cell frequency *in vitro* observed in the presence of CTLA-4Ig and MR1 is dependent on the concentration of the blocking agents. While a low dose of CTLA-4Ig and MR1, ranging between 0.125 µg/mL and 4 µg/mL, resulted in a concentration dependent increase in the frequency of Treg cells, a higher dose (between 8 µg/mL and 32 µg/mL) resulted in a concentration dependent decrease in the frequency of the Treg cells (manuscript in preparation). Thus, at a very high dose of costimulatory blocking agents, Treg cells also seem to be affected. We further explored this issue in a mouse model of GvHD. A treatment regime using 500 µg (per mouse) of CTLA-4Ig (in combination with MR1) was equally effective as a

10 times lower dose in preventing the disease. However, intermediate doses had no effect on survival. Again, the treatment with a low dose of CTLA-4Ig, but not with a high dose, was followed by an increase in Treg cell frequency (manuscript in preparation). This observation can potentially be explained assuming two separate mechanisms of action (Figure 2): treatment with a high dose blocks all the Teff cells (but also the Treg cells) and therefore prevents the disease. At a low dose, however, not all the Teff cells are blocked, but Treg cells remain activated and are able to suppress the remaining Teff cells. Intermediate doses are not effective, most likely because not all the Teff cells are blocked while at the same time Treg cells are affected and therefore not able to suppress Teff cells. It is possible that Treg cells need the same costimulatory signals as Teff cells, but have a lower threshold for activation. Another possibility is that a low dose of CTLA-4Ig and MR1 only partially blocks the Teff cells, which produce low amounts of IL-2. As Treg cells can take up IL-2 more efficiently than Teff cells due to the constitutive expression of the high affinity IL-2 receptor (CD25)^[95], the low amounts of IL-2 might be sufficient to maintain Treg cells but not enough to allow for Teff cell priming and activation. This issue will have to be examined more closely in the future. If IL-2 and not costimulation is the limiting factor for Treg cell activation, expansion of Treg cells can be facilitated by adding exogenous IL-2.

Other costimulatory pathways have been suggested to be relevant for Treg cell activation and function. Triggering GITR on Treg cells increases their proliferation and enforces their suppressive activity^[158]. Blocking the

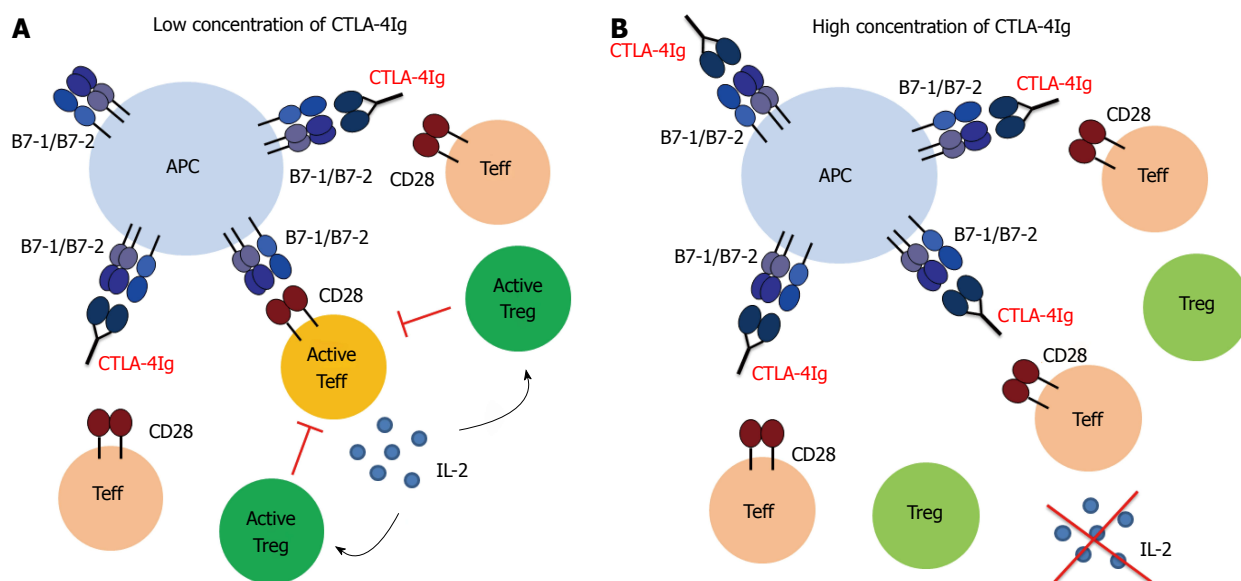


Figure 2 The potential effects of a cytotoxic T lymphocyte-associated antigen-4Ig treatment started before T cell priming. If cytotoxic T lymphocyte antigen (CTLA)-4Ig is given before T cell priming at a low dose, it reduces CD28 mediated T cell activation. Since blockade of B7 molecules is not complete, some Teff cells are still activated and produce interleukin (IL)-2. Treg cell-activation is less or not dependent on CD28 signalling and the low amounts of IL-2 produced by the Teff cells are sufficient to maintain Treg cell activation. In that way activated Treg cells further suppress the remaining Teff cells. The effect of CTLA-4Ig is in this situation based on down-regulation of Teff cells and maintenance of Treg activity (A). If CTLA-4Ig is given at a high concentration, Teff and Treg cells are equally suppressed due to missing costimulation and/or IL-2. The effect of CTLA-4Ig is in this case based on reduced Teff cell activity (B). APC: Antigen presenting cell, Teff: effector T cell, Treg: Regulatory T cell.

ICOS/ICOSL interactions in a model of ovalbumin (OVA) induced airway inflammation^[159] and EAE^[30] abrogated Treg activity *in vitro* and *in vivo*. An antagonistic anti-PD-1 antibody can prevent the induction of Treg cells from naïve CD4⁺ T cell *in vitro*, which suggests that PD-1 signalling is important in this process^[160]. Defects in or blockade of CTLA-4 leads to uncontrolled expansion of Treg cells, which suggests a cell-intrinsic effect of CTLA-4 triggering on Treg cells and an important role for CTLA-4 in regulating Treg generation in the thymus and in the periphery^[161,162]. Also, CTLA-4 regulates the TCR specificity during thymic development as over-expression of CTLA-4 leads to a self-skewed TCR repertoire whereas deficiency of CTLA-4 prevents the development of a self-skewed TCR repertoire^[163]. There is also evidence that CTLA-4 signalling is involved in the induction of Foxp3 in naïve T cells and promotes generation of iTreg cells in the periphery^[164]. In addition, CTLA-4 is a key mediator in suppression by Treg cells as described before. Recently, the OX40/OX40L pathway has come into focus with regard to Treg cell activation and proliferation. OX40 triggering acts in concert with IL-2 and leads to extensive Treg cell expansion. In the presence of IL-2, these cells are stable and show potent suppressive activity^[165].

The effect of CD40/CD40L blockade on Treg cells

A large body of evidence including our own studies suggests that Treg cells are not affected by CD40/CD40L blockade^[120,136,143-147,155]. Although Treg cells require CD40L signalling during their development in the thymus^[152,153], only about 4%-9% of Treg cells express

CD40L in the periphery^[166]. Up-regulation of CD40L in Treg cells upon activation is delayed compared to Teff cells, which express CD40L within the first 5 to 15 min after activation^[136]. This fast up-regulation is made possible through the storage of preformed CD40L (pCD40L). Treg cells, on the other hand, are incapable of storing pCD40L and consequently have to generate it *de novo*^[42,166]. Altogether this suggests that Treg cells are indeed not dependent on CD40L signalling concerning their activation. Therefore, CD40L blockade provides a promising target to modulate the balance between Treg cells and Teff cells in favour of Treg cell activity.

The effect of CTLA-4Ig on Treg cells

CTLA-4Ig has been proven to be very effective as an immunosuppressive treatment in various animal models and is successfully used in the clinic to treat rheumatoid arthritis (abatacept) and rejection after renal transplantation (belatacept)^[110,111]. However, recent findings have raised concern about the use of CTLA-4Ig in systems where Treg cells are crucial for the success of the therapy. Riella and co-workers showed that CTLA-4Ig accelerates transplant rejection in a MHC class II mismatch model, in which tolerance induction and graft survival is crucially dependent on Treg cell function^[167]. Furthermore, in a study in which rejection of a skin transplant could be prevented by expansion of Treg cells using IL-2/anti-IL-2 complexes, simultaneous administration of CTLA-4Ig could break tolerance induction^[168]. As mentioned before, we have observed a dose dependent effect of CTLA-4Ig on Treg cells (manuscript in preparation). It is possible that the amount of CTLA-4Ig applied was indeed high

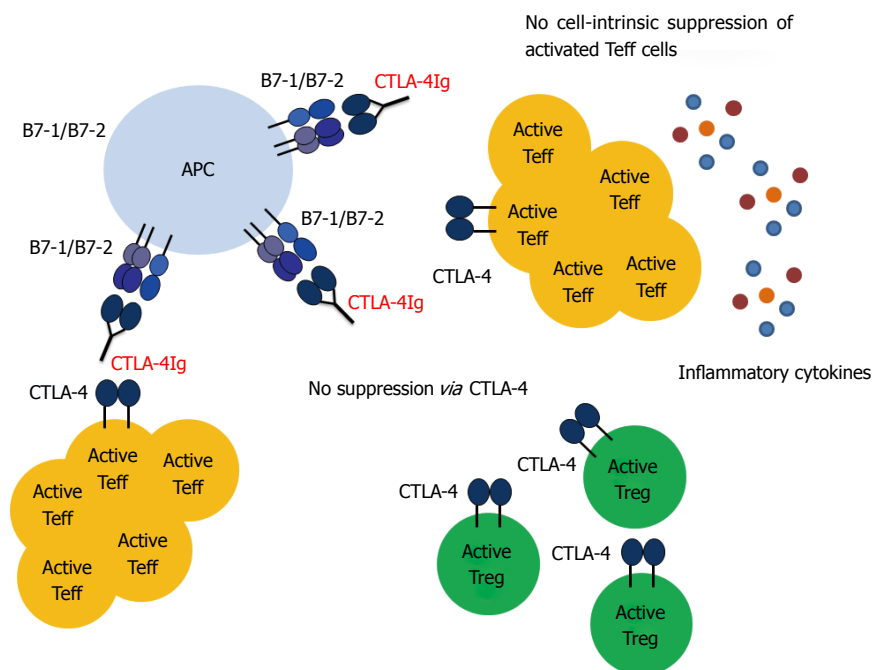


Figure 3 The potential effects of cytolytic T lymphocyte-associated antigen-4Ig treatment given after T cell priming. If cytolytic T lymphocyte antigen (CTLA)-4Ig is applied after priming when T cells are already activated, it mainly interferes with the CTLA-4/B7 interaction. This interaction is important for cell-intrinsic down-regulation of Teff cells. In the absence of this cell-intrinsic regulation, activated Teff cells proliferate more strongly and secrete more inflammatory cytokines. Furthermore, Treg cells are not able to exert their suppressive function mediated via CTLA-4. Both effects will potentially result in further increase of the T cell mediated inflammation. APC: Antigen presenting cell; Teff: Effector T cell; Treg: Regulatory T cell.

enough to interfere with the Treg cells. Especially in a model where Treg cells are crucial for the outcome of the disease, a high dose might be less effective than a low dose which spares the Treg cells.

The differential sensitivity of Treg cells vs Teff cells to CD28/CTLA-4/B7 blockade is certainly not the only problem that might arise from CTLA-4Ig treatment. Another factor that has to be considered is that CTLA-4Ig does not only interfere with the CD28/B7 signaling but also with the CTLA-4/B7 signaling (Figure 3). CTLA-4 is expressed on activated Teff cells and constitutively on Treg cells, and triggering of membrane CTLA-4 leads to suppression of the corresponding T cell^[18,20]. This holds true for Teff cells as well as for Treg cells^[169]. Since Treg cells express CTLA-4 constitutively, CTLA-4Ig administration during priming will presumably prevent CTLA-4 mediated cell-intrinsic suppression of Treg cells and will therefore enhance their activity. In addition, CTLA-4Ig engagement to the B7 ligands leads to reverse signalling to the APCs, which results in IDO production^[141]. Both mechanisms thus result in the creation of a suppressive environment. However, CTLA-4 is also a key molecule for Treg cell function^[81]. Our above mentioned data argue against interference of CTLA-4Ig with Treg cell activation, but do not exclude interference with Treg function or induction. In this context, blockade of the B7 molecules with CTLA-4Ig prevents CTLA-4 mediated trans-endocytosis and degradation of the B7 molecules by Treg cells as well as “reverse signalling” via CTLA-4/B7 signalling and IDO production. Moreover, if CTLA-4Ig is given after T cell priming, Teff cells will also have up-

regulated CTLA-4 and by blocking B7 molecules, the cell-intrinsic suppression of Teff cells might be blocked. This is not relevant in a setting of transplantation, when it is exactly known when T cell priming occurs. However, for patients with autoimmune diseases such as multiple sclerosis (MS), the situation is different. It is not possible to predict disease onset or a relapse episode and therefore it is not known when auto-reactive T cells are primed and activated. In such settings it might be dangerous to apply CTLA-4Ig treatment. Indeed, we have found in a model of experimental autoimmune encephalomyelitis (EAE), the mouse model for the human disease MS, that treatment with CTLA-4Ig after T cell priming leads to exacerbation of the disease. This is most likely due to interference with the CTLA-4/B7 mediated suppression (manuscript in preparation). Further studies will be required to examine if this exacerbation is a result of missing cell-intrinsic suppression of the Teff cells, interference with Treg cell function and *de novo* induction or both.

CONCLUSION

Based on the above discussed studies and our own results we believe that it can be possible to modulate costimulation in such a way that Teff cell activation is prevented but Treg cells can still be activated. Especially blockade of the CD40/CD40L pathway provides a promising target to manipulate the Teff/Treg cell balance in favor of Treg cell activity. However, blockade of the CD40/CD40L interaction alone is not always sufficient to guar-

antee full protection. Therefore, CD40/CD40L blockade must be combined with CTLA-4Ig in order to prevent CD28 mediated activation. Several factors have to be taken into account when using CTLA-4Ig as a treatment option. First, if CTLA-4Ig is given before T cell priming (*e.g.*, in a transplant setting), the dose of the reagent is an important factor. A high dose of CTLA-4Ig can also affect the Treg cells. Careful titration is required to find the optimal dose that blocks Teff cells but spares the Treg cells (Figure 2). This might be of great importance if Treg cells are crucial for the success of the therapy. Second, it has to be considered whether CTLA-4Ig is given before or after T cell priming. CTLA-4Ig treatment after T cell priming might be dangerous as it can interfere with CTLA-4 mediated suppression (Figure 3). This can affect cell-intrinsic suppression of the Teff cells and/or affect Treg cell function and induction. Third, knowing the pathophysiology of the disease (especially concerning involvement of Treg cells) is crucial in order to find a balance between maximal suppression of Teff cells and minimal interference with Treg cells.

It will be important to more closely study the costimulatory requirements of Treg cells and the effect of blocking those signals on their activity. This will help to improve the success of a therapy involving costimulation blockade. Especially when using CTLA-4Ig, it will be necessary to know exactly which effect the treatment has in the corresponding disease setting in order to prevent undesired effects. Furthermore, the finding that Treg cells and Teff cells respond differently to costimulation blockade can potentially be exploited in a context of Treg cells based therapy. Treg cells can be expanded *in vitro* or perhaps even *in vivo*, while the outgrowth of Teff cells is prevented under the cover of costimulation blockade.

REFERENCES

- 1 **Starr TK**, Jameson SC, Hogquist KA. Positive and negative selection of T cells. *Annu Rev Immunol* 2003; **21**: 139-176 [PMID: 12414722 DOI: 10.1146/annurev.immunol.21.120601.141107]
- 2 **Hogquist KA**, Baldwin TA, Jameson SC. Central tolerance: learning self-control in the thymus. *Nat Rev Immunol* 2005; **5**: 772-782 [PMID: 16200080 DOI: 10.1038/nri1707]
- 3 **Schwartz RH**. T cell anergy. *Annu Rev Immunol* 2003; **21**: 305-334 [PMID: 12471050 DOI: 10.1146/annurev.immunol.21.120601.141110]
- 4 **Grillot DA**, Merino R, Núñez G. Bcl-XL displays restricted distribution during T cell development and inhibits multiple forms of apoptosis but not clonal deletion in transgenic mice. *J Exp Med* 1995; **182**: 1973-1983 [PMID: 7500043]
- 5 **Lenardo MJ**. Interleukin-2 programs mouse alpha beta T lymphocytes for apoptosis. *Nature* 1991; **353**: 858-861 [PMID: 1944559 DOI: 10.1038/353858a0]
- 6 **Bretscher PA**. A two-step, two-signal model for the primary activation of precursor helper T cells. *Proc Natl Acad Sci USA* 1999; **96**: 185-190 [PMID: 9874793]
- 7 **Liu Y**, Linsley PS. Costimulation of T-cell growth. *Curr Opin Immunol* 1992; **4**: 265-270 [PMID: 1418704]
- 8 **Mueller DL**, Jenkins MK, Schwartz RH. Clonal expansion versus functional clonal inactivation: a costimulatory signaling pathway determines the outcome of T cell antigen receptor occupancy. *Annu Rev Immunol* 1989; **7**: 445-480 [PMID: 2653373 DOI: 10.1146/annurev.iy.07.040189.002305]
- 9 **Sharpe AH**, Freeman GJ. The B7-CD28 superfamily. *Nat Rev Immunol* 2002; **2**: 116-126 [DOI: 10.1038/nri727]
- 10 **Lenschow DJ**, Su GH, Zuckerman LA, Nabavi N, Jellis CL, Gray GS, Miller J, Bluestone JA. Expression and functional significance of an additional ligand for CTLA-4. *Proc Natl Acad Sci USA* 1993; **90**: 11054-11058 [PMID: 7504292]
- 11 **Hathcock KS**, Laszlo G, Pucillo C, Linsley P, Hodes RJ. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J Exp Med* 1994; **180**: 631-640 [PMID: 7519245]
- 12 **McAdam AJ**, Schweitzer AN, Sharpe AH. The role of B7 co-stimulation in activation and differentiation of CD4+ and CD8+ T cells. *Immunol Rev* 1998; **165**: 231-247 [PMID: 9850864]
- 13 **Jenkins MK**, Taylor PS, Norton SD, Urdahl KB. CD28 delivers a costimulatory signal involved in antigen-specific IL-2 production by human T cells. *J Immunol* 1991; **147**: 2461-2466 [PMID: 1717561]
- 14 **Appelmann LJ**, Berezovskaya A, Grass I, Boussiotis VA. CD28 costimulation mediates T cell expansion via IL-2-independent and IL-2-dependent regulation of cell cycle progression. *J Immunol* 2000; **164**: 144-151 [PMID: 10605005]
- 15 **Viola A**, Lanzavecchia A. T cell activation determined by T cell receptor number and tunable thresholds. *Science* 1996; **273**: 104-106 [PMID: 8658175]
- 16 **Boise LH**, Minn AJ, Noel PJ, June CH, Accavitti MA, Lindsten T, Thompson CB. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-XL. *Immunity* 1995; **3**: 87-98 [PMID: 7621080]
- 17 **Verbinnen B**, Van Gool SW, Ceuppens JL. Blocking costimulatory pathways: prospects for inducing transplantation tolerance. *Immunotherapy* 2010; **2**: 497-509 [PMID: 20636004 DOI: 10.2217/imt.10.31]
- 18 **Walunas TL**, Lenschow DJ, Bakker CY, Linsley PS, Freeman GJ, Green JM, Thompson CB, Bluestone JA. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994; **1**: 405-413 [PMID: 7882171]
- 19 **Qureshi OS**, Kaur S, Hou TZ, Jeffery LE, Poulter NS, Briggs Z, Kenefick R, Willox AK, Royle SJ, Rappoport JZ, Sansom DM. Constitutive clathrin-mediated endocytosis of CTLA-4 persists during T cell activation. *J Biol Chem* 2012; **287**: 9429-9440 [PMID: 22262842 DOI: 10.1074/jbc.M111.304329]
- 20 **Thompson CB**, Allison JP. The emerging role of CTLA-4 as an immune attenuator. *Immunity* 1997; **7**: 445-450 [PMID: 9354465]
- 21 **Carreno BM**, Bennett F, Chau TA, Ling V, Luxenberg D, Jussif J, Baroja ML, Madrenas J. CTLA-4 (CD152) can inhibit T cell activation by two different mechanisms depending on its level of cell surface expression. *J Immunol* 2000; **165**: 1352-1356 [PMID: 10903737]
- 22 **Collins AV**, Brodie DW, Gilbert RJ, Iaboni A, Manso-Sancho R, Walse B, Stuart DI, van der Merwe PA, Davis SJ. The interaction properties of costimulatory molecules revisited. *Immunity* 2002; **17**: 201-210 [PMID: 12196291]
- 23 **Waterhouse P**, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 1995; **270**: 985-988 [PMID: 7481803]
- 24 **Mandelbrot DA**, McAdam AJ, Sharpe AH. B7-1 or B7-2 is required to produce the lymphoproliferative phenotype in mice lacking cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). *J Exp Med* 1999; **189**: 435-440 [PMID: 9892625]
- 25 **Tai X**, Van Laethem F, Sharpe AH, Singer A. Induction of autoimmune disease in CTLA-4-/- mice depends on a specific CD28 motif that is required for *in vivo* costimulation. *Proc Natl Acad Sci USA* 2007; **104**: 13756-13761 [PMID: 17702861 DOI: 10.1073/pnas.0706509104]
- 26 **Bour-Jordan H**, Esensten JH, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone JA. Intrinsic and extrinsic control

- of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. *Immunol Rev* 2011; **241**: 180-205 [PMID: 21488898 DOI: 10.1111/j.1600-065X.2011.01011.x]
- 27 **Krummel MF**, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med* 1995; **182**: 459-465 [PMID: 7543139]
 - 28 **Hutloff A**, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R, Anagnostopoulos I, Kroczeck RA. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature* 1999; **397**: 263-266 [PMID: 9930702 DOI: 10.1038/16717]
 - 29 **Nurieva RI**. Regulation of immune and autoimmune responses by ICOS-B7h interaction. *Clin Immunol* 2005; **115**: 19-25 [PMID: 15870016 DOI: 10.1016/j.clim.2005.02.010]
 - 30 **Dong C**, Temann UA, Flavell RA. Cutting edge: critical role of inducible costimulator in germinal center reactions. *J Immunol* 2001; **166**: 3659-3662 [PMID: 11238604]
 - 31 **McAdam AJ**, Greenwald RJ, Levin MA, Chernova T, Malenkovich N, Ling V, Freeman GJ, Sharpe AH. ICOS is critical for CD40-mediated antibody class switching. *Nature* 2001; **409**: 102-105 [PMID: 11343122 DOI: 10.1038/35051107]
 - 32 **Agata Y**, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, Honjo T. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol* 1996; **8**: 765-772 [PMID: 8671665]
 - 33 **Freeman GJ**, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000; **192**: 1027-1034 [PMID: 11015443]
 - 34 **Latchman Y**, Wood CR, Chernova T, Chaudhary D, Borde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, Greenfield EA, Bourque K, Boussiotis VA, Carter LL, Carreno BM, Malenkovich N, Nishimura H, Okazaki T, Honjo T, Sharpe AH, Freeman GJ. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol* 2001; **2**: 261-268 [PMID: 11224527 DOI: 10.1038/85330]
 - 35 **Nishimura H**, Honjo T, Minato N. Facilitation of beta selection and modification of positive selection in the thymus of PD-1-deficient mice. *J Exp Med* 2000; **191**: 891-898 [PMID: 10704469]
 - 36 **van Kooten C**, Banchereau J. CD40-CD40 ligand. *J Leukoc Biol* 2000; **67**: 2-17 [PMID: 10647992]
 - 37 **Bishop GA**, Moore CR, Xie P, Stunz LL, Kraus ZJ. TRAF proteins in CD40 signaling. *Adv Exp Med Biol* 2007; **597**: 131-151 [PMID: 17633023 DOI: 10.1007/978-0-387-70630-6_11]
 - 38 **Mackey MF**, Barth RJ, Noelle RJ. The role of CD40/CD154 interactions in the priming, differentiation, and effector function of helper and cytotoxic T cells. *J Leukoc Biol* 1998; **63**: 418-428 [PMID: 9544571]
 - 39 **Karpusas M**, Hsu YM, Wang JH, Thompson J, Lederman S, Chess L, Thomas D. 2 A crystal structure of an extracellular fragment of human CD40 ligand. *Structure* 1995; **3**: 1031-1039 [PMID: 8589998]
 - 40 **Stark R**, Hartung A, Zehn D, Frentsch M, Thiel A. IL-12-mediated STAT4 signaling and TCR signal strength cooperate in the induction of CD40L in human and mouse CD8+ T cells. *Eur J Immunol* 2013; **43**: 1511-1517 [PMID: 23765345 DOI: 10.1002/eji.201243218]
 - 41 **Frentsch M**, Stark R, Matzmohr N, Meier S, Durlanik S, Schulz AR, Stervbo U, Jürchott K, Gebhardt F, Heine G, Reuter MA, Betts MR, Busch D, Thiel A. CD40L expression permits CD8+ T cells to execute immunologic helper functions. *Blood* 2013; **122**: 405-412 [PMID: 23719298 DOI: 10.1182/blood-2013-02-483586]
 - 42 **Koguchi Y**, Buenafe AC, Thauland TJ, Gardell JL, Bivins-Smith ER, Jacoby DB, Slifka MK, Parker DC. Preformed CD40L is stored in Th1, Th2, Th17, and T follicular helper cells as well as CD4+ 8- thymocytes and invariant NKT cells but not in Treg cells. *PLoS One* 2012; **7**: e31296 [PMID: 22363608 DOI: 10.1371/journal.pone.0031296]
 - 43 **Banchereau J**, Bazan F, Blanchard D, Brière F, Galizzi JP, van Kooten C, Liu YJ, Rousset F, Saeland S. The CD40 antigen and its ligand. *Annu Rev Immunol* 1994; **12**: 881-922 [PMID: 7516669 DOI: 10.1146/annurev.ry.12.040194.004313]
 - 44 **Foy TM**, Aruffo A, Bajorath J, Buhlmann JE, Noelle RJ. Immune regulation by CD40 and its ligand GP39. *Annu Rev Immunol* 1996; **14**: 591-617 [PMID: 8717526 DOI: 10.1146/annurev.immunol.14.1.591]
 - 45 **Van Kooten C**, Banchereau J. CD40-CD40 ligand: a multifunctional receptor-ligand pair. *Adv Immunol* 1996; **61**: 1-77 [PMID: 8834494]
 - 46 **Banchereau J**, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; **392**: 245-252 [PMID: 9521319 DOI: 10.1038/32588]
 - 47 **Rousset F**, Garcia E, Banchereau J. Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. *J Exp Med* 1991; **173**: 705-710 [PMID: 1705282]
 - 48 **Kawabe T**, Naka T, Yoshida K, Tanaka T, Fujiwara H, Sue-matsu S, Yoshida N, Kishimoto T, Kikutani H. The immune responses in CD40-deficient mice: impaired immunoglobulin class switching and germinal center formation. *Immunity* 1994; **1**: 167-178 [PMID: 7534202]
 - 49 **Grewal IS**, Flavell RA. The role of CD40 ligand in costimulation and T-cell activation. *Immunol Rev* 1996; **153**: 85-106 [PMID: 9010720]
 - 50 **Bennett SR**, Carbone FR, Karamalis F, Flavell RA, Miller JF, Heath WR. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 1998; **393**: 478-480 [PMID: 9624004 DOI: 10.1038/30996]
 - 51 **Croft M**. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol* 2009; **9**: 271-285 [PMID: 19319144 DOI: 10.1038/nri2526]
 - 52 **Seko Y**, Ishiyama S, Nishikawa T, Kasajima T, Hiroe M, Suzuki S, Ishiwata S, Kawai S, Tanaka Y, Azuma M, Kobata T, Yagita H, Okumura K, Nagai R. Expression of tumor necrosis factor ligand superfamily costimulatory molecules CD27L, CD30L, OX40L and 4-1BBL in the heart of patients with acute myocarditis and dilated cardiomyopathy. *Cardiovasc Pathol* 2002; **11**: 166-170 [PMID: 12031769]
 - 53 **Burgess JK**, Carlin S, Pack RA, Arndt GM, Au WW, Johnson PR, Black JL, Hunt NH. Detection and characterization of OX40 ligand expression in human airway smooth muscle cells: a possible role in asthma? *J Allergy Clin Immunol* 2004; **113**: 683-689 [PMID: 15100674 DOI: 10.1016/j.jaci.2003.12.311]
 - 54 **Wing K**, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat Immunol* 2010; **11**: 7-13 [PMID: 20016504 DOI: 10.1038/ni.1818]
 - 55 **Sakaguchi S**. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005; **6**: 345-352 [PMID: 15785760 DOI: 10.1038/ni1178]
 - 56 **Fontenot JD**, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 2005; **22**: 329-341 [PMID: 15780990 DOI: 10.1016/j.immuni.2005.01.016]
 - 57 **Fontenot JD**, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; **4**: 330-336 [PMID: 12612578 DOI: 10.1038/ni904]
 - 58 **Brunkow ME**, Jeffery EW, Hjerrild KA, Paepers B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001; **27**: 68-73 [PMID: 11138001 DOI: 10.1038/83784]
 - 59 **Wildin RS**, Ramsdell F, Peake J, Faravelli F, Casanova JL,

- Buist N, Levy-Lahad E, Mazzella M, Goulet O, Perroni L, Bricarelli FD, Byrne G, McEuen M, Proll S, Appleby M, Brunkow ME. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001; **27**: 18-20 [PMID: 11137992 DOI: 10.1038/83707]
- 60 **Chen W**, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. Conversion of peripheral CD4+CD25-naïve T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med* 2003; **198**: 1875-1886 [PMID: 14676299 DOI: 10.1084/jem.20030152]
- 61 **Fantini MC**, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. Cutting edge: TGF-beta induces a regulatory phenotype in CD4+CD25- T cells through Foxp3 induction and down-regulation of Smad7. *J Immunol* 2004; **172**: 5149-5153 [PMID: 15100250]
- 62 **Knoechel B**, Lohr J, Kahn E, Bluestone JA, Abbas AK. Sequential development of interleukin 2-dependent effector and regulatory T cells in response to endogenous systemic antigen. *J Exp Med* 2005; **202**: 1375-1386 [PMID: 16287710 DOI: 10.1084/jem.20050855]
- 63 **Petrusch U**, Jensen SM, Twitty C, Poehlein CH, Haley DP, Walker EB, Fox BA. Disruption of TGF-beta signaling prevents the generation of tumor-sensitized regulatory T cells and facilitates therapeutic antitumor immunity. *J Immunol* 2009; **183**: 3682-3689 [PMID: 19692636 DOI: 10.4049/jimmunol.0900560]
- 64 **Sawamukai N**, Satake A, Schmidt AM, Lamborn IT, Ojha P, Tanaka Y, Kambayashi T. Cell-autonomous role of TGFβ and IL-2 receptors in CD4+ and CD8+ inducible regulatory T-cell generation during GVHD. *Blood* 2012; **119**: 5575-5583 [PMID: 22496155 DOI: 10.1182/blood-2011-07-367987]
- 65 **Maldonado RA**, von Andrian UH. How tolerogenic dendritic cells induce regulatory T cells. *Adv Immunol* 2010; **108**: 111-165 [PMID: 21056730 DOI: 10.1016/B978-0-12-380995-7.0004-5]
- 66 **Elias KM**, Laurence A, Davidson TS, Stephens G, Kanno Y, Shevach EM, O'Shea JJ. Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway. *Blood* 2008; **111**: 1013-1020 [PMID: 17951529 DOI: 10.1182/blood-2007-06-096438]
- 67 **Xiao S**, Jin H, Korn T, Liu SM, Oukka M, Lim B, Kuchroo VK. Retinoic acid increases Foxp3+ regulatory T cells and inhibits development of Th17 cells by enhancing TGF-beta-driven Smad3 signaling and inhibiting IL-6 and IL-23 receptor expression. *J Immunol* 2008; **181**: 2277-2284 [PMID: 18684916]
- 68 **Bilate AM**, Lafaille JJ. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. *Annu Rev Immunol* 2012; **30**: 733-758 [PMID: 22224762 DOI: 10.1146/annurev-immunol-020711-075043]
- 69 **Thornton AM**, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, Shevach EM. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *J Immunol* 2010; **184**: 3433-3441 [PMID: 20181882 DOI: 10.4049/jimmunol.0904028]
- 70 **Verhagen J**, Wraith DC. Comment on "Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells". *J Immunol* 2010; **185**: 7129; author reply 7130 [PMID: 21127313 DOI: 10.4049/jimmunol.1090105]
- 71 **Akimova T**, Beier UH, Wang L, Levine MH, Hancock WW. Helios expression is a marker of T cell activation and proliferation. *PLoS One* 2011; **6**: e24226 [PMID: 21918685 DOI: 10.1371/journal.pone.0024226]
- 72 **Weiss JM**, Bilate AM, Gobert M, Ding Y, Curotto de Lafaille MA, Parkhurst CN, Xiong H, Dolpady J, Frey AB, Ruocco MG, Yang Y, Floess S, Huehn J, Oh S, Li MO, Niec RE, Rudensky AY, Dustin ML, Littman DR, Lafaille JJ. Neuro-pilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. *J Exp Med* 2012; **209**: 1723-1742, S1 [PMID: 22966001 DOI: 10.1084/jem.20120914]
- 73 **Groux H**, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; **389**: 737-742 [PMID: 9338786 DOI: 10.1038/39614]
- 74 **Miller A**, Lider O, Roberts AB, Sporn MB, Weiner HL. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor beta after antigen-specific triggering. *Proc Natl Acad Sci USA* 1992; **89**: 421-425 [PMID: 1370356]
- 75 **Weiner HL**. Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol Rev* 2001; **182**: 207-214 [PMID: 11722636]
- 76 **Thornton AM**, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998; **188**: 287-296 [PMID: 9670041]
- 77 **Takahashi T**, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* 1998; **10**: 1969-1980 [PMID: 9885918]
- 78 **Yamazaki S**, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K, Steinman RM. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J Exp Med* 2003; **198**: 235-247 [PMID: 12874257 DOI: 10.1084/jem.20030422]
- 79 **Tonkin DR**, He J, Barbour G, Haskins K. Regulatory T cells prevent transfer of type 1 diabetes in NOD mice only when their antigen is present in vivo. *J Immunol* 2008; **181**: 4516-4522 [PMID: 18802054]
- 80 **Wing K**, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. *Science* 2008; **322**: 271-275 [PMID: 18845758 DOI: 10.1126/science.1160062]
- 81 **Takahashi T**, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000; **192**: 303-310 [PMID: 10899917]
- 82 **Qureshi OS**, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, Baker J, Jeffery LE, Kaur S, Briggs Z, Hou TZ, Futter CE, Anderson G, Walker LS, Sansom DM. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* 2011; **332**: 600-603 [PMID: 21474713 DOI: 10.1126/science.1202947]
- 83 **Munn DH**, Sharma MD, Mellor AL. Ligation of B7-1/B7-2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. *J Immunol* 2004; **172**: 4100-4110 [PMID: 15034022]
- 84 **Dejean AS**, Beisner DR, Ch'en IL, Kerdiles YM, Babour A, Arden KC, Castrillon DH, DePinho RA, Hedrick SM. Transcription factor Foxo3 controls the magnitude of T cell immune responses by modulating the function of dendritic cells. *Nat Immunol* 2009; **10**: 504-513 [PMID: 19363483 DOI: 10.1038/ni.1729]
- 85 **Veldhoen M**, Moncrieffe H, Hocking RJ, Atkins CJ, Stockinger B. Modulation of dendritic cell function by naïve and regulatory CD4+ T cells. *J Immunol* 2006; **176**: 6202-6210 [PMID: 16670330]
- 86 **Asseman C**, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999; **190**: 995-1004 [PMID: 10510089]
- 87 **Li MO**, Sanjabi S, Flavell RA. Transforming growth factor-

- beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. *Immunity* 2006; **25**: 455-471 [PMID: 16973386 DOI: 10.1016/j.immuni.2006.07.011]
- 88 **Collison LW**, Workman CJ, Kuo TT, Boyd K, Wang Y, Vignali KM, Cross R, Sehy D, Blumberg RS, Vignali DA. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 2007; **450**: 566-569 [PMID: 18033300 DOI: 10.1038/nature06306]
 - 89 **Cao X**, Cai SF, Fehniger TA, Song J, Collins LI, Piwnicka-Worms DR, Ley TJ. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. *Immunity* 2007; **27**: 635-646 [PMID: 17919943 DOI: 10.1016/j.immuni.2007.08.014]
 - 90 **Andersson J**, Tran DQ, Pesu M, Davidson TS, Ramsey H, O'Shea JJ, Shevach EM. CD4⁺ FoxP3⁺ regulatory T cells confer infectious tolerance in a TGF-beta-dependent manner. *J Exp Med* 2008; **205**: 1975-1981 [PMID: 18710931 DOI: 10.1084/jem.20080308]
 - 91 **Bopp T**, Becker C, Klein M, Klein-Hessling S, Palmethofer A, Serfling E, Heib V, Becker M, Kubach J, Schmitt S, Stoll S, Schild H, Staeger MS, Stassen M, Jonuleit H, Schmitt E. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. *J Exp Med* 2007; **204**: 1303-1310 [PMID: 17502663 DOI: 10.1084/jem.20062129]
 - 92 **Ernst PB**, Garrison JC, Thompson LF. Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology. *J Immunol* 2010; **185**: 1993-1998 [PMID: 20686167 DOI: 10.4049/jimmunol.1000108]
 - 93 **Pace KE**, Lee C, Stewart PL, Baum LG. Restricted receptor segregation into membrane microdomains occurs on human T cells during apoptosis induced by galectin-1. *J Immunol* 1999; **163**: 3801-3811 [PMID: 10490978]
 - 94 **Pandiyar P**, Zheng L, Ishihara S, Reed J, Lenardo MJ. CD4⁺CD25⁺Foxp3⁺ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4⁺ T cells. *Nat Immunol* 2007; **8**: 1353-1362 [PMID: 17982458 DOI: 10.1038/ni1536]
 - 95 **Yu A**, Zhu L, Altman NH, Malek TR. A low interleukin-2 receptor signaling threshold supports the development and homeostasis of T regulatory cells. *Immunity* 2009; **30**: 204-217 [PMID: 19185518 DOI: 10.1016/j.immuni.2008.11.014]
 - 96 **Papiernik M**, de Moraes ML, Pontoux C, Vasseur F, Pénit C. Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. *Int Immunol* 1998; **10**: 371-378 [PMID: 9620592]
 - 97 **Wing JB**, Sakaguchi S. Multiple treg suppressive modules and their adaptability. *Front Immunol* 2012; **3**: 178 [PMID: 22754556 DOI: 10.3389/fimmu.2012.00178]
 - 98 **Read S**, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, Sharpe AH, Powrie F. Blockade of CTLA-4 on CD4⁺CD25⁺ regulatory T cells abrogates their function in vivo. *J Immunol* 2006; **177**: 4376-4383 [PMID: 16982872]
 - 99 **Suri-Payer E**, Cantor H. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by CD4⁺CD25⁺ T cells. *J Autoimmun* 2001; **16**: 115-123 [PMID: 11247637 DOI: 10.1006/jaut.2000.0473]
 - 100 **Vincenti F**. Costimulation blockade in autoimmunity and transplantation. *J Allergy Clin Immunol* 2008; **121**: 299-306; quiz 307-308 [PMID: 18269922 DOI: 10.1016/j.jaci.2008.01.002]
 - 101 **Lenschow DJ**, Zeng Y, Thistlethwaite JR, Montag A, Brady W, Gibson MG, Linsley PS, Bluestone JA. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science* 1992; **257**: 789-792 [PMID: 1323143]
 - 102 **Lin H**, Bolling SF, Linsley PS, Wei RQ, Gordon D, Thompson CB, Turka LA. Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. *J Exp Med* 1993; **178**: 1801-1806 [PMID: 8228826]
 - 103 **Blazar BR**, Taylor PA, Linsley PS, Valleria DA. In vivo blockade of CD28/CTLA4: B7/BB1 interaction with CTLA4-Ig reduces lethal murine graft-versus-host disease across the major histocompatibility complex barrier in mice. *Blood* 1994; **83**: 3815-3825 [PMID: 7515723]
 - 104 **Pearson TC**, Alexander DZ, Winn KJ, Linsley PS, Lowry RP, Larsen CP. Transplantation tolerance induced by CTLA4-Ig. *Transplantation* 1994; **57**: 1701-1706 [PMID: 8016872]
 - 105 **Cross AH**, Girard TJ, Giacchetto KS, Evans RJ, Keeling RM, Lin RF, Trotter JL, Karr RW. Long-term inhibition of murine experimental autoimmune encephalomyelitis using CTLA-4-Fc supports a key role for CD28 costimulation. *J Clin Invest* 1995; **95**: 2783-2789 [PMID: 7539461 DOI: 10.1172/JCI117982]
 - 106 **Lenschow DJ**, Ho SC, Sattar H, Rhee L, Gray G, Nabavi N, Herold KC, Bluestone JA. Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med* 1995; **181**: 1145-1155 [PMID: 7532678]
 - 107 **Finck BK**, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA4Ig. *Science* 1994; **265**: 1225-1227 [PMID: 7520604]
 - 108 **Davies JK**, Gribben JG, Brennan LL, Yuk D, Nadler LM, Guinan EC. Outcome of alloanergized haploidentical bone marrow transplantation after ex vivo costimulatory blockade: results of 2 phase 1 studies. *Blood* 2008; **112**: 2232-2241 [PMID: 18617635 DOI: 10.1182/blood-2008-03-143636]
 - 109 **Koura DT**, Horan JT, Langston AA, Qayed M, Mehta A, Khoury HJ, Harvey RD, Suessmuth Y, Couture C, Carr J, Grizzle A, Johnson HR, Cheeseman JA, Conger JA, Robertson J, Stempora L, Johnson BE, Garrett A, Kirk AD, Larsen CP, Waller EK, Kean LS. In vivo T cell costimulation blockade with abatacept for acute graft-versus-host disease prevention: a first-in-disease trial. *Biol Blood Marrow Transplant* 2013; **19**: 1638-1649 [PMID: 24047754 DOI: 10.1016/j.bbmt.2013.09.003]
 - 110 **Genovese MC**, Becker JC, Schiff M, Luggen M, Sherrer Y, Kremer J, Birbara C, Box J, Natarajan K, Nuamah I, Li T, Aranda R, Hagerty DT, Dougados M. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med* 2005; **353**: 1114-1123 [PMID: 16162882 DOI: 10.1056/NEJMoa050524]
 - 111 **Charpentier B**. Belatacept: a novel immunosuppressive agent for kidney transplant recipients. *Expert Rev Clin Immunol* 2012; **8**: 719-728 [PMID: 23167683 DOI: 10.1586/eci.12.79]
 - 112 **Blazar BR**, Taylor PA, Panoskaltsis-Mortari A, Buhlman J, Xu J, Flavell RA, Korngold R, Noelle R, Valleria DA. Blockade of CD40 ligand-CD40 interaction impairs CD4⁺ T cell-mediated alloreactivity by inhibiting mature donor T cell expansion and function after bone marrow transplantation. *J Immunol* 1997; **158**: 29-39 [PMID: 8977172]
 - 113 **Larsen CP**, Alexander DZ, Hollenbaugh D, Elwood ET, Ritchie SC, Aruffo A, Hendrix R, Pearson TC. CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation* 1996; **61**: 4-9 [PMID: 8560571]
 - 114 **Bumgardner GL**, Li J, Heininger M, Orosz CG. Costimulation pathways in host immune responses to allogeneic hepatocytes. *Transplantation* 1998; **66**: 1841-1845 [PMID: 9884287]
 - 115 **Molano RD**, Berney T, Li H, Cattani P, Pileggi A, Vizzardelli C, Kenyon NS, Ricordi C, Burckly LC, Inverardi L. Prolonged islet graft survival in NOD mice by blockade of the CD40-CD154 pathway of T-cell costimulation. *Diabetes* 2001; **50**: 270-276 [PMID: 11272136]
 - 116 **Tung TH**, Mackinnon SE, Mohanakumar T. Long-term limb allograft survival using anti-CD40L antibody in a murine model. *Transplantation* 2003; **75**: 644-650 [PMID: 12640303 DOI: 10.1097/01.TP.0000053756.90975.8E]
 - 117 **Law CL**, Grewal IS. Therapeutic interventions targeting CD40L (CD154) and CD40: the opportunities and challenges. *Adv Exp Med Biol* 2009; **647**: 8-36 [PMID: 19760064 DOI: 10.1007/978-0-387-89520-8_2]
 - 118 **Sidiropoulos PI**, Boumpas DT. Lessons learned from anti-

- CD40L treatment in systemic lupus erythematosus patients. *Lupus* 2004; **13**: 391-397 [PMID: 15230298]
- 119 CDP7657, a Monovalent Fab PEG Anti-CD40L Antibody, Inhibits Immune Responses in Both HuSCID Mice and Non-Human Primates. *Arthritis Rheum* 2010; Suppl 10: 1245 [DOI: 10.1002/art.29011]
- 120 Pinelli DF, Wagener ME, Liu D, Yamniuk A, Tamura J, Grant S, Larsen CP, Suri A, Nadler SG, Ford ML. An anti-CD154 domain antibody prolongs graft survival and induces Foxp3(+) iTreg in the absence and presence of CTLA-4 Ig. *Am J Transplant* 2013; **13**: 3021-3030 [PMID: 24007441 DOI: 10.1111/ajt.12417]
- 121 Daley SR, Cobbold SP, Waldmann H. Fc-disabled anti-mouse CD40L antibodies retain efficacy in promoting transplantation tolerance. *Am J Transplant* 2008; **8**: 2265-2271 [PMID: 18782294 DOI: 10.1111/j.1600-6143.2008.02382.x]
- 122 Margolles-Clark E, Umland O, Kenyon NS, Ricordi C, Buchwald P. Small-molecule costimulatory blockade: organic dye inhibitors of the CD40-CD154 interaction. *J Mol Med (Berl)* 2009; **87**: 1133-1143 [PMID: 19707732 DOI: 10.1007/s00109-009-0519-3]
- 123 Allen SD, Rawale SV, Whitacre CC, Kaumaya PT. Therapeutic peptidomimetic strategies for autoimmune diseases: costimulation blockade. *J Pept Res* 2005; **65**: 591-604 [PMID: 15885118 DOI: 10.1111/j.1399-3011.2005.00256.x]
- 124 Tai YT, Li X, Tong X, Santos D, Otsuki T, Catley L, Tournilhac O, Podar K, Hideshima T, Schlossman R, Richardson P, Munshi NC, Luqman M, Anderson KC. Human anti-CD40 antagonist antibody triggers significant antitumor activity against human multiple myeloma. *Cancer Res* 2005; **65**: 5898-5906 [PMID: 15994968 DOI: 10.1158/0008-5472.CAN-04-4125]
- 125 Luqman M, Klabunde S, Lin K, Georgakis GV, Cherukuri A, Holash J, Goldbeck C, Xu X, Kadel EE, Lee SH, Aukerman SL, Jallal B, Aziz N, Weng WK, Wierda W, O'Brien S, Younes A. The antileukemia activity of a human anti-CD40 antagonist antibody, HCD122, on human chronic lymphocytic leukemia cells. *Blood* 2008; **112**: 711-720 [PMID: 18497318 DOI: 10.1182/blood-2007-04-084756]
- 126 Byrd JC, Kipps TJ, Flinn IW, Cooper M, Odenike O, Bendiske J, Rediske J, Bilic S, Dey J, Baeck J, O'Brien S. Phase I study of the anti-CD40 humanized monoclonal antibody lucatumumab (HCD122) in relapsed chronic lymphocytic leukemia. *Leuk Lymphoma* 2012; **53**: 2136-2142 [PMID: 22475052 DOI: 10.3109/10428194.2012.681655]
- 127 Boon L, Brok HP, Bauer J, Ortiz-Bujsse A, Schellekens MM, Ramdien-Murli S, Blezer E, van Meurs M, Ceuppens J, de Boer M, 't Hart BA, Laman JD. Prevention of experimental autoimmune encephalomyelitis in the common marmoset (*Callithrix jacchus*) using a chimeric antagonist monoclonal antibody against human CD40 is associated with altered B cell responses. *J Immunol* 2001; **167**: 2942-2949 [PMID: 11509643]
- 128 Kasran A, Boon L, Wortel CH, Hogezaand RA, Schreiber S, Goldin E, Boer M, Geboes K, Rutgeerts P, Ceuppens JL. Safety and tolerability of antagonist anti-human CD40 Mab ch5D12 in patients with moderate to severe Crohn's disease. *Aliment Pharmacol Ther* 2005; **22**: 111-122 [PMID: 16011669 DOI: 10.1111/j.1365-2036.2005.02526.x]
- 129 Kopf M, Coyle AJ, Schmitz N, Barner M, Oxenius A, Galimore A, Gutierrez-Ramos JC, Bachmann MF. Inducible costimulator protein (ICOS) controls T helper cell subset polarization after virus and parasite infection. *J Exp Med* 2000; **192**: 53-61 [PMID: 10880526]
- 130 Howland KC, Ausubel LJ, London CA, Abbas AK. The roles of CD28 and CD40 ligand in T cell activation and tolerance. *J Immunol* 2000; **164**: 4465-4470 [PMID: 10779746]
- 131 Larsen CP, Elwood ET, Alexander DZ, Ritchie SC, Hendrix R, Tucker-Burden C, Cho HR, Aruffo A, Hollenbaugh D, Linsley PS, Winn KJ, Pearson TC. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* 1996; **381**: 434-438 [PMID: 8632801 DOI: 10.1038/381434a0]
- 132 Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH, Knechtle SJ. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci USA* 1997; **94**: 8789-8794 [PMID: 9238056]
- 133 Ito D, Ogasawara K, Iwabuchi K, Inuyama Y, Onoé K. Induction of CTL responses by simultaneous administration of liposomal peptide vaccine with anti-CD40 and anti-CTLA-4 mAb. *J Immunol* 2000; **164**: 1230-1235 [PMID: 10640735]
- 134 Schaub M, Issazadeh S, Stadlbauer TH, Peach R, Sayegh MH, Houry SJ. Costimulatory signal blockade in murine relapsing experimental autoimmune encephalomyelitis. *J Neuroimmunol* 1999; **96**: 158-166 [PMID: 10337914]
- 135 Wang X, Huang W, Mihara M, Sinha J, Davidson A. Mechanism of action of combined short-term CTLA4Ig and anti-CD40 ligand in murine systemic lupus erythematosus. *J Immunol* 2002; **168**: 2046-2053 [PMID: 11823542]
- 136 Verbinnen B, Billiau AD, Vermeiren J, Galicia G, Bullens DM, Boon L, Cadot P, Hens G, Dewolf-Peeters C, Van Gool SW, Ceuppens JL. Contribution of regulatory T cells and effector T cell deletion in tolerance induction by costimulation blockade. *J Immunol* 2008; **181**: 1034-1042 [PMID: 18606655]
- 137 Wekerle T, Sachs DH, Sykes M. Mixed chimerism for the induction of tolerance: potential applicability in clinical composite tissue grafting. *Transplant Proc* 1998; **30**: 2708-2710 [PMID: 9745551]
- 138 Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nat Med* 1999; **5**: 1298-1302 [PMID: 10545997 DOI: 10.1038/15256]
- 139 Wells AD, Li XC, Li Y, Walsh MC, Zheng XX, Wu Z, Nuñez G, Tang A, Sayegh M, Hancock WW, Strom TB, Turka LA. Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. *Nat Med* 1999; **5**: 1303-1307 [PMID: 10545998 DOI: 10.1038/15260]
- 140 Li XC, Li Y, Dodge I, Wells AD, Zheng XX, Turka LA, Strom TB. Induction of allograft tolerance in the absence of Fas-mediated apoptosis. *J Immunol* 1999; **163**: 2500-2507 [PMID: 10452986]
- 141 Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, Candeloro P, Belladonna ML, Bianchi R, Fioretti MC, Puccetti P. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat Immunol* 2002; **3**: 1097-1101 [PMID: 12368911 DOI: 10.1038/ni846]
- 142 Kurtz J, Ito H, Wekerle T, Shaffer J, Sykes M. Mechanisms involved in the establishment of tolerance through costimulatory blockade and BMT: lack of requirement for CD40L-mediated signaling for tolerance or deletion of donor-reactive CD4+ cells. *Am J Transplant* 2001; **1**: 339-349 [PMID: 12099378]
- 143 Honey K, Cobbold SP, Waldmann H. CD40 ligand blockade induces CD4+ T cell tolerance and linked suppression. *J Immunol* 1999; **163**: 4805-4810 [PMID: 10528180]
- 144 Graca L, Honey K, Adams E, Cobbold SP, Waldmann H. Cutting edge: anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. *J Immunol* 2000; **165**: 4783-4786 [PMID: 11045999]
- 145 Taylor PA, Noelle RJ, Blazar BR. CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J Exp Med* 2001; **193**: 1311-1318 [PMID: 11390438]
- 146 Quezada SA, Bennett K, Blazar BR, Rudensky AY, Sakaguchi S, Noelle RJ. Analysis of the underlying cellular mechanisms of anti-CD154-induced graft tolerance: the interplay of clonal anergy and immune regulation. *J Immunol* 2005; **175**: 771-779 [PMID: 16002673]

- 147 **Jiang X**, Sun W, Guo D, Cui Z, Zhu L, Lin L, Tang Y, Wang X, Liang J. Cardiac allograft acceptance induced by blockade of CD40-CD40L costimulation is dependent on CD4+CD25+ regulatory T cells. *Surgery* 2011; **149**: 336-346 [PMID: 20875655 DOI: 10.1016/j.surg.2010.08.012]
- 148 **Kurtz J**, Shaffer J, Lie A, Anosova N, Benichou G, Sykes M. Mechanisms of early peripheral CD4 T-cell tolerance induction by anti-CD154 monoclonal antibody and allogeneic bone marrow transplantation: evidence for anergy and deletion but not regulatory cells. *Blood* 2004; **103**: 4336-4343 [PMID: 14962909 DOI: 10.1182/blood-2003-08-2642]
- 149 **Tang Q**, Henriksen KJ, Boden EK, Tooley AJ, Ye J, Subudhi SK, Zheng XX, Strom TB, Bluestone JA. Cutting edge: CD28 controls peripheral homeostasis of CD4+CD25+ regulatory T cells. *J Immunol* 2003; **171**: 3348-3352 [PMID: 14500627]
- 150 **Salomon B**, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000; **12**: 431-440 [PMID: 10795741]
- 151 **Pacholczyk R**, Kern J. The T-cell receptor repertoire of regulatory T cells. *Immunology* 2008; **125**: 450-458 [PMID: 19128356 DOI: 10.1111/j.1365-2567.2008.02992.x]
- 152 **Martín-Gayo E**, Sierra-Filardi E, Corbí AL, Toribio ML. Plasmacytoid dendritic cells resident in human thymus drive natural Treg cell development. *Blood* 2010; **115**: 5366-5375 [PMID: 20357241 DOI: 10.1182/blood-2009-10-248260]
- 153 **McHugh RS**, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 2002; **16**: 311-323 [PMID: 11869690]
- 154 **Vermeiren J**, Ceuppens JL, Van Ghelue M, Witters P, Bullens D, Mages HW, Kroczeck RA, Van Gool SW. Human T cell activation by costimulatory signal-deficient allogeneic cells induces inducible costimulator-expressing anergic T cells with regulatory cell activity. *J Immunol* 2004; **172**: 5371-5378 [PMID: 15100277]
- 155 **Vogel I**, Verbinen B, Maes W, Boon L, Van Gool SW, Ceuppens JL. Foxp3+ regulatory T cells are activated in spite of B7-CD28 and CD40-CD40L blockade. *Eur J Immunol* 2013; **43**: 1013-1023 [PMID: 23348953 DOI: 10.1002/eji.201242737]
- 156 **Coenen JJ**, Koenen HJ, van Rijssen E, Hilbrands LB, Joosten I. Tolerizing effects of co-stimulation blockade rest on functional dominance of CD4+CD25+ regulatory T cells. *Transplantation* 2005; **79**: 147-156 [PMID: 15665762]
- 157 **Poirier N**, Azimzadeh AM, Zhang T, Dilek N, Mary C, Nguyen B, Tillou X, Wu G, Reneaudin K, Hervouet J, Martinet B, Coulon F, Allain-Launay E, Karam G, Soullillou JP, Pierson RN, Blanche G, Vanhove B. Inducing CTLA-4-dependent immune regulation by selective CD28 blockade promotes regulatory T cells in organ transplantation. *Sci Transl Med* 2010; **2**: 17ra10 [PMID: 20371478 DOI: 10.1126/scitranslmed.3000116]
- 158 **Igarashi H**, Cao Y, Iwai H, Piao J, Kamimura Y, Hashiguchi M, Amagasa T, Azuma M. GITR ligand-costimulation activates effector and regulatory functions of CD4+ T cells. *Biochem Biophys Res Commun* 2008; **369**: 1134-1138 [PMID: 18346459 DOI: 10.1016/j.bbrc.2008.03.024]
- 159 **Akbari O**, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyper-reactivity. *Nat Med* 2002; **8**: 1024-1032 [PMID: 12145647 DOI: 10.1038/nm745]
- 160 **Wang C**, Li Y, Proctor TM, Vandenbark AA, Offner H. Down-modulation of programmed death 1 alters regulatory T cells and promotes experimental autoimmune encephalomyelitis. *J Neurosci Res* 2010; **88**: 7-15 [PMID: 19642196 DOI: 10.1002/jnr.22181]
- 161 **Verhagen J**, Gabrysová L, Minaee S, Sabatos CA, Anderson G, Sharpe AH, Wraith DC. Enhanced selection of FoxP3+ T-regulatory cells protects CTLA-4-deficient mice from CNS autoimmune disease. *Proc Natl Acad Sci USA* 2009; **106**: 3306-3311 [PMID: 19218450 DOI: 10.1073/pnas.0803186106]
- 162 **Tang AL**, Teijaro JR, Njau MN, Chandran SS, Azimzadeh A, Nadler SG, Rothstein DM, Farber DL. CTLA4 expression is an indicator and regulator of steady-state CD4+ FoxP3+ T cell homeostasis. *J Immunol* 2008; **181**: 1806-1813 [PMID: 18641318]
- 163 **Yamaguchi T**, Kishi A, Osaki M, Morikawa H, Prieto-Martin P, Wing K, Saito T, Sakaguchi S. Construction of self-recognizing regulatory T cells from conventional T cells by controlling CTLA-4 and IL-2 expression. *Proc Natl Acad Sci USA* 2013; **110**: E2116-E2125 [PMID: 23690575 DOI: 10.1073/pnas.1307185110]
- 164 **Barnes MJ**, Griseri T, Johnson AM, Young W, Powrie F, Izcue A. CTLA-4 promotes Foxp3 induction and regulatory T cell accumulation in the intestinal lamina propria. *Mucosal Immunol* 2013; **6**: 324-334 [PMID: 22910217 DOI: 10.1038/mi.2012.75]
- 165 **Xiao X**, Gong W, Demirci G, Liu W, Spoerl S, Chu X, Bishop DK, Turka LA, Li XC. New insights on OX40 in the control of T cell immunity and immune tolerance in vivo. *J Immunol* 2012; **188**: 892-901 [PMID: 22147766 DOI: 10.4049/jimmunol.1101373]
- 166 **Li W**, Carlson TL, Green WR. Stimulation-dependent induction of CD154 on a subset of CD4+ FoxP3+ T-regulatory cells. *Int Immunopharmacol* 2011; **11**: 1205-1210 [PMID: 21496498 DOI: 10.1016/j.intimp.2011.03.021] Available]
- 167 **Riella LV**, Liu T, Yang J, Chock S, Shimizu T, Mfarrej B, Batal I, Xiao X, Sayegh MH, Chandraker A. Deleterious effect of CTLA4-Ig on a Treg-dependent transplant model. *Am J Transplant* 2012; **12**: 846-855 [PMID: 22300534 DOI: 10.1111/j.1600-6143.2011.03929.x]
- 168 **Charbonnier LM**, Vokaer B, Lemaître PH, Field KA, Leo O, Le Moine A. CTLA4-Ig restores rejection of MHC class-II mismatched allografts by disabling IL-2-expanded regulatory T cells. *Am J Transplant* 2012; **12**: 2313-2321 [PMID: 22759373 DOI: 10.1111/j.1600-6143.2012.04184.x]
- 169 **Tai X**, Van Laethem F, Pobezinsky L, Guinter T, Sharrow SO, Adams A, Granger L, Kruhlak M, Lindsten T, Thompson CB, Feigenbaum L, Singer A. Basis of CTLA-4 function in regulatory and conventional CD4(+) T cells. *Blood* 2012; **119**: 5155-5163 [PMID: 22403258 DOI: 10.1182/blood-2011-11-388918]

P- Reviewer: Camara NOS, Chui YL, Haque A **S- Editor:** Ji FF
L- Editor: A **E- Editor:** Wang CH



Immunopathogenesis of reactive arthritis: Role of the cytokines

Ricardo Javier Eliçabe, María Silvia Di Genaro

Ricardo Javier Eliçabe, María Silvia Di Genaro, Division of Immunology, Faculty of Chemistry, Biochemistry and Pharmacy, National University of San Luis, San Luis 5700, Argentine
 Ricardo Javier Eliçabe, María Silvia Di Genaro, Laboratory of Immunopathology, Multidisciplinary Institute of Biological Investigations San Luis, San Luis 5700, Argentine

Author contributions: Eliçabe RJ and Di Genaro MS designed the review and wrote the paper; Eliçabe RJ made the figure and contributed to the editing of the paper.

Supported by Agencia Nacional de Promoción Científica y Tecnológica, No. PICT 2008-763, PICT 2011-732; by the National University of San Luis (Project 0401); by the Scientific Career of National Council of Scientific and Technical Investigations; and by National Council of Scientific and Technical Investigations

Correspondence to: María Silvia Di Genaro, Professor, PhD, Laboratory of Immunopathology, Multidisciplinary Institute of Biological Investigations San Luis, 950 Ejército de los Andes Street, San Luis 5700, Argentina. sdigena@unsl.edu.ar

Telephone: +54-0266-4520300 Fax: +54-0266-4422644

Received: March 15, 2014 Revised: May 24, 2014

Accepted: June 14, 2014

Published online: July 27, 2014

Abstract

Reactive arthritis (ReA), also known as sterile postinfectious arthritis, belongs to the group of related arthropathies known as spondyloarthritis (SpA). ReA can arise 1-4 wk after a gastrointestinal or genitourinary infection, but once arthritis develops, the microorganism is not found in the joint. The classical microbes associated with ReA development include Gram-negative aerobic or microaerophilic bacteria containing LPS in their outer membrane. The immunopathogenic mechanisms involved in ReA development are still unknown. A hypothesis suggested that the bacteria probably persist outside the joint, at sites such as gut mucosa or lymph nodes, and bacterial antigens might then be transported to the joints. On the other hand, an altered immune response and the unbalanced production of cytokines have been reported in subjects with ReA. Cur-

rently, there is increased evidence to suggest that both mechanisms would operate in the immunopathogenesis of ReA. In this review we highlight recent advances on the role of cytokines in the ReA. Particularly, we discuss the roles of some pro- and anti-inflammatory cytokines involved in the immunopathogenesis of ReA.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Reactive arthritis; Cytokines; Immunopathogenesis; Infection; Interleukin-17; Interleukin-12; Interleukin-23; Interleukin-6; Tumor necrosis factor- α ; Interleukin-10

Core tip: The immunopathogenic mechanisms involved in reactive arthritis (ReA) development are still unknown. However, in the last years, increased evidence suggests that the immune response in particular certain cytokines could be involved in the pathogenesis of ReA. Currently, the use of biological agents that block the action of certain cytokines has contributed to improving the treatment of some rheumatic pathology. Understanding the role of cytokines in the pathogenesis of ReA could contribute to the development of future treatments. In this review, we highlight recent advances on the role of certain cytokines in the pathogenesis of ReA.

Eliçabe RJ, Di Genaro MS. Immunopathogenesis of reactive arthritis: Role of the cytokines. *World J Immunol* 2014; 4(2): 78-87 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/78.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.78>

INTRODUCTION

Reactive arthritis (ReA), also known as sterile postinfectious arthritis, belongs to the group of related arthropathies known as spondyloarthritis (SpA)^[1]. This group

also includes undifferentiated SpA, psoriatic arthritis (PsA), arthritis associated with inflammatory bowel disease and ankylosing spondylitis (AS). The SpA arthropathies have common several epidemiological, pathological, clinical and radiological features. ReA, as with other SpA, exhibits an absence of rheumatoid factor and has a genetic association with the molecule HLA-B27^[1-3]. ReA can arise 1-4 wk after a gastrointestinal or genitourinary infection, but once arthritis develops, the microorganism is not found in the joint^[2]. The ReA symptoms were recognized and studied in 1942 by Bauer and Engelmann, who associated these symptoms with those described in 1916 by the German physician Hans Reiter. At that time, Reiter described the clinical triad: arthritis, non-gonococcal urethritis and conjunctivitis in a German soldier after an episode of bloody diarrhea. So, Bauer and Engelmann coined the term Reiter's syndrome to describe this new pathology^[2]. However, most patients do not have the complete triad of symptoms. These observations drove Ahvonen to propose the name of ReA as the term most adapted to describe the "arthritis that happens during or after an infection in another site of the body without evidence of microorganisms in the joint"^[4]. Yet, this operational definition of ReA has led to uncertain diagnosis in different clinical settings. Thus, several attempts have been made to create classification criteria; however, lack of consensus has led to a failure to achieve any universally validated diagnostic criteria. Based on discussions at the 4th International Workshop on ReA, this term should be used only in patients with clinical features of ReA and in cases where a pathogen known to cause ReA is implicated^[5].

CLINICAL FEATURES

ReA most commonly affects young adults aged 20 to 40 years old and is rare in children^[6-8]. Both sexes are equally affected by ReA after a gastrointestinal infection, while ReA is more frequent in men when triggered by a urogenital tract infection^[3]. The presence of the HLA-B27 allele does not seem to be related to the onset of ReA; however, HLA-B27 positive patients have more severe arthritis with a tendency to progress to a chronic stage and they also have a greater chance of developing extra-articular symptoms. One hypothesis suggests that this molecule favors the cross-reaction between antigen and host, or it might be itself a target of the immune response^[9].

The symptoms of ReA typically start between 1 to 4 wk after the gastrointestinal infection. However, the triggering infection could be asymptomatic, such as *Chlamydia*-induced ReA, resulting in underdiagnosis^[2]. Clinical features of ReA are characterized by asymmetrical oligoarthritis, often in large joints of the lower extremities or in the upper extremities. A mild polyarticular form, particularly in the small joints, can also occur. Patients can have dactylitis. The typical extra-articular manifestations

are enthesitis, tendinitis and bursitis. ReA share these clinical characteristics and inflammatory back pain with other members of SpA, such as AS and PsA^[1]. Other extra-articular features include eye disease, where conjunctivitis is most prevalent, followed by acute anterior uveitis, and skin changes, such as erythema nodosum, keratoderma blennorrhagica and circinate balanitis^[3].

The clinical diagnosis is made based on the clinical symptoms. Evidence for infection triggering the arthropathy is most convincing when microbe isolation or antigen detection is successful. In this respect, fecal culture of enteric pathogens associated with ReA or the finding of *Chlamydia trachomatis* nucleic acids in urine, cervical or urethral swabs are secondary criteria used to confirm the diagnosis.

Animal models

Animal models of ReA have complemented studies in human materials. However, these animal models are limited since even when they are developed after bacterial infection as in human ReA, in some of them the route of infection was intravenous instead of oral. Table 1 shows animal models of ReA similar to the human form of the disease^[10-17]. We have described an experimental model useful for studying the pathogenesis of *Yersinia enterocolitica* (*Y. enterocolitica*) ReA. In our model, TNFRp55 deficient mice develop ReA after oral infection with *Y. enterocolitica* O: 3, the most common serotype associated with human ReA. *TNFRp55*^{-/-} mice exhibited macroscopic signs of severe and progressive arthritis with significantly higher clinical score compared with wild-type mice from d 14 to 56 after infection^[14]. Extensively, increased scores for inflammation and bone/cartilage degradation resulted when histopathological changes were analyzed in the joints. In these animals, we observed luminal disorganization of the synovial membrane, which was densely infiltrated with various types of leucocytes, sometimes concomitant with follicle formation. The articular cartilage and bone were degraded. Proliferation of synovial lining cells was also detected^[14,15]. This evidence and the data presented in Table 1 indicate ReA development in animal models that resemble this disease in humans. Nevertheless, the convergence of these models with human studies will contribute to understand the pathogenic mechanisms of ReA.

TRIGGERING BACTERIAL AND PATHOPHYSIOLOGY

The classical bacteria associated with gastrointestinal ReA are *Yersinia*, *Salmonella*, *Shigella* and *Campylobacter*, while *C. trachomatis* is by far the most common cause of ReA associated with genital infection^[3,18]. All these pathogens are Gram-negative aerobic or microaerophilic bacteria containing LPS in their outer membrane.

The immunopathogenic mechanisms involved in ReA development are still unknown. Even when bacterial

Table 1 Animal models of reactive arthritis similar to the human form of the disease

Animal	Bacteria	Route of infection	Arthritis onset/ remission	Clinical symptoms	Cytokine involved	Ref.
Lewis rats	<i>Y. enterocolitica</i> O:8 ¹	<i>iv</i> ¹	1 wk/6 wk	Polyarticular arthritis, erythema	ND	Hill <i>et al</i> ^[10]
DBA/2 and BDF1 mice	<i>Y. enterocolitica</i> O:8 plasmid cured ¹	<i>iv</i> ¹	Day 31/3 wk	Polyarticular arthritis	ND	Yong <i>et al</i> ^[11]
SHR rats	<i>Y. enterocolitica</i> O:8 ¹	<i>iv</i> ¹	1-4 wk/7-25 wk	Polyarticular arthritis, erythema, swelling and impaired movement of the joint	ND	Merilahti-Palo <i>et al</i> ^[12]
Swiss, BALB/ c and C3H/ HeJ mice	<i>Y. enterocolitica</i> O:3	<i>iv</i> ¹ /Oral	1-3 wk/2-8 mo	Monoarticular arthritis, swelling redness, deformations and conjunctivitis	ND	de los Toyos <i>et al</i> ^[13]
C57BL/6 <i>TNFRp55</i> ^{-/-} mice	<i>Y. enterocolitica</i> O:3	<i>ig</i>	2 wk/chronic until 8 wk	Polyarticular arthritis, swelling, erythema	IL-17 IFN- γ IL-6 IL-1 β	Di Genaro <i>et al</i> ^[14] Eliçabe <i>et al</i> ^[15]
BALB/c mice	<i>S. enteritidis</i>	<i>ig</i>	1 wk/ND	Synovial inflammation	TNF- α IL-17	Noto Llana <i>et al</i> ^[16] Noto Llana <i>et al</i> ^[17]

¹Different to the human form of the disease. ND: Not determined; *iv*: Intravenous infection; *ig*: Intragastric infection; IL: Interleukin; TNF: Tumor necrosis factor.

cultures of synovial fluids are negative in ReA, bacterial antigens have been found in the joints of patients. In *Chlamydia*-induced ReA, bacterial DNA and RNA have been detected in the joint, suggesting that live *Chlamydia* are present^[19-21]. Positive reaction of antibodies specific to *Salmonella* and *Yersinia* antigens in synovial fluid cells of ReA patients suggests the presence of bacterial antigen in the joint^[22,23]. Based on these findings, some authors have suggested that the bacteria probably persist outside the joint at sites such as gut mucosa or lymph nodes, and bacterial antigens might then be transported by monocytes to the joints^[24,25]. On the other hand, an altered immune response and the unbalanced production of cytokines have been reported in subjects with ReA^[26,27]. This altered immune response benefits the bacterial persistence and disfavors the elimination of the antigen by the host.

In this review, we highlight recent advances on the role of cytokines in ReA. Particularly, we discuss the roles of pro- and anti-inflammatory cytokines, especially interleukin (IL)-17, IL-12, IL-23, IL-6, tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ) as well as IL-10 in the pathophysiology of the ReA. Finally, we discuss the latest advances in the treatment of ReA based on the use of biological agents that neutralize the functions of certain cytokines, such as TNF- α or IL-6.

ROLE OF THE CYTOKINES IN ReA

Conflicting data have been reported on the production of cytokines in ReA patients. CD4⁺ T cells mediate immunity as a balance between different lineages of T helper (Th)-1, Th2, Th3 and Th17 which secrete IFN- γ , IL-4, TGF- γ and IL-17, respectively, as the main cytokine for each profile. Some studies revealed low levels of Th1 cytokines in ReA, especially of TNF- α but also of IFN- γ in peripheral blood and synovium^[28-34]. Since Th1 cells secreting IFN- γ and TNF- α have been proposed for bac-

terial clearance, defective Th1 response may contribute to bacterial persistence. Other data suggest that a Th2 cytokine profile and Th3 response with expression of TGF- β is common in ReA^[32]. Temporal relationships of these different Th1 and Th2 cytokines or blunting of initial cytokine response might also be important in the disease manifestations and its maintenance. On the other hand, the discovery of Th17 cells and their importance in the pathogenesis of chronic inflammatory diseases suggested that these cells may have a pathogenic role in ReA. However, the available studies are not large enough to support the role of certain cytokines in the pathogenesis of ReA.

NOVEL CYTOKINES IMPLICATED IN PATHOGENESIS OF ReA

IL-17

IL-17 is a 15-20-kDa glycoprotein produced by a novel subset of Th cells, termed Th17 cells, and to a lesser extent by innate lymphoid cells, including T-cells, innate-like lymphoid cells, mast cells and neutrophils^[35]. Th17 cells are critical in the pathogenesis of the arthritis, as demonstrated in several animal models^[36-38]. Th17 differentiation, survival and expansion depend on a variety of cytokines and transcription factors that work in concert to drive the induction of increased Th17 numbers. TGF- β in synergy with IL-6 has been described as the central factor involved in generating Th17 cells in mice. It has been shown in humans that TGF- β , IL-1 β and IL-6, combined with IL-21 or IL-23, can induce Th17 differentiation^[39]. IL-17 binds to IL-17RA/IL-17RC, which is expressed by a variety of cells, such as monocytes, lymphocytes, lymphoid tissue inducer cells, epithelial cells, synoviocytes, fibroblasts and keratinocytes^[35].

Th17 cell responses and IL-17 expression provide protection against bacterial and fungal pathogens through production and induction of inflammatory cytokines

and granulopoiesis, or by the recruitment of neutrophils. However, Th17 cells producing IL-17 have been suggested as the central effector lineage involved in the pathogenicity of ReA^[40]. Thus, it has been shown that ReA patients have elevated levels of IL-17 in synovial fluid and that this cytokine contributes to the development of joint inflammation^[40,41]. Furthermore, high expression of IL-17 was found in the synovial fluid of patients with SpA and an increased number of circulating memory Th17 cells has been recently reported in these patients^[42,43]. Moreover, in patients with *C. trachomatis*-induced ReA, increased percentages of IL-17-positive CD4⁺ T cells^[44] and higher IL-17 concentrations were detected in synovial fluid^[45].

Recent works suggest that *Salmonella*-induced ReA in mice dependent on CD4⁺ T cells secreting IL-17^[17]. Interestingly, these authors observed that the expression of IL-17 in the large intestine and in mesenteric lymph nodes (MLN) resembles that of popliteal and inguinal lymph nodes (ILN)^[17]. Accordingly, previous results from our laboratory demonstrated that IL-17 plays a major role in *Yersinia*-induced ReA^[15]. Furthermore, we detected a strong correlation among IL-17 levels in MLNs, ILNs and joints from *TNFRp55*^{-/-} mice with arthritis, supporting a link between the intestinal mucosa and the articular immune response. In addition, we observed that neutralization of IL-17 resulted in the abrogation of synovitis^[15]. In line with these results, other authors have reported recently that modulating intestinal IL-23/IL-17 expression by consumption of *Lactobacillus casei* prior to *Salmonella* infection in mice abolishes intestinal and joint inflammation^[46].

These data in animal models and patients support the hypothesis that Th17 cells may be involved in ReA pathogenesis. However, there are few reports for understanding and elucidating the true role of IL-17 in the pathogenesis of ReA.

IL-12 and IL-23

IL-12 and IL-23 are heterodimeric cytokines that share subunits and have important roles in autoimmunity. These IL-12 family cytokines share some biological characteristics but have functional differences. IL-12 is composed of two covalently linked subunits, IL-12p35 and IL-12p40, while IL-23 is composed of two covalently linked subunits, IL-23p19, which is distantly related to IL-12p35, and the IL-12p40 subunit^[47,48]. Furthermore, the receptors of IL-23 and IL-12 are also heterodimers that share the receptor 1 chain and have unique 2 chains^[49]. IL-12 is released by antigen presenting cells such as dendritic cells (DCs) and monocytes/macrophages in response to bacterial products and immune signals. Furthermore, IL-12 is the main stimulator of IFN- γ production by inducing development of Th1 responses^[49,50]. In addition, IL-23 is produced by macrophages and activated DCs and plays a crucial role in the generation of the Th17 cells. Since IL-12 has the ability to orchestrate the Th1 response, this cytokine plays a crucial role in the protective immunity

against many pathogens associated with ReA. Thus, the low concentrations of IL-12 have been linked to the bacterial persistence hypothesis and then to the pathogenesis of ReA^[28]. On the other hand, data on IL-23 concentrations in synovial fluid or serum of patients with ReA are limited, but high levels of IL-17 found in synovial fluids and sera of patients with ReA may reflect IL-23 activity. Moreover, abnormality of IL-12p40 gene expression in humans has been reported and IL-12 deficiency has been detected in patients with ReA^[51,52]. Yin *et al.*^[28] found that the balance of anti-inflammatory cytokines (IL-10) and IL-12 in the synovial fluid is also important. This may contribute to the decreased clearance of the bacteria or their components from the joint and lead to ReA^[28]. In relation to these findings, a recent study has shown that monocyte-derived macrophages from subjects with a history of ReA show low IL-12 and IL-23 production^[53]. Conversely, some authors have reported that IL-12/23p40 levels in synovial fluids of patients with ReA and other SpA are higher compared to synovial fluids of patients with osteoarthritis (OA) used as control^[41,54].

Interestingly, we demonstrated that the p40-deficient mice develop acute ReA after oral infection with *Y. enterocolitica*, suggesting that IL-12 or IL-23 could exert a protective effect on the development of ReA^[55]. However, we have observed elevated levels of p40 in regional lymph nodes to joints of *TNFRp55*^{-/-} mice with *Yersinia*-induced ReA. This effect has been accompanied by high levels of IFN- γ and IL-17 in affected joints^[15]. These results are in accordance with the concept that the IL-12/IL-23 pathway plays a dual role protecting from infection and eliciting tissue damage, and support future study to determine whether IL-12/23p40 could be a possible target for ReA treatment.

IL-6

IL-6 is a pleiotropic cytokine that is involved in numerous biological processes. The pleiotropy and redundancy of IL-6 functions have been identified by characterizing a unique receptor system comprising two functional proteins: a receptor specific for IL-6 (IL-6R)^[56] and gp130, the common signal transducer of cytokines related to IL-6, including the IL-12 family cytokines IL-27 and IL-35^[57,58]. In the early phase of infectious inflammation, IL-6 is produced by monocytes and macrophages immediately after the stimulation with distinct pathogen-associated molecular patterns. In noninfectious inflammation, damage-associated molecular patterns from damaged or dying cells stimulate monocytes and macrophages to produce IL-6. The pathogenic role of IL-6 in rheumatic diseases like rheumatoid arthritis (RA) has been well established. The critical role for IL-6 in the pathogenesis of RA is provided by clinical trials, in which tocilizumab, a humanized mAb specific for IL-6R, has been shown to suppress disease activity and erosive progression in patients with RA^[59]. In ReA, elevated IL-6 concentrations in the plasma and sera of the patients has been reported^[60,61]. Moreover, synovial fluid concentrations of

IL-6 were higher in patients with ReA^[41]. Interestingly, we found that mice TNFRp55-deficient macrophages are hyperactivated to secrete common pro-inflammatory mediators such as NO and IL-6 following stimulation with *Yersinia* antigens. The higher concentrations of IL-6 production detected in stimulated TNFRp55^{-/-} macrophages may be associated with our previous *in vivo* results demonstrating the increased susceptibility of TNFRp55^{-/-} mice to *Yersinia*-induced ReA^[14]. Furthermore, higher concentrations of IL-6 were detected in the joints of these mice which showed a severe chronic synovitis^[15]. This data suggests that over-synthesis of IL-6 may be related to the development of ReA.

TNF- α

TNF- α is a cytokine prototype of a large family of over 40, known as TNF superfamily, and TNF receptor (TNFR) proteins. TNF- α is a cytokine with pleiotropic functions produced by a large number of cells, but are monocytic lineage cells (macrophages, astroglia, microglia, Kupffer cells and alveolar macrophages) major sources. Initially, this cytokine is produced as a pro-TNF and is expressed on the cell surface. Subsequently it is cleaved by the action of a metalloproteinase (TACE) and released into the extracellular medium as a soluble protein^[62]. Often, TNF- α is not detected in high concentrations in serum or tissues, but increases intensively on various inflammatory and infectious conditions. Two receptors, TNF-R1 (TNF receptor type 1; CD120a; p55/60) and TNF-R2 (TNF receptor type 2; CD120b; p75/80) bind to membrane-integrated TNF (memTNF) as well as soluble TNF- α (sTNF- α). In the vast majority of cells, TNF-R1 appears to be the key mediator of TNF- α signaling, whereas in the lymphoid system, TNF-R2 seems to play a major role. Low TNF- α secretion by blood mononuclear cells may be related to ReA development since TNF- α deficiency may interfere with eradication of bacterial infection in its early stages^[34,63-66]. However, other studies suggest that TNF- α could have a pathogenic role during the chronic stage of ReA in line with the role of this cytokine in RA. In this regard, some studies have revealed significant increase of TNF- α production in chronic ReA compared with acute ReA^[66]. These data support the possibility that anti-TNF- α treatment in ReA during the chronic phase of the disease could be beneficial. However, considering that TNF- α may be required for the elimination of ReA-associated bacteria, anti-TNF- α biologics might favor bacteria growth. Results obtained in our laboratory showed that TNFRp55 deficiency favors the development of ReA after infection with *Y. enterocolitica*^[14]. These data support the idea that the relative lack of TNF- α may play a protective role in ReA at acute phase of disease. On the other hand, we have demonstrated an *in vivo* regulatory role for TNFRp55 signaling in fine-tuning of Th17 and Th1 programs during bacterial-induced ReA through modulation of the common p40 subunit of IL-23 and IL-12^[15]. This evidence suggests that TNF- α might have a dual role in ReA, playing a protective role first and during the initial

stage. However, during the chronic stage of the disease, TNF- α would act as a pro-inflammatory cytokine.

IFN- γ

IFN- γ is produced mainly by natural killer (NK) cells and a particular subset of T cells, namely Th1 cells^[67]. As previously mentioned, IL-12 is the main stimulator of IFN- γ production^[47,50]. Thus, IL-12 and IFN- γ coordinate the link between pathogen recognition by innate immune cells and the induction of specific immunity by mediating a positive feedback loop to amplify the Th1 response. The functional IFN-receptor (IFN-R) consists of 2 ligand-binding IFNGR1 chains and 2 signal-transducing IFNGR2 chains^[68]. Mice deficient in IFN- γ or its receptor are susceptible to an array of intracellular pathogens^[69-71]. It was thought that Th1 cells cause damage in the joints mainly through IFN- γ driven inflammatory mechanisms. However, similar to TNF- α , conflicting data have been reported about the role of IFN- γ in ReA. As previously mentioned, some authors have reported an aberrant lower production of IFN- γ in patients with ReA^[28-34,52]. In contrast, in patients with *C. trachomatis*-induced ReA, the synovial fluid concentrations of IFN- γ were significantly higher than in OA patients but no significant differences were found between ReA and RA patients^[45]. Similar results were reported by Singh *et al*^[41]. Other studies have shown that the percentages of IFN- γ positive CD3⁺ cells were significantly higher in peripheral blood and synovial fluid of chronic ReA patients^[66]. These data support the idea that, as with TNF- α , IFN- γ may play a significant protective role in ReA in the acute phase of disease. However, in the chronic phase, this cytokine, as in RA, could play a pathogenic role in ReA.

IL-10

IL-10 is an anti-inflammatory cytokine with a major role in preventing inflammatory and autoimmune pathologies^[72]. Based on a large body of evidence, T cells are thought to be the main source of IL-10 *in vivo*. Regulatory T (Treg) subsets are also a key source of IL-10 *in vivo* and play a central role in mediating the inflammation control. However, it is now accepted that IL-10 is expressed by subsets of all CD4⁺ T helper populations, including Th1, Th2 and Th17^[73]. Nevertheless, this cytokine is also expressed by B cells and cells of the innate immune system (DCs, stimulated macrophages, mast cells, NK cells, eosinophils and neutrophils)^[74]. This cytokine binds to IL-10 receptor (IL-10R), which consists of two subunits. They are members of the interferon receptor family and belong to JAK/STAT3 class of receptors^[74]. Extensive studies have demonstrated that IL-10 inhibits the production of pro-inflammatory cytokines and chemokines in activated monocytes/macrophages and inhibits proliferation of CD4⁺ T cells^[75]. However, the role of IL-10 in ReA is less clear. Appel *et al*^[32] reported that the amount of IL-10 and TGF- β secreting cells was higher in ReA than in RA patients. This result was accompanied by a lower level of TNF- α secretion in ReA patients. Interest-

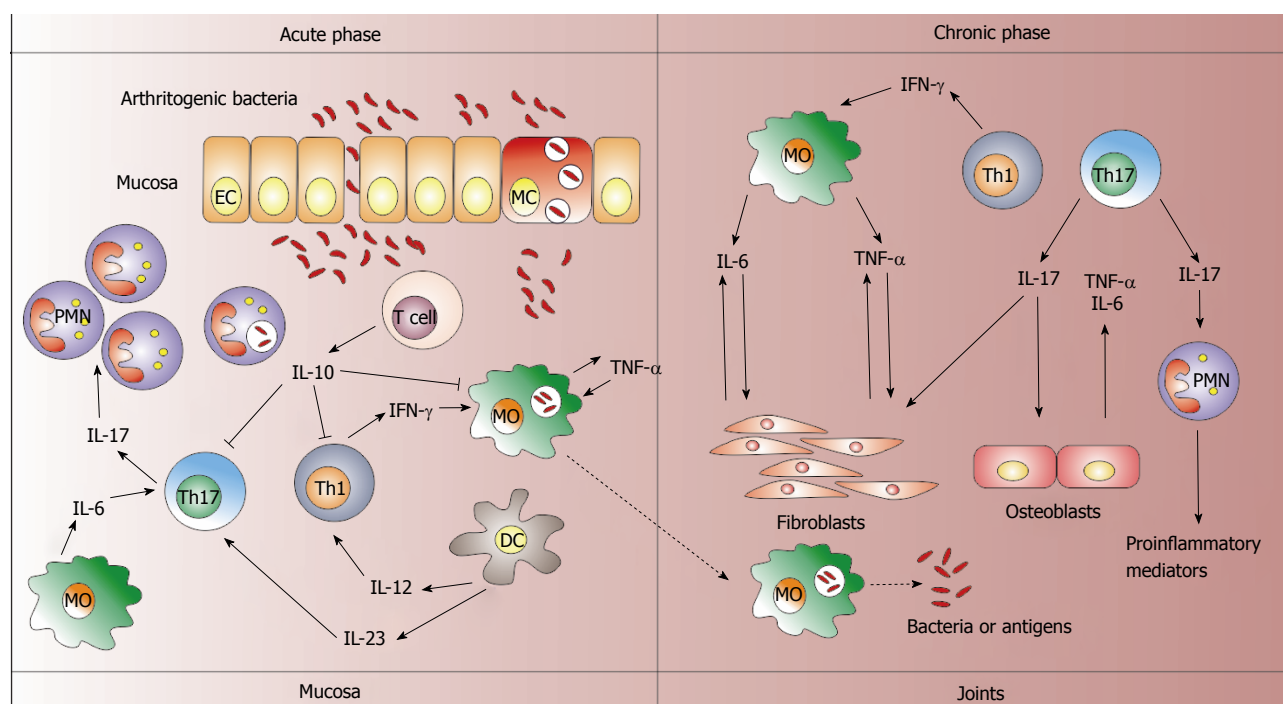


Figure 1 The role of cytokines in reactive arthritis depending on the state of disease. Arthritogenic bacteria enter through the gastrointestinal or genitourinary mucosa using different strategies (M cells; epithelial cells) and induce an inflammatory response. During the acute stage, interleukin (IL)-12 and IL-23 plus IL-6 promote the development of Th1 and Th17 cells, respectively. These cells are a major source of interferon (IFN)- γ and IL-17, favoring the bacterial clearance. The IFN- γ activates macrophages to kill phagocytosed bacteria and secrete tumor necrosis factor (TNF)- α . IL-17 induces the migration of polymorphonuclear cells to the site of infection. However, this effect could be disrupted by the action of regulatory T cells producing IL-10. This regulatory event contributes to the bacterial persistence in the mucosa. Then, the bacteria could reach the joint transported by macrophages. In chronic stages, IL-6, TNF- α , IFN- γ and IL-17 exert pro-inflammatory roles in the joint. These cytokines stimulate articular cells (e.g., fibroblasts, osteoblast) and immune cells to produce more cytokines and pro-inflammatory mediators that contribute to chronic articular inflammation. These effects may be enhanced by the presence of bacteria or bacterial antigens in the joint. EC: Epithelial cell; MC: M cell; MO: Macrophage; PMN: Polymorphonuclear cell; DC: Dendritic cell.

ingly, all ReA patients had a disease course of less than 6 mo. These authors suggest that this cytokine milieu might contribute to the lack of elimination of the triggering agent. Similar results were reported by Yin *et al.*^[28]. These authors found that synovial fluid mononuclear cells secreted low amounts of IFN- γ and TNF- α , but high amounts of IL-10 upon stimulation with specific bacteria, which was responsible for the suppression of IFN- γ and TNF- α ^[28]. There is also evidence indicating association of the IL-10 promoter region with the development of ReA. This raises the possibility that high levels of IL-10 in the joints of patients with ReA may be genetically determined, making these individuals more prone to the persistence of arthritogenic bacteria^[76].

Despite these clinical findings suggesting a pathogenic role of IL-10 in human ReA, IL-10 depletion and IL-10 treatment in other types of arthritis models have demonstrated the anti-inflammatory properties of IL-10 in arthritis^[77-80]. Results obtained in our laboratory showed that the number of Treg cells as well as the *FoxP3* mRNA expression and IL-10 levels were significantly decreased in joint regional lymph nodes of *TNFRp55*^{-/-} mice at the arthritis onset^[81]. These results would indicate that IL-10 plays a protective role during the acute phase of arthritis. However, the clinical evidence suggests that high levels of IL-10 could promote bacterial persistence, favoring

the development of ReA.

TREATMENT BASED ON BIOLOGICAL AGENTS

IL-6 antagonists

Published data on the effects of IL-6 blockade in patients with SpA are very scarce. Thus, in 1996 a report describes a patient with ReA who received a murine anti-IL-6 antibody^[82] and, in 2009, tocilizumab was reported to be successful in another patient with ReA^[83]. Only two injections of tocilizumab led to complete clinical remission from symptoms caused by ReA^[83]. Recently, Kwan *et al.*^[84] reported successful results of tocilizumab in the treatment of a case of ReA precipitated by intravesical bacillus Calmette-Guérin (BCG) which did not respond completely to disease modifying antirheumatic drugs (DMARDs). As previously mentioned, IL-6 is one of the cytokines that favor the differentiation of naïve T cells into Th17 cells^[39]. Therefore, it is possible that the inhibitory action of tocilizumab is exerted indirectly interfering with the differentiation of Th17 cells. These data indicate that IL-6 may play a pivotal role directly or indirectly in the pathogenesis of ReA and tocilizumab treatment can be an option for an alternative treatment.

TNF-antagonists

The pathogenic role during the critical stage of the disease supports the idea that TNF- α blocking agents could be an effective treatment for patients with ReA who develop severe arthritis that does not respond to conventional lines of treatment. Thus, Kaipainen-Seppönen *et al.*^[85] reported two cases of ReA post *Y. enterocolitica* treated early with infliximab (an anti-TNF- α antibody). One patient that received this treatment within 2 mo after the disease onset exhibited an improvement after the third infusion. The second patient that was treated after one month of evolution showed an immediate clinical improvement with almost complete regression after 15 d^[85]. Recently, Thomas-Pohl *et al.*^[86] obtained the same result in one patient with ReA triggered by a gastrointestinal infection. Similar results were reported by Edrees in a patient with a severe case of *C. trachomatis*-related ReA that was successfully treated with etanercept (a fusion protein of TNFRp75)^[87]. Thus, anti-TNF- α therapy has proved efficacious in some cases. However, sufficient data are lacking and theoretical concerns with their use remain. Large controlled trials are needed to evaluate the role of TNF- α blocking agents in ReA.

CONCLUSION

The network of cytokines is complex with feedback regulatory circuits that make it difficult to elucidate the role of a particular cytokine in ReA. In addition, the clinical reports of cytokine levels in patients with ReA have included patients in different stages of the disease or they are not large enough to support the role of different cytokines in ReA development. However, the current evidence in patients with anti-cytokine treatments suggests that IL-6 and TNF- α may play central roles in ReA pathogenesis. Furthermore, the IL-17/23 axis should be considered in the picture of ReA development, although further investigations are necessary for these cytokines. According to the presented evidence in this review, Figure 1 shows the different functions of the cytokines in ReA depending on the disease phases. Moreover, animal models may contribute to provide insight into the immunopathogenic mechanisms mediated by a particular cytokine in ReA and to support anti-cytokine treatments.

REFERENCES

- 1 Townes JM. Reactive arthritis after enteric infections in the United States: the problem of definition. *Clin Infect Dis* 2010; **50**: 247-254 [PMID: 20025528 DOI: 10.1086/649540]
- 2 Carter JD, Hudson AP. Reactive arthritis: clinical aspects and medical management. *Rheum Dis Clin North Am* 2009; **35**: 21-44 [PMID: 19480995 DOI: 10.1016/j.rdc.2009.03.010]
- 3 Hannu T. Reactive arthritis. *Best Pract Res Clin Rheumatol* 2011; **25**: 347-357 [PMID: 22100285 DOI: 10.1016/j.berh.2011.01.018]
- 4 Leirisalo-Repo M. Reactive arthritis. *Scand J Rheumatol* 2005; **34**: 251-259 [PMID: 16195157 DOI: 10.1080/03009740500202540]
- 5 Braun J, Kingsley G, van der Heijde D, Sieper J. On the difficulties of establishing a consensus on the definition of and diagnostic investigations for reactive arthritis. Results and discussion of a questionnaire prepared for the 4th International Workshop on Reactive Arthritis, Berlin, Germany, July 3-6, 1999. *J Rheumatol* 2000; **27**: 2185-2192 [PMID: 10990232]
- 6 Rudwaleit M, Richter S, Braun J, Sieper J. Low incidence of reactive arthritis in children following a salmonella outbreak. *Ann Rheum Dis* 2001; **60**: 1055-1057 [PMID: 11602478 DOI: 10.1136/ard.60.11.1055]
- 7 Mattila L, Leirisalo-Repo M, Koskimies S, Granfors K, Siitonen A. Reactive arthritis following an outbreak of Salmonella infection in Finland. *Br J Rheumatol* 1994; **33**: 1136-1141 [PMID: 8000742 DOI: 10.1093/rheumatology/33.12.1136]
- 8 Mattila L, Leirisalo-Repo M, Pelkonen P, Koskimies S, Granfors K, Siitonen A. Reactive arthritis following an outbreak of Salmonella Bovismorbificans infection. *J Infect* 1998; **36**: 289-295 [PMID: 9661939 DOI: 10.1016/S0163-4453(98)94243-8]
- 9 Colmegna I, Cuchacovich R, Espinoza LR. HLA-B27-associated reactive arthritis: pathogenetic and clinical considerations. *Clin Microbiol Rev* 2004; **17**: 348-369 [PMID: 15084505 DOI: 10.1128/CMR.17.2.348-369.2004]
- 10 Hill JL, Yu DT. Development of an experimental animal model for reactive arthritis induced by Yersinia enterocolitica infection. *Infect Immun* 1987; **55**: 721-726 [PMID: 3493220]
- 11 Yong Z, Hill JL, Hirofuji T, Mander M, Yu DT. An experimental mouse model of Yersinia-induced reactive arthritis. *Microb Pathog* 1988; **4**: 305-310 [PMID: 3200165 DOI: 10.1016/0882-4010(88)90091-5]
- 12 Merilahti-Palo R, Gripenberg-Lerche C, Söderström KO, Toivanen P. Long term follow up of SHR rats with experimental yersinia associated arthritis. *Ann Rheum Dis* 1992; **51**: 91-96 [PMID: 1540047]
- 13 de los Toyos JR, Vázquez J, Sampedro A, Hardisson C. Yersinia enterocolitica serotype O: 3 is arthritogenic for mice. *Microb Pathog* 1990; **8**: 363-370 [PMID: 2215184 DOI: 10.1016/0882-4010(90)90095-8]
- 14 Di Genaro MS, Cargnelutti DE, Eliçabe JR, Lacoste MG, Valdez S, Gómez N, de Guzmán AM. Role of TNFRp55 in Yersinia enterocolitica O: 3-induced arthritis: triggering bacterial antigens and articular immune response. *Rheumatology (Oxford)* 2007; **46**: 590-596 [PMID: 17043042 DOI: 10.1093/rheumatology/kel348]
- 15 Eliçabe RJ, Cargnelutti E, Serer MI, Stege PW, Valdez SR, Toscano MA, Rabinovich GA, Di Genaro MS. Lack of TNFRp55 results in heightened expression of IFN- γ and IL-17 during the development of reactive arthritis. *J Immunol* 2010; **185**: 4485-4495 [PMID: 20810989 DOI: 10.4049/jimmunol.0902245]
- 16 Noto Llana M, Sarnacki SH, Giacomodonato MN, Caccuri RL, Blanco GA, Cerquetti MC. Sublethal infection with Salmonella Enteritidis by the natural route induces intestinal and joint inflammation in mice. *Microbes Infect* 2009; **11**: 74-82 [PMID: 19022393 DOI: 10.1016/j.micinf.2008.10.010]
- 17 Noto Llana M, Sarnacki SH, Vázquez MV, Gartner AS, Giacomodonato MN, Cerquetti MC. Salmonella enterica induces joint inflammation and expression of interleukin-17 in draining lymph nodes early after onset of enterocolitis in mice. *Infect Immun* 2012; **80**: 2231-2239 [PMID: 22493084 DOI: 10.1128/IAI.00324-12]
- 18 Rich E, Hook EW, Alarcón GS, Moreland LW. Reactive arthritis in patients attending an urban sexually transmitted diseases clinic. *Arthritis Rheum* 1996; **39**: 1172-1177 [PMID: 8670327 DOI: 10.1002/art.1780390715]
- 19 Schumacher HR, Gérard HC, Arayssi TK, Pando JA, Brangan PJ, Saaibi DL, Hudson AP. Lower prevalence of Chlamydia pneumoniae DNA compared with Chlamydia trachomatis DNA in synovial tissue of arthritis patients. *Arthritis Rheum* 1999; **42**: 1889-1893 [PMID: 10513803]
- 20 Bas S, Griffais R, Kvien TK, Glennäs A, Melby K, Vischer TL. Amplification of plasmid and chromosome Chlamydia

- DNA in synovial fluid of patients with reactive arthritis and undifferentiated seronegative oligoarthritis. *Arthritis Rheum* 1995; **38**: 1005-1013 [PMID: 7612032 DOI: 10.1002/art.1780380718]
- 21 **Gérard HC**, Carter JD, Hudson AP. Chlamydia trachomatis is present and metabolically active during the remitting phase in synovial tissues from patients with chronic Chlamydia-induced reactive arthritis. *Am J Med Sci* 2013; **346**: 22-25 [PMID: 23792903 DOI: 10.1097/MAJ.0b013e3182648740]
 - 22 **Granfors K**, Jalkanen S, von Essen R, Lahesmaa-Rantala R, Isomäki O, Pekkola-Heino K, Merilahti-Palo R, Saario R, Isomäki H, Toivanen A. Yersinia antigens in synovial-fluid cells from patients with reactive arthritis. *N Engl J Med* 1989; **320**: 216-221 [PMID: 2643047 DOI: 10.1056/NEJM198901263200404]
 - 23 **Granfors K**, Jalkanen S, Lindberg AA, Mäki-Ikola O, von Essen R, Lahesmaa-Rantala R, Isomäki H, Saario R, Arnold WJ, Toivanen A. Salmonella lipopolysaccharide in synovial cells from patients with reactive arthritis. *Lancet* 1990; **335**: 685-688 [PMID: 1690327 DOI: 10.1016/0140-6736(90)90804-E]
 - 24 **Zhang Y**, Gripenberg-Lerche C, Söderström KO, Toivanen A, Toivanen P. Antibiotic prophylaxis and treatment of reactive arthritis. Lessons from an animal model. *Arthritis Rheum* 1996; **39**: 1238-1243 [PMID: 8670337 DOI: 10.1002/art.1780390725]
 - 25 **Granfors K**, Merilahti-Palo R, Luukkainen R, Möttönen T, Lahesmaa R, Probst P, Märker-Hermann E, Toivanen P. Persistence of Yersinia antigens in peripheral blood cells from patients with Yersinia enterocolitica O: 3 infection with or without reactive arthritis. *Arthritis Rheum* 1998; **41**: 855-862 [PMID: 9588737]
 - 26 **Gracey E**, Inman RD. Chlamydia-induced ReA: immune imbalances and persistent pathogens. *Nat Rev Rheumatol* 2012; **8**: 55-59 [PMID: 22105240 DOI: 10.1038/nrrheum.2011.173]
 - 27 **Anttonen K**, Orpana A, Leirisalo-Repo M, Repo H. Aberrant TNF secretion by whole blood in healthy subjects with a history of reactive arthritis: time course in adherent and non-adherent cultures. *Ann Rheum Dis* 2006; **65**: 372-378 [PMID: 16107515 DOI: 10.1136/ard.2005.035972]
 - 28 **Yin Z**, Braun J, Neure L, Wu P, Liu L, Eggens U, Sieper J. Crucial role of interleukin-10/interleukin-12 balance in the regulation of the type 2 T helper cytokine response in reactive arthritis. *Arthritis Rheum* 1997; **40**: 1788-1797 [PMID: 9336412 DOI: 10.1002/art.1780401010]
 - 29 **Simon AK**, Seipelt E, Sieper J. Divergent T-cell cytokine patterns in inflammatory arthritis. *Proc Natl Acad Sci USA* 1994; **91**: 8562-8566 [PMID: 8078923 DOI: 10.1073/pnas.91.18.8562]
 - 30 **Smeets TJ**, Dolhain RJ, Breedveld FC, Tak PP. Analysis of the cellular infiltrates and expression of cytokines in synovial tissue from patients with rheumatoid arthritis and reactive arthritis. *J Pathol* 1998; **186**: 75-81 [PMID: 9875143]
 - 31 **Kotake S**, Schumacher HR, Arayssi TK, Gérard HC, Branigan PJ, Hudson AP, Yarboro CH, Klippel JH, Wilder RL. Gamma interferon and interleukin-10 gene expression in synovial tissues from patients with early stages of Chlamydia-associated arthritis and undifferentiated oligoarthritis and from healthy volunteers. *Infect Immun* 1999; **67**: 2682-2686 [PMID: 10225943]
 - 32 **Appel H**, Neure L, Kuhne M, Braun J, Rudwaleit M, Sieper J. An elevated level of IL-10- and TGFbeta-secreting T cells, B cells and macrophages in the synovial membrane of patients with reactive arthritis compared to rheumatoid arthritis. *Clin Rheumatol* 2004; **23**: 435-440 [PMID: 15459815 DOI: 10.1007/s10067-004-0916-5]
 - 33 **van Holten J**, Smeets TJ, Blankert P, Tak PP. Expression of interferon beta in synovial tissue from patients with rheumatoid arthritis: comparison with patients with osteoarthritis and reactive arthritis. *Ann Rheum Dis* 2005; **64**: 1780-1782 [PMID: 15878901 DOI: 10.1136/ard.2005.040477]
 - 34 **Braun J**, Yin Z, Spiller I, Siebert S, Rudwaleit M, Liu L, Radbruch A, Sieper J. Low secretion of tumor necrosis factor alpha, but no other Th1 or Th2 cytokines, by peripheral blood mononuclear cells correlates with chronicity in reactive arthritis. *Arthritis Rheum* 1999; **42**: 2039-2044 [PMID: 10524674]
 - 35 **Suzuki E**, Mellins ED, Gershwin ME, Nestle FO, Adamopoulos IE. The IL-23/IL-17 axis in psoriatic arthritis. *Autoimmun Rev* 2014; **13**: 496-502 [PMID: 24424175 DOI: 10.1016/j.autrev.2014.01.050]
 - 36 **Hickman-Brecks CL**, Racz JL, Meyer DM, LaBranche TP, Allen PM. Th17 cells can provide B cell help in autoantibody induced arthritis. *J Autoimmun* 2011; **36**: 65-75 [PMID: 21075597 DOI: 10.1016/j.jaut.2010.10.007]
 - 37 **Koenders MI**, Lubberts E, Oppers-Walgreen B, van den Bersselaar L, Helsen MM, Di Padova FE, Boots AM, Gram H, Joosten LA, van den Berg WB. Blocking of interleukin-17 during reactivation of experimental arthritis prevents joint inflammation and bone erosion by decreasing RANKL and interleukin-1. *Am J Pathol* 2005; **167**: 141-149 [PMID: 15972960 DOI: 10.1016/S0002-9440(10)62961-6]
 - 38 **Nakae S**, Saijo S, Horai R, Sudo K, Mori S, Iwakura Y. IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proc Natl Acad Sci USA* 2003; **100**: 5986-5990 [PMID: 12721360 DOI: 10.1073/pnas.1035999100]
 - 39 **Bedoya SK**, Lam B, Lau K, Larkin J. Th17 cells in immunity and autoimmunity. *Clin Dev Immunol* 2013; **2013**: 986789 [PMID: 24454481 DOI: 10.1155/2013/986789]
 - 40 **Singh AK**, Misra R, Aggarwal A. Th-17 associated cytokines in patients with reactive arthritis/undifferentiated spondyloarthritis. *Clin Rheumatol* 2011; **30**: 771-776 [PMID: 21181220 DOI: 10.1007/s10067-010-1646-5]
 - 41 **Singh R**, Aggarwal A, Misra R. Th1/Th17 cytokine profiles in patients with reactive arthritis/undifferentiated spondyloarthritis. *J Rheumatol* 2007; **34**: 2285-2290 [PMID: 17937463 DOI: 10.3899/jrheum.110849]
 - 42 **Jandus C**, Bioley G, Rivals JP, Dudler J, Speiser D, Romero P. Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. *Arthritis Rheum* 2008; **58**: 2307-2317 [PMID: 18668556 DOI: 10.1002/art.23655]
 - 43 **Wendling D**, Cedoz JP, Racadot E, Dumoulin G. Serum IL-17, BMP-7, and bone turnover markers in patients with ankylosing spondylitis. *Joint Bone Spine* 2007; **74**: 304-305 [PMID: 17369068 DOI: 10.1016/j.jbspin.2006.11.005]
 - 44 **Shen H**, Goodall JC, Gaston JS. Frequency and phenotype of T helper 17 cells in peripheral blood and synovial fluid of patients with reactive arthritis. *J Rheumatol* 2010; **37**: 2096-2099 [PMID: 20634245 DOI: 10.3899/jrheum.100146]
 - 45 **Bas S**, Neff L, Viatte S, Vuillet M, Spenato U, Guerne PA, Michel M, Tiercy JM, Butrimiene I, Gabay C. Relationship between gamma-interferon and interleukin-17 in Chlamydia trachomatis reactive arthritis. *Clin Exp Rheumatol* 2009; **27**: 885-886 [PMID: 19917178]
 - 46 **Noto Llana M**, Sarnacki SH, Aya Castañeda Mdel R, Bernal MI, Giacomodonato MN, Cerquetti MC. Consumption of Lactobacillus casei fermented milk prevents Salmonella reactive arthritis by modulating IL-23/IL-17 expression. *PLoS One* 2013; **8**: e82588 [PMID: 24340048 DOI: 10.1371/journal.pone.0082588]
 - 47 **Trinchieri G**. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat Rev Immunol* 2003; **3**: 133-146 [PMID: 12563297 DOI: 10.1038/nri1001]
 - 48 **Oppmann B**, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu M, Gorman D, Wagner J, Zurawski S, Liu Y, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* 2000; **13**: 715-725 [PMID: 11114383 DOI: 10.1016/

- S1074-7613(00)00070-4]
- 49 **Parham C**, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F, To W, Wagner J, O'Farrell AM, McClanahan T, Zurawski S, Hannum C, Gorman D, Rennick DM, Kastelein RA, de Waal Malefyt R, Moore KW. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J Immunol* 2002; **168**: 5699-5708 [PMID: 12023369]
 - 50 **Chan SH**, Perussia B, Gupta JW, Kobayashi M, Pospisil M, Young HA, Wolf SF, Young D, Clark SC, Trinchieri G. Induction of interferon gamma production by natural killer cell stimulatory factor: characterization of the responder cells and synergy with other inducers. *J Exp Med* 1991; **173**: 869-879 [PMID: 1672545]
 - 51 **Haraguchi S**, Day NK, Nelson RP, Emmanuel P, Duplantier JE, Christodoulou CS, Good RA. Interleukin 12 deficiency associated with recurrent infections. *Proc Natl Acad Sci USA* 1998; **95**: 13125-13129 [PMID: 9789052 DOI: 10.1073/pnas.95.22.13125]
 - 52 **Bas S**, Kvien TK, Buchs N, Fulpius T, Gabay C. Lower level of synovial fluid interferon-gamma in HLA-B27-positive than in HLA-B27-negative patients with Chlamydia trachomatis reactive arthritis. *Rheumatology (Oxford)* 2003; **42**: 461-467 [PMID: 12626797 DOI: 10.1093/rheumatology/keg163]
 - 53 **Välimäki E**, Aittomäki S, Karenko L, Kantonen J, Pettersson T, Turunen U, Matikainen S, Leirisalo-Repo M, Repo H. Normal inflammasome activation and low production of IL-23 by monocyte-derived macrophages from subjects with a history of reactive arthritis. *Scand J Rheumatol* 2013; **42**: 294-298 [PMID: 23425136 DOI: 10.3109/03009742.2012.754940]
 - 54 **Wendling D**, Cedoz JP, Racadot E. Serum and synovial fluid levels of p40 IL12/23 in spondyloarthropathy patients. *Clin Rheumatol* 2009; **28**: 187-190 [PMID: 18827961 DOI: 10.1007/s10067-008-1011-0]
 - 55 **Di Genaro MS**, Cargnelutti DE, Castro DO, Eliçabe RJ, Gutiérrez JV, Correa SG, de Guzmán AM. Yersinia-triggered arthritis in IL-12p40-deficient mice: relevant antigens and local expression of Toll-like receptor mRNA. *Scand J Rheumatol* 2007; **36**: 28-35 [PMID: 17454932 DOI: 10.1080/03009740600906651]
 - 56 **Yamasaki K**, Taga T, Hirata Y, Yawata H, Kawanishi Y, Seed B, Taniguchi T, Hirano T, Kishimoto T. Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor. *Science* 1988; **241**: 825-828 [PMID: 3136546 DOI: 10.1126/science.3136546]
 - 57 **Hibi M**, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T. Molecular cloning and expression of an IL-6 signal transducer, gp130. *Cell* 1990; **63**: 1149-1157 [PMID: 2261637 DOI: 10.1016/0092-8674(90)90411-7]
 - 58 **Taga T**, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T, Kishimoto T. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell* 1989; **58**: 573-581 [PMID: 2788034 DOI: 10.1016/0092-8674(89)90438-8]
 - 59 **Smolen JS**, Beaulieu A, Rubbert-Roth A, Ramos-Remus C, Rovensky J, Alecock E, Woodworth T, Alten R. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet* 2008; **371**: 987-997 [PMID: 18358926 DOI: 10.1016/S0140-6736(08)60453-5]
 - 60 **Metsärinne KP**, Nordström DC, Kontinen YT, Teppo AM, Fyhrquist FY. Plasma interleukin-6 and renin substrate in reactive arthritis, rheumatoid arthritis, and systemic lupus erythematosus. *Rheumatol Int* 1992; **12**: 93-96 [PMID: 1384103 DOI: 10.1007/BF00290261]
 - 61 **Straub RH**, Paimela L, Peltomaa R, Schölmerich J, Leirisalo-Repo M. Inadequately low serum levels of steroid hormones in relation to interleukin-6 and tumor necrosis factor in untreated patients with early rheumatoid arthritis and reactive arthritis. *Arthritis Rheum* 2002; **46**: 654-662 [PMID: 11920401 DOI: 10.1002/art.10177]
 - 62 **Wajant H**, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. *Cell Death Differ* 2003; **10**: 45-65 [PMID: 12655295 DOI: 10.1038/sj.cdd.4401189]
 - 63 **Westendorp RG**, Langermans JA, de Bel CE, Meinders AE, Vandenbroucke JP, van Furth R, van Dissel JT. Release of tumor necrosis factor: an innate host characteristic that may contribute to the outcome of meningococcal disease. *J Infect Dis* 1995; **171**: 1057-1060 [PMID: 7706790 DOI: 10.1093/infdis/171.4.1057]
 - 64 **Netea MG**, van der Meer JW, van Deuren M, Kullberg BJ. Proinflammatory cytokines and sepsis syndrome: not enough, or too much of a good thing? *Trends Immunol* 2003; **24**: 254-258 [PMID: 12738419 DOI: 10.1016/S1471-4906(03)00079-6]
 - 65 **Saleh M**, Vaillancourt JP, Graham RK, Huyck M, Srinivasa SM, Alnemri ES, Steinberg MH, Nolan V, Baldwin CT, Hotchkiss RS, Buchman TG, Zehnbauser BA, Hayden MR, Farrer LA, Roy S, Nicholson DW. Differential modulation of endotoxin responsiveness by human caspase-12 polymorphisms. *Nature* 2004; **429**: 75-79 [PMID: 15129283 DOI: 10.1038/nature02451]
 - 66 **Butrimiene I**, Jarmalaite S, Ranceva J, Venalis A, Jasiuleviciute L, Zvirbliene A. Different cytokine profiles in patients with chronic and acute reactive arthritis. *Rheumatology (Oxford)* 2004; **43**: 1300-1304 [PMID: 15266062 DOI: 10.1093/rheumatology/keh323]
 - 67 **Frucht DM**, Fukao T, Bogdan C, Schindler H, O'Shea JJ, Koyasu S. IFN-gamma production by antigen-presenting cells: mechanisms emerge. *Trends Immunol* 2001; **22**: 556-560 [PMID: 11574279 DOI: 10.1016/S1471-4906(01)02005-1]
 - 68 **Schroder K**, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol* 2004; **75**: 163-189 [PMID: 14525967 DOI: 10.1189/jlb.0603252]
 - 69 **Flynn JL**, Chan J, Triebold KJ, Dalton DK, Stewart TA, Bloom BR. An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. *J Exp Med* 1993; **178**: 2249-2254 [PMID: 7504064 DOI: 10.1084/jem.178.6.2249]
 - 70 **Huang S**, Hendriks W, Althage A, Hemmi S, Bluethmann H, Kamijo R, Vilcek J, Zinkernagel RM, Aguet M. Immune response in mice that lack the interferon-gamma receptor. *Science* 1993; **259**: 1742-1745 [PMID: 8456301 DOI: 10.1126/science.8456301]
 - 71 **Harty JT**, Bevan MJ. Specific immunity to Listeria monocytogenes in the absence of IFN gamma. *Immunity* 1995; **3**: 109-117 [PMID: 7621071 DOI: 10.1016/1074-7613(95)90163-9]
 - 72 **O'Garra A**, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C. Strategies for use of IL-10 or its antagonists in human disease. *Immunol Rev* 2008; **223**: 114-131 [PMID: 18613832 DOI: 10.1111/j.1600-065X.2008.00635.x]
 - 73 **Hedrich CM**, Bream JH. Cell type-specific regulation of IL-10 expression in inflammation and disease. *Immunol Res* 2010; **47**: 185-206 [PMID: 20087682 DOI: 10.1007/s12026-009-8150-5]
 - 74 **Yao Y**, Simard AR, Shi FD, Hao J. IL-10-producing lymphocytes in inflammatory disease. *Int Rev Immunol* 2013; **32**: 324-336 [PMID: 23617759 DOI: 10.3109/08830185.2012.762361]
 - 75 **Moore KW**, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; **19**: 683-765 [PMID: 11244051 DOI: 10.1146/annurev.immunol.19.1.683]
 - 76 **Kaluza W**, Leirisalo-Repo M, Märker-Hermann E, Westman P, Reuss E, Hug R, Mastrovic K, Stradmann-Bellinghausen B, Granfors K, Galle PR, Höhler T. IL10.G microsatellites mark promoter haplotypes associated with protection against the

- development of reactive arthritis in Finnish patients. *Arthritis Rheum* 2001; **44**: 1209-1214 [PMID: 11352256]
- 77 **Finnegan A**, Kaplan CD, Cao Y, Eibel H, Glant TT, Zhang J. Collagen-induced arthritis is exacerbated in IL-10-deficient mice. *Arthritis Res Ther* 2003; **5**: R18-R24 [PMID: 12716449 DOI: 10.1186/ar601]
 - 78 **Finnegan A**, Mikecz K, Tao P, Glant TT. Proteoglycan (aggrecan)-induced arthritis in BALB/c mice is a Th1-type disease regulated by Th2 cytokines. *J Immunol* 1999; **163**: 5383-5390 [PMID: 10553063]
 - 79 **Fellowes R**, Etheridge CJ, Coade S, Cooper RG, Stewart L, Miller AD, Woo P. Amelioration of established collagen induced arthritis by systemic IL-10 gene delivery. *Gene Ther* 2000; **7**: 967-977 [PMID: 10849557]
 - 80 **Kasama T**, Strieter RM, Lukacs NW, Lincoln PM, Burdick MD, Kunkel SL. Interleukin-10 expression and chemokine regulation during the evolution of murine type II collagen-induced arthritis. *J Clin Invest* 1995; **95**: 2868-2876 [PMID: 7769128 DOI: 10.1172/JCI117993]
 - 81 **Cargnelutti E**, Di Genaro MS. Reactive Arthritis: from clinical features to pathogenesis. *Int J Clin Med* 2013; **4**: 20-30 [DOI: 10.4236/ijcm.2013.412A2004]
 - 82 **Wendling D**, Racadot E, Toussirot E, Wijdenes J. Combination therapy of anti-CD4 and anti-IL6 monoclonal antibodies in a case of severe spondylarthropathy. *Br J Rheumatol* 1996; **35**: 1330 [PMID: 9010072 DOI: 10.1093/rheumatology/35.12.1330]
 - 83 **Tanaka T**, Kuwahara Y, Shima Y, Hirano T, Kawai M, Ogawa M, Arimitsu J, Hagihara K, Narazaki M, Ogata A, Kawase I, Kishimoto T. Successful treatment of reactive arthritis with a humanized anti-interleukin-6 receptor antibody, tocilizumab. *Arthritis Rheum* 2009; **61**: 1762-1764 [PMID: 19950316 DOI: 10.1002/art.24899]
 - 84 **Kwan K**, Bharadwaj S, Inderjeeth C. Response to treatment with tocilizumab of reactive arthritis induced by intravesical bacillus Calmette-Guérin unresponsive to DMARDs. *Int J Rheum Dis* 2012; **15**: e73-e75 [PMID: 22898231 DOI: 10.1111/j.1756-185X.2012.01735.x]
 - 85 **Kaipiainen-Seppönen O**, Niinisalo H, Korpilähde T, Viro-lainen J. Treatment of reactive arthritis with infliximab. *Scand J Rheumatol* 2003; **32**: 122-124 [PMID: 12737333 DOI: 10.1080/03009740310000157]
 - 86 **Thomas-Pohl M**, Tissot A, Banal F, Lechevalier D. Spectacular evolution of reactive arthritis after early treatment with infliximab. *Joint Bone Spine* 2012; **79**: 524 [PMID: 22542050 DOI: 10.1016/j.jbspin.2012.03.001]
 - 87 **Edrees A**. Successful use of etanercept for the treatment of Reiter's syndrome: a case report and review of the literature. *Rheumatol Int* 2012; **32**: 1-3 [PMID: 21785961 DOI: 10.1007/s00296-011-2000-1]

P- Reviewer: Gazouli M, Konttinen Yrj, Pixley J **S- Editor:** Ji FF
L- Editor: Roemmele A **E- Editor:** Wang CH





Update on pythiosis immunobiology and immunotherapy

Érico S Loreto, Juliana SM Tondolo, Régis A Zanette, Sydney H Alves, Janio M Santurio

Érico S Loreto, Juliana SM Tondolo, Régis A Zanette, Sydney H Alves, Janio M Santurio, Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil

Érico S Loreto, Juliana SM Tondolo, Régis A Zanette, Sydney H Alves, Janio M Santurio, Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil

Author contributions: Loreto ÉS, Tondolo JSM, Zanette RA, Alves SH and Santurio JM solely contributed to this paper.

Supported by The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil, No. CAPES-AUX PE-PNPD 743/2012

Correspondence to: Dr. Janio M Santurio, Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Av. Roraima nº 1000, Prédio 20, sala 4139, Santa Maria, RS 97105-900, Brazil. janio.santurio@gmail.com

Telephone: +55-55-32208906 Fax: +55-55-32208906

Received: March 27, 2014 Revised: May 6, 2014

Accepted: June 10, 2014

Published online: July 27, 2014

Abstract

Pythiosis is an invasive, ulcerative, pyogranulomatous disease caused by *Pythium insidiosum*, a fungus-like oomycete that has been reported to affect humans, horses, dogs, and other mammals mainly in tropical and subtropical areas of the world. The disease is characterized by an eosinophilic granulomatous and a Th2 immune response which in turn helps to protect the fungus from the host cells. Pythiosis can present clinically in subcutaneous, gastrointestinal, and vascular tissues or in a systemically disseminated form depending on the species and site of infection. Changes in iron metabolism and anemia are commonly observed. The diagnosis is accomplished through clinical and pathological features, laboratory characteristics of cultures, serological and molecular tests. Treatment includes radical surgery, antimicrobial drugs, immunotherapy or a combination of these treatments. Immunotherapy is a practical and non-invasive alternative for treating pythiosis which is believed to promote a switch from a Th2 to Th1 immune response, resulting in a favorable

clinical response. This therapy has demonstrated cure rates above 70% and 55% in horses and humans but low cure rates in dogs and cats. Despite the curative properties of this type of immunotherapy, the antibodies that are produced do not prevent host reinfection. Thus, development of effective adjuvants and new diagnostic techniques for early disease diagnosis are of utmost importance. The aim of this review was to promote pythiosis awareness and to provide an update about the immunotherapy and immunobiology of this disease.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: *Pythium insidiosum*; Treatment; Pythiosis; Immunotherapy; Pathogenesis

Core tip: Pythiosis is a life-threatening disease for which there is no gold standard chemotherapy. Immunotherapy derived from killed mycelium from *Pythium insidiosum* is a non-invasive therapy that has demonstrated cure rates above 90% when associated with the surgical removal of the lesions and early disease diagnosis.

Loreto ÉS, Tondolo JSM, Zanette RA, Alves SH, Santurio JM. Update on pythiosis immunobiology and immunotherapy. *World J Immunol* 2014; 4(2): 88-97 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/88.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.88>

INTRODUCTION

Pythium insidiosum (*P. insidiosum*, an oomycete also known as water mold) is a filamentous microorganism that shares many characteristics with fungi (*i.e.*, it grows by polarized hyphal extension, engages in an absorptive mode of nutrition, and it can form spores for reproduction). However, *P. insidiosum* is classified in a completely different taxonomic group, namely the Stramenopiles, together with diatoms and brown algae^[1]. This classifica-

tion is the first essential information for understanding pythiosis in humans and other mammals; infections have similar clinical and histopathological characteristics as those of certain mycoses. Conversely, treating pythiosis with antifungal drugs is generally inefficient because the oomycetes do not synthesize ergosterol, which is a component of the plasma membrane of true fungi and the primary target of those drugs^[2]. Similarly, the immune response of animals with pythiosis presents similar features to those of fungal infections, and there are peculiarities found only in infections caused by this oomycete^[3]. The challenge of treating pythiosis is characterized by the severity of the disease in mammals and by the absence of a gold standard chemotherapy. Nevertheless, immunotherapy is a practical and non-invasive alternative for treating pythiosis, and there is a favorable clinical response. In this context, the aim of this review was to promote pythiosis awareness and to provide an update about the immunotherapy and immunobiology of this disease.

THE EPIDEMIOLOGY, IMMUNOBIOLOGY AND PATHOGENESIS OF PYTHIOSIS

The proposed life cycle of *P. insidiosum* is characterized by the colonization of aquatic plants and the soil of wetlands or swampy areas, which serve as a substrate for mycelial vegetative growth and the asexual formation of mobile biflagellate zoospores that move through the water, find another host, encyst and form a new mycelium that can then start a new colonization^[4-6].

In view of this biological cycle, pythiosis cases are associated with human or animal contact with areas in which zoospore-containing water accumulates (such as wetlands and lakes) and environmental temperatures range between 30 °C and 40 °C. Most reports of animal pythiosis are described in horses that live in swampy areas or periodically enter ponds or lakes. These cases are distributed primarily between the peak months of the rainy season in each region. A higher human disease frequency has been observed in thalassemic patients in Thailand, where it is common for people to work in flooded rice cultivation areas^[7-9].

Human and animal pythiosis cases have been described in tropical and subtropical climatic regions. Although these cases are most often diagnosed in Australia, Asia, Latin America and United States, some cases have originated from temperate areas of Japan, South Korea, Oceania and Africa. There are no reports of animal-animal or animal-human transmission^[10,11].

P. insidiosum hyphae do not exert sufficient pressure to penetrate undamaged skin through mechanical means alone^[12], and they must effect a decisive reduction in tissue strength by proteinase secretion or by finding prior skin damage to invade their host. Indeed, *P. insidiosum* possesses a strong tropism for mammalian injured tissue^[10]. Interestingly, *P. insidiosum* has been recovered from a mosquito larva (*Culex quinquefasciatus*)^[13] and hematophagous insects prefer the same anatomical areas for blood feeding in which pythiosis lesions are more prevalent in

horses^[9]. Given this information, future studies should investigate if insect bites could favor the penetration of zoospores into injured skin or if infected mosquitoes could directly transmit the disease.

Once inside the host, pythiosis pathogenesis involves the Splendore-Hoeppli phenomenon (reaction), which is characterized by the presence of radiating, star-like asteroid or club-shaped eosinophilic material around the infectious agent^[14]. Thus, *P. insidiosum* triggers an eosinophilic granulomatous reaction similar to other fungi, such as *Basidiobolus* spp. and *Conidiobolus* spp.^[15], with characteristic histopathological features depending on the species and the clinical form^[16].

Mendoza *et al.*^[17] proposed that the antigens released by *P. insidiosum* hyphae modulate the host's immune response and may be responsible by keeping an eosinophilic granulomatous response, locking the immune system into a Th2 immune response through the continuous stimulatory production of more eosinophils and mast cells, which in turn helps to protect the fungus from the host cells and leads to a worsening condition, and if not treated properly, can lead to host death. As a consequence, the *P. insidiosum* hyphae surrounded by degranulated eosinophils would be camouflaged inside the eosinophilic micro-abscesses, preventing their full presentation to the immune system and thereby ensuring their viable presence in infected tissues. These features and the subsequent finding of elevated IgE levels in humans and horses with the disease strongly validated the concept of a Th2 modulation by this pathogen during natural infection^[3,17]. High Th2 interleukin levels [interleukin (IL)-4, IL-5 and IL-10] have also been detected in human patients with pythiosis, confirming a Th2 immune response^[18,19].

The eosinophil degranulation in equids and camels with pythiosis is remarkable, forming around the hyphae cores of necrotic yellow-gray and firm materials called *kunkers*, which are easily shed from lesions^[4,15,20,21]. The *kunkers* range from 2 to 10 mm in diameter, have an irregular shape and sandy aspect, may be branched and invade the granulation tissue within the sinus formed along its trajectory. This pronounced degranulation is also associated with extensive tissue damage and with the tumor-like appearance of lesions that can reach over 50 cm in diameter^[22]. Horses are the mammals that are most affected by pythiosis, with no predisposition according to their age, race or sex. However, although young animals are also susceptible, the disease is rarely observed in animals under one year of age, and the bodily lesions are predominant in dark pigmented areas^[9].

The lesions are subcutaneous and present primarily in the distal extremities, the ventral portion of the thoraco-abdominal wall and face, which represent anatomical structures that remain in contact with contaminated water containing *P. insidiosum* zoospores^[7,23,24]. The *kunkers* are considered to be pathognomonic of pythiosis in equids, and they have also been described in camels with vulvar pythiosis^[21] and in a case of equine conidiobolo-

mycosis^[25]. The disease was also described in cattle^[26-29], cats^[27,30], dogs^[16,17,27,31-39], sheep^[40,41] and occasionally in animals kept in captivity in zoos^[42,43] and birds^[44].

P. insidiosum can cause superficial infections in humans, namely keratitis with corneal involvement^[45-47]; cutaneous and subcutaneous infections^[48]; orbital pythiosis and bone involvement^[49,50]; and systemic infections, namely arteritis of the lower limbs and/or dissemination^[8,19,51]. Although pythiosis can affect apparently healthy individuals^[48,50,51], most cases are reported in patients with thalassemia and other hematological diseases^[8]. The same authors have argued that iron overload, which is a marked characteristic of patients with thalassemia, could increase host susceptibility to pythiosis by promoting the infectivity of the pathogen or by impairing host immunity.

In fact, both iron overload and deficiency can weaken the immune system^[52-54]. Additionally, many microorganisms are known to be avid for iron during infection^[53,55] and changes in iron metabolism may increase host susceptibility to infection by *P. insidiosum*. Krajaeun *et al*^[56] described that *P. insidiosum* expresses a gene encoding a ferrochelatase and Krajaeun *et al*^[57] reported, through the transcriptome analysis of this species, an extensive repertoire of proteins that may be involved as virulence factors during infection. Although the role of iron in pythiosis has not been fully explained, the disease is more frequently found in human patients with thalassemia and with other hemolytic anemias^[18,19,58]. Anemia as a consequence of the disease has already been described in horses^[59-65], dogs^[33,34,37,39,66], cats^[39,67], camels^[21,68] and in a jaguar^[42] and Bengal tiger^[69]. Santos *et al*^[9] also argue that the iron deficiency is common in lactating foals (< 1-year-old and, which are less susceptible to pythiosis) because of the low iron levels in the milk. In contrast, iron deficiency is uncommon in adult horses, and they may have increased levels of circulating iron, especially in the Brazilian Pantanal, which contains high levels of this mineral in the soil, plants and water^[70], and where it is observed a high incidence of pythiosis.

Loreto *et al*^[71] reported an increase in the unsaturated iron binding capacity (UIBC) in rabbits experimentally infected with *P. insidiosum*, suggesting that there was an increase in the transferrin concentration and/or an increase in the number of transferrin iron receptors, which is compatible with a physiological decrease in the iron availability. Similar results were observed by Zanette *et al*^[72], who noted that rabbits experimentally infected with pythiosis presented decreased serum iron levels, increased transferrin levels with low saturations (increased UIBC) and markedly decreased levels of stainable iron in the hepatocytes, which suggests an affinity for iron by *P. insidiosum*.

DIAGNOSIS AND HUMORAL RESPONSE

A classical pythiosis diagnosis is accomplished through clinical and pathological features, in addition to cultural, morphological and reproductive characteristics *in vitro*. A differential diagnosis includes habronemiasis, neoplasms,

exuberant granulation tissue, and fungal or bacterial granulomas^[73,74]. Microscopic evaluations using 10% KOH (direct examination) can reveal *P. insidiosum* hyaline hyphae and eventually septate-morphology, depending on the clinical material evaluated. This material can easily be confused with filamentous fungi, particularly those of the orders Entomofthorales and Mucorales^[11]. A culture from *kunkers* or biopsies can usually be performed on V8 agar, corn meal agar and Sabouraud dextrose agar.

Hyphal growth can be observed after 24 h of incubation at 37 °C when submerged in culture medium, and it exhibits a hyaline or white color^[75]. Because *P. insidiosum* does not produce reproductive structures in traditional culture media, the induction of zoosporogenesis (asexual zoospore formation) can be obtained by cultivating *P. insidiosum* in sterile blades of grass that are then transferred to a mineral solution^[76]. However, the correct identification of this species should be confirmed by molecular methods^[77-81].

The production of anti-*P. insidiosum* antibodies was one of the first immunological features described for pythiosis, and these antibodies were easily detected by immunodiffusion and complement fixation tests with antigens that were extracted from the pathogen^[82,83]. Studies then confirmed that humans and animals suffering from pythiosis exhibited a humoral immune response upon host-pathogen interaction^[3,36,77,84-86], but this response was not sufficient to clear the infection^[19,50,87,88]. However, the serological tests developed for detecting antibodies, such as agar gel immunodiffusion, enzyme-linked immunosorbent assay (ELISA), Western blot, latex agglutination and immunochromatographic tests^[77-80,89-93], are highly useful for the early diagnosis of pythiosis. In equine pythiosis cases in which the animal is far from reference laboratories, sending serum for ELISA and collecting *kunkers* and tissues for microbiological culture and histopathological analysis are among the primary forms of diagnosis. An early pythiosis diagnosis can also be performed through immunohistochemical^[116,94] and molecular methods^[80,95].

TREATMENT

Antimicrobial and surgical treatment

Because primary antifungal drugs act directly or indirectly on ergosterol and *Pythium* spp. are unable to synthesize any sterols, it is understandable that pythiosis cases do not respond satisfactorily to antifungal treatments. However, contradictory results have been reported in the use of antifungal agents to treat pythiosis^[8,10,49,96].

P. insidiosum isolates have varying *in vitro* susceptibility to antifungal compounds^[97,98]. Reviews of antifungal drug associations show that *in vitro* synergism occurs in AmB + terbinafine^[99], terbinafine + azole antifungals and terbinafine + caspofungin associations^[100]. Additionally, some antibacterial drugs that act as protein synthesis inhibitors (macrolides, tetracyclines and glycylcycline) have been shown to inhibit the *in vitro* growth of *P. insidiosum*^[101,102]; nonetheless, experimental *in vivo* tests have not been con-

ducted to demonstrate the clinical effectiveness of these antibiotics.

Successes and failures of pythiosis treatment cases have been reported with combinations of antifungal therapies. The surgical removal of the lesion, the amputation of the affected limb or the enucleation of the affected eye represents the last resort in human pythiosis treatment. However, recurrence rates of 40% have been observed, which illustrates the difficulty of controlling this disease^[11]. The implementation of surgical treatment with antifungal drugs or potassium iodide was described in cases of therapeutic healing^[73].

Surgically removing all affected tissue is the traditional and most commonly used method for equine pythiosis treatment. The surgery yields good results for small and superficial lesions. However, removing the lesion with a safety margin to avoid recurrences is often hampered by the anatomical regions that are typically involved (distal extremities and the ventral portion of the thoraco-abdominal wall)^[73].

Immunotherapy

Although the antigens used in vaccine preparation (usually from the infectious agent itself) are intended to trigger a protective response in the host immune system (antibody production), the aim of immunotherapy (antibodies or antigens from the infectious agent) is the objective modification of the host immune response to mount an effective response against a disease that is already present. Despite the fact that a protective vaccine against pythiosis does not currently exist, the immunotherapy developed from protein extracts of *P. insidiosum* cultures is a non-invasive alternative for treating this disease in humans and animals.

Immunotherapy was discovered by serendipity when investigators were working on a skin test for pythiosis in horses, and they found that almost half the animals were cured upon inoculation with *P. insidiosum* immunogen^[88,103,104]. The first investigator to use a culture-derived antigen for a skin test was Witkamp^[83], but he did not report cure rates in his experiments. Miller^[103] was the first researcher to report the use of *P. insidiosum* antigens (sonicated hyphae) with therapeutic potential when injected into horses ($n = 30$), resulting in 53% healing in the animals with pythiosis (Table 1). During the following year, the same author observed an immunotherapeutic efficiency ratio of 75% when associated with surgical removal^[105]. Subsequent studies showed that lesions presenting with more than two months of progress in cases of chronic pythiosis had cure rates of approximately 20%-40% with immunotherapy, and cure rates of 100% were obtained when the lesions had less than 20 d of evolution^[17,88,104].

In addition to the lesion evolution time, the manner by which the *P. insidiosum* mycelium is broken to obtain the antigens is also associated with immunotherapy efficacy. In this context, modifications to the original technique as described by Miller^[103] have been developed with

the aim of increasing the effectiveness and safety of immunotherapy.

Mendoza *et al.*^[104] tested two immunotherapies by using the cell mass or a concentrated soluble antigen as an antigen, and they observed efficacies of 60% and 70%, respectively, when treating 71 horses. Mendoza *et al.*^[17] reported that immunotherapy derived from the soluble antigen and sonicated hyphae of *P. insidiosum* cured 72% of the horses ($n = 18$) with pythiosis.

Santurio *et al.*^[106] compared the immunotherapy obtained from sonication, maceration (or liquidification) or the combination of these two techniques in experimental pythiosis cases in rabbits and observed that the macerated immunotherapy had a higher efficiency, with a reduction of 71.8% in the lesion sizes and the clinical cure of two rabbits ($n = 5$). This macerated immunotherapy was lyophilized, and it was valid for more than one year without refrigeration^[106]. This treatment exhibited a cure rate of 50% to 83% ($n = 19$)^[107], or 75% ($n = 8$)^[7] and 90% when combined with surgical excision ($n = 11$)^[24] in horses in the Brazilian Pantanal. The best results are typically observed when the disease is in its early stages.

Despite the good immunotherapy performance in equines, immunotherapy in cats and dogs has been disappointing^[17,27,31]. One explanation for this failure might be that most dogs and cats with pythiosis are diagnosed several months after the initial onset of infection, resulting in animals with weakened immune systems that respond poorly to immunotherapy^[3]. However, the healing of a dog was demonstrated by the combination of immunotherapy and antifungal therapy^[108].

The immunotherapy treatment period (no. of doses) is related to the size, location, time of lesion development, and individual patient response. Santos *et al.*^[24] reported that a horse with 90 d of disease evolution required five months of treatment (eight doses) for complete lesion healing, and they noted that the slowness in the immunotherapy response cannot be interpreted as refractory and in turn end in the premature withdrawal of treatment. Conversely, only two to three doses promoted the effective healing of four horses bearing lesions with seven and 45 d of development.

Field tests with macerated immunotherapy have demonstrated that the efficacy of this treatment is directly associated with early diagnosis. The borderline between a clinical cure and an unsatisfactory response or even non-responsive cases seems to be 60 d from the appearance of lesions in horses^[3]. The treatment consists of subcutaneous applications at 14-d intervals until the complete healing of the granulomatous ulcerative tissue. A mild reaction at the injection site is often observed, and in most cases, it subsides in a few weeks. The number of doses is variable, and some animals respond better to weekly applications. In fact, the only disadvantage of this treatment is the production of protective IgG classes, which impairs serodiagnostic tests such as ELISA and immunochromatography. In this context, blood collection for serological diagnosis of pythiosis should be performed

Table 1 Review of animal pythiosis cases reported in the literature when treated with immunotherapy

Species/ <i>n</i>	Lesions	Adjunctive therapy	Immunotherapy type ³ , doses	Outcome	Ref.
<i>Horses</i>					
40	Various ⁴	No or surgery	UF, 3 ¹ doses at 7-d intervals	C (53%), I (33%)	[103]
5	Limbs	ATM, surgery	UF, 3 doses at 7-d intervals	C (20%), 60(E), 20 (D)	[60]
5	Various ⁴	No	SA, 2 doses at 15-d intervals	C (60%)	[88]
1	Limb, bones	No	SA, 2 doses at 7-d intervals	E	[109]
71	Various ⁴	Nr	FH or SA, 1 or 2 doses at 7-d intervals	C (66%)	[104]
1	Limb, bones	Surgery, ATM	Nr, 3 doses postsurgical	D	[110]
2	Abdomen	Surgery, ATM	SH, 3 doses at 7-d intervals	C (50%), E (50%)	[111]
19	Various ⁴	No	LMH, 3 to 9 doses at 14-d intervals	C (50%-83%)	[107]
18	Various ⁴	Surgery, ATM	SA + SH, 2 ¹ doses at 15-d intervals	C (72%)	[17]
1	Limbs, sub-maxillary	Surgery	Nr	E	[23]
1	Limb	ATM	LMH, 7 doses at 14-d intervals	D	[112]
1	Hind pastern, fetlock	ATB	SA, 3 doses at 1, 7 and 21 d	E	[113]
1	Face	Surgery, ATM	LMH, 5 doses at 14-d intervals	E	[114]
1	Face	ATM	LMH, 5 doses at 14-d intervals	C	[64]
1 ²	Limb, abdomen	No	LMH, 4-5 doses at 14-d intervals	C	[115]
11	Limbs, abdomen	No or surgery	LMH, 2-5 doses at 14-d intervals	C (70%-90%)	[24]
8	Limbs, abdomen	No or surgery	LMH, Nr	C (75%)	[7]
47	Various ⁴	No or surgery	LMH, Nr	C (79%-84%)	[9]
<i>Dogs</i>					
1	Cutaneous	AMB, surgery	UF, 1 dose	C	[38]
6	Cutaneous, intestinal	ATM, surgery	SA + SH, 2 ¹ doses at 15-d intervals	C (33%)	[17]
2	Cutaneous	Itraconazole	SA, 1 or 2 doses at 7-d intervals	E	[31]
1	Cutaneous	No	SA, 2 doses at 14-d intervals	C	[35]
1	Gastrointestinal	ATF, surgery	Nr, 3 doses at 1, 7 and 21 d	C	[32]
1	Gastrointestinal	ATF	SA, 6 doses at 15-d intervals	C	[108]
<i>Camels</i>					
1	Face, stomach	Surgery, ATM	SA + SH, 2 ¹ doses at 14-d intervals	D	[68]
2	Vulvar	Surgery, ATM	SA, 3 doses at 1, 10, 17 d	C (50%)	[21]
<i>Sheep</i>					
6	Oronasal	No	LMH, 1-5 doses at 14-d intervals	C (16.7%)	[41]

¹At least; ²Same animal, cured twice with immunotherapy with reinfection within an interval of two years; ³Manufacturing process for immunotherapy; ⁴Not reported individually (subcutaneous). Nr: Not reported; AMB: Amphotericin B; UF: Ultrasonication of hyphae; SA: Soluble antigens; SH: Sonicated hyphae; FH: Fragmented hyphae; LMH: Lyophilized macerated hyphae; C: Cured; I: Clinically improved; D: Died; E: Euthanized; ATM: Antimicrobials; ATB: Antibacterials; ATF: Antifungals.

Table 2 Review of human pythiosis cases reported in the literature when treated with immunotherapy

<i>n</i>	Lesions	Adjunctive therapy	Immunotherapy type ² , doses	Outcome	Ref.
1	Vascular	ATM, surgery	SA, 2 doses at 14-d intervals	C	[19]
8	Vascular	Surgery/amputation, ATF	SA, 2 ¹ doses at 14-d intervals	C (50%)	[18]
1	Vascular	Above-knee amputation	SA, Nr	C	[116]
1	Vascular	ATM, limb amputation	SA, Nr	D	[117]
1	Ocular	ATM, enucleation	Nr	D	[118]
1	Vascular	Above-knee amputation	Nr	C	[119]
1	Vascular	ATM, above-knee amputations	Nr	C	[120]
1	Vascular	ATM, above-the-knee-amputation	Nr, 4 doses at 7-d intervals	C	[95]
1	Vascular/disseminated	ATM	SA, 2 doses at 7-d intervals	D	[121]
3	Ocular	ATM, surgery	Nr, 3 doses	C (66%)	[122]

¹At least; ²Manufacturing process for immunotherapy. Nr: Not reported; SA: Soluble antigens; C: Cured; D: Died; ATM: Antimicrobials; ATF: Antifungals.

before the application of immunotherapy, thus preventing false-positive results.

Because of the higher incidence of pythiosis in horses, most data on the efficacy of immunotherapy are described in this animal species^[7,9,17,23,24,60,64,88,103,104,107,109-115]. However, there are also descriptions of its use in dogs^[17,31,32,35,38,108], camels^[21,68] and sheep^[41] (Table 1). Human immunotherapy was described for both successful and failed treatments in association with surgical proce-

dures and the use of various antimicrobials^[18,19,95,116-122] (Table 2). These studies suggest that the injection of *P. insidiosum* immunogens in the form of immunotherapy make antigens available to the host immune system that are not produced during active infection, stimulating a healing response and the formation of immune responses with the presence of mononuclear cells and the disappearance of the eosinophilic reaction around the hyphae.

The proposed mechanism for immunotherapy success is based on a change in the type of cellular response. The immune response observed during pythiosis involves eosinophilic inflammation and the expression of T helper lymphocyte type 2 (Th2) with the release of interleukins 4 and 5 and the mobilization of eosinophils and mast cells. However, the expression of T helper lymphocyte type 1 (Th1) occurs after the immunotherapeutic treatment with the release of interleukin 2 and $\text{INF-}\gamma$ and the mobilization of T lymphocytes and macrophages, which destroy the *P. insidiosum* cells^[3]. This approach was observed for the immune response to human pythiosis when interleukin 4 and 5 production was detected in association with high IgE titers; a large amount of inflammatory cells (eosinophils and mast cells) was identified, which indicated a Th2 response during the infection. After immunotherapy, the patients presented high blood levels of interleukin 2 and $\text{INF-}\gamma$ with a mononuclear immune response, which is typical of a Th1 response^[18,19]. Additionally, an increase in the enzyme activity of ecto-adenosine deaminase (E-ADA) was observed in a rabbit model of experimental pythiosis, which is also associated with the switch from a Th2 to a Th1 response^[123].

Despite the curative properties of this type of immunotherapy, the antibodies that are produced do not prevent host reinfection^[2,115]. Santos *et al.*^[115] described a case of reinfection that occurred two years after the end of a successful immunotherapy treatment against pythiosis. Reinfection occurred at a different anatomical site than the initial infection (abdomen versus left pelvic limb), and although the new lesion was larger (60 cm perilesional edema and ulcerated lesions with approximately 20 cm in diameter), a cure was achieved with four immunotherapy doses (versus the five doses needed in the primary treatment). It is important to note that the levels of antibody's anti-*P. insidiosum* are associated with the response to treatment. Antibody titers are stable or increase in cases of unsuccessful treatment or when there is a persistent or recurrent infection. In cases of healing, substantial reductions of antibody's titers are seen during the subsequent months after the resolution of the infection^[35].

Given the above information, we can conclude that effective immunotherapy treatment can be obtained in association with a rapid and accurate diagnosis, and it may or may not be associated with surgical excision.

CONCLUSION

In summary, although the current immunotherapies used for treating pythiosis make use of crude *P. insidiosum* antigens, some studies have described the identification of immunodominant antigens^[124,125], and the best aspects of these immunotherapeutic elements could lead to a new vaccination strategy that is more effective and protective. A recent description of the *P. insidiosum* transcriptome^[57] uncovered many putative virulence proteins, and it provided a set of candidate targets for the development of better pythiosis diagnosis and treatment modalities.

Because the production of IgG by stimulated B cells is known to protect the host for short periods of time^[2,115], the development of effective adjuvants and new diagnostic techniques for early disease diagnosis are of utmost importance, primarily for animal and human use in endemic areas.

REFERENCES

- 1 **Beakes GW**, Glockling SL, Sekimoto S. The evolutionary phylogeny of the oomycete "fungi". *Protoplasma* 2012; **249**: 3-19 [PMID: 21424613 DOI: 10.1007/s00709-011-0269-2]
- 2 **Gaastra W**, Lipman LJ, De Cock AW, Exel TK, Pegge RB, Scheurwater J, Vilela R, Mendoza L. *Pythium insidiosum*: an overview. *Vet Microbiol* 2010; **146**: 1-16 [PMID: 20800978 DOI: 10.1016/j.vetmic.2010.07.019]
- 3 **Mendoza L**, Newton JC. Immunology and immunotherapy of the infections caused by *Pythium insidiosum*. *Med Mycol* 2005; **43**: 477-486 [PMID: 16320491 DOI: 10.1080/13693780500279882]
- 4 **Fonseca AO**, Botton Sde A, Nogueira CE, Corrêa BF, Silveira Jde S, de Azevedo MI, Maroneze BP, Santurio JM, Pereira DI. In vitro reproduction of the life cycle of *Pythium insidiosum* from kunkers' equine and their role in the epidemiology of pythiosis. *Mycopathologia* 2014; **177**: 123-127 [PMID: 24326464 DOI: 10.1007/s11046-013-9720-6]
- 5 **Mendoza L**, Hernandez F, Ajello L. Life cycle of the human and animal oomycete pathogen *Pythium insidiosum*. *J Clin Microbiol* 1993; **31**: 2967-2973 [PMID: 8263182]
- 6 **Vanittanakom N**, Szekely J, Khanthawong S, Sawutdechakul P, Vanittanakom P, Fisher MC. Molecular detection of *Pythium insidiosum* from soil in Thai agricultural areas. *Int J Med Microbiol* 2014; **304**: 321-326 [PMID: 24444720 DOI: 10.1016/j.ijmm.2013.11.016]
- 7 **Santos CEP**, Santurio JM, Marques LC. Pythiosis of livestock in the Pantanal, Mato Grosso, Brazil. *Pesq Vet Bras* 2011; **31**: 1083-1089 [DOI: 10.1590/S0100-736X2011001200008]
- 8 **Krajaeun T**, Sathapatayavongs B, Pracharktam R, Nitiyanant P, Leelachaikul P, Wanachiwanawin W, Chaiprasert A, Assanasen P, Saipetch M, Mootsikapun P, Chetchotisakd P, Lekhakula A, Mitarnun W, Kalnauwakul S, Supparatpinyo K, Chaiwarith R, Chiewchanvit S, Tananuvat N, Srisiri S, Suankratay C, Kulwichit W, Wongsaisuan M, Somkaew S. Clinical and epidemiological analyses of human pythiosis in Thailand. *Clin Infect Dis* 2006; **43**: 569-576 [PMID: 16886148 DOI: 10.1086/506353]
- 9 **Santos CEP**, Ubiali DG, Pescador CA, Zanette RA, Santurio JM, Marques LC. Epidemiological survey of equine pythiosis in the Brazilian Pantanal and nearby areas: results of 76 Cases. *J Equine Vet Sci* 2014; **34**: 270-274 [DOI: 10.1016/j.jevs.2013.06.003]
- 10 **Mendoza L**. *Pythium insidiosum* and mammalian hosts. In: Lamour K, Kamoun S, eds. *Oomycete Genetics and Genomics: Diversity, Interactions and Research Tools*. Hoboken, N.J.: John Wiley and Sons, 2009: 387-405
- 11 **Mendoza L**, Vilela R. Anomalous fungal and fungal-like infections: lacaziosis, pythiosis, and rhinosporidiosis. In: Anaisie EJ, McGinnis MR, Pfaller MA, editors. *Clinical mycology*. Edinburgh: Churchill Livingstone/Elsevier, 2009: 403-415
- 12 **Ravishankar JP**, Davis CM, Davis DJ, MacDonald E, Makseian SD, Millward L, Money NP. Mechanics of solid tissue invasion by the mammalian pathogen *Pythium insidiosum*. *Fungal Genet Biol* 2001; **34**: 167-175 [PMID: 11728155 DOI: 10.1006/fgbi.2001.1304]
- 13 **Schurko A**, Mendoza L, de Cock AW, Klassen GR. Evidence for geographic clusters: Molecular genetic differences among strains of *Pythium insidiosum* from Asia, Australia and the Americas are explored. *Mycologia* 2003; **95**: 200-208 [PMID: 12798882]

- 21156606]
- 14 **Hussein MR.** Mucocutaneous Splendore-Hoeppli phenomenon. *J Cutan Pathol* 2008; **35**: 979-988 [PMID: 18976399 DOI: 10.1111/j.1600-0560.2008.01045.x]
- 15 **Miller RI, Campbell RS.** The comparative pathology of equine cutaneous phycomycosis. *Vet Pathol* 1984; **21**: 325-332 [PMID: 6730223]
- 16 **Martins TB, Kommers GD, Trost ME, Inkelmann MA, Figuera RA, Schild AL.** A comparative study of the histopathology and immunohistochemistry of pythiosis in horses, dogs and cattle. *J Comp Pathol* 2012; **146**: 122-131 [PMID: 21824626 DOI: 10.1016/j.jcpa.2011.06.006]
- 17 **Mendoza L, Mandy W, Glass R.** An improved Pythium insidiosum-vaccine formulation with enhanced immunotherapeutic properties in horses and dogs with pythiosis. *Vaccine* 2003; **21**: 2797-2804 [PMID: 12798620 DOI: 10.1016/S0264-410X(03)00225-1]
- 18 **Wanachiwanawin W, Mendoza L, Visuthisakchai S, Mutsikapan P, Sathapatayavongs B, Chaiprasert A, Suwanagool P, Manuskiatti W, Ruangsetakit C, Ajello L.** Efficacy of immunotherapy using antigens of Pythium insidiosum in the treatment of vascular pythiosis in humans. *Vaccine* 2004; **22**: 3613-3621 [PMID: 15315840 DOI: 10.1016/j.vaccine.2004.03.031]
- 19 **Thitithanyanont A, Mendoza L, Chuansumrit A, Prachartam R, Laothamatas J, Sathapatayavongs B, Lolekha S, Ajello L.** Use of an immunotherapeutic vaccine to treat a life-threatening human arteritic infection caused by Pythium insidiosum. *Clin Infect Dis* 1998; **27**: 1394-1400 [PMID: 9868649]
- 20 **Álvarez JAC, Viloria MIV, Ayola SCP.** Clinical and histopathological evaluation of cutaneous pythiosis in donkeys (Equus asinus). *Rev Med Vet (Bogota)* 2013; **25**: 9-19
- 21 **Videla R, van Amstel S, O'Neill SH, Frank LA, Newman SJ, Vilela R, Mendoza L.** Vulvar pythiosis in two captive camels (Camelus dromedarius). *Med Mycol* 2012; **50**: 219-224 [PMID: 21696258 DOI: 10.3109/13693786.2011.588970]
- 22 **Leal ABM, Leal AT, Santurio JM, Kommers GD, Catto JB.** Equine pythiosis in the Brazilian Pantanal region: Clinical and pathological findings of typical and atypical cases. *Pesq Vet Bras* 2001; **21**: 151-156
- 23 **Reis JL, de Carvalho EC, Nogueira RH, Lemos LS, Mendoza L.** Disseminated pythiosis in three horses. *Vet Microbiol* 2003; **96**: 289-295 [PMID: 14559176 DOI: 10.1016/j.vetmic.2003.07.005]
- 24 **Santos CEP, Santurio JM, Colodel EM, Juliano RS, Silva JA, Marques LC.** Contribution to the study of cutaneous pythiosis in equidae from northern Pantanal, Brazil. *Ars Vet* 2011; **27**: 134-140
- 25 **Humber RA, Brown CC, Kornegay RW.** Equine zygomycosis caused by Conidiobolus lamprauges. *J Clin Microbiol* 1989; **27**: 573-576 [PMID: 2715329]
- 26 **Santurio JM, Monteiro AB, Leal AT, Kommers GD, de Sousa RS, Catto JB.** Cutaneous Pythiosis insidiosi in calves from the Pantanal region of Brazil. *Mycopathologia* 1998; **141**: 123-125 [PMID: 9755503]
- 27 **Thomas RC, Lewis DT.** Pythiosis in dogs and cats. *Comp Cont Educ Pract Vet* 1998; **20**: 63-75
- 28 **Gabriel AL, Kommers GD, Trost ME, Barros CSL, Pereira DB, Schwendler SE, Santurio JM.** Outbreak of cutaneous pythiosis in cattle. *Pesq Vet Bras* 2008; **28**: 583-587
- 29 **Grecco FB, Schild AL, Quevedo P, Assis-Brasil ND, Kommers GD, Marcolongo-Pereira C, Soares MP.** Cutaneous pythiosis in cattle in the Southern region of Rio Grande do Sul, Brazil. *Pesq Vet Bras* 2009; **29**: 938-942
- 30 **Cardona Álvarez JA, Vargas Viloria M, Perdomo SC.** Frequency of presentation of bovine cutaneous pythiosis (Pythium insidiosum) in three cattle farms in Córdoba, Colombia. *CES Med Vet Zoot* 2012; **7**: 47-54
- 31 **Dykstra MJ, Sharp NJ, Olivry T, Hillier A, Murphy KM, Kaufman L, Kunkle GA, Pucheu-Haston C.** A description of cutaneous-subcutaneous pythiosis in fifteen dogs. *Med Mycol* 1999; **37**: 427-433 [PMID: 10647124]
- 32 **Schmiedt CW, Stratton-Phelps M, Torres BT, Bell D, Uhl EW, Zimmerman S, Epstein J, Cornell KK.** Treatment of intestinal pythiosis in a dog with a combination of marginal excision, chemotherapy, and immunotherapy. *J Am Vet Med Assoc* 2012; **241**: 358-363 [PMID: 22812473 DOI: 10.2460/javma.241.3.358]
- 33 **Fernandes CP, Giordani C, Grecco FB, V Sallis ES, R Stainki D, Gaspar LF, Garcez Ribeiro CL, Nobre MO.** Gastric pythiosis in a dog. *Rev Iberoam Micol* 2012; **29**: 235-237 [PMID: 22306044 DOI: 10.1016/j.riam.2012.01.002]
- 34 **Berryessa NA, Marks SL, Pesavento PA, Krasnansky T, Yoshimoto SK, Johnson EG, Grooters AM.** Gastrointestinal pythiosis in 10 dogs from California. *J Vet Intern Med* 2008; **22**: 1065-1069 [PMID: 18647164 DOI: 10.1111/j.1939-1676.2008.0123.x]
- 35 **Hensel P, Greene CE, Medleau L, Latimer KS, Mendoza L.** Immunotherapy for treatment of multicentric cutaneous pythiosis in a dog. *J Am Vet Med Assoc* 2003; **223**: 215-28, 197 [PMID: 12875449]
- 36 **Grooters AM, Leise BS, Lopez MK, Gee MK, O'Reilly KL.** Development and evaluation of an enzyme-linked immunosorbent assay for the serodiagnosis of pythiosis in dogs. *J Vet Intern Med* 2002; **16**: 142-146 [PMID: 11899028]
- 37 **Cooper RC, Allison N, Boring JG.** Apparent successful surgical treatment of intestinal pythiosis with vascular invasion in a dog. *Canine Pract* 1991; **16**: 9-12
- 38 **Foil CSO, Short BG, Fadok VA, Kunkle GA.** A report of subcutaneous pythiosis in 5 dogs and a review of the etiologic agent Pythium spp. *J Am Anim Hosp Assoc* 1984; **20**: 959-966
- 39 **Ader PL.** Phycomycosis in fifteen dogs and two cats. *J Am Vet Med Assoc* 1979; **174**: 1216-1223 [PMID: 438051]
- 40 **Tabosa IM, Riet-Correa F, Nobre VM, Azevedo EO, Reis-Júnior JL, Medeiros RM.** Outbreaks of pythiosis in two flocks of sheep in northeastern Brazil. *Vet Pathol* 2004; **41**: 412-415 [PMID: 15232143]
- 41 **Carrera MV, Peixoto RM, Gouveia GV, Pessoa CRM, Jesus FPK, Santurio JM, Botton SA, Costa MM.** Pythiosis in sheep from Pernambuco and Bahia States, Brazil. *Pesq Vet Bras* 2013; **33**: 476-482 [DOI: 10.1590/S0100-736X2013000400011]
- 42 **Camus AC, Grooters AM, Aquilar RE.** Granulomatous pneumonia caused by Pythium insidiosum in a central American jaguar, Panthera onca. *J Vet Diagn Invest* 2004; **16**: 567-571 [PMID: 15586573]
- 43 **Grooters AM.** Pythiosis, lagenidiosis, and zygomycosis in small animals. *Vet Clin North Am Small Anim Pract* 2003; **33**: 695-720, v [PMID: 12910739 DOI: 10.1016/S0195-5616(03)00034-2]
- 44 **Pesavento PA, Barr B, Riggs SM, Eigenheer AL, Pamma R, Walker RL.** Cutaneous pythiosis in a nestling white-faced ibis. *Vet Pathol* 2008; **45**: 538-541 [PMID: 18587102 DOI: 10.1354/vp.45-4-538]
- 45 **Badenoch PR, Coster DJ, Wetherall BL, Brettig HT, Rozenbils MA, Drenth A, Wagels G.** Pythium insidiosum keratitis confirmed by DNA sequence analysis. *Br J Ophthalmol* 2001; **85**: 502-503 [DOI: 10.1136/bjo.85.4.496g]
- 46 **Murdoch D, Parr D.** Pythium insidiosum keratitis. *Aust N Z J Ophthalmol* 1997; **25**: 177-179 [PMID: 9267609 DOI: 10.1111/j.1442-9071.1997.tb01304.x]
- 47 **Virgile R, Perry HD, Pardanani B, Szabo K, Rahn EK, Stone J, Salkin I, Dixon DM.** Human infectious corneal ulcer caused by Pythium insidiosum. *Cornea* 1993; **12**: 81-83 [PMID: 8458239 DOI: 10.1097/00003226-199301000-00015]
- 48 **Bosco Sde M, Bagagli E, Araújo JP, Candeias JM, de Franco MF, Alencar Marques ME, Mendoza L, de Camargo RP, Alencar Marques S.** Human pythiosis, Brazil. *Emerg Infect Dis* 2005; **11**: 715-718 [PMID: 15890126 DOI: 10.3201/eid1105.040943]
- 49 **Shenep JL, English BK, Kaufman L, Pearson TA, Thompson JW, Kaufman RA, Frisch G, Rinaldi MG.** Successful medical

- therapy for deeply invasive facial infection due to *Pythium insidiosum* in a child. *Clin Infect Dis* 1998; **27**: 1388-1393 [PMID: 9868648 DOI: 10.1086/515042]
- 50 **Triscott JA**, Weedon D, Cabana E. Human subcutaneous pythiosis. *J Cutan Pathol* 1993; **20**: 267-271 [PMID: 8366216 DOI: 10.1111/j.1600-0560.1993.tb00654.x]
- 51 **Thianprasit M**, Chaiprasert A, Imwidthaya P. Human pythiosis. *Curr Top Med Mycol* 1996; **7**: 43-54 [PMID: 9504058]
- 52 **Walker EM**, Walker SM. Effects of iron overload on the immune system. *Ann Clin Lab Sci* 2000; **30**: 354-365 [PMID: 11045759]
- 53 **Jurado RL**. Iron, infections, and anemia of inflammation. *Clin Infect Dis* 1997; **25**: 888-895 [PMID: 9356804 DOI: 10.1086/515549]
- 54 **Ekiz C**, Agaoglu L, Karakas Z, Gurel N, Yalcin I. The effect of iron deficiency anemia on the function of the immune system. *Hematol J* 2005; **5**: 579-583 [PMID: 15692603 DOI: 10.1038/sj.thj.6200574]
- 55 **Johnson L**. Iron and siderophores in fungal-host interactions. *Mycol Res* 2008; **112**: 170-183 [PMID: 18280720 DOI: 10.1016/j.mycres.2007.11.012]
- 56 **Krajaejun T**, Khositnithikul R, Lerksuthirath T, Lowhnoo T, Rujirawat T, Petchthong T, Yingyong W, Suriyaphol P, Smittipat N, Juthayothin T, Phuntumart V, Sullivan TD. Expressed sequence tags reveal genetic diversity and putative virulence factors of the pathogenic oomycete *Pythium insidiosum*. *Fungal Biol* 2011; **115**: 683-696 [PMID: 21724174 DOI: 10.1016/j.funbio.2011.05.001]
- 57 **Krajaejun T**, Lerksuthirath T, Garg G, Lowhnoo T, Yingyong W, Khositnithikul R, Tangphatsornruang S, Suriyaphol P, Ranganathan S, Sullivan TD. Transcriptome analysis reveals pathogenicity and evolutionary history of the pathogenic oomycete *Pythium insidiosum*. *Fungal Biol* 2014 [DOI: 10.1016/j.funbio.2014.01.009]
- 58 **Sathapatayavongs B**, Leelachaikul P, Prachaktam R, Atichartakarn V, Sriphojanart S, Trairatvorakul P, Jirasiritham S, Nontasut S, Eurvilaichit C, Flegel T. Human pythiosis associated with thalassemia hemoglobinopathy syndrome. *J Infect Dis* 1989; **159**: 274-280 [PMID: 2644370 DOI: 10.1093/infdis/159.2.274]
- 59 **González Charry H**, Trheebilcock Perna E, Montaña Aguirre J, León J. Potassium iodine (K.I.) as treatment for subcutaneous equine phycomycosis. *Revista ICA (Colombia)* 1979; **14**: 115-122
- 60 **Miller RI**, Wold D, Lindsay WA, Beadle RE, McClure JJ, McClure JR, McCoy DJ. Complications associated with immunotherapy of equine phycomycosis. *J Am Vet Med Assoc* 1983; **182**: 1227-1229 [PMID: 6863139]
- 61 **Morton LD**, Morton DG, Baker GJ, Gelberg HB. Chronic eosinophilic enteritis attributed to *Pythium* sp. in a horse. *Vet Pathol* 1991; **28**: 542-544 [PMID: 1771746 DOI: 10.1177/030098589102800615]
- 62 **Chaffin MK**, Schumacher J, Hooper N. Multicentric cutaneous pythiosis in a foal. *J Am Vet Med Assoc* 1992; **201**: 310-312 [PMID: 1500331]
- 63 **Worster AA**, Lillich JD, Cox JH, Rush BR. Pythiosis with bone lesions in a pregnant mare. *J Am Vet Med Assoc* 2000; **216**: 1795-1798, 1760 [PMID: 10844973]
- 64 **Santos CEP**, Juliano RS, Santurio JM, Marques LC. Efficacy of immunotherapy in the treatment of facial horse pythiosis. *Acta Sci Vet* 2011; **39**: 955
- 65 **Mosbah E**, Karrouf GIA, Younis EA, Saad HS, Ahdy A, Zaghloul AE. Diagnosis and surgical management of pythiosis in draft horses: report of 33 cases in Egypt. *J Equine Vet Sci* 2012; **32**: 164-169 [DOI: 10.1016/j.jevs.2011.08.014]
- 66 **Liljebjelke KA**, Abramson C, Brockus C, Greene CE. Duodenal obstruction caused by infection with *Pythium insidiosum* in a 12-week-old puppy. *J Am Vet Med Assoc* 2002; **220**: 1188-1191, 1162 [PMID: 11990966]
- 67 **Rakich PM**, Grooters AM, Tang KN. Gastrointestinal pythiosis in two cats. *J Vet Diagn Invest* 2005; **17**: 262-269 [PMID: 15945385 DOI: 10.1177/104063870501700310]
- 68 **Wellehan JF**, Farina LL, Keoughan CG, Lafortune M, Grooters AM, Mendoza L, Brown M, Terrell SP, Jacobson ER, Heard DJ. Pythiosis in a dromedary camel (*Camelus dromedarius*). *J Zoo Wildl Med* 2004; **35**: 564-568 [PMID: 15732604 DOI: 10.1638/03-098]
- 69 **Buergelt C**, Powe J, White T. Abdominal pythiosis in a Bengal tiger (*Panthera tigris tigris*). *J Zoo Wildl Med* 2006; **37**: 186-189 [PMID: 17312799 DOI: 10.1638/05-003.1]
- 70 **Santos SA**. Recomendações sobre manejo nutricional para equinos criados em pastagens nativas no Pantanal. Corumbá: EMBRAPA-CPAP, 1997
- 71 **Loreto ES**, Alves SH, Santurio JM, Nogueira CW, Zeni G. Diphenyl diselenide in vitro and in vivo activity against the oomycete *Pythium insidiosum*. *Vet Microbiol* 2012; **156**: 222-226 [PMID: 22055205 DOI: 10.1016/j.vetmic.2011.10.008]
- 72 **Zanette RA**, Bitencourt PE, Alves SH, Figuera RA, Flores MM, Wolkmer P, Hecktheuer PA, Thomas LR, Pereira PL, Loreto ES, Santurio JM. Insights into the pathophysiology of iron metabolism in *Pythium insidiosum* infections. *Vet Microbiol* 2013; **162**: 826-830 [PMID: 23182911 DOI: 10.1016/j.vetmic.2012.10.036]
- 73 **Santurio JM**, Ferreira L. Pitiose: uma abordagem micológica e terapêutica. Porto Alegre: Editora da UFRGS, 2008
- 74 **Leal AT**, Leal ABM, Flores EF, Santurio JM. *Pythiosis*. *Cienc Rural* 2001; **31**: 735-743
- 75 **Grooters AM**, Whittington A, Lopez MK, Borroughs MN, Roy AF. Evaluation of microbial culture techniques for the isolation of *Pythium insidiosum* from equine tissues. *J Vet Diagn Invest* 2002; **14**: 288-294 [PMID: 12152807]
- 76 **Pereira DIB**, Santurio JM, Alves SH, Argenta JS, Cavalheiro AS, Ferreira L. In vitro zoosporogenesis among oomycetes *Pythium insidiosum* isolates. *Cienc Rural* 2008; **38**: 143-147
- 77 **Grooters AM**, Gee MK. Development of a nested polymerase chain reaction assay for the detection and identification of *Pythium insidiosum*. *J Vet Intern Med* 2002; **16**: 147-152
- 78 **Schurko AM**, Mendoza L, de Cock AW, Bedard JE, Klassen GR. Development of a species-specific probe for *Pythium insidiosum* and the diagnosis of pythiosis. *J Clin Microbiol* 2004; **42**: 2411-2418 [PMID: 15184412]
- 79 **Vanittanakom N**, Supabandhu J, Khamwan C, Preparattapan J, Thirach S, Prasertwitayakij N, Louthrenoo W, Chiewchanvit S, Tananuvat N. Identification of emerging human-pathogenic *Pythium insidiosum* by serological and molecular assay-based methods. *J Clin Microbiol* 2004; **42**: 3970-3974 [PMID: 15364977 DOI: 10.1128/Jcm.42.9.3970-3974.2004]
- 80 **Botton SA**, Pereira DI, Costa MM, Azevedo MI, Argenta JS, Jesus FP, Alves SH, Santurio JM. Identification of *Pythium insidiosum* by nested PCR in cutaneous lesions of Brazilian horses and rabbits. *Curr Microbiol* 2011; **62**: 1225-1229 [PMID: 21188592 DOI: 10.1007/s00284-010-9781-4]
- 81 **Thongsri Y**, Wonglakorn L, Chaiprasert A, Svobodova L, Hamal P, Pakarasang M, Prariyachatigul C. Evaluation for the clinical diagnosis of *Pythium insidiosum* using a single-tube nested PCR. *Mycopathologia* 2013; **176**: 369-376 [PMID: 23948967 DOI: 10.1007/s11046-013-9695-3]
- 82 **Miller RI**, Campbell RS. Immunological studies on equine phycomycosis. *Aust Vet J* 1982; **58**: 227-231 [PMID: 6814414]
- 83 **Witkamp J**. Bijdrage tot de kennis van de Hyphomycosis destruens. *Nederlandsh-Indisch blande voor Diergeneeskunde en Dierenteelt* 1924; **36**: 229-345
- 84 **Mendoza L**, Nicholson V, Prescott JF. Immunoblot analysis of the humoral immune response to *Pythium insidiosum* in horses with pythiosis. *J Clin Microbiol* 1992; **30**: 2980-2983 [PMID: 1452669]
- 85 **Mendoza L**, Kaufman L, Standard PG. Immunodiffusion test for diagnosing and monitoring pythiosis in horses. *J Clin Microbiol* 1986; **23**: 813-816 [PMID: 3086368]
- 86 **Imwidthaya P**, Srimuang S. Immunodiffusion test for diag-

- nosing human pythiosis. *Mycopathologia* 1989; **106**: 109-112 [PMID: 2507920]
- 87 **Miller RI**, Campbell RS. Haematology of horses with phycomycosis. *Aust Vet J* 1983; **60**: 28-29 [PMID: 6830547]
- 88 **Mendoza L**, Alfaro AA. Equine pythiosis in Costa Rica: report of 39 cases. *Mycopathologia* 1986; **94**: 123-129 [PMID: 3088454]
- 89 **Krajaejun T**, Kunakorn M, Niemhom S, Chongtrakool P, Prachartam R. Development and evaluation of an in-house enzyme-linked immunosorbent assay for early diagnosis and monitoring of human pythiosis. *Clin Diagn Lab Immunol* 2002; **9**: 378-382 [PMID: 11874882 DOI: 10.1128/Cdli.9.2.378-382.2002]
- 90 **Santurio JM**, Leal AT, Leal ABM, Alves SH, Lubeck I, Griebeler J, Copetti MV. Indirect ELISA for the serodiagnosis of pythiosis. *Pesq Vet Bras* 2006; **26**: 47-50
- 91 **Jindayok T**, Piromsontikorn S, Srimuang S, Khupulsup K, Krajaejun T. Hemagglutination test for rapid serodiagnosis of human pythiosis. *Clin Vaccine Immunol* 2009; **16**: 1047-1051 [PMID: 19494087 DOI: 10.1128/Cvi.00113-09]
- 92 **Krajaejun T**, Imkhieo S, Intaramat A, Ratanabanangkoon K. Development of an immunochromatographic test for rapid serodiagnosis of human pythiosis. *Clin Vaccine Immunol* 2009; **16**: 506-509 [PMID: 19225072 DOI: 10.1128/Cvi.00276-08]
- 93 **Supabandhu J**, Vanittanakom P, Laohapensang K, Vanittanakom N. Application of immunoblot assay for rapid diagnosis of human pythiosis. *J Med Assoc Thai* 2009; **92**: 1063-1071 [PMID: 19694332]
- 94 **Trost ME**, Gabriel AL, Masuda EK, Figuera RA, Irigoyen LF, Kommers GD. Clinical, morphologic and immunohistochemical aspects of canine gastrointestinal pythiosis. *Pesq Vet Bras* 2009; **29**: 673-679
- 95 **Salipante SJ**, Hoogestraat DR, SenGupta DJ, Murphey D, Panayides K, Hamilton E, Castañeda-Sánchez I, Kennedy J, Monsaas PW, Mendoza L, Stephens K, Dunn JJ, Cookson BT. Molecular diagnosis of subcutaneous *Pythium insidiosum* infection by use of PCR screening and DNA sequencing. *J Clin Microbiol* 2012; **50**: 1480-1483 [PMID: 22205808 DOI: 10.1128/JCM.06126-11]
- 96 **Mendoza L**, Prasla SH, Ajello L. Orbital pythiosis: a non-fungal disease mimicking orbital mycotic infections, with a retrospective review of the literature. *Mycoses* 2004; **47**: 14-23 [PMID: 14998394]
- 97 **Argenta JS**, Santurio JM, Alves SH, Pereira DI, Cavalheiro AS, Spanemberg A, Ferreiro L. In vitro activities of voriconazole, itraconazole, and terbinafine alone or in combination against *Pythium insidiosum* isolates from Brazil. *Antimicrob Agents Chemother* 2008; **52**: 767-769 [PMID: 18056274 DOI: 10.1128/Aac.01075-07]
- 98 **Pereira DI**, Santurio JM, Alves SH, Argenta JS, Pötter L, Spanemberg A, Ferreiro L. Caspofungin in vitro and in vivo activity against Brazilian *Pythium insidiosum* strains isolated from animals. *J Antimicrob Chemother* 2007; **60**: 1168-1171 [PMID: 17785281 DOI: 10.1093/Jac/Dkm332]
- 99 **Cavalheiro AS**, Zanette RA, Spader TB, Lovato L, Azevedo MI, Botton S, Alves SH, Santurio JM. In vitro activity of terbinafine associated to amphotericin B, fluvastatin, rifampicin, metronidazole and ibuprofen against *Pythium insidiosum*. *Vet Microbiol* 2009; **137**: 408-411 [PMID: 19269752 DOI: 10.1016/j.vetmic.2009.01.036]
- 100 **Cavalheiro AS**, Maboni G, de Azevedo MI, Argenta JS, Pereira DI, Spader TB, Alves SH, Santurio JM. In Vitro activity of terbinafine combined with caspofungin and azoles against *Pythium insidiosum*. *Antimicrob Agents Chemother* 2009; **53**: 2136-2138 [PMID: 19289531 DOI: 10.1128/Aac.01506-08]
- 101 **Loreto ES**, Mario DA, Denardi LB, Alves SH, Santurio JM. In vitro susceptibility of *Pythium insidiosum* to macrolides and tetracycline antibiotics. *Antimicrob Agents Chemother* 2011; **55**: 3588-3590 [PMID: 21537028 DOI: 10.1128/Aac.01586-10]
- 102 **Mahl DL**, de Jesus FP, Loreto É, Zanette RA, Ferreiro L, Pilotto MB, Alves SH, Santurio JM. In vitro susceptibility of *Pythium insidiosum* isolates to aminoglycoside antibiotics and tigecycline. *Antimicrob Agents Chemother* 2012; **56**: 4021-4023 [PMID: 22508303 DOI: 10.1128/Aac.00073-12]
- 103 **Miller RI**. Treatment of equine phycomycosis by immunotherapy and surgery. *Aust Vet J* 1981; **57**: 377-382 [PMID: 7342944]
- 104 **Mendoza L**, Villalobos J, Calleja CE, Solis A. Evaluation of two vaccines for the treatment of pythiosis insidiosi in horses. *Mycopathologia* 1992; **119**: 89-95 [PMID: 1435952]
- 105 **Miller RI**, Campbell RS. Clinical observations on equine phycomycosis. *Aust Vet J* 1982; **58**: 221-226 [PMID: 6890342]
- 106 **Santurio JM**, Leal AT, Leal AB, Festugatto R, Lubeck I, Sallis ES, Copetti MV, Alves SH, Ferreiro L. Three types of immunotherapies against pythiosis insidiosi developed and evaluated. *Vaccine* 2003; **21**: 2535-2540 [PMID: 12744888 DOI: 10.1016/S0264-410X(03)00035-5]
- 107 **Monteiro AB**. Immunotherapy of equine pythiosis: testing the efficacy of a biological and evaluation of the leukocytic response to the treatment in horses naturally infected with *Pythium insidiosum*. Master in Veterinary Medicine. Santa Maria, RS, Brazil: Federal University of Santa Maria, 1999
- 108 **Pereira DI**, Botton SA, Azevedo MI, Motta MA, Lobo RR, Soares MP, Fonseca AO, Jesus FP, Alves SH, Santurio JM. Canine gastrointestinal pythiosis treatment by combined antifungal and immunotherapy and review of published studies. *Mycopathologia* 2013; **176**: 309-315 [PMID: 23918089 DOI: 10.1007/s11046-013-9683-7]
- 109 **Mendoza L**, Alfaro AA, Villalobos J. Bone lesions caused by *Pythium insidiosum* in a horse. *J Med Vet Mycol* 1988; **26**: 5-12 [PMID: 3379540]
- 110 **Eaton SA**. Osseous involvement by *Pythium insidiosum*. *Comp Cont Educ Pract Vet* 1993; **15**: 485-488
- 111 **Fisher EM**. Cutaneous phycomycosis in two horses. *Aust Vet J* 2000; **78**: 257 [PMID: 10840572 DOI: 10.1111/j.1751-0813.1999.tb12942.x]
- 112 **Maciel ICD**, Silveira JT, Maia CA, Sousa RM, Oliveira NJF, Duarte ER. Fatal pythiosis in horse initially treated to cutaneous habronemiasis. *Acta Sci Vet* 2008; **36**: 293-297
- 113 **White SD**, Ghoddusi M, Grooters AM, Jones K. Cutaneous pythiosis in a nontravelled California horse. *Vet Dermatol* 2008; **19**: 391-394 [PMID: 18699814 DOI: 10.1111/j.1365-3164.2008.00690.x]
- 114 **Bandeira A**, Santos JdA, Melo Cd, Andrade V, Dantas A, Araujo J. Equine cutaneous pythiosis in Sergipe State, Brazil. *Cienc Vet Trop* 2009; **12**: 46-54
- 115 **Santos CE**, Marques LC, Zanette RA, Jesus FP, Santurio JM. Does immunotherapy protect equines from reinfection by the oomycete *Pythium insidiosum*? *Clin Vaccine Immunol* 2011; **18**: 1397-1399 [PMID: 21715582 DOI: 10.1128/Cvi.05150-11]
- 116 **Laohapensang K**, Rerkasem K, Supabandhu J, Vanittanakom N. Necrotizing arteritis due to emerging *Pythium insidiosum* infection in patients with thalassemia: rapid diagnosis with PCR and serological tests-case reports. *Int J Angiol* 2005; **14**: 123-128 [DOI: 10.1007/s00547-005-2012-3]
- 117 **Pupaibool J**, Chindamporn A, Patrakul K, Suankratay C, Sindhuphak W, Kulwichit W. Human pythiosis. *Emerg Infect Dis* 2006; **12**: 517-518 [PMID: 16710978]
- 118 **Lekhanont K**, Chuckpaiwong V, Chongtrakool P, Aroonroch R, Vongthongsri A. *Pythium insidiosum* keratitis in contact lens wear: a case report. *Cornea* 2009; **28**: 1173-1177 [PMID: 19730096 DOI: 10.1097/ICO.0b013e318199fa41]
- 119 **Sudjaritruk T**, Sirisanthana V. Successful treatment of a child with vascular pythiosis. *BMC Infect Dis* 2011; **11**: 33 [PMID: 21276255 DOI: 10.1186/1471-2334-11-33]
- 120 **Keoprasom N**, Chularojanamontri L, Chaikulkeeree M, Chairprasert A, Wanachiwanawin W, Ruangsetakit C. Vascular pythiosis in a thalassemic patient presenting as bilateral leg ulcers. *Med Mycol Case Rep* 2012; **2**: 25-28 [PMID:

- 24432209 DOI: 10.1016/j.mmcr.2012.12.002]
- 121 **Schloemer NJ**, Lincoln AH, Mikhailov TA, Collins CL, Di Rocco JR, Kehl SC, Chusid MJ. Fatal disseminated *Pythium insidiosum* infection in a child with Diamond-Blackfan anemia. *Infect Dis Clin Pract* 2013; **21**: e24-e26 [DOI: 10.1097/IPC.0b013e318278f3b5]
 - 122 **Thanathane O**, Enkvetchakul O, Rangsin R, Waraasawapati S, Samerpitak K, Suwan-apichon O. Outbreak of *Pythium* keratitis during rainy season: a case series. *Cornea* 2013; **32**: 199-204 [PMID: 22902492 DOI: 10.1097/Ico.0b013e3182535841]
 - 123 **Bach BC**, Leal DB, Jaques JA, Souza Vdo C, Ruchel JB, Schlemmer KB, Zanette RA, Hecktheuer PA, de Lima Pereira P, Casali EA, Alves SH, Santurio JM. E-ADA activity in lymphocytes of an experimental model of pythiosis treated with immunotherapy. *Cell Biochem Funct* 2013; **31**: 476-481 [PMID: 23086808 DOI: 10.1002/cbf.2921]
 - 124 **Krajaejun T**, Kunakorn M, Prachartam R, Chongtrakool P, Sathapatayavongs B, Chaiprasert A, Vanittanakom N, Chindamporn A, Mootsikapun P. Identification of a novel 74-kiloDalton immunodominant antigen of *Pythium insidiosum* recognized by sera from human patients with pythiosis. *J Clin Microbiol* 2006; **44**: 1674-1680 [PMID: 16672392 DOI: 10.1128/Jcm.44.5.1674-1680.2006]
 - 125 **Krajaejun T**, Keeratijarut A, Sriwanichrak K, Lowhnoo T, Rujirawat T, Petchthong T, Yingyong W, Kalambaheti T, Smittipat N, Juthayothin T, Sullivan TD. The 74-kilodalton immunodominant antigen of the pathogenic oomycete *Pythium insidiosum* is a putative exo-1,3-beta-glucanase. *Clin Vaccine Immunol* 2010; **17**: 1203-1210 [PMID: 20237199 DOI: 10.1128/Cvi.00515-09]

P- Reviewer: dos Santos CEP, Prariyachatigul C, Sahu RP, Wang ZX **S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Wang CH





GM3-containing nanoparticles in immunosuppressed hosts: Effect on myeloid-derived suppressor cells

Audry Fernández, Liliana Oliver, Rydell Alvarez, Luis E Fernández, Circe Mesa

Audry Fernández, Liliana Oliver, Rydell Alvarez, Circe Mesa, Immunobiology Division, Center of Molecular Immunology, Havana 11600, Cuba

Luis E Fernández, Innovation Division, Center of Molecular Immunology, Havana 11600, Cuba

Author contributions: Fernández A, Oliver L, Alvarez R, Fernández LE and Mesa C drafted the review and wrote the paper.

Supported by Center of Molecular Immunology

Correspondence to: Circe Mesa, PhD, Director of Immunobiology Division, Center of Molecular Immunology, 216 St. and 15th Avenue, Atabey, Playa, PO Box 16040, Havana 11600, Cuba. circe@cim.sld.cu

Telephone: +53-7-2143161 Fax: +53-7-2720644

Received: March 8, 2014 Revised: June 12, 2014

Accepted: June 27, 2014

Published online: July 27, 2014

Abstract

Cancer vaccines to date have not broadly achieved a significant impact on the overall survival of patients. The negative effect on the immune system of the tumor itself and conventional anti-tumor treatments such as chemotherapy is, undoubtedly, a key reason for these disappointing results. Myeloid-derived suppressor cells (MDSCs) are considered a central node of the immunosuppressive network associated with tumors. These cells inhibit the effector function of natural killer and CD8⁺ T cells, expand regulatory T cells and can differentiate into tumor-associated macrophages within the tumor microenvironment. Thus, overcoming the suppressive effects of MDSCs is likely to be critical for cancer immunotherapy to generate effective anti-tumor immune responses. However, the capacity of cancer vaccines and particularly their adjuvants to overcome this inhibitory population has not been well characterized. Very small size proteoliposomes (VSSP) is a nanoparticulated adjuvant specifically designed to be formulated with vaccines used in the treatment of immunocompromised patients. This adjuvant contains immunostimulatory bacterial signals together with GM3

ganglioside. VSSP promotes dendritic cell maturation, antigen cross-presentation to CD8⁺ T cells, Th1 polarization, and enhances CD8⁺ T cell response in tumor-free mice. Currently, four cancer vaccines using VSSP as the adjuvant are in Phase I and II clinical trials. In this review, we summarize our work characterizing the unique ability of VSSP to stimulate antigen-specific CD8⁺ T cell responses in two immunocompromised scenarios; in tumor-bearing mice and during chemotherapy-induced leukopenia. Particular emphasis has been placed on the interaction of these nanoparticles with MDSCs, as well as comparison with other cancer vaccine adjuvants currently in preclinical or clinical studies.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Very small size proteoliposomes; Adjuvants; Tumors; Myeloid-derived suppressor cells; Leukopenia; Chemotherapy

Core tip: Very small size proteoliposomes (VSSP) is a nanoparticulated adjuvant being used in the formulation of several cancer vaccines that are currently in clinical trials. In this review we summarize the unique ability of VSSP to stimulate antigen-specific CD8⁺ T cell responses in tumor-bearing mice and in mice with chemotherapy-induced leukopenia, both immunosuppressive scenarios frequently found in cancer patients. As a possible mechanism of this efficacy, we have focused on the modulation of myeloid-derived suppressor cells (MDSCs) by these nanoparticles, in the context of the current knowledge about the interaction of cancer vaccine adjuvants with MDSCs.

Fernández A, Oliver L, Alvarez R, Fernández LE, Mesa C. GM3-containing nanoparticles in immunosuppressed hosts: Effect on myeloid-derived suppressor cells. *World J Immunol* 2014; 4(2): 98-106 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/98.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.98>

INTRODUCTION

The central importance and complexity of the interactions between tumors and the immune system has only recently been recognized, with rapidly expanding investigations in the last decade. Tumors are not only shaped by the immune system^[1,2] but actively induce impairment of antigen-presenting cells (APCs) as well as effector T lymphocytes^[3,4], contributing significantly to both tumor progression and metastasis. One of the key cellular mediators of tumor-induced immunosuppression are myeloid-derived suppressor cells (MDSCs), which not only are the manifestation of the myeloid differentiation block that causes loss of mature APCs, but also actively and directly inhibit the lytic activity of both CD8⁺ T cells^[5,6] and NK cells^[7].

MDSCs are currently thought of as a heterogeneous population of immature myeloid cells with suppressive activity. In mice these cells are routinely identified by the co-expression of CD11b and Gr1 markers. More recently, two subpopulations of MDSCs have been identified with different phenotypes and mechanisms of suppression: monocytic (Mo-MDSCs) and granulocytic (G-MDSCs)^[8-11]. In tumor-bearing mice, as well as in cancer patients, the G-MDSCs constitute 70%-80% of overall MDSCs, whereas Mo-MDSCs represent only 20%-30%^[11-14]. Mo-MDSCs (CD11b⁺Ly6C^{hi}Ly6G⁻) are highly immunosuppressive and exert their suppression *via* antigen-independent mechanisms^[15-18]. In comparison, G-MDSCs (CD11b⁺Ly6C^{lo}Ly6G⁺) are moderately immunosuppressive, release reactive oxygen species (ROS) and require antigen-specific interaction with T cells to induce tolerance^[9,11,19,20]. Several mechanisms of MDSC-mediated suppression have been described and are extensively detailed in other reviews^[3,21]. Among these, the depletion of L-arginine, production of nitric oxide (NO) and generation of ROS/reactive nitrogen species have been linked to the overexpression of arginase 1 (ARG1), inducible nitric oxide synthase (NOS2) and NADPH oxidase^[3,13,22]. MDSCs are also able to expand regulatory T cells (Tregs) populations^[23,24] and can differentiate into tumor-associated macrophages within the tumor microenvironment^[25,26]—both regulatory populations that play an important role in tumor-induced immunosuppression. Recent findings suggest that MDSCs can also facilitate tumor-progression and metastasis by increasing angiogenesis^[27,28], *via* secretion of matrix metalloproteinases^[29,30] and by aiding in the formation of the metastatic niche^[27,31].

Given the pro-tumor importance of MDSCs, many efforts have been undertaken to find drugs capable of reducing the number of circulating MDSCs, abrogate MDSCs suppressive function or differentiate these cells into mature APCs. For instance, it has been demonstrated that 25-hydroxy vitamin D3 and all-trans retinoic acid reduce the frequency of MDSCs by inducing their differentiation towards HLA-DR⁺ cells and dendritic cells (DCs), respectively, in patients with advance head and neck squamous cell carcinoma and metastatic renal cell

carcinoma (RCC)^[32-34]. Sunitinib, a pan-receptor tyrosine kinase inhibitor, and chemotherapeutic agents (taxanes, gemcitabine and 5-fluorouracil) also decrease circulating MDSCs in patients with RCC, melanoma, pancreatic and esophagogastric cancer^[35,36]. Finally, the phosphodiesterase-5 inhibitor sildenafil diminishes the suppressive function of human MDSCs^[37].

Although the pharmacological modulation of MDSCs represents a potentially important strategy for cancer treatment, none of these drugs detailed above have thus far improved the clinical outcome in cancer patients. These data suggest that inhibiting MDSCs alone (unlike the T cell checkpoint inhibitors) is not sufficient to achieve an effective anti-tumor response, and that combination with strategies to specifically activate immune responses against the cancer are needed. However, most cancer vaccines have not shown significant objective responses in clinical trials. But, the unimpressive clinical impact of active immunotherapy in cancer patients may be in turn tied to the immunosuppressive environment generated by tumors^[3,4,21] as well as the aggressive chemotherapeutic treatments used in patients, which frequently induce leukopenia^[38-40]. Thus, the combination of cancer vaccines with agents interfering with MDSCs number/function may be an effective approach to generate fully functional tumor-specific immune effectors. Even more desirable would be to find agents that are capable of simultaneously activating tumor-specific effector cells, inhibiting the suppressive function of MDSCs, and diminishing leukopenic period after chemotherapy. As detailed below, these are all properties of the VSSP adjuvant.

Adjuvants are critical but largely unappreciated components of vaccine formulations, necessary to potentiate the immune response specific for the nominal antigen. This is particularly important in cancer, where the vaccine antigen is often a self protein for which self-tolerance needs to be broken. In recent years the interaction of adjuvants with regulatory cells, and particularly MDSCs, have begun to be studied^[41-45]. This field is still in its infancy however, and there is only strong evidence for the modulation of tumor-induced MDSCs by synthetic oligodeoxynucleotides containing unmethylated CpG motifs (CpG)^[44], formalin-inactivated Herpes Simplex Virus^[43] and VSSP^[42], while indirect evidence suggests that other adjuvants may expand MDSCs once inoculated in the hosts. Therefore, the selection of suitable adjuvants for cancer vaccines is a very complex matter, and needs to be based in the ability to overcome the immunosuppression generated by tumors and chemotherapy. In this review we summarize the immunomodulatory properties of VSSP, a novel adjuvant for cancer immunotherapy.

GENERAL PROPERTIES OF VSSP

VSSP is a nanoparticulated adjuvant obtained through the hydrophobic incorporation of the GM3 ganglioside into outer membrane vesicles (OMVs) from *Neisseria meningitidis*^[46]. It has been shown that VSSP contains TLR4

Table 1 Modulation caused by very small size proteoliposomes on different immune cell populations

Immune cell	Effect of VSSP	Ref.
DCs	Increases costimulation and MHCII expression	[47]
	Enhances production of IL-12, IL-6, IL-18, IL-1 β and reduces secretion of IL-10	[47,48]
	Induces Th1-polarizing capacity	[47]
	Facilitates cross-presentation of protein antigens	[50]
MDSCs	Expands poorly suppressive MDSCs	[42]
	Reduces the suppressive function of tumor-induced MDSCs	[42]
	Impairs migration of tumor-induced MDSCs towards the tumor microenvironment	[42]
	Promotes differentiation of tumor-induced MDSCs into mature DCs	[42,59]
	Reduces the suppressive function of MDSCs generated during chemotherapy-induced leukopenia	[62]
	Induces Th1 polarization	[47]
CD4 ⁺ T cells	Potentiates CTL responses in healthy mice.	[50]
CTL	Primary expansion independent of CD4 ⁺ T cell help	
	Generates similar CTL responses in tumor-free and tumor-bearing mice	[42]
	Increases CD8 ⁺ T cell counts, with memory phenotype, and protects CTL response in leukopenic mice	[62]

VSSP: Very small size proteoliposomes; MDSCs: Myeloid-derived suppressor cells; DCs: Dendritic cells; CTL: Cytotoxic T lymphocytes.

and TLR2 ligands, which play an important role in the immunomodulatory properties of this compound^[47,48]. Immunization of mice, monkeys and humans with VSSP generated IgM and IgG antibodies specific for both GM3 and OMPs^[46,49]. This adjuvant also induced DC maturation, as evidenced by the increased expression of MHCII and CD40, CD80 and CD86 costimulatory molecules (Table 1)^[47]. Additionally, VSSP-treated DCs secreted inflammatory cytokines such as IL-12p40/70 and IL-6^[47]. DCs from healthy donors treated *in vitro* with VSSP produced not only higher levels of IL-6 but also decreased amount of IL-10, in comparison to lipopolysaccharide [LPS, the prototypic TLR4 agonist (Table 1)]^[48]. Experiments with antigen-specific transgenic T cells demonstrated that VSSP-treated DCs induced a Th1 phenotype in stimulated naïve CD4⁺ T cells^[47]. Furthermore, VSSP expanded CD8⁺ T cells specific for the co-injected antigen and promoted an effective *in vivo* cytotoxic T lymphocytes (CTL) response^[50]. In the latter case, CD8⁺ T cell activation was mediated by the cross-presentation of exogenous antigens and did not require help from CD4⁺ T cells (Table 1)^[50].

More recently, we have found that VSSP treatment of naïve mice (without a vaccine antigen) significantly increased the frequency of splenic CD11b⁺Gr1⁺ cells^[42]. However, these CD11b⁺Gr1⁺ cells were poorly suppressive on both antigen-specific and allogeneic CTL assays (Table 1). The residual suppressive capacity of VSSP-derived MDSCs depended on NOS but not ARG, which was associated with a significant increase of NOS3 en-

zyme. Although VSSP contains TLR2 and TLR4 ligands, the interaction of these particles with the immune system appears to be more complex than can be explained by just TLR activation. For example, OMPs containing the same TLR ligands induced a significantly lower expansion of CD11b⁺Gr1⁺ cells than did VSSP, indicating that the presence of the GM3 ganglioside is also relevant for the immunomodulatory properties of this compound.

VSSP-induced expansion of MDSC numbers is not entirely unexpected, as MDSCs have also been reported to accumulate in mice treated with granulocyte and macrophage colony-stimulating factor (GM-CSF)^[51,52], LPS^[41], CpG^[53], complete Freund's adjuvant^[45] and Bacillus Calmette-Guérin from *Mycobacterium bovis*^[54]. Similar MDSCs expansion has been described for other conditions involving major inflammatory responses, such as superantigen vaccination^[55], polymicrobial sepsis^[56], after burn^[57] and traumatic injuries^[58]. These findings are consistent with a physiological role of MDSCs as a counterbalancing mechanism to inflammation, preventing collateral damage to the tissue caused by activated T cells once the "dangerous" antigen has been eliminated.

EFFECT OF VSSP ON TUMOR-BEARING IMMUNOCOMPROMISED HOSTS

The effect of VSSP on the phenotype, suppressive function and differentiation status of tumor-induced MDSCs has been evaluated in mice bearing C26GM, EL4, EG.7 and MCA203 tumors (Table 1)^[42]. Splenic MDSCs derived from VSSP-treated tumor-bearing mice (MDSCs-T+V) contained a higher frequency of CD11b⁺Gr1^{hi} and Ly6C^{lo}Ly6G⁺ G-MDSCs than untreated tumor-bearing counterparts (MDSCs-T). In addition, IL-4R α is down-regulated on MDSCs-T+V, and these cells showed an increase of the homing molecule CD62L. Consistent with our *in vitro* studies, the suppressive function of tumor-induced splenic MDSCs was significantly reduced when VSSP is given *in vivo*. Several different findings support this effect of VSSP. First, MDSCs-T+V were unable to suppress the hemagglutinin (HA) peptide-specific proliferation of CD8⁺ T cells from CL4 TCR transgenic mice, in the same experimental setting where equal number of MDSCs-T were significantly inhibitory. *In vitro* ⁵¹Cr release CTL assays demonstrated that, as expected, MDSCs-T completely suppressed both antigen-specific and alloantigen-specific lytic activity of CD8⁺ T cells. In contrast, MDSCs-T+V isolated from EL4 and C26GM tumor-bearing mice only marginally affected the generation of the CTL.

The effect of VSSP on MDSCs *in vivo* was further examined in adoptive transfer experiments. In the first approach, MDSCs-T and MDSC-T+V were adoptively transferred into CD45.1⁺ B6 congenic mice, which previously received the transference of ovalbumin (OVA)-specific CD8⁺ T cells from OTI transgenic mice, and vaccinated with the immunodominant OVA₂₅₇₋₂₆₄ (SIINFEKL) peptide emulsified in incomplete Freund's adju-

vant (IFA). Similar frequencies of IFN- γ ⁺ antigen-specific CD8⁺ T cells were found in recipient mice transferred with MDSCs-T+V compared to control mice receiving no MDSCs, whereas transfer of MDSCs-T significantly impaired the activation of OTI lymphocytes. Additional experiments were performed to compare VSSP with other adjuvants or well-established vaccination systems. On this regard, we found that VSSP-based vaccines are more efficient than vaccination with DCs or vaccines employing the adjuvant polyinosinic:polycytidylic acid (polyI:C) in activating antigen-specific CTL responses in the presence of MDSCs-T. In fact, vaccination of BALB/c mice, which had been adoptively transferred with both congenic antigen-specific CD8⁺ T cells and MDSCs-T, with HA peptide in VSSP adjuvant prevented the MDSCs-T-mediated suppression of CD8⁺ T cell responses that was observed in mice vaccinated with HA-pulsed DCs. Also congenic OTI CD8⁺ T cells transferred to EG.7 tumor-bearing mice produce IFN- γ in response to VSSP admixed with SIINFEKL peptide- but not to a vaccine consisting of SIINFEKL-pulsed DCs. Importantly, the OVA-specific *in vivo* CTL response generated in mice with EL4 tumors by the administration of OVA/VSSP was comparable to that observed in tumor-free mice, whereas vaccination with OVA/polyI:C was unable to overcome the tumor-induced impairment of the CTL response.

In addition to TCR transgenic T cell responses to a model antigen, we have found that VSSP blunts MDSC-mediated suppression of endogenous T cell responses to native tumor antigen, by measuring the inhibition of tumor-specific CD8⁺ T cells by MDSCs in an ELISPOT assay. CD8⁺ T cells isolated from MCA203 tumor-bearing mice did not release IFN- γ when stimulated with MCA203 tumor cells, irrespective of the presence of MDSCs. In contrast, a significant frequency of CD8⁺ T cells derived from VSSP-treated tumor-bearing mice were activated by tumor cells and produced IFN- γ , even when MDSCs-T+V were added to the culture. Importantly, MDSCs-T maintained their ability to suppress tumor-specific CTL in this experiment.

Within the tumor microenvironment itself, VSSP treatment did not change the phenotype and functional capacity of CD11b⁺ sorted MDSCs. However, adoptively transferred congenic MDSCs-T had a reduced ability to infiltrate tumors in EL4 tumor-bearing mice treated with VSSP. More importantly, in these VSSP-treated mice, tumor-infiltrating transferred MDSCs-T were more differentiated into CD11c⁺MHCII⁺CD11b⁻ phenotype characteristic of DCs, and did not differentiate towards MHCII⁺F4/80⁺ macrophages. A similar differentiation pattern was observed *in vivo* in the spleen and lymph nodes from VSSP-inoculated tumor-bearing mice. In a more recent work, it was demonstrated that *in vitro* treatment with VSSP of tumor-induced MDSCs was sufficient to differentiate this immature population towards phenotypically mature DCs and, more importantly, causes the loss of their suppressive function^[59]. Since VSSP contains a TLR4 ligand, a comparison with

LPS was done in the same experimental setting. Interestingly, incubation with LPS fails to differentiate tumor-induced MDSCs into DCs and, consequently, these cells retain their inhibitory activity^[59]. In agreement with these results, Greifenberg *et al.*^[60] have shown that incubation of bone marrow (BM)-derived MDSCs with the combination of LPS and IFN- γ increases NO secretion, enhancing the suppressive activity of these MDSCs and impairing their maturation into DCs. These findings further suggest that VSSP's effect on MDSCs is not a shared characteristic of all TLR4 agonists, but is a unique property of VSSP. Other authors have reported that TLR4 signaling is involved in the promotion of tumor growth associated with the recruitment of G-MDSCs, through the interaction with S100A9 protein^[61]. VSSP also expands G-MDSCs subpopulation in tumor-bearing mice, however it also potentiates CTL responses and anti-tumor activity on those mice^[42]. Therefore, the complexity of signals in the structure of VSSP (TLR2 agonist, GM3 ganglioside, *etc.*) likely makes these particles distinct from single TLR4 agonists. In fact, VSSP can induce activation of BM-derived DCs obtained from LPS hyporesponsive mice (C3H/HeJ)^[47].

It has been shown in the literature that other adjuvants can also reduce the suppressive function of tumor-recruited MDSCs. For instance, intratumoral injection of CpG reduces the suppressive function of Mo-MDSCs and induces their differentiation towards macrophages with tumoricidal capability^[44]. However, CpG does not modify G-MDSCs, and intratumoral injections in patients may be difficult to impossible. Formalin-inactivated Herpes Simplex Virus also decreases the suppressive function of MDSCs-T, but whether this adjuvant is able to differentiate MDSCs has not been addressed^[43].

INFLUENCE OF VSSP ON CHEMOTHERAPY-ASSOCIATED IMMUNOSUPPRESSED HOSTS

The ability of VSSP to rescue the number and functionality of relevant immune populations on mice undergoing chemotherapy-induced leukopenia has been also tested (Table 1)^[62]. The widely used chemotherapy agent cyclophosphamide (CY) was used to induce the leukopenic setting for these studies. In this model, VSSP accelerated the recovery of specific leukocytes population when administered in the early stages of leukopenia. Splenic CD4⁺ and CD8⁺ T cells (with a memory CD4⁺CD44^{hi} and CD8⁺CD44^{hi} phenotype) and CD11c⁺CD11b⁺ DCs were some of the populations most enhanced by VSSP in leukopenic mice. Interestingly, MDSCs were also significantly expanded. However, similar to what was seen in the tumor-mediated immunosuppression setting, MDSCs from leukopenic mice treated with VSSP showed a reduced capacity to suppress T cell responses, compared to CY-induced MDSCs (Table 1). Importantly, in the same experimental setting, we found that polyI:C treat-

ment induced none of the effects observed with VSSP inoculation.

The ability of VSSP to activate antigen-specific CD8⁺ T cells was also tested in leukopenic mice. In this immunocompromised scenario, vaccination with a single dose of OVA/VSSP, at the time point corresponding to the lowest CD8 counts, induced significant antigen-specific CTL responses. In comparison, vaccination with three doses of OVA/polyI:C was not capable of inducing antigen-specific effector CD8⁺ T cell activation. Furthermore, VSSP treatment of OVA/polyI:C vaccinated animals restored the dampened CTL responses in polyI:C-treated leukopenic mice, indicating that VSSP can function as an immunomodulator as well. This effect could be associated to the capacity of VSSP, different from polyI:C, to accelerate the recovery of effector CD8⁺ memory T cells and to induce the expansion of DCs and less suppressive MDSCs.

Granulocyte colony-stimulating factor (G-CSF) is the standard growth factor used in the clinic to revert chemotherapy-induced leukopenia, but also has been reported to be a tumor-derived factor that induces MDSCs generation and recruitment^[63]. Therefore we assessed whether treatment with recombinant G-CSF could restore the *in vivo* CTL response barely induced by OVA/polyI:C vaccine in CY-treated mice^[62]. Administration of G-CSF has no impact in the impaired antigen-specific CTL response, possibly due to the expansion of MDSCs but also *via* G-CSF-induced Th2 responses^[64] and the resulting differentiation of Tregs that may impair effector T lymphocyte proliferation^[65]. However, when VSSP was given with G-CSF, the ability of VSSP to restore CD8⁺ T cell function was not affected, which opens the possibility for their concomitant use in the clinic. Moreover, the functionality of MDSCs recruited in these experiments was additionally evaluated. As expected from previous reports, our data also demonstrated that, in leukopenic mice treated with G-CSF, the induced MDSCs were highly suppressive. Importantly, the concomitant treatment with VSSP dampened the inhibitory function of MDSCs expanded after G-CSF injection. To our knowledge, no other adjuvant has been tested in this immunosuppressive leukopenic scenario induced by chemotherapy.

ANTI-TUMOR ACTIVITY OF VSSP

Several pre-clinical studies support the anti-tumor efficacy of VSSP, whether used alone or in combination with other tumor-associated antigens different from the GM3 ganglioside. The combination of surgery and VSSP alone prevented tumor recurrence and improved survival in melanoma B16F10 tumor-bearing mice^[66]. In a different tumor model, treatment of mice bearing MCA203 tumors with three doses of VSSP was sufficient to significantly delay tumor growth^[42]. Of interest, GM3 ganglioside, an important component of VSSP, is highly expressed on both melanoma B16F10 and MCA203 sar-

coma. Particularly in MCA203 tumor-bearing mice, treatment with VSSP alone caused a significant increase in the frequency of classical IFN- γ -producing CD8⁺ T cells specific for MCA203 antigens, suggesting an antigen-spreading likely induced by the initial response against the GM3 ganglioside^[42]. Moreover, VSSP-adjuvanted vaccines (both peptides and whole proteins) have shown anti-tumor activity. For instance, a vaccine containing the extracellular domain of murine epidermal growth factor receptor (EGFR) and VSSP has a potent anti-metastatic effect in the Lewis lung carcinoma model^[67]. In a mouse model of cervical cancer induced by Human Papilloma Virus (HPV), the immunization with an E7-derived CTL peptide from HPV 16 mixed with VSSP induced regression of established tumors^[68]. Therapeutic vaccination of EG.7 tumor-bearing mice with OVA or SIINFEKL peptide adjuvanted in VSSP, but not SIINFEKL emulsified in IFA, caused a significant reduction of tumor growth^[42]. However, VSSP administration alone to EL4 and C26GM tumor-bearing mice, with the same schedule associated with the inhibition of MDSCs suppressive function, does not delay tumor growth. One possible explanation for the absence of an anti-tumor effect of VSSP alone in these models is the lack of a tumor-associated antigen during treatment, and consequently, the absence of antigen-specific CD8⁺ T cell activation. In fact, EL4 tumors express low levels of GM3 whereas an inappropriate exposure of this ganglioside on the surface of C26GM tumor cells has been observed^[42]. Altogether, these data strongly suggest that the best induction of anti-tumor responses requires combining the abrogation of tumor-induced MDSCs with a specific stimulation of T lymphocytes, which can be successfully done by mixing a proper tumor-associated antigen with VSSP.

Finally, four therapeutic cancer vaccines employing VSSP as adjuvant are in clinical trials. An EGFR-based vaccine^[67] is currently in Phase I clinical trials. A Phase I clinical trial in patients with advanced solid tumors using a formulation of a mutated vascular endothelial growth factor^[69] and VSSP has been recently completed. In this trial, the most common adverse events were Grade 1 pain and erythema at injection site and Grade 1 fever^[70]. Additionally, a gonadotropin releasing hormone-based vaccine^[71] and a HPV-derived peptidic vaccine^[72] are currently in Phase II trials in prostate cancer patients and women with high-grade cervical intraepithelial neoplasia, respectively. Both vaccines have previously shown to be safe and immunogenic. The most frequent adverse event in patients receiving the HPV vaccine was local pain at the vaccination site, whereas fever, tremors and cramps were seen in few cases, but none exceeded Grade 1^[72]. Another Phase I trial using VSSP alone in metastatic melanoma patients demonstrated the safety of this preparation even in the presence of Montanide ISA 51, with toxicity consisting of local reaction at the site of injection and mild fever and chills^[49]. In this trial both humoral and cellular responses were induced by the VSSP treatment. Additionally, an ongoing physician-lead trial is evaluat-

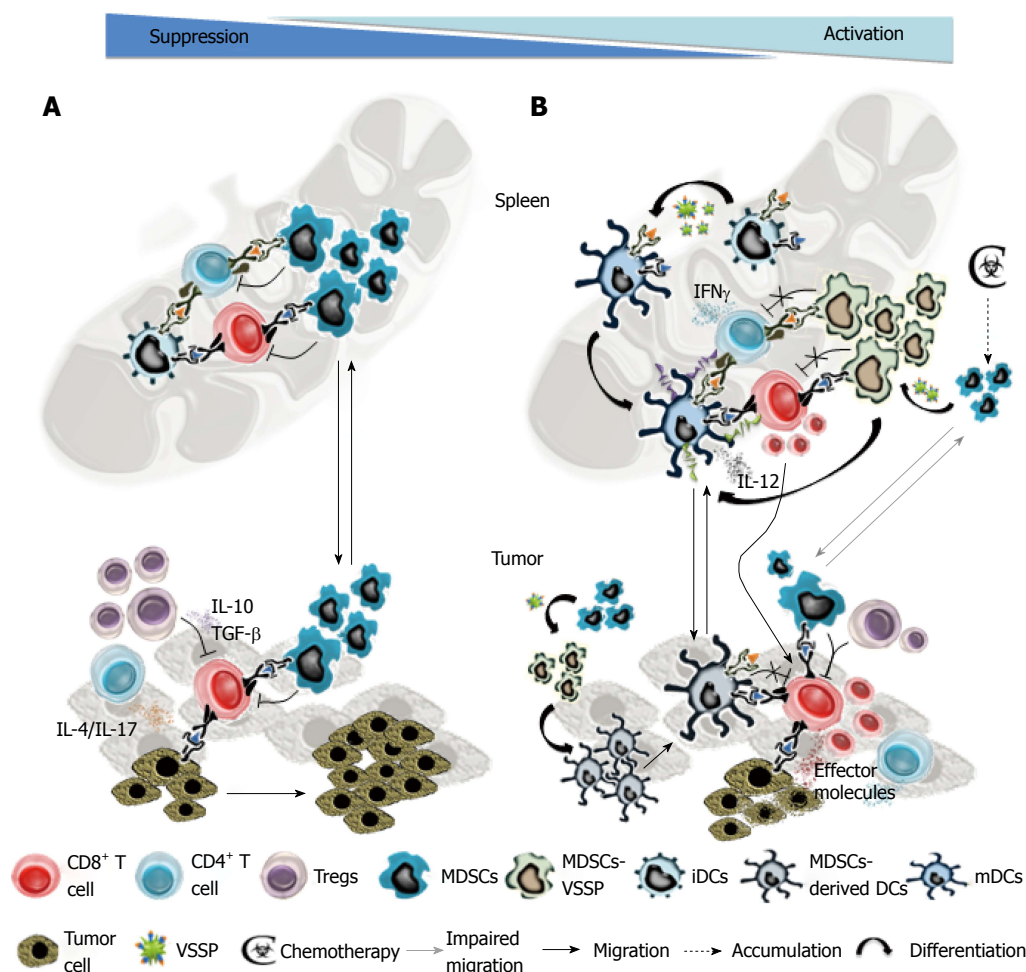


Figure 1 Schematic of potential immunomodulatory effects of very small size proteoliposomes in tumor-bearing hosts. A: Tumor-associated immunosuppressive networks prevent the elimination of neoplastic cells by specific T cells, thus contributing to tumor growth and metastasis; B: VSSP administration reduces the suppressive function of tumor-induced MDSCs, impairs their migration to the tumor microenvironment and promotes their differentiation towards DCs, both at the tumor and secondary lymphoid organs. VSSP also stimulates the activation and effector function of tumor-specific CTL, and combined with the concomitant reduction in the frequency of suppressive MDSCs and Tregs at the tumor site, further enhances elimination of neoplastic cells. An accelerated recovery from chemotherapy-induced leukopenia with VSSP treatment also contributes to a better anti-tumor response. VSSP: Very small size proteoliposomes; MDSCs: Myeloid-derived suppressor cells; DCs: Dendritic cells; CTL: Cytotoxic T lymphocytes; IL: Interleukin; TGF: Transforming growth factor.

ing the modulation of tumor-induced MDSCs by VSSP treatment alone in RCC patients.

CONCLUSION

The immunomodulatory and anti-tumor properties of VSSP are summarized in Figure 1. In tumor-bearing mice, activation and effector function of tumor-specific CD8 $^{+}$ and CD4 $^{+}$ T cells are impaired, among other factors, due to ineffective antigenic presentation by immature DCs and through multiple suppressive mechanisms exerted by MDSCs. Experimental evidence suggest that VSSP-based vaccines could promote cross-presentation of the formulated antigen by DCs, drive the full maturation of the DCs and, simultaneously, inhibit tumor-induced MDSCs immunosuppression. In addition, VSSP could induce Th1 polarization on tumor-specific CD4 $^{+}$ T cells. All these effects may significantly enhance the proliferation and activation of tumor-specific CD8 $^{+}$ T cells, thus eliciting robust anti-tumor immunity. VSSP

also diminishes the migration of MDSCs towards the tumor site and promotes their differentiation into DCs. Tumor-infiltrating MDSCs have been related with the recruitment and expansion of Tregs^[23,24,73], in addition to an impaired migration of effector T cells^[74]. Thus, within the tumor microenvironment, VSSP treatment may tip the balance between functional T cells vs suppressive MDSCs/Tregs to favor the immune effectors that ultimately lead to an anti-tumor response. The higher frequency of DCs could additionally contribute to activate T cells specific for other tumor antigens by capturing, processing and presenting the proteins released by dying tumor cells. In chemotherapy-treated individuals, VSSP also accelerates the homeostatic recovery of CD8 $^{+}$ T cells and DCs, whereas the suppressive function of chemotherapy-induced MDSCs is abrogated. Altogether, these elements support the use of VSSP as a novel adjuvant or immunomodulator for active immunotherapy and, particularly, for the combination with chemotherapy in the clinical setting.

ACKNOWLEDGMENTS

We are grateful to Dr. Kelvin P (Roswell Park Cancer Institute, Buffalo, United States) for the language correction and critical review of the scientific content of this manuscript.

REFERENCES

- Kim R, Emi M, Tanabe K. Cancer immunoediting from immune surveillance to immune escape. *Immunology* 2007; **121**: 1-14 [PMID: 17386080 DOI: 10.1111/j.1365-2567.2007.02587]
- Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011; **331**: 1565-1570 [PMID: 21436444 DOI: 10.1126/science.1203486]
- Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* 2012; **12**: 253-268 [PMID: 22437938 DOI: 10.1038/nri3175]
- Talmadge JE, Gabrilovich DI. History of myeloid-derived suppressor cells. *Nat Rev Cancer* 2013; **13**: 739-752 [PMID: 24060865 DOI: 10.1038/nrc3581]
- Gallina G, Dolcetti L, Serafini P, De Santo C, Marigo I, Colombo MP, Basso G, Brombacher F, Borrello I, Zanovello P, Biciatto S, Bronte V. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8+ T cells. *J Clin Invest* 2006; **116**: 2777-2790 [PMID: 17016559 DOI: 10.1172/JCI28828]
- Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, Herber DL, Schneck J, Gabrilovich DI. Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat Med* 2007; **13**: 828-835 [PMID: 17603493 DOI: 10.1038/nm1609]
- Liu C, Yu S, Kappes J, Wang J, Grizzle WE, Zinn KR, Zhang HG. Expansion of spleen myeloid suppressor cells represses NK cell cytotoxicity in tumor-bearing host. *Blood* 2007; **109**: 4336-4342 [PMID: 17244679 DOI: 10.1182/blood-2006-09-046201]
- Dolcetti L, Peranzoni E, Ugel S, Marigo I, Fernandez Gomez A, Mesa C, Geilich M, Winkels G, Traggiai E, Casati A, Grassi F, Bronte V. Hierarchy of immunosuppressive strength among myeloid-derived suppressor cell subsets is determined by GM-CSF. *Eur J Immunol* 2010; **40**: 22-35 [PMID: 19941314 DOI: 10.1002/eji.200939903]
- Movahedi K, Guillemins M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, De Baetselier P, Van Ginderachter JA. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood* 2008; **111**: 4233-4244 [PMID: 18272812 DOI: 10.1182/blood-2007-07-099226]
- Peranzoni E, Zilio S, Marigo I, Dolcetti L, Zanovello P, Mandruzzato S, Bronte V. Myeloid-derived suppressor cell heterogeneity and subset definition. *Curr Opin Immunol* 2010; **22**: 238-244 [PMID: 20171075 DOI: 10.1016/j.coi.2010.01.021]
- Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol* 2008; **181**: 5791-5802 [PMID: 18832739 DOI: 10.4049/jimmunol.181.8.5791]
- Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, Padhya T, McCaffrey TV, McCaffrey JC, Gabrilovich DI. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. *J Immunol* 2009; **182**: 5693-5701 [PMID: 19380816 DOI: 10.4049/jimmunol.0900092]
- Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol* 2009; **9**: 162-174 [PMID: 19197294 DOI: 10.1038/nri2506]
- Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of t-cell function in advanced cancer patients. *Cancer Res* 2001; **61**: 4756-4760 [PMID: 11406548]
- Cuervo H, Guerrero NA, Carbajosa S, Beschin A, De Baetselier P, Gironès N, Fresno M. Myeloid-derived suppressor cells infiltrate the heart in acute Trypanosoma cruzi infection. *J Immunol* 2011; **187**: 2656-2665 [PMID: 21804013 DOI: 10.4049/jimmunol.1002928]
- Lesokhin AM, Hohl TM, Kitano S, Cortez C, Hirschhorn-Cymerman D, Avogadri F, Rizzuto GA, Lazarus JJ, Pamer EG, Houghton AN, Merghoub T, Wolchok JD. Monocytic CCR2(+) myeloid-derived suppressor cells promote immune escape by limiting activated CD8 T-cell infiltration into the tumor microenvironment. *Cancer Res* 2012; **72**: 876-886 [PMID: 22174368 DOI: 10.1158/0008-5472.CAN-11-1792]
- Nausch N, Galani IE, Schlecker E, Cerwenka A. Mononuclear myeloid-derived "suppressor" cells express RAE-1 and activate natural killer cells. *Blood* 2008; **112**: 4080-4089 [PMID: 18753637 DOI: 10.1182/blood-2008-03-143776]
- Qin A, Cai W, Pan T, Wu K, Yang Q, Wang N, Liu Y, Yan D, Hu F, Guo P, Chen X, Chen L, Zhang H, Tang X, Zhou J. Expansion of monocytic myeloid-derived suppressor cells dampens T cell function in HIV-1-seropositive individuals. *J Virol* 2013; **87**: 1477-1490 [PMID: 23152536 DOI: 10.1128/JVI.01759-12]
- Choi J, Suh B, Ahn YO, Kim TM, Lee JO, Lee SH, Heo DS. CD15+/CD16low human granulocytes from terminal cancer patients: granulocytic myeloid-derived suppressor cells that have suppressive function. *Tumour Biol* 2012; **33**: 121-129 [PMID: 22081309 DOI: 10.1007/s13277-011-0254-6]
- Lechner MG, Liebertz DJ, Epstein AL. Characterization of cytokine-induced myeloid-derived suppressor cells from normal human peripheral blood mononuclear cells. *J Immunol* 2010; **185**: 2273-2284 [PMID: 20644162 DOI: 10.4049/jimmunol.1000901]
- Serafini P. Myeloid derived suppressor cells in physiological and pathological conditions: the good, the bad, and the ugly. *Immunol Res* 2013; **57**: 172-184 [PMID: 24203443 DOI: 10.1007/s12026-013-8455-2]
- Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* 2005; **5**: 641-654 [PMID: 16056256 DOI: 10.1038/nri1668]
- Pan PY, Ma G, Weber KJ, Ozao-Choy J, Wang G, Yin B, Divino CM, Chen SH. Immune stimulatory receptor CD40 is required for T-cell suppression and T regulatory cell activation mediated by myeloid-derived suppressor cells in cancer. *Cancer Res* 2010; **70**: 99-108 [PMID: 19996287 DOI: 10.1158/0008-5472.CAN-09-1882]
- Serafini P, Mgebrouff S, Noonan K, Borrello I. Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells. *Cancer Res* 2008; **68**: 5439-5449 [PMID: 18593947 DOI: 10.1158/0008-5472.CAN-07-6621]
- Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T, McCaffrey TV, McCaffrey JC, Gabrilovich DI. HIF-1 α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med* 2010; **207**: 2439-2453 [PMID: 20876310 DOI: 10.1084/jem.20100587]
- Doedens AL, Stockmann C, Rubinstein MP, Liao D, Zhang N, DeNardo DG, Coussens LM, Karin M, Goldrath AW, Johnson RS. Macrophage expression of hypoxia-inducible factor-1 α suppresses T-cell function and promotes tumor progression. *Cancer Res* 2010; **70**: 7465-7475 [PMID: 20841473 DOI: 10.1158/0008-5472.CAN-10-1439]
- Chioda M, Peranzoni E, Desantis G, Papalini F, Falisi E, Solito S, Mandruzzato S, Bronte V. Myeloid cell diversification and complexity: an old concept with new turns in oncology. *Cancer Metastasis Rev* 2011; **30**: 27-43 [PMID: 21267772 DOI: 10.1007/s10555-011-9268-1]
- Yang L, DeBusk LM, Fukuda K, Fingleton B, Green-Jarvis

- B, Shyr Y, Matrisian LM, Carbone DP, Lin PC. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 2004; **6**: 409-421 [PMID: 15488763 DOI: 10.1016/j.ccr.2004.08.031]
- 29 **Du R**, Lu KV, Petritsch C, Liu P, Ganss R, Passequé E, Song H, Vandenberg S, Johnson RS, Werb Z, Bergers G. HIF1 α induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell* 2008; **13**: 206-220 [PMID: 18328425 DOI: 10.1016/j.ccr.2008.01.034]
- 30 **Pahler JC**, Tazzyman S, Erez N, Chen YY, Murdoch C, Nozawa H, Lewis CE, Hanahan D. Plasticity in tumor-promoting inflammation: impairment of macrophage recruitment evokes a compensatory neutrophil response. *Neoplasia* 2008; **10**: 329-340 [PMID: 18392134]
- 31 **Yan HH**, Pickup M, Pang Y, Gorska AE, Li Z, Chytil A, Geng Y, Gray JW, Moses HL, Yang L. Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. *Cancer Res* 2010; **70**: 6139-6149 [PMID: 20631080 DOI: 10.1158/0008-5472.CAN-10-0706]
- 32 **Kusmartsev S**, Su Z, Heiser A, Dannull J, Eruslanov E, Kübler H, Yancey D, Dahm P, Vieweg J. Reversal of myeloid cell-mediated immunosuppression in patients with metastatic renal cell carcinoma. *Clin Cancer Res* 2008; **14**: 8270-8278 [PMID: 19088044 DOI: 10.1158/1078-0432.CCR-08-0165]
- 33 **Lathers DM**, Clark JL, Achille NJ, Young MR. Phase 1B study to improve immune responses in head and neck cancer patients using escalating doses of 25-hydroxyvitamin D3. *Cancer Immunol Immunother* 2004; **53**: 422-430 [PMID: 14648070 DOI: 10.1007/s00262-003-0459-7]
- 34 **Mirza N**, Fishman M, Fricke I, Dunn M, Neuger AM, Frost TJ, Lush RM, Antonia S, Gabrilovich DI. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. *Cancer Res* 2006; **66**: 9299-9307 [PMID: 16982775 DOI: 10.1158/0008-5472.CAN-06-1690]
- 35 **Ko JS**, Zea AH, Rini BI, Ireland JL, Elson P, Cohen P, Golshayan A, Rayman PA, Wood L, Garcia J, Dreicer R, Bukowski R, Finke JH. Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. *Clin Cancer Res* 2009; **15**: 2148-2157 [PMID: 19276286 DOI: 10.1158/1078-0432.CCR-08-1332]
- 36 **Montero AJ**, Diaz-Montero CM, Kyriakopoulos CE, Bronte V, Mandruzzato S. Myeloid-derived suppressor cells in cancer patients: a clinical perspective. *J Immunother* 2012; **35**: 107-115 [PMID: 22306898 DOI: 10.1097/CJI.0b013e318242169f]
- 37 **Serafini P**, Meckel K, Kelso M, Noonan K, Califano J, Koch W, Dolcetti L, Bronte V, Borrello I. Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *J Exp Med* 2006; **203**: 2691-2702 [PMID: 17101732 DOI: 10.1084/jem.20061104]
- 38 **Crawford J**, Dale DC, Kuderer NM, Culakova E, Poniewierski MS, Wolff D, Lyman GH. Risk and timing of neutropenic events in adult cancer patients receiving chemotherapy: the results of a prospective nationwide study of oncology practice. *J Natl Compr Canc Netw* 2008; **6**: 109-118 [PMID: 18319047]
- 39 **Pardoll D**. Does the immune system see tumors as foreign or self? *Annu Rev Immunol* 2003; **21**: 807-839 [PMID: 12615893 DOI: 10.1146/annurev.immunol.21.120601.141135]
- 40 **Todryk S**. A sense of tumour for the immune system. *Immunology* 2002; **107**: 1-4 [PMID: 12225356 DOI: 10.1046/j.1365-2567.2002.01506.x]
- 41 **De Wilde V**, Van Rompaey N, Hill M, Lebrun JF, Lemaître P, Lhomme F, Kubjak C, Vokaer B, Oldenhove G, Charbonnier LM, Cuturi MC, Goldman M, Le Moine A. Endotoxin-induced myeloid-derived suppressor cells inhibit alloimmune responses via heme oxygenase-1. *Am J Transplant* 2009; **9**: 2034-2047 [PMID: 19681826 DOI: 10.1111/j.1600-6143.2009.02757]
- 42 **Fernández A**, Mesa C, Marigo I, Dolcetti L, Clavell M, Oliver L, Fernández LE, Bronte V. Inhibition of tumor-induced myeloid-derived suppressor cell function by a nanoparticulated adjuvant. *J Immunol* 2011; **186**: 264-274 [PMID: 21135171 DOI: 10.4049/jimmunol.1001465]
- 43 **Ohkusu-Tsukada K**, Ohta S, Kawakami Y, Toda M. Adjuvant effects of formalin-inactivated HSV through activation of dendritic cells and inactivation of myeloid-derived suppressor cells in cancer immunotherapy. *Int J Cancer* 2011; **128**: 119-131 [PMID: 20323389 DOI: 10.1002/ijc.25319]
- 44 **Shirota Y**, Shirota H, Klinman DM. Intratumoral injection of CpG oligonucleotides induces the differentiation and reduces the immunosuppressive activity of myeloid-derived suppressor cells. *J Immunol* 2012; **188**: 1592-1599 [PMID: 22231700 DOI: 10.4049/jimmunol.1101304]
- 45 **Wang Z**, Jiang J, Li Z, Zhang J, Wang H, Qin Z. A myeloid cell population induced by Freund adjuvant suppresses T-cell-mediated antitumor immunity. *J Immunother* 2010; **33**: 167-177 [PMID: 20145547 DOI: 10.1097/CJI.0b013e3181bed2ba]
- 46 **Estevez F**, Carr A, Solorzano L, Valiente O, Mesa C, Barroso O, Sierra GV, Fernandez LE. Enhancement of the immune response to poorly immunogenic gangliosides after incorporation into very small size proteoliposomes (VSSP). *Vaccine* 1999; **18**: 190-197 [PMID: 10501249 DOI: 10.1016/S0264-410X(99)00219-4]
- 47 **Mesa C**, De León J, Rigley K, Fernández LE. Very small size proteoliposomes derived from *Neisseria meningitidis*: an effective adjuvant for Th1 induction and dendritic cell activation. *Vaccine* 2004; **22**: 3045-3052 [PMID: 15297054 DOI: 10.1016/j.vaccine.2004.02.010]
- 48 **Venier C**, Guthmann MD, Fernández LE, Fainboim L. Innate-immunity cytokines induced by very small size proteoliposomes, a *Neisseria*-derived immunological adjuvant. *Clin Exp Immunol* 2007; **147**: 379-388 [PMID: 17223981 DOI: 10.1111/j.1365-2249.2006.03297.x]
- 49 **Guthmann MD**, Bitton RJ, Carnero AJ, Gabri MR, Cinat G, Koliren L, Lewi D, Fernandez LE, Alonso DF, Gómez DE, Fainboim L. Active specific immunotherapy of melanoma with a GM3 ganglioside-based vaccine: a report on safety and immunogenicity. *J Immunother* 2004; **27**: 442-451 [PMID: 15534488 DOI: 10.1097/00002371-200411000-00004]
- 50 **Mesa C**, de León J, Fernández LE. Very small size proteoliposomes derived from *Neisseria meningitidis*: An effective adjuvant for generation of CTL responses to peptide and protein antigens. *Vaccine* 2006; **24**: 2692-2699 [PMID: 16316710 DOI: 10.1016/j.vaccine.2005.08.111]
- 51 **Parmiani G**, Castelli C, Pilla L, Santinami M, Colombo MP, Rivoltini L. Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. *Ann Oncol* 2007; **18**: 226-232 [PMID: 17116643 DOI: 10.1093/annonc/mdl158]
- 52 **Serafini P**, Carbley R, Noonan KA, Tan G, Bronte V, Borrello I. High-dose granulocyte-macrophage colony-stimulating factor-producing vaccines impair the immune response through the recruitment of myeloid suppressor cells. *Cancer Res* 2004; **64**: 6337-6343 [PMID: 15342423 DOI: 10.1158/0008-5472.CAN-04-0757]
- 53 **Morecki S**, Gelfand Y, Yacovlev E, Eizik O, Shabat Y, Slavin S. CpG-induced myeloid CD11b+Gr-1+ cells efficiently suppress T cell-mediated immunoreactivity and graft-versus-host disease in a murine model of allogeneic cell therapy. *Biol Blood Marrow Transplant* 2008; **14**: 973-984 [PMID: 18721760 DOI: 10.1016/j.bbmt.2008.06.018]
- 54 **Martino A**, Badell E, Abadie V, Balloy V, Chignard M, Mistou MY, Combadière B, Combadière C, Winter N. Mycobacterium bovis bacillus Calmette-Guérin vaccination mobilizes innate myeloid-derived suppressor cells restraining in vivo T cell priming via IL-1R-dependent nitric oxide production. *J Immunol* 2010; **184**: 2038-2047 [PMID: 20083674 DOI: 10.4049/jimmunol.0903348]
- 55 **Cauley LS**, Miller EE, Yen M, Swain SL. Superantigen-induced CD4 T cell tolerance mediated by myeloid cells and

- IFN-gamma. *J Immunol* 2000; **165**: 6056-6066 [PMID: 11086037 DOI: 10.4049/jimmunol.165.11.6056]
- 56 **Scumpia PO**, Kelly-Scumpia KM, Delano MJ, Weinstein JS, Cuenca AG, Al-Quran S, Bovio I, Akira S, Kumagai Y, Moldawer LL. Cutting edge: bacterial infection induces hematopoietic stem and progenitor cell expansion in the absence of TLR signaling. *J Immunol* 2010; **184**: 2247-2251 [PMID: 20130216 DOI: 10.4049/jimmunol.0903652]
- 57 **Murphey ED**, Lin CY, McGuire RW, Toliver-Kinsky T, Herndon DN, Sherwood ER. Diminished bacterial clearance is associated with decreased IL-12 and interferon-gamma production but a sustained proinflammatory response in a murine model of postseptic immunosuppression. *Shock* 2004; **21**: 415-425 [PMID: 15087817 DOI: 10.1097/00024382-200405000-00004]
- 58 **Makarenkova VP**, Bansal V, Matta BM, Perez LA, Ochoa JB. CD11b+/Gr-1+ myeloid suppressor cells cause T cell dysfunction after traumatic stress. *J Immunol* 2006; **176**: 2085-2094 [PMID: 16455964 DOI: 10.4049/jimmunol.176.4.2085]
- 59 **Fernández A**, Oliver L, Alvarez R, Hernández A, Raymond J, Fernández LE, Mesa C. Very small size proteoliposomes abrogate cross-presentation of tumor antigens by myeloid-derived suppressor cells and induce their differentiation to dendritic cells. *J Immunother Cancer* 2014; **2**: 5 [PMID: 24829762 DOI: 10.1186/2051-1426-2-5]
- 60 **Greifenberg V**, Ribechini E, Rössner S, Lutz MB. Myeloid-derived suppressor cell activation by combined LPS and IFN-gamma treatment impairs DC development. *Eur J Immunol* 2009; **39**: 2865-2876 [PMID: 19637228 DOI: 10.1002/eji.200939486]
- 61 **Källberg E**, Vogl T, Liberg D, Olsson A, Björk P, Wikström P, Bergh A, Roth J, Ivars F, Leanderson T. S100A9 interaction with TLR4 promotes tumor growth. *PLoS One* 2012; **7**: e34207 [PMID: 22470535 DOI: 10.1371/journal.pone.0034207]
- 62 **Oliver L**, Fernández A, Raymond J, López-Requena A, Fernández LE, Mesa C. Very small size proteoliposomes derived from *Neisseria meningitidis*: an effective adjuvant for antigen-specific cytotoxic T lymphocyte response stimulation under leukopenic conditions. *Vaccine* 2012; **30**: 2963-2972 [PMID: 22391399 DOI: 10.1016/j.vaccine.2012.02.054]
- 63 **Waight JD**, Hu Q, Miller A, Liu S, Abrams SI. Tumor-derived G-CSF facilitates neoplastic growth through a granulocytic myeloid-derived suppressor cell-dependent mechanism. *PLoS One* 2011; **6**: e27690 [PMID: 22110722 DOI: 10.1371/journal.pone.0027690]
- 64 **Pan L**, Delmonte J, Jalonen CK, Ferrara JL. Pretreatment of donor mice with granulocyte colony-stimulating factor polarizes donor T lymphocytes toward type-2 cytokine production and reduces severity of experimental graft-versus-host disease. *Blood* 1995; **86**: 4422-4429 [PMID: 8541530]
- 65 **Rutella S**, Pierelli L, Bonanno G, Sica S, Ameglio F, Capoluongo E, Mariotti A, Scambia G, d'Onofrio G, Leone G. Role for granulocyte colony-stimulating factor in the generation of human T regulatory type 1 cells. *Blood* 2002; **100**: 2562-2571 [PMID: 12239170 DOI: 10.1182/blood-2001-12-0291]
- 66 **Gabri MR**, Mazorra Z, Ripoll GV, Mesa C, Fernandez LE, Gomez DE, Alonso DF. Complete antitumor protection by perioperative immunization with GM3/VSSP vaccine in a preclinical mouse melanoma model. *Clin Cancer Res* 2006; **12**: 7092-7098 [PMID: 17145833 DOI: 10.1158/1078-0432.CCR-06-1075]
- 67 **Ramírez BS**, Pestana ES, Hidalgo GG, García TH, Rodríguez RP, Ullrich A, Fernández LE. Active antimetastatic immunotherapy in Lewis lung carcinoma with self EGFR extracellular domain protein in VSSP adjuvant. *Int J Cancer* 2006; **119**: 2190-2199 [PMID: 16841332 DOI: 10.1002/ijc.22085]
- 68 **Torréns I**, Mendoza O, Batte A, Reyes O, Fernández LE, Mesa C, Guillén G. Immunotherapy with CTL peptide and VSSP eradicated established human papillomavirus (HPV) type 16 E7-expressing tumors. *Vaccine* 2005; **23**: 5768-5774 [PMID: 16112257 DOI: 10.1016/j.vaccine.2005.07.049]
- 69 **Bequet-Romero M**, Morera Y, Ayala-Ávila M, Ancizar J, Soria Y, Blanco A, Suárez-Alba J, Gavilondo JV. CIGB-247: a VEGF-based therapeutic vaccine that reduces experimental and spontaneous lung metastasis of C57Bl/6 and BALB/c mouse tumors. *Vaccine* 2012; **30**: 1790-1799 [PMID: 22240345 DOI: 10.1016/j.vaccine.2012.01.006]
- 70 **Gavilondo JV**, Hernández-Bernal F, Ayala-Ávila M, de la Torre AV, de la Torre J, Morera-Díaz Y, Bequet-Romero M, Sánchez J, Valenzuela CM, Martín Y, Selman-Housein KH, Garabito A, Lazo OC. Specific active immunotherapy with a VEGF vaccine in patients with advanced solid tumors. results of the CENTAURO antigen dose escalation phase I clinical trial. *Vaccine* 2014; **32**: 2241-2250 [PMID: 24530151 DOI: 10.1016/j.vaccine.2013.11.102]
- 71 **Aguilar FF**, Barranco JJ, Fuentes EB, Aguilera LC, Sáez YL, Santana MD, Vázquez EP, Baker RB, Acosta OR, Pérez HG, Nieto GG. Very small size proteoliposomes (VSSP) and Montanide combination enhance the humoral immune response in a GnRH based vaccine directed to prostate cancer. *Vaccine* 2012; **30**: 6595-6599 [PMID: 22921738 DOI: 10.1016/j.vaccine.2012.08.020]
- 72 **Solares AM**, Baladron I, Ramos T, Valenzuela C, Borbon Z, Fanjull S, Gonzalez L, Castillo D, Esmir J, Granadillo M, Batte A, Cintado A, Ale M, Fernandez de Cossio ME, Ferrer A, Torrens I, Lopez-Saura P. Safety and Immunogenicity of a Human Papillomavirus Peptide Vaccine (CIGB-228) in Women with High-Grade Cervical Intraepithelial Neoplasia: First-in-Human, Proof-of-Concept Trial. *ISRN Obstet Gynecol* 2011; **2011**: 292951 [PMID: 21748025 DOI: 10.5402/2011/292951]
- 73 **Schlecker E**, Stojanovic A, Eisen C, Quack C, Falk CS, Umanisky V, Cerwenka A. Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. *J Immunol* 2012; **189**: 5602-5611 [PMID: 23152559 DOI: 10.4049/jimmunol.1201018]
- 74 **Molon B**, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, De Palma A, Mauri P, Monegal A, Rescigno M, Savino B, Colombo P, Jonjic N, Pecanic S, Lazzarato L, Fruttero R, Gasco A, Bronte V, Viola A. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. *J Exp Med* 2011; **208**: 1949-1962 [PMID: 21930770 DOI: 10.1084/jem.20101956]

P- Reviewer: Fukuda S, Magnusson LU **S- Editor:** Song XX
L- Editor: A **E- Editor:** Wang CH



Role of host immune responses in sequence variability of HIV-1 Vpu

Zafrul Hasan, Doreen Kamori, Takamasa Ueno

Zafrul Hasan, Doreen Kamori, Takamasa Ueno, Center for AIDS Research, Kumamoto University, Kumamoto 860-0811, Japan
 Takamasa Ueno, International Research Center for Medical Sciences (IRCMS), Kumamoto University, Kumamoto 860-0811, Japan

Author contributions: Hasan Z and Kamori D contributed equally to this work; Hasan Z and Kamori D generated the figures and wrote the manuscript; Ueno T designed the study and contributed to the writing of the manuscript.

Supported by A Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture (MEXT) of Japan; and A Grant-in-Aid for AIDS Research from the Ministry of Health, Labor, and Welfare of Japan; The Scholarship for the International Priority Graduate Programs, to Hasan Z and Kamori D; Advanced Graduate Courses for International Students (Doctoral Course), MEXT, Japan, to Hasan Z and Kamori D

Correspondence to: Takamasa Ueno, PhD, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan. uenotaka@kumamoto-u.ac.jp

Telephone: +81-96-3736826 Fax: +81-96-3736825

Received: March 7, 2014 Revised: April 19, 2014

Accepted: June 14, 2014

Published online: July 27, 2014

Abstract

Viral protein U (Vpu) is an accessory protein associated with two main functions important in human immunodeficiency virus type 1 (HIV-1) replication and dissemination; these are down-regulation of CD4 receptor through mediating its proteasomal degradation and enhancement of virion release by antagonizing tetherin/BST2. It is also well established that Vpu is one of the most highly variable proteins in the HIV-1 proteome. However it is still unclear what drives Vpu sequence variability, whether Vpu acquires polymorphisms as a means of immune escape, functional advantage, or otherwise. It is assumed that the host-pathogen interaction is a cause of polymorphic phenotype of Vpu and that the resulting functional heterogeneity of Vpu may have critical significance *in vivo*. In order to comprehensively understand Vpu variability, it is important to integrate at the population level the genetic association

approaches to identify specific amino acid residues and the immune escape kinetics which may impose Vpu functional constraints *in vivo*. This review will focus on HIV-1 accessory protein Vpu in the context of its sequence variability at population level and also bring forward evidence on the role of the host immune responses in driving Vpu sequence variability; we will also highlight the recent findings that illustrate Vpu functional implication in HIV-1 pathogenesis.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Human immunodeficiency virus type 1; Vpu; Sequence variability; Immune responses; Human leukocyte antigen class I

Core tip: Viral protein U (Vpu) is a highly polymorphic human immunodeficiency virus type 1 (HIV-1) accessory protein; however factors that are attributable to Vpu sequence variability are not well defined. In this review we have focused on the immune responses both innate (natural killer cells) and adaptive (cellular and humoral) immunity that are directed towards HIV-1 Vpu and we also show the interaction between Vpu and host cellular factors. We also highlight evidence that suggests interaction between the host immune responses and Vpu may contribute to Vpu sequence variability. Finally we have summarized the current knowledge on HIV-1 Vpu functions including Vpu evasion activities from the host immune surveillance.

Hasan Z, Kamori D, Ueno T. Role of host immune responses in sequence variability of HIV-1 Vpu. *World J Immunol* 2014; 4(2): 107-115 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/107.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.107>

INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1) demonstrates a significant genetic diversity due to its high

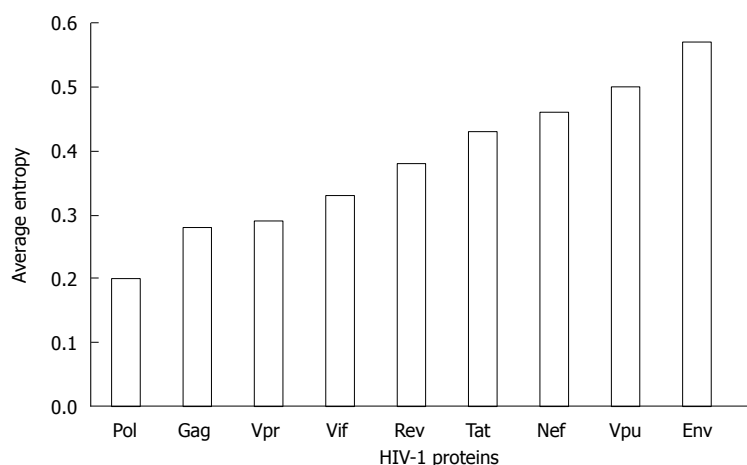


Figure 1 Sequence variability of human immunodeficiency virus type 1 proteins. The sequence variability of nine proteins of human immunodeficiency virus type 1 (HIV-1) shown in the graph was determined by using Shannon entropy approach^[24,90]. The full genome clade B sequences of the individual patients were retrieved from Los Alamos database ($n = 544$). Vpu: Viral protein U.

mutation rate; so far this extraordinary diversity has been a major setback in development of vaccine and antiretroviral drugs. Low fidelity of reverse transcriptase that give rise to error prone replication process, high progeny production, turnover rates and recombination of circulating HIV-1 strains are some of the viral factors that contributes to HIV-1 diversity^[1-3]. The adaptive potential of HIV-1 is shaped by both virus and the host immune factors, in other words both the diversifying and purifying selection factors influence HIV-1 diversity. In fact, strong evidence has also indicated that the host immune responses influence HIV-1 diversity by selection of escape mutations^[4-6]. Thus a comprehensive analysis of the dynamics of polymorphisms in HIV-1 proteins is a powerful tool to reveal actual interactions between HIV-1 and the host immune system^[7-9].

HIV-1 viral protein U (Vpu) is a 16-kDa accessory protein^[10] responsible for various functions such as CD4 down-regulation^[11-13] and enhancement of virion release by antagonizing tetherin/BST2^[14-17]. Interestingly, functionally competent Vpu (with respect to BST-2 antagonistic activity) were only found in the pandemic group M subtypes, suggesting that Vpu functional adaptation may confer pandemic spread of this HIV-1 subtype^[18]. In general, the host genetic factor is one of the main driving force of sequence polymorphism in HIV-1^[18], as evidenced in HIV-1 Nef^[7,19-21] and Env^[22,23] proteins whose highly polymorphic phenotype is mostly attributed by the host immune responses such as HLA class I-restricted CD8⁺ T lymphocytes and neutralizing antibodies, respectively. However, it is still unclear to what extent the host immune responses influence Vpu sequence variation. This review focuses on the role of host immune responses in Vpu sequence variability. Briefly, we also discuss the current understanding of Vpu functions including evasion of the immune system and their implication in viral pathogenesis.

SEQUENCE VARIABILITY OF VPU

Vpu exhibit a stable reading frame *in vivo* despite being a highly variable protein, suggesting functional importance of Vpu in HIV-1 replication and persistence. Further-

more, it has evidently been shown that only HIV-1 strains of the pandemic M group evolved a fully functional Vpu that efficiently antagonizes human tetherin/BST-2; this suggests that Vpu evolutionary adaptation may be associated with the pandemic spread of HIV-1^[18]. Several studies have demonstrated the extent of Vpu sequence variability both at inter- and intra-patient level. By using the 101 aligned amino acid sequences of entire HIV-1 genome, one study showed that Vpu had the highest average entropy score in comparison to other proteins in HIV-1 genome^[24]. Another study analyzing the intra-patient diversity and adaptation of non-structural genes in primary HIV-1 subtype C infection reported that *vpu* compared to *vif*, *vpr*, *tat exon 1* and *rev exon 1* genes has the highest mean of intra-patient diversity that increased gradually^[25]. We retrieved full lengths clade B sequences ($n = 544$) of HIV-1 proteins (Gag, Pol, Env, Nef, Vif, Vpu, Vpr, Tat and Rev) from Los Alamos database and the average entropy score of each protein was determined. Vpu was observed to be one of the proteins with the highest average entropy score (Figure 1), confirming the highly variable nature of Vpu at population level. However, interestingly, a recent study has shown that despite extensive Vpu sequence variation in HIV-1 infected individuals, Vpu functions (CD4 cell surface downregulation and tetherin counteraction activity) were maintained^[26].

IMMUNE RESPONSES TOWARDS VPU

Humoral immunity

Several studies have reported Vpu-specific humoral immune responses during HIV-1 infection^[27-31]. However there has been some controversy on correlation between the presence of anti-Vpu Ab responses in HIV-infected patients' sera and clinical outcome. Some studies have indicated that anti-Vpu Ab responses may influence the clinical outcomes in HIV-1 infected individuals^[27,28,30,31], while on the other hand other studies have showed no correlation^[29]. These findings indicate that Vpu is indeed a target of antibodies although no evidence yet support that such antibody responses influence the Vpu variability. The epitopic regions for such antibodies reported include 37-50^[30] and 68-81^[28] of Vpu; nonetheless there

is no specific Vpu activity mapped to these regions so far. However, considering that Vpu is a small protein (81 amino acids); it is intriguing to test whether such Vpu-specific antibodies can inhibit Vpu functions and subvert viral replication.

Cellular immunity

A growing number of clinical evidence has suggested that HLA-restricted, HIV-specific CD8+ cytotoxic T lymphocytes (CTL) is mainly involved in controlling HIV-1 replication^[32-34]. CTL responses have been well appreciated in SIV-infected macaque's model^[32,33] and in HIV-1 infected patients of both acute^[35,36] and chronic^[37] phases as well as in elite controllers who spontaneously suppress viral replication below detection limit^[38,39]. HLA-restricted CTL responses are thought to be the main driving force of HIV-1 control and viral evolution^[40-43]. The viral polymorphism in response to immune selective pressures follows predictable patterns and kinetics at the population and these immune "footprints/landscape" could be predictable based on the autologous viral sequences and the host immune genetics^[9,42,44]. However, Vpu has been reported to be a poor target for CD8+ T cells as revealed by interferon (IFN)- γ Elispot assay^[45], because only some few epitopes were identified and less than 3% of patients showed detectable Vpu-specific CD8+ T cell responses. Although several HLA-restricted CTL epitopes of Vpu are reported^[45-49], this protein is less targeted by CTLs at least compared to the Nef protein. Consistently, our previous study showed only three HLA-associated polymorphisms in Vpu at Glu-5 with HLA-C*03 and Arg-37, Lys-37 with HLA-A*3303 in a chronic HIV-infected patient cohort in Japan ($n = 216$), indicating that the HLA class I has minor contribution (2% of the total codons) towards Vpu variability^[50]. The increased numbers of subjects to 516 showed similar results (DK, ZH, and TU: unpublished observation). Furthermore, an international large IHAC cohort (International HIV Adaptation Collaborative, $n = 1888$) identified that only 26.3% of the highly variable Vpu codons exhibited statistically significant HLA class I associations^[20]. Although the HLA class I-associated viral polymorphisms observed in the two cohorts suggested to be influenced by several factors such as the host genetic profiles, mixture of multiethnic populations, studied sample size, geographical location and circulating HIV-strains, these results suggest that HLA-associated polymorphisms are only partly attributable to the Vpu variability (Figure 2). However, it is of note that the low CTL responses observed in the previous studies^[45,51] and subtle numbers of HLA-associated polymorphisms^[20,50] may be an underestimation due to the current technical limitation toward a highly variable protein, even though a number of studies reported a plenty of CTL targeting^[52,53] and HLA-associated polymorphisms in Nef^[19,20,42], which showed comparable variability with Vpu at a population level (Figure 1).

Natural killer cells

A number of evidence suggests that natural killer (NK)

cells have an important role in control of HIV-1 infection^[54-56]. Assuming that NK cells may act as a selective force, as similar to CTLs, HIV-1 may leave footprints as viral polymorphisms in association with polymorphic NK cell ligand such as killer-cell immunoglobulin-like receptors (KIR). In fact, one study identified 22 amino-acid polymorphisms within the HIV-1 clade B sequence that are significantly associated with the expression of specific KIR genes in chronically HIV-1 infected, treatment naïve patients ($n = 91$)^[44]. Three (13.6%) of these KIR associated polymorphisms were located in Vpu at positions Ser-3 and Vpu-Env overlapping region (Met-71 and His-74) (Figure 2)^[44]. In addition, the HIV-1-specific antibody-dependent NK cell cytotoxicity is identified towards a 13-mer Vpu peptide (⁶⁹EMGHHAPWDVDL⁸¹)^[57]. Such responses are also observed toward Env^[58] and Nef^[59] in HIV-1 infected patients as well. However, there is no evidence at the moment that show antibody-dependent NK cell cytotoxicity associates with viral polymorphisms.

VPU FUNCTIONALITY INCLUDING IMMUNE EVASION ACTIVITY

In order to conquer the hostile host environment, viruses need to evolve and develop critical interactions with the host cellular factors. Vpu does not only play important role in HIV-1 pathogenesis through CD4 receptor degradation^[11] and enhancement of virion release from infected cells by antagonizing tetherin/BST-2^[60-62]; but Vpu has also evolved to interact with and modulate other host surface receptors and factors (Figure 3).

Vpu induces CD4 receptor degradation

Vpu induces the rapid degradation of newly synthesized CD4 receptor molecules that are retained together with Env precursor protein (gp160) in the endoplasmic reticulum^[13]. The cytoplasmic domain of Vpu and the DSGxxS motif are critical in interaction with and degradation of CD4, respectively^[12,63] (Figure 2). The degradation process is achieved by Vpu recruiting β -TrCP and then interacts with CD4 cytoplasmic domain and subsequently subject CD4 to degradation by the ubiquitin-proteasome pathway^[11,64]. In doing so Vpu contributes to the suppression of HIV-1 primary receptor at the surface of the infected cell.

Vpu enhances virion release

Enhancement of virion release by Vpu has been shown to be achieved through antagonizing tetherin/BST-2, an IFN regulated host restriction factor. BST-2 directly binds to virions and hence retains them on the surface of infected cells^[61,62]. Vpu through AxxxAxxx motif in transmembrane domain directly interacts with BST-2 transmembrane domain, the Vpu DSGxxS and [D/E]XXXL[L/I/V] motifs in the cytoplasmic domain also play crucial role in ensuring BST-2 downmodulation^[15,65,66] (Figure 2). Previous studies indicated BST-2

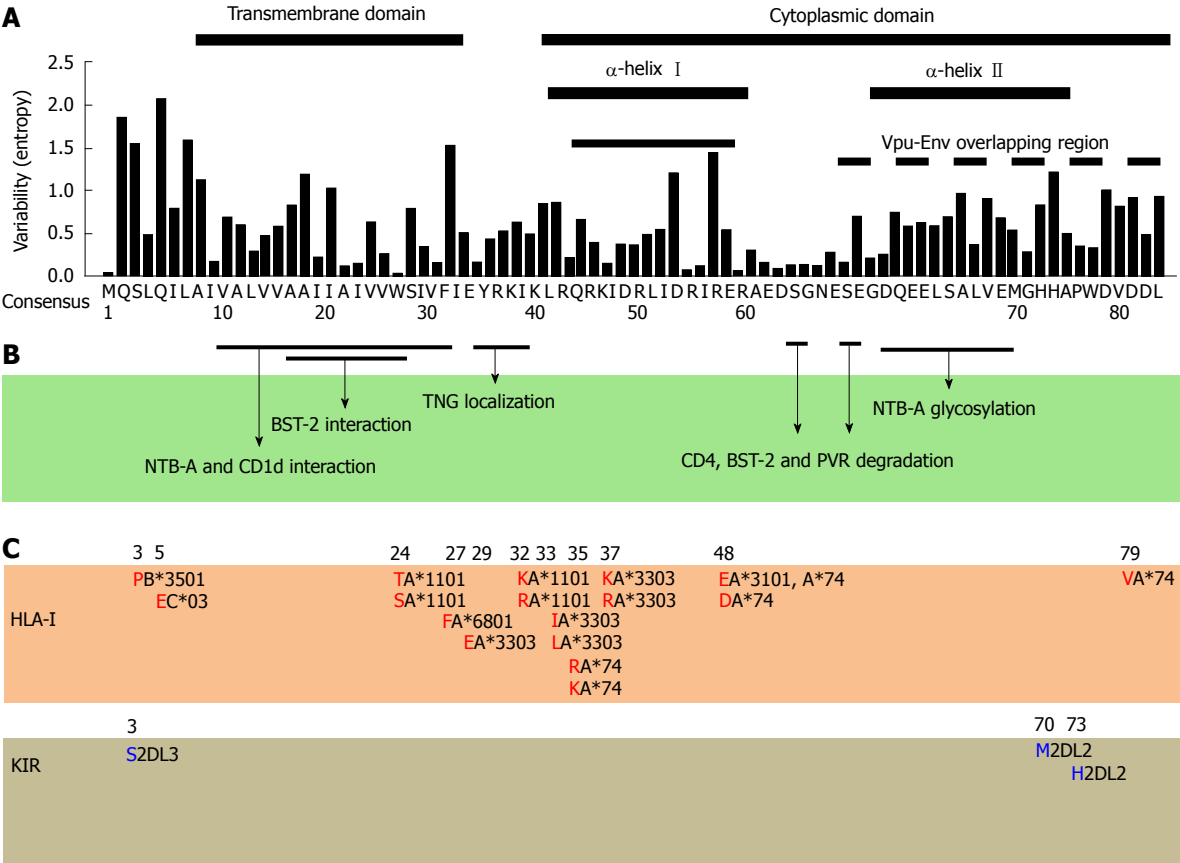


Figure 2 Correlation among amino-acid codon variability, functional regions, and host-mediated immune escape map of human immunodeficiency virus type 1 viral protein U. **A:** Amino acid codon variability is assigned to each position of Vpu using Shannon entropy approach^[24,90]. Sequences were retrieved from Los Alamos database ($n=1139$), the consensus subtype B sequence is indicated as a reference; **B:** Interacting positions and domains responsible for the indicated functions of human immunodeficiency virus type 1 (HIV-1) Vpu are shown^[14,62,91]; **C:** Immune escape map shows amino acid codons and residues (red and blue) associated with HLA-I alleles^[20,45,50] and killer-cell immunoglobulin-like receptors (KIR)^[44], respectively. The specific alleles are indicated in black adjacent to the amino acid.

downmodulation is through β -TrCP-dependent proteasomal degradation pathway^[67] while others suggested the β -TrCP-dependent endo-lysosomal pathway^[63,68]. In contrast, recent studies showed that BST-2 antagonistic activity by Vpu takes place in the trans-Golgi networks (TGN)^[14]. Vpu interferes with anterograde transport of BST-2 to the cell surface subsequently leading to BST-2 trapping in the TGN^[15-17,69].

Vpu modulation of other cell surface receptors and host factors

Recent studies have indicated that Vpu is emerging as a viral factor with a range of activities devoted to counteracting host innate and adaptive immunity including the modulation of NK cell co-activation ligand NK-T and B cell antigen (NTB-A)^[70], PVR activating ligand of NK cells^[71], and CD1d^[72,73] (Figure 3).

NTB-A triggering is necessary for induction of efficient lysis of target cells upon engagement of the activating receptor NKG2D^[74]. The Ser-52 and Ser-56 residues important for CD4 and BST-2 degradation did not affect NTB-A expression, indicating that the down modulation of NTB-A by Vpu is mediated by different domains^[70]. A recent study has shown that downmodulation of NTB-A is achieved by Vpu interfering with the anterograde trans-

port of NTB-A by retaining it within the Golgi compartment and hence affects its glycosylation pattern that subsequently reduces surface expression of NTB-A^[75].

PVR (CD155, Necl-5) is a ligand for the activating receptor DNAM-1 (CD226) expressed by NK cells^[76,77]. PVR downmodulation by Nef and Vpu is another strategy evolved by HIV-1 to avoid NK cell-mediated lysis of infected cells^[71]. PVR downregulation alters multiple important PVR-mediated innate cellular immune processes such as adhesion and migration, and therefore may influence HIV-1 pathogenesis.

CD1d molecules are important in dendritic cells for lipid antigen presentation to CD1d-restricted NKT cells^[78,79]. CD1d and CD1d-restricted NKT cells are present at pathogen entry sites thus play a crucial role in early immune responses^[80]. Vpu has been shown to be the major viral factor that inhibit recycling of CD1d from the endosomal compartment back to cell surface through retaining CD1d in early endosomes^[72].

Vpu has also been implicated in inhibition of ubiquitination and degradation of p53 (a substrate of SCF ^{β -TrCP} ligase complex). The successful interaction of SCF ^{β -TrCP} complex with β -TrCP binding motif (DS₅₂GNE₅₆) present in Vpu has been shown to be essential^[81]. It was observed that Vpu mutants with alanine substitutions

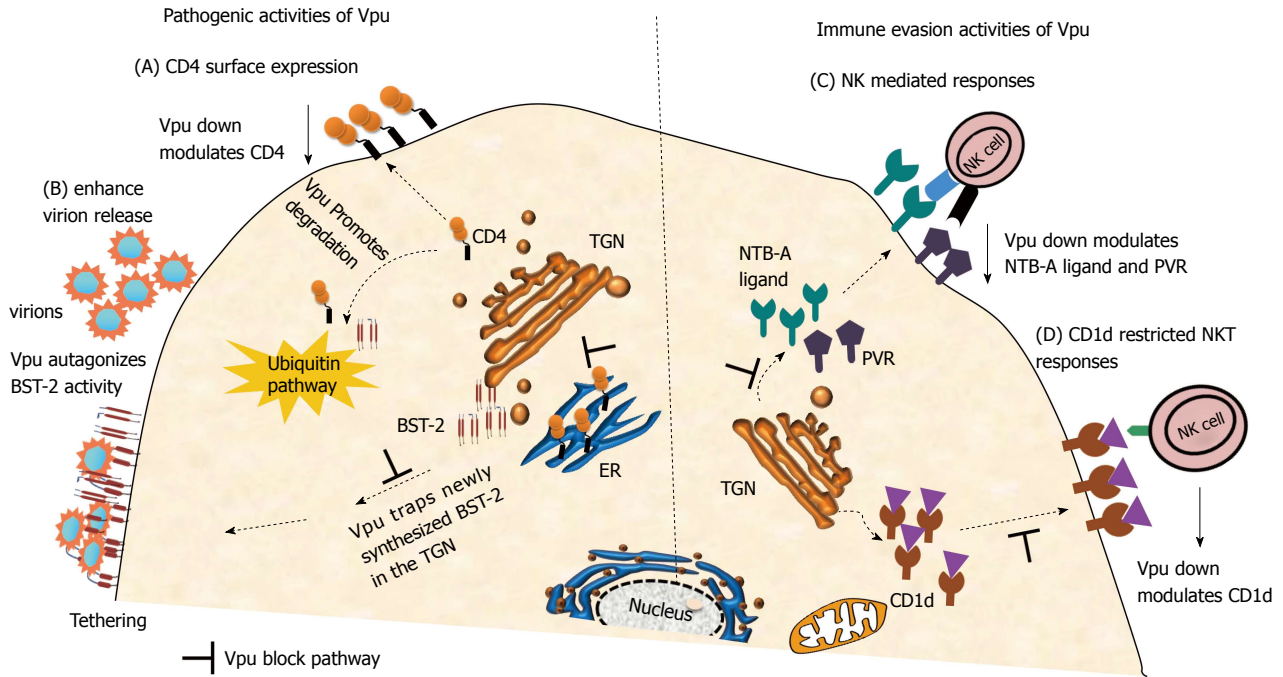


Figure 3 Viral protein U functionality including immune evasion activity. The schematic representation of the cell illustrates some key functions of viral protein U (Vpu) including immune evasion activities. (A): Panel A illustrates CD4 down regulation by Vpu through degradation in a β -TrCP dependent ubiquitination pathway^[11,12,64], (B): Panel B demonstrates enhancement of virion release by Vpu through antagonizing BST-2, which is achieved through direct interaction with BST-2 which subsequently leads to trapping of BST-2 in the trans-Golgi networks^[14-16] and also indicates β -TrCP dependent ubiquitination of BST-2^[62,65,66]; (C): Panel C demonstrates Vpu evasion of NK cell recognition through down modulation of NTB-A ligand^[70] and PVR^[71]; D: Panel D shows down modulation of CD1d from cell surface hence avoid CD1d-restricted NKT cell responses^[72,73]. NK: Natural killer; NKT: Natural killer T.

(DA₅₂GNEA₅₆) failed to stabilize p53 and did not prevent its ubiquitination. This suggested that Vpu is able to achieve modulation of p53 through competing efficiently with p53 protein for the β -TrCP subunit of the SCF complex and hence inhibits subsequent ubiquitination of p53 protein. The modulation of p53 positively correlated with apoptosis during the late stages of HIV-1 infection^[81].

Finally, although Vpu showed multiple functions *in vitro* and *ex vivo*, it is yet clear how and what functions of Vpu are important in viral pathogenesis *in vivo*.

CONCLUSION

The current knowledge on factors that are attributed to Vpu polymorphism has not been quite sufficient; therefore this prompt for further analysis to reveal the unresolved questions of why Vpu is so variable and what factors drive Vpu polymorphism. In order to define the complex dynamics of HIV-1 Vpu evolution, immune escape patterns, and functional adaptation during the course of infection, further insight is needed on the role of host genetics and other immune selection pressures towards shaping HIV-1 Vpu diversity. The emergence of advanced DNA sequencing technologies such as ultra-deep sequence which is superior and more sensitive than Sanger sequence methods has made it possible to accurately detect and analyze minor variants of HIV-1 within a host^[82-85]. Furthermore, the establishment of different contemporaneous cohorts of HIV-1-infected individuals worldwide enables us to examine to what extent the host

immune components play a role on viral adaptation and/or evolution at both intra- and inter-patients' level.

So far the current studies have indicated that the host immune responses directed towards Vpu is not entirely attributable to HIV-1 Vpu variation (Figure 2), it is therefore crucial to apprehend other factors that may explain Vpu variation. Of note previous studies have identified immune responses directed towards Vpu, using peptides of HIV-1 consensus sequences^[45,57]. However, ironically due to Vpu polymorphic nature itself, these results may mask the exact extent to which immune responses contribute to Vpu sequence variation. Alternatively, HIV-1 like other RNA viruses has evolved to shorten its genome length through overlapping its genes^[86]. The overlapping region of Vpu and Env is one of promising aspect to consider when we focus on Vpu variation. Because host immune responses (neutralizing antibodies) contribute to Env polymorphic nature^[87,88], it is enticing to assume that immune responses directed towards Env may influence Vpu polymorphisms through Vpu-Env overlapping region. KIR associated polymorphisms within Vpu-Env overlapping region have been reported previously^[44]. Although it is still unknown whether NK cells recognize Vpu or Env protein, nonetheless these findings indicate the importance of this region for Vpu variability. Furthermore, it is reported that X4- and R5-tropic HIV-1 showed differential amino acid polymorphisms in Vpu^[89], suggesting that cellular compartment influences Vpu variability.

The current increase in number of new findings of

Vpu from pandemic HIV-1 group M strain and other HIV-1 strains, enlighten us the precise role or mechanisms of how Vpu degrade the viral receptor CD4, antagonize tetherin/BST-2, enhance p53 stability and modulate NK-cell activities through modulation of PVR, NTB-A and CD1d receptors (Figure 3). Understanding the mode of action of Vpu and association of the immune factors certainly open plenty of new windows to deciphering the intricate mechanisms associated with HIV-1 immune pathogenesis *in vivo*. Also, understanding pathways of Vpu intra- and inter-patients sequence variability and adaptation may provide us with an alternative approach for prospects of viral persistence and Vpu contributions *in vivo*.

ACKNOWLEDGMENTS

The authors wish to thank the Ministry of Education, Science, Sports, and Culture (MEXT) of Japan and the Ministry of Health, Labor, and Welfare of Japan for their grant-in aid for the AIDS research. We also wish to thank Dr. J Carlson (Microsoft Research, Redmond, Washington, United States) and M. Mahiti (Kumamoto University, Japan) for their helpful discussion.

REFERENCES

- Ji JP, Loeb LA. Fidelity of HIV-1 reverse transcriptase copying RNA in vitro. *Biochemistry* 1992; **31**: 954-958 [PMID: 1370910]
- Shriner D, Rodrigo AG, Nickle DC, Mullins JI. Pervasive genomic recombination of HIV-1 in vivo. *Genetics* 2004; **167**: 1573-1583 [PMID: 15342499 DOI: 10.1534/genetics.103.023382]
- Ramratnam B, Bonhoeffer S, Binley J, Hurley A, Zhang L, Mittler JE, Markowitz M, Moore JP, Perelson AS, Ho DD. Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. *Lancet* 1999; **354**: 1782-1785 [PMID: 10577640 DOI: 10.1016/S0140-6736(99)02035-8]
- Snoeck J, Fellay J, Bartha I, Douek DC, Telenti A. Mapping of positive selection sites in the HIV-1 genome in the context of RNA and protein structural constraints. *Retrovirology* 2011; **8**: 87 [PMID: 22044801 DOI: 10.1186/1742-4690-8-87]
- O'Brien SJ, Nelson GW. Human genes that limit AIDS. *Nat Genet* 2004; **36**: 565-574 [DOI: 10.1038/Ng1369]
- An P, Winkler CA. Host genes associated with HIV/AIDS: advances in gene discovery. *Trends Genet* 2010; **26**: 119-131 [PMID: 20149939 DOI: 10.1016/j.tig.2010.01.002]
- Brumme ZL, Walker BD. Tracking the culprit: HIV-1 evolution and immune selection revealed by single-genome amplification. *J Exp Med* 2009; **206**: 1215-1218 [PMID: 19487418]
- Brumme ZL, Art FYP, Jonathan MC, Walkerd BD. Identifying HLA-Associated Polymorphisms in HIV-1. HIV Molecular Immunology 2010. Los Alamos, New Mexico: Los Alamos National Laboratory, Theoretical Biology and Biophysics, 2010: 3-16
- Moore CB, John M, James IR, Christiansen FT, Witt CS, Mallal SA. Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. *Science* 2002; **296**: 1439-1443 [PMID: 12029127 DOI: 10.1126/science.1069660]
- Maldarelli F, Chen MY, Willey RL, Strebel K. Human immunodeficiency virus type 1 Vpu protein is an oligomeric type I integral membrane protein. *J Virol* 1993; **67**: 5056-5061 [PMID: 8331740]
- Schubert U, Antón LC, Bacák I, Cox JH, Bour S, Bennink JR, Orlowski M, Strebel K, Yewdell JW. CD4 glycoprotein degradation induced by human immunodeficiency virus type 1 Vpu protein requires the function of proteasomes and the ubiquitin-conjugating pathway. *J Virol* 1998; **72**: 2280-2288 [PMID: 9499087]
- Tiganos E, Yao XJ, Friborg J, Daniel N, Cohen EA. Putative alpha-helical structures in the human immunodeficiency virus type 1 Vpu protein and CD4 are involved in binding and degradation of the CD4 molecule. *J Virol* 1997; **71**: 4452-4460 [PMID: 9151836]
- Willey RL, Maldarelli F, Martin MA, Strebel K. Human immunodeficiency virus type 1 Vpu protein regulates the formation of intracellular gp160-CD4 complexes. *J Virol* 1992; **66**: 226-234 [PMID: 1727486]
- Dubé M, Roy BB, Guiot-Guillain P, Mercier J, Binette J, Leung G, Cohen EA. Suppression of Tetherin-restricting activity upon human immunodeficiency virus type 1 particle release correlates with localization of Vpu in the trans-Golgi network. *J Virol* 2009; **83**: 4574-4590 [PMID: 19244337]
- Dubé M, Roy BB, Guiot-Guillain P, Binette J, Mercier J, Chiasson A, Cohen EA. Antagonism of tetherin restriction of HIV-1 release by Vpu involves binding and sequestration of the restriction factor in a perinuclear compartment. *PLoS Pathog* 2010; **6**: e1000856 [PMID: 20386718 DOI: 10.1371/journal.ppat.1000856]
- Dube M, Paquay C, Roy BB, Bego MG, Mercier J, Cohen EA. HIV-1 Vpu Antagonizes BST-2 by Interfering Mainly with the Trafficking of Newly Synthesized BST-2 to the Cell Surface. *Traffic* 2011; **12**: 1714-1729 [DOI: 10.1111/j.1600-0854.2011.01277.x]
- Schmidt S, Fritz JV, Bitzegeio J, Fackler OT, Keppler OT. HIV-1 Vpu blocks recycling and biosynthetic transport of the intrinsic immunity factor CD317/tetherin to overcome the virion release restriction. *MBio* 2011; **2**: e00036-e00011 [PMID: 21610122 DOI: 10.1128/mBio.00036-11]
- Sauter D, Unterwiesing D, Vogl M, Usmani SM, Heigle A, Kluge SF, Hermkes E, Moll M, Barker E, Peeters M, Learn GH, Bibollet-Ruche F, Fritz JV, Fackler OT, Hahn BH, Kirchhoff F. Human tetherin exerts strong selection pressure on the HIV-1 group N Vpu protein. *PLoS Pathog* 2012; **8**: e1003093 [PMID: 23308067 DOI: 10.1371/journal.ppat.1003093]
- Brumme ZL, John M, Carlson JM, Brumme CJ, Chan D, Brockman MA, Swenson LC, Tao I, Szeto S, Rosato P, Sela J, Kadie CM, Frahm N, Brander C, Haas DW, Riddler SA, Haubrich R, Walker BD, Harrigan PR, Heckerman D, Mallal S. HLA-associated immune escape pathways in HIV-1 subtype B Gag, Pol and Nef proteins. *PLoS One* 2009; **4**: e6687 [PMID: 19690614 DOI: 10.1371/journal.pone.0006687]
- Carlson JM, Brumme CJ, Martin E, Listgarten J, Brockman MA, Le AQ, Chui CK, Cotton LA, Knapp DJ, Riddler SA, Haubrich R, Nelson G, Pfeifer N, Deziel CE, Heckerman D, Apps R, Carrington M, Mallal S, Harrigan PR, John M, Brumme ZL. Correlates of protective cellular immunity revealed by analysis of population-level immune escape pathways in HIV-1. *J Virol* 2012; **86**: 13202-13216 [PMID: 23055555]
- Ueno T, Motozono C, Dohki S, Mwimanzu P, Rauch S, Fackler OT, Oka S, Takiguchi M. CTL-mediated selective pressure influences dynamic evolution and pathogenic functions of HIV-1 Nef. *J Immunol* 2008; **180**: 1107-1116 [PMID: 18178851]
- Wei X, Decker JM, Wang S, Hui H, Kappes JC, Wu X, Salazar-Gonzalez JF, Salazar MG, Kilby JM, Saag MS, Komarova NL, Nowak MA, Hahn BH, Kwong PD, Shaw GM. Antibody neutralization and escape by HIV-1. *Nature* 2003; **422**: 307-312 [PMID: 12646921 DOI: 10.1038/nature01470]
- Richman DD, Wrinn T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci USA* 2003; **100**: 4144-4149 [PMID: 12644702 DOI: 10.1073/pnas.0630530100]

- 24 **Yusim K**, Kesmir C, Gaschen B, Addo MM, Altfeld M, Brunak S, Chigaev A, Detours V, Korber BT. Clustering patterns of cytotoxic T-lymphocyte epitopes in human immunodeficiency virus type 1 (HIV-1) proteins reveal imprints of immune evasion on HIV-1 global variation. *J Virol* 2002; **76**: 8757-8768 [PMID: 12163596]
- 25 **Rossenkhani R**, Novitsky V, Sebulana TK, Musonda R, Gashe BA, Essex M. Viral diversity and diversification of major non-structural genes vif, vpr, vpu, tat exon 1 and rev exon 1 during primary HIV-1 subtype C infection. *PLoS One* 2012; **7**: e35491 [PMID: 22590503 DOI: 10.1371/journal.pone.0035491]
- 26 **Pickering S**, Hué S, Kim EY, Reddy S, Wolinsky SM, Neil SJ. Preservation of tetherin and CD4 counter-activities in circulating Vpu alleles despite extensive sequence variation within HIV-1 infected individuals. *PLoS Pathog* 2014; **10**: e1003895 [PMID: 24465210 DOI: 10.1371/journal.ppat.1003895]
- 27 **Matsuda Z**, Chou MJ, Matsuda M, Huang JH, Chen YM, Redfield R, Mayer K, Essex M, Lee TH. Human Immunodeficiency Virus Type-1 Has an Additional Coding Sequence in the Central Region of the Genome. *P Natl Acad Sci USA* 1988; **85**: 6968-6972 [DOI: 10.1073/pnas.85.18.6968]
- 28 **Schneider T**, Hildebrandt P, Ronspeck W, Weigelt W, Pauli G. The antibody response to the HIV-1 specific "out" (vpu) protein: identification of an immunodominant epitope and correlation of antibody detectability to clinical stages. *AIDS Res Hum Retroviruses* 1990; **6**: 943-950 [PMID: 1697179]
- 29 **Reiss P**, Lange JMA, Deronde A, Dewolf F, Dekker J, Daner SA, Deboucq C, Goudsmit J. Antibody-Response to Viral Protein-U (Vpu) and Protein-R (Vpr) in Hiv-1-Infected Individuals. *J Acq Immun Def Syndr* 1990; **3**: 115-122
- 30 **Kusk P**, Lindhardt BO, Bugge TH, Holmbäck K, Hulgaard EF. Mapping of a new immunodominant human linear B-cell epitope on the vpu protein of the human immunodeficiency virus type 1. *J Acquir Immune Defic Syndr* 1993; **6**: 334-338 [PMID: 7681110]
- 31 **Chen YM**, Rey WY, Lan YC, Lai SF, Huang YC, Wu SI, Liu TT, Hsiao KJ. Antibody reactivity to HIV-1 Vpu in HIV-1/AIDS patients on highly active antiretroviral therapy. *J Biomed Sci* 2003; **10**: 266-275 [PMID: 12595763]
- 32 **Jin X**, Bauer DE, Tuttleton SE, Lewin S, Gettie A, Blanchard J, Irwin CE, Safritz JT, Mittler J, Weinberger L, Kostrikis LG, Zhang L, Perelson AS, Ho DD. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 1999; **189**: 991-998 [PMID: 10075982]
- 33 **Schmitz JE**, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallan BJ, Ghayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 1999; **283**: 857-860 [PMID: 9933172]
- 34 **Altfeld M**, Rosenberg ES. The role of CD4(+) T helper cells in the cytotoxic T lymphocyte response to HIV-1. *Curr Opin Immunol* 2000; **12**: 375-380 [PMID: 10899028]
- 35 **Strebeck H**, Nixon DF. T cell immunity in acute HIV-1 infection. *J Infect Dis* 2010; **202** Suppl 2: S302-S308 [PMID: 20846037 DOI: 10.1086/655652]
- 36 **Liu Y**, McNevin JP, Holte S, McElrath MJ, Mullins JI. Dynamics of viral evolution and CTL responses in HIV-1 infection. *PLoS One* 2011; **6**: e15639 [PMID: 21283794 DOI: 10.1371/journal.pone.0015639]
- 37 **Klein MR**, van Baalen CA, Holwerda AM, Kerkhof Garde SR, Bende RJ, Keet IP, Eeftink-Schattenkerk JK, Osterhaus AD, Schuitemaker H, Miedema F. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. *J Exp Med* 1995; **181**: 1365-1372 [PMID: 7699324]
- 38 **Deeks SG**, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* 2007; **27**: 406-416 [PMID: 17892849]
- 39 **Miura T**, Brockman MA, Schneidewind A, Lobritz M, Pereyra F, Rathod A, Block BL, Brumme ZL, Brumme CJ, Baker B, Rothchild AC, Li B, Trocha A, Cutrell E, Frahm N, Brander C, Toth I, Arts EJ, Allen TM, Walker BD. HLA-B57/B*5801 human immunodeficiency virus type 1 elite controllers select for rare gag variants associated with reduced viral replication capacity and strong cytotoxic T-lymphocyte [corrected] recognition. *J Virol* 2009; **83**: 2743-2755 [PMID: 19116253]
- 40 **Messaoudi I**, Guevara Patiño JA, Dyal R, LeMaout J, Nikolich-Zugich J. Direct link between mhc polymorphism, T cell avidity, and diversity in immune defense. *Science* 2002; **298**: 1797-1800 [PMID: 12459592]
- 41 **Brumme ZL**, Brumme CJ, Chui C, Mo T, Wynhoven B, Woods CK, Henrick BM, Hogg RS, Montaner JS, Harrigan PR. Effects of human leukocyte antigen class I genetic parameters on clinical outcomes and survival after initiation of highly active antiretroviral therapy. *J Infect Dis* 2007; **195**: 1694-1704 [PMID: 17471440]
- 42 **Brumme ZL**, Brumme CJ, Heckerman D, Korber BT, Daniels M, Carlson J, Kadie C, Bhattacharya T, Chui C, Szinger J, Mo T, Hogg RS, Montaner JS, Frahm N, Brander C, Walker BD, Harrigan PR. Evidence of differential HLA class I-mediated viral evolution in functional and accessory/regulatory genes of HIV-1. *PLoS Pathog* 2007; **3**: e94 [PMID: 17616974]
- 43 **Sewell AK**, Price DA, Oxenius A, Kelleher AD, Phillips RE. Cytotoxic T lymphocyte responses to human immunodeficiency virus: control and escape. *Stem Cells* 2000; **18**: 230-244 [PMID: 10924089 DOI: 10.1634/stemcells.18-4-230]
- 44 **Alter G**, Heckerman D, Schneidewind A, Fadda L, Kadie CM, Carlson JM, Oniangue-Ndza C, Martin M, Li B, Khakoo SI, Carrington M, Allen TM, Altfeld M. HIV-1 adaptation to NK-cell-mediated immune pressure. *Nature* 2011; **476**: 96-100 [PMID: 21814282]
- 45 **Addo MM**, Altfeld M, Rathod A, Yu M, Yu XG, Goulder PJ, Rosenberg ES, Walker BD. HIV-1 Vpu represents a minor target for cytotoxic T lymphocytes in HIV-1-infection. *AIDS* 2002; **16**: 1071-1073 [PMID: 11953475]
- 46 **Addo MM**, Yu XG, Rosenberg ES, Walker BD, Altfeld M. Cytotoxic T-lymphocyte (CTL) responses directed against regulatory and accessory proteins in HIV-1 infection. *DNA Cell Biol* 2002; **21**: 671-678 [PMID: 12396610 DOI: 10.1089/104454902760330219]
- 47 **Kloverpris H**, Karlsson I, Bonde J, Thorn M, Vinner L, Pedersen AE, Hentze JL, Andresen BS, Svane IM, Gerstoft J, Kronborg G, Fomsgaard A. Induction of novel CD8+ T-cell responses during chronic untreated HIV-1 infection by immunization with subdominant cytotoxic T-lymphocyte epitopes. *AIDS* 2009; **23**: 1329-1340 [PMID: 19528789 DOI: 10.1097/QAD.0b013e32832d9b00]
- 48 **Corbet S**, Nielsen HV, Vinner L, Lauemoller S, Therrien D, Tang S, Kronborg G, Mathiesen L, Chaplin P, Brunak S, Buus S, Fomsgaard A. Optimization and immune recognition of multiple novel conserved HLA-A2, human immunodeficiency virus type 1-specific CTL epitopes. *J Gen Virol* 2003; **84**: 2409-2421 [PMID: 12917462]
- 49 **Kiepiela P**, Ngumbela K, Thobakgale C, Ramduth D, Honeyborne I, Moodley E, Reddy S, de Pierres C, Mncube Z, Mkhwanazi N, Bishop K, van der Stok M, Nair K, Khan N, Crawford H, Payne R, Leslie A, Prado J, Prendergast A, Frater J, McCarthy N, Brander C, Learn GH, Nickle D, Rousseau C, Coovadia H, Mullins JI, Heckerman D, Walker BD, Goulder P. CD8(+) T-cell responses to different HIV proteins have discordant associations with viral load. *Nat Med* 2007; **13**: 46-53 [DOI: 10.1038/Nm1520]
- 50 **Hasan Z**, Carlson JM, Gatanaga H, Le AQ, Brumme CJ, Oka S, Brumme ZL, Ueno T. Minor contribution of HLA class I-associated selective pressure to the variability of HIV-1 ac-

- cessory protein Vpu. *Biochem Biophys Res Commun* 2012; **421**: 291-295 [PMID: 22503975]
- 51 **Altfeld M**, Addo MM, Eldridge RL, Yu XG, Thomas S, Khatri A, Strick D, Phillips MN, Cohen GB, Islam SA, Kallams SA, Brander C, Goulder PJR, Rosenberg ES, Walker BD, Collaboration HS. Vpr is preferentially targeted by CTL during HIV-1 infection. *J Immunol* 2001; **167**: 2743-2752
- 52 **Lucchiari M**, Niedermann G, Leipner C, Meyerhans A, Eichmann K, Maier B. Human immune response to HIV-1-Nef. I. CD45RO- T lymphocytes of non-infected donors contain cytotoxic T lymphocyte precursors at high frequency. *Int Immunol* 1994; **6**: 1739-1749 [PMID: 7865467]
- 53 **Novitsky V**, Cao H, Rybak N, Gilbert P, McLane MF, Gaolekwe S, Peter T, Thior I, Ndung'u T, Marlink R, Lee TH, Essex M. Magnitude and frequency of cytotoxic T-lymphocyte responses: identification of immunodominant regions of human immunodeficiency virus type 1 subtype C. *J Virol* 2002; **76**: 10155-10168 [PMID: 12239290]
- 54 **Alter G**, Martin MP, Teigen N, Carr WH, Suscovich TJ, Schneidewind A, Streeck H, Waring M, Meier A, Brander C, Lifson JD, Allen TM, Carrington M, Altfeld M. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. *J Exp Med* 2007; **204**: 3027-3036 [PMID: 18025129 DOI: 10.1084/jem.20070695]
- 55 **Funke J**, Dürr R, Dietrich U, Koch J. Natural killer cells in HIV-1 infection: a double-edged sword. *AIDS Rev* 2011; **13**: 67-76 [PMID: 21587340]
- 56 **Alter G**, Altfeld M. NK cells in HIV-1 infection: evidence for their role in the control of HIV-1 infection. *J Intern Med* 2009; **265**: 29-42 [DOI: 10.1111/j.1365-2796.2008.02045.x]
- 57 **Stratov I**, Chung A, Kent SJ. Robust NK cell-mediated human immunodeficiency virus (HIV)-specific antibody-dependent responses in HIV-infected subjects. *J Virol* 2008; **82**: 5450-5459 [PMID: 18353957]
- 58 **Alsmadi O**, Herz R, Murphy E, Pinter A, Tilley SA. A novel antibody-dependent cellular cytotoxicity epitope in gp120 is identified by two monoclonal antibodies isolated from a long-term survivor of human immunodeficiency virus type 1 infection. *J Virol* 1997; **71**: 925-933 [PMID: 8995609]
- 59 **Yamada T**, Watanabe N, Nakamura T, Iwamoto A. Antibody-dependent cellular cytotoxicity via humoral immune epitope of Nef protein expressed on cell surface. *J Immunol* 2004; **172**: 2401-2406 [PMID: 14764710]
- 60 **Nomaguchi M**, Fujita M, Adachi A. Role of HIV-1 Vpu protein for virus spread and pathogenesis. *Microbes Infect* 2008; **10**: 960-967 [PMID: 18672082]
- 61 **Dubé M**, Bego MG, Paquay C, Cohen ÉA. Modulation of HIV-1-host interaction: role of the Vpu accessory protein. *Retrovirology* 2010; **7**: 114 [PMID: 21176220]
- 62 **Neil SJ**, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 2008; **451**: 425-430 [PMID: 18200009]
- 63 **Vincent MJ**, Raja NU, Jabbar MA. Human immunodeficiency virus type 1 Vpu protein induces degradation of chimeric envelope glycoproteins bearing the cytoplasmic and anchor domains of CD4: role of the cytoplasmic domain in Vpu-induced degradation in the endoplasmic reticulum. *J Virol* 1993; **67**: 5538-5549 [PMID: 8350411]
- 64 **Bour S**, Schubert U, Strebel K. The human immunodeficiency virus type 1 Vpu protein specifically binds to the cytoplasmic domain of CD4: implications for the mechanism of degradation. *J Virol* 1995; **69**: 1510-1520 [PMID: 7853484]
- 65 **Mitchell RS**, Katsura C, Skasko MA, Fitzpatrick K, Lau D, Ruiz A, Stephens EB, Margottin-Goguet F, Benarous R, Guatelli JC. Vpu antagonizes BST-2-mediated restriction of HIV-1 release via beta-TrCP and endo-lysosomal trafficking. *PLoS Pathog* 2009; **5**: e1000450 [PMID: 19478868 DOI: 10.1371/journal.ppat.1000450]
- 66 **Rong L**, Zhang J, Lu J, Pan Q, Lorgeoux RP, Aloysius C, Guo F, Liu SL, Wainberg MA, Liang C. The transmembrane domain of BST-2 determines its sensitivity to down-modulation by human immunodeficiency virus type 1 Vpu. *J Virol* 2009; **83**: 7536-7546 [PMID: 19474106 DOI: 10.1128/JVI.00620-09]
- 67 **Mangeat B**, Gers-Huber G, Lehmann M, Zufferey M, Luban J, Pignatelli B. HIV-1 Vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its beta-TrCP2-dependent degradation. *PLoS Pathog* 2009; **5**: e1000574 [PMID: 19730691 DOI: 10.1371/journal.ppat.1000574]
- 68 **Mangeat B**, Gers-Huber G, Lehmann M, Zufferey M, Luban J, Pignatelli B. HIV-1 Vpu neutralizes the antiviral factor Tetherin/BST-2 by binding it and directing its beta-TrCP2-dependent degradation. *PLoS Pathog* 2009; **5**: e1000574 [PMID: 19730691 DOI: 10.1371/journal.ppat.1000574]
- 69 **Hauser H**, Lopez LA, Yang SJ, Oldenburg JE, Exline CM, Guatelli JC, Cannon PM. HIV-1 Vpu and HIV-2 Env counteract BST-2/tetherin by sequestration in a perinuclear compartment. *Retrovirology* 2010; **7**: 51 [PMID: 20529266 DOI: 10.1186/1742-4690-7-51]
- 70 **Shah AH**, Sowrirajan B, Davis ZB, Ward JP, Campbell EM, Planelles V, Barker E. Degranulation of natural killer cells following interaction with HIV-1-infected cells is hindered by downmodulation of NTB-A by Vpu. *Cell Host Microbe* 2010; **8**: 397-409 [PMID: 21075351]
- 71 **Matusali G**, Potestà M, Santoni A, Cerboni C, Doria M. The human immunodeficiency virus type 1 Nef and Vpu proteins downregulate the natural killer cell-activating ligand PVR. *J Virol* 2012; **86**: 4496-4504 [PMID: 22301152]
- 72 **Moll M**, Andersson SK, Smed-Sörensen A, Sandberg JK. Inhibition of lipid antigen presentation in dendritic cells by HIV-1 Vpu interference with CD1d recycling from endosomal compartments. *Blood* 2010; **116**: 1876-1884 [PMID: 20530791]
- 73 **Kelly H**, Mandraju R, Coelho-dos-Reis JG, Tsuji M. Effects of HIV-1-induced CD1c and CD1d modulation and endogenous lipid presentation on CD1c-restricted T-cell activation. *BMC Immunol* 2013; **14**: 4 [PMID: 23347583 DOI: 10.1186/1471-2172-14-4]
- 74 **Flaig RM**, Stark S, Watzl C. Cutting edge: NTB-A activates NK cells via homophilic interaction. *J Immunol* 2004; **172**: 6524-6527 [PMID: 15153464]
- 75 **Bolduan S**, Hubel P, Reif T, Lodermeier V, Höhne K, Fritz JV, Sauter D, Kirchhoff F, Fackler OT, Schindler M, Schubert U. HIV-1 Vpu affects the anterograde transport and the glycosylation pattern of NTB-A. *Virology* 2013; **440**: 190-203 [PMID: 23528733 DOI: 10.1016/j.virol.2013.02.021]
- 76 **Shibuya A**, Campbell D, Hannum C, Yssel H, Franz-Bacon K, McClanahan T, Kitamura T, Nicholl J, Sutherland GR, Lanier LL, Phillips JH. DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. *Immunity* 1996; **4**: 573-581 [PMID: 8673704]
- 77 **Takai Y**, Miyoshi J, Ikeda W, Ogita H. Nectins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. *Nat Rev Mol Cell Biol* 2008; **9**: 603-615 [PMID: 18648374 DOI: 10.1038/nrm2457]
- 78 **Mattner J**, Debord KL, Ismail N, Goff RD, Cantu C, Zhou D, Saint-Mezard P, Wang V, Gao Y, Yin N, Hoebe K, Schneewind O, Walker D, Beutler B, Teyton L, Savage PB, Bendelac A. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 2005; **434**: 525-529 [PMID: 15791258 DOI: 10.1038/nature03408]
- 79 **Tupin E**, Kinjo Y, Kronenberg M. The unique role of natural killer T cells in the response to microorganisms. *Nat Rev Microbiol* 2007; **5**: 405-417 [PMID: 17487145 DOI: 10.1038/nrmicro1657]
- 80 **Kawana K**, Matsumoto J, Miura S, Shen L, Kawana Y, Nagamatsu T, Yasugi T, Fujii T, Yang H, Quayle AJ, Taketani Y, Schust DJ. Expression of CD1d and ligand-induced cytokine production are tissue specific in mucosal epithelia of the human lower reproductive tract. *Infect Immun* 2008; **76**: 3011-3018 [PMID: 18458073 DOI: 10.1128/IAI.01672-07]

- 81 **Verma S**, Ali A, Arora S, Banerjee AC. Inhibition of β -TrCP-dependent ubiquitination of p53 by HIV-1 Vpu promotes p53-mediated apoptosis in human T cells. *Blood* 2011; **117**: 6600-6607 [PMID: 21521785]
- 82 **Bushman FD**, Hoffmann C, Ronen K, Malani N, Minkah N, Rose HM, Tebas P, Wang GP. Massively parallel pyrosequencing in HIV research. *AIDS* 2008; **22**: 1411-1415 [PMID: 18614863 DOI: 10.1097/QAD.0b013e3282fc972e]
- 83 **Bimber BN**, Burwitz BJ, O'Connor S, Detmer A, Gostick E, Lank SM, Price DA, Hughes A, O'Connor D. Ultradeep pyrosequencing detects complex patterns of CD8+ T-lymphocyte escape in simian immunodeficiency virus-infected macaques. *J Virol* 2009; **83**: 8247-8253 [PMID: 19515775]
- 84 **Henn MR**, Boutwell CL, Charlebois P, Lennon NJ, Power KA, Macalalad AR, Berlin AM, Malboeuf CM, Ryan EM, Gnerre S, Zody MC, Erlich RL, Green LM, Berical A, Wang Y, Casali M, Streeck H, Bloom AK, Dudek T, Tully D, Newman R, Axten KL, Gladden AD, Battis L, Kemper M, Zeng Q, Shea TP, Gujja S, Zedlack C, Gasser O, Brander C, Hess C, Günthard HF, Brumme ZL, Brumme CJ, Bazner S, Rychert J, Tinsley JP, Mayer KH, Rosenberg E, Pereyra F, Levin JZ, Young SK, Jessen H, Altfeld M, Birren BW, Walker BD, Allen TM. Whole genome deep sequencing of HIV-1 reveals the impact of early minor variants upon immune recognition during acute infection. *PLoS Pathog* 2012; **8**: e1002529 [PMID: 22412369 DOI: 10.1371/journal.ppat.1002529]
- 85 **Henn MR**, Boutwell CL, Charlebois P, Lennon NJ, Power KA, Macalalad AR, Berlin AM, Malboeuf CM, Ryan EM, Gnerre S, Zody MC, Erlich RL, Green LM, Berical A, Wang Y, Casali M, Streeck H, Bloom AK, Dudek T, Tully D, Newman R, Axten KL, Gladden AD, Battis L, Kemper M, Zeng Q, Shea TP, Gujja S, Zedlack C, Gasser O, Brander C, Hess C, Günthard HF, Brumme ZL, Brumme CJ, Bazner S, Rychert J, Tinsley JP, Mayer KH, Rosenberg E, Pereyra F, Levin JZ, Young SK, Jessen H, Altfeld M, Birren BW, Walker BD, Allen TM. Whole genome deep sequencing of HIV-1 reveals the impact of early minor variants upon immune recognition during acute infection. *PLoS Pathog* 2012; **8**: e1002529 [PMID: 22412369 DOI: 10.1371/journal.ppat.1002529]
- 86 **Chirico N**, Vianelli A, Belshaw R. Why genes overlap in viruses. *Proc Biol Sci* 2010; **277**: 3809-3817 [PMID: 20610432]
- 87 **Fenyö EM**, Albert J, McKeating J. The role of the humoral immune response in HIV infection. *AIDS* 1996; **10** Suppl A: S97-106 [PMID: 8883616]
- 88 **McKeating JA**. Biological consequences of human immunodeficiency virus type 1 envelope polymorphism: does variation matter? 1995 Fleming Lecture. *J Gen Virol* 1996; **77** (Pt 12): 2905-2919 [PMID: 9000081]
- 89 **Dimonte S**, Babakir-Mina M, Aquaro S, Perno CF. Specific VpU codon changes were significantly associated with gp120 V3 tropic signatures in HIV-1 B-subtype. *Virol Sin* 2012; **27**: 360-368 [PMID: 23271577 DOI: 10.1007/s12250-012-3287-0]
- 90 **Efron B**, Tibshirani R. Statistical data analysis in the computer age. *Science* 1991; **253**: 390-395 [PMID: 17746394]
- 91 **Schubert U**, Strebel K. Differential activities of the human immunodeficiency virus type 1-encoded Vpu protein are regulated by phosphorylation and occur in different cellular compartments. *J Virol* 1994; **68**: 2260-2271 [PMID: 8139011]

P- Reviewer: Brett TJ, Fackler OT **S- Editor:** Wen LL
L- Editor: A **E- Editor:** Wang CH



Targeting TLR4/MAPKs signaling pathway: A better option for therapeutic inhibition of atherosclerosis

Janeesh Plakkal Ayyappan, Annie Abraham

Janeesh Plakkal Ayyappan, Annie Abraham, Department of Biochemistry, School of Life sciences, University of Kerala, Kariavattom Campus, Thiruvananthapuram 695 581, Kerala, India
 Author contributions: All authors contributed to this paper.

Supported by University of Kerala, India for the study
 Correspondence to: Dr. Annie Abraham, Director, Professor of Biochemistry, Department of Biochemistry, School of Life sciences, University of Kerala, Kariavattom Campus, Thiruvananthapuram 695 581, Kerala, India. annieab2001@gmail.com
 Telephone: +91-471-2308078 Fax: +91-471-2308614

Received: March 28, 2014 Revised: May 10, 2014
 Accepted: June 27, 2014

Published online: July 27, 2014

Abstract

Cardiovascular diseases, especially atherosclerosis, found to be the dreadful diseases worldwide. There are diverse pathways associated with the progression of atherosclerosis. One of the important signaling pathways to target atherosclerotic plaque rupture is toll-like receptor 4 (TLR4) Pathway. Several studies are available for illustrating the role of TLR4 in health and diseases. Different types of immune cell are activated in atherosclerosis but primary cells that are activated by the TLR4 signaling are macrophages and endothelial cells. Mechanisms by which macrophages uptake lipids are diverse and it is very important to target signaling pathway responsible for controlling foam cell formation. The process of macrophages transformed foam cell formation is the critical event in progression of atherosclerotic lesion and TLR4 found to have actively participate in the event through mitogen activated protein kinases (MAPKs) activation. The activation of MAPKs signaling pathway leads to the accumulation of cholesterol in the macrophages and also contribute to the dissociation of $I\kappa B$ and the nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κB) p65 subunit, thereby activating key inflammatory cascade

activation by MAPKs/NF- κB signaling pathway to induce toxicity by activating different inflammatory parameters. Hence, the review focussed on exploring the role of TLR4/MAPKs signaling pathway for the therapeutic inhibition of atherosclerosis.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Atherosclerosis; Toll-like receptor 4; Mitogen activated protein kinase; Foam cells; Inflammatory markers

Core tip: The inhibition of atherosclerosis is one of primary target for the therapeutics of cardiovascular diseases, which is the eminent health problem worldwide. The important function of toll-like receptor 4 (TLR4) in the activation and progression of atherosclerosis is justified here. The TLR4 in turn activates the mitogen activated protein kinases (MAPKs) and nuclear factor kappa-light-chain-enhancer of activated B cells which are responsible for most of the inflammatory events. Hence, therapeutic inhibition of TLR4/MAPKs signaling pathway is one of the best method of inhibiting atherosclerosis.

Plakkal Ayyappan J, Abraham A. Targeting TLR4/MAPKs signaling pathway: A better option for therapeutic inhibition of atherosclerosis. *World J Immunol* 2014; 4(2): 116-121 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/116.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.116>

INTRODUCTION

Cardiovascular disease, especially atherosclerosis is a main health problem worldwide and it is a disease characterised by the deposition of lipid in the blood vessels. There are several studies undertaken to know the proximal role of

immune system in atherosclerosis^[1]. Macrophages are the primary cells which are present in atherosclerotic lesions and they uptake lipids and get transformed to foam cells. These foam cells are risky and contribute to the development of atherosclerotic plaque rupture.

It was known that inflammatory process and its further cascade by activating immune system may contribute to the development of inflammation related atherosclerosis^[2]. Usually the luminal side of the blood vessel walls are prone to atherosclerotic injury^[3]. The presence of human histocompatibility leukocyte antigen is widely upregulated as the result of inflammatory processes^[4]. There are studies reporting the role of variety of Infectious organisms and HSP60 as trigger of atherosclerosis^[5].

It is very important to know the mechanism by which macrophages uptakes lipid and transformed get into foam cells. Targeting of macrophages transformed to foam cells are very important therapeutic strategies^[6]. The studies on mechanism by which macrophages accumulate OxLDL and its further activation cascades are very important. It usually activates further cascades by activating components like polyoxygenated cholesteryl ester hydroperoxides and in turn activates toll-like receptor 4 (TLR4)^[6].

It was suggested that TLR4 act as a link between inflammation and atherosclerosis^[7]. TLR4 found to have an active participation in the progression of atherosclerotic diseases. It can also interferes with the cholesterol metabolic machinery in macrophages^[8]. The research in TLR4 shown that, silencing of TLR4 gene seems to have reduced the size of atherosclerotic lesion, lipid content and macrophage polarisation in mice fed a high cholesterol diet for continuous six months^[9].

TLR4 found to have act as an important receptor for arterial remodelling^[10]. The activation of TLR4 receptor leads to the further activation of MYD88 protein and through protein cascade further activates mitogen activated protein kinases (MAPKs). The activation of MAPKs are essential for the secretion of chemoattract protein to direct monocytes to the atherosclerotic site^[11]. The study on inhibition of tyrosine phosphatases like MAPKs found to have demolished the atherosclerotic lesion size in mice^[11].

The phosphorylation of MAPKs triggers the activation of several downstream proteins and further activates the nuclear factor translocation (NF- κ B) which ultimately leading to the progression and rupture of atherosclerotic plaque^[12]. Hence, the review focussed on exploring the role of TLR4/MAPKs signaling pathway in therapeutic inhibition of atherosclerosis.

ATHEROSCLEROSIS AND ITS ACTIVATION BY IMMUNE SYSTEM

The immune system is considered to be the guardian of host and its activation as a result to solve the denudation of endothelium. If the immune system unable to control this activation, then it will result in the chronic immune

reaction and can result in the development of atherosclerotic plaque formation^[13]. The regions of atherosclerotic lesions are usually crowded with macrophages and T cells which usually plays an adequate role in innate and acquired immune reactions. It was known in atherosclerotic disease condition there is an clonal expansion of differentiated T cells, which are common in all adaptive immune reactions^[14].

TOLL-LIKE RECEPTOR-4

Toll-like receptor-4 (TLR4) pattern-recognition receptors are found to have an important role in the immune function. TLRs resides in the family of type I transmembrane receptor which consists of intracellular domain and an extracellular leucine repeat domain^[15-17]. It was known that human TLR4 was the first characterised form of mammalian toll^[15]. TLR4 is expressed in different types of cells, among them most abundant cell type is macrophages and dendric cells^[15]. Usually, it is an membrane receptor which act as a signal transducing agent in different inflammatory insult condition like LPS induced^[18-21].

The extensive research in the field of TLRs resulted in knowing mechanism of immune response induced by TLR4, it is by recognition the pathogen associated molecular pattern. The recent studies using mouse knock out genes demonstrated the active role of TLR4 in triggering and development of atherosclerotic plaque^[15].

TLR4 IN HEALTH AND DISEASES

Among the toll like receptors, the best characterised form is the TLR4, which has found to have prominent role in the atherosclerosis^[22]. The tissue slice from aorta of atherosclerotic plaque area showed an prominent expression of TLR4 by immunohistochemical analysis^[23].

The research studies on cardiovascular diseases shown that infection associated with C pneumonia found to have role in the progression of atherosclerotic diseases^[24]. It usually triggers the diseases by activating TLR4 receptor to induce the migration and proliferation of smooth muscle cells^[25]. The patient with up regulated expression of human TLR4 results in the elevation of IL-12 expression on the downstream activation of TLR4^[22].

Lipopolysaccharide are released upon microbial infection and might triggers the plaque cells to promote the production of different cytokines which initiates the progression of plaque and its rupture which results in severe complications^[26]. The up regulated expression of hTLR4 in patients results in the enhanced expression of MYD88 protein level^[27]. Extensive genetic study on TLR4 gene showed that any polymorphism in TLR4 gene found to have slow down the progress of atherosclerosis. It is due to the mutation on TLR4 (Asp 299 Gly and Thr 399ile) residues. The analysis on TLR4 polymorphism in different patient showed that the patient with acute coronary syndrome showed less polymorphism were as healthy old people showed least polymorphism^[28].

ACTIVATION OF IMMUNE CELLS BY TLR4 SIGNALING

Macrophages and the endothelial cells are the main two types of cells which primary respond to the microbial infection. TLR4 expression in macrophages triggers the local differentiation of these cells to antigen presenting one^[29,30]. Finally it act as the bridge between innate and adaptive immune response to local antigen such as heat shock proteins and OxLDL^[31].

TLR4 AND ITS ROLE IN CHOLESTEROL METABOLISM

TLR4 has active role in cholesterol metabolism in macrophages^[8], which elucidates the process by which TLR4 affect the disease pathology. It has been found that deficiency in TLR4 gene was associated with reduction in the atherosclerotic lesion in cholesterol fed mice for six months^[9]. The gene polymorphism in TLR4 results in the 25% reduction in plaque of double mutant mice. The levels of plasma cholesterol didn't affect significantly on TLR4 deficiency. Over all the genetic polymorphism in TLR4 results in the reduction in levels of cholesterol, conforming the active role of TLR4 in atherosclerosis.

TLR4 SIGNALING IN ATHEROSCLEROSIS

The innate immune system can be activated by variety of pathogen by TLR4 signaling pathways^[16,18]. Lipopolysaccharide can specifically activates TLR4 ligand^[31], which is the major component of gram negative bacteria. Cholesterol induced toxicity causes tissue injury and which releases cellular fibronectin and HSP60 which triggers the activation of TLR4 receptor and results in the atherosclerotic progression^[32,33].

The activation of TLR4 leads to the accumulation of different cells in the atherosclerotic walls like endothelial cells^[20,30], macrophages^[7,20,30], adventitial fibroblast^[20,34] and dendritic cells^[20,35,36]. TLRs have two important domains like extracellular leucine rich (LRR) domain and intracellular domain (TIR). When the TLR4 receptor stimulates, the TIR domain bind to TIR domain adaptor protein MYD88, then to adaptor protein (AD) to form TIRAP complex which is known as MYD88-MAIL and TIR domain consist of adaptor inducing IFN- β (TRIF), the TRIF-related adaptor molecule (TRAM) resulting in two distinct signaling mechanism. MyD88-dependent and the MyD88-independent/TRIF-dependent pathways^[37].

MAPKS ACTIVATION BY TLR4 SIGNALING

TLR4 is widely expressed in atherosclerotic plaques and results in the activation of macrophages and endothelial cells. There comes a link between TLR4/MAPKs/NF- κ B pathway in inducing inflammatory stress and ultimately

resulting in atherosclerotic plaque rupture^[30]. Upon activation TLR4 receptor leads to the activation of IRAK associated protein TRAF6 which induces activation of TAK1 and MKK6 *via* JNK/p38 to activates NF- κ B and resulting in the activation of downstream signaling to promote the progression of the disease^[38,39].

TLR4/MAPKS SIGNALING PATHWAY AS A THERAPEUTIC TARGET

TLR4 found to have an eminent role in the innate immune system. When it comes in with microbial product TLR4 activates intracellular signaling pathway. The execution of the mechanism is through NF- κ B signaling pathway. It is known that TLR4 induced NF- κ B activation is an critical component in ancient host defence system, which is phylogenetically conserved in most of insects and mammals^[40].

The alterations in the mechanisms regulating the activation of MAPKs and NF- κ B are responsible for the most of inflammatory events^[12]. In normal cells the NF- κ B resides in the cytoplasm and usually associated with I κ B, a family of inhibitory proteins, which usually binds to NF- κ B and inhibits the nuclear translocation^[41]. NF- κ B usually regulates the cell survival and inflammatory stress on the active κ B binding sites called the promoter gene^[12]. Active NF- κ B complexes are dimers of combinations of Rel family polypeptides (p50, p52 and p65) that respond to a wide variety of stimuli. The NF- κ B subunit determines the biological effect by nuclear translocation and further binding to κ B-regulatory elements^[42,43].

Research study on MAPKs pathway suggests the active participation of MAPKs in the translocation of NF- κ B subunits. Upon inflammatory stress the cells elicits inflammatory responses *via* MAPKs signaling pathway. It regulates various cellular activities like gene expression, mitosis, programmed cell death, *etc.* The phosphorylation of MAPKs act as switch for tuning the activation of target protein on/off^[44,45].

Natural products have long been recognized as an important source of therapeutically effective medicines. It is recognized that natural-product structures have great chemical diversity, biochemical specificity and other molecular properties that make them favourable lead structures^[46]. There are several plant compounds which can be used to target this pathway. We have recently published our research paper on Robinin a bioflavonoid from *Vigna unguiculata* leaf^[47,48] which selectively modulates TLR/NF- κ B signaling pathway in oxidized LDL induced human peripheral blood mononuclear cells^[49]. Targeting of TLR4/MAPKs signaling pathway (Figure 1) is very essential for the therapeutic inhibition of atherosclerosis. The activation of TLR4 in turn activates cascades of proteins and IKK dependent phosphorylation of I κ B. There is also an activation of MAPKs which contribute to the dissociation of I κ B and the nuclear translocation of NF- κ B p65 subunit (Figure 1) resulting in the activation of key

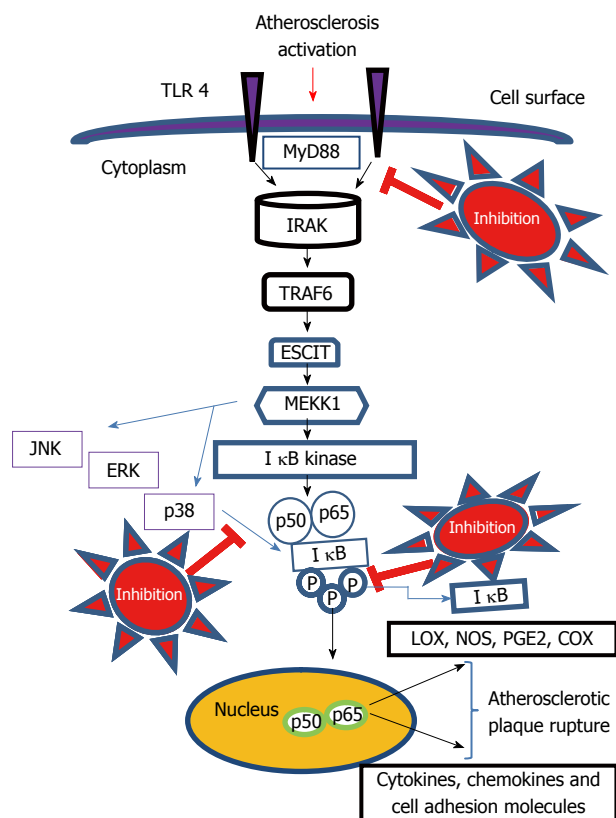


Figure 1 Proposed mechanism of toll-like receptor 4/mitogen activated protein kinases signaling pathway and its suitable targets for therapeutic inhibition of atherosclerosis. The activation of toll-like receptor 4 (TLR4) receptor by various external stimulus leads to the transmittance of signal from cell surface to interior. The TLR4 in turn activates cascades of proteins and finally activates the IKK dependent phosphorylation of I κ B and also there is an activation of mitogen activated protein kinase (MAPKs) which also contribute to the dissociation of I κ B and the nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) p65 subunit, thereby activating key inflammatory cascade activation by MAPKs/NF- κ B signaling pathway to induce toxicity by activating different inflammatory parameters. Hence we can target the TLR4/MAPKs signaling pathway at different places in the signaling pathway which indicated in the diagram. IKK: I κ B kinase; JNK- c-Jun N-terminal kinases; ERK: Extracellular signal regulated kinases; MEKK1: Mitogen-activated protein kinase kinase kinase 1.

inflammatory cascade through MAPKs/NF- κ B signaling pathway. Hence we can target the TLR4/MAPKs signaling pathway at different places in the signaling pathway as indicated in the proposed mechanism in Figure 1. Hence, Identification of naturally occurring phytocompounds that can suppress or downregulate TLR4/MAPKs signaling pathway would be an efficient strategy for inhibition of atherosclerosis

CONCLUSION

The inhibition of atherosclerosis is one of primary target for the therapeutics of atherosclerosis, the leading cause of death worldwide. The important role of TLR4 in the activation and progression of atherosclerosis is justified here. The TLR4 in turn activates the MAPKs and NF- κ B which are responsible for most of inflammatory events. Hence, therapeutic inhibition of TLR4/MAPKs signal-

ing pathway is one of the best method for inhibiting atherosclerosis.

REFERENCES

- Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol* 2011; **12**: 204-212 [PMID: 21321594 DOI: 10.1038/ni.2001]
- Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999; **340**: 115-126 [PMID: 9887164 DOI: 10.1056/NEJM199901143400207]
- Laine P, Kaartinen M, Penttilä A, Panula P, Paavonen T, Kovanen PT. Association between myocardial infarction and the mast cells in the adventitia of the infarct-related coronary artery. *Circulation* 1999; **99**: 361-369 [PMID: 9918522 DOI: 10.1161/01.CIR.99.3.361]
- Ramshaw AL, Parums DV. Immunohistochemical characterization of inflammatory cells associated with advanced atherosclerosis. *Histopathology* 1990; **17**: 543-552 [PMID: 2076887 DOI: 10.1111/j.1365-2559.1990.tb00794.x]
- Xu Q, Schett G, Li C, Hu Y, Wick G. Mechanical stress-induced heat shock protein 70 expression in vascular smooth muscle cells is regulated by Rac and Ras small G proteins but not mitogen-activated protein kinases. *Circ Res* 2000; **86**: 1122-1128 [PMID: 10850962 DOI: 10.1161/01.CIR.102.1.14]
- Miller YI, Choi SH, Fang L, Harkewicz R. Toll-like receptor-4 and lipoprotein accumulation in macrophages. *Trends Cardiovasc Med* 2009; **19**: 227-232 [PMID: 20382346 DOI: 10.1016/j.tcm.2010.02.001]
- Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, Luthringer D, Xu XP, Rajavashisth TB, Yano J, Kaul S, Arditi M. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and up-regulated by oxidized LDL. *Circulation* 2001; **104**: 3103-3108 [PMID: 11748108 DOI: 10.1161/hc5001.100631]
- Castrillo A, Joseph SB, Vaidya SA, Haberland M, Fogelman AM, Cheng G, Tontonoz P. Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. *Mol Cell* 2003; **12**: 805-816 [PMID: 14580333 DOI: 10.1016/S1097-2765(03)00384-8]
- Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, Akira S, Rajavashisth TB, Arditi M. Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. *Proc Natl Acad Sci USA* 2004; **101**: 10679-10684 [PMID: 15249654 DOI: 10.1073/pnas.0403249101]
- Hollestelle SC, De Vries MR, Van Keulen JK, Schoneveld AH, Vink A, Strijder CF, Van Middelaa BJ, Pasterkamp G, Quax PH, De Kleijn DP. Toll-like receptor 4 is involved in outward arterial remodeling. *Circulation* 2004; **109**: 393-398 [PMID: 14699006 DOI: 10.1161/01.CIR.0000109140.51366.72]
- Imaizumi S, Grijalva V, Priceman S, Wu L, Su F, Farias-Eisner R, Hama S, Navab M, Fogelman AM, Reddy ST. Mitogen-activated protein kinase phosphatase-1 deficiency decreases atherosclerosis in apolipoprotein E null mice by reducing monocyte chemoattractant protein-1 levels. *Mol Genet Metab* 2010; **101**: 66-75 [PMID: 20619710]
- Garcia-Garcia FJ, Mullol J, Perez-Gonzalez M, Pujols L, Alobid I, Roca-Ferrer J, Picado C. Signal transduction pathways (MAPKs, NF- κ B, and C/EBP) regulating COX-2 expression in nasal fibroblasts from asthma patients with aspirin intolerance. *PLoS One* 2012; **7**: e51281 [PMID: 23240010 DOI: 10.1371/journal.pone.0051281]
- Barbic J, Leef MF, Burns DL, Shahin RD. Role of gamma interferon in natural clearance of Bordetella pertussis infection. *Infect Immun* 1997; **65**: 4904-4908 [PMID: 9393774]
- Hansson GK, Libby P, Schönbeck U, Yan ZQ. Innate and adaptive immunity in the pathogenesis of atherosclerosis.

- Circ Res* 2002; **91**: 281-291 [PMID: 12193460 DOI: 10.1161/01.RES.0000029784.15893.10]
- 15 **Medzhitov R**, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997; **388**: 394-397 [PMID: 9237759]
- 16 **Hashimoto C**, Hudson KL, Anderson KV. The Toll gene of Drosophila, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell* 1988; **52**: 269-279 [PMID: 2449285 DOI: 10.1016/0092-8674(88)90516-8]
- 17 **Rock FL**, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to Drosophila Toll. *Proc Natl Acad Sci USA* 1998; **95**: 588-593 [PMID: 9435236]
- 18 **Poltorak A**, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998; **282**: 2085-2088 [PMID: 9851930 DOI: 10.1126/science.282]
- 19 **Qureshi ST**, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4) *J Exp Med* 1999; **189**: 615-625 [PMID: 9989976 DOI: 10.1084/jem.189.4.615]
- 20 **Pasterkamp G**, Van Keulen JK, De Kleijn DP. Role of Toll-like receptor 4 in the initiation and progression of atherosclerotic disease. *Eur J Clin Invest* 2004; **34**: 328-334 [PMID: 15147329 DOI: 10.1111/j.1365-2362.2004.01338.x]
- 21 **Hoshino K**, Tsutsui H, Kawai T, Takeda K, Nakanishi K, Takeda Y, Akira S. Cutting edge: generation of IL-18 receptor-deficient mice: evidence for IL-1 receptor-related protein as an essential IL-18 binding receptor. *J Immunol* 1999; **162**: 5041-5044 [PMID: 10227969]
- 22 **Andrea D**, Tamara A, Edo D, Snjezana D, Jerko B. 5 Toll-like receptors and atherosclerosis. *Med Glas* 2009; **6**: 23-31
- 23 **Yamamoto M**, Sato S, Hemmi H, Sanjo H, Uematsu S, Kaisho T, Hoshino K, Takeuchi O, Kobayashi M, Fujita T, Takeda K, Akira S. Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4. *Nature* 2002; **420**: 324-329 [PMID: 12447441 DOI: 10.1038/nature01182]
- 24 **Saikk P**. Epidemiologic association of Chlamydia pneumoniae and atherosclerosis: the initial serologic observation and more. *J Infect Dis* 2000; **181** Suppl 3: S411-S413 [PMID: 10839725 DOI: 10.1086/315625]
- 25 **Sasu S**, LaVerda D, Qureshi N, Golenbock DT, Beasley D. Chlamydia pneumoniae and chlamydial heat shock protein 60 stimulate proliferation of human vascular smooth muscle cells via toll-like receptor 4 and p44/p42 mitogen-activated protein kinase activation. *Circ Res* 2001; **89**: 244-250 [PMID: 11485974 DOI: 10.1161/hh1501.094184]
- 26 **Libby P**, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. *Circulation* 1997; **96**: 4095-4103 [PMID: 9403635 DOI: 10.1161/01.CIR.96.11.4095]
- 27 **Björkbacka H**, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, Means T, Halmen K, Luster AD, Golenbock DT, Freeman MW. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. *Nat Med* 2004; **10**: 416-421 [PMID: 15034566 DOI: 10.1038/nm1008]
- 28 **Balistreri CR**, Candore G, Colonna-Romano G, Lio D, Caruso M, Hoffmann E, Franceschi C, Caruso C. Role of Toll-like receptor 4 in acute myocardial infarction and longevity. *JAMA* 2004; **292**: 2339-2340 [PMID: 15547160 DOI: 10.1001/jama.292.19.2339]
- 29 **Hertz CJ**, Kiertcher SM, Godowski PJ, Bouis DA, Norgard MV, Roth MD, Modlin RL. Microbial lipopeptides stimulate dendritic cell maturation via Toll-like receptor 2. *J Immunol* 2001; **166**: 2444-2450 [PMID: 11160304]
- 30 **Edfeldt K**, Swedenborg J, Hansson GK, Yan ZQ. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. *Circulation* 2002; **105**: 1158-1161 [PMID: 11889007]
- 31 **Kobe B**, Deisenhofer J. Proteins with leucine-rich repeats. *Curr Opin Struct Biol* 1995; **5**: 409-416 [PMID: 7583641]
- 32 **Aravind L**, Dixit VM, Koonin EV. Apoptotic molecular machinery: vastly increased complexity in vertebrates revealed by genome comparisons. *Science* 2001; **291**: 1279-1284 [PMID: 11181990 DOI: 10.1126/science.291.5507.1279]
- 33 **Muzio M**, Ni J, Feng P, Dixit VM. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science* 1997; **278**: 1612-1615 [PMID: 9374458]
- 34 **Vink A**, Schoneveld AH, van der Meer JJ, van Middelaar BJ, Sluiter JP, Smeets MB, Quax PH, Lim SK, Borst C, Pasterkamp G, de Kleijn DP. In vivo evidence for a role of toll-like receptor 4 in the development of intimal lesions. *Circulation* 2002; **106**: 1985-1990 [PMID: 12370224 DOI: 10.1161/01.CIR.0000032146]
- 35 **Bobryshev YV**, Ikezawa T, Watanabe T. Formation of Birbeck granule-like structures in vascular dendritic cells in human atherosclerotic aorta. Lag-antibody to epidermal Langerhans cells recognizes cells in the aortic wall. *Atherosclerosis* 1997; **133**: 193-202 [PMID: 9298679]
- 36 **Hoshino K**, Kaisho T, Iwabe T, Takeuchi O, Akira S. Differential involvement of IFN-beta in Toll-like receptor-stimulated dendritic cell activation. *Int Immunol* 2002; **14**: 1225-1231 [PMID: 12356687 DOI: 10.1093/intimm/14.10.1225]
- 37 **Falck-Hansen M**, Kassiteridi C, Monaco C. Toll-like receptors in atherosclerosis. *Int J Mol Sci* 2013; **14**: 14008-14023 [PMID: 23880853 DOI: 10.3390/ijms140714008]
- 38 **Cao Z**, Henzel WJ, Gao X. IRAK: a kinase associated with the interleukin-1 receptor. *Science* 1996; **271**: 1128-1131 [PMID: 8599092 DOI: 10.1126/science.271.5252.1128]
- 39 **Wang C**, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 2001; **412**: 346-351 [PMID: 11460167 DOI: 10.1038/35085597]
- 40 **Zhang G**, Ghosh S. Toll-like receptor-mediated NF-kappaB activation: a phylogenetically conserved paradigm in innate immunity. *J Clin Invest* 2001; **107**: 13-19 [PMID: 11134172]
- 41 **Baldwin AS**. The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 1996; **14**: 649-683 [PMID: 8717528 DOI: 10.1146/annurev.immunol.14.1.649]
- 42 **Chun KS**, Surh YJ. Signal transduction pathways regulating cyclooxygenase-2 expression: potential molecular targets for chemoprevention. *Biochem Pharmacol* 2004; **68**: 1089-1100 [PMID: 15313405]
- 43 **Syeda F**, Grosjean J, Houliston RA, Keogh RJ, Carter TD, Paleolog E, Wheeler-Jones CP. Cyclooxygenase-2 induction and prostacyclin release by protease-activated receptors in endothelial cells require cooperation between mitogen-activated protein kinase and NF-kappaB pathways. *J Biol Chem* 2006; **281**: 11792-11804 [PMID: 16467309 DOI: 10.1074/jbc.M509292200]
- 44 **Dong C**, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* 2002; **20**: 55-72 [PMID: 11861597 DOI: 10.1146/annurev.immunol.20.091301.131133]
- 45 **Raman M**, Chen W, Cobb MH. Differential regulation and properties of MAPKs. *Oncogene* 2007; **26**: 3100-3112 [PMID: 17496909 DOI: 10.1038/sj.onc.1210392]
- 46 **Souto AL**, Tavares JF, da Silva MS, Diniz Mde F, de Athaydes-Filho PF, Barbosa Filho JM. Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. *Molecules* 2011; **16**: 8515-8534 [PMID: 21989312 DOI: 10.3390/molecules16108515]
- 47 **Janeesh PA**, Abraham A. Amelioration of cholesterol induced atherosclerosis by normalizing gene expression, cholesterol profile and antioxidant enzymes by Vigna unguiculata. *Plant Foods Hum Nutr* 2013; **68**: 118-123 [PMID: 23475595]

DOI: 10.1007/s11130-013-0345-1]

- 48 **Janeesh PA**, Abraham A. Vigna unguiculata modulates cholesterol induced cardiac markers, genotoxicity and gene expressions profile in an experimental rabbit model. *Food Funct* 2013; **4**: 568-574 [PMID: 23641512 DOI: 10.1039/c3fo30194j]

- 49 **Janeesh PA**, Sasikala V, Dhanya CR, Abraham A. Robinin modulates TLR/NF- κ B signaling pathway in oxidized LDL induced human peripheral blood mononuclear cells. *Int Immunopharmacol* 2014; **18**: 191-197 [PMID: 24295649 DOI: 10.1016/j.intimp.2013.11.023]

P- Reviewer: Amiya E, Lin GM, Shi GY **S- Editor:** Ji FF
L- Editor: A **E- Editor:** Wang CH



Biologic response modifiers in retinal vasculitis

Sandeep Saxena, Khushboo Srivastav

Sandeep Saxena, Khushboo Srivastav, Retina Service, Department of Ophthalmology, King George's Medical University, Lucknow 226003, India

Author contributions: Saxena S and Srivastav K solely contributed to this paper.

Correspondence to: Sandeep Saxena, MS, FRCS, Retina Service, Department of Ophthalmology, King George's Medical University, Chowk, Lucknow 226003, India. sandeepsaxena2020@yahoo.com

Telephone: +91-94-15160528 Fax: +91-94-15160528

Received: March 29, 2014 Revised: May 11, 2014

Accepted: June 18, 2014

Published online: July 27, 2014

Core tip: Corticosteroids play a pivotal role in the treatment of intraocular inflammation. Lately, therapy by immunosuppression has taken the center stage for patients with severe intraocular inflammation. However, biologic response modifiers specifically targeting suppression of the immune effector responses have revolutionized the treatment of intraocular inflammation.

Saxena S, Srivastav K. Biologic response modifiers in retinal vasculitis. *World J Immunol* 2014; 4(2): 122-129 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i2/122.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i2.122>

Abstract

Intraocular inflammation is an important cause of blindness both in the developing and developed world. Corticosteroids play a pivotal role in the treatment of intraocular inflammation. Lately, therapy by immunosuppression has taken the center stage for patients with severe intraocular inflammation. However, the side effects of immunosuppressive drugs are oncogenic, infectious, and hematological. Recently, biologic response modifiers specifically targeting suppression of the immune effector responses have revolutionized the treatment of intraocular inflammation. Anti-tumour necrosis factor agents are etanercept, infliximab, and adalimumab. Newer drugs include certolizumab and golimumab. Infliximab has been found to be superior to corticosteroids in treating retinal vasculitis. Anti-interleukin therapies include rituximab, daclizumab, anakinra, tocilizumab and secukinumab. Rituximab has been proven to be quite effective. Other biologics used are interferons and abatacept. However, there are several limitations and side effects associated with their use.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Uveitis; Immunotherapy; Biologic response modifiers; Retinal vasculitis; Non-infectious uveitis

INTRODUCTION

Intraocular inflammation accounts for 5% to 20% of blindness in the developed world and 25% in the developing world^[1]. Though the prevalence of retinal vasculitis is less, still the complexity and heterogeneity of the disease makes it unique. The etiology of most of them is unknown. Uveitogenic proteins that can incite intraocular inflammation include rhodopsin, retinal arrestin, recoverin, phosducin, retinal pigment epithelium derived (RPE-65) and inter-photoreceptor retinoid binding protein. These uveitogenic retinal antigens incite innate immunity by antigen mimicry and have been found to be associated in patients with intraocular inflammatory disease by numerous studies. Involved immunogenic pathway is similar for all types of intraocular inflammation^[2,3]. Over the last two decades, laboratory diagnostic tools have entered into an era of molecular diagnostic tests. With the advent of experimental and cellular biology, several biomarkers are being identified. Many uveitic diseases are known to be strongly associated with particular human leucocyte antigen (HLA) haplotypes. It has largely been supported by continued development of experimental models of autoimmune uveitis along with improved molecular biologic techniques. Novel sophisticated technolo-

gies such as multiplex bead assays have revolutionized the management of complex refractory uveitis. Despite the varied immune etiology, intraocular inflammation poses a significant therapeutic challenge given the heterogeneity of the retinal vasculitis spectrum along with the pressing need and increasing expectations for personalised care. This review attempts to present the current concepts of immunotherapy in retinal vasculitis.

PHYSIOLOGICAL AND PATHOLOGICAL MECHANISM

Immune privilege guards the eye by mechanical sequestration behind an efficient blood-retinal barrier, local inhibition of activation and functioning of adaptive and innate immune cells, and systemic regulation by induction of T regulatory cells^[2]. On the other hand, it leaves the eye vulnerable to an autoimmune attack by lymphocytes primed elsewhere in the body by chance encounter with a self or with mimic antigens.

Immunohistologically, retinal vasculitis is characterized by an infiltration of mainly cluster differentiation 4 (CD4⁺) T cells. Posterior uveitis in humans is considered to be a T cell-mediated autoimmune disease. Importance of T cells is highlighted by the fact that cyclosporin A can be effective in arresting the disease progression in many cases^[4]. In an experimental model, the ability to adaptively transfer disease using activated retinal antigen-specific CD4⁺ T cells is further evidence of CD4⁺ T cell-mediated processes inducing the irreversible destruction of the photoreceptor cells of the retina^[5]. The CD4 interacts directly with major histocompatibility complex (MHC) class II molecules on the surface of the antigen-presenting cell. Recognition of the MHC peptide complex by CD4⁺ T cells leads to secretion of cytokines. T Helper cells (Th) were divided into two subsets: Th1 and Th2. Th1 subset secretes Interferon- γ (IFN- γ) and Interleukin-2 (IL-2) responsible for cellular anti-viral immunity, and a Th2 subset secretes IL-4 required for blood borne parasitic responses. CD4⁺ Th1 cells and IFN- γ are considered to be the major effectors in the pathogenesis of experimental autoimmune uveitis^[6]. Another subset of regulatory CD4⁺ T cells that secrete IL-10 and transforming growth factor- β (TGF- β) was added^[7]. However, the presence of inflammatory diseases in IFN- γ -deficient mice indicated existence of other Th cell subsets and led to the discovery of the Th17 subset secreting IL-17 and IL-23^[8]. Recently, other Th cell subsets have been assigned on the basis of the secretion of IL-9 (Th9) or IL-21 (T follicular helper)^[9].

CYTOKINE PROFILE IN RETINAL VASCULITIS

Ooi *et al.*^[10] conducted a systematic review on inflammatory cytokines in uveitis of various etiologies. Few studies were conducted by us to ascertain the cytokine profile in Eales' disease. Following is a description of the

cytokines involved in some of the important causes of retinal vasculitis.

Eales' disease (retinal periphlebitis)

Eales' disease is an idiopathic obliterative vasculopathy that primarily affects the peripheral retina of young adults. Role of tumor necrosis factor-alpha (TNF- α) in Eales' disease was evaluated by us in several studies. In one such study, quantification of the TNF- α levels was carried out in young adults with Eales' disease and healthy controls of similar age. TNF- α level was found to be significantly raised in cases as compared with controls. It was also observed that higher levels of TNF- α were associated with increased severity of Eales' disease which was graded according to a new grading system based on severity of inflammation^[11]. In another study, we evaluated the levels of TNF in the serum of 52 patients with proliferative stage of Eales' disease and in 32 healthy controls to study its relation with the area of retinal capillary non-perfusion (ischemic retina). TNF levels were significantly increased in the proliferative stage of the disease as compared to controls and higher levels were associated with an increased area of retinal capillary non-perfusion on fluorescein angiography. It was concluded that increased TNF level in proliferative Eales' disease is related to retinal cell death signaling^[12]. We conducted another study in which we for the first time evaluated IL-1 β , IL-6, IL-10, and TNF- α in the serum of 45 consecutive patients with Eales' disease and in 28 healthy controls. It was found that levels of IL-1 β , IL-6, IL-10, and TNF- α were significantly increased in the inflammatory stage of Eales' disease as compared to controls. Also it was observed that IL-1 β levels decreased significantly and TNF- α levels increased significantly during the proliferative stage of the disease as compared to the inflammatory stage. It was concluded that for controlling inflammatory activity and/or the associated long-term sequelae related to angiogenesis in Eales' disease, IL-1 system and TNF- α represent novel target for immunotherapy^[13].

Behcet's disease

It is a systemic vasculitis with recurrent ocular involvement as uveitis and retinal vasculitis. HLA-B51 phenotype association has been found. Raised intraocular levels of the following immune factors have been found: IL-2, IL-6, IFN- γ and TNF- α . Recurrent episodes of Behcet's disease-related uveitis has been found to be positively correlated with serum TNF- α levels^[14].

Sarcoidosis

An acute or chronic granulomatous uveitis of unknown etiology involving the anterior, intermediate or posterior uveal layers. The aqueous immune profile of patients with sarcoidosis revealed elevated levels of IL-1 α , IL-6 and IL-8^[10,15].

Vogt-koyanagi-harada disease

A multisystem chronic granulomatous disorder associated

with HLA-DR1 and HLA-DR4 phenotype with ocular manifestation as a chronic, bilateral panuveitis. Raised intraocular levels of the following immune factors have been identified: IL-6, IL-8 and IFN- γ ^[16].

Fuchs' heterochromic iridocyclitis

A chronic typically unilateral anterior uveitis syndrome with or without associated glaucoma. One study found IFN- γ to be raised in aqueous samples of patients with FHC when compared to patients with idiopathic uveitis. Higher levels of IL-10 was found in larger number of FHC samples than of idiopathic uveitis (not statistically significant)^[17].

Idiopathic uveitis

The commonest form of uveitis and has been found to be associated with increased intraocular levels of IL-1 β , IL-2, TNF- α , IFN- γ , IL-6, IL-8 and MCP-1^[16,18].

Ankylosing spondylitis

A chronic inflammatory disorder of the axial skeleton with a strong association with HLA-B27 phenotype which manifests in the eye as severe acute anterior uveitis. Reports have revealed elevated intraocular levels of IL-2, IFN- γ , IL-6 and TNF- α ^[15].

IMMUNOTHERAPY

Corticosteroids and immunosuppressants

Corticosteroids played a pivotal role in the treatment of intraocular inflammation in the early 1950s, later on therapy by immunosuppression took the center stage for patients with severe intraocular inflammation. Now with the proteomic labeling, we can target specific cytokine pathway and deliver targeted therapy for patients with intraocular inflammation. We have now probably embarked on much specialised stratified care^[4,5,18-23]. The treatment of noninfectious posterior uveitis can lead to severe vision loss, and the first-line conventional treatment includes systemic steroids. When the prednisone doses necessary to control intraocular inflammation are above 0.3 mg/d, a therapeutic association is proposed in order to lower the daily prednisone dose. The combined drugs are immunosuppressive or immunomodulative. The side effects of immunosuppressive drugs are oncogenic, infectious, hematological and can involve reproductive troubles, associated with specific toxic effects depending on the drug used. We undertook a tertiary care center-based prospective interventional study to evaluate the response time and safety profile of low-dose oral methotrexate pulsed therapy in Eales' disease. Twenty one consecutive patients with idiopathic retinal periphlebitis were administered 12.5 mg methotrexate as a single oral dose, once per week for 12 wk. Drug safety was monitored by various laboratory tests that included twice-weekly white blood cells and differential counts, twice-weekly platelet counts, and monthly liver function tests for a mean follow-up period of 6 mo. It was found that all patients

showed improvement in visual acuity. All the side effects of methotrexate were mild to moderate in severity and rapidly reversible on dose reduction or discontinuation. We concluded that low dose oral methotrexate pulse therapy (at a dose of 12.5 mg/wk) is clinically effective within 4 wk and is associated with an acceptable safety profile^[24]. Conventional therapy with corticosteroids and immunosuppressive agents (such as methotrexate, azathioprine, mycophenolate mofetil and cyclosporine) may not be sufficient to control ocular inflammation or prevent non-ophthalmic complications in refractory patients. In a study conducted by us, efficacy of combined oral corticosteroid and low-dose oral methotrexate pulsed therapy in Eales' disease was evaluated prospectively based on weighted visual morbidity scale for disease activity and visual acuity grading in 36 consecutive cases. Oral corticosteroids in a weekly tapering dose for 4 wk and 12.5 mg methotrexate as a single oral dose, once per week for 12 wk were administered simultaneously. We concluded that this combined oral therapy is clinically effective with an acceptable safety profile^[25].

Biologic response modifiers

Biologics specifically target inflammatory cytokines and cause suppression of the immune effector responses that are responsible for damaging tissues. They were first used for ocular inflammation in 1990s. Commonly used biologics are anti-TNF agents and anti-interleukins. Now we have entered into an era of recombinant cytokines.

Anti TNF- α agents

TNF- α is a pleiotropic inflammatory cytokine. It plays a pivotal role in down-regulating both inflammatory and the immune response. Thus, blockade with anti-TNF agents has turned into the most important tool in the management of retinal vasculitis. The three most commonly used TNF inhibitors in the US are infliximab, etanercept and adalimumab. Newer drugs include certolizumab and golimumab.

Infliximab: Infliximab is a chimeric immunoglobulin G1 (IgG1) monoclonal antibody with the antigen-binding region derived from a mouse antibody and the constant region from a human antibody^[26]. It binds to TNF- α with high affinity thereby blocking the binding of TNF- α to its receptor. One of the considerations in giving infliximab is that it can potentially induce antinuclear antibody and anti-double stranded DNA on long term therapy^[27,28]. Early monitoring and optimizing dose regimens can be useful in patients on long term infliximab therapy. Side effects are autoimmune diseases which improve on stopping the drug, blood dyscrasias, allergies secondary to infusion, fever, fatigue, upper respiratory chest infection, headache, gastrointestinal upset, headache.

Adalimumab: Adalimumab is a fully humanized recombinant IgG1 monoclonal antibody with high binding to

human TNF- α . Side effects are gastrointestinal disturbances including haemorrhage, hyperlipidaemia, hypertension, chest pain, tachycardia, cough, dyspnea, mood changes, paraesthesia, haematuria, renal impairment, electrolyte disturbances, hyperuricaemia, musculoskeletal pain, eye disorders (visual impairment, conjunctivitis, blepharitis, eye swelling), rash, dermatitis.

Etanercept: Etanercept is a soluble fusion protein and prevents both TNF- α and TNF- β from interacting with receptors. It consists of 2 dimers of higher affinity type 2 TNF receptors. Side effects include headache, infection like upper respiratory tract infections, urinary tract infections, butterfly rash on cheeks, dizziness, fatigue, swelling of the arms/legs, unusual bruising/bleeding, severe headache, mental/mood changes, seizures, unexplained muscle weakness, numbness/tingling of the hands/feet, unsteadiness, vision changes, severe stomach/abdominal pain.

Golimumab: Golimumab is a novel fully humanized anti-TNF α monoclonal antibody. Side effects include body aches or pain, chills, cough, difficulty with breathing, ear congestion, fever, headache, loss of voice, muscle aches, sneezing, sore throat, stuffy or runny nose, unusual tiredness or weakness. Blurred vision, burning, crawling, itching, numbness, prickling, “pins and needles”, or tingling feelings, congestion cough with mucus diarrhea, dizziness, general feeling of discomfort or illness, hoarseness, joint pain, loss of appetite, muscle aches and pains, nausea, nervousness, pain or tenderness around the eyes and cheek bones, painful cold sores or blisters on the lips, pounding in the ears, shivering, shortness of breath or troubled breathing, slow or fast heartbeat, sweating, tender/swollen glands in the neck.

Anti-TNF- α agents have improved the treatment armamentarium for refractory immune-mediated uveitis particularly in Behçet disease-associated uveitis. A prospective observational study of patients with panuveitis was undertaken in which 19 eyes received an infliximab infusion, 8 eyes received high-dose methylprednisolone intravenously and 8 eyes received intravitreal triamcinolone acetonide at attack's onset. Unchanged baseline maintenance therapy was continued for 30 d. Visual acuity, anterior chamber cells, vitreous cells and inflammation of the posterior eye segment were assessed at baseline and at days 1, 7, 14 and 29 post-treatment. Infliximab was superior to corticosteroids in treating retinal vasculitis as well as in resolution of retinitis and cystoid macular oedema^[29]. A study was conducted in which anti-TNF α therapy was administered in 15 patients of chronic non-infectious uveitis when no response had been obtained with classical immunosuppressive therapies or in the presence of severe rheumatoid disease. Mean duration of ocular disease was 8 years. Treatment was initiated with infliximab, etanercept, and adalimumab. It was concluded that anti-TNF- α therapy is effective and safe^[30]. Importance of TNF- α in the pathophysiology of

multi-systemic sarcoidosis and refractory retinal vasculitis was emphasized in a case report in which 2 patients experienced an excellent response to infliximab^[31]. A retrospective noncomparative case series was conducted on 6 pediatric patients with uveitis refractory to methotrexate, cyclosporine, mycophenolate mofetil, etanercept, daclizumab and topical steroids. These patients initially received infliximab at doses between 5 and 10 mg/kg at 2 to 4 wk interval and then were maintained at 4 to 8 wk interval at doses of 5 to 18 mg/kg. Reduction in intraocular inflammation after infliximab therapy initiation was seen in all the patients. The only adverse reactions seen were vitreous hemorrhage in 1 patient and a case of transient upper respiratory infusion reaction. It was concluded that for the treatment of refractory pediatric uveitis, infliximab seems to be an effective agent without apparent serious toxicity^[32]. To evaluate the clinical response after switching from infliximab to adalimumab, a prospective, longitudinal and observational study was conducted in 69 patients with Behçet's disease. Seventeen patients were switched to adalimumab for lack or loss of efficacy or infusion reactions to infliximab. Of the 17 treated patients, 9 showed sustained remission of the disease and 3 showed good response. No side effects were observed in any patient. They concluded that adalimumab can be used to treat patients with Behçet's disease showing a scarce response or adverse events to infliximab^[33]. A study was conducted to alert physician for timely recognition and to evaluate current treatment of recurrent hypopyon iridocyclitis or panuveitis in Behçet's disease. It was found that for the control of acute panuveitis, a single infliximab infusion should be considered, whereas in reducing the number of episodes in refractory uveoretinitis with faster regression and for complete remission of cystoid macular edema, repeated long-term infliximab infusions proved to be more effective^[34]. Rifkin *et al*^[35] studied current status of three of the five commercially available TNF inhibitors-etanercept, infliximab, and adalimumab for their efficacy in treatment of ocular inflammation. They found etanercept to be inadequate in controlling ocular inflammation. Infliximab and adalimumab, however, showed encouraging results in multiple trials^[35]. There are only two reports in the literature about the use of golimumab in uveitis, describing four patients with juvenile idiopathic arthritis-associated uveitis and a case of idiopathic retinal vasculitis. Mesquida *et al*^[36] first reported about the use of golimumab in Behçet's disease. William *et al*^[37] reported good outcomes using golimumab in three patients with juvenile idiopathic arthritis.

Anti-interleukin therapies

Rituximab: Rituximab (first used in the treatment of Non Hodgkin's B cell lymphoma) is a recombinant chimeric monoclonal antibody with binding efficacy to CD20. It works by blocking CD20-bearing B cells. Side effects are severe stomach pain with constipation, bloody or tarry stools, coughing up blood or vomit that looks like coffee grounds, painful blistering skin rash with

burning, itching, or tingly feeling, or upper stomach pain, vomiting, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes), runny or stuffy nose, sinus pain, sore throat, headache, dizziness, itching, or mild stomach cramps.

Daclizumab: Daclizumab is a recombinant monoclonal antibody of the human IgG1 isotype composed of 90% human and 10% mouse antibody sequences that bind to CD25 with high affinity and inhibit IL-2-mediated responses of activated T cells. It was withdrawn in 2009 based on a report by Wroblewski *et al*^[38] according to which four of 39 patients developed solid malignant tumor while on daclizumab over a follow up period of 11 years. Side effects include poor wound healing, unusual growths/lumps, swollen glands (*e.g.*, on the neck, in the armpits), unexplained weight loss, night sweats, easy bruising/bleeding, abdominal pain/swelling, unusual tiredness. A very serious allergic reaction to this drug is rare.

Anakinra: Anakinra is a recombinant non-glycosylated homologue of HuIL1Ra, a natural immunomodulating molecule, which competitively inhibits binding of IL1 α and IL1 β to the IL1 receptor type 1. Side effects are infections, nausea or diarrhea, headache, sinus infection, or redness, bruising, pain, or swelling at the injection site.

Tocilizumab: Tocilizumab is a recombinant humanized monoclonal antibody and inhibits IL-6 mediated responses by binding to both membrane-bound and soluble IL-6 receptors with high affinity.

Secukinumab: Secukinumab is a fully humanized IgG1k monoclonal antibody neutralizing IL-17A.

Sadreddini *et al*^[39] reported treating a patient with visual loss due to retinal vasculitis resistant to prednisolone and azathioprine with rituximab successfully with a sustained remission of 24 mo of follow-up. Severe retinal vasculitis is a potentially blinding complication of patients with systemic lupus erythematosus (SLE). Hickman *et al*^[40] first reported that rituximab can be used to treat severe bilateral SLE-associated retinal vasculitis. This case suggested that rituximab-induced B-cell depletion may provide an important new therapeutic option in such refractory cases. A study was conducted to evaluate the efficacy of rituximab in patients with retinal vasculitis and edema, resistant to cytotoxic drugs. Twenty patients were randomized to a rituximab group or cytotoxic combination therapy group. Rituximab was given in two 1000-mg courses (15-d interval). Subjects received methotrexate (15 mg/weekly) with prednisolone (0.5 mg/kg per day). The cytotoxic combination therapy group received pulse cyclophosphamide (1000 mg/monthly), azathioprine (2-3 mg/kg per day) and prednisolone (0.5 mg/kg per day). It was concluded that rituximab was efficient in severe ocular manifestations of Behcet's disease as significant improvement after 6 mo was seen with rituximab, but

not with cytotoxic drugs^[41]. A pilot study aimed to evaluate the safety, pharmacokinetics and clinical activity of gevokizumab in Behcet's disease patients with uveitis was conducted. Patients with acute posterior or panuveitis and/or retinal vasculitis, receiving 10 mg/d or less of prednisolone and resistant to azathioprine and/or cyclosporin were enrolled into the study. Patients received a single infusion of gevokizumab (0.3 mg/kg) and immunosuppressive agents were discontinued at baseline. On evaluation of the safety and uveitis status and pharmacokinetics of gevokizumab, it was found that no treatment-related adverse event was observed and rapid and durable clinical response was seen in all patients. Complete resolution of intraocular inflammation was achieved in 4-21 d, with a median duration of response of 49 d. Moreover, despite discontinuation of immunosuppressive agents and without the need to increase corticosteroid dosages, the effect was observed^[42]. In addition, a clinical trial is underway for the use of anakinra in Behcet's disease (clinical trial reference number NCT01441076). Muselier *et al*^[43] showed tocilizumab to be effective in treatment of refractory uveitis. Secukinumab has proved to be quite effective in the treatment of patients with anterior and posterior uveitis with no serious adverse effects^[44].

Interferons

(1) IFN α ; (2) Recombinant IFN α -2a (Roferon-A); (3) Recombinant IFN α -2b (Intron A); and (4) Pegylated interferons.

Interferon- α : Interferon α -2A and Interferon α -2B are human recombinant interferons manufactured using recombinant DNA technology with *E. coli* to produce human proteins. It is a type I interferon and has been used in the treatment of uveitis due to its anti-proliferative, anti-angiogenic, apoptotic effects and the ability to activate dendritic, cytolytic T and natural killer cells. A prospective, open clinical trial was conducted to study long term effects of interferon α -2A on panuveitis in seven patients with Behcet's disease. IFN α -2A was given for a mean duration of 23.6 mo in seven patients. Initial dose of IFN α -2A was 6×10^6 IU/d, followed by 3×10^6 IU/d after 1 mo and 3×10^6 IU every other day after 3 mo. Additionally in the beginning of the therapy, two patients received low dose prednisolone (between 0.2 and 0.4 mg/kg per body weight). In three patients complete cessation of IFN α -2A was possible (observation period was 22, 6, and 4 mo). Six patients who had ocular manifestations of Behcet's disease for the first time or with minor damage during their course of chronic relapsing panuveitis showed marked improvement. New relapses were prevented in one patient with advanced ocular Behcet's disease. Resolution of retinal infiltrates occurred within 2 wk and retinal vasculitis within 4 wk. It was found that complete remission of retinal vasculitis occurred in all patients treated with IFN α -2A alone or in combination with low dose steroids. It was concluded that retinal or optic nerve damage due to vascular occlu-

sion can be prevented by treatment with IFN α -2A. No severe side effects were found^[45]. Evaluation of the efficacy of interferon needs to be done in other etiologies of retinal vasculitis through randomized studies^[46,47]. Another study was conducted to evaluate the long-term development of visual acuity in patients with severe ocular Behcet's disease who were treated with IFN α -2A. Fifteen eyes of 9 patients with an active panuveitis and/or retinal vasculitis due to Behcet's disease refractory to immunosuppressive treatment were included. Visual acuity before initiation of IFN was compared to visual acuity at the end of the follow-up. Increase in visual acuity of two lines or more was seen in 10 eyes during the follow-up. In 5 eyes visual acuity remained stable. No decrease of visual acuity in any eye was seen. In the presence of macular edema, quick response to IFN α -2A was seen. It was concluded that IFN α -2A seems to be much more effective to prevent a loss or decrease of visual acuity over a long period of time in patients with severe ocular Behcet's disease compared to conventional immunosuppressants^[48].

Fusion protein of cytotoxic T-lymphocyte antigen 4

Abatacept: It is a fusion protein that prevents activation of T cells by barring antigen presenting cells from delivering the co-stimulatory signals. There are case reports and case control studies reporting on the effectiveness of abatacept in the treatment of refractory uveitis in patients with juvenile idiopathic arthritis^[49].

IMPORTANT CONSIDERATION

These drugs are contraindicated in patients with tuberculosis or any active infection and in patients with pregnancy or breast feeding. Patients should be instructed to avoid pregnancy till 5 mo after stopping last dose of biologics. Before prescribing them, malignant conditions should be ruled out. Baseline blood counts, liver function tests and Glucose should be measured and subsequently at every 4 wk for three months followed by every 6 wk. If patient develops fever, sore throat or bleeding then examination by a physician needs to be done. Demyelinating diseases should be ruled out before starting these drugs as TNF- α agents can aggravate multiple sclerosis. Caution should be taken as reduced immunity can lead to increased risk of infection including flare up of latent tuberculosis. Also, worsening of heart failure can occur if already present.

LIMITATIONS

There is no proven causal relationship as yet with any of these novel biomarkers, though there is association of these biomarkers with some specific uveitis entities. Whether it is the disease leading to release of a specific biomarker or is it the inflammatory cytokine causing the disease is yet to be determined in future research. In addition, biologic response modifiers are expensive and with

life threatening risks. Hence, a specialist experienced with immunology and the pathophysiology of inflammatory diseases has to supervise. Strict monitoring with awareness of the adverse effects is needed in rendering this specific therapy in refractory uveitis patients.

CONCLUSION

With the advent of experimental and cellular biology, cytokines are increasingly being recognized as biological markers in intraocular inflammatory diseases. Several experimental models and improved molecular biologic techniques have supported it. Biologics provide customized ocular therapy. As shown by various studies and randomized controlled trials, they have been found to be effective in several systemic diseases. Many biologic agents have been found to be efficacious in refractory anterior and posterior uveitis, particularly Behcet's disease. With the advent of novel and advanced sophisticated techniques, newer cytokines are being found. The efficacy of biologic therapies and their comparison with each other are being studied in various randomized controlled trials. In future, evidence based medicine will pave way for tailored treatment by specific biologic regime.

REFERENCES

- 1 **Gritz DC, Wong IG.** Incidence and prevalence of uveitis in Northern California; the Northern California Epidemiology of Uveitis Study. *Ophthalmology* 2004; **111**: 491-500; discussion 500 [PMID: 15019324 DOI: 10.1016/j.ophtha.2003.06.014]
- 2 **Caspi R.** Autoimmunity in the immune privileged eye: pathogenic and regulatory T cells. *Immunol Res* 2008; **42**: 41-50 [PMID: 18629448 DOI: 10.1007/s12026-008-8031-3]
- 3 **Luger D, Caspi RR.** New perspectives on effector mechanisms in uveitis. *Semin Immunopathol* 2008; **30**: 135-143 [PMID: 18317764 DOI: 10.1007/s00281-008-0108-5]
- 4 **Dick AD.** Immune mechanisms of uveitis: insights into disease pathogenesis and treatment. *Int Ophthalmol Clin* 2000; **40**: 1-18 [PMID: 10791254 DOI: 10.1097/00004397-200004000-00003]
- 5 **Dick AD, Carter DA.** Cytokines and immunopathogenesis of intraocular posterior segment inflammation. *Ocul Immunol Inflamm* 2003; **11**: 17-28 [PMID: 12854024 DOI: 10.1076/ocui.11.1.17.15575]
- 6 **Abbas AK, Murphy KM, Sher A.** Functional diversity of helper T lymphocytes. *Nature* 1996; **383**: 787-793 [PMID: 8893001]
- 7 **Sakaguchi S.** Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 2004; **22**: 531-562 [PMID: 15032588 DOI: 10.1002/ijc.25429]
- 8 **Korn T, Bettelli E, Oukka M, Kuchroo VK.** IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; **27**: 485-517 [PMID: 19132915 DOI: 10.1146/annurev.immunol.021908.132710]
- 9 **Akdis M.** The cellular orchestra in skin allergy; are differences to lung and nose relevant? *Curr Opin Allergy Clin Immunol* 2010; **10**: 443-451 [PMID: 20736733 DOI: 10.1097/ACI.0b013e32833d7d48]
- 10 **Ooi KG, Galatowicz G, Calder VL, Lightman SL.** Cytokines and chemokines in uveitis: is there a correlation with clinical phenotype? *Clin Med Res* 2006; **4**: 294-309 [PMID: 17210978 DOI: 10.3121/cm.4.4.294]
- 11 **Saxena S, Pant AB, Khanna VK, Singh K, Shukla RK, Meyer**

- CH, Singh VK. Tumor necrosis factor- α -mediated severity of idiopathic retinal periphlebitis in young adults (Eales' disease): implication for anti-TNF- α therapy. *J Ocul Biol Dis Infor* 2010; **3**: 35-38 [PMID: 21139707 DOI: 10.1007/s12177-010-9053-3]
- 12 **Saxena S**, Khanna VK, Pant AB, Meyer CH, Singh VK. Elevated tumor necrosis factor in serum is associated with increased retinal ischemia in proliferative eales' disease. *Pathobiology* 2011; **78**: 261-265 [PMID: 21849807 DOI: 10.1159/000329589]
 - 13 **Saxena S**, Pant AB, Khanna VK, Agarwal AK, Singh K, Kumar D, Singh VK. Interleukin-1 and tumor necrosis factor- α : novel targets for immunotherapy in Eales disease. *Ocul Immunol Inflamm* 2009; **17**: 201-206 [PMID: 19585364 DOI: 10.1080/09273940902731015]
 - 14 **Ahn JK**, Yu HG, Chung H, Park YG. Intraocular cytokine environment in active Behçet uveitis. *Am J Ophthalmol* 2006; **142**: 429-434 [PMID: 16935587 DOI: 10.1016/j.ajo.2006.04.016]
 - 15 **Ooi KG**, Galatowicz G, Towler HM, Lightman SL, Calder VL. Multiplex cytokine detection versus ELISA for aqueous humor: IL-5, IL-10, and IFN γ profiles in uveitis. *Invest Ophthalmol Vis Sci* 2006; **47**: 272-277 [PMID: 16384973]
 - 16 **El-Asrar AM**, Struyf S, Kangave D, Al-Obeidan SS, Opdenakker G, Geboes K, Van Damme J. Cytokine profiles in aqueous humor of patients with different clinical entities of endogenous uveitis. *Clin Immunol* 2011; **139**: 177-184 [PMID: 21334264 DOI: 10.1016/j.clim.2011.01.014]
 - 17 **Curnow SJ**, Falciani F, Durrani OM, Cheung CM, Ross EJ, Wloka K, Rauz S, Wallace GR, Salmon M, Murray PI. Multiplex bead immunoassay analysis of aqueous humor reveals distinct cytokine profiles in uveitis. *Invest Ophthalmol Vis Sci* 2005; **46**: 4251-4259 [PMID: 16249505 DOI: 10.1167/iops.05-0444]
 - 18 **Atan D**, Fraser-Bell S, Plskova J, Kuffova L, Hogan A, Tufail A, Kilmartin DJ, Forrester JV, Bidwell J, Dick AD, Churchill AJ. Cytokine polymorphism in noninfectious uveitis. *Invest Ophthalmol Vis Sci* 2010; **51**: 4133-4142 [PMID: 20335604 DOI: 10.1167/iops.09-4583]
 - 19 **Takeuchi H**, Remington G. A systematic review of reported cases involving psychotic symptoms worsened by aripiprazole in schizophrenia or schizoaffective disorder. *Psychopharmacology (Berl)* 2013; **228**: 175-185 [PMID: 23736279 DOI: 10.1007/s00213-013-3154-1]
 - 20 **Dick AD**. Experimental approaches to specific immunotherapies in autoimmune disease: future treatment of endogenous posterior uveitis? *Br J Ophthalmol* 1995; **79**: 81-88 [PMID: 7880799 DOI: 10.1136/bjo.79.1.81]
 - 21 **Imrie FR**, Dick AD. Biologics in the treatment of uveitis. *Curr Opin Ophthalmol* 2007; **18**: 481-486 [PMID: 18163000 DOI: 10.1097/ICU.0b013e3282f03d42]
 - 22 **Sharma SM**, Dick AD, Ramanan AV. Non-infectious pediatric uveitis: an update on immunomodulatory management. *Paediatr Drugs* 2009; **11**: 229-241 [PMID: 19566107 DOI: 10.2165/00148581-200911040-00002]
 - 23 **Willermain F**, Rosenbaum JT, Bodaghi B, Rosenzweig HL, Childers S, Behrend T, Wildner G, Dick AD. Interplay between innate and adaptive immunity in the development of non-infectious uveitis. *Prog Retin Eye Res* 2012; **31**: 182-194 [PMID: 22120610 DOI: 10.1016/j.preteyeres.2011.11.004]
 - 24 **Bali T**, Saxena S, Kumar D, Nath R. Response time and safety profile of pulsed oral methotrexate therapy in idiopathic retinal periphlebitis. *Eur J Ophthalmol* 2005; **15**: 374-378 [PMID: 15945007]
 - 25 **Saxena S**. Combined oral corticosteroid-methotrexate therapy in Eales' disease. *Ann Ophthalmol (Skokie)* 2009; **41**: 93-97 [PMID: 19845224]
 - 26 **Takeuchi M**. A systematic review of biologics for the treatment of noninfectious uveitis. *Immunotherapy* 2013; **5**: 91-102 [PMID: 23256801 DOI: 10.2217/imt.12.134]
 - 27 **De Rycke L**, Baeten D, Kruithof E, Van den Bosch F, Veys EM, De Keyser F. Infliximab, but not etanercept, induces IgM anti-double-stranded DNA autoantibodies as main anti-nuclear reactivity: biologic and clinical implications in autoimmune arthritis. *Arthritis Rheum* 2005; **52**: 2192-2201 [PMID: 15986349 DOI: 10.1002/art.21190]
 - 28 **Iwata D**, Namba K, Mizuuchi K, Kitaichi N, Kase S, Takemoto Y, Ohno S, Ishida S. Correlation between elevation of serum antinuclear antibody titer and decreased therapeutic efficacy in the treatment of Behçet's disease with infliximab. *Graefes Arch Clin Exp Ophthalmol* 2012; **250**: 1081-1087 [PMID: 22234352 DOI: 10.1007/s00417-011-1908-1]
 - 29 **Markomichelakis N**, Delicha E, Masselos S, Fragiadaki K, Kaklamanis P, Sfrikakis PP. A single infliximab infusion vs corticosteroids for acute panuveitis attacks in Behçet's disease: a comparative 4-week study. *Rheumatology (Oxford)* 2011; **50**: 593-597 [PMID: 21097877 DOI: 10.1093/rheumatology/keq366]
 - 30 **Petropoulos IK**, Vaudaux JD, Guex-Crosier Y. Anti-TNF- α therapy in patients with chronic non-infectious uveitis: the experience of Jules Gonin Eye Hospital. *Klin Monbl Augenheilkd* 2008; **225**: 457-461 [PMID: 18454398 DOI: 10.1055/s-2008-1027361]
 - 31 **Cruz BA**, Reis DD, Araujo CA. Refractory retinal vasculitis due to sarcoidosis successfully treated with infliximab. *Rheumatol Int* 2007; **27**: 1181-1183 [PMID: 17520259 DOI: 10.1097/01.rhu.0000217187.70246.2b]
 - 32 **Rajaraman RT**, Kimura Y, Li S, Haines K, Chu DS. Retrospective case review of pediatric patients with uveitis treated with infliximab. *Ophthalmology* 2006; **113**: 308-314 [PMID: 16406545 DOI: 10.1016/j.ophttha.2005.09.037]
 - 33 **Olivieri I**, Leccese P, D'Angelo S, Padula A, Nigro A, Palazzi C, Coniglio G, Latanza L. Efficacy of adalimumab in patients with Behçet's disease unsuccessfully treated with infliximab. *Clin Exp Rheumatol* 2011; **29**: S54-S57 [PMID: 21968237]
 - 34 **Evereklioglu C**. Ocular Behçet disease: current therapeutic approaches. *Curr Opin Ophthalmol* 2011; **22**: 508-516 [PMID: 21897239 DOI: 10.1016/j.survophthal.2005.04.009]
 - 35 **Rifkin LM**, Birnbaum AD, Goldstein DA. TNF inhibition for ophthalmic indications: current status and outlook. *BioDrugs* 2013; **27**: 347-357 [PMID: 23568177 DOI: 10.1007/s40259-013-0022-9]
 - 36 **Mesquida M**, Victoria Hernández M, Llorenç V, Pelegrín L, Espinosa G, Dick AD, Adán A. Behçet disease-associated uveitis successfully treated with golimumab. *Ocul Immunol Inflamm* 2013; **21**: 160-162 [PMID: 23252659 DOI: 10.3109/09273948.2012.741744]
 - 37 **William M**, Faez S, Papaliodis GN, Lobo AM. Golimumab for the treatment of refractory juvenile idiopathic arthritis-associated uveitis. *J Ophthalmic Inflamm Infect* 2012; **2**: 231-233 [PMID: 22581347 DOI: 10.1007/s12348-012-0081-y]
 - 38 **Wroblewski K**, Sen HN, Yeh S, Faia L, Li Z, Sran P, Gangaputra S, Vitale S, Sherry P, Nussenblatt R. Long-term dalcizumab therapy for the treatment of noninfectious ocular inflammatory disease. *Can J Ophthalmol* 2011; **46**: 322-328 [PMID: 21816251 DOI: 10.1016/j.cjco.2011.06.008]
 - 39 **Sadreddini S**, Noshad H, Molaeeard M, Noshad R. Treatment of retinal vasculitis in Behçet's disease with rituximab. *Mod Rheumatol* 2008; **18**: 306-308 [PMID: 18438602 DOI: 10.3109/s10165-008-0057-9]
 - 40 **Hickman RA**, Denniston AK, Yee CS, Toescu V, Murray PI, Gordon C. Bilateral retinal vasculitis in a patient with systemic lupus erythematosus and its remission with rituximab therapy. *Lupus* 2010; **19**: 327-329 [PMID: 19900982 DOI: 10.1177/0961203309347332]
 - 41 **Davatchi F**, Shams H, Rezaipoor M, Sadeghi-Abdollahi B, Shahram F, Nadji A, Chams-Davatchi C, Akhlaghi M, Faezi T, Naderi N. Rituximab in intractable ocular lesions of Behçet's disease: randomized single-blind control study (pilot study). *Int J Rheum Dis* 2010; **13**: 246-252 [PMID: 20704622 DOI: 10.1111/j.1756-185X.2010.01546.x]

- 42 **Gül A**, Tugal-Tutkun I, Dinarello CA, Reznikov L, Esen BA, Mirza A, Scannon P, Solinger A. Interleukin-1 β -regulating antibody XOMA 052 (gevokizumab) in the treatment of acute exacerbations of resistant uveitis of Behcet's disease: an open-label pilot study. *Ann Rheum Dis* 2012; **71**: 563-566 [PMID: 22084392 DOI: 10.1136/annrheumdis-2011-155143]
- 43 **Muselier A**, Bielefeld P, Bidot S, Vinit J, Besancenot JF, Bron A. Efficacy of tocilizumab in two patients with anti-TNF-alpha refractory uveitis. *Ocul Immunol Inflamm* 2011; **19**: 382-383 [PMID: 21970668 DOI: 10.3109/09273948.2011.606593]
- 44 **Hueber W**, Patel DD, Dryja T, Wright AM, Koroleva I, Bruin G, Antoni C, Draelos Z, Gold MH, Durez P, Tak PP, Gomez-Reino JJ, Foster CS, Kim RY, Samson CM, Falk NS, Chu DS, Callanan D, Nguyen QD, Rose K, Haider A, Di Padova F. Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. *Sci Transl Med* 2010; **2**: 52ra72 [PMID: 20926833 DOI: 10.1126/scitranslmed.3001107]
- 45 **Kötter I**, Eckstein AK, Stübiger N, Zierhut M. Treatment of ocular symptoms of Behçet's disease with interferon alpha 2a: a pilot study. *Br J Ophthalmol* 1998; **82**: 488-494 [PMID: 9713053 DOI: 10.1136/bjo.87.4.423]
- 46 **Fardeau C**. [Interferon and retinal vasculitis]. *J Fr Ophthalmol* 2006; **29**: 392-397 [PMID: 16885805 DOI: 10.1016/S0181-5512(06)77697-5]
- 47 **Kötter I**, Zierhut M, Eckstein A, Vonthein R, Ness T, Günaydin I, Grimbacher B, Blaschke S, Peter HH, Kanz L, Stübiger N. Human recombinant interferon-alpha2a (rhIFN alpha2a) for the treatment of Behçet's disease with sight-threatening retinal vasculitis. *Adv Exp Med Biol* 2003; **528**: 521-523 [PMID: 12918755 DOI: 10.1007/0-306-48382-3_104]
- 48 **Deuter CM**, Kötter I, Günaydin I, Zierhut M, Stübiger N. [Ocular involvement in Behçet's disease: first 5-year-results for visual development after treatment with interferon alfa-2a]. *Ophthalmologe* 2004; **101**: 129-134 [PMID: 14991308 DOI: 10.1007/s00347-003-0927-7]
- 49 **Kenawy N**, Cleary G, Mewar D, Beare N, Chandna A, Pearce I. Abatacept: a potential therapy in refractory cases of juvenile idiopathic arthritis-associated uveitis. *Graefes Arch Clin Exp Ophthalmol* 2011; **249**: 297-300 [PMID: 20922440 DOI: 10.1007/s00417-010-1523-6]

P- Reviewer: Issa SA, Machida S **S- Editor:** Ji FF

L- Editor: A **E- Editor:** Wang CH



World Journal of *Immunology*

World J Immunol 2014 November 27; 4(3): 130-198

Volume End



**REVIEW**

- 130 Subcutaneous and sublingual immunotherapy: Where do we stand?
Yukselen A, Kendirli SG
- 141 Transgenic plants for allergen-specific immunotherapy
Saeki M, Kaminuma O, Nishimura T, Takaiwa F, Hiroi T
- 149 Immunological aspects of drug-induced liver injury
Tajiri K, Shimizu Y
- 158 Apoptotic signaling through reactive oxygen species in cancer cells
Kim D, Park GB, Hur DY
- 174 Recent advances of cluster of differentiation 74 in cancer
Liu YH, Lin JY

MINIREVIEWS

- 185 Modulation of monocyte subsets in infectious diseases
Ka MB, Olive D, Mege JL

CASE REPORT

- 194 Autoimmune hepatitis in a patient infected by HIV-1 and under highly active antiretroviral treatment: Case report and literature review
Casal Moura M, Pereira E, Braz V, Eloy C, Lopes J, Carneiro F, Araújo JP

Contents

World Journal of Immunology
Volume 4 Number 3 November 27, 2014

APPENDIX I-V Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Immunology*, Guillaume Vogt, MD, PhD, Research Associate, INSERM U980, Necker Medical School, University Paris Descartes, Paris, France

AIM AND SCOPE *World Journal of Immunology* (*World J Immunol*, *WJI*, online ISSN 2219-2824, DOI: 10.5411) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJI covers a wide range of subjects including: (1) autoimmune diseases such as type 1 diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, myasthenia gravis, in both humans and animal models of disease, with an interest on aspects including the etiology, pathogenesis, mechanisms of disease induction, maintenance and progression; (2) tumor immunology including immunosurveillance, immunoediting and immunotherapies in animal models and in humans; (3) clinical immunology in humans and animal models including mechanisms of disease, regulation and therapy and immunodeficiencies; (4) innate immunity including cell subsets, receptors and soluble mediators, complement and inflammation; (5) adaptive immune mechanisms and cells including soluble mediators and antibodies; (6) immune cell development, differentiation, maturation; (7) control mechanisms for immune cells including immune tolerance and apoptosis; (8) immune cell interactions and immune cell receptors; (9) immunological methods and techniques; (10) immune cell activation including cell signaling pathways, biochemical and pharmacologic modulation studies; (11) infection; (12) different modalities of vaccination including gene therapy; (13) hypersensitivity and allergy; (14) transplantation.

We encourage authors to submit their manuscripts to *WJI*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING *World Journal of Immunology* is now indexed in Digital Object Identifier.

FLYLEAF I-III Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Ya-Jing Lu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Immunology

ISSN
ISSN 2219-2824 (online)

LAUNCH DATE
December 27, 2011

FREQUENCY
Four-monthly

EDITORS-IN-CHIEF
Antonio La Cava, MD, PhD, Professor, Department of Medicine, University of California Los Angeles, Los Angeles, CA 90095-1670, United States

Seung-Yong Seong, MD, PhD, Professor, Department of Microbiology and Immunology, 103 Daehag-ro, Jongno-gu, Seoul 110-799, South Korea

EDITORIAL OFFICE
Jin-Lei Wang, Director

Xiu-Xia Song, Vice Director
World Journal of Immunology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
<http://www.wjgnet.com>

PUBLICATION DATE
November 27, 2014

COPYRIGHT

© 2014 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS

Full instructions are available online at http://www.wjgnet.com/2219-2824/g_info_20100722180909.htm.

ONLINE SUBMISSION
<http://www.wjgnet.com/esps/>

Subcutaneous and sublingual immunotherapy: Where do we stand?

Ayfer Yukselen, Seval Guneser Kendirli

Ayfer Yukselen, Clinic of Pediatric Allergy and Immunology, Gaziantep Children Hospital, 27560 Gaziantep, Turkey
 Seval Guneser Kendirli, Clinic of Pediatric Allergy and Immunology, Cukurova University Faculty of Medicine, 01330 Balcali, Adana, Turkey

Author contributions: The collection of documents, the review of articles published up to day about immunotherapy and writing of the manuscript were made by Yukselen A; Kendirli SG consulted and contributed to the manuscript with her valuable comments.

Correspondence to: Ayfer Yukselen, MD, Clinic of Pediatric Allergy and Immunology, Gaziantep Children Hospital, Osman-gazi mah., Sehitkamil, 27560 Gaziantep, Turkey. ayfyukselen@gmail.com

Telephone: +90-342-2200072 Fax: +90-342-2200072

Received: March 29, 2014 Revised: May 1, 2014

Accepted: July 12, 2014

Published online: November 27, 2014

be faced during this long-term treatment, recent investigations have been focused on the implementation of allergens in quite efficacious and safer ways.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Asthma; Efficacy; Rhinitis; Safety; Subcutaneous immunotherapy; Sublingual immunotherapy

Core tip: Specific allergen immunotherapy is the unique treatment method capable of changing the natural course of allergic disease. Both Subcutaneous (SCIT) and sublingual (SLIT) may act as efficient treatment options in patients with allergic rhinoconjunctivitis and asthma. In this paper, we reviewed clinical efficacy and safety of both SCIT and SLIT in allergic respiratory diseases by discussing recent studies.

Abstract

Though symptoms of allergic diseases can be reduced by the use of drugs such as corticosteroids, antihistamines or leukotrien antagonists, the only treatment directed to change the natural course of allergic disease is allergen-specific immunotherapy (SIT). Its efficacy can last years after the cessation of the treatment. SIT brings on regulatory T cells with the capacity to generate interleukin-10 and transforming growth factor- β , restricts activation of mast cells and basophils, and shifts antibody isotype from IgE to the noninflammatory type immunoglobulin G4. Subcutaneous (SCIT) and sublingual (SLIT) immunotherapy are the two most used ways at the present for applying SIT. These two treatments were demonstrated to be effective on reducing symptoms and medication use, in prevention of new sensitizations and in protecting from progression of rhinitis to asthma. The safety of SLIT appears to be better than SCIT although there have been a few head to head comparisons. In order to overcome compliance problems or possible systemic side effects which may

Yukselen A, Kendirli SG. Subcutaneous and sublingual immunotherapy: Where do we stand? *World J Immunol* 2014; 4(3): 130-140
 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i3/130.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i3.130>

INTRODUCTION

The number of allergic respiratory diseases such as allergic rhinitis (AR) and asthma has gone up in the past twenty years in both children and adults around the world^[1]. The estimation reveals that up to 20% of the United States and Western Europe populations are likely to be affected by allergic respiratory diseases^[2]. These diseases may impact the quality of life, work and educational performance, which can lead to an important individual and economic lost. Pharmacotherapy provides symptomatic relief and is effective in most cases, however, no sustainable benefit is provided when the treatment is ended. Moreover, some patients fail tolerating pharmacotherapy in both rhinitis and asthma, and some various publica-

tions have reported only limited control of symptoms^[3,4].

Allergen-specific immunotherapy (SIT) was first implemented by Noon^[5] in 1911, and represents till now the sole treatment targeted to address the cause of IgE-mediated allergic diseases^[6]. It is also the unique treatment which is able to shift the natural course of the respiratory allergic diseases by ameliorating symptoms^[7], lessening the need to medications^[7] and preventing progression from rhinitis to asthma^[8,9]. In addition, it offers permanent benefit years after the treatment is stopped. The basic principle of SIT is to induce immune tolerance to allergens by administering them to patients in repeated, increasing doses^[10].

The effectiveness of the most used routes, subcutaneous (SCIT) and sublingual (SLIT) immunotherapy, is referenced for perennial along with seasonal allergic respiratory diseases by systematic reviews and meta-analyses^[7,11-13].

There is not any specific criteria that can help to identify which one of these routes should be selected. The first used method of administration was subcutaneous. But, lots of research data encourage the use of SLIT because of the discomfort of repeated injections and higher risk of adverse reactions. Recently, allergy immunotherapy tablets have also been used for patients with respiratory allergies and sold in some countries for both adults and children. The most common indoor and outdoor allergens covered by allergy immunotherapy tablets may replace sublingual drops in the near future.

This review will be focus primarily on the clinical efficacy and safety of both SCIT and SLIT in allergic respiratory diseases, particularly in asthma and AR, in the light of recent literature.

Induction of tolerance and immunologic changes during SIT

The main mechanism of action of SIT involves alterations in the configuration of allergen-specific memory T and B cell reactions, the synthesis of particular antibody isotypes that incline the immunologic response towards non inflammatory patterns, along with reducing activation, tissue migration and degranulation of effector cells including mast cells, basophiles and eosinophiles^[14]. Early suppression of innate effector cells of allergic inflammation, regulation of Th2 type responses have been demonstrated to occur both in the tissue and in the peripheral blood during SIT^[6,14,15].

Another significant mechanism linked to the use of SCIT and SLIT is the appearance and activity of FOXP3+ CD25+ Treg cells. These cells can produce IL-10 and transforming growth factor- β (TGF- β) to inhibit activity of allergen-specific Th2 cells with the following recruitment of other inflammatory effector cells. In addition, the production of IL-10 and TGF- β from Treg cells stimulates B cells to undergo class switching and produce the noninflammatory antibodies IgG4 and IgA2^[16-18]. IL-10-secreting Breg cells can inhibit effector T cells and selectively induced IgG4 antibodies to con-

tribute to allergen tolerance^[14,16].

Unlike SCIT, SLIT is demonstrated to work slightly different. Actual models of SLIT proposes an uptake of allergen by antigen-presenting cells in the oral mucosa, pursued by migration to regional lymph nodes^[19]. Subsets of dendritic cells found in epithelium and subepithelium of oral mucosa were presented to be effective at allergen uptake *in vitro* and capable of inducing T cells secreting IFN- γ and/or IL-10 with production of IgG1 and IgG4 antibodies^[20].

During SIT, an increment have also been observed in IL-12 which is a potent Th1 cell cytokine^[21]. Consequently, these events lead to shifting from a Th2 cell pattern of response to more of a Th1 and Treg cell pattern which also reflects allergic tolerance and thus clinical improvement in allergic diseases^[22]. Both early and late-phase allergic reactions can be inhibited in peripheral tissue such as skin, nose or lungs by SIT^[23,24].

An important increment in serum-specific IgG4 and serum allergen-specific IgA, in addition increases in IL-10 and TGF- β are some alterations demonstrated after allergen specific immunotherapy^[25]. Moreover, the important role of T-regulatory cells in the induction of allergen-specific tolerance was also confirmed by the local presentation of FOXP3+CD25+ T-cells in the nasal and sublingual mucosa after immunotherapy^[22].

Clinical effectiveness of SCIT in rhinitis

Frew *et al*^[26] demonstrated that one season of immunotherapy with grass pollen decreased symptoms and medication use and ameliorated the quality of life of patients with moderately severe allergic rhinitis.

A Cochrane review of SCIT in seasonal AR due to tree, weed or grass pollens involved 51 studies based on 2871 individuals both adults and children^[11]. Symptom scores from 15 studies showed an important reduction in the SCIT group [SMD-0.73 (95%CI: -0.97 to -0.50, $P < 0.00001$)], and medication use from 13 studies demonstrated a significant decrease in the group of SIT [SMD of -0.57 (95%CI: -0.82 to -0.33, $P < 0.00001$)]. Furthermore, most of these studies included in this review, reported that nasal and bronchial symptoms along with quality of life scores, and all of the clinical parameters improved in favor of the immunotherapy groups.

In 2007, the update of Global European Allergy and Asthma Network stated that SCIT studies carried on the last 10 years confirmed these results and declared that SCIT was particularly efficacious in improving of symptoms and decreasing of medication consumption in grass, birch, *Parietaria*, mite and ragweed allergy^[27].

The significant improvement in symptoms and quality of life as well as reduction in seasonal bronchial hyper-responsiveness were also reported in various studies of grass-pollen SCIT^[28,29].

In a meta-analyses included 44 studies of house dust mite (HDM) immunotherapy for AR and asthma, it was stated that, though SCIT was found effective, the magnitude of effect varied greatly from one study to another^[30].

Three studies performed in patients with HDM-induced AR demonstrated a significant difference between active and placebo, in terms of symptom scores^[31,32] and nasal VAS after one-year treatment^[33].

It was also reported that SCIT with animal dander, especially in patients with rhinoconjunctivitis due to cat allergy is capable to reduce symptom scores and decrease skin test responses^[34].

Clinical effectiveness of SLIT in rhinitis

The first meta-analysis on SLIT for the treatment of allergic rhinitis involved 979 patients in 22 trials which all of them were double-blind, placebo-controlled and published up to 2002^[35]. Six of these SLIT studies were performed in patients sensitized with house dust mite, five with grass pollen and *Parietaria*, two with olive and one with respectively ragweed, cat, tree and cupressus. This meta-analysis revealed that SLIT was significantly effective in comparison to placebo regarding the decrease in both symptoms and medication use.

A meta-analysis which was in the framework of Cochrane review included 49 studies which 23 of them for grass, nine trees, five for *Parietaria*, two for ragweed, eight for dust mites, one for cat, and one for mixed pollens^[12]. There was 2333 patients receiving SLIT and overall, there was significant decrease in symptoms (SMD, -0.49; 95%CI: -0.64 to -0.34) and medication requirements (SMD, -0.32; 95%CI: -0.43 to -0.21). As individual allergens were evaluated, there was significant improvement in symptoms for house-dust mites, grass pollen, ragweed, *Parietaria*, and trees.

Another meta-analysis, included four studies for mites, three for grass, one respectively for *Parietaria* and olive, and one for pollen mix and totally 484 patients (most of them are children)^[36]. A considerable reduction in both symptoms and rescue medication use was detected. This meta-analysis showed that, treatment duration of > 18 mo and SLIT with pollen extracts were more beneficial than shorter treatment durations and dust-mite antigens.

In GA2LEN meta-analysis of SLIT for house-dust mite allergic rhinitis demonstrated significant symptom and medication reduction in 194 active SLIT-treated patients in comparison to 188 placebo participants^[13].

Recently, allergy immunotherapy tablets have been marketed for using in patients with allergic respiratory diseases. The studies conducting to investigate the efficacy of grass pollen tablets in allergic rhinitis revealed significant decrease in symptom and medication scores during pollen season^[37-40]. In a study involved 509 adult patients with HDM-allergic rhinitis published recently, it was reported that twelve months of treatment with sublingual tablets of HDM allergen extracts was effective and well tolerated^[41].

Clinical effectiveness of SCIT in asthma

There are lots of studies which assessed the effectiveness of SCIT in asthma in the literature. The first results of these studies was published in 1995 by Abramson^[42] and

then updated several times in the framework of Cochrane review^[7,43,44].

In a recent Cochrane review, 88 trials of SCIT were evaluated^[7]. The studies included in this review involved 3459 patients suffering from asthma and reported the results of SCIT for dust mites (42 studies), pollen (27 studies), animal dander (10 studies), molds (2 studies), latex (2 studies), and multiple allergens (6 studies). It was concluded that SCIT improved asthma symptoms, reduced drug requirement and diminished bronchial hyperresponsiveness. Additionally, it was noted that the reduction in symptoms was more pronounced by both mite and pollen immunotherapy.

There are also other SCIT studies with dust mites in adult and pediatric patients also showed amelioration in symptoms, decreasing in medication requirements and BHR^[31,45-47].

Several studies of SCIT, particularly with mites^[48,49] or mixed-allergen up to seven aeroallergens^[50] showed minimal improvement in medication scores, symptom scores and PEF. Although significant steroid-sparing effect of immunotherapy was observed in moderate persistent asthmatics included in those studies, it is important to maintain asthma control during the study in order to obtain maximum benefit from the immunotherapy.

Clinical effectiveness of SLIT in asthma

The effectiveness of SLIT in asthma has been evaluated in many studies and meta-analyses. However, in most of these studies asthma assessment was performed in combination of rhinitis and rarely was the primary outcome. Therefore, we need to carefully designed studies of SLIT carried particularly on asthmatic patients^[51].

In 2009, the World Allergy Organization Position Paper on Sublingual Immunotherapy discussed a number of important points regarding the current status of SLIT efficacy^[52]. It has been stated that although SLIT meta-analyses have shown effective to address allergic rhinitis in adults, allergic rhinitis and asthma in children, there are limitations about the conclusions of these meta-analyses because of the significant heterogeneity between the studies included in them.

A meta-analysis in asthma involving 25 studies based on 1706 participants of about whom eight trials were for mites, 14 trials for pollen, one trial for latex, and two for mixed allergens showed an important effect of SLIT for symptoms and medication requirements when all allergic symptoms and medication use for both allergic rhinoconjunctivitis and asthma were evaluated together^[53]. But, when we analyse the asthmatic symptoms and decrease in use of specific asthma medication as constant outcomes, it appears that this decreases is not remarkable (SMD, -0.38 and -0.91). The authors then suggested that even though the evidence is not very strong, SLIT ameliorated some parameters of asthma, may be in a lesser proportion than SCIT.

Another meta-analysis of SLIT in asthma involved nine studies on 441 participants whom ages vary from 3

to 18 years. Within this trials, six included dust mites- and three included pollen- allergic patients. When compared with placebo, a considerable reduction in symptom (SMD, -1.14; 95%CI: -2.10 to -0.18) and medication scores (SMD, -1.63; 95%CI: - 2.83 to -0.44) was noted with SLIT^[54].

A different meta-analysis evaluated nine trials about 452 both adults and children with asthma treated with house dust mite SLIT. This meta-analysis demonstrated notable improvement in symptom and medication scores^[13].

Another meta-analysis of seven trials conducted on 256 children showed significant decreases both in symptoms and medication use related to asthma; the authors deduced that sublingual immunotherapy is a safe and effective treatment option in respiratory allergies^[55].

Additionally, an important finding observed in some pollen studies is the delay in positive results to the second year of treatment^[56,57].

Recently, in a study involving 602 asthmatic patients who are sensitized to house dust mites, it was reported that daily treatment with SLIT tablet reduced inhaled budesonide more than 80 ug/d in comparison to placebo after 1 year^[58]. Similarly, the steroid sparing effect of SLIT was also shown in birch pollen allergic patients with asthma^[59].

Long-term effects of SCIT and SLIT

SIT provides both clinical and immunologic tolerance as specified by the persistence of clinical improvement and associated long-term immunological parameters after stopping the treatment. Additionally, long-term benefits of SIT include prevention of new sensitizations in monosensitized patients and progression from rhinitis to asthma particularly in children.

A study with grass pollen immunotherapy showed that there is no remarkable difference in symptoms and medication use in the following three years after 3-4 years of SCIT^[60].

A recent HDM study^[61] evaluated the long-term effect of either 3 or 5 years time duration of subcutaneous immunotherapy in 240 patients. The first year of this study was a double-blind placebo-controlled phase; after treatment of 3 years with HDM SCIT, one group was then followed for 2 years without any treatment, while the other group kept being under treatment for 5 years. When the patients were assessed after a period of 3 and 5 years of treatment, both groups had considerable amelioration of symptoms compared to baseline, revealing more than 70% reduction in rhinitis symptoms in the 5-years group while 50% reduction in the 3-years treatment group.

There are also some studies supporting the persistence of improvement in symptoms along with preventive effects on new sensitizations and asthma development that continued for years after ending of treatment in children with allergic rhinitis given 3-years immunotherapy^[62,63].

It has been documented that SCIT with a single allergen has a preventive effect against sensitization to differ-

ent inhalant allergens^[64-67].

Recent studies have shown such effects with SLIT. One of them is an open, randomized study involved 216 children with allergic rhinitis by Marogna *et al*^[68]. This study showed significant reduction in development of new sensitizations in children receiving SLIT (3.1%) when compared with controls.

A SLIT study included 257 patients with grass pollen allergy by Durham *et al*^[69] demonstrated persistence in reduction of rhinoconjunctivitis scores related to symptoms and medication use in the SLIT group at the 1-year period after ending of 3-year SLIT^[69]. Finally, Marogna *et al* have noted that clinical benefit persists for 8 years after SLIT treatment is given for a 4- to 5-year duration; new sensitizations were also reduced in SLIT groups^[70].

SAFETY

Safety of SCIT

Patients treated with SCIT have run a risk of both local and systemic adverse reactions but, in most cases, symptoms are reversible if they are diagnosed early and treated immediately. All allergen preparations such as standardized extracts^[27], allergoids^[71] or recombinant allergens^[72] may lead to side effects during treatment.

The incidence of systemic reactions of SCIT varies between 0.06% and 1.01% in those receiving injections^[73]. The vast majority of reactions occurred during SCIT were reported as mild and death is infrequent (*i.e.*, incidence is about one per million to one per 2 million injections)^[73].

A recent Cochrane review revealed that epinephrine was administered in 0.13% of injections in the SCIT group while this rate was 0.01% in the placebo group. No fatalities was reported in this review. Local reactions were seen frequently in the SCIT group in comparison to placebo (92% *vs* 33%)^[11].

Almost all cases of fatality due to SCIT reported previously were patients having asthma that was frequently poorly controlled^[74]. Therefore it should be kept in mind that uncontrolled asthma is a contraindication to initiation of SCIT as stated in guidelines.

Safety of SLIT

The safety of SLIT seems to be better than that of subcutaneous immunotherapy regarding the occurrence of severe systemic reactions. The serious adverse effects such as anaphylaxis described during sublingual treatment are rare^[75-79]. A recent meta-analysis for SLIT in AR showed that there are no cases of severe systemic reaction or anaphylaxis, and there was no need to use epinephrine for any of the systemic reactions^[12].

Indications to SLIT were extended in some official documents to "Patients with systemic reactions after subcutaneous immunotherapy"^[80]. However, there are also some reports on patients who had ceased this treatment since adverse reactions and severe anaphylactic reactions to SLIT^[79]. Therefore, it has been recommended that immunotherapy should be customized for each patient

based on the intensity of sensitization, accompanying allergies, environmental exposures, and other risk factors.

Local side effects such as perioral itching or mild swelling are seen particularly in the early phase of SLIT and encountered in about three-fourths of patients. Nausea, abdominal pain mainly in children, rhinitis, conjunctivitis, headache, urticaria, cough and bronchospasm are other infrequent side effects which may occur during SLIT^[81].

Head-to-head studies

There are a few studies which compare SCIT and SLIT directly^[31,81-88]. A summary of the characteristics of SCIT *vs* SLIT comparison studies is shown in Table 1.

The study of Mungan *et al*^[83], consisted of 36 adults with HDM-allergic rhinitis and asthma; they randomized to treat with SCIT, SLIT or placebo. It was found that one-year SCIT improved symptom scores of both rhinitis and asthma when SLIT was effective only for symptoms of rhinitis. However, they reported that though no notable alteration was recorded in placebo group in terms of symptom and medication scores, drug requirement was significantly reduced in both SCIT and SLIT groups.

A placebo-controlled double-blind double-dummy study (all patients received both sublingual medication and subcutaneous injections) carried on 71 adults with allergic rhinitis sensitized to birch pollen was reported by Quirino *et al*^[82] in 2004. This particular study showed that both routes of treatment were effective in the reduction of symptoms and medication use when compared with placebo arm. They concluded that SLIT decreased the median disease severity to one-half and SCIT to one-third of placebo treatment. There was not found statistically significant difference between SCIT and SLIT.

Another study compared SCIT with SLIT in patients sensitized to grass pollen was also designed in double-blind double-dummy manner^[84]. This study demonstrated that both SCIT and SLIT meet the same effectiveness according to subjective clinical outcomes. Both treatment mode reduced significantly symptoms and drug usage ($P = 0.002$ for symptoms and drugs in SLIT-treated patients; $P = 0.002$ for symptoms and $P = 0.0039$ for drugs in patients given SCIT). But, in this study, alteration in immunologic outcomes (total specific IgG, specific IgG4, skin reactivity) was observed only in SCIT group.

A study published in 2007 by Mauro *et al*^[86] included patients with allergic rhinitis sensitized to *Betulaceae* and showed that there was no significant difference between patients received SCIT and SLIT in terms of symptom scores and medication consumption. It was also noted that although the increment in Bet v 1 specific IgG4 was observed in both treatment arms, it reached statistically significant levels only in patients received SCIT.

Eifan *et al*^[85] published the results of a study which conducted in an open design and included 48 children with asthma/rhinitis sensitized to HDM. The patients were randomized to receive either SCIT, SLIT or pharmacotherapy. Both SLIT and SCIT demonstrated signifi-

cant improvement in symptom and medication scores as well as in visual analog scores for both rhinitis and asthma, in severity of skin and nasal sensitization to specific allergen in comparison to the pharmacotherapy group. In this study, both SCIT and SLIT had decreased disease severity more than half than the severity observed in pharmacotherapy group. The authors concluded that SCIT and SLIT are equally effective in the control of the disease severity.

Another study which evaluate the efficacy of three-years SCIT and SLIT in total 193 HDM allergic patients with perennial rhinitis showed that although both treatment mode effective, greater improvement was observed in SCIT group in comparison to the SLIT group^[88].

In a recent open-scheme, prospective study involving 60 children (5-12 years of age) with asthma/rhinitis sensitized to HDM, patients were randomized to receive either SCIT, SCIT plus SLIT, SLIT or pharmacotherapy^[87]. Children were evaluated for symptom/medication scores, allergen-specific nasal reactivity and Der p 1-driven cytokine responses at baseline, 1, 4 and 12 mo. The improvement in symptom and medication scores was observed earlier in the SCIT group than the SLIT group (4 mo *vs* 12 mo). This study concluded that subcutaneous route of immunotherapy appeared more effective in comparison to the sublingual route since it provided earlier clinical efficacy along with earlier induction of regulatory cytokines and production of IgG4 antibodies. Nevertheless, combining these two routes of immunotherapy looks promising particularly in children because of obtaining significant clinical efficacy with the advantage of fewer injections.

A randomized, placebo-controlled, double-dummy trial investigating the efficacy of SCIT and SLIT in children with asthma and/or rhinitis sensitized to HDM was published in 2012^[31]. This particular study indicated that one-year SCIT reduced significantly symptoms and medication consumption related to both rhinitis and asthma. SLIT decreased symptoms of rhinitis and asthma in addition to medication scores for rhinitis, but this lessening was not found significant in comparison to the placebo group. Only SCIT was recognized to have a superior effect to placebo on reduction of rhinitis and asthma symptoms after one-year of treatment. The same cohort was then followed for the one subsequent year in an open scheme and the placebo group was randomized to have SCIT or SLIT, and for 1 year all patients received active treatment with SCIT or SLIT^[89]. This latter study demonstrated that the effect of SLIT on symptoms and drug usage related to asthma was less prominent than SCIT in the first year, but it increased in the second year of SLIT. The conclusion of this study is, although both clinical and immunologic improvement with SCIT begins from the first year of immunotherapy, it requires longer treatment with SLIT in HDM-sensitized children with rhinitis and asthma.

The fact of immunotherapy has also some placebo effect has been accepted since long time. Although there

Table 1 Head-to-head study characteristics with subcutaneous and sublingual

Ref.	Year	Allergen	Study design	No. of patients	Findings
Quirino <i>et al</i> ^[84]	1996	Grass pollen	Double-blind, double-dummy	SCIT (<i>n</i> = 10) SLIT (<i>n</i> = 10) No placebo group	Significant reduction in symptoms and medications for SCIT and SLIT groups ↑ Total specific IgG, ↑ specific IgG4 and ↓ skin reactivity for SCIT only
Mungan <i>et al</i> ^[83]	1999	Dust mite	Single-blind, placebo controlled	SCIT (<i>n</i> = 10) SLIT (<i>n</i> = 15) Placebo (<i>n</i> = 11)	↓ Rhinitis symptoms with SLIT ↓ Skin reactivity with SCIT ↑ Specific IgG4 with SCIT
Khinchi <i>et al</i> ^[82]	2004	Birch pollen	Randomized, double-blind, double-dummy, placebo-controlled	SCIT (<i>n</i> = 21) SLIT (<i>n</i> = 18) Placebo (<i>n</i> = 19)	Significant reduction in symptoms and medications for SCIT <i>vs</i> placebo and SLIT <i>vs</i> placebo No difference between SCIT and SLIT groups
Mauro <i>et al</i> ^[86]	2007	Birch pollen	Randomized, double-blind, double-dummy	SCIT (<i>n</i> = 19) SLIT (<i>n</i> = 15)	No difference in mean symptom and medication score between SCIT and SLIT Specific IgG4 with SCIT
Tahamiler <i>et al</i> ^[88]	2008	Dust mite	Open label, randomized	SCIT (<i>n</i> = 96) SLIT (<i>n</i> = 97)	↓ Rhinitis and conjunctivitis symptoms scores and nasal provocation score with SCIT and SLIT (greater improvement with SCIT)
Eifan <i>et al</i> ^[85]	2010	Dust mite	Open label, randomized, controlled	SCIT (<i>n</i> = 16) SLIT (<i>n</i> = 16) Pharmacotherapy (<i>n</i> = 16)	↓ Rhinitis and asthma symptom score, total medication score ↓ Skin reactivity with SCIT and SLIT ↓ Specific IgE with SCIT and SLIT
Keles <i>et al</i> ^[87]	2011	Dust mite	Open label, randomized, controlled	SCIT (<i>n</i> = 11) SLIT (<i>n</i> = 13) SCIT plus SLIT (<i>n</i> = 14) Pharmacotherapy (<i>n</i> = 12)	Reduction in total symptom score and total medication score in all immunotherapy groups ↓ Skin reactivity with SCIT ↑ Specific IgG4 for SCIT and SCIT plus SLIT
Yukselen <i>et al</i> ^[89]	2012	Dust mite	Randomized, double-blind, double-dummy, placebo-controlled	SCIT (<i>n</i> = 10) SLIT (<i>n</i> = 11) Placebo (<i>n</i> = 10)	Significant reduction in rhinitis and asthma symptom score with SCIT Skin reactivity with SCIT and SLIT ↑ Specific IgG4 with SCIT

SCIT: Subcutaneous immunotherapy; SLIT: Sublingual immunotherapy.

is heterogeneity between many immunotherapy trials, most of them showed significant improvement in clinical outcomes and immunologic parameters in comparison to the placebo. Both SCIT and SLIT have proven to be effective in both rhinitis and asthma. However, the two trials^[31,82] (one in birch pollen -allergic adults and another in mite- allergic children) designed with double-dummy arms as recommended to obtain more valuable results showed greater efficacy of SCIT than SLIT for clinical improvement of rhinitis.

A systematic review of trials involving direct comparison of SCIT and SLIT regarding the efficacy and safety in the treatment of allergic rhinitis and asthma was published recently^[90]. It included 8 randomized controlled trials with 555 subjects published between 1989 and 2011, comparing the effectiveness of SCIT with SLIT^[81,82,84-88,90]. Three studies included only adults^[82,83,86] and 2 included both adults and children^[87,90]. The mean age of the subjects ranged between 6 and 40 years. Three studies had only SCIT and SLIT arms^[86,88,91]. In addition to SCIT and SLIT arms, 3 studies had a placebo arm^[31,81,82] and 2 studies had a pharmacotherapy arm^[85,87]. Two trials included patients with allergic rhinoconjunctivitis and rhinitis to tree pollen^[82,86]. The remaining 6 trials studied dust mite immunotherapy, 2 of which were exclusively in patients with rhinitis^[88,91] and 4 in patients with rhinitis and/or asthma^[31,82,84,86]. As the result of systematic analysing of all these head-to-head studies, it was noticed that low-

grade evidence confirms more pronounced efficacy of SCIT for asthma symptom reduction and also for decreasing of symptoms and medication use related to rhinitis in comparison to SLIT; there was also moderate-grade evidence which supports better efficacy of SCIT than SLIT for reduction of nasal and/or eye symptoms. More studies are needed to fortify this evidence so as to make clinical decision.

CONCLUSION

SIT is an immunologically based treatment which can modify the natural course of IgE-mediated allergic respiratory diseases. Despite the significant heterogeneity in study design, there is considerable evidence to defend the whole efficacy and safety of both SCIT and SLIT for treating allergic rhinoconjunctivitis and asthma.

Although the two routes proved equivalent in terms of efficacy in some head-to head comparisons, the question of “which one of these routes should be preferred in allergic diseases?” may be discussed. When this recommendation has been made, it should be considered not only the clinical effectiveness together with the quality of evidence, but also safety, costs, and patient's preference and adherence. There are also some limited rate patients shifted from SCIT to SLIT or vice versa. The most common reasons reported in allergic children who shifted from SLIT to SCIT are a perceived low efficacy of the

treatment and local side effects. On the other hand, frequent discomfort and side effects caused by injections are main causes of interrupting of SCIT. The patient's adherence to treatment mode is also an important factor in choosing the the route of immunotherapy. The improved adherence is expected in SLIT, because it does not require much treatment-related patient time. Similarly, SLIT's favorable safety profile which allows home administration is expected to improve the convenience of immunotherapy and to rise the rate of patients taking this treatment mode. However, several studies have indicated that SLIT adherence is equally as poor as SCIT. Therefore, the treatment mode of immunotherapy should be individualized for each patient according to patient's perception, adherence and preference. Additionally, in multiple allergen sensitization, it may be more convenient to prefer the SCIT to SLIT.

Because of long-term duration of treatment and possible side effects with SIT, novel safer and faster methods or administration routes have been investigated. Different approaches have been performed to improve the safety and efficacy by adding adjuvants, like Monophosphoryl lipid A (MPL), DNA sequences or bacteriophage combined with cytosine phosphodiester guanine (CpG) oligodeoxynucleotides (ODN), or by modifying the allergen itself, or using recombinant allergens. In these cases, T-cell epitopes should ideally be preserved so that the resulting hypoallergen will still be able to modify the allergen-specific immune response^[92].

In addition to allergen modification, recombinant allergens and adding adjuvants, the trials have concentrated on the ways of administration. A newly described procedure is engineering modular antigen translocating (MAT) molecules for intracellular targeting of allergens to the major histocompatibilityclass- II (MHC- II) presentation pathway to reinforce antigen presentation. MAT-allergen fusions are capable of quickly translocating into the cytoplasm of PBMCs, gather intracellularly and bring on potent proliferation of PBMC cultures showing an increased presentation through the MHC- II presentation pathway. In PBMC cultures of allergic donors, MAT vaccines lead to change in cytokine profile from Th2 to Th1, and reduce the secretion of IL-4, IL-5 and IL-2 in comparison to those induced by the corresponding recombinant allergens^[93]. As a result, MAT molecules represent promising compounds for the development of strong allergy vaccines.

There is a growing interest in intralymphatic allergen specific immunotherapy (ILIT) because it is a highly efficacious and safe treatment route that requires only 3 injections. Recently, the results of ILIT performed by guidance of ultrasonography in humans was reported^[94]. This trial was designed as a double-blind, placebo controlled manner using the recombinant major cat dander allergen Fel d 1-MAT molecule. After only three injections, it was shown significant increment in nasal reactivity to the allergen in the ILIT in comparison to the placebo group. It was also noted that there was pronounced responses in T

regulatory cells and IL-10 in the ILIT group.

One more ILIT study reported also recently was a double-blind, placebo-controlled trial, and included patients having allergic rhinitis sensitized to birch or grass pollen^[95]. This study showed that three intralymphatic inguinal injections of pollen induced significant reduction in nasal symptoms after nasal provocation.

Epicutaneous immunotherapy (EPIT) is one more new way of administering in SIT. In a recent placebo controlled, double-blind study involved 132 patients with grass pollen-induced rhinoconjunctivitis, it was demonstrated that EPIT which performed as 6 weekly patches, decreased symptoms significantly both during the pollen period and subsequent year^[96]. Epicutaneous allergen-specific immunotherapy is a promising way of administration since its ability to provide a safe, needle-free, and self-administrable treatment option. Further well-designed controlled studies will help discover the optimal regimen for SIT efficacy and safety.

REFERENCES

- 1 **Eder W**, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006; **355**: 2226-2235 [PMID: 17124020 DOI: 10.1056/NEJMra054308]
- 2 **Bauchau V**, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 2004; **24**: 758-764 [PMID: 15516669 DOI: 10.1183/09031936.04.00013904]
- 3 **Soyer OU**, Beyhun NE, Demir E, Yildirim S, Boz AB, Altinel N, Cevit O, Karakas T, Anlar Y, Sögüt A, Altıntaş D, Canitez Y, Büyükdere Z, Sekerel BE. A multicenter survey of childhood asthma in Turkey. II: Utilization of asthma drugs, control levels and their determinants. *Pediatr Allergy Immunol* 2009; **20**: 172-179 [PMID: 18823358 DOI: 10.1111/j.1399-3038.2008.00769]
- 4 **Demoly P**, Gueron B, Annunziata K, Adamek L, Walters RD. Update on asthma control in five European countries: results of a 2008 survey. *Eur Respir Rev* 2010; **19**: 150-157 [PMID: 20956184 DOI: 10.1183/09059180.00002110]
- 5 **Noon L**. Prophylactic inoculation against hay fever. *Lancet* 1911; **177**: 1572-1573
- 6 **Akdis CA**, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2011; **127**: 18-27; quiz 28-29 [PMID: 21211639 DOI: 10.1016/j.jaci.2010.11.030]
- 7 **Abramson MJ**, Puy RM, Weiner JM. Injection allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2010; **(8)**: CD001186 [PMID: 20687065 DOI: 10.1002/14651858]
- 8 **Bacharier LB**, Boner A, Carlsen KH, Eigenmann PA, Frischer T, Götz M, Helms PJ, Hunt J, Liu A, Papadopoulos N, Platts-Mills T, Pohunek P, Simons FE, Valovirta E, Wahn U, Wildhaber J. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. *Allergy* 2008; **63**: 5-34 [PMID: 18053013 DOI: 10.1111/j.1398-9995.2007.01586]
- 9 **Möller C**, Dreborg S, Ferdousi HA, Halken S, Høst A, Jacobsen L, Koivikko A, Koller DY, Niggemann B, Norberg LA, Urbanek R, Valovirta E, Wahn U. Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J Allergy Clin Immunol* 2002; **109**: 251-256 [PMID: 11842293 DOI: 10.1067/mai.2002.121317]
- 10 **Akdis CA**. Therapies for allergic inflammation: refining strategies to induce tolerance. *Nat Med* 2012; **18**: 736-749 [PMID: 22561837 DOI: 10.1038/nm.2754]
- 11 **Calderon MA**, Alves B, Jacobsen M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for season-

- nal allergic rhinitis. *Cochrane Database Syst Rev* 2007; **(1)**: CD001936 [PMID:17253469 DOI: 10.1002/14651858]
- 12 **Radulovic S**, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev* 2010; **(12)**: CD002893 [PMID: 21154351 DOI: 10.1002/14651858]
 - 13 **Compalati E**, Passalacqua G, Bonini M, Canonica GW. The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA2LEN meta-analysis. *Allergy* 2009; **64**: 1570-1579 [PMID: 19796205 DOI: 10.1111/j.1398-9995.2009.02129]
 - 14 **Akdis M**, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol* 2014; **133**: 621-631 [PMID: 24581429 DOI: 10.1016/j.jaci.2013.12.1088]
 - 15 **Larché M**, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006; **6**: 761-771 [PMID: 16998509 DOI: 10.1038/nri1934]
 - 16 **Francis JN**, Till SJ, Durham SR. Induction of IL-10+CD4+CD25+ T cells by grass pollen immunotherapy. *J Allergy Clin Immunol* 2003; **111**: 1255-1261 [PMID: 12789226 DOI: 10.1067/mai.2003.1570]
 - 17 **Jutel M**, Akdis CA. T-cell regulatory mechanisms in specific immunotherapy. *Chem Immunol Allergy* 2008; **94**: 158-177 [PMID: 18802346 DOI: 10.1159/000155000]
 - 18 **Jutel M**, Van de Veen W, Agache I, Azkur KA, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy and novel ways for vaccine development. *Allergol Int* 2013; **62**: 425-433 [PMID: 24153333 DOI: 10.2332/allergolint.13-RAI-0608]
 - 19 **Moingeon P**, Batard T, Fadel R, Frati F, Sieber J, Van Overtvelt L. Immune mechanisms of allergen-specific sublingual immunotherapy. *Allergy* 2006; **61**: 151-165 [PMID: 16409190 DOI: 10.1111/j.1398-9995.2006.01002]
 - 20 **Mascarelli L**, Lombardi V, Louise A, Saint-Lu N, Chabre H, Moussu H, Betbeder D, Balazuc AM, Van Overtvelt L, Moingeon P. Oral dendritic cells mediate antigen-specific tolerance by stimulating TH1 and regulatory CD4+ T cells. *J Allergy Clin Immunol* 2008; **122**: 603-9.e5 [PMID: 18774396 DOI: 10.1016/j.jaci.2008.06.034]
 - 21 **Hamid QA**, Schotman E, Jacobson MR, Walker SM, Durham SR. Increases in IL-12 messenger RNA+ cells accompany inhibition of allergen-induced late skin responses after successful grass pollen immunotherapy. *J Allergy Clin Immunol* 1997; **99**: 254-260 [PMID: 9042055 DOI: 10.1016/S0091-6749(97)70106-4]
 - 22 **Radulovic S**, Jacobson MR, Durham SR, Nouri-Aria KT. Grass pollen immunotherapy induces Foxp3-expressing CD4+ CD25+ cells in the nasal mucosa. *J Allergy Clin Immunol* 2008; **121**: 1467-1472, 1472.e1 [PMID: 18423565 DOI: 10.1016/j.jaci.2008.03.013]
 - 23 **Iliopoulos O**, Proud D, Adkinson NF, Creticos PS, Norman PS, Kagey-Sobotka A, Lichtenstein LM, Naclerio RM. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol* 1991; **87**: 855-866 [PMID: 2013680 DOI: 10.1016/0091-6749(91)90134-A]
 - 24 **Pienkowski MM**, Norman PS, Lichtenstein LM. Suppression of late-phase skin reactions by immunotherapy with ragweed extract. *J Allergy Clin Immunol* 1985; **76**: 729-734 [PMID: 4056258 DOI: 10.1016/0091-6749(85)90679-7]
 - 25 **Jutel M**, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszczyk M, Blaser K, Akdis CA. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; **33**: 1205-1214 [PMID: 12731045 DOI: 10.1002/eji.200322919]
 - 26 **Frew AJ**, Powell RJ, Corrigan CJ, Durham SR. Efficacy and safety of specific immunotherapy with SQ allergen extract in treatment-resistant seasonal allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2006; **117**: 319-325 [PMID: 16461133 DOI: 10.1016/j.jaci.2005.11.014]
 - 27 **Passalacqua G**, Durham SR. Allergic rhinitis and its impact on asthma update: allergen immunotherapy. *J Allergy Clin Immunol* 2007; **119**: 881-891 [PMID: 17418661 DOI: 10.1016/j.jaci.2007.01.045]
 - 28 **Walker SM**, Pajno GB, Lima MT, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. *J Allergy Clin Immunol* 2001; **107**: 87-93 [PMID: 11149996 DOI: 10.1067/mai.2001.112027]
 - 29 **Powell RJ**, Frew AJ, Corrigan CJ, Durham SR. Effect of grass pollen immunotherapy with Alutard SQ on quality of life in seasonal allergic rhinoconjunctivitis. *Allergy* 2007; **62**: 1335-1338 [PMID: 17714551 DOI: 10.1111/j.1398-9995.2007.01455]
 - 30 **Calderon MA**, Casale TB, Nelson HS, Demoly P. An evidence-based analysis of house dust mite allergen immunotherapy: a call for more rigorous clinical studies. *J Allergy Clin Immunol* 2013; **132**: 1322-1336 [PMID: 24139829 DOI: 10.1016/j.jaci.2013.09.004]
 - 31 **Yukselen A**, Kendirli SG, Yilmaz M, Altintas DU, Karakoc GB. Effect of one-year subcutaneous and sublingual immunotherapy on clinical and laboratory parameters in children with rhinitis and asthma: a randomized, placebo-controlled, double-blind, double-dummy study. *Int Arch Allergy Immunol* 2012; **157**: 288-298 [PMID: 22041501 DOI: 10.1159/000327566]
 - 32 **Riechelmann H**, Schmutzhard J, van der Werf JF, Distler A, Kleinjans HA. Efficacy and safety of a glutaraldehyde-modified house dust mite extract in allergic rhinitis. *Am J Rhinol Allergy* 2010; **24**: e104-e109 [PMID: 21244725 DOI: 10.2500/ajra.2010.24.3508]
 - 33 **Pichler CE**, Marquardsen A, Sparholt S, Löwenstein H, Bircher A, Bischof M, Pichler WJ. Specific immunotherapy with Dermatophagoides pteronyssinus and D. farinae results in decreased bronchial hyperreactivity. *Allergy* 1997; **52**: 274-283 [PMID: 9140517 DOI: 10.1111/j.1398-9995.1997.tb00991]
 - 34 **Varney VA**, Edwards J, Tabbah K, Brewster H, Mavroleon G, Frew AJ. Clinical efficacy of specific immunotherapy to cat dander: a double-blind placebo-controlled trial. *Clin Exp Allergy* 1997; **27**: 860-867 [PMID: 9291281 DOI: 10.1111/j.1365-2222.1997.tb01225]
 - 35 **Moline ML**, Pollak CP, Monk TH, Lester LS, Wagner DR, Zendell SM, Graeber RC, Salter CA, Hirsch E. Age-related differences in recovery from simulated jet lag. *Sleep* 1992; **15**: 28-40 [PMID: 1557592 DOI: 10.1111/j.1398-9995.2005.00699]
 - 36 **Penagos M**, Compalati E, Tarantini F, Baena-Cagnani R, Huerta J, Passalacqua G, Canonica GW. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: a meta-analysis of randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol* 2006; **97**: 141-148 [PMID: 16937742 DOI: 10.1016/S1081-1206(10)60004]
 - 37 **Dahl R**, Kapp A, Colombo G, de Monchy JG, Rak S, Emminger W, Riis B, Grönager PM, Durham SR. Sublingual grass allergen tablet immunotherapy provides sustained clinical benefit with progressive immunologic changes over 2 years. *J Allergy Clin Immunol* 2008; **121**: 512-518.e2 [PMID: 18155284 DOI: 10.1016/j.jaci.2007.10.039]
 - 38 **Didier A**, Mallin HJ, Worm M, Horak F, Jäger S, Montagut A, André C, de Beaumont O, Melac M. Optimal dose, efficacy, and safety of once-daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. *J Allergy Clin Immunol* 2007; **120**: 1338-1345 [PMID: 17935764 DOI: 10.1016/j.jaci.2007.07.046]
 - 39 **Bufe A**, Eberle P, Franke-Beckmann E, Funck J, Kimmig M, Klimek L, Knecht R, Stephan V, Tholstrup B, Weisshaar C, Kaiser F. Safety and efficacy in children of an SQ-standardized grass allergen tablet for sublingual immunotherapy. *J Allergy Clin Immunol* 2009; **123**: 167-173.e7 [PMID: 19130937]

- DOI: 10.1016/j.jaci.2008.10.044]
- 40 **Wahn U**, Tabar A, Kuna P, Halken S, Montagut A, de Beaumont O, Le Gall M. Efficacy and safety of 5-grass-pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2009; **123**: 160-166.e3 [PMID: 19046761 DOI: 10.1016/j.jaci.2008.10.009]
 - 41 **Bergmann KC**, Demoly P, Worm M, Fokkens WJ, Carrillo T, Tabar AI, Nguyen H, Montagut A, Zeldin RK. Efficacy and safety of sublingual tablets of house dust mite allergen extracts in adults with allergic rhinitis. *J Allergy Clin Immunol* 2013; **S0091-6749(13)01773-9** [PMID: 24388010 DOI: 10.1016/j.jaci.2013.11.012]
 - 42 **Abramson MJ**, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med* 1995; **151**: 969-974 [PMID: 7697274 DOI: 10.1164/ajrccm/151.4.969]
 - 43 **Abramson MJ**, Puy RM, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2000; **(2)**: CD001186 [PMID: 10796617 DOI: 10.1002/1465185]
 - 44 **Abramson M**, Puy R, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev* 2003; **(4)**: CD001186 [PMID: 14583928 DOI: 10.1002/14651858]
 - 45 **García-Robaina JC**, Sánchez I, de la Torre F, Fernández-Caldas E, Casanovas M. Successful management of mite-allergic asthma with modified extracts of Dermatophagoides pteronyssinus and Dermatophagoides farinae in a double-blind, placebo-controlled study. *J Allergy Clin Immunol* 2006; **118**: 1026-1032 [PMID: 17088125 DOI: 10.1016/j.jaci.2006.07.043]
 - 46 **Pifferi M**, Baldini G, Marrazzini G, Baldini M, Ragazzo V, Pietrobelli A, Boner AL. Benefits of immunotherapy with a standardized Dermatophagoides pteronyssinus extract in asthmatic children: a three-year prospective study. *Allergy* 2002; **57**: 785-790 [PMID: 12169173 DOI: 10.1034/j.1398-9995.2002.23498]
 - 47 **Basomba A**, Tabar AI, de Rojas DH, García BE, Alamar R, Olaguibel JM, del Prado JM, Martín S, Rico P. Allergen vaccination with a liposome-encapsulated extract of Dermatophagoides pteronyssinus: a randomized, double-blind, placebo-controlled trial in asthmatic patients. *J Allergy Clin Immunol* 2002; **109**: 943-948 [PMID: 12063522 DOI: 10.1067/mai.2002.124465]
 - 48 **Blumberg G**, Groes L, Haugaard L, Dahl R. Steroid-sparing effect of subcutaneous SQ-standardised specific immunotherapy in moderate and severe house dust mite allergic asthmatics. *Allergy* 2006; **61**: 843-848 [PMID: 16792582 DOI: 10.1111/j.1398-9995.2006.01088]
 - 49 **Maestrelli P**, Zanolla L, Pozzan M, Fabbri LM. Effect of specific immunotherapy added to pharmacologic treatment and allergen avoidance in asthmatic patients allergic to house dust mite. *J Allergy Clin Immunol* 2004; **113**: 643-649 [PMID: 15100667 DOI: 10.1016/j.jaci.2003.12.586]
 - 50 **Adkinson NF**, Eggleston PA, Eney D, Goldstein EO, Schuberth KC, Bacon JR, Hamilton RG, Weiss ME, Arshad H, Meinert CL, Tonascia J, Wheeler B. A controlled trial of immunotherapy for asthma in allergic children. *N Engl J Med* 1997; **336**: 324-331 [PMID: 9011784 DOI: 10.1056/NEJM199701303360502]
 - 51 **Calderón MA**, Boyle RJ, Penagos M, Sheikh A. Immunotherapy: The meta-analyses. What have we Learned? *Immunol Allergy Clin North Am* 2011; **31**: 159-173, vii [PMID: 21530812 DOI: 10.1016/j.iac.2011.02.002]
 - 52 **Canonica GW**, Bousquet J, Casale T, Lockett RF, Baena-Cagnani CE, Pawankar R, Potter PC, Bousquet PJ, Cox LS, Durham SR, Nelson HS, Passalacqua G, Ryan DP, Brozek JL, Compalati E, Dahl R, Delgado L, van Wijk RG, Gower RG, Ledford DK, Filho NR, Valovirta EJ, Yusuf OM, Zuberbier T. Sub-lingual Immunotherapy. World Allergy Organization Position Paper 2009. *World Allergy Organ J* 2009; **2**: 233-281 [PMID: 20041860 DOI: 10.1002/14651858]
 - 53 **Calamita Z**, Saconato H, Pelá AB, Atallah AN. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane Collaboration method. *Allergy* 2006; **61**: 1162-1172 [PMID: 16942563 DOI: 10.1111/j.1398-9995.2006.01205]
 - 54 **Penagos M**, Passalacqua G, Compalati E, Baena-Cagnani CE, Orozco S, Pedroza A, Canonica GW. Metaanalysis of the efficacy of sublingual immunotherapy in the treatment of allergic asthma in pediatric patients, 3 to 18 years of age. *Chest* 2008; **133**: 599-609 [PMID: 17951626 DOI: 10.1097/01.WOX.0000301999.54398.13]
 - 55 **Olaguibel JM**, Alvarez Puebla MJ. Efficacy of sublingual allergen vaccination for respiratory allergy in children. Conclusions from one meta-analysis. *J Investig Allergol Clin Immunol* 2005; **15**: 9-16 [PMID: 15864877]
 - 56 **Pradaliere A**, Basset D, Claudel A, Couturier P, Wessel F, Galvain S, André C. Sublingual-swallow immunotherapy (SLIT) with a standardized five-grass-pollen extract (drops and sublingual tablets) versus placebo in seasonal rhinitis. *Allergy* 1999; **54**: 819-828 [PMID: 10485385 DOI: 10.1034/j.1398-9995.1999.00077]
 - 57 **La Rosa M**, Ranno C, André C, Carat F, Tosca MA, Canonica GW. Double-blind placebo-controlled evaluation of sublingual-swallow immunotherapy with standardized Parietaria judaica extract in children with allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 1999; **104**: 425-432 [PMID: 10452766 DOI: 10.1016/S0091-6749(99)70388]
 - 58 **Mosbech H**. Tolerability and efficacy of house dust mite AIT. *Allergy* 2011; **66** Suppl 95: 55-56 [PMID: 21668857 DOI: 10.1111/j.1398-9995.2011.02641]
 - 59 **Marogna M**, Braidì C, Bruno ME, Colombo C, Colombo F, Massolo A, Palumbo L, Compalati E. The contribution of sublingual immunotherapy to the achievement of control in birch-related mild persistent asthma: a real-life randomised trial. *Allergol Immunopathol (Madr)* 2013; **41**: 216-224 [PMID: 23141837 DOI: 10.1016/j.aller.2012.07.004]
 - 60 **Durham SR**, Walker SM, Varga EM, Jacobson MR, O'Brien F, Noble W, Till SJ, Hamid QA, Nouri-Aria KT. Long-term clinical efficacy of grass-pollen immunotherapy. *N Engl J Med* 1999; **341**: 468-475 [PMID: 10441602 DOI: 10.1056/NEJM199908123410702]
 - 61 **Tabar AI**, Arroabarren E, Echechipia S, García BE, Martín S, Alvarez-Puebla MJ. Three years of specific immunotherapy may be sufficient in house dust mite respiratory allergy. *J Allergy Clin Immunol* 2011; **127**: 57-63, 63.e1-3 [PMID: 21211641 DOI: 10.1016/j.jaci.2010.10.025]
 - 62 **Jacobsen L**, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Høst A, Koivikko A, Norberg LA, Valovirta E, Wahn U, Möller C. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 2007; **62**: 943-948 [PMID: 17620073 DOI: 10.1111/j.1398-9995.2007.01451]
 - 63 **Eng PA**, Borer-Reinhold M, Heijnen IA, Gnehm HP. Twelve-year follow-up after discontinuation of preseasonal grass pollen immunotherapy in childhood. *Allergy* 2006; **61**: 198-201 [PMID: 16409196 DOI: 10.1111/j.1398-9995.2006.01011]
 - 64 **Des Roches A**, Paradis L, Menardo JL, Bouges S, Daurés JP, Bousquet J. Immunotherapy with a standardized Dermatophagoides pteronyssinus extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J Allergy Clin Immunol* 1997; **99**: 450-453 [PMID: 9111487 DOI: 10.1016/S0091-6749(97)70069-1]
 - 65 **Pajno GB**, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin Exp Allergy* 2001; **31**: 1392-1397 [PMID: 11591189 DOI: 10.1046/j.1365-2222.2001.01161]
 - 66 **Purello-D'Ambrosio F**, Gangemi S, Merendino RA, Isola S, Puccinelli P, Parmiani S, Ricciardi L. Prevention of new sensitizations in monosensitized subjects submitted to spe-

- cific immunotherapy or not. A retrospective study. *Clin Exp Allergy* 2001; **31**: 1295-1302 [PMID: 11529901 DOI: 10.1046/j.1365-2222.2001.01027]
- 67 **Inal A**, Altintas DU, Yilmaz M, Karakoc GB, Kendirli SG, Sertdemir Y. Prevention of new sensitizations by specific immunotherapy in children with rhinitis and/or asthma monosensitized to house dust mite. *J Invest Allergol Clin Immunol* 2007; **17**: 85-91 [PMID: 17460946]
 - 68 **Marogna M**, Tomassetti D, Bernasconi A, Colombo F, Massolo A, Businco AD, Canonica GW, Passalacqua G, Tripodi S. Preventive effects of sublingual immunotherapy in childhood: an open randomized controlled study. *Ann Allergy Asthma Immunol* 2008; **101**: 206-211 [PMID: 18727478 DOI: 10.1016/S1081-1206(10)60211-6]
 - 69 **Durham SR**, Emminger W, Kapp A, Colombo G, de Monchy JG, Rak S, Scadding GK, Andersen JS, Riis B, Dahl R. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 2010; **125**: 131-138. e1-131-8.e7 [PMID: 20109743 DOI: 10.1016/j.jaci.2009.10.035]
 - 70 **Marogna M**, Spadolini I, Massolo A, Canonica GW, Passalacqua G. Long-lasting effects of sublingual immunotherapy according to its duration: a 15-year prospective study. *J Allergy Clin Immunol* 2010; **126**: 969-975 [PMID: 20934206 DOI: 10.1016/j.jaci.2010.08.030]
 - 71 **Corrigan CJ**, Kettner J, Doemer C, Cromwell O, Narkus A. Efficacy and safety of preseasonal-specific immunotherapy with an aluminium-adsorbed six-grass pollen allergoid. *Allergy* 2005; **60**: 801-807 [PMID: 15876311 DOI: 10.1111/j.1398-9995.2005.00790]
 - 72 **Jutel M**, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 2005; **116**: 608-613 [PMID: 16159631 DOI: 10.1016/j.jaci.2005.06.004]
 - 73 **Bernstein DI**, Epstein T, Murphy-Berendts K, Liss GM. Surveillance of systemic reactions to subcutaneous immunotherapy injections: year 1 outcomes of the ACAAI and AAAAI collaborative study. *Ann Allergy Asthma Immunol* 2010; **104**: 530-535 [PMID: 20568387 DOI: 10.1016/j.anai.2010.04.008]
 - 74 **Bousquet J**, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998; **102**: 558-562 [PMID: 9802362 DOI: 10.1016/S0091-6749(98)70271-4]
 - 75 **André C**, Fadel R. Anaphylaxis caused by allergen sublingual immunotherapy? *Allergy* 2007; **62**: 1220-1221 [PMID: 17845597 DOI: 10.1111/j.1398-9995.2007.01512]
 - 76 **Dunsky EH**, Goldstein MF, Dvorin DJ, Belecanech GA. Anaphylaxis to sublingual immunotherapy. *Allergy* 2006; **61**: 1235 [PMID: 16942576 DOI: 10.1111/j.1398-9995.2006.01137]
 - 77 **Eifan AO**, Keles S, Bahceciler NN, Barlan IB. Anaphylaxis to multiple pollen allergen sublingual immunotherapy. *Allergy* 2007; **62**: 567-568 [PMID: 17313400 DOI: 10.1111/j.1398-9995.2006.01301]
 - 78 **de Groot H**, Bijl A. Anaphylactic reaction after the first dose of sublingual immunotherapy with grass pollen tablet. *Allergy* 2009; **64**: 963-964 [PMID: 19222420 DOI: 10.1111/j.1398-9995.2009.01998]
 - 79 **Burastero S**. Regarding Gidaro GB, Marcucci F, Sensi L, Incorvaia C, Frati F, Ciprandi G. The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin Exp Allergy* 2005; **35**: 1407-1408; author reply 1409 [PMID: 16238803 DOI: 10.1111/j.1365-2222.2005.02366]
 - 80 **Alvarez-Cuesta E**, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy. *Allergy* 2006; **61** Suppl 82: 1-20 [PMID: 16930249 DOI: 10.1111/j.1398-9995.2006.01219]
 - 81 **Rodríguez-Pérez N**, Ambríz-Moreno Mde J, Canonica GW, Penagos M. Frequency of acute systemic reactions in patients with allergic rhinitis and asthma treated with sublingual immunotherapy. *Ann Allergy Asthma Immunol* 2008; **101**: 304-310 [PMID: 18814454 DOI: 10.1016/S1081-1206(10)60496-6]
 - 82 **Khinchin MS**, Poulsen LK, Carat F, André C, Hansen AB, Malling HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* 2004; **59**: 45-53 [PMID: 14674933 DOI: 10.1046/j.1398-9995.2003.00387]
 - 83 **Mungan D**, Misirligil Z, Gürbüz L. Comparison of the efficacy of subcutaneous and sublingual immunotherapy in mite-sensitive patients with rhinitis and asthma—a placebo controlled study. *Ann Allergy Asthma Immunol* 1999; **82**: 485-490 [PMID: 10353581 DOI: 10.1016/S1081-1206(10)62726-3]
 - 84 **Quirino T**, Iemoli E, Siciliani E, Parmiani S, Milazzo F. Sublingual versus injective immunotherapy in grass pollen allergic patients: a double blind (double dummy) study. *Clin Exp Allergy* 1996; **26**: 1253-1261 [PMID: 8955574 DOI: 10.1046/j.1365-2222.1996.d01-280]
 - 85 **Eifan AO**, Akkoc T, Yildiz A, Keles S, Ozdemir C, Bahceciler NN, Barlan IB. Clinical efficacy and immunological mechanisms of sublingual and subcutaneous immunotherapy in asthmatic/rhinitis children sensitized to house dust mite: an open randomized controlled trial. *Clin Exp Allergy* 2010; **40**: 922-932 [PMID: 20100188 DOI: 10.1111/j.1365-2222.2009.03448]
 - 86 **Mauro M**, Russello M, Incorvaia C, Gazzola GB, Di Cara G, Frati F. Comparison of efficacy, safety and immunologic effects of subcutaneous and sublingual immunotherapy in birch pollinosis: a randomized study. *Eur Ann Allergy Clin Immunol* 2007; **39**: 119-122 [PMID: 17523385]
 - 87 **Keles S**, Karakoc-Aydiner E, Ozen A, Izgi AG, Tevetoglu A, Akkoc T, Bahceciler NN, Barlan I. A novel approach in allergen-specific immunotherapy: combination of sublingual and subcutaneous routes. *J Allergy Clin Immunol* 2011; **128**: 808-815. e7 [PMID: 21641635 DOI: 10.1016/j.jaci.2011.04.033]
 - 88 **Tahamiler R**, Saritzali G, Canakcioglu S, Ozcara E, Dirican A. Comparison of the long-term efficacy of subcutaneous and sublingual immunotherapies in perennial rhinitis. *ORL J Otorhinolaryngol Relat Spec* 2008; **70**: 144-150 [PMID: 18391573 DOI: 10.1159/000124286]
 - 89 **Yukselen A**, Kendirli SG, Yilmaz M, Altintas DU, Karakoc GB. Two year follow-up of clinical and inflammation parameters in children monosensitized to mites undergoing subcutaneous and sublingual immunotherapy. *Asian Pac J Allergy Immunol* 2013; **31**: 233-241 [PMID: 24053706 DOI: 10.12932/AP0276.31.3.2013]
 - 90 **Chelladurai Y**, Suarez-Cuervo C, Erekosima N, Kim JM, Ramanathan M, Segal JB, Lin SY. Effectiveness of subcutaneous versus sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. *J Allergy Clin Immunol Pract* 2013; **1**: 361-369 [PMID: 24565541 DOI: 10.1016/j.jaip.2013.04.005]
 - 91 **Piazza I**, Bizzaro N. Humoral response to subcutaneous, oral, and nasal immunotherapy for allergic rhinitis due to *Dermatophagoides pteronyssinus*. *Ann Allergy* 1993; **71**: 461-469 [PMID: 7755664]
 - 92 **El-Quotob D**, Mencia G, Fernandez-Caldas E. Recent advances in immunotherapy for allergic diseases. *Recent Pat Inflamm Allergy Drug Discov* 2014; **8**: 24-35 [PMID: 24237114 DOI: 10.2174/1872213x0766613111311159]
 - 93 **Cramer R**, Flückiger S, Daigle I, Kündig T, Rhyner C. Design, engineering and in vitro evaluation of MHC class-II targeting allergy vaccines. *Allergy* 2007; **62**: 197-206 [PMID: 17298430 DOI: 10.1111/j.1398-9995.2006.01292]
 - 94 **Senti G**, Cramer R, Kuster D, Johansen P, Martinez-Gomez JM, Graf N, Steiner M, Hothorn LA, Grönlund H, Tivig C, Zaleska A, Soyer O, van Hage M, Akdis CA, Akdis M, Rose H, Kündig TM. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. *J Allergy*

- Clin Immunol* 2012; **129**: 1290-1296 [PMID: 22464647 DOI: 10.1016/j.jaci.2012.02.026]
- 95 **Bruzik KS**, Salamonczyk GM, Sobon B. 13C CP-MAS study of the gel phases of 1,2-dipalmitoylphosphatidylcholine. *Biochim Biophys Acta* 1990; **1023**: 143-146 [PMID: 2337426 DOI: 10.1016/j.jaci.2012.10.056]
- 96 **Senti G**, von Moos S, Tay F, Graf N, Sonderegger T, Johansen P, Kündig TM. Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: A double-blind, placebo-controlled dose escalation study. *J Allergy Clin Immunol* 2012; **129**: 128-135 [PMID: 21996342 DOI: 10.1016/j.jaci.2011.08.036]

P- Reviewer: Sahu RP, Wang ZX **S- Editor:** Ji FF **L- Editor:** A
E- Editor: Lu YJ



Transgenic plants for allergen-specific immunotherapy

Mayumi Saeki, Osamu Kaminuma, Tomoe Nishimura, Fumio Takaiwa, Takachika Hiroi

Mayumi Saeki, Osamu Kaminuma, Tomoe Nishimura, Takachika Hiroi, Allergy and Immunology Project, Tokyo Metropolitan Institute of Medical Science, Tokyo 156-8506, Japan
 Fumio Takaiwa, Genetically Modified Organism Research Center, National Institute of Agrobiological Sciences, Ibaraki 305-8602, Japan

Author contributions: Saeki M designed and wrote the paper; Kaminuma O, Nishimura T, Takaiwa F and Hiroi T contributed to editing of the paper.

Supported by Grant-in-Aid for Agri-health Project from the Ministry of Agriculture, Forestry and Fisheries of Japan to Takaiwa F and Hiroi T, JSPS KAKENHI to Saeki M No. 24791817 and 26860122; and that to Kaminuma O, No. 24500501; and Grant-in-Aid of Mishima Kaiun Memorial Foundation to Kaminuma O.
Correspondence to: Takachika Hiroi, DDS, PhD, Allergy and Immunology Project, Tokyo Metropolitan Institute of Medical Science, 2-1-6, Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan. hiro-i-tk@igakuken.or.jp

Telephone: +81-3-53163266 Fax: +81-3-53163153

Received: March 28, 2014 Revised: July 29, 2014

Accepted: September 4, 2014

Published online: November 27, 2014

doses. In addition, the amount, distribution, and allergenicity of the expressed allergen have been improved in our Tg rice. Rice-based oral IT is a promising new concept in IT for the treatment of allergic diseases.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Allergic disease; Asthma; Oral immunotherapy; Rhinitis; Transgenic rice

Core tip: We aim to establish clinically applicable oral immunotherapy by employing transgenic rice seeds (Tg rice) in which allergen epitopes are expressed. We have identified a suitable allergen packaging system, modified allergens to reduce their allergenicity, selected high allergen-producing lines, and evaluated their efficacy in allergic disease models. We are thus nearly ready to start clinical trials of our Tg rice for the treatment of Japanese cedar pollinosis.

Saeki M, Kaminuma O, Nishimura T, Takaiwa F, Hiroi T. Transgenic plants for allergen-specific immunotherapy. *World J Immunol* 2014; 4(3): 141-148 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i3/141.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i3.141>

Abstract

Allergen-specific immunotherapy (IT) is an effective treatment for allergic diseases. Although subcutaneous and sublingual ITs are currently used, safer, easier, and more effective IT is under development. Induction of immune tolerance by oral administration of allergen has been proven, though oral IT has not been applied clinically. It is mainly because a large amount of purified allergen is required to induce oral tolerance. To overcome this problem, plants, peculiarly rice, have been investigated as allergen vehicles for oral IT. Rice can store a considerable amount of expressed allergen in its seeds and the accumulated allergen is stable and resistant to gastrointestinal digestion. Therefore, we have developed transgenic rice seeds (Tg rice) in which major epitopes of cedar pollen or house dust mites are expressed. We are establishing Tg rice with demonstrated efficacy in murine models of allergic rhinitis and bronchial asthma by oral administration at practical

INTRODUCTION

The prevalence of allergic diseases such as allergic rhinitis and bronchial asthma has markedly increased in industrialized countries, and it is a major health concern^[1-3]. Allergen-specific immunotherapy (IT) leads to immune tolerance, a state of immune unresponsiveness, by repeated allergen administration, and has been recognized as an effective therapeutic method^[4,5]. Oral IT, which is based on oral allergen administration, is a relatively conventional method to induce allergen-specific immune tolerance. However, this IT has yet to be applied clinically because of several problems^[6-8], particularly the lack of an efficient oral allergen delivery system. We recently

showed that rice seeds can be used as a carrier for oral IT. The factors favoring their use are ease of consumption for individuals of all ages, the ability to store a considerable amount of allergen, stability at room temperature for 2-3 years, resistance to digestive enzymes and low pH^[9,12]. Therefore, to establish clinically applicable oral IT, we have been developing transgenic rice seeds (Tg rice) expressing full-length allergen or epitopes of Japanese cedar pollen or house dust mites. Oral IT with Tg rice is set to become one of the most useful tools to treat allergic diseases. This review summarizes the progression and prospects of allergen-specific ITs for allergic diseases, with a focus on Tg rice-based oral IT.

SUBCUTANEOUS AND SUBLINGUAL IMMUNOTHERAPY

Currently, subcutaneous IT (SCIT) and sublingual IT (SLIT) are two major IT modes used for clinical treatment of pollen- or house dust-mediated allergic diseases^[13-16]. Since SCIT was initially described by Noon in 1911^[17], it has been the most popular mode of IT for allergic diseases. Although the immunological mechanisms by which SCIT induces immune tolerance are not completely understood, researchers have demonstrated the production of IgE-blocking antibody, alteration of the Th1/Th2 balance in favor of Th1 responses, and the induction of regulatory T cells by this therapy^[18-22]. Nevertheless, repeated systemic injection of allergens by SCIT is inconvenient and heightens the risk of severe side effects, including anaphylactic shock^[23,24]. Therefore, SLIT has been developed as an alternative, noninvasive IT^[25,26]. Since it was initially described by Scadding *et al.*^[27] in 1986, its clinical market share has become approximately 45% of the total allergen-specific IT treatments performed in Europe^[28]. Although the incidence of anaphylaxis is reduced in SLIT, several side effects associated with allergenicity have been reported^[23,29]. In addition, SLIT requires the patient to retain the allergen solution or tablet under the tongue, something that is difficult for small children. Therefore, several trials are in progress to develop safer, easier, and more efficient IT by modifying allergens, adjuvants, and routes of administration^[30-34]. For example, to avoid IgE-dependent side effects, peptide IT using dominant T-cell epitopes has been proposed in both SCIT and SLIT^[35,36], and various alternative routes of allergen administration such as oral^[37-39], epicutaneous^[40], and intralymphatic^[41,42] have been investigated.

ORAL IMMUNOTHERAPY FOR ALLERGIC DISEASES

Induction of immune tolerance by oral administration of specific allergen has been recognized for a century^[43-45]. Even though the mechanisms of oral tolerance have not been fully defined, many studies have suggested that

low-dose allergens induce cellular activation, which was adoptively transferable *in vivo*, and that high-dose allergens induce nontransferable clonal anergy and/or deletion^[46,50]. The characteristics of the immune system in the intestinal mucosa suggest some patients unresponsive to SCIT or SLIT are curable by oral IT^[51,52]. Thus, several attempts have been made to use oral IT to treat allergic diseases since the 1920s^[53]. Recently, clinical trials of oral IT for patients with autoimmune diseases have also been performed^[37-39], although this therapy is yet to be used for human diseases. The greatest obstacle to its clinical application is the amount of allergen required. Oral IT requires about 200 times larger quantities of allergens to induce immune tolerance in comparison to SCIT^[54]. Therefore, researchers hope to develop systems for effective allergen delivery to the intestinal mucosa to achieve clinical applications of oral IT. In this regard, there is concern that oral administration of a large amount of allergen may lead adverse effects such as impaired clearance of secondary bacterial infections due to excess suppression of immune function. However, almost all of adverse effects reported in clinical studies of oral IT were relatively mild, and no systemic immunological suppression have been observed^[43,44,54]. Therefore, such risks in oral IT seem to be negligible.

PLANT-BASED VACCINES

In order to meet the requirement of large amounts of allergen in oral IT, several allergen-expressing plants have been developed. During early studies in the 1990s, tobacco was used as a model transgenic plant for expressing allergen^[55]. Since then, the feasibility of plants such as potato, banana, tomato, and rice has been explored by introducing heat-labile enterotoxin, hepatitis B antigen, respiratory syncytial virus antigen, and cholera toxin^[9,56-59]. The use of plants as allergen vehicles offers several advantages over genetically engineered and/or purified allergens. The cost of production is lower, no refrigeration is required for storage, and contamination with mammalian pathogens hardly occurs in transgenic plants^[60]. In addition, the walls of plant cells in which the expressed allergen accumulate are resistant to the acidic environment of the stomach^[60]. The edible parts of plants such as fruits and crop seeds are easy to administer to people of all ages.

RICE SEED AS AN ALLERGEN CARRIER

Rice is the major food staple commonly eaten daily throughout Asia. Allergens expressed in rice seeds are stable at room temperature for 2-3 years^[9,10]. Rice seeds mainly express three endogenous proteins: alcohol-soluble prolamin, acid- and alkaline-soluble glutelin, and saline-soluble globulin. Two characteristic organs, protein body (PB)- I and PB- II, mediate storage of these proteins in rice seeds^[61-63]. Prolamin is composed of three isoforms (10, 13, and 16-kDa) synthesized in the endo-

plasmic reticulum (ER) and retained in the ER lumen; then, it forms the smooth and spherical protein body PB-I^[61,64]. Four glutelin isoforms (GluA, GluB, GluC, and GluD) are initially synthesized in the ER, and then they are transported to the protein storage vacuole, PB-II^[65,66]. The α -globulin is also deposited in PB-II. It has been reported that exogenous proteins can be made to accumulate in PB-I and/or PB-II of rice seeds by expressing them with the promoters of glutelin (GluB-1, GluB-4), 26-kDa globulin, or 10-kDa and 16-kDa prolamin^[67]. Allergen accumulated in PBs is protected from the gastrointestinal digestive enzymes and low pH environment^[10,12,13]. In particular, PB-I is characterized by higher digestion resistance and it seems to be an ideal capsule for efficient allergen delivery to the intestinal immune system^[68]. For these reasons, rice seeds have been recognized as one of the most feasible allergen carriers for oral IT.

RICE SEED-BASED ORAL IMMUNOTHERAPY FOR ALLERGIC RHINITIS

Seasonal allergic rhinitis caused by Japanese cedar pollen is a serious health concern in Japan. Over 26% of the Japanese population has this disease^[69-71]. Two major allergens, *Cryptomeria japonica* (Cry j) 1 and Cry j 2, have been identified in the pollen^[72,73]. To avoid the IgE-mediated side effects seen with SCIT and SLIT, we have developed several types of Tg rice that accumulate modified fusion proteins of Cry j 1 and Cry j 2 epitope peptides (Table 1).

Cry j Tg rice

First, we established Tg rice expressing a fusion protein of mouse T-cell epitope peptides in Cry j 1 and Cry j 2, and a soybean storage protein, glycinin A1aB1b (Cry j Tg rice)^[74]. A1aB1b is located in PB-II when expressed in Tg rice seeds with the GluB-1 promoter^[75]; the fusion protein was expected to be accumulated in PB-II^[74]. Oral administration of Cry j Tg rice to cedar pollen-immunized mice inhibited allergen-induced IgE and IgG responses, CD4⁺ T-cell proliferation, and T helper 2 (Th2) cytokine synthesis (IL-4, IL-5, and IL-13). In addition, allergen-induced serum histamine elevation and sneezing response were suppressed^[74].

7Crp Tg rice

Since Cry j Tg rice was effective in the mouse model of allergic rhinitis, we developed Tg rice for application in the human body. Seven dominant human T-cell epitopes were identified in Cry j 1 and Cry j 2^[76-78]. We have demonstrated that 92% of 48 patients with Japanese cedar pollinosis showed positive T-cell responses to the fusion protein in which the seven epitopes were linked (7Crp). This 7Crp was not reactive to cedar pollen-specific IgE in the patients' sera^[79]. These findings suggest 7Crp has the potential to modulate cedar pollen-mediated T-cell responses without inducing IgE-dependent side effects. We

developed three lines of Tg rice accumulating 7Crp^[79]. The first line expressed 7Crp under GluB-1 promoter regulation without its signal peptide and failed to express the allergen protein, although its transcript was detectable. We concluded that absence of the signal peptide caused 7Crp instability. By adding GluB-1 signal peptide, 7Crp was accumulated in the Tg rice. In addition, high 7Crp expression in the Tg rice was achieved by adding a C-terminal ER retention signal (KDEL sequence). Finally, we established Tg rice expressing 7Crp with GluB-1 signal peptide as well as the KDEL sequence (7Crp Tg rice). After verifying accumulation of 7Crp in PB-I and PB-II, the effect of 7Crp Tg rice on allergic responses was examined in the mouse model^[79]. Oral administration of 7Crp Tg rice to B10.S mice that recognize one of the seven epitopes suppressed allergen-induced IgE and T-cell responses^[79].

Shuffled Cry j Tg rice

There are inter-individual differences in the sequence recognition of T-cell epitopes because of a variety of MHC class II haplotypes^[80-82]. Therefore, peptide IT with only major T-cell epitopes does not seem to be effective for all patients. The molecular shuffling method has been used to preserve immunogenicity/tolerogenicity (T-cell reactivity) and reduce allergenicity (IgE reactivity)^[32,83]. Based on this strategy, we improved our Tg rice to induce immune tolerance in a more efficient manner. Cry j 1 was divided into three overlapped fragments to disrupt its tertiary structure, and these fragments were shuffled and inserted into the middle of glutelins. The tertiary structure of Cry j 2 was also destroyed following its reconstruction to be a mosaic molecule by insertion of the KDEL sequence. After the abrogation of allergenicity in these fragments was verified^[84], Tg rice accumulating three Cry j 1/glutelin fusions and one reconstructed Cry j 2 was established (shuffled Cry j Tg rice)^[84]. The expressed allergens were successfully localized in PB-I. Oral administration of shuffled Cry j Tg rice to cedar pollen-immunized mice inhibited allergen-induced IgE and IgG responses, CD4⁺ T-cell proliferation, and Th2 cytokine synthesis. Consistent with this finding, allergen-induced sneezing response, serum histamine elevation, and infiltration of eosinophils in the nose were attenuated^[84].

RICE SEED-BASED ORAL IMMUNOTHERAPY FOR BRONCHIAL ASTHMA

House dust mites (HDM) are strongly associated with the development of allergic diseases, such as bronchial asthma, allergic rhinitis and atopic dermatitis^[85,86]. Specifically, *Dermatophagoides pteronyssinus* (Der p)- and *Dermatophagoides farinae* (Der f)-derived allergens are important components of indoor allergens associated with bronchial asthma^[87,88]. The major HDM allergens are classified into two groups: group 1 (Der p 1 and Der f 1), mainly derived

Table 1 Characteristics of transgenic rices for oral immunotherapy against allergic rhinitis and bronchial asthma

Tg rice name	Target disease	Target allergen	Expression plasmid construction			Allergen accumulation		Pharmacological effect	Ref.
			Promoter	Coding protein	KDEL sequence	Localization	Yield		
Cry j	Allergic rhinitis	<i>Cryptomeria Japonica</i> pollen (Japanese cedar pollen)	GluB-1	Fusion protein of mouse T-cell epitopes in Cry j 1 and Cry j 2, and glycinin A1aB1b	absence	presumably PB-II	7 µg/grain	IgE and IgG CD4+ T-cell proliferation Th2 cytokine Histamine Sneezing	[74]
7Crp	Allergic rhinitis	<i>Cryptomeria Japonica</i> pollen (Japanese cedar pollen)	GluB-1	Fusion protein of seven human T-cell epitopes in Cry j 1 and Cry j 2	presence	PB-I and PB-II	62 µg/grain	CD4+ T-cell proliferation IgE	[79]
Shuffled Cry j	Allergic rhinitis	<i>Cryptomeria Japonica</i> pollen (Japanese cedar pollen)	Prolamin (16-kDa) GluB Prolamin (10-kDa) GluB-1	Fusion protein of deconstructed full-length Cry j 1, Cry j 2, and glutelins	presence	PB-I	10-25 µg/grain	IgE and IgG CD4+ T-cell proliferation Th2 cytokine Histamine Sneezing Eosinophilia	[84]
Der p 1	Bronchial asthma	<i>Dermatophagoides Pteronyssinus</i> (House dust mite)	GluB-1	Human and mouse T-cell epitopes in Der p 1	absence	PB-I	90 µg/grain	IgE and IgG CD4+ T-cell proliferation Th2 cytokine Eosinophilia Bronchial hyperreactivity	[89-91]
Der f 2	Bronchial asthma	<i>Dermatophagoides Farinae</i> (House dust mite)	GluB-1	Cysteine residue-mutated full-length Der f 2	presence	Der f 2 body	15-30 µg/grain	IgE and IgG	[99]

from feces, and group 2 (Der p 2 and Der f 2) derived from the bodies. Therefore, for application to treatment for bronchial asthma, two types of Tg rice accumulating modified Der p 1 and Der f 2 peptides have been established (Table 1).

Der p 1 Tg rice

First, we created Tg rice expressing both human and mouse T-cell epitopes of Der p 1 (Der p 1 Tg rice)^[89,90]. By using the technique established to develop Cry j-related Tg rice, the expressed allergen was efficiently deposited in PB-I^[90]. Oral administration of Der p 1 Tg rice to Der p 1-immunized mice suppressed allergen-induced IgE and IgG responses, CD4⁺ T-cell proliferation, and Th2 cytokine synthesis. In addition, allergen-induced eosinophil infiltration into the lungs and bronchial hyperreactivity were diminished^[90], suggesting that rice seed-based oral IT is useful for the treatment of allergic rhinitis and bronchial asthma.

Administration of lower doses (approximately 5 g/kg per day) also suppressed allergen-induced lung eosinophilia^[91]. Interestingly, the production of allergen-specific IgE was not affected unlike in the high-dose experiment (approximately 50 g/kg per day)^[91]. Although we cannot explain why the low-dose Der p 1 Tg rice was not effective for IgE production, it was suggested that the efficacy

of oral IT is not mainly caused by suppression of IgE responses. Most importantly, effective attenuation of allergic inflammation was accomplished at a dose of Tg rice that is achievable through daily consumption.

Der f 2 Tg rice

Der f 2 contains three disulfide bonds (Cys8-Cys119, Cys21-Cys27, and Cys73-Cys78), two of which (Cys8-Cys119 and Cys73-Cys78) are critical for IgE-binding^[92-96]. In contrast, T-cell epitopes of the group 2 allergens are distributed over the entire protein^[94,97]. We constructed three Der f 2 derivatives in which cysteine residues were mutated: ΔC lacked all three disulfide bonds, C8/119S lacked the Cys8-Cys119 bond, and 8-119C lacked the Cys21-Cys27 and Cys73-Cys78 bonds^[98]. Binding activity with HDM-specific IgE was markedly decreased in ΔC, followed by C8/119S and 8-119C. Then, three lines of Tg rice expressing these Der f 2 derivatives were established (Der f 2 Tg rice). The localization of the expressed allergen in the Tg rice was unique. Thus, Der f 2 derivatives aggregated and formed a PB-like structure, named the Der f 2 body, distinguishable from PB-I and PB-II by its electron density. Oral administration of Der f 2 Tg rice containing C8/119S, 8-119C, or both to Der f 2-immunized mice inhibited allergen-induced IgE and IgG responses^[98], suggesting the potential of this Tg rice

to treat HDM-mediated allergic responses. Interestingly, IgE and IgG responses were not affected by Der f 2 Tg rice containing ΔC . ΔC was water-soluble and rapidly degraded by digestive enzymes in comparison to other Der f 2 derivatives^[98]. These data strongly suggest allergen digestibility is critical for the efficacy of oral IT using Tg rice. Further studies are needed to investigate the effectiveness of Der f 2 Tg rice in treating asthma symptoms.

SAFETY OF RICE SEED-BASED ORAL IMMUNOTHERAPY

So far a series of oral IT trials have raised several safety concerns including gastrointestinal symptoms^[43,44,54]. However, such adverse effects were not observed in cynomolgus macaques at least by daily oral administration of high-dose 7Crp Tg rice for 26 wk^[99]. Although further investigation into its safety is required, Tg rice-based oral IT may be safer than other modes of IT.

CONCLUSION

We have demonstrated the potential of Tg rice-based oral IT for treating allergic diseases such as allergic rhinitis and bronchial asthma in preclinical animal studies. Because rice-based oral IT is highly effective and does not produce side effects, this mode of treatment promises to become a new approach to oral IT that will improve the quality of treatment for allergic diseases, replacing established drug therapies and other types of IT. A clinical trial of Tg rice is under development to evaluate the efficacy and safety of Tg rice-based oral IT in human subjects.

REFERENCES

- 1 Devereux G. The increase in the prevalence of asthma and allergy: food for thought. *Nat Rev Immunol* 2006; **6**: 869-874 [PMID: 17063187]
- 2 Subbarao P, Mandhane PJ, Sears MR. Asthma: epidemiology, etiology and risk factors. *CMAJ* 2009; **181**: E181-E190 [PMID: 19752106 DOI: 10.1503/cmaj.080612]
- 3 Latvala J, von Hertzen L, Lindholm H, Haahela T. Trends in prevalence of asthma and allergy in Finnish young men: nationwide study, 1966-2003. *BMJ* 2005; **330**: 1186-1187 [PMID: 15849204]
- 4 Moingeon P, Mascarell L. Induction of tolerance via the sublingual route: mechanisms and applications. *Clin Dev Immunol* 2012; **2012**: 623474 [PMID: 22110534 DOI: 10.1155/2012/623474]
- 5 Larché M. Specific immunotherapy. *Br Med Bull* 2000; **56**: 1019-1036 [PMID: 11359635]
- 6 Sánchez-García S, Rodríguez del Río P, Escudero C, García-Fernández C, Ramírez A, Ibáñez MD. Efficacy of oral immunotherapy protocol for specific oral tolerance induction in children with cow's milk allergy. *Isr Med Assoc J* 2012; **14**: 43-47 [PMID: 22624442]
- 7 Calvani M, Giorgio V, Miceli Sopo S. Specific oral tolerance induction for food. A systematic review. *Eur Ann Allergy Clin Immunol* 2010; **42**: 11-19 [PMID: 20355360]
- 8 Staden U, Rolinck-Werninghaus C, Brewé F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. *Allergy* 2007; **62**: 1261-1269 [PMID: 17919140]
- 9 Tokuhara D, Yuki Y, Nochi T, Kodama T, Mejima M, Kurokawa S, Takahashi Y, Nanno M, Nakanishi U, Takaiwa F, Honda T, Kiyono H. Secretory IgA-mediated protection against *V. cholerae* and heat-labile enterotoxin-producing enterotoxigenic *Escherichia coli* by rice-based vaccine. *Proc Natl Acad Sci USA* 2010; **107**: 8794-8799 [PMID: 20421480 DOI: 10.1073/pnas.0914121107]
- 10 Nochi T, Takagi H, Yuki Y, Yang L, Masumura T, Mejima M, Nakanishi U, Matsumura A, Uozumi A, Hiroi T, Morita S, Tanaka K, Takaiwa F, Kiyono H. Rice-based mucosal vaccine as a global strategy for cold-chain- and needle-free vaccination. *Proc Natl Acad Sci USA* 2007; **104**: 10986-10991 [PMID: 17573530]
- 11 Streatfield SJ. Mucosal immunization using recombinant plant-based oral vaccines. *Methods* 2006; **38**: 150-157 [PMID: 16431131]
- 12 Takaiwa F. Seed-based oral vaccines as allergen-specific immunotherapies. *Hum Vaccin* 2011; **7**: 357-366 [PMID: 21358268]
- 13 Lin SY, Ereksomima N, Kim JM, Ramanathan M, Suarez-Cuervo C, Chelladurai Y, Ward D, Segal JB. Sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. *JAMA* 2013; **309**: 1278-1288 [PMID: 23532243 DOI: 10.1001/jama.2013.2049]
- 14 Chelladurai Y, Suarez-Cuervo C, Ereksomima N, Kim JM, Ramanathan M, Segal JB, Lin SY. Effectiveness of subcutaneous versus sublingual immunotherapy for the treatment of allergic rhinoconjunctivitis and asthma: a systematic review. *J Allergy Clin Immunol Pract* 2013; **1**: 361-369 [PMID: 24565541 DOI: 10.1016/j.jaip.2013.04.005]
- 15 Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of subcutaneous and sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a meta-analysis-based comparison. *J Allergy Clin Immunol* 2012; **130**: 1097-1107.e2 [PMID: 23021885 DOI: 10.1016/j.jaci.2012.08.012]
- 16 Khinchi MS, Poulsen LK, Carat F, André C, Hansen AB, Malling HJ. Clinical efficacy of sublingual and subcutaneous birch pollen allergen-specific immunotherapy: a randomized, placebo-controlled, double-blind, double-dummy study. *Allergy* 2004; **59**: 45-53 [PMID: 14674933]
- 17 Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911; **177**: 1572-1573
- 18 Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, Staple SQ, Aalberse RC, Till SJ, Durham SR. Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. *J Immunol* 2004; **172**: 3252-3259 [PMID: 14978133]
- 19 Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszczyk M, Blaser K, Akdis CA. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003; **33**: 1205-1214 [PMID: 12731045]
- 20 Pilette C, Nouri-Aria KT, Jacobson MR, Wilcock LK, Detry B, Walker SM, Francis JN, Durham SR. Grass pollen immunotherapy induces an allergen-specific IgA2 antibody response associated with mucosal TGF-beta expression. *J Immunol* 2007; **178**: 4658-4666 [PMID: 17372025]
- 21 Ebner C, Siemann U, Bohle B, Willheim M, Wiedermann U, Schenk S, Klotz F, Ebner H, Kraft D, Scheiner O. Immunological changes during specific immunotherapy of grass pollen allergy: reduced lymphoproliferative responses to allergen and shift from TH2 to TH1 in T-cell clones specific for Phl p 1, a major grass pollen allergen. *Clin Exp Allergy* 1997; **27**: 1007-1015 [PMID: 9678832]
- 22 Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol* 2005; **5**: 271-283 [PMID: 15775993]
- 23 John M. Eisenberg Center for Clinical Decisions and Communications Science. Subcutaneous and sublingual im-

- munotherapy to treat allergic rhinitis/rhinoconjunctivitis and asthma. Comparative Effectiveness Review Summary Guides for Clinicians. Rockville (MD): Agency for Healthcare Research and Quality, 2013 [PMID: 24049847]
- 24 **Stewart GE**, Lockey RF. Systemic reactions from allergen immunotherapy. *J Allergy Clin Immunol* 1992; **90**: 567-578 [PMID: 1401640]
- 25 **Yukselen A**, Kendirli SG, Yilmaz M, Altintas DU, Karakoc GB. Two year follow-up of clinical and inflammation parameters in children monosensitized to mites undergoing subcutaneous and sublingual immunotherapy. *Asian Pac J Allergy Immunol* 2013; **31**: 233-241 [PMID: 24053706 DOI: 10.12932/AP0276.31.3.2013]
- 26 **Saporta D**. Efficacy of sublingual immunotherapy versus subcutaneous injection immunotherapy in allergic patients. *J Environ Public Health* 2012; **2012**: 492405 [PMID: 22523505 DOI: 10.1155/2012/492405]
- 27 **Scadding GK**, Brostoff J. Low dose sublingual therapy in patients with allergic rhinitis due to house dust mite. *Clin Allergy* 1986; **16**: 483-491 [PMID: 3536171]
- 28 **Cox L**, Jacobsen L. Comparison of allergen immunotherapy practice patterns in the United States and Europe. *Ann Allergy Asthma Immunol* 2009; **103**: 451-459; quiz 459-461, 495 [PMID: 20084837 DOI: 10.1016/S1081-1206(10)60259-1]
- 29 **Canonica GW**, Bousquet J, Casale T, Lockey RF, Baena-Cagnani CE, Pawankar R, Potter PC, Bousquet PJ, Cox LS, Durham SR, Nelson HS, Passalacqua G, Ryan DP, Brozek JL, Compalati E, Dahl R, Delgado L, van Wijk RG, Gower RG, Ledford DK, Filho NR, Valovirta EJ, Yusuf OM, Zuberbier T, Akhanda W, Almarales RC, Ansotegui I, Bonifazi F, Ceuppens J, Chivato T, Dimova D, Dumitrascu D, Fontana L, Katelaris CH, Kaulsay R, Kuna P, Larenas-Linnemann D, Manoussakis M, Nekam K, Nunes C, O'Hehir R, Olaguibel JM, Onder NB, Park JW, Priftanji A, Puy R, Sarmiento L, Scadding G, Schmid-Grendelmeier P, Seberova E, Sepiashvili R, Solé D, Togias A, Tomino C, Toskala E, Van Beever H, Vieths S. Sub-lingual immunotherapy: World Allergy Organization Position Paper 2009. *Allergy* 2009; **64** Suppl 91: 1-59 [PMID: 20041860 DOI: 10.1111/j.1398-9995.2009.02309.x]
- 30 **Ye YL**, Chuang YH, Chiang BL. Strategies of mucosal immunotherapy for allergic diseases. *Cell Mol Immunol* 2011; **8**: 453-461 [PMID: 21666705 DOI: 10.1038/cmi.2011.17]
- 31 **Klimek L**, Pfaar O. A comparison of immunotherapy delivery methods for allergen immunotherapy. *Expert Rev Clin Immunol* 2013; **9**: 465-474; quiz 475 [PMID: 23634740 DOI: 10.1586/eci.13.25]
- 32 **Valenta R**. The future of antigen-specific immunotherapy of allergy. *Nat Rev Immunol* 2002; **2**: 446-453 [PMID: 12093011]
- 33 **Casale TB**, Stokes JR. Future forms of immunotherapy. *J Allergy Clin Immunol* 2011; **127**: 8-15; quiz 16-17 [PMID: 21094518 DOI: 10.1016/j.jaci.2010.10.034]
- 34 **Cox L**, Compalati E, Kundig T, Larche M. New directions in immunotherapy. *Curr Allergy Asthma Rep* 2013; **13**: 178-195 [PMID: 23315329 DOI: 10.1007/s11882-012-0335-7]
- 35 **Johansen P**, von Moos S, Mohanan D, Kundig TM, Senti G. New routes for allergen immunotherapy. *Hum Vaccin Immunother* 2012; **8**: 1525-1533 [PMID: 23095873 DOI: 10.4161/hv.21948]
- 36 **Jutel M**, Solarewicz-Madejek K, Smolinska S. Recombinant allergens: the present and the future. *Hum Vaccin Immunother* 2012; **8**: 1534-1543 [PMID: 23095874 DOI: 10.4161/hv.22064]
- 37 **Anagnostou K**, Islam S, King Y, Foley L, Pasea L, Bond S, Palmer C, Deighton J, Ewan P, Clark A. Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. *Lancet* 2014; **383**: 1297-1304 [PMID: 24485709 DOI: 10.1016/S0140-6736(13)62301-6]
- 38 **Patriarca G**, Nucera E, Roncallo C, Pollastrini E, Bartolozzi F, De Pasquale T, Buonomo A, Gasbarrini G, Di Campli C, Schiavino D. Oral desensitizing treatment in food allergy: clinical and immunological results. *Aliment Pharmacol Ther* 2003; **17**: 459-465 [PMID: 12562461]
- 39 **Meglio P**, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. *Allergy* 2004; **59**: 980-987 [PMID: 15291907]
- 40 **Senti G**, von Moos S, Tay F, Graf N, Sonderegger T, Johansen P, Kundig TM. Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: A double-blind, placebo-controlled dose escalation study. *J Allergy Clin Immunol* 2012; **129**: 128-135 [PMID: 21996342 DOI: 10.1016/j.jaci.2011.08.036]
- 41 **Hylander T**, Latif L, Petersson-Westin U, Cardell LO. Intralymphatic allergen-specific immunotherapy: an effective and safe alternative treatment route for pollen-induced allergic rhinitis. *J Allergy Clin Immunol* 2013; **131**: 412-420 [PMID: 23374268 DOI: 10.1016/j.jaci.2012.10.056]
- 42 **Senti G**, Cramer R, Kuster D, Johansen P, Martinez-Gomez JM, Graf N, Steiner M, Hothorn LA, Grönlund H, Tivig C, Zaleska A, Soyer O, van Hage M, Akdis CA, Akdis M, Rose H, Kundig TM. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. *J Allergy Clin Immunol* 2012; **129**: 1290-1296 [PMID: 22464647 DOI: 10.1016/j.jaci.2012.02.026]
- 43 **Sudo K**, Taniuchi S, Takahashi M, Soejima K, Hatano Y, Nakano K, Shimo T, Koshino H, Kaneko K. Home-based oral immunotherapy (OIT) with an intermittent loading protocol in children unlikely to outgrow egg allergy. *Allergy Asthma Clin Immunol* 2014; **10**: 11 [PMID: 24572125 DOI: 10.1186/1710-1492-10-11]
- 44 **Murakami D**, Kubo K, Sawatsubashi M, Kikkawa S, Ejima M, Saito A, Kato A, Komune S. Phase I/II study of oral immunotherapy with Cry j1-galactomannan conjugate for Japanese cedar pollinosis. *Auris Nasus Larynx* 2014; **41**: 350-358 [PMID: 24698163 DOI: 10.1016/j.anl.2014.02.010]
- 45 **Weiner HL**. Oral tolerance, an active immunologic process mediated by multiple mechanisms. *J Clin Invest* 2000; **106**: 935-937 [PMID: 11032852]
- 46 **Friedman A**, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci USA* 1994; **91**: 6688-6692 [PMID: 8022835]
- 47 **Gregerson DS**, Obritsch WF, Donoso LA. Oral tolerance in experimental autoimmune uveoretinitis. Distinct mechanisms of resistance are induced by low dose vs high dose feeding protocols. *J Immunol* 1993; **151**: 5751-5761 [PMID: 7693817]
- 48 **Chen Y**, Kuchroo VK, Inobe J, Hafler DA, Weiner HL. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 1994; **265**: 1237-1240 [PMID: 7520605]
- 49 **Whitacre CC**, Gienapp IE, Orosz CG, Bitar DM. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. *J Immunol* 1991; **147**: 2155-2163 [PMID: 1717550]
- 50 **Chen Y**, Inobe J, Marks R, Gonnella P, Kuchroo VK, Weiner HL. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995; **376**: 177-180 [PMID: 7603570]
- 51 **Mowat AM**. Anatomical basis of tolerance and immunity to intestinal antigens. *Nat Rev Immunol* 2003; **3**: 331-341 [PMID: 12669023]
- 52 **Weiner HL**, da Cunha AP, Quintana F, Wu H. Oral tolerance. *Immunol Rev* 2011; **241**: 241-259 [PMID: 21488901 DOI: 10.1111/j.1600-065X.2011.01017.x]
- 53 **Pollia JA**. Dermatitis, Urticaria, and Aggravation of Nasal Symptoms from Oral Administration of Pollen Suspensions. *Cal West Med* 1939; **50**: 25 [PMID: 18744992]
- 54 **Taudorf E**, Laursen LC, Lanner A, Björkstén B, Dreborg S, Søbørg M, Weeke B. Oral immunotherapy in birch pollen

- hay fever. *J Allergy Clin Immunol* 1987; **80**: 153-161 [PMID: 3301985]
- 55 **Mason HS**, Lam DM, Arntzen CJ. Expression of hepatitis B surface antigen in transgenic plants. *Proc Natl Acad Sci USA* 1992; **89**: 11745-11749 [PMID: 1465391]
 - 56 **Sandhu JS**, Krasnyanski SF, Domier LL, Korban SS, Osadjan MD, Buetow DE. Oral immunization of mice with transgenic tomato fruit expressing respiratory syncytial virus-F protein induces a systemic immune response. *Transgenic Res* 2000; **9**: 127-135 [PMID: 10951696]
 - 57 **Kumar GB**, Ganapathi TR, Revathi CJ, Srinivas L, Bapat VA. Expression of hepatitis B surface antigen in transgenic banana plants. *Planta* 2005; **222**: 484-493 [PMID: 15918027]
 - 58 **Haq TA**, Mason HS, Clements JD, Arntzen CJ. Oral immunization with a recombinant bacterial antigen produced in transgenic plants. *Science* 1995; **268**: 714-716 [PMID: 7732379]
 - 59 **Korban SS**, Krasnyanski SF, Buetow DE. Foods as production and delivery vehicles for human vaccines. *J Am Coll Nutr* 2002; **21**: 212S-217S [PMID: 12071307]
 - 60 **Walmsley AM**, Arntzen CJ. Plants for delivery of edible vaccines. *Curr Opin Biotechnol* 2000; **11**: 126-129 [PMID: 10753769]
 - 61 **Yamagata H**, Tanaka K. The site of synthesis and accumulation of rice storage proteins. *Plant and cell physiology* 1986; **27**: 135-145
 - 62 **Krishnan HB**, Franceschi VR, Okita TW. Immunochemical studies on the role of the Golgi complex in protein-body formation in rice seeds. *Planta* 1986; **169**: 471-480 [PMID: 24232753 DOI: 10.1007/BF00392095]
 - 63 **Oparka KJ**, Harris N. Rice protein-body formation: all types are initiated by dilation of the endoplasmic reticulum. *Planta* 1982; **154**: 184-188 [PMID: 24275981 DOI: 10.1007/BF00387914]
 - 64 **Saito Y**, Shigemitsu T, Yamasaki R, Sasou A, Goto F, Kishida K, Kuroda M, Tanaka K, Morita S, Satoh S, Masumura T. Formation mechanism of the internal structure of type I protein bodies in rice endosperm: relationship between the localization of prolamin species and the expression of individual genes. *Plant J* 2012; **70**: 1043-1055 [PMID: 22348505 DOI: 10.1111/j.1365-3113X.2012.04947.x]
 - 65 **Takahashi H**, Saito Y, Kitagawa T, Morita S, Masumura T, Tanaka K. A novel vesicle derived directly from endoplasmic reticulum is involved in the transport of vacuolar storage proteins in rice endosperm. *Plant Cell Physiol* 2005; **46**: 245-249 [PMID: 15659439]
 - 66 **Chargelegue D**, Obregon P, Drake PM. Transgenic plants for vaccine production: expectations and limitations. *Trends Plant Sci* 2001; **6**: 495-496 [PMID: 11701351]
 - 67 **Qu le Q**, Takaiwa F. Evaluation of tissue specificity and expression strength of rice seed component gene promoters in transgenic rice. *Plant Biotechnol J* 2004; **2**: 113-125 [PMID: 17147604]
 - 68 **Takagi H**, Hiroi T, Hirose S, Yang L, Takaiwa F. Rice seed ER-derived protein body as an efficient delivery vehicle for oral tolerogenic peptides. *Peptides* 2010; **31**: 1421-1425 [PMID: 20457197 DOI: 10.1016/j.peptides.2010.04.032]
 - 69 **PG-MARJ Committee**. Practical Guideline for the Management of Allergic Rhinitis in Japan—Perennial Rhinitis and Pollinosis-2009 Edition. 6th ed. Tokyo: Life Science, 2009 (in Japanese)
 - 70 **Okamoto Y**, Horiguchi S, Yamamoto H, Yonekura S, Hanazawa T. Present situation of cedar pollinosis in Japan and its immune responses. *Allergol Int* 2009; **58**: 155-162 [PMID: 19307773 DOI: 10.2332/allergolint.08-RAI-0074]
 - 71 **Tanihara S**, Oki I, Ojima T, Nakamura Y, Yanagawa H. Process and current status of the epidemiologic studies on cedar pollinosis in Japan. *J Epidemiol* 1999; **9**: 20-26 [PMID: 10098349]
 - 72 **Yasueda H**, Yui Y, Shimizu T, Shida T. Isolation and partial characterization of the major allergen from Japanese cedar (*Cryptomeria japonica*) pollen. *J Allergy Clin Immunol* 1983; **71**: 77-86 [PMID: 6822692]
 - 73 **Sakaguchi M**, Inouye S, Taniai M, Ando S, Usui M, Matuhashi T. Identification of the second major allergen of Japanese cedar pollen. *Allergy* 1990; **45**: 309-312 [PMID: 2382797]
 - 74 **Takagi H**, Hiroi T, Yang L, Tada Y, Yuki Y, Takamura K, Ishimitsu R, Kawauchi H, Kiyono H, Takaiwa F. A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. *Proc Natl Acad Sci USA* 2005; **102**: 17525-17530 [PMID: 16278301]
 - 75 **Katsube T**, Kurisaka N, Ogawa M, Maruyama N, Ohtsuka R, Utsumi S, Takaiwa F. Accumulation of soybean glycinin and its assembly with the glutelins in rice. *Plant Physiol* 1999; **120**: 1063-1074 [PMID: 10444090]
 - 76 **Hirahara K**, Saito S, Serizawa N, Sasaki R, Sakaguchi M, Inouye S, Taniguchi Y, Kaminogawa S, Shiraishi A. Oral administration of a dominant T-cell determinant peptide inhibits allergen-specific TH1 and TH2 cell responses in Cry j 2-primed mice. *J Allergy Clin Immunol* 1998; **102**: 961-967 [PMID: 9847437]
 - 77 **Hirahara K**, Tatsuta T, Takatori T, Ohtsuki M, Kirinaka H, Kawaguchi J, Serizawa N, Taniguchi Y, Saito S, Sakaguchi M, Inouye S, Shiraishi A. Preclinical evaluation of an immunotherapeutic peptide comprising 7 T-cell determinants of Cry j 1 and Cry j 2, the major Japanese cedar pollen allergens. *J Allergy Clin Immunol* 2001; **108**: 94-100 [PMID: 11447388]
 - 78 **Sone T**, Morikubo K, Miyahara M, Komiyama N, Shimizu K, Tsunoo H, Kino K. T cell epitopes in Japanese cedar (*Cryptomeria japonica*) pollen allergens: choice of major T cell epitopes in Cry j 1 and Cry j 2 toward design of the peptide-based immunotherapeutics for the management of Japanese cedar pollinosis. *J Immunol* 1998; **161**: 448-457 [PMID: 9647255]
 - 79 **Takagi H**, Hirose S, Yasuda H, Takaiwa F. Biochemical safety evaluation of transgenic rice seeds expressing T cell epitopes of Japanese cedar pollen allergens. *J Agric Food Chem* 2006; **54**: 9901-9905 [PMID: 17177518]
 - 80 **Sone T**, Dairiki K, Morikubo K, Shimizu K, Tsunoo H, Mori T, Kino K. Recognition of T cell epitopes unique to Cha o 2, the major allergen in Japanese cypress pollen, in allergic patients cross-reactive to Japanese cedar and Japanese cypress pollen. *Allergol Int* 2009; **58**: 237-245 [PMID: 19307778 DOI: 10.2332/allergolint.08-OA-0027]
 - 81 **Immonen A**, Farci S, Taivainen A, Partanen J, Pouvell-Moratille S, Närvänen A, Kinnunen T, Saarelainen S, Rytönen-Nissinen M, Maillere B, Virtanen T. T cell epitope-containing peptides of the major dog allergen Can f 1 as candidates for allergen immunotherapy. *J Immunol* 2005; **175**: 3614-3620 [PMID: 16148105]
 - 82 **Masuyama K**, Chikamatsu K, Ikagawa S, Matsuoka T, Takahashi G, Yamamoto T, Endo S. Analysis of helper T cell responses to Cry j 1-derived peptides in patients with nasal allergy: candidate for peptide-based immunotherapy of Japanese cedar pollinosis. *Allergol Int* 2009; **58**: 63-70 [PMID: 19050376 DOI: 10.2332/allergolint.08-OA-0008]
 - 83 **Gafvelin G**, Parmley S, Neimert-Andersson T, Blank U, Eriksson TL, van Hage M, Punnonen J. Hypoallergens for allergen-specific immunotherapy by directed molecular evolution of mite group 2 allergens. *J Biol Chem* 2007; **282**: 3778-3787 [PMID: 17170112]
 - 84 **Wakasa Y**, Takagi H, Hirose S, Yang L, Saeki M, Nishimura T, Kaminuma O, Hiroi T, Takaiwa F. Oral immunotherapy with transgenic rice seed containing destructured Japanese cedar pollen allergens, Cry j 1 and Cry j 2, against Japanese cedar pollinosis. *Plant Biotechnol J* 2013; **11**: 66-76 [PMID: 23066780 DOI: 10.1111/pbi.12007]
 - 85 **Thomas WR**, Smith WA, Hales BJ, Mills KL, O'Brien RM. Characterization and immunobiology of house dust mite allergens. *Int Arch Allergy Immunol* 2002; **129**: 1-18 [PMID: 12098349]

- 12372994]
- 86 **Platt-Mills TA**, Sporik RB, Chapman MD, Heymann PW. The role of indoor allergens in asthma. *Allergy* 1995; **50**: 5-12 [PMID: 7793537]
- 87 **Platts-Mills TA**, Vervloet D, Thomas WR, Aalberse RC, Chapman MD. Indoor allergens and asthma: report of the Third International Workshop. *J Allergy Clin Immunol* 1997; **100**: S2-24 [PMID: 9438476]
- 88 **Kawamoto S**, Aki T, Yamashita M, Tategaki A, Fujimura T, Tsuboi S, Katsutani T, Suzuki O, Shigeta S, Murooka Y, Ono K. Toward elucidating the full spectrum of mite allergens-state of the art. *J Biosci Bioeng* 2002; **94**: 285-298 [PMID: 16233305]
- 89 **Suzuki K**, Kaminuma O, Yang L, Motoi Y, Takai T, Ichikawa S, Okumura K, Ogawa H, Mori A, Takaiwa F, Hiroi T. Development of transgenic rice expressing mite antigen for a new concept of immunotherapy. *Int Arch Allergy Immunol* 2009; **149** Suppl 1: 21-24 [PMID: 19494500 DOI: 10.1159/000210649]
- 90 **Suzuki K**, Kaminuma O, Yang L, Takai T, Mori A, Umezugoto M, Ohtomo T, Ohmachi Y, Noda Y, Hirose S, Okumura K, Ogawa H, Takada K, Hirasawa M, Hiroi T, Takaiwa F. Prevention of allergic asthma by vaccination with transgenic rice seed expressing mite allergen: induction of allergen-specific oral tolerance without bystander suppression. *Plant Biotechnol J* 2011; **9**: 982-990 [PMID: 21447056 DOI: 10.1111/j.1467-7652.2011.00613.x]
- 91 **Saeki M**, Nishimura T, Kaminuma O, Suzuki K, Takai T, Mori A, Takada K, Takaiwa F, Hiroi T. Inhibition of allergen-induced airway inflammation by low-dose oral immunotherapy with transgenic rice seeds independently of immunoglobulin e synthesis. *Int Arch Allergy Immunol* 2012; **158** Suppl 1: 66-69 [PMID: 22627369 DOI: 10.1159/000337771]
- 92 **Johannessen BR**, Skov LK, Kastrup JS, Kristensen O, Bolwig C, Larsen JN, Spangfort M, Lund K, Gajhede M. Structure of the house dust mite allergen Der f 2: implications for function and molecular basis of IgE cross-reactivity. *FEBS Lett* 2005; **579**: 1208-1212 [PMID: 15710415]
- 93 **Nishiyama C**, Fukada M, Usui Y, Iwamoto N, Yuuki T, Okumura Y, Okudaira H. Analysis of the IgE-epitope of Der f 2, a major mite allergen, by in vitro mutagenesis. *Mol Immunol* 1995; **32**: 1021-1029 [PMID: 8544851]
- 94 **Inoue R**, Matsuoka T, Kondo N, Nishimura Y, Matsushita S. Identification of Dermatophagoides farinae-2-derived peptides and class II HLA molecules recognized by T cells from atopic individuals. *Int Arch Allergy Immunol* 1997; **114**: 354-360 [PMID: 9414139]
- 95 **O'Brien RM**, Thomas WR, Nicholson I, Lamb JR, Tait BD. An immunogenetic analysis of the T-cell recognition of the major house dust mite allergen Der p 2: identification of high- and low-responder HLA-DQ alleles and localization of T-cell epitopes. *Immunology* 1995; **86**: 176-182 [PMID: 7490115]
- 96 **Takai T**, Yokota T, Yasue M, Nishiyama C, Yuuki T, Mori A, Okudaira H, Okumura Y. Engineering of the major house dust mite allergen Der f 2 for allergen-specific immunotherapy. *Nat Biotechnol* 1997; **15**: 754-758 [PMID: 9255789]
- 97 **Joost van Neerven R**, van t'Hof W, Ringrose JH, Jansen HM, Aalberse RC, Wierenga EA, Kapsenberg ML. T cell epitopes of house dust mite major allergen Der p II. *J Immunol* 1993; **151**: 2326-2335 [PMID: 7688399]
- 98 **Yang L**, Hirose S, Suzuki K, Hiroi T, Takaiwa F. Expression of hypoallergenic Der f 2 derivatives with altered intramolecular disulphide bonds induces the formation of novel ER-derived protein bodies in transgenic rice seeds. *J Exp Bot* 2012; **63**: 2947-2959 [PMID: 22378952 DOI: 10.1093/jxb/ers006]
- 99 **Domon E**, Takagi H, Hirose S, Sugita K, Kasahara S, Ebinuma H, Takaiwa F. 26-Week oral safety study in macaques for transgenic rice containing major human T-cell epitope peptides from Japanese cedar pollen allergens. *J Agric Food Chem* 2009; **57**: 5633-5638 [PMID: 19462978 DOI: 10.1021/jf900371u]

P- Reviewer: Huang L, Yeligar SM S- Editor: Song XX
L- Editor: A E- Editor: Lu YJ





Immunological aspects of drug-induced liver injury

Kazuto Tajiri, Yukihiro Shimizu

Kazuto Tajiri, The Third Department of Internal Medicine, Toyama University Hospital, Toyama 930-0194, Japan

Yukihiro Shimizu, Gastroenterology Unit, Nanto Municipal Hospital, Nanto, Toyama, 932-0211, Japan

Author contributions: Both Tajiri K and Shimizu Y wrote the manuscript.

Correspondence to: Yukihiro Shimizu, MD, PhD, Gastroenterology Unit, Nanto Municipal Hospital, Nanto, Toyama 932-0211, Japan. rsf14240@nifty.com

Telephone: +81-763-821475 Fax: +81-763-821853

Received: March 5, 2014 Revised: May 17, 2014

Accepted: July 17, 2014

Published online: November 27, 2014

and immunological reactions, may be associated with adduct formation and the onset of DILI. This review summarizes current knowledge on the immunological aspects of DILI, including its pathogenesis, diagnosis and treatment.

Tajiri K, Shimizu Y. Immunological aspects of drug-induced liver injury. *World J Immunol* 2014; 4(3): 149-157 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i3/149.htm>
DOI: <http://dx.doi.org/10.5411/wji.v4.i3.149>

Abstract

Drug induced liver injury (DILI) is a common condition of increasing incidence. Many environmental and genetic factors are involved in its pathogenesis, and immunological mechanisms are also thought to contribute to the development and severity of DILI. This review summarizes current understanding of the immunological pathogenesis of DILI and discusses the perspective for clinical applications.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Immune cells; Cytokine; Human leukocyte antigen; Innate immunity; Drug-induced liver injury

Core tip: Drug-induced liver injury (DILI) is a common liver disease that occurs frequently after drug ingestion. DILI can be classified as predictable or idiosyncratic. The former is dose dependent, has a short latency period, and results from direct toxicity of the drug or its metabolite(s). Idiosyncratic DILI may be more problematic because it is usually unpredictable and is found more frequently in the clinical setting. This type of DILI is due to an allergic reaction or the toxicity of metabolites generated *via* individual drug metabolism reactions. Various factors, such as environmental, genetic

INTRODUCTION

Drug-induced liver injury (DILI) is a common liver disease that occurs frequently after drug ingestion including herbal medicine. Its incidence is reported to be increasing^[1]. Clinical manifestations of DILI vary from transient mild elevation of liver enzymes to fulminant liver failure leading to death. DILI can be divided into three types according to the pattern of liver injury; hepatocellular [alanine aminotransferase (ALT) $\geq$ 3 upper limit of normal (ULN) and ALT/alkaline phosphatase (ALP) ratio $\geq$ 5], cholestatic (ALP $\geq$ 2 ULN and ALT/ALP ratio $\leq$ 2), and mixed type (2 < ALT/ALP ratio < 5)^[2]. In addition, DILI can be classified as predictable or idiosyncratic^[3]. The former is dose dependent, has a short latency period, and results from direct toxicity of the drug or its metabolite(s); *e.g.*, acetaminophen. Idiosyncratic DILI may be more problematic because it is usually unpredictable and is found more frequently in the clinical setting. This type of DILI is due to an allergic reaction or the toxicity of metabolites generated *via* individual drug metabolism reactions. Various factors, such as environmental, genetic and immunological reactions, may be associated with adduct formation and the onset of DILI. This review will summarize current knowledge on the immunological aspects of DILI, including its pathogenesis, diagnosis and treatment.

IMMUNOLOGICAL PATHOGENESIS OF DRUG-INDUCED LIVER INJURY

DILI is thought to occur through direct toxic or allergic effects of drugs. However, there is another mechanism, in which DILI is induced by toxic or reactive metabolites produced through abnormal metabolism. Most drugs are fat-soluble, with the liver being the main organ for their biotransformation and elimination. Drug elimination by the liver may be determined by several reactions, such as hepatic blood flow, hepatic metabolism and biliary extraction. Drug metabolism in the liver largely depends on the hydroxylation activity of cytochrome P (CYP) 450 enzymes (Phase 1 reaction); the products are unstable or reactive metabolites; and the process is affected by various factors, such as genetic alterations^[4]. The hydroxylated metabolites are then conjugated by glucuronization, sulfonation, glutathionization, or acetylation (Phase 2 reaction), followed by their transport to the extracellular space as water-soluble, stable and detoxified products (Phase 3 reaction). These final metabolites are excreted into the urine or bile juice. During this process, most of the targets for immune attack are thought to be unstable and reactive metabolites conjugated with intracellular proteins or macromolecules. These newly formed antigens are recognized by immune cells. Activation of the immune system generates autoantibodies and cell-mediated immune responses, leading to injury to hepatocytes^[5] (Figure 1).

Animal models of the immunological pathogenesis of DILI

Immunological mechanisms leading to DILI have been widely explored using experimental animal models and predictable drugs such as acetaminophen. In DILI, the balance between pro- and anti-inflammatory responses resulting from the activation of the innate immune system in the liver determines tissue susceptibility and the severity of liver injury^[6].

Model of acetaminophen-induced liver injury in mice

Acetaminophen induced liver injury (AILI) involves glutathione depletion and covalent binding of a metabolite of acetaminophen to mitochondrial protein, both essential mechanisms of hepatocyte death. Acetaminophen hepatotoxicity is dependent upon its metabolic transformation to the reactive metabolite N-acetyl-*p*-benzoquinone imine (NAPQI) by CYP450^[7]. Following the phase 2 process of conjugation detoxification, NAPQI becomes covalently bound to hepatic mitochondrial protein, leading to cell death. Cell damage induces the release of damage-associated molecular patterns (DAMPs), such as HMGB-1 and heat shock proteins (HSP), which then activate the innate immune system^[8].

In animal models of AILI, innate immune cells such as macrophages and NK/NKT cells play a pivotal role in protection against acetaminophen^[9,11]. Depletion or inactivation of hepatic macrophages markedly reduced

the severity of AILI by inhibiting cytokine production, especially of tumor necrosis factor (TNF)- α , soon after acetaminophen administration^[12], but induced more severe liver injury at later times, possibly due to the suppression of delayed production of prostaglandin by hepatic macrophages^[9]. These macrophages were shown to be derived from circulating monocytes that infiltrate the liver, but not from resident Kupffer cells^[13], with TNF- α regarded as an essential participant in the macrophage dependent etiology of AILI^[14-16].

In contrast, the administration of anti-NK1.1 antibody to deplete NK and NKT cells protected mice from AILI, probably due to a reduction in IFN- γ concentrations^[10]. Starvation-induced ketone production has been observed in NKT cell-deficient mice, resulting in increases in CYP2E1-mediated reactive metabolites and enhanced susceptibility to AILI^[11]. More recently, however, NK and NKT cells were shown to contribute to protection against AILI only in the presence of dimethyl sulfoxide^[17], suggesting NK and NKT cells may have little involvement in protecting these mice against AILI. NK/NKT cells are generally regarded as being involved in the immunopathogenesis of AILI, with IFN- γ being a key molecule. Various cytokines and chemokines are produced in response to IFN- γ , with these factors promoting neutrophil infiltration^[10,18]. NKT cells have also been reported involved in a mouse model of halothane-induced liver injury by recruiting neutrophils to the liver, with these neutrophils playing a pathogenic role in liver injury^[19]. In contrast, eosinophil depletion was found to reduce the severity of halothane-induced liver injury in a mouse model, whereas depletion of neutrophils failed to reduce the severity of liver injury^[20]. This study found that eosinophils accumulated exclusively around areas of hepatocellular necrosis, suggesting a pathogenic role of eosinophils in liver injury similar to that observed in many patients with DILI^[20]. Recently, regulatory T-cells were also reported to reduce the severity of trifluoroacetyl chloride-induced DILI by decreasing the hepatic levels of pro-inflammatory cytokines^[21].

Dendritic cells (DCs) have also been reported to play a protective role in AILI. Hepatic DCs have been reported to suppress the severity of AILI, at least in part, by preventing NK cell activation and inducing neutrophil apoptosis^[22].

In contrast, interleukin (IL)-10 null mice were found to be more susceptible to AILI, suggesting that a counter-regulatory anti-inflammatory response also modulates liver injury^[23]. Furthermore, receptors involved in the innate immune system, such as toll-like receptors (TLR)-4 and 9 have been found to play roles in promoting inflammatory responses and subsequent pathogenesis of AILI^[24,25]. Collectively, the innate immune system is closely associated with the onset and severity of AILI, which may explain, at least in part, the pathogenesis of AILI in mice.

Mouse DILI model except for acetaminophen toxicity

Cytokines and immune cells have been reported associated with the pathogenesis of DILI but not of AILI. For

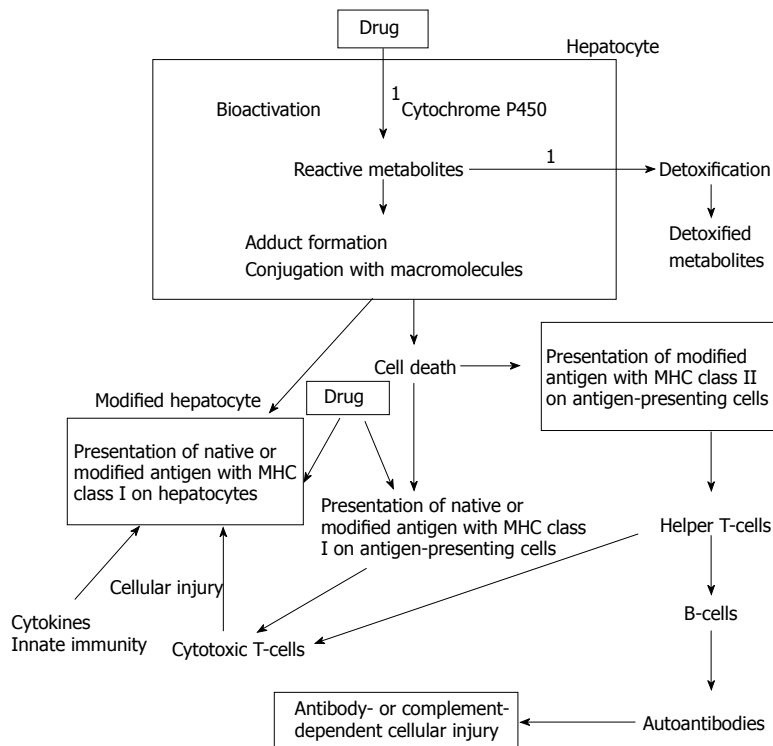


Figure 1 Hypothetical schema of the immunological pathogenesis of drug induced liver injury (ref. [5] with modifications). ¹Genetic polymorphism.

example, IL-4 was reported to mediate dicloxacillin-induced liver injury in a mouse model^[26], and IL-17 and IL-1 β were found to be involved in the onset of diclofenac-induced liver injury in mice^[27]. Diclofenac has been reported to inhibit TNF- α induced survival signals through nuclear factor- κ B and to sensitize hepatocytes to apoptosis^[28]. Thus, the activation of the innate immune system in the liver may play an important role in immune-mediated idiosyncratic DILI^[6,29].

Although experimental data support the hypothesis that DILIs are induced through the activation of the innate immune system within the liver, different cell populations may be involved in each DILI. Moreover, these data are derived from mice with a uniform genetic background and environment.

Immunological pathogenesis of DILI in humans

In contrast to mice, genetic background and environment are diverse in humans, and most patients who develop this disease are receiving multiple drugs. The conditions under which DILI develops are therefore more complex in humans, making the diagnosis of DILI and the analysis of immunological mechanisms more difficult.

The pathogenesis of DILI in humans may be influenced by a variety of genetic and environmental effects on drug metabolism and immunological responses. These factors may, in turn, be associated with the onset and severity of DILI in humans (Figure 2)^[30].

Cytokines

Pro- and anti-inflammatory responses have been observed in patients with DILI. For example, a study of 111 pa-

tients with DILI due to acetaminophen overdose included measurements of the plasma concentrations of cytokines such as IL-6, IL-8, IL-10 and monocyte chemoattractant protein (MCP)-1^[31]. In that study, the concentrations of IL-6, IL-8, and MCP-1 were elevated in patients with elevated serum ALT^[31], with MCP-1 concentration most closely associated with the severity of toxicity. Thus, the hepatotoxicity in patients with acetaminophen overdose is thought to be due to the direct toxicity of the acetaminophen metabolite, whereas the severity of toxicity may be immunologically determined.

The expression of various cytokines and their association with the severity of liver injury have been reported in patients with acute liver failure^[32]. For example, IL-6 and TNF- α have been associated with disease progression, whereas IL-10 was associated with disease protection. A study of 39 patients with acute liver failure due to DILI found that serum IL-17 and IL-21 concentrations were elevated^[33] and that autoantibodies were more frequently found in patients with than without DILI, suggesting that autoimmune responses are involved in its pathogenesis^[34]. Elevation of the serum cytokines, IL-1 β , IL-10, IL-12, IL-13, and TNF- α preceded an increase in liver enzymes in a patient with DILI^[35]. Moreover, CD8+ T cells were reported to produce IFN- γ when peripheral mononuclear cells of patients with the hepatocellular type of DILI were stimulated with the causative drug (Figure 3A and B). In contrast, CD14+ monocytes from patients with the cholestatic type of DILI were found to produce TNF- α upon stimulation by lysates of HepG2 cells that were incubated with the causative drug (Figure

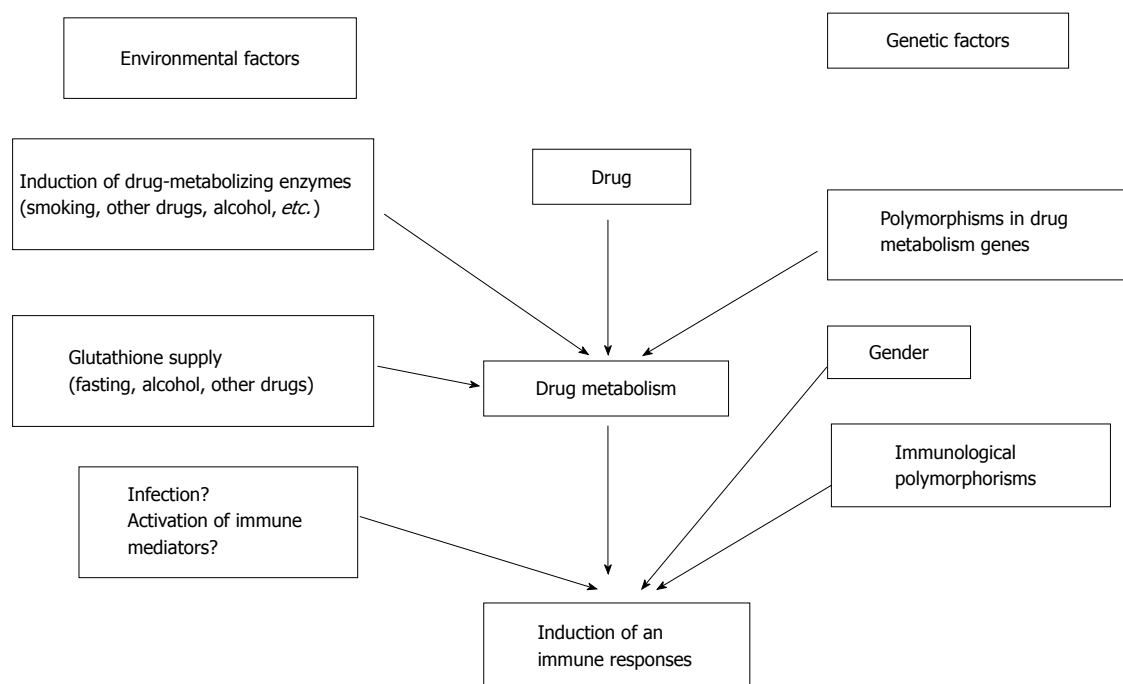


Figure 2 Contribution of environmental and genetic factors to drug metabolism and immune response in drug induced liver injury^[30].

3C and D)^[36]. Thus, the mechanisms responsible for two types of DILI, hepatocellular and cholestatic, may be different, with the immune system activated by both the drug itself and its reactive metabolites.

Assessments of genetic factors in patients with DILI found that the low IL-10 producing haplotype was more prevalent, but genetic polymorphisms in IL-10, IL-4 and TNF- α were not related to the risk of developing DILI^[37]. In contrast, IL-10 polymorphisms were reported associated with the incidence of docetaxel-induced liver injury^[38]. Thus, the balance between pro- and anti-inflammatory cytokines plays a significant role in the pathogenesis of DILI, and the pattern of pro- and anti-inflammatory cytokines may be a candidate biomarker of DILI.

Autoantibody production

Inhalation of the anesthetic halothane has been explored as an immunoallergic response model. Patients with halothane-induced liver injury were found to produce autoantibodies that recognize autoantigens and neoantigens created by trifluoroacetylation of hepatic proteins. Incubation of halothane-pretreated rabbit hepatocytes with the sera of patients with halothane-induced fulminant hepatitis increased susceptibility to lymphocyte cytotoxicity^[39]. Halothane is metabolized by CYP2E1 to form the reactive metabolite acyl halide, which may trigger immune responses. Anti-CYP autoantibodies induced by drugs have also been found in patients with idiosyncratic drug reactions to dihydralazine^[40] and tienilic acid^[41].

Monocyte activation

Recently, human monocytic cells have been reported activated by hepatotoxic drugs, such as amiodarone and

its metabolite, when co-incubated with CYP3A4 superosomes^[42].

HLA genotype and T cell response

Since antigenic peptides are presented on human leukocyte antigen (HLA) molecules, HLA haplotype has been shown associated with the development of various diseases. For example, 57% of Belgian patients with amoxicillin-clavulanate-induced liver injury had the *DRB1*1501* allele compared with 11% of the general population^[43], with the same allele found in 70% of Scottish patients with amoxicillin-clavulanate-induced liver injury^[44]. Furthermore, 53% of patients in the United Kingdom had the HLA-*DRB1*15* allele, compared with 30% of the general population^[45]. Conversely, 9.8% of patients with DILI had the *DRB1*07* allele, compared with 29% of the general population^[45]. Study of a Spanish cohort did not show a significant association between DILI and the *DRB1*15* allele, but found that the frequency of *DQB1*06* was significantly higher in these patients than in the general population^[46]. A recent genome-wide association study in a European cohort with amoxicillin-clavulanate-induced liver injury showed that multiple HLA genotypes could affect susceptibility to the onset of the disease^[47]. Although several single nucleotide polymorphisms (SNPs) within the HLA were found associated with DILI, the strongest association was observed in SNPs within HLA-*DRB1* and an independent association was found with HLA-*A*0201*. Moreover, HLA-*A*0201*, *DQB1*0602* and amoxicillin-clavulanate-induced liver injury were found to be associated with *PTPN22*, a gene associated with the development of various autoimmune diseases^[47]. Importantly, only SNPs inside the HLA re-

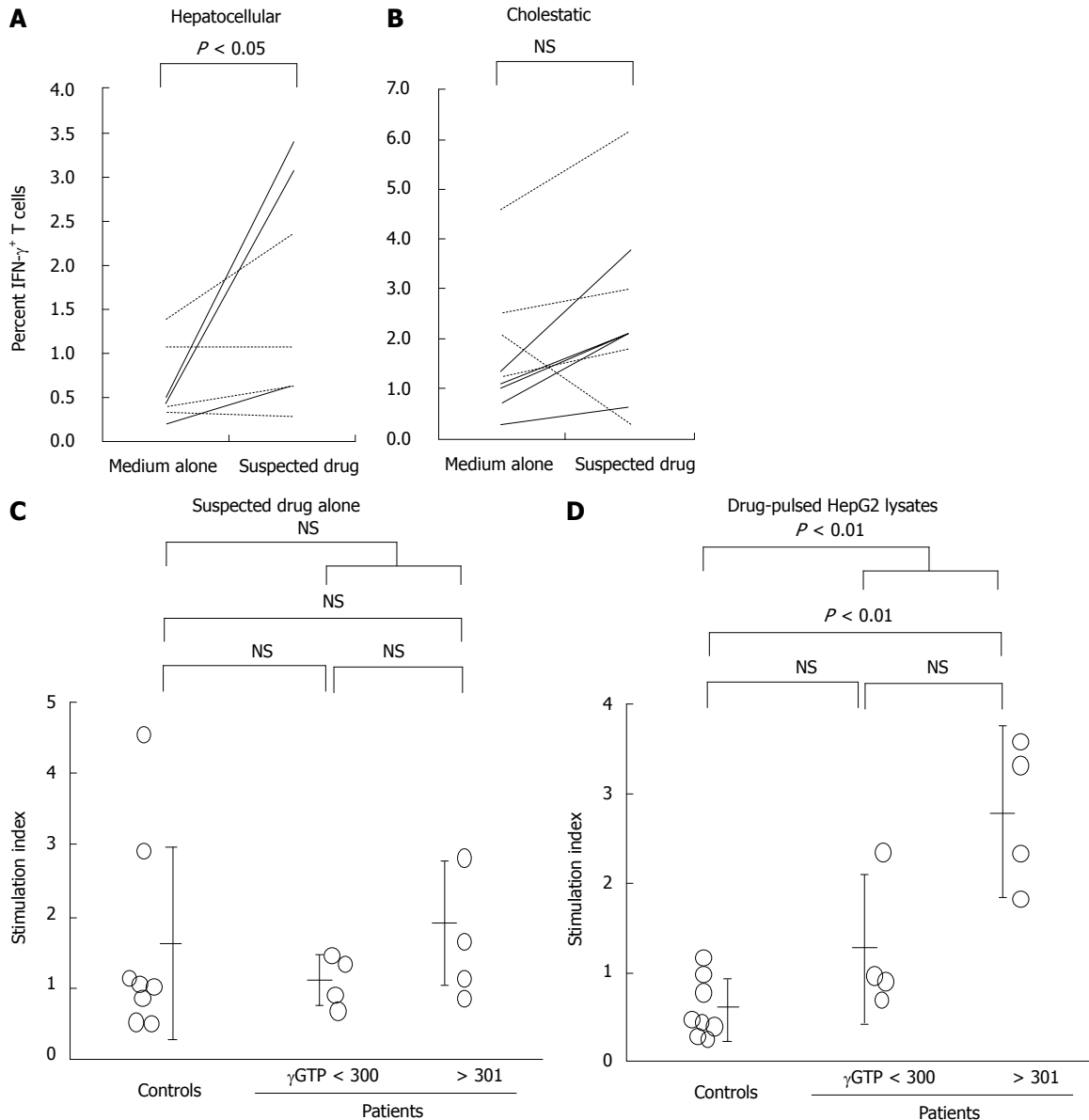


Figure 3 Cytokine production by immune cells after stimulation with suspected drug alone or suspected drug-pulsed HepG2 lysates in patients with drug induced liver injury^[36]. A, B: The percentages of CD8⁺ cells that are IFN- γ ⁺ after stimulation with the suspected drug alone in patients with hepatocellular type (A) and cholestatic type (B) liver injury; C, D: Fold expansions of TNF- α ⁺ cells (stimulation index) in CD14⁺ cells after stimulation with untreated HepG2 lysates (C) or the suspected drug-pulsed HepG2 lysates (D) in patients with cholestatic type liver injury. NS: Not significant.

gion were significantly associated with amoxicillin-clavulanate-DILI.

Recently, activation of T-cells with a particular HLA genotype has been explored in patients with DILI. Flu-cloxacillin (Flux)-induced liver injury has been strongly associated with the HLA-B*5701 allele, with approximately 85% of these patients having at least one copy of HLA-B*5701^[48]. Flux has been reported to activate naïve CD8⁺ T-cells when DCs present the drug antigen in patients with HLA-B*5701. Following drug stimulation, T-cells expressing CCR4 and CCR9 were found to secrete IFN- γ , T-helper 2 cytokines, perforin, granzyme B, and FasL^[49], with the activation of CD8⁺ T cells restricted to those with the HLA-B*5701 allele^[50]. Hypersensitivity reactions without the formation of conjugates, in which drugs can activate the immune system directly and phar-

macologically, have been observed in these types of allergic reaction^[51,52], consistent with earlier results^[37]. These findings suggest that the HLA genotype and its SNPs contribute significantly to susceptibility to DILI, possibly through abnormal antigen presentation to T cells.

Collectively, various immunological mechanisms, including innate and acquired immunity, are involved in the pathogenesis of DILI. The balance between pro- and anti-inflammatory cytokines may affect the onset and severity of DILI.

IMMUNOLOGICAL DIAGNOSIS OF DRUG-INDUCED LIVER INJURY

Because there are no standard criteria for the diagnosis

of DILI, various clinical scales have been developed. The Naranjo Adverse Drug Reactions Probability Scale (NADRPS) was proposed for the assessment of adverse drug reactions in 1981^[53]. The NADRPS has been widely used to diagnose DILI due to its simplicity and wide applicability, despite it not being developed specifically for the diagnosis of DILI. The Roussel Uclaf Causality Assessment Method (RUCAM) diagnostic scale, first proposed in 1993^[2], has been used to classify the pattern of liver injury into hepatocellular, cholestatic, or mixed type. The RUCAM is based on seven criteria, including temporal relationship, clinical course (response after withdrawal of drug), risk factors, concomitant drugs, exclusion of other non-drug etiologies, likelihood of a reaction based on package labeling, and re-challenge. This method has been widely used as a standardized scale with high reliability, reproducibility and specificity.

The more recently described Maria and Victorino clinical diagnostic scale (CDS) simplified the RUCAM scale by using only five criteria^[54]. This scale emphasizes immunoallergic reactions, such as extrahepatic manifestations^[55]. Lymphocyte proliferation in response to drugs was observed in over 50% of patients with DILI^[54]. In Japan, the diagnostic scale similar to the RUCAM scale includes drug-lymphocyte stimulation tests (DLST) in the diagnosis^[56]. Thus, although the diagnosis of DILI is mainly dependent on the course of the disease and the causative drug, some immunological methods have been utilized in its diagnosis because immunological mechanisms are involved in its pathogenesis.

Although these methods of causality assessment for DILI are useful in most of the clinical setting, there may be some pitfalls in the diagnosis of DILI induced by herbal medicine^[57,58]. Hepatotoxicity due to herbs has not been fully recognized and the risk may be underestimated. There is a variety of the quality of herbal products, which may makes the evaluation of the causality for DILI by herbs complicated^[58]. Furthermore, various extracts from herbs have been reported to have immunomodulatory effects *via* cytokine production, toll-like receptor binding, and induction of signal transduction *via* T-cell receptor or dendritic cell maturation^[59-62], all of which could be associated with the immunological basis for DILI caused by herbs.

One of the most popular additional diagnostic tests for DILI is the DLST^[63]. In this test, lymphocytes collected from the heparinized peripheral blood of patients are incubated with dilutions of the suspected drug, with lymphocyte proliferation evaluated by ³H-thymidine uptake. DLST is widely used in Japan and is incorporated in Japanese diagnostic criteria for DILI (DDW-J scale). However, the sensitivity of this test is below 50% and the lymphocyte response to the suspected drug may not necessarily be related to the liver injury. Another test using the peripheral blood of patients is the leukocyte migration test (LMT), which has been reported more useful than DLST^[64]. This test measures the chemotaxis of granulocytes in response to chemotactic factors pro-

duced by mononuclear cells after incubation with the suspected drug. Furthermore, a cytokine production test showed high diagnostic sensitivity^[36]. In this analysis, a mixture of a HepG2 cell extract and culture medium, which retain metabolic enzyme activities such as CYP450 are incubated with dilutions of the suspected drug, followed by incubation with peripheral blood lymphocytes isolated from patients suspected of having DILI. Intracytoplasmic cytokines, such as IFN- γ , TNF- α and IL-2, of the lymphocytes are finally evaluated by flow cytometry. Although these tests are useful for the diagnosis or identification of a single causative drug, they are not simple to perform and may not be suitable for routine examinations.

Thus, immunological methods detecting lymphocyte reactivity against a causative drug or its derivatives may be helpful for the diagnosis of DILI. At this time, however, this method is not applicable to all types of DILI.

IMMUNOLOGICAL TREATMENT OF DRUG-INDUCED LIVER INJURY

The main treatment strategy for DILI is cessation of the causative drug. Early recognition of the adverse effects of a drug is most important in managing DILI and in preventing severe liver injury. Although few specific treatments for DILI have proven beneficial, there are two exceptions; N-acetylcysteine for acetaminophen toxicity and L-carnitine for valproic acid overdose^[65,66].

Patients should be assessed carefully by serial biochemical tests. Severe hepatocellular injury may develop into acute liver failure, and the only effective therapy for the latter may be liver transplantation. Patients with severe liver injury, particularly those with jaundice, should be managed carefully and considered for referral to a liver transplant specialist. Some treatments that can modulate immune functions have been tried in clinical settings, although there is no consensus on their use.

Corticosteroids are of unproven benefit for DILI, but may be used to treat patients with hypersensitivity reactions^[67]. The combination of a corticosteroid and ursodeoxycholic acid has been reported safe for patients with DILI, leading to a more rapid reduction in bilirubin and transaminases^[68].

CONCLUSION

Various immunological mechanisms are involved in the pathogenesis of the unpredictable type of DILI. To date, however, analyses of these mechanisms have been unsatisfactory, and additional studies are required to better understand the pathogenesis of DILI and its diagnosis, to predict the extent of injury in each subject and to manage these patients.

REFERENCES

- 1 Verma S, Kaplowitz N. Diagnosis, management and pre-

- vention of drug-induced liver injury. *Gut* 2009; **58**: 1555-1564 [PMID: 19834119 DOI: 10.1136/gut.2008.163675]
- 2 **Danan G**, Benichou C. Causality assessment of adverse reactions to drugs--I. A novel method based on the conclusions of international consensus meetings: application to drug-induced liver injuries. *J Clin Epidemiol* 1993; **46**: 1323-1330 [PMID: 8229110]
 - 3 **Kaplowitz N**. Drug-induced liver injury. *Clin Infect Dis* 2004; **38** Suppl 2: S44-S48 [PMID: 14986274]
 - 4 **Andrade RJ**, Robles M, Ulzurrun E, Lucena MI. Drug-induced liver injury: insights from genetic studies. *Pharmacogenomics* 2009; **10**: 1467-1487 [PMID: 19761370 DOI: 10.2217/pgs.09.111]
 - 5 **Liu ZX**, Kaplowitz N. Immune-mediated drug-induced liver disease. *Clin Liver Dis* 2002; **6**: 755-774 [PMID: 12362579]
 - 6 **Kaplowitz N**. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov* 2005; **4**: 489-499 [PMID: 15931258]
 - 7 **Raucy JL**, Lasker JM, Lieber CS, Black M. Acetaminophen activation by human liver cytochromes P450IIE1 and P450IA2. *Arch Biochem Biophys* 1989; **271**: 270-283 [PMID: 2729995]
 - 8 **Laverty HG**, Antoine DJ, Benson C, Chaponda M, Williams D, Kevin Park B. The potential of cytokines as safety biomarkers for drug-induced liver injury. *Eur J Clin Pharmacol* 2010; **66**: 961-976 [PMID: 20694460 DOI: 10.1007/s00228-010-0862-x]
 - 9 **Ju C**, Reilly TP, Bourdi M, Radonovich MF, Brady JN, George JW, Pohl LR. Protective role of Kupffer cells in acetaminophen-induced hepatic injury in mice. *Chem Res Toxicol* 2002; **15**: 1504-1513 [PMID: 12482232]
 - 10 **Liu ZX**, Govindarajan S, Kaplowitz N. Innate immune system plays a critical role in determining the progression and severity of acetaminophen hepatotoxicity. *Gastroenterology* 2004; **127**: 1760-1774 [PMID: 15578514]
 - 11 **Martin-Murphy BV**, Kominsky DJ, Orlicky DJ, Donohue TM, Ju C. Increased susceptibility of natural killer T-cell-deficient mice to acetaminophen-induced liver injury. *Hepatology* 2013; **57**: 1575-1584 [PMID: 23150232 DOI: 10.1002/hep.26134]
 - 12 **Goldin RD**, Ratnayaka ID, Breach CS, Brown IN, Wickramasinghe SN. Role of macrophages in acetaminophen (paracetamol)-induced hepatotoxicity. *J Pathol* 1996; **179**: 432-435 [PMID: 8869293]
 - 13 **Holt MP**, Cheng L, Ju C. Identification and characterization of infiltrating macrophages in acetaminophen-induced liver injury. *J Leukoc Biol* 2008; **84**: 1410-1421 [PMID: 18713872 DOI: 10.1189/jlb.0308173]
 - 14 **Gardner CR**, Laskin JD, Dambach DM, Sacco M, Durham SK, Bruno MK, Cohen SD, Gordon MK, Gerecke DR, Zhou P, Laskin DL. Reduced hepatotoxicity of acetaminophen in mice lacking inducible nitric oxide synthase: potential role of tumor necrosis factor- α and interleukin-10. *Toxicol Appl Pharmacol* 2002; **184**: 27-36 [PMID: 12392966]
 - 15 **Boess F**, Bopst M, Althaus R, Polsky S, Cohen SD, Eugster HP, Boelsterli UA. Acetaminophen hepatotoxicity in tumor necrosis factor/lymphotoxin- α gene knockout mice. *Hepatology* 1998; **27**: 1021-1029 [PMID: 9537442]
 - 16 **Ishida Y**, Kondo T, Tsuneyama K, Lu P, Takayasu T, Mukaida N. The pathogenic roles of tumor necrosis factor receptor p55 in acetaminophen-induced liver injury in mice. *J Leukoc Biol* 2004; **75**: 59-67 [PMID: 14557383]
 - 17 **Masson MJ**, Carpenter LD, Graf ML, Pohl LR. Pathogenic role of natural killer T and natural killer cells in acetaminophen-induced liver injury in mice is dependent on the presence of dimethyl sulfoxide. *Hepatology* 2008; **48**: 889-897 [PMID: 18712839]
 - 18 **Ishida Y**, Kondo T, Ohshima T, Fujiwara H, Iwakura Y, Mukaida N. A pivotal involvement of IFN- γ in the pathogenesis of acetaminophen-induced acute liver injury. *FASEB J* 2002; **16**: 1227-1236 [PMID: 12153990]
 - 19 **Cheng L**, You Q, Yin H, Holt MP, Ju C. Involvement of natural killer T cells in halothane-induced liver injury in mice. *Biochem Pharmacol* 2010; **80**: 255-261 [PMID: 20359463 DOI: 10.1016/j.bcp.2010.03.025]
 - 20 **Proctor WR**, Chakraborty M, Chea LS, Morrison JC, Berkson JD, Semple K, Bourdi M, Pohl LR. Eosinophils mediate the pathogenesis of halothane-induced liver injury in mice. *Hepatology* 2013; **57**: 2026-2036 [PMID: 23238640 DOI: 10.1002/hep.26196]
 - 21 **Cho J**, Kim L, Li Z, Rose NR, Talor MV, Njoku DB. Sex bias in experimental immune-mediated, drug-induced liver injury in BALB/c mice: suggested roles for Tregs, estrogen, and IL-6. *PLoS One* 2013; **8**: e61186 [PMID: 23577207 DOI: 10.1371/journal.pone.0061186]
 - 22 **Connolly MK**, Ayo D, Malhotra A, Hackman M, Bedrosian AS, Ibrahim J, Cieza-Rubio NE, Nguyen AH, Henning JR, Dorvil-Castro M, Pachter HL, Miller G. Dendritic cell depletion exacerbates acetaminophen hepatotoxicity. *Hepatology* 2011; **54**: 959-968 [PMID: 21574173 DOI: 10.1002/hep.24429]
 - 23 **Bourdi M**, Masubuchi Y, Reilly TP, Amouzadeh HR, Martin JL, George JW, Shah AG, Pohl LR. Protection against acetaminophen-induced liver injury and lethality by interleukin 10: role of inducible nitric oxide synthase. *Hepatology* 2002; **35**: 289-298 [PMID: 11826401]
 - 24 **Yohe HC**, O'Hara KA, Hunt JA, Kitzmiller TJ, Wood SG, Bement JL, Bement WJ, Szakacs JG, Wrighton SA, Jacobs JM, Kostrubsky V, Sinclair PR, Sinclair JF. Involvement of Toll-like receptor 4 in acetaminophen hepatotoxicity. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1269-G1279 [PMID: 16439473]
 - 25 **Imaeda AB**, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Sutterwala FS, Flavell RA, Mehal WZ. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. *J Clin Invest* 2009; **119**: 305-314 [PMID: 19164858 DOI: 10.1172/JCI35958]
 - 26 **Higuchi S**, Kobayashi M, Yoshikawa Y, Tsuneyama K, Fukami T, Nakajima M, Yokoi T. IL-4 mediates dicloxacillin-induced liver injury in mice. *Toxicol Lett* 2011; **200**: 139-145 [PMID: 21094227 DOI: 10.1016/j.toxlet.2010.11.006]
 - 27 **Yano A**, Higuchi S, Tsuneyama K, Fukami T, Nakajima M, Yokoi T. Involvement of immune-related factors in diclofenac-induced acute liver injury in mice. *Toxicology* 2012; **293**: 107-114 [PMID: 22285467 DOI: 10.1016/j.tox.2012.01.008]
 - 28 **Fredriksson L**, Herpers B, Benedetti G, Matadin Q, Puigvert JC, de Bont H, Dragovic S, Vermeulen NP, Commandeur JN, Danen E, de Graauw M, van de Water B. Diclofenac inhibits tumor necrosis factor- α -induced nuclear factor- κ B activation causing synergistic hepatocyte apoptosis. *Hepatology* 2011; **53**: 2027-2041 [PMID: 21433042 DOI: 10.1002/hep.24314]
 - 29 **Aithal GP**, Ramsay L, Daly AK, Sonchit N, Leathart JB, Alexander G, Kenna JG, Caldwell J, Day CP. Hepatic adducts, circulating antibodies, and cytokine polymorphisms in patients with diclofenac hepatotoxicity. *Hepatology* 2004; **39**: 1430-1440 [PMID: 15122773]
 - 30 **Obermayer-Straub P**, Manns MP. Immunological mechanisms in liver injury. In: Drug-induced liver disease, 1st ed. In: Kaplowitz N, DeLeve LD, editors. New York: Marcel Dekker, 2003: 125-149
 - 31 **James LP**, Simpson PM, Farrar HC, Kearns GL, Wasserman GS, Blumer JL, Reed MD, Sullivan JE, Hinson JA. Cytokines and toxicity in acetaminophen overdose. *J Clin Pharmacol* 2005; **45**: 1165-1171 [PMID: 16172181]
 - 32 **Berry PA**, Antoniadis CG, Hussain MJ, McPhail MJ, Bernal W, Vergani D, Wendon JA. Admission levels and early changes in serum interleukin-10 are predictive of poor outcome in acute liver failure and decompensated cirrhosis. *Liver Int* 2010; **30**: 733-740 [PMID: 20456041 DOI: 10.1111/j.1478-3231.2010.02219.x]
 - 33 **Li J**, Zhu X, Liu F, Cai P, Sanders C, Lee WM, Uetrecht J.

- Cytokine and autoantibody patterns in acute liver failure. *J Immunotoxicol* 2010; **7**: 157-164 [PMID: 20039781 DOI: 10.3109/15476910903501748]
- 34 **Adams DH**, Ju C, Ramaiah SK, Uetrecht J, Jaeschke H. Mechanisms of immune-mediated liver injury. *Toxicol Sci* 2010; **115**: 307-321 [PMID: 20071422 DOI: 10.1093/toxsci/kfq009]
 - 35 **Kakisaka K**, Takikawa Y. Elevation of serum cytokines preceding elevation of liver enzymes in a case of drug-induced liver injury. *Hepatol Res* 2013; **44**: E284-E289 [PMID: 24119001 DOI: 10.1111/hepr.12253]
 - 36 **Murata H**, Shimizu Y, Okada K, Higuchi K, Watanabe A. Detection and analysis of intracytoplasmic cytokines in peripheral blood mononuclear cells in patients with drug-induced liver injury. *J Hepatol* 2003; **38**: 573-582 [PMID: 12713867]
 - 37 **Pachkoria K**, Lucena MI, Crespo E, Ruiz-Cabello F, Lopez-Ortega S, Fernandez MA, Romero-Gomez M, Madrazo A, Durán JA, de Dios AM, Borraz Y, Navarro JM, Andrade RJ. Analysis of IL-10, IL-4 and TNF-alpha polymorphisms in drug-induced liver injury (DILI) and its outcome. *J Hepatol* 2008; **49**: 107-114 [PMID: 18485518 DOI: 10.1016/j.jhep.2008.03.017]
 - 38 **Liang X**, Zhang J, Zhu Y, Lu Y, Zhou X, Wang Z, Yu J, Yan Y, Di L, Che L, Jiang H, Shao B, Wang X, Yang H, Lysterly HK, Ren J. Specific genetic polymorphisms of IL10-592 AA and IL10-819 TT genotypes lead to the key role for inducing docetaxel-induced liver injury in breast cancer patients. *Clin Transl Oncol* 2013; **15**: 331-334 [PMID: 23143946 DOI: 10.1007/s12094-012-0936-6]
 - 39 **Vergani D**, Mieli-Vergani G, Alberti A, Neuberger J, Edleston AL, Davis M, Williams R. Antibodies to the surface of halothane-altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. *N Engl J Med* 1980; **303**: 66-71 [PMID: 6991940]
 - 40 **Bourdi M**, Tinell M, Beaune PH, Pessayre D. Interactions of dihydralazine with cytochromes P4501A: a possible explanation for the appearance of anti-cytochrome P4501A2 autoantibodies. *Mol Pharmacol* 1994; **45**: 1287-1295 [PMID: 8022422]
 - 41 **Lecoeur S**, André C, Beaune PH. Tienilic acid-induced autoimmune hepatitis: anti-liver and-kidney microsomal type 2 autoantibodies recognize a three-site conformational epitope on cytochrome P4502C9. *Mol Pharmacol* 1996; **50**: 326-333 [PMID: 8700140]
 - 42 **Endo S**, Toyoda Y, Fukami T, Nakajima M, Yokoi T. Stimulation of human monocytic THP-1 cells by metabolic activation of hepatotoxic drugs. *Drug Metab Pharmacokinet* 2012; **27**: 621-630 [PMID: 22785256]
 - 43 **Hautekeete ML**, Horsmans Y, Van Waeyenberge C, Demanet C, Henrion J, Verbist L, Brenard R, Sempoux C, Michielsen PP, Yap PS, Rahier J, Geubel AP. HLA association of amoxicillin-clavulanate-induced hepatitis. *Gastroenterology* 1999; **117**: 1181-1186 [PMID: 10535882]
 - 44 **O'Donohue J**, Oien KA, Donaldson P, Underhill J, Clare M, MacSween RN, Mills PR. Co-amoxiclav jaundice: clinical and histological features and HLA class II association. *Gut* 2000; **47**: 717-720 [PMID: 11034591]
 - 45 **Donaldson PT**, Daly AK, Henderson J, Graham J, Pirmohamed M, Bernal W, Day CP, Aithal GP. Human leucocyte antigen class II genotype in susceptibility and resistance to co-amoxiclav-induced liver injury. *J Hepatol* 2010; **53**: 1049-1053 [PMID: 20800921 DOI: 10.1016/j.jhep.2010.05.033]
 - 46 **Andrade RJ**, Lucena MI, Alonso A, García-Cortes M, García-Ruiz E, Benítez R, Fernández MC, Pelaez G, Romero M, Corpas R, Durán JA, Jiménez M, Rodrigo L, Nogueras F, Martín-Vivaldi R, Navarro JM, Salmerón J, de la Cuesta FS, Hidalgo R. HLA class II genotype influences the type of liver injury in drug-induced idiosyncratic liver disease. *Hepatology* 2004; **39**: 1603-1612 [PMID: 15185301]
 - 47 **Lucena MI**, Molokhia M, Shen Y, Urban TJ, Aithal GP, Andrade RJ, Day CP, Ruiz-Cabello F, Donaldson PT, Stephens C, Pirmohamed M, Romero-Gomez M, Navarro JM, Fontana RJ, Miller M, Groome M, Bondon-Guitton E, Conforti A, Stricker BH, Carvajal A, Ibanez L, Yue QY, Eichelbaum M, Floratos A, Pe'er I, Daly MJ, Goldstein DB, Dillon JF, Nelson MR, Watkins PB, Daly AK. Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. *Gastroenterology* 2011; **141**: 338-347 [PMID: 21570397 DOI: 10.1053/j.gastro.2011.04.001]
 - 48 **Daly AK**, Donaldson PT, Bhatnagar P, Shen Y, Pe'er I, Floratos A, Daly MJ, Goldstein DB, John S, Nelson MR, Graham J, Park BK, Dillon JF, Bernal W, Cordell HJ, Pirmohamed M, Aithal GP, Day CP. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet* 2009; **41**: 816-819 [PMID: 19483685 DOI: 10.1038/ng.379]
 - 49 **Monshi MM**, Faulkner L, Gibson A, Jenkins RE, Farrell J, Earnshaw CJ, Alfrevic A, Cederbrant K, Daly AK, French N, Pirmohamed M, Park BK, Naisbitt DJ. Human leukocyte antigen (HLA)-B*57: 01-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury. *Hepatology* 2013; **57**: 727-739 [PMID: 22987284 DOI: 10.1002/hep.26077]
 - 50 **Wuillemin N**, Adam J, Fontana S, Krähenbühl S, Pichler WJ, Yerly D. HLA haplotype determines hapten or p-i T cell reactivity to flucloxacillin. *J Immunol* 2013; **190**: 4956-4964 [PMID: 23596311 DOI: 10.4049/jimmunol.1202949]
 - 51 **Pichler WJ**. The p-i Concept: Pharmacological Interaction of Drugs With Immune Receptors. *World Allergy Organ J* 2008; **1**: 96-102 [PMID: 23282405 DOI: 10.1097/WOX.0b013e3181778282]
 - 52 **Pichler WJ**, Naisbitt DJ, Park BK. Immune pathomechanism of drug hypersensitivity reactions. *J Allergy Clin Immunol* 2011; **127**: S74-S81 [PMID: 21354503 DOI: 10.1016/j.jaci.2010.11.048]
 - 53 **Naranjo CA**, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janacek E, Domecq C, Greenblatt DJ. A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981; **30**: 239-245 [PMID: 7249508]
 - 54 **Maria VA**, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. *Hepatology* 1997; **26**: 664-669 [PMID: 9303497]
 - 55 **Kaplowitz N**. Causality assessment versus guilt-by-association in drug hepatotoxicity. *Hepatology* 2001; **33**: 308-310 [PMID: 11124850]
 - 56 **Watanabe M**, Shibuya A. Validity study of a new diagnostic scale for drug-induced liver injury in Japan-comparison with two previous scales. *Hepatol Res* 2004; **30**: 148-154 [PMID: 15588780]
 - 57 **Teschke R**, Frenzel C, Schulze J, Eickhoff A. Herbal hepatotoxicity: challenges and pitfalls of causality assessment methods. *World J Gastroenterol* 2013; **19**: 2864-2882 [PMID: 23704820 DOI: 10.3748/wjg.v19.i19.2864]
 - 58 **Teschke R**, Wolff A, Frenzel C, Schwarzenboeck A, Schulze J, Eickhoff A. Drug and herb induced liver injury: Council for International Organizations of Medical Sciences scale for causality assessment. *World J Hepatol* 2014; **6**: 17-32 [DOI: 10.4254/wjh.v6.i1.17]
 - 59 **Burns JJ**, Zhao L, Taylor EW, Spelman K. The influence of traditional herbal formulas on cytokine activity. *Toxicology* 2010; **278**: 140-159 [PMID: 19818374 DOI: 10.1016/j.tox.2009.09.020]
 - 60 **Liang Q**, Wu Q, Jiang J, Duan J, Wang C, Smith MD, Lu H, Wang Q, Nagarkatti P, Fan D. Characterization of sparstonin B, a Chinese herb-derived compound, as a selective Toll-like receptor antagonist with potent anti-inflammatory properties. *J Biol Chem* 2011; **286**: 26470-26479 [PMID: 21665946 DOI: 10.1074/jbc.M111.227934]
 - 61 **Reid-Adam J**, Yang N, Song Y, Cravedi P, Li XM, Heeger P. Immunosuppressive effects of the traditional Chinese herb Qu Mai on human alloreactive T cells. *Am J Transplant* 2013;

- 13: 1159-1167 [PMID: 23433080 DOI: 10.1111/ajt.12180]
- 62 **Kim ME**, Kim HK, Park HY, Kim DH, Chung HY, Lee JS. Baicalin from *Scutellaria baicalensis* impairs Th1 polarization through inhibition of dendritic cell maturation. *J Pharmacol Sci* 2013; **121**: 148-156 [PMID: 23419270]
- 63 **Takikawa H**, Takamori Y, Kumagi T, Onji M, Watanabe M, Shibuya A, Hisamochi A, Kumashiro R, Ito T, Mitsumoto Y, Nakamura A, Sakaguchi T. Assessment of 287 Japanese cases of drug induced liver injury by the diagnostic scale of the International Consensus Meeting. *Hepatol Res* 2003; **27**: 192-195 [PMID: 14585395]
- 64 **Usui K**, Oda Y, Kubota R, Negishi K, Uno K, Tsunematsu S, Kumagai N, Komiyama T. Clinical application of the leukocyte migration test and new diagnostic criteria for identifying causative agents in patients with drug-induced liver injury. *Hepato-gastroenterology* 2007; **54**: 1752-1757 [PMID: 18019711]
- 65 **Polson J**, Lee WM. AASLD position paper: the management of acute liver failure. *Hepatology* 2005; **41**: 1179-1197 [PMID: 15841455]
- 66 **Bohan TP**, Helton E, McDonald I, König S, Gazitt S, Sugimoto T, Scheffner D, Cusmano L, Li S, Koch G. Effect of L-carnitine treatment for valproate-induced hepatotoxicity. *Neurology* 2001; **56**: 1405-1409 [PMID: 11376200]
- 67 **Giannattasio A**, D'Ambrosi M, Volpicelli M, Iorio R. Steroid therapy for a case of severe drug-induced cholestasis. *Ann Pharmacother* 2006; **40**: 1196-1199 [PMID: 16720710]
- 68 **Wree A**, Dechêne A, Herzer K, Hilgard P, Syn WK, Gerken G, Canbay A. Steroid and ursodesoxycholic Acid combination therapy in severe drug-induced liver injury. *Digestion* 2011; **84**: 54-59 [PMID: 21304237 DOI: 10.1159/000322298]

P- Reviewer: Han T, He JY, Suk KT **S- Editor:** Wen LL
L- Editor: A **E- Editor:** Lu YJ





Apoptotic signaling through reactive oxygen species in cancer cells

Daejin Kim, Ga Bin Park, Dae Young Hur

Daejin Kim, Ga Bin Park, Dae Young Hur, Department of Anatomy and Research Center for Tumor Immunology, Inje University College of Medicine, Busan 614-735, South Korea
Author contributions: Kim D and Park GB designed the review and wrote the paper; Hur DY contributed to the editing of whole review.

Correspondence to: Dae Young Hur, MD, PhD, Department of Anatomy and Research Center for Tumor Immunology, Inje University College of Medicine, Bokjiro-75, Jin-gu, Busan 614-735, South Korea. dyhur@inje.ac.kr
Telephone: +82-51-8906796 Fax: +82-51-8966634
Received: March 27, 2014 Revised: June 3, 2014
Accepted: October 14, 2014
Published online: November 27, 2014

Abstract

Reactive oxygen species (ROS) take part in diverse biological processes like cell growth, programmed cell death, cell senescence, and maintenance of the transformed state through regulation of signal transduction. Cancer cells adapt to new higher ROS circumstance. Sometimes, ROS induce cancer cell proliferation. Meanwhile, elevated ROS render cancer cells vulnerable to oxidative stress-induced cell death. However, this prominent character of cancer cells allows acquiring a resistance to oxidative stress conditions relative to normal cells. Activated signaling pathways that increase the level of intracellular ROS in cancer cells not only render up-regulation of several genes involved in cellular proliferation and evasion of apoptosis but also cause cancer cells and cancer stem cells to develop a high metabolic rate. In over the past several decades, many studies have indicated that ROS play a critical role as the secondary messenger of tumorigenesis and metastasis in cancer from both *in vitro* and *in vivo*. Here we summarize the role of ROS and anti-oxidants in contributing to or preventing cancer. In addition, we review the activated signaling pathways that make cancer cells susceptible to death.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Oxidative stress; Reactive oxygen species; Carcinogenesis; Apoptosis; Signal transduction; Antioxidants

Core tip: Reactive oxygen species originally used to induce injurious cellular effects are now recognized as key physiological molecules for the induction of host defense genes, activation of transcription factors, and regulation of signal transduction. Tumorigenic cells can induce a new redox balance, resulting in cellular adaptation and proliferation. Here, we review the role of oxidative stress in cancer cells using a pathophysiological view.

Kim D, Park GB, Hur DY. Apoptotic signaling through reactive oxygen species in cancer cells. *World J Immunol* 2014; 4(3): 158-173 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i3/158.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i3.158>

INTRODUCTION

The high intracellular ROS levels are related to various human diseases, including neurodegenerative disease^[1-7], inflammatory disease^[8,9], cardiovascular disease^[10,11], immune system dysfunctions^[12], obesity^[13], and diabetes^[14,15]. Survival of tumor cells is greatly dependent on their capacity to control expression of endogenous antioxidants to maintain the upper standard level of ROS below the threshold that will induce tumor cell death^[16,17]. ROS could contribute to the initiation of cancer by accelerating tumorigenic signaling pathways, increasing DNA mutations and changing the activity of the tyrosine phosphatases superfamily^[18-21]. For example, cancer inactivates the tumor suppressor phosphatase and tensin homolog (PTEN) by oxidation^[22,23] and inhibits the

mitogen-activated protein kinase (MAPK) phosphatase by ROS, which in turn induces activation of extracellular signal-regulated kinases (ERK). Although greater oxidative stresses activate nuclear factor-kappa B (NF- κ B) for growth or survival, high intracellular ROS levels also lead to activation of c-Jun N-terminal kinase (JNK) and p38 kinases, and their activities often facilitate cell apoptosis^[24,25]. Normally, the inhibition of PTEN by ROS activates the phosphoinositide-3 kinase (PI3K)/Akt signaling pathway and blocks cell apoptosis^[26,27]. In contrast to the apoptotic death, necrosis induces *via* mitochondrial production of ROS after signaling from tumor necrosis factor- α (TNF- α) or death receptor^[28,29]. Interestingly, apoptotic cells inhibit ERK1/2 but induce p38 and JNK inside macrophage, while necrotic cells induce macrophage ERK1/2^[30-32]. ROS-mediated signaling has received more attention in oncological studies than ROS-mediated cellular stress and damage of cancer cells. In this article, we present and summarize the interaction between redox status or redox signaling systems and apoptosis in tumor cell death and anti-cancer treatments.

ROS CELLULAR SOURCES AND DETOXIFICATION

ROS are mainly generated from mitochondrial electron transfer complex (ETC) during the reduction of oxygen. Superoxide anion ($\cdot\text{O}_2^-$) generated by O_2 from the mitochondrial electron transport chain, which is usually changed into hydrogen peroxide (H_2O_2) by several cytoprotective enzymes, including superoxide dismutase (SOD)^[33,34]. Although scientists are now considering the consequences of different levels of oxidative stress, ROS formation in cells can inflict serious hazards and was originally known for their ability to induce injurious cellular effects.

Sources of oxidative stress (internal and external)

Reactive oxygen species (ROS) are the most abundantly produced oxygen species in mitochondria. Reactive nitrogen species (RNS) are also produced during intracellular metabolic processes in mitochondrial ETC. Extracellular ROS can be also found in a variety of natural or acquired environment. NAD(P)H oxidase (NOX) can be found in cell membrane phagosomes in neutrophil. The NOX complex is composed of seven members, NOX1-5, and two dual oxidases (Duox), Duox1 and Duox2^[35]. Although activation mechanisms and tissue distribution are significantly different, all these enzymes, including cytochrome c oxidase and cyclo-oxygenase (COX) are able to generate superoxide anion^[36,37]. Nitric oxide (NO) is produced from arginine catalyzed by a nitric oxide synthase (NOS). Fast reaction between $\cdot\text{O}_2^-$ and NO gives rise to peroxynitrite (ONOO^-) and ONOO^- is oxidizing molecule that connected to cancer. NO is finally converted into a hydroxyl radical and nitrite anion (NO_2^-)^[38,39]. Numerous agents, including anti-cancer drugs, have been shown to induce proliferation or apoptosis through ROS

production in various cancer types. Low sodium arsenite induces MCF-7 epithelial breast cancer cell proliferation by ROS production, activation of NF- κ B, and increases in c-Myc and heme oxygenase-1 (HO-1)^[40]. ROS-enhancing compound, such as piperlongumine, is insufficient to induce death of cancer cell lines including osteosarcoma cells, breast, and glioblastoma cancer cells, but not in normal cells^[41-43].

Natural defense mechanisms of antioxidants

Although the ROS levels modestly increases in tumorigenic cells, intracellular ROS is maintained below a toxic level in normal cells by various scavengers and antioxidative enzymes. Besides mitochondrial superoxide dismutases (SODs), catalase, glutathione (GSH), peroxidase (GPx), and peroxiredoxin also modulate oxidative status^[44]. SODs are metalloenzymes which catalyze the dismutation of $\cdot\text{O}_2^-$ to O_2 and H_2O_2 . They ubiquitously exist in eukaryotes and prokaryotes. SODs also play a critical role in inhibiting oxidative inactivation of NO, thereby preventing ONOO^- formation and mitochondrial dysfunction^[45]. SODs utilize metal ions such as copper (Cu^{2+}), zinc (Zn^{2+}), manganese (Mn^{2+}) or iron (Fe^{2+}) as cofactors. Ferric ions catalyze hydrogen peroxide, which is the Fenton reaction^[46]. Catalase, which is located in peroxisomes, facilitates the decomposition of H_2O_2 to water and oxygen and protects cells from H_2O_2 produced within the cell^[46]. GPx catalyzes the reduction of hydrogen peroxide using cellular GSH as the reducing reagent. GPx converts H_2O_2 to H_2O + O_2 ^[47]. Peroxiredoxins are thioredoxin peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides and peroxynitrite^[48]. In neutrophil, many species of bacteria are killed readily by a myeloperoxidase/hydrogen peroxide/chloride system. HOCl, oxidizing chloride ions, is the most bactericidal oxidant produced by myeloperoxidase^[49].

THE ROLE OF ROS IN CANCER CELLS

Besides, genetic factors have important roles in the transforming events that lead to carcinogenesis. The enhanced oxidative stress is generally associated with cancer promotion and progression. Meanwhile, high levels of ROS are less harmful in cancer cells than they would be in normal cells because cancer cells have developed mechanisms to keep themselves from intrinsic oxidative stress through regulation of antioxidant functions and pro-survival molecules^[16,17]; however, oxidative stress still has a negative impact on various types of cancer cells as well^[50,51]. The identification of specific alterations in critical cellular components by ROS can provide evidences for early detection, prevention of cancer.

ROS in chronic inflammation associated with cancer

Over the several years' studies about the cytokine, inflammatory cells and cytokines found in neoplastic tissues seems to contribute to tumor growth, progression, and immunosuppression. ROS induced by these cells and

cytokines facilitate cancer growth, invasion, and metastasis through DNA damage or inhibition of DNA repair. Chronic inflammation predisposes cells for an oncogenic transformation through overproduction of ROS, increased COX-2, and aberrant NF- κ B expression^[52,53]. Defective mitochondria have also been characterized by excessive ROS production in several chronic human diseases associated with inflammation. ROS derived from mitochondria (mtROS) enhance signaling pathways to produce pro-inflammatory cytokine subsets. mtROS activates NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome-dependent pro-inflammatory cytokine production^[18,54].

Oxidative stress can activate various transcription factors including NF- κ B, activating protein-1 (AP-1), p53, and hypoxia inducible factor-1 α (HIF-1 α). Activation of these transcription factors can result in the expression of numerous different genes, including cytokines and chemokines^[55,56]. Nuclear factor erythroid-related factor 2 (Nrf2) is one of the master transcription regulators in controlling antioxidant responses. Nrf2 controls the expression of hundreds of genes, including NAD(P)H:quinone oxidoreductase 1 (NQO1), Glutathione S-transferase (GST), GPx and oxidoreductases for inflammatory responses, tissue remodeling and fibrosis, carcinogenesis, and metastasis^[57-59]. Kelch-like protein 1 (Keap1), a suppressor protein anchored in the cytoplasm that physically binds Nrf2, controls the access of Nrf2 to promoters of Antioxidant Response Element (ARE)-regulated antioxidant enzymes^[60,61]. MAPK, PI3K, atypical protein kinase C (PKC), and other pathways are also found as alternative pathways for Nrf2 activation^[62,63]. Importantly, somatic mutations that disrupt the Nrf2-Keap1 interaction are identified in cancer patients. In non-small-cell lung cancer (NSCLC) cells with Keap1 gene mutations, Nrf2 is constitutively activated and cells proliferate independently of epidermal growth factor receptor (EGFR) signaling^[64-66]. Although the above-mentioned studies show the effects of ROS-mediated inflammation in carcinogenesis, It is contrast with blockade of NF- κ B predisposes murine skin to squamous cell carcinoma. RelA subunit of NF- κ B has tumor suppressing activity under some circumstances^[67-71]. Because NF- κ B is modulated by ROS, the effects of ROS on carcinogenesis may be unfavorable to certain type of cells and conditions.

Role of ROS in mitochondrial DNA mutations

Although the significance of ROS and antioxidant systems in carcinogenesis is still controversial, substantial evidence suggests that an increase of intracellular ROS might contribute to carcinogenesis^[72-74]. ROS also might stimulate the expansion of initiated cell clones through stimulation of cell proliferation and suppression of apoptosis^[72]. The involvement of mitochondria in disease has been largely recognized to their essential role in production of ROS and to the damaging effect of chemical agents or pathological conditions on these organelles^[33,52]. Recently, several studies have reported that tumorigenic

mitochondrial DNA (mtDNA) mutations affect respiratory chain complexes. Decreased mitochondrial activity is considered to be tumorigenic, mainly because of the enhanced ROS production. H₂O₂ exported to the nucleus enhances the transcription of selected genes that favor tumor progression^[75,76]. Depletion of mtDNA, especially encoded OXPHOS genes, plays a key role in transformation of breast epithelial cells. Breast epithelial cells results in *in vitro* tumorigenic phenotype as well as breast tumorigenesis in a xenograft model^[77]. Claudin-1 and 7 in p53 network of breast epithelial cells are down-regulated in tumorigenesis^[77]. In humans, mtDNA mutations coding (ND1, ND4, ND5, and cytochrome b genes) or noncoding regions are frequently detected in breast cancer tissue^[78,79]. However, the pathological relevance of mtDNA mutations in cancer cells is still controversial^[80]. Nonetheless, a clear-cut correlation between the occurrence of pathogenic mtDNA mutations and mitochondrial energetic impairment is a well-demonstrated feature of oncocyomas, characterized by disruptive mutations of mtDNA, especially in complex I subunits^[81]. Initial enhanced ROS generation may induce supercomplex disorganization, eventually leading to a possible decrease of complex I assembly^[82]. Dissociation of the supercomplex might further induce ROS generation and have harmful consequences, such as disassembly of complex I and III^[83,84]. However, ROS measurements in tumor biopsies are not practicable currently in oncocyoma. Further studies are needed to understand whether ROS may influence the proliferative potential and accumulation of mutations in oncocyoma. Because elevated ROS have been proposed to induce apoptosis, additional studies are required to determine the role of apoptosis in regulating the survival and proliferation of oncocyotic cells. It has been reported that the cell line carrying the heteroplasmic ND5 mtDNA mutation showed progressively decline of respiratory function and significantly enhancement of dependence on glucose in tumor growth, while cells with homoplasmic ND5 mutation inhibited tumor formation^[85,86].

SIGNALING PATHWAYS REGULATED BY ROS IN CANCER

ROS-related pathways are considerably activated in many types of cancers. In particular, transient formation of H₂O₂, as second messengers, participates in growth, proliferation, and metabolism^[87]. The level of intracellular ROS has a considerable influence on various signal pathways, including MAPK signaling cascades^[88,89], PI3K/Akt signaling cascades^[90,91], and I κ B kinase/NF- κ B signaling pathway^[40,92]. Oxidative stress-mediated signaling involves all characters of cancer cell behaviors, such as cell survival, apoptosis, energy metabolism, angiogenesis, metastasis, and cancer stem cell generation. Researchers have noticed that some cancer cells show death or arrested growth when exposed to increased ROS, whereas others are able to eliminate even high levels of ROS for survival^[93,94]. Emerging research indicates that modest in-

creases in ROS are oncogenic, whereas dramatic increases in ROS seem to suppress tumors^[95]. GTPase Rac1 in the cytoplasm activates NF- κ B and markedly blocks the activity of caspase-3 and TNF-induced apoptosis, whereas mitochondria-derived ROS promote TNF-induced apoptosis^[96]. ROS generated by the newly described NOX5 are essential for prostate cancer growth^[97]. NOX4-mediated ROS generation in extracellular matrix of cancer partially transfers cell survival signals through the Akt/apoptosis signal-regulating kinase 1 (ASK1) pathway in pancreatic cancer cells^[98]. Inhibiting ROS with the antioxidants, NOX4 antisense, or MnSOD overexpression efficiently stimulates apoptosis in pancreatic cancer cells^[99]. ROS produced by NADPH oxidase also inhibit protein tyrosine phosphatases (PTPs) and sustain the activation of Janus kinase 2 (Jak2)^[91,100].

Proliferation and survival

The high intracellular ROS levels in cancer cells are largely the byproducts of the highly metabolic nature of these cells. These ROS levels could be protumorigenic, but also increase the susceptibility of cancer cells to cell death. High levels of ROS in cancer cells indicate hyperactive PI3K/Akt signaling generated by increased mitochondrial metabolism and by the suppression of antioxidant gene expression, through the inhibition of forkhead box O (FOXO) transcription factor^[101]. In addition to elevating SOD2 and catalase, FOXO induces the expression of Sestrin3^[102,103]. Sestrin3 is a member of a family of proteins that includes Sestrin1 and Sestrin2, which were originally identified as antioxidants induced by the tumor suppressor p53^[104,105]. Thus, the suppression of Sestrins expression in cancer cells could increase intracellular ROS and activate mammalian target of rapamycin complex 1 (mTORC1). ROS produced by reactive oxygen species modulator 1 (Romo1), a mitochondria-localized protein^[106-108], are necessary to the ERK-dependent proliferation of lung cancer cells^[108]. Similarly, high intracellular ROS levels generated by inactivation of antioxidant mechanisms has been connected with increased proliferation of breast^[109] and ovarian^[110] cancer cells.

Methionine sulfoxide reductase A (MsrA), a ROS scavenger, is down-regulated in a number of breast cancers. Moreover, reduction of MsrA levels results in increased ROS levels, which reduces the PTEN activity and activates PI3K pathway and leads to increased cell proliferation and a more aggressive cellular phenotype consequently^[109]. Cancer cells adopt alternative mechanisms of antioxidation in order to maintain the intracellular level of ROS below a toxic threshold level^[109-111]. Forkhead box M1 (FOXO1) is expressed at low levels in normal cells, but its expression is markedly elevated in cancer cells^[111]. FOXO1 controls multiple pro-tumorigenic activities, but also reduces ROS levels through the transcriptional induction of SOD2, catalase, and mitochondrial-dependent peroxide reductase^[112]. In addition, the expression of detoxifying enzymes such as GST and NQO1 are elevated in cancer cells^[113,114]. The high intracellular ROS levels

generated by exogenous administration of ROS enhance the proliferation of several cancer types. Akt activity and cell growth are significantly stimulated by treating hepatoma cells with low concentration of ROS, which could be abolished by adding antioxidants. PI3K inhibitor, wortmannin, inhibits Akt phosphorylation induced by ROS^[115]. In another study, monomethylarsonous acid (MMAIII), ROS inducer, induces proliferation and activation of MAPK pathway as well as up-regulation of COX-2 and EGFR in human urothelium cells^[116]. Generally, COX-2 expression is induced by NF- κ B, not CCAAT/enhancer binding protein (C/EBP) in chronic gastritis and gastric cancer. Sphingosine kinase 1 and Sphingosine-1-phosphate are required for TNF-induced COX-2 induction in lung cancer^[117,118].

Apoptosis and necroptosis

Elevated intracellular ROS levels in cancer cells render these cells more vulnerable to oxidative stress-induced cell death. Therefore, cancer cells can be selectively killed without harming normal cells. Intracellular ROS levels in tumor cells are more likely to reach a threshold that triggers death after exposing to exogenous ROS-producing or -stimulating agents^[119-121]. Apoptosis is prompted through extrinsic and intrinsic pathways^[122]. In the extrinsic pathway, ROS are generated by ligation of cell surface death receptors [CD95(Fas), TNFR1, death receptor 3 (DR3), and DR5]. In turn, death receptor-ligand interaction leads to the subsequently activation of Fas-associated protein with death domain (FADD) and caspase-8^[123-126]. In the intrinsic pathway, apoptosis is induced by mitochondria membrane disruption without involving death receptors. Subsequently, ROS activated by Bcl-2 family members, which are located in the outer mitochondrial membrane make pore. That results in cytochrome c release, apoptosome formation, and activation of caspases-3 and -7^[127]. Exogenous administration of H₂O₂ induces apoptosis in various cancer cells, including lymphoma cells^[128], leukemia cells^[129,130], hepatoma cells^[42,131], and bladder cancer cells^[132] through the activation of MAPK signaling pathways.

ROS have been paid little attention in adaptive immunity because ROS production by the transformed and primary human B cells is very low compared to the levels of ROS are released by phagocytes^[133,134]. However, later studies showed T cell receptor (TCR) or B cell receptor (BCR) engagement elicits ROS production transiently and superoxide controls pro-apoptotic and proliferative signal transduction, respectively^[135,136]. In previous reports, we have shown that ROS might regulate apoptosis of lymphocytes directly or indirectly using EBV-transformed B cells as lymphoma or using an activated B cell model. Engagement of B7-H4, a negative regulator of T-cell mediated responses, induces the high levels of intracellular ROS and the expression of FasL. B7-H4 ligation induces Fas/FasL-mediated apoptosis through activation of caspase. Subsequently, cytochrome c, apoptosis-inducing factor (AIF), and endonuclease G (EndoG) are

released from the mitochondria on EBV-transformed B cells after stimulation of B7-H4^[137]. B7-H1 stimulation in EBV-transformed B cells also induces both transcription and translation of FasL. B7-H1 stimulation activated the phosphorylation of JNK and down-regulated ERK1/2 and p-Akt. N-acetylcysteine (NAC), ROS scavenger, and SP600125 completely blocked the induction of FasL and activation of JNK. B7-H1-mediated apoptosis on EBV-transformed B cells may be involved in the induction of FasL, which is evoked by ROS generation and JNK activation after cross-linking of B7-H1^[138]. Ligation of CD70, the ligand for CD27, expressed on EBV-transformed B cells induced production of ROS and triggered ER stress-mediated apoptosis *via* ROS generation and MAPK pathway activation. These reports suggest that ROS-mediated alternate signaling pathways induce apoptosis and provide information supporting ROS as a target against EBV-related tumors^[139,140]. The present paradigm of cell death is caspase-dependent apoptosis, whereas necroptosis is a regulated through caspase-independent manner^[141-143]. TNF α , FasL, and Trail, the same ligands that can initiate apoptosis also trigger the necroptosis. Receptor-interacting protein-1 (RIP-1) and RIP-3 play a critical role in TNF-induced necroptosis^[144]. TNF-mediated ROS generation and their lethal action are confined to the inner mitochondrial membrane in L929 cells^[145,146]. ROS scavenger butylated hydroxyanisole (BHA) efficiently blocks TNF-induced necroptosis. Interestingly, inhibitors of NF- κ B facilitate TNF-induced necrotic cell death, suggesting that NF- κ B suppresses the necrotic cell death pathway^[147]. However, antioxidant treatment against ROS is unable to protect all cell lines from necroptosis^[141,148]. Recent work revealed the critical role of RIP-3 kinase activity in linking TNFR1/RIP-1-associated events. RIP-3 binds RIP-1 through this unique C-terminal segment to inhibit RIP- and TNF receptor-1-mediated NF- κ B activation and necrostatin-1, RIP-1 kinase inhibitor, prevents RIP-1/RIP-3 interaction from necroptosis^[149,150].

Role of ROS in tumor cell dissemination

ROS mediate induction of matrix metalloproteinases (MMPs) involved in cancer invasion and metastasis. ROS have been reported to cause a significant increase in the production and expression of MMP-7. MMP-7 expression after H₂O₂ exposure is mediated by AP-1-dependent MAPKs in colorectal cancer cells^[151]. ROS also up-regulate Akt and CXCR4 expression as well as inactivating PTEN. ROS mediate CXCR4-dependent cell migration cancer progression in prostate cancer cells^[152]. Meanwhile, Hydrogen peroxide and hydroxyl radical prevent migration of non-small cell lung cancer cell from inhibiting Caveolin-1 down-regulation^[153]. TNF or ROS can induce p38 MAPK- and MMP-9-dependent angiogenesis of endothelial cells^[154,155]. Furthermore, oxidative stress induced by H₂O₂ stimulates angiogenesis and tumor progression by altering the gene expression of CXC chemokine ligand 14 (CXCL14) and IL-8 through the EGFR/MAPK signaling pathway^[156]. EGF treatment induces

H₂O₂ production, leading to activation of the Akt and vascular endothelial growth factor (VEGF) expression for angiogenesis in ovarian cancer cells^[90]. Stimulation of tumor angiogenesis is connected with intracellular level of ROS. ROS regulate HIF-1 α and VEGF expression^[157]. Antioxidant N-acetyl-L-aspartate (NAC) decreased vessels number *via* suppressing HIF-1 α expression in colon^[158], liver^[159] cancer cells. Conditions of energetic stress could lead to oxidative stress. Cancer cells that consume high levels of glucose create energetic stress during the formation of solid tumors. Higher levels of stress may occur when cells detach from the matrix and translocate to the lumen or during metastasis^[160]. Decreased glucose uptake during these processes suppresses ATP production and activates AMP-activated protein kinase (AMPK), but also inhibits the generation of NADPH *via* the pentose phosphate pathway. Reduced levels of NADPH result in increased intracellular ROS, which could eventually cause cell death^[161,162]. However, the concomitant activation of AMPK elicits alternative mechanisms that maintain intracellular NADPH levels.

Several studies have reported that cancer-associated fibroblasts (CAFs) play a critical role in the metastatic spread of cancer cells^[163,164]. ROS-controlled signaling mechanisms involved in myofibroblast differentiation have diverse cellular effects. Recent data from human breast cancers and animal models established that myofibroblasts are derived from bone marrow derived cells such as fibrocytes or mesenchymal stem cells^[165,166]. Moreover, various mesenchymal cell types including endothelial cells, pericytes, or pre-adipocytes can also be converted into myofibroblasts in breast carcinomas as well as local resident fibroblasts^[167-169]. Mitochondrial ROS generation results in expression of NOX4, an enzyme that is required for TGF- β -driven conversion of fibroblasts into myofibroblasts^[170]. In addition, fibroblasts suffering from chronic oxidative stress exhibit properties normally found in myofibroblasts^[171]. Indeed, fibroblasts derived from mouse models exhibiting chronic oxidative stress (such as JunD^{-/-} or NRF-2^{-/-} mice, depleted for key anti-oxidant transcription factors) are converted into myofibroblasts^[172]. Cancer cells themselves produce H₂O₂, which is a highly diffusible species. NOX enzymes, located at the plasma membrane in various carcinomas, might contribute to the production of H₂O₂ and the conversion of surrounding fibroblasts into myofibroblasts^[173]. The increase of ROS in the stromal fibroblasts results in the promotion of tumor cell motility and neo-angiogenesis, further increasing metastatic dissemination^[174,175]. CAFs, similar to fibroblasts exposed to chronic oxidative stress, express genes that encode for proteases involved in extracellular matrix (ECM) remodeling, including collagens, cell adhesion molecules, and MMPs^[171,173]. ROS remodel the ECM and create tracks for collective migration of tumor cells through the activation of Rho-dependent pathways^[176]. Improved understanding of ROS functions in cancer progression or metastasis could therefore help pave the way for new concepts in therapy.

ROS in hypoxia and tumor metabolism

As a cancerous tumor grows, the cancer cells repeatedly face limited oxygen supply due to the imbalance between growth rate and neovascularization. Cancer cells are able to adapt to oxidative stress and switch to glycolysis. Tumors utilize the Warburg effect, relying on glycolysis to supply energy for cancer cell survival^[177]. The ROS released from mitochondria during the hypoxia act as signaling molecules that initiate diverse functional responses^[178]. The increased ROS under low oxygen conditions, HIF-1 α becomes transcriptionally active and accumulates at low levels of manganese (Mn)-containing SOD (Mn-SOD) activity. However, at moderate levels of MnSOD activity, hypoxic induction of VEGF and HIF-1 α protein are suppressed in human breast carcinoma cells. This suggests that superoxide may contribute to accumulation of HIF-1 α ^[179].

HIF-1 α expression has been correlated with poor prognosis and increased cancer cell invasiveness and recent studies have also shown that the antitumorigenic effect of antioxidants is HIF-dependent^[180]. HIF-1 α regulates glycolysis-related genes in response to hypoxia and leads to glycolytic ATP generation^[181,182]. However, it is not yet clear why tumor cells rely on glycolysis in the presence of oxygen and whether cellular ROS involved in regulation of glucose metabolism. ROS Accumulation and HIF-1 stabilization in CAFs results from the down-regulation of sirtuin-3 (SIRT3), a mitochondrial NAD-dependent deacetylase. HIF-1 α can work with SIRT3 to regulate CAF metabolism, driving to metabolic reprogramming towards glycolysis^[183]. In addition, hydrogen peroxide stabilizes HIF-1 α thus leading to transcription of genes that code for signaling stromal cells such as macrophages and fibroblasts to support an invasive tumor cell phenotype. ROS-mediated HIF-1 α helps the tumor cell convert energy production from OXPHOS to glycolysis, a metabolic switch that has been associated with increased metastatic potential^[184].

Generation of cancer stem cells using ROS

Stem cells can be difficult to obtain, which makes it challenging to directly evaluate the role of ROS and the regulatory mechanism in stem cells^[185]. However, interesting research has been conducted using Hematopoietic stem cells (HSCs) in the bone marrow. HSCs are principally located in a low-oxygen environment, which allows long-term protection from ROS-related oxidative stress. The ROS^{low} population has a higher self-renewal potential. In contrast, ROS^{high} population expresses high levels of the activated p38 MAPK and mammalian target of rapamycin (mTOR)^[186]. ROS production and NF- κ B activation triggered by GTPase Rac 1 are critical events for facilitating tumorigenesis after APC loss^[187]. In comparison with cancer cells, cancer stem cells (CSCs) also have a lower intracellular ROS content than non-CSCs, which is similar to HSCs and may be caused by the increased expression of antioxidant systems^[38,188].

ROS^{low} breast cancer cells are predominantly in qui-

escent phase of the cell cycle compared with ROS^{high} cells. The expression of ESR1 or MYC, which are both necessary for MCF7 proliferation in ROS^{low} breast cancer cells do not change^[189]. Since ROS are critical mediators of ionizing radiation induced-therapy and chemotherapy, the expression of antioxidants in CSCs prevented DNA damage and protected cells from irradiation- or drug-induced cell death^[38]. Due to high levels of antioxidant signaling, cancer stem cells also may not be responsive to other (chemotherapeutic) treatments that target cancer cells by increasing intracellular ROS levels^[52]. Niclosamide, antihelminthic agent, increases induces apoptosis through up-regulation of ROS in progenitor/stem cells from acute myelogenous leukemia (AML) patients as well as Niclosamide inhibits the transcription and DNA binding of NF- κ B^[190]. Niclosamide is synergistic with the frontline chemotherapeutic agents, such as cytarabine, etoposide, and daunorubicin^[190]. The peroxisome proliferator-activated receptor γ (PPAR γ) is a ligand-dependent transcription factor belonging to the nuclear hormone receptor superfamily^[191]. PPAR γ agonists inhibit the stem cell-like features and repress tumor growth of human hepatocellular carcinoma (HCC) cells through NOX2-mediated ROS generation^[192]. To date, it is unclear how ROS kill CSCs, although it does appear that ROS levels in normal or cancer environment may influence development and differentiation of stem cells^[193].

ROS in autophagy

Autophagy is activated under stress conditions as protective process for the cell and in various pathological conditions, including cancer and neurodegenerative diseases^[194-196]. One major breakthrough in both the understanding of autophagy regulation and its implication in cancer was the discovery of Beclin-1. Mutation of Beclin-1 is detected in human breast, ovarian, and prostate cancer^[197]. Beclin-1^(-/-) mutant mice die in early embryonic stage and Beclin-1^(+/-) mutant mice have shown the decreased autophagy formation and suffer from a high incidence of spontaneous tumors^[198]. Beclin-1 is the first identified tumor suppressor protein that functions in the lysosomal degradation pathway of autophagy. Bcl-2, a specific inhibitor for apoptosis, inhibits starvation-induced autophagy in cancer cells and mice through interacting with Beclin-1^[199,200]. In addition, oncogenic signaling molecules, including class I PI3K, Akt, and mTOR suppress the macroautophagic pathway. However, PTEN and p53 stimulate autophagy^[201-203]. Autophagy-related genes (Atg) 4, a direct target for oxidation by Intracellular H₂O₂ generated during starvation, is regulated by conjugating Atg8 at the site of autophagosome formation *via* the lipidation of Atg8^[204]. Low levels of ROS modify Atg4 and HMGB1 proteins, which activate AMPK and ASK1/JNK pathways or transactivate various proteins that could up-regulate autophagy, leading to reductions in apoptosis^[205]. MAPKs, such as JNK and p38 MAPK, play a critical role in ROS-mediated autophagy events^[206,207]. Meanwhile, activated ERK and JNK are also upstream

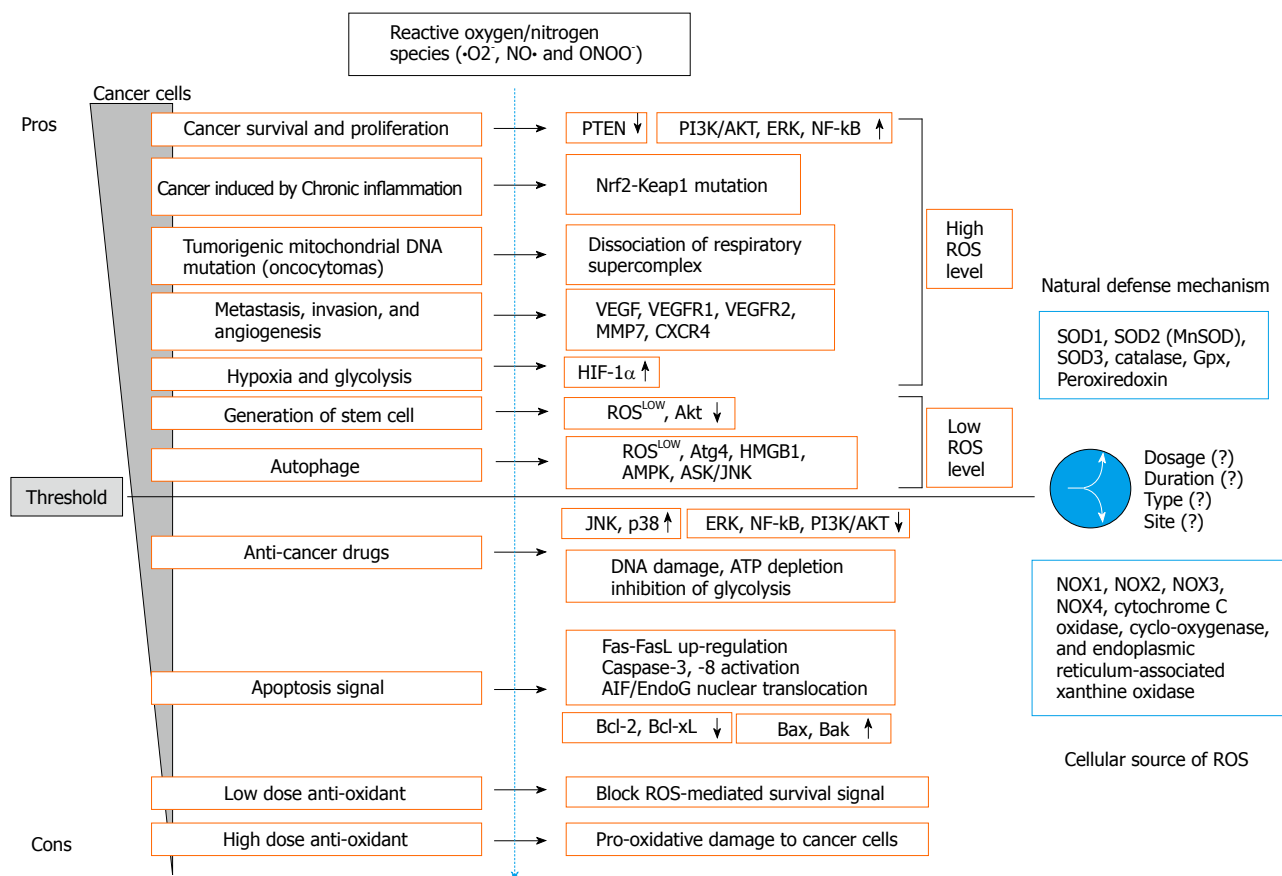


Figure 1 The role of reactive oxygen species on cancer cells to explain the different effects at each condition. Reactive oxygen species (ROS) level inside cancer cell appears varied even within a given type of cancer cells. A so-called “double-edged sword strategy” uses to manipulate the opposite role of ROS sequentially to kill cancer cells more effectively. VEGF: Vascular endothelial growth factor; PI3K: Phosphoinositide-3 kinase; PTEN: Phosphatase and tensin homolog; HIF-1 α : Hypoxia inducible factor-1 α ; JNK: C-Jun N-terminal kinase; AMPK: AMP-activated protein kinase; SOD: superoxide dismutase; NOX: NAD(P)H oxidase.

effectors controlling both autophagy and apoptosis in response to elevated intracellular ROS^[208]. Antioxidant, N-acetyl-L-cysteine (NAC), clearly reduces K-Ras-induced Atg5 and Atg7 induction, autophagy, and malignant cell transformation^[209]. Starvation-induced production of $\cdot\text{O}_2\cdot$ induces autophagy and cell death. Exogenous H_2O_2 is effectively converted to intracellular $\cdot\text{O}_2\cdot$ leading to autophagy-induced cell death. Overexpression of SOD2 and 3-methyladenine, autophagy inhibitor, attenuate starvation-induced autophagy^[210]. 2-Methoxyestradiol inhibits SODs and induces autophagy-mediated cell death in the transformed or cancer cell lines, but not in astrocytes^[210]. From these results, understanding the mechanisms that regulate the crosstalk between ROS and autophagy regulation is important for various disorders, including neurodegenerative diseases and cancer.

ROS AS A THERAPEUTIC TARGET FOR CANCER TREATMENT AND PREVENTION

Many chemotherapeutic drugs are designed to increase cellular ROS levels with the goal of inducing irreversible damage, consequently resulting in tumor cell apoptosis. For example, intracellular ROS levels increase in a dose-dependent manner in A549 human lung cancer cells after

treatment with paclitaxel. Addition of NAC or GSH, two H_2O_2 scavengers, induces a four-times increase in paclitaxel IC₅₀^[211]. Combined treatment with trichostatin A and gemcitabine synergistically inhibits growth of pancreatic adenocarcinoma cell lines and induces apoptosis through the induction of ROS by gemcitabine^[212]. Wogonin, a flavonoid isolated from *Scutellaria baicalensis*, synergistically sensitizes cancer cells to TNF-induced apoptosis through inhibition of catalase activity and an increase of cellular H_2O_2 . Wogonin-induced ROS inhibit TNF-induced phosphorylation on the NF- κB p65 subunit^[213]. Mutations in mitochondrial genes (mtDNA), such as the gene encoding cytochrome c oxidase II, are associated with increased ROS generation and involved in cancer initiation and progression^[74-76]. However, the susceptibility of mitochondrial DNA to ROS-induced mutation may also be utilized for therapy^[214]. Low levels of exogenous H_2O_2 or H_2O_2 produced by mitochondria induce a modest drop in ATP level, delayed toxicity, and G₂/M arrest without affecting cell viability. Concomitant inhibition of glycolysis was found to markedly sensitize cells to death in the presence of nontoxic concentrations of H_2O_2 ^[215]. Another approach to regulating intracellular ROS levels is the use of antioxidants to prevent tumor cells from entering the ROS-mediated survival signaling pathway. ROS, as sec-

ond messengers in signaling pathways, regulate not only kinase phosphorylation (MAPK, Rho kinase) but also transcription factors (NF- κ B, AP-1, and HIF-1). ROS also up-regulate proto-oncogene and pro-inflammatory gene expression and activity^[216,217]. The protective effects of antioxidants have generated significant interest in developing synthetic and natural antioxidants as therapeutic agents to prevent and/or treat patients with cancer.

Several clinical trials have been published regarding the effects of antioxidant vitamins on the risk of cardiovascular disease. However, clinical trial data has been inconsistent and inconclusive, until now^[218]. Although oxidative stress against cancer cells induces apoptosis, several exogenous antioxidants also produce favorable effects in various cancer patients^[219]. The major antioxidant vitamin systems include vitamin E, vitamin C, and GSH^[220]. Vitamin C (ascorbic acid) is a water-soluble antioxidant involved in the reduction of radicals by recycling radicals produced by oxidation of vitamin E. A 20 μ mol/L rise in plasma ascorbic acid concentration is associated with an approximately 20% reduction in the risk of all-cause mortality. Ascorbic acid was inversely related to cancer mortality in men but not women^[221]. In a study conducted in Japan, Vitamin C was found to reduce oxidative stress among subjects with atrophic gastritis^[222]. However, Vitamins E and C, at high concentrations, also function as pro-oxidants causing cell damage^[223]. Unfortunately, one report showed Vitamin E supplement significantly increased the risk of prostate cancer compared with placebo group and selenium combination group^[224]. There is possibility that the bioavailability of anti-oxidants may be insufficient after oral administration, or that they may be inaccessible to the source of free radicals, particularly if ROS are generated in specific cellular compartments and organelles^[225]. In addition, antioxidants do not inhibit the production of ROS; rather, they scavenge ROS after ROS has been generated. Selenium supplementation has been shown to reduce total and prostate cancer incidence but was not significantly associated with lung and colorectal cancer incidence^[226]. In addition, selenium shows the most prominent protective effect on former male smokers^[226]. However, the benefits of selenium were only observed in those patients with the lowest baseline blood selenium levels^[226]. A phase II clinical trial about the effect of pomegranate for men reveals that prostate-specific antigen (PSA) doubling time is significantly extended after treatment with pomegranate juice in patients received surgery or radiotherapy. Further, a decrease in cell proliferation and an increase in apoptosis is observed in patients who consumed pomegranate^[227]. Green tea is popular because it contains epigallocatechin gallate, a polyphenolic compound that provides potential benefits for prostate cancer control^[228,229].

Lycopene in tomato decreases serum prostate-specific antigen levels and oxidative DNA damage and increases apoptotic cells in carcinomas^[230]. In another study, tomato sauce consumption suppresses the progression of prostate carcinoma^[231]. Curcumin is also demonstrated

the inhibitory effects in colon carcinogenesis^[232].

CONCLUSION

Daily fruits and vegetables intake has been inversely correlated to the risk of the development of chronic diseases, including cancer. Cancer is caused by both internal factors and environmental factors^[233]. The link between diet and cancer indicates that cancer is a disease which can be prevented largely by lifestyle changes. In addition, ROS have plenty roles in carcinogenesis or anti-tumor effects though numerous pathways (Figure 1). Since ROS are involved in the transformation of nonmalignant cells to malignant cells, regulation of ROS can be a critical approach to prevent cancer development. ROS act as the second messenger for generating further intracellular events. ROS play a crucial role in tumorigenesis and cancer cell survival as well as apoptotic signaling in cancer cells. In biological systems, enzymatic and nonenzymatic systems have evolved to protect against oxidative damage. The potential of pro-oxidants or antioxidants in treating cancer associated with oxidative stress is reinforced by experimental researches, several clinical studies, and epidemiological data. Therefore, future studies should continue to clarify the different roles of ROS in cancer cells.

REFERENCES

- 1 **Bruce-Keller AJ**, Gupta S, Knight AG, Beckett TL, McMul-len JM, Davis PR, Murphy MP, Van Eldik LJ, St Clair D, Keller JN. Cognitive impairment in humanized APP $\times$ PS1 mice is linked to A β (1-42) and NOX activation. *Neurobiol Dis* 2011; **44**: 317-326 [PMID: 21798347 DOI: 10.1016/j.nbd.2011.07.012]
- 2 **Shimohama S**, Tanino H, Kawakami N, Okamura N, Kodama H, Yamaguchi T, Hayakawa T, Nunomura A, Chiba S, Perry G, Smith MA, Fujimoto S. Activation of NADPH oxidase in Alzheimer's disease brains. *Biochem Biophys Res Commun* 2000; **273**: 5-9 [PMID: 10873554]
- 3 **Murakami K**, Murata N, Noda Y, Tahara S, Kaneko T, Kinoshita N, Hatsuta H, Murayama S, Barnham KJ, Irie K, Shirasawa T, Shimizu T. SOD1 (copper/zinc superoxide dismutase) deficiency drives amyloid β protein oligomerization and memory loss in mouse model of Alzheimer disease. *J Biol Chem* 2011; **286**: 44557-44568 [PMID: 22072713 DOI: 10.1074/jbc.M111.279208]
- 4 **Umeda-Kameyama Y**, Tsuda M, Ohkura C, Matsuo T, Namba Y, Ohuchi Y, Aigaki T. Thioredoxin suppresses Parkin-associated endothelin receptor-like receptor-induced neurotoxicity and extends longevity in *Drosophila*. *J Biol Chem* 2007; **282**: 11180-11187 [PMID: 17301052]
- 5 **Hu X**, Weng Z, Chu CT, Zhang L, Cao G, Gao Y, Signore A, Zhu J, Hastings T, Greenamyre JT, Chen J. Peroxiredoxin-2 protects against 6-hydroxydopamine-induced dopaminergic neurodegeneration via attenuation of the apoptosis signal-regulating kinase (ASK1) signaling cascade. *J Neurosci* 2011; **31**: 247-261 [PMID: 21209210 DOI: 10.1523/JNEUROSCI.4589-10.2011]
- 6 **Sherer TB**, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, Miller GW, Yagi T, Matsuno-Yagi A, Greenamyre JT. Mechanism of toxicity in rotenone models of Parkinson's disease. *J Neurosci* 2003; **23**: 10756-10764 [PMID: 14645467]
- 7 **Rosen DR**, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX. Mu-

- tations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993; **362**: 59-62 [PMID: 8446170]
- 8 **Blake DR**, Hall ND, Treby DA, Halliwell B, Gutteridge JM. Protection against superoxide and hydrogen peroxide in synovial fluid from rheumatoid patients. *Clin Sci (Lond)* 1981; **61**: 483-486 [PMID: 7285498]
 - 9 **Mazzetti I**, Grigolo B, Borzi RM, Meliconi R, Facchini A. Serum copper/zinc superoxide dismutase levels in patients with rheumatoid arthritis. *Int J Clin Lab Res* 1996; **26**: 245-249 [PMID: 9007615]
 - 10 **Nakazono K**, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M. Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci USA* 1991; **88**: 10045-10048 [PMID: 1658794]
 - 11 **Rajagopalan S**, Kurz S, Münzel T, Tarpey M, Freeman BA, Griending KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 1996; **97**: 1916-1923 [PMID: 8621776]
 - 12 **Krause KH**, Bedard K. NOX enzymes in immuno-inflammatory pathologies. *Semin Immunopathol* 2008; **30**: 193-194 [PMID: 18560833 DOI: 10.1007/s00281-008-0127-2]
 - 13 **Krieger-Brauer HI**, Medda PK, Kather H. Insulin-induced activation of NADPH-dependent H₂O₂ generation in human adipocyte plasma membranes is mediated by Galphai2. *J Biol Chem* 1997; **272**: 10135-10143 [PMID: 9092559]
 - 14 **Aggeli IK**, Theofilatos D, Beis I, Gaitanaki C. Insulin-induced oxidative stress up-regulates heme oxygenase-1 via diverse signaling cascades in the C2 skeletal myoblast cell line. *Endocrinology* 2011; **152**: 1274-1283 [PMID: 21325398]
 - 15 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400]
 - 16 **Farber E**, Rubin H. Cellular adaptation in the origin and development of cancer. *Cancer Res* 1991; **51**: 2751-2761 [PMID: 2032214]
 - 17 **Huang P**, Feng L, Oldham EA, Keating MJ, Plunkett W. Superoxide dismutase as a target for the selective killing of cancer cells. *Nature* 2000; **407**: 390-395 [PMID: 11014196]
 - 18 **Reuter S**, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* 2010; **49**: 1603-1616 [PMID: 20840865 DOI: 10.1016/j.freeradbiomed.2010.09.006]
 - 19 **Visconti R**, Grieco D. New insights on oxidative stress in cancer. *Curr Opin Drug Discov Devel* 2009; **12**: 240-245 [PMID: 19333869]
 - 20 **Waris G**, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog* 2006; **5**: 14 [PMID: 16689993]
 - 21 **Tonks NK**. Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* 2006; **7**: 833-846 [PMID: 17057753]
 - 22 **Cao J**, Schulte J, Knight A, Leslie NR, Zagazdzon A, Bronson R, Manevich Y, Beeson C, Neumann CA. Prdx1 inhibits tumorigenesis via regulating PTEN/AKT activity. *EMBO J* 2009; **28**: 1505-1517 [PMID: 19369943 DOI: 10.1038/emboj.2009.101]
 - 23 **Silva A**, Yunes JA, Cardoso BA, Martins LR, Jotta PY, Abecasis M, Nowill AE, Leslie NR, Cardoso AA, Barata JT. PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. *J Clin Invest* 2008; **118**: 3762-3774 [PMID: 18830414 DOI: 10.1172/JCI34616]
 - 24 **Marshall HE**, Hess DT, Stamler JS. S-nitrosylation: physiological regulation of NF-kappaB. *Proc Natl Acad Sci USA* 2004; **101**: 8841-8842 [PMID: 15187230]
 - 25 **Davis RJ**. Signal transduction by the JNK group of MAP kinases. *Cell* 2000; **103**: 239-252 [PMID: 11057897]
 - 26 **Hay N**. The Akt-mTOR tango and its relevance to cancer. *Cancer Cell* 2005; **8**: 179-183 [PMID: 16169463]
 - 27 **Manning BD**, Cantley LC. AKT/PKB signaling: navigating downstream. *Cell* 2007; **129**: 1261-1274 [PMID: 17604717]
 - 28 **Li M**, Carpio DF, Zheng Y, Bruzzo P, Singh V, Ouaz F, Medzhitov RM, Beg AA. An essential role of the NF-kappa B/Toll-like receptor pathway in induction of inflammatory and tissue-repair gene expression by necrotic cells. *J Immunol* 2001; **166**: 7128-7135 [PMID: 11390458]
 - 29 **Zong WX**, Thompson CB. Necrotic death as a cell fate. *Genes Dev* 2006; **20**: 1-15 [PMID: 16391229]
 - 30 **Sen P**, Wallet MA, Yi Z, Huang Y, Henderson M, Mathews CE, Earp HS, Matsushima G, Baldwin AS, Tisch RM. Apoptotic cells induce Mer tyrosine kinase-dependent blockade of NF-kappaB activation in dendritic cells. *Blood* 2007; **109**: 653-660 [PMID: 17008547]
 - 31 **Ren G**, Su J, Zhao X, Zhang L, Zhang J, Roberts AI, Zhang H, Das G, Shi Y. Apoptotic cells induce immunosuppression through dendritic cells: critical roles of IFN-gamma and nitric oxide. *J Immunol* 2008; **181**: 3277-3284 [PMID: 18713999]
 - 32 **Martinon F**, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 2004; **117**: 561-574 [PMID: 15163405]
 - 33 **Forman HJ**, Fukuto JM, Miller T, Zhang H, Rinna A, Levy S. The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. *Arch Biochem Biophys* 2008; **477**: 183-195 [PMID: 18602883 DOI: 10.1016/j.ab.2008.06.011]
 - 34 **Pervaiz S**, Taneja R, Ghaffari S. Oxidative stress regulation of stem and progenitor cells. *Antioxid Redox Signal* 2009; **11**: 2777-2789 [PMID: 19650689 DOI: 10.1089/ars.2009.2804]
 - 35 **Bedard K**, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev* 2007; **87**: 245-313 [PMID: 17237347]
 - 36 **del Río LA**, Sandalio LM, Palma JM, Bueno P, Corpas FJ. Metabolism of oxygen radicals in peroxisomes and cellular implications. *Free Radic Biol Med* 1992; **13**: 557-580 [PMID: 1334030]
 - 37 **Inoue M**, Sato EF, Nishikawa M, Park AM, Kira Y, Imada I, Utsumi K. Mitochondrial generation of reactive oxygen species and its role in aerobic life. *Curr Med Chem* 2003; **10**: 2495-2505 [PMID: 14529465]
 - 38 **Diehn M**, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M, Joshua B, Kaplan MJ, Wapnir I, Dirbas FM, Somlo G, Garberoglio C, Paz B, Shen J, Lau SK, Quake SR, Brown JM, Weissman IL, Clarke MF. Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature* 2009; **458**: 780-783 [PMID: 19194462 DOI: 10.1038/nature07733]
 - 39 **Chen W**, Druhan LJ, Chen CA, Hemann C, Chen YR, Berka V, Tsai AL, Zweier JL. Peroxynitrite induces destruction of the tetrahydrobiopterin and heme in endothelial nitric oxide synthase: transition from reversible to irreversible enzyme inhibition. *Biochemistry* 2010; **49**: 3129-3137 [PMID: 20184376 DOI: 10.1021/bi9016632]
 - 40 **Ruiz-Ramos R**, Lopez-Carrillo L, Rios-Perez AD, De Vizcaya-Ruiz A, Cebrian ME. Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-Myc proteins, NF-kappaB activation and cell proliferation in human breast cancer MCF-7 cells. *Mutat Res* 2009; **674**: 109-115 [PMID: 18996220 DOI: 10.1016/j.mrgentox.2008.09.021]
 - 41 **Adams DJ**, Boskovic ZV, Theriault JR, Wang AJ, Stern AM, Wagner BK, Shamji AF, Schreiber SL. Discovery of small-molecule enhancers of reactive oxygen species that are non-toxic or cause genotype-selective cell death. *ACS Chem Biol* 2013; **8**: 923-929 [PMID: 23477340 DOI: 10.1021/cb300653v]
 - 42 **Raj L**, Ide T, Gurkar AU, Foley M, Schenone M, Li X, Tolliday NJ, Golub TR, Carr SA, Shamji AF, Stern AM, Mandino-

- va A, Schreiber SL, Lee SW. Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature* 2011; **475**: 231-234 [PMID: 21753854 DOI: 10.1038/nature10167]
- 43 **Liu JM**, Pan F, Li L, Liu QR, Chen Y, Xiong XX, Cheng K, Yu SB, Shi Z, Yu AC, Chen XQ. Piperlongumine selectively kills glioblastoma multiforme cells via reactive oxygen species accumulation dependent JNK and p38 activation. *Biochem Biophys Res Commun* 2013; **437**: 87-93 [PMID: 23796709 DOI: 10.1016/j.bbrc.2013.06.042]
 - 44 **Fridovich I**. Superoxide anion radical (O₂⁻), superoxide dismutases, and related matters. *J Biol Chem* 1997; **272**: 18515-18517 [PMID: 9228011]
 - 45 **Fukai T**, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxid Redox Signal* 2011; **15**: 1583-1606 [PMID: 21473702 DOI: 10.1089/ars.2011.3999]
 - 46 **Goyal MM**, Basak A. Human catalase: looking for complete identity. *Protein Cell* 2010; **1**: 888-897 [PMID: 21204015 DOI: 10.1007/s13238-010-0113-z]
 - 47 **Hofmann B**, Hecht HJ, Flohé L. Peroxiredoxins. *Biol Chem* 2002; **383**: 347-364 [PMID: 12033427]
 - 48 **Trachootham D**, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov* 2009; **8**: 579-591 [PMID: 19478820 DOI: 10.1038/nrd2803]
 - 49 **Hampton MB**, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 1998; **92**: 3007-3017 [PMID: 9787133]
 - 50 **Storz P**. Reactive oxygen species in tumor progression. *Front Biosci* 2005; **10**: 1881-1896 [PMID: 15769673]
 - 51 **Gupta A**, Rosenberger SF, Bowden GT. Increased ROS levels contribute to elevated transcription factor and MAP kinase activities in malignantly progressed mouse keratinocyte cell lines. *Carcinogenesis* 1999; **20**: 2063-2073 [PMID: 10545407]
 - 52 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545 [PMID: 11229684]
 - 53 **Schetter AJ**, Heegaard NH, Harris CC. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. *Carcinogenesis* 2010; **31**: 37-49 [PMID: 19955394 DOI: 10.1093/carcin/bgp272]
 - 54 **Naik E**, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. *J Exp Med* 2011; **208**: 417-420 [PMID: 21357740 DOI: 10.1084/jem.20110367]
 - 55 **Hussain SP**, Harris CC. Inflammation and cancer: an ancient link with novel potentials. *Int J Cancer* 2007; **121**: 2373-2380 [PMID: 17893866]
 - 56 **Morgan MJ**, Liu ZG. Crosstalk of reactive oxygen species and NF- κ B signaling. *Cell Res* 2011; **21**: 103-115 [PMID: 21187859 DOI: 10.1038/cr.2010.178]
 - 57 **Hybertson BM**, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol Aspects Med* 2011; **32**: 234-246 [PMID: 22020111 DOI: 10.1016/j.mam.2011]
 - 58 **Banning A**, Deubel S, Kluth D, Zhou Z, Brigelius-Flohé R. The Gl-GPx gene is a target for Nrf2. *Mol Cell Biol* 2005; **25**: 4914-4923 [PMID: 15923610]
 - 59 **Köhle C**, Bock KW. Coordinate regulation of Phase I and II xenobiotic metabolisms by the Ah receptor and Nrf2. *Biochem Pharmacol* 2007; **73**: 1853-1862 [PMID: 17266942]
 - 60 **Itoh K**, Wakabayashi N, Katoh Y, Ishii T, Igarashi K, Engel JD, Yamamoto M. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev* 1999; **13**: 76-86 [PMID: 9887101]
 - 61 **Dinkova-Kostova AT**, Holtzclaw WD, Cole RN, Itoh K, Wakabayashi N, Katoh Y, Yamamoto M, Talalay P. Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci USA* 2002; **99**: 11908-11913 [PMID: 12193649]
 - 62 **Yu R**, Lei W, Mandlekar S, Weber MJ, Der CJ, Wu J, Kong AN. Role of a mitogen-activated protein kinase pathway in the induction of phase II detoxifying enzymes by chemicals. *J Biol Chem* 1999; **274**: 27545-27552 [PMID: 10488090]
 - 63 **Kang KW**, Lee SJ, Park JW, Kim SG. Phosphatidylinositol 3-kinase regulates nuclear translocation of NF-E2-related factor 2 through actin rearrangement in response to oxidative stress. *Mol Pharmacol* 2002; **62**: 1001-1010 [PMID: 12391262]
 - 64 **Yamadori T**, Ishii Y, Homma S, Morishima Y, Kurishima K, Itoh K, Yamamoto M, Minami Y, Noguchi M, Hizawa N. Molecular mechanisms for the regulation of Nrf2-mediated cell proliferation in non-small-cell lung cancers. *Oncogene* 2012; **31**: 4768-4777 [PMID: 22249257 DOI: 10.1038/onc.2011.628]
 - 65 **Kim YR**, Oh JE, Kim MS, Kang MR, Park SW, Han JY, Eom HS, Yoo NJ, Lee SH. Oncogenic NRF2 mutations in squamous cell carcinomas of oesophagus and skin. *J Pathol* 2010; **220**: 446-451 [PMID: 19967722 DOI: 10.1002/path.2653]
 - 66 **Singh A**, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, Herman JG, Baylin SB, Sidransky D, Gabrielson E, Brock MV, Biswal S. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. *PLoS Med* 2006; **3**: e420 [PMID: 17020408]
 - 67 **Dajee M**, Lazarov M, Zhang JY, Cai T, Green CL, Russell AJ, Marinkovich MP, Tao S, Lin Q, Kubo Y, Khavari PA. NF- κ B blockade and oncogenic Ras trigger invasive human epidermal neoplasia. *Nature* 2003; **421**: 639-643 [PMID: 12571598]
 - 68 **Gapuzan ME**, Yufit PV, Gilmore TD. Immortalized embryonic mouse fibroblasts lacking the RelA subunit of transcription factor NF- κ B have a malignantly transformed phenotype. *Oncogene* 2002; **21**: 2484-2492 [PMID: 11971183]
 - 69 **Ricca A**, Biroccio A, Trisciuglio D, Cippitelli M, Zupi G, Del Bufalo D. RelA over-expression reduces tumorigenicity and activates apoptosis in human cancer cells. *Br J Cancer* 2001; **85**: 1914-1921 [PMID: 11747334]
 - 70 **Seitz CS**, Lin Q, Deng H, Khavari PA. Alterations in NF- κ B function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF- κ B. *Proc Natl Acad Sci USA* 1998; **95**: 2307-2312 [PMID: 9482881]
 - 71 **van Hogerlinden M**, Rozell BL, Ahrlund-Richter L, Toftgård R. Squamous cell carcinomas and increased apoptosis in skin with inhibited Rel/nuclear factor- κ B signaling. *Cancer Res* 1999; **59**: 3299-3303 [PMID: 10416581]
 - 72 **Halliwell B**. Oxidative stress and cancer: have we moved forward? *Biochem J* 2007; **401**: 1-11 [PMID: 17150040]
 - 73 **Klaunig JE**, Kamendulis LM. The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* 2004; **44**: 239-267 [PMID: 14744246]
 - 74 **Karihtala P**, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS* 2007; **115**: 81-103 [PMID: 17295675]
 - 75 **Wallace DC**. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 2005; **39**: 359-407 [PMID: 16285865]
 - 76 **Wallace DC**. Mitochondrial DNA mutations in disease and aging. *Environ Mol Mutagen* 2010; **51**: 440-450 [PMID: 20544884 DOI: 10.1002/em.20586]
 - 77 **Kulawiec M**, Safina A, Desouki MM, Still I, Matsui S, Bakin A, Singh KK. Tumorigenic transformation of human breast epithelial cells induced by mitochondrial DNA depletion. *Cancer Biol Ther* 2008; **7**: 1732-1743 [PMID: 19151587]
 - 78 **Parrella P**, Xiao Y, Fliss M, Sanchez-Céspedes M, Mazzarelli P, Rinaldi M, Nicol T, Gabrielson E, Cuomo C, Cohen D, Pandit S, Spencer M, Rabitti C, Fazio VM, Sidransky D. Detection of mitochondrial DNA mutations in primary

- breast cancer and fine-needle aspirates. *Cancer Res* 2001; **61**: 7623-7626 [PMID: 11606403]
- 79 **Wang CY**, Wang HW, Yao YG, Kong QP, Zhang YP. Somatic mutations of mitochondrial genome in early stage breast cancer. *Int J Cancer* 2007; **121**: 1253-1256 [PMID: 17514652]
 - 80 **Frezza C**, Gottlieb E. Mitochondria in cancer: not just innocent bystanders. *Semin Cancer Biol* 2009; **19**: 4-11 [PMID: 19101633 DOI: 10.1016/j.semcancer.2008.11.008]
 - 81 **Bonora E**, Porcelli AM, Gasparre G, Biondi A, Ghelli A, Carelli V, Baracca A, Tallini G, Martinuzzi A, Lenaz G, Rugolo M, Romeo G. Defective oxidative phosphorylation in thyroid oncocyctic carcinoma is associated with pathogenic mitochondrial DNA mutations affecting complexes I and III. *Cancer Res* 2006; **66**: 6087-6096 [PMID: 16778181]
 - 82 **Porcelli AM**, Angelin A, Ghelli A, Mariani E, Martinuzzi A, Carelli V, Petronilli V, Bernardi P, Rugolo M. Respiratory complex I dysfunction due to mitochondrial DNA mutations shifts the voltage threshold for opening of the permeability transition pore toward resting levels. *J Biol Chem* 2009; **284**: 2045-2052 [PMID: 19047048 DOI: 10.1074/jbc.M807321200]
 - 83 **Lenaz G**, Genova ML. Kinetics of integrated electron transfer in the mitochondrial respiratory chain: random collisions vs. solid state electron channeling. *Am J Physiol Cell Physiol* 2007; **292**: C1221-C1239 [PMID: 17035300]
 - 84 **Zimmermann FA**, Mayr JA, Neureiter D, Feichtinger R, Alinger B, Jones ND, Eder W, Sperl W, Kofler B. Lack of complex I is associated with oncocyctic thyroid tumours. *Br J Cancer* 2009; **100**: 1434-1437 [PMID: 19352385 DOI: 10.1038/sj.bjc.6605028]
 - 85 **Shidara Y**, Yamagata K, Kanamori T, Nakano K, Kwong JQ, Manfredi G, Oda H, Ohta S. Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. *Cancer Res* 2005; **65**: 1655-1663 [PMID: 15753359]
 - 86 **Park JS**, Sharma LK, Li H, Xiang R, Holstein D, Wu J, Lechleiter J, Naylor SL, Deng JJ, Lu J, Bai Y. A heteroplasmic, not homoplasmic, mitochondrial DNA mutation promotes tumorigenesis via alteration in reactive oxygen species generation and apoptosis. *Hum Mol Genet* 2009; **18**: 1578-1589 [PMID: 19208652 DOI: 10.1093/hmg/ddp069]
 - 87 **Chiarugi P**, Fiaschi T. Redox signalling in anchorage-dependent cell growth. *Cell Signal* 2007; **19**: 672-682 [PMID: 17204396]
 - 88 **Liou GY**, Storz P. Reactive oxygen species in cancer. *Free Radic Res* 2010; **44**: 479-496 [PMID: 20370557 DOI: 10.3109/10715761003667554]
 - 89 **Cosentino C**, Grieco D, Costanzo V. ATM activates the pentose phosphate pathway promoting anti-oxidant defence and DNA repair. *EMBO J* 2011; **30**: 546-555 [PMID: 21157431 DOI: 10.1038/emboj.2010.330]
 - 90 **Liu LZ**, Hu XW, Xia C, He J, Zhou Q, Shi X, Fang J, Jiang BH. Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1 α expression through activation of AKT and P70S6K1 in human ovarian cancer cells. *Free Radic Biol Med* 2006; **41**: 1521-1533 [PMID: 17045920]
 - 91 **Mochizuki T**, Furuta S, Mitsushita J, Shang WH, Ito M, Yokoo Y, Yamaura M, Ishizone S, Nakayama J, Konagai A, Hirose K, Kiyosawa K, Kamata T. Inhibition of NADPH oxidase 4 activates apoptosis via the AKT/apoptosis signal-regulating kinase 1 pathway in pancreatic cancer PANC-1 cells. *Oncogene* 2006; **25**: 3699-3707 [PMID: 16532036]
 - 92 **Manna SK**, Zhang HJ, Yan T, Oberley LW, Aggarwal BB. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *J Biol Chem* 1998; **273**: 13245-13254 [PMID: 9582369]
 - 93 **Kong Q**, Beel JA, Lillehei KO. A threshold concept for cancer therapy. *Med Hypotheses* 2000; **55**: 29-35 [PMID: 11021322]
 - 94 **Schafer FQ**, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 2001; **30**: 1191-1212 [PMID: 11368918]
 - 95 **Ramsey MR**, Sharpless NE. ROS as a tumour suppressor? *Nat Cell Biol* 2006; **8**: 1213-1215 [PMID: 17077852]
 - 96 **Deshpande SS**, Angkeow P, Huang J, Ozaki M, Irani K. Rac1 inhibits TNF-alpha-induced endothelial cell apoptosis: dual regulation by reactive oxygen species. *FASEB J* 2000; **14**: 1705-1714 [PMID: 10973919]
 - 97 **Brar SS**, Corbin Z, Kennedy TP, Hemendinger R, Thornton L, Bommarius B, Arnold RS, Whorton AR, Sturrock AB, Huecksteadt TP, Quinn MT, Krenitsky K, Ardie KG, Lambeth JD, Hoidal JR. NOX5 NAD(P)H oxidase regulates growth and apoptosis in DU 145 prostate cancer cells. *Am J Physiol Cell Physiol* 2003; **285**: C353-C369 [PMID: 12686516]
 - 98 **Edderkaoui M**, Hong P, Vaquero EC, Lee JK, Fischer L, Friess H, Buchler MW, Lerch MM, Pandolfi SJ, Gukovskaya AS. Extracellular matrix stimulates reactive oxygen species production and increases pancreatic cancer cell survival through 5-lipoxygenase and NADPH oxidase. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G1137-G1147 [PMID: 16037546]
 - 99 **Vaquero EC**, Edderkaoui M, Pandolfi SJ, Gukovsky I, Gukovskaya AS. Reactive oxygen species produced by NAD(P)H oxidase inhibit apoptosis in pancreatic cancer cells. *J Biol Chem* 2004; **279**: 34643-34654 [PMID: 15155719]
 - 100 **Lee JK**, Edderkaoui M, Truong P, Ohno I, Jang KT, Berti A, Pandolfi SJ, Gukovskaya AS. NADPH oxidase promotes pancreatic cancer cell survival via inhibiting JAK2 dephosphorylation by tyrosine phosphatases. *Gastroenterology* 2007; **133**: 1637-1648 [PMID: 17983808]
 - 101 **Nogueira V**, Park Y, Chen CC, Xu PZ, Chen ML, Tonic I, Unterman T, Hay N. Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. *Cancer Cell* 2008; **14**: 458-470 [PMID: 19061837 DOI: 10.1016/j.ccr.2008.11.003]
 - 102 **Chen CC**, Jeon SM, Bhaskar PT, Nogueira V, Sundararajan D, Tonic I, Park Y, Hay N. FoxOs inhibit mTORC1 and activate Akt by inducing the expression of Sestrin3 and Rictor. *Dev Cell* 2010; **18**: 592-604 [PMID: 20412774 DOI: 10.1016/j.devcel.2010.03.008]
 - 103 **Budanov AV**, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* 2008; **134**: 451-460 [PMID: 18692468 DOI: 10.1016/j.cell.2008.06.028]
 - 104 **Kopnin PB**, Agapova LS, Kopnin BP, Chumakov PM. Repression of sestrin family genes contributes to oncogenic Ras-induced reactive oxygen species up-regulation and genetic instability. *Cancer Res* 2007; **67**: 4671-4678 [PMID: 17510393]
 - 105 **Sablina AA**, Budanov AV, Ilyinskaya GV, Agapova LS, Kravchenko JE, Chumakov PM. The antioxidant function of the p53 tumor suppressor. *Nat Med* 2005; **11**: 1306-1313 [PMID: 16286925]
 - 106 **Chung YM**, Kim JS, Yoo YD. A novel protein, Romo1, induces ROS production in the mitochondria. *Biochem Biophys Res Commun* 2006; **347**: 649-655 [PMID: 16842742]
 - 107 **Hwang IT**, Chung YM, Kim JJ, Chung JS, Kim BS, Kim HJ, Kim JS, Yoo YD. Drug resistance to 5-FU linked to reactive oxygen species modulator 1. *Biochem Biophys Res Commun* 2007; **359**: 304-310 [PMID: 17537404]
 - 108 **Na AR**, Chung YM, Lee SB, Park SH, Lee MS, Yoo YD. A critical role for Romo1-derived ROS in cell proliferation. *Biochem Biophys Res Commun* 2008; **369**: 672-678 [PMID: 18313394 DOI: 10.1016/j.bbrc.2008.02.098]
 - 109 **De Luca A**, Sanna F, Salles M, Ruggiero C, Grossi M, Sacchetta P, Rossi C, De Laurenzi V, Di Ilio C, Favaloro B. Me-

- thionine sulfoxide reductase A down-regulation in human breast cancer cells results in a more aggressive phenotype. *Proc Natl Acad Sci USA* 2010; **107**: 18628-18633 [PMID: 20937881 DOI: 10.1073/pnas.1010171107]
- 110 **Hu Y**, Rosen DG, Zhou Y, Feng L, Yang G, Liu J, Huang P. Mitochondrial manganese-superoxide dismutase expression in ovarian cancer: role in cell proliferation and response to oxidative stress. *J Biol Chem* 2005; **280**: 39485-39492 [PMID: 16179351]
 - 111 **Pilarsky C**, Wenzig M, Specht T, Saeger HD, Grützmann R. Identification and validation of commonly overexpressed genes in solid tumors by comparison of microarray data. *Neoplasia* 2004; **6**: 744-750 [PMID: 15720800]
 - 112 **Park HJ**, Carr JR, Wang Z, Nogueira V, Hay N, Tyner AL, Lau LF, Costa RH, Raychaudhuri P. FoxM1, a critical regulator of oxidative stress during oncogenesis. *EMBO J* 2009; **28**: 2908-2918 [PMID: 19696738]
 - 113 **Belinsky M**, Jaiswal AK. NAD(P)H: quinone oxidoreductase1 (DT-diaphorase) expression in normal and tumor tissues. *Cancer Metastasis Rev* 1993; **12**: 103-117 [PMID: 8375015]
 - 114 **Di Pietro G**, Magno LA, Rios-Santos F. Glutathione S-transferases: an overview in cancer research. *Expert Opin Drug Metab Toxicol* 2010; **6**: 153-170 [PMID: 20078251 DOI: 10.1517/17425250903427980]
 - 115 **Dong-Yun S**, Yu-Ru D, Shan-Lin L, Ya-Dong Z, Lian W. Redox stress regulates cell proliferation and apoptosis of human hepatoma through Akt protein phosphorylation. *FEBS Lett* 2003; **542**: 60-64 [PMID: 12729898]
 - 116 **Eblin KE**, Jensen TJ, Wnek SM, Buffington SE, Futscher BW, Gandolfi AJ. Reactive oxygen species regulate properties of transformation in UROtsa cells exposed to monomethylarsonous acid by modulating MAPK signaling. *Toxicology* 2009; **255**: 107-114 [PMID: 19014992 DOI: 10.1016/j.tox.2008.10.007]
 - 117 **Pettus BJ**, Bielawski J, Porcelli AM, Reames DL, Johnson KR, Morrow J, Chalfant CE, Obeid LM, Hannun YA. The sphingosine kinase 1/sphingosine-1-phosphate pathway mediates COX-2 induction and PGE2 production in response to TNF- α . *FASEB J* 2003; **17**: 1411-1421 [PMID: 12890694]
 - 118 **Seo JH**, Kim H, Kim KH. Cyclooxygenase-2 expression by transcription factors in *Helicobacter pylori*-infected gastric epithelial cells: comparison between HP 99 and NCTC 11637. *Ann N Y Acad Sci* 2002; **973**: 477-480 [PMID: 12485915]
 - 119 **Yi J**, Yang J, He R, Gao F, Sang H, Tang X, Ye RD. Emodin enhances arsenic trioxide-induced apoptosis via generation of reactive oxygen species and inhibition of survival signaling. *Cancer Res* 2004; **64**: 108-116 [PMID: 14729614]
 - 120 **Jing Y**, Yang J, Wang Y, Li H, Chen Y, Hu Q, Shi G, Tang X, Yi J. Alteration of subcellular redox equilibrium and the consequent oxidative modification of nuclear factor kappaB are critical for anticancer cytotoxicity by emodin, a reactive oxygen species-producing agent. *Free Radic Biol Med* 2006; **40**: 2183-2197 [PMID: 16785032]
 - 121 **Trachootham D**, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, Huang P. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer Cell* 2006; **10**: 241-252 [PMID: 16959615]
 - 122 **Ozben T**. Oxidative stress and apoptosis: impact on cancer therapy. *J Pharm Sci* 2007; **96**: 2181-2196 [PMID: 17593552]
 - 123 **Denning TL**, Takaishi H, Crowe SE, Boldogh I, Jevnikar A, Ernst PB. Oxidative stress induces the expression of Fas and Fas ligand and apoptosis in murine intestinal epithelial cells. *Free Radic Biol Med* 2002; **33**: 1641-1650 [PMID: 12488132]
 - 124 **Medan D**, Wang L, Toledo D, Lu B, Stehlik C, Jiang BH, Shi X, Rojanasakul Y. Regulation of Fas (CD95)-induced apoptotic and necrotic cell death by reactive oxygen species in macrophages. *J Cell Physiol* 2005; **203**: 78-84 [PMID: 15368542]
 - 125 **Reinehr R**, Becker S, Eberle A, Grether-Beck S, Häussinger D. Involvement of NADPH oxidase isoforms and Src family kinases in CD95-dependent hepatocyte apoptosis. *J Biol Chem* 2005; **280**: 27179-27194 [PMID: 15917250]
 - 126 **Uchikura K**, Wada T, Hoshino S, Nagakawa Y, Aiko T, Bulkley GB, Klein AS, Sun Z. Lipopolysaccharides induced increases in Fas ligand expression by Kupffer cells via mechanisms dependent on reactive oxygen species. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G620-G626 [PMID: 15087279]
 - 127 **Martindale JL**, Holbrook NJ. Cellular response to oxidative stress: signaling for suicide and survival. *J Cell Physiol* 2002; **192**: 1-15 [PMID: 12115731]
 - 128 **Hampton MB**, Orrenius S. Dual regulation of caspase activity by hydrogen peroxide: implications for apoptosis. *FEBS Lett* 1997; **414**: 552-556 [PMID: 9323034]
 - 129 **Bejarano I**, Espino J, Marchena AM, Barriga C, Paredes SD, Rodríguez AB, Pariente JA. Melatonin enhances hydrogen peroxide-induced apoptosis in human promyelocytic leukaemia HL-60 cells. *Mol Cell Biochem* 2011; **353**: 167-176 [PMID: 21431366 DOI: 10.1007/s11010-011-0783-8]
 - 130 **Price M**, Terlecky SR, Kessel D. A role for hydrogen peroxide in the pro-apoptotic effects of photodynamic therapy. *Photochem Photobiol* 2009; **85**: 1491-1496 [PMID: 19659920 DOI: 10.1111/j.1751-1097.2009.00589.x]
 - 131 **Valdameri G**, Trombetta-Lima M, Worfel PR, Pires AR, Martinez GR, Noleto GR, Cadena SM, Sogayar MC, Winnischofer SM, Rocha ME. Involvement of catalase in the apoptotic mechanism induced by apigenin in HepG2 human hepatoma cells. *Chem Biol Interact* 2011; **193**: 180-189 [PMID: 21756884 DOI: 10.1016/j.cbi.2011.06.009]
 - 132 **Choudhary S**, Wang KK, Wang HC. Oncogenic H-Ras, FK228, and exogenous H₂O₂ cooperatively activated the ERK pathway in selective induction of human urinary bladder cancer J82 cell death. *Mol Carcinog* 2011; **50**: 215-219 [PMID: 21344509 DOI: 10.1002/mc.20708]
 - 133 **Maly FE**, Nakamura M, Gauchat JF, Urwyler A, Walker C, Dahinden CA, Cross AR, Jones OT, de Weck AL. Superoxide-dependent nitroblue tetrazolium reduction and expression of cytochrome b-245 components by human tonsillar B lymphocytes and B cell lines. *J Immunol* 1989; **142**: 1260-1267 [PMID: 2536769]
 - 134 **Maly FE**, Cross AR, Jones OT, Wolf-Vorbeck G, Walker C, Dahinden CA, De Weck AL. The superoxide generating system of B cell lines. Structural homology with the phagocytic oxidase and triggering via surface Ig. *J Immunol* 1988; **140**: 2334-2339 [PMID: 2832475]
 - 135 **Devadas S**, Zaritskaya L, Rhee SG, Oberley L, Williams MS. Discrete generation of superoxide and hydrogen peroxide by T cell receptor stimulation: selective regulation of mitogen-activated protein kinase activation and fas ligand expression. *J Exp Med* 2002; **195**: 59-70 [PMID: 11781366]
 - 136 **Singh DK**, Kumar D, Siddiqui Z, Basu SK, Kumar V, Rao KV. The strength of receptor signaling is centrally controlled through a cooperative loop between Ca²⁺ and an oxidant signal. *Cell* 2005; **121**: 281-293 [PMID: 15851034]
 - 137 **Song H**, Park G, Kim YS, Hur I, Kim H, Ryu JW, Lee HK, Cho DH, Choi IH, Lee WJ, Hur DY. B7-H4 reverse signaling induces the apoptosis of EBV-transformed B cells through Fas ligand up-regulation. *Cancer Lett* 2008; **266**: 227-237 [PMID: 18417276 DOI: 10.1016/j.canlet.2008.02.067]
 - 138 **Kim YS**, Park GB, Lee HK, Song H, Choi IH, Lee WJ, Hur DY. Cross-linking of B7-H1 on EBV-transformed B cells induces apoptosis through reactive oxygen species production, JNK signaling activation, and fasL expression. *J Immunol* 2008; **181**: 6158-6169 [PMID: 18941206]
 - 139 **Hintzen RQ**, Lens SM, Koopman G, Pals ST, Spits H, van Lier RA. CD70 represents the human ligand for CD27. *Int Immunol* 1994; **6**: 477-480 [PMID: 8186199]
 - 140 **Park GB**, Kim YS, Lee HK, Song H, Cho DH, Lee WJ, Hur

- DY. Endoplasmic reticulum stress-mediated apoptosis of EBV-transformed B cells by cross-linking of CD70 is dependent upon generation of reactive oxygen species and activation of p38 MAPK and JNK pathway. *J Immunol* 2010; **185**: 7274-7284 [PMID: 21078900 DOI: 0.4049/jimmunol.1001547]
- 141 **Degterev A**, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol* 2005; **1**: 112-119 [PMID: 16408008]
 - 142 **Hitomi J**, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ, Yuan J. Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell* 2008; **135**: 1311-1323 [PMID: 19109899 DOI: 10.1016/j.cell.2008.10.044]
 - 143 **Vercammen D**, Brouckaert G, Denecker G, Van de Craen M, Declercq W, Fiers W, Vandenabeele P. Dual signaling of the Fas receptor: initiation of both apoptotic and necrotic cell death pathways. *J Exp Med* 1998; **188**: 919-930 [PMID: 9730893]
 - 144 **Holler N**, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nat Immunol* 2000; **1**: 489-495 [PMID: 11101870]
 - 145 **Festjens N**, Vanden Berghe T, Cornelis S, Vandenabeele P. RIP1, a kinase on the crossroads of a cell's decision to live or die. *Cell Death Differ* 2007; **14**: 400-410 [PMID: 17301840]
 - 146 **Goossens V**, De Vos K, Vercammen D, Steemans M, Vancampennolle K, Fiers W, Vandenabeele P, Grooten J. Redox regulation of TNF signaling. *Biofactors* 1999; **10**: 145-156 [PMID: 10609876]
 - 147 **Lin Y**, Choksi S, Shen HM, Yang QF, Hur GM, Kim YS, Tran JH, Nedospasov SA, Liu ZG. Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. *J Biol Chem* 2004; **279**: 10822-10828 [PMID: 14701813]
 - 148 **He S**, Wang L, Miao L, Wang T, Du F, Zhao L, Wang X. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF- α . *Cell* 2009; **137**: 1100-1111 [PMID: 19524512 DOI: 10.1016/j.cell.2009.05.021]
 - 149 **Sun X**, Yin J, Starovasnik MA, Fairbrother WJ, Dixit VM. Identification of a novel homotypic interaction motif required for the phosphorylation of receptor-interacting protein (RIP) by RIP3. *J Biol Chem* 2002; **277**: 9505-9511 [PMID: 11734559]
 - 150 **Zhang DW**, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* 2009; **325**: 332-336 [PMID: 19498109 DOI: 10.1126/science.1172308]
 - 151 **Ho BY**, Wu YM, Chang KJ, Pan TM. Dimeric acid inhibits SW620 cell invasion by attenuating H₂O₂-mediated MMP-7 expression via JNK/C-Jun and ERK/C-Fos activation in an AP-1-dependent manner. *Int J Biol Sci* 2011; **7**: 869-880 [PMID: 21814482]
 - 152 **Chetram MA**, Don-Salu-Hewage AS, Hinton CV. ROS enhances CXCR4-mediated functions through inactivation of PTEN in prostate cancer cells. *Biochem Biophys Res Commun* 2011; **410**: 195-200 [PMID: 21627959 DOI: 10.1016/j.bbrc.2011.05.074]
 - 153 **Rungtabnana P**, Nimmannit U, Halim H, Rojanasakul Y, Chanvorachote P. Hydrogen peroxide inhibits non-small cell lung cancer cell anoikis through the inhibition of caveolin-1 degradation. *Am J Physiol Cell Physiol* 2011; **300**: C235-C245 [PMID: 21148404 DOI: 10.1152/ajpcell.00249.2010]
 - 154 **Rajashekhar G**, Kamocka M, Marin A, Suckow MA, Wolter WR, Badve S, Sanjeevaiah AR, Pumiglia K, Rosen E, Clauss M. Pro-inflammatory angiogenesis is mediated by p38 MAP kinase. *J Cell Physiol* 2011; **226**: 800-808 [PMID: 20803566 DOI: 10.1002/jcp.22404]
 - 155 **Yasuda M**, Ohzeki Y, Shimizu S, Naito S, Ohtsuru A, Yamamoto T, Kuroiwa Y. Stimulation of in vitro angiogenesis by hydrogen peroxide and the relation with ETS-1 in endothelial cells. *Life Sci* 1999; **64**: 249-258 [PMID: 10027759]
 - 156 **Maehata Y**, Ozawa S, Kobayashi K, Kato Y, Yoshino F, Miyamoto C, Izukuri K, Kubota E, Hata R, Lee MC. Reactive oxygen species (ROS) reduce the expression of BRAK/CXCL14 in human head and neck squamous cell carcinoma cells. *Free Radic Res* 2010; **44**: 913-924 [PMID: 20815772 DOI: 10.3109/10715762.2010.490836]
 - 157 **Xia C**, Meng Q, Liu LZ, Rojanasakul Y, Wang XR, Jiang BH. Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. *Cancer Res* 2007; **67**: 10823-10830 [PMID: 18006827]
 - 158 **Khromova NV**, Kopnin PB, Stepanova EV, Agapova LS, Kopnin BP. p53 hot-spot mutants increase tumor vascularization via ROS-mediated activation of the HIF1/VEGF-A pathway. *Cancer Lett* 2009; **276**: 143-151 [PMID: 19091459 DOI: 10.1016/j.canlet.2008.10.049]
 - 159 **Lin CC**, Huang CY, Mong MC, Chan CY, Yin MC. Antiangiogenic potential of three triterpenic acids in human liver cancer cells. *J Agric Food Chem* 2011; **59**: 755-762 [PMID: 21175131 DOI: 10.1021/jf103904b]
 - 160 **Schafer ZT**, Grassian AR, Song L, Jiang Z, Gerhart-Hines Z, Irie HY, Gao S, Puigserver P, Brugge JS. Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment. *Nature* 2009; **461**: 109-113 [PMID: 19693011 DOI: 10.1038/nature08268]
 - 161 **Nagalingam A**, Arbiser JL, Bonner MY, Saxena NK, Sharma D. Honokiol activates AMP-activated protein kinase in breast cancer cells via an LKB1-dependent pathway and inhibits breast carcinogenesis. *Breast Cancer Res* 2012; **14**: R35 [PMID: 22353783]
 - 162 **Jeon SM**, Chandel NS, Hay N. AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. *Nature* 2012; **485**: 661-665 [PMID: 22660331 DOI: 10.1038/nature11066]
 - 163 **Joyce JA**, Pollard JW. Microenvironmental regulation of metastasis. *Nat Rev Cancer* 2009; **9**: 239-252 [PMID: 19279573 DOI: 10.1038/nrc2618]
 - 164 **Karnoub AE**, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007; **449**: 557-563 [PMID: 17914389]
 - 165 **Paunescu V**, Bojin FM, Tatu CA, Gavriluc OI, Rosca A, Gruia AT, Tanasie G, Bunu C, Crisnic D, Gherghiceanu M, Tatu FR, Tatu CS, Vermesan S. Tumour-associated fibroblasts and mesenchymal stem cells: more similarities than differences. *J Cell Mol Med* 2011; **15**: 635-646 [PMID: 20184663 DOI: 10.1111/j.1582-4934.2010.01044.x]
 - 166 **Quante M**, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, Baik GH, Shibata W, Diprete B, Betz KS, Friedman R, Varro A, Tycko B, Wang TC. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell* 2011; **19**: 257-272 [PMID: 21316604 DOI: 10.1016/j.ccr.2011.01.020]
 - 167 **Dirat B**, Bochet L, Dabek M, Daviaud D, Dauvillier S, Majed B, Wang YY, Meulle A, Salles B, Le Gonidec S, Garrido I, Escourrou G, Valet P, Muller C. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. *Cancer Res* 2011; **71**: 2455-2465 [PMID: 21459803 DOI: 10.1158/0008-5472.CAN-10-3323]
 - 168 **Kidd S**, Spaeth E, Watson K, Burks J, Lu H, Klopp A, Andreeff M, Marini FC. Origins of the tumor microenvironment: quantitative assessment of adipose-derived and bone marrow-derived stroma. *PLoS One* 2012; **7**: e30563 [PMID: 22363446 DOI: 10.1371/journal.pone.0030563]
 - 169 **Kojima Y**, Acar A, Eaton EN, Mellody KT, Scheel C, Ben-

- Porath I, Onder TT, Wang ZC, Richardson AL, Weinberg RA, Orimo A. Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. *Proc Natl Acad Sci USA* 2010; **107**: 20009-20014 [PMID: 21041659 DOI: 10.1073/pnas.1013805107]
- 170 Jain M, Rivera S, Monclus EA, Synenki L, Zirk A, Eisenbart J, Feghali-Bostwick C, Mutlu GM, Budinger GR, Chandel NS. Mitochondrial reactive oxygen species regulate transforming growth factor- β signaling. *J Biol Chem* 2013; **288**: 770-777 [PMID: 23204521 DOI: 10.1074/jbc.M112.431973]
- 171 Artaud-Macari E, Goven D, Brayer S, Hamimi A, Besnard V, Marchal-Somme J, Ali ZE, Crestani B, Kerdine-Römer S, Boutten A, Bonay M. Nuclear factor erythroid 2-related factor 2 nuclear translocation induces myofibroblastic dedifferentiation in idiopathic pulmonary fibrosis. *Antioxid Redox Signal* 2013; **18**: 66-79 [PMID: 22703534 DOI: 10.1089/ars.2011.4240]
- 172 Lee JM, Calkins MJ, Chan K, Kan YW, Johnson JA. Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *J Biol Chem* 2003; **278**: 12029-12038 [PMID: 12556532]
- 173 Toullec A, Gerald D, Despouy G, Bourachot B, Cardon M, Lefort S, Richardson M, Rigai G, Parrini MC, Lucchesi C, Bellanger D, Stern MH, Dubois T, Sastre-Garau X, Delattre O, Vincent-Salomon A, Mechta-Grigoriou F. Oxidative stress promotes myofibroblast differentiation and tumour spreading. *EMBO Mol Med* 2010; **2**: 211-230 [PMID: 20535745 DOI: 10.1002/emmm.201000073]
- 174 Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, DeLaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005; **121**: 335-348 [PMID: 15882617]
- 175 Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG, Moses HL. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* 2004; **303**: 848-851 [PMID: 14764882]
- 176 Sanz-Moreno V, Gaggioli C, Yeo M, Albrengues J, Wallberg F, Viros A, Hooper S, Mitter R, Féral CC, Cook M, Larkin J, Marais R, Meneguzzi G, Sahai E, Marshall CJ. ROCK and JAK1 signaling cooperate to control actomyosin contractility in tumor cells and stroma. *Cancer Cell* 2011; **20**: 229-245 [PMID: 21840487 DOI: 10.1016/j.ccr.2011.06.018]
- 177 Dang CV, Semenza GL. Oncogenic alterations of metabolism. *Trends Biochem Sci* 1999; **24**: 68-72 [PMID: 10098401]
- 178 Guzy RD, Schumacker PT. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. *Exp Physiol* 2006; **91**: 807-819 [PMID: 16857720]
- 179 Wang M, Kirk JS, Venkataraman S, Domann FE, Zhang HJ, Schafer FQ, Flanagan SW, Weydert CJ, Spitz DR, Buettner GR, Oberley LW. Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1 α and vascular endothelial growth factor. *Oncogene* 2005; **24**: 8154-8166 [PMID: 16170370]
- 180 Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, Raman V, Bhujwalla ZM, Felsner DW, Cheng L, Pevsner J, Lee LA, Semenza GL, Dang CV. HIF-dependent antitumorigenic effect of antioxidants in vivo. *Cancer Cell* 2007; **12**: 230-238 [PMID: 17785204]
- 181 Tacchini L, Dansi P, Matteucci E, Desiderio MA. Hepatocyte growth factor signalling stimulates hypoxia inducible factor-1 (HIF-1) activity in HepG2 hepatoma cells. *Carcinogenesis* 2001; **22**: 1363-1371 [PMID: 11532856]
- 182 Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* 2006; **441**: 437-443 [PMID: 16724055]
- 183 Fiaschi T, Chiarugi P. Oxidative stress, tumor microenvironment, and metabolic reprogramming: a diabolic liaison. *Int J Cell Biol* 2012; **2012**: 762825 [PMID: 22666258 DOI: 10.1155/2012/762825]
- 184 Chi JT, Wang Z, Nuyten DS, Rodriguez EH, Schaner ME, Salim A, Wang Y, Kristensen GB, Helland A, Børresen-Dale AL, Giaccia A, Longaker MT, Hastie T, Yang GP, van de Vijver MJ, Brown PO. Gene expression programs in response to hypoxia: cell type specificity and prognostic significance in human cancers. *PLoS Med* 2006; **3**: e47 [PMID: 16417408]
- 185 Abbott A. Stem cells: The cell division. *Nature* 2011; **480**: 310-312 [PMID: 22170661 DOI: 10.1038/480310a]
- 186 Jang YY, Sharkis SJ. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. *Blood* 2007; **110**: 3056-3063 [PMID: 17595331]
- 187 Myant KB, Cammareri P, McGhee EJ, Ridgway RA, Huels DJ, Cordero JB, Schwitala S, Kalna G, Ogg EL, Athineos D, Timpson P, Vidal M, Murray GI, Greten FR, Anderson KL, Sansom OJ. ROS production and NF- κ B activation triggered by RAC1 facilitate WNT-driven intestinal stem cell proliferation and colorectal cancer initiation. *Cell Stem Cell* 2013; **12**: 761-773 [PMID: 23665120 DOI: 10.1016/j.stem.2013.04.006]
- 188 Ye XQ, Li Q, Wang GH, Sun FF, Huang GJ, Bian XW, Yu SC, Qian GS. Mitochondrial and energy metabolism-related properties as novel indicators of lung cancer stem cells. *Int J Cancer* 2011; **129**: 820-831 [PMID: 21520032 DOI: 10.1002/ijc.25944]
- 189 Dey-Guha I, Wolfer A, Yeh AC, G Albeck J, Darp R, Leon E, Wulfschuhle J, Petricoin EF, Wittner BS, Ramaswamy S. Asymmetric cancer cell division regulated by AKT. *Proc Natl Acad Sci USA* 2011; **108**: 12845-12850 [PMID: 21757645 DOI: 10.1073/pnas.1109632108]
- 190 Jin Y, Lu Z, Ding K, Li J, Du X, Chen C, Sun X, Wu Y, Zhou J, Pan J. Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: inactivation of the NF- κ B pathway and generation of reactive oxygen species. *Cancer Res* 2010; **70**: 2516-2527 [PMID: 20215516 DOI: 10.1158/0008-5472.CAN-09-3950]
- 191 Wang T, Xu J, Yu X, Yang R, Han ZC. Peroxisome proliferator-activated receptor gamma in malignant diseases. *Crit Rev Oncol Hematol* 2006; **58**: 1-14 [PMID: 16388966]
- 192 Liu L, Yang Z, Xu Y, Li J, Xu D, Zhang L, Sun J, Xia S, Zou F, Liu Y. Inhibition of oxidative stress-elicited AKT activation facilitates PPAR γ agonist-mediated inhibition of stem cell character and tumor growth of liver cancer cells. *PLoS One* 2013; **8**: e73038 [PMID: 24023668 DOI: 10.1371/journal.pone.0073038]
- 193 Abdel-Wahab O, Levine RL. Metabolism and the leukemic stem cell. *J Exp Med* 2010; **207**: 677-680 [PMID: 20368582 DOI: 10.1084/jem.20100523]
- 194 Cecconi F, Levine B. The role of autophagy in mammalian development: cell makeover rather than cell death. *Dev Cell* 2008; **15**: 344-357 [PMID: 18804433 DOI: 10.1016/j.devcel.2008.08.012]
- 195 Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. *Nature* 2008; **451**: 1069-1075 [PMID: 18305538 DOI: 10.1038/nature06639]
- 196 Kundu M, Thompson CB. Autophagy: basic principles and relevance to disease. *Annu Rev Pathol* 2008; **3**: 427-455 [PMID: 18039129]
- 197 Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, Cattoretti G, Levine B. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest* 2003; **112**: 1809-1820 [PMID: 14638851]
- 198 Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development,

- is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci USA* 2003; **100**: 15077-15082 [PMID: 14657337]
- 199 **Pattingre S**, Levine B. Bcl-2 inhibition of autophagy: a new route to cancer? *Cancer Res* 2006; **66**: 2885-2888 [PMID: 16540632]
 - 200 **Pattingre S**, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 2005; **122**: 927-939 [PMID: 16179260]
 - 201 **Petiot A**, Ogier-Denis E, Blommaert EF, Meijer AJ, Codogno P. Distinct classes of phosphatidylinositol 3'-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J Biol Chem* 2000; **275**: 992-998 [PMID: 10625637]
 - 202 **Arico S**, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, Ogier-Denis E. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. *J Biol Chem* 2001; **276**: 35243-35246 [PMID: 11477064]
 - 203 **Feng Z**, Zhang H, Levine AJ, Jin S. The coordinate regulation of the p53 and mTOR pathways in cells. *Proc Natl Acad Sci USA* 2005; **102**: 8204-8209 [PMID: 15928081]
 - 204 **Scherz-Shouval R**, Shvets E, Fass E, Shorer H, Gil L, Elazar Z. Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. *EMBO J* 2007; **26**: 1749-1760 [PMID: 17347651]
 - 205 **Kaminsky VO**, Zhivotovsky B. Free radicals in cross talk between autophagy and apoptosis. *Antioxid Redox Signal* 2014; **21**: 86-102 [PMID: 24359220 DOI: 10.1089/ars.2013.5746]
 - 206 **Baregamian N**, Song J, Bailey CE, Papaconstantinou J, Evers BM, Chung DH. Tumor necrosis factor- α and apoptosis signal-regulating kinase 1 control reactive oxygen species release, mitochondrial autophagy, and c-Jun N-terminal kinase/p38 phosphorylation during necrotizing enterocolitis. *Oxid Med Cell Longev* 2009; **2**: 297-306 [PMID: 20716917 DOI: 10.4161/oxim.2.5.9541]
 - 207 **Wu H**, Wang MC, Bohmann D. JNK protects Drosophila from oxidative stress by transcriptionally activating autophagy. *Mech Dev* 2007; **126**: 624-637 [PMID: 19540338 DOI: 10.1016/j.mod.2009.06.1082]
 - 208 **Wong CH**, Iskandar KB, Yadav SK, Hirpara JL, Loh T, Pervaiz S. Simultaneous induction of non-canonical autophagy and apoptosis in cancer cells by ROS-dependent ERK and JNK activation. *PLoS One* 2010; **5**: e9996 [PMID: 20368806 DOI: 10.1371/journal.pone.0009996]
 - 209 **Kim MJ**, Woo SJ, Yoon CH, Lee JS, An S, Choi YH, Hwang SG, Yoon G, Lee SJ. Involvement of autophagy in oncogenic K-Ras-induced malignant cell transformation. *J Biol Chem* 2011; **286**: 12924-12932 [PMID: 21300795 DOI: 10.1074/jbc.M110.138958]
 - 210 **Chen Y**, Azad MB, Gibson SB. Superoxide is the major reactive oxygen species regulating autophagy. *Cell Death Differ* 2009; **16**: 1040-1052 [PMID: 19407826 DOI: 10.1038/cdd.2009.49]
 - 211 **Alexandre J**, Batteux F, Nicco C, Chéreau C, Laurent A, Guillemin L, Weill B, Goldwasser F. Accumulation of hydrogen peroxide is an early and crucial step for paclitaxel-induced cancer cell death both in vitro and in vivo. *Int J Cancer* 2006; **119**: 41-48 [PMID: 16450384]
 - 212 **Donadelli M**, Costanzo C, Beghelli S, Scupoli MT, Dandrea M, Bonora A, Piacentini P, Budillon A, Caraglia M, Scarpa A, Palmieri M. Synergistic inhibition of pancreatic adenocarcinoma cell growth by trichostatin A and gemcitabine. *Biochim Biophys Acta* 2007; **1773**: 1095-1106 [PMID: 17555830]
 - 213 **Yang L**, Zheng XL, Sun H, Zhong YJ, Wang Q, He HN, Shi XW, Zhou B, Li JK, Lin Y, Zhang L, Wang X. Catalase suppression-mediated H₂O₂ accumulation in cancer cells by wogonin effectively blocks tumor necrosis factor-induced NF- κ B activation and sensitizes apoptosis. *Cancer Sci* 2011; **102**: 870-876 [PMID: 21244577 DOI: 10.1111/j.1349-7006.2011.01874.x]
 - 214 **Carew JS**, Zhou Y, Albitar M, Carew JD, Keating MJ, Huang P. Mitochondrial DNA mutations in primary leukemia cells after chemotherapy: clinical significance and therapeutic implications. *Leukemia* 2003; **17**: 1437-1447 [PMID: 12886229]
 - 215 **Panieri E**, Gogvadze V, Norberg E, Venkatesh R, Orrenius S, Zhivotovsky B. Reactive oxygen species generated in different compartments induce cell death, survival, or senescence. *Free Radic Biol Med* 2013; **57**: 176-187 [PMID: 23295411 DOI: 10.1016/j.freeradbiomed.2012.12.024]
 - 216 **Al Ghouleh I**, Khoo NK, Knaus UG, Griendling KK, Touyz RM, Thannickal VJ, Barchowsky A, Nauseef WM, Kelley EE, Bauer PM, Darley-Usmar V, Shiva S, Cifuentes-Pagano E, Freeman BA, Gladwin MT, Pagano PJ. Oxidases and peroxidases in cardiovascular and lung disease: new concepts in reactive oxygen species signaling. *Free Radic Biol Med* 2011; **51**: 1271-1288 [PMID: 21722728 DOI: 10.1016/j.freeradbiomed.2011.06.011]
 - 217 **Zinkevich NS**, Gutterman DD. ROS-induced ROS release in vascular biology: redox-redox signaling. *Am J Physiol Heart Circ Physiol* 2011; **301**: H647-H653 [PMID: 21685266 DOI: 10.1152/ajpheart.01271.2010]
 - 218 **Kizhakekuttu TJ**, Widlansky ME. Natural antioxidants and hypertension: promise and challenges. *Cardiovasc Ther* 2010; **28**: e20-e32 [PMID: 20370791 DOI: 10.1111/j.1755-5922.2010.00137.x]
 - 219 **Lamson DW**, Brignall MS. Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. *Altern Med Rev* 1999; **4**: 304-329 [PMID: 10559547]
 - 220 **Traber MG**, Stevens JF. Vitamins C and E: beneficial effects from a mechanistic perspective. *Free Radic Biol Med* 2011; **51**: 1000-1013 [PMID: 21664268 DOI: 10.1016/j.freeradbiomed.2011.05.017]
 - 221 **Khaw KT**, Bingham S, Welch A, Luben R, Wareham N, Oakes S, Day N. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. European Prospective Investigation into Cancer and Nutrition. *Lancet* 2001; **357**: 657-663 [PMID: 11247548]
 - 222 **Sasazuki S**, Hayashi T, Nakachi K, Sasaki S, Tsubono Y, Okubo S, Hayashi M, Tsugane S. Protective effect of vitamin C on oxidative stress: a randomized controlled trial. *Int J Vitam Nutr Res* 2008; **78**: 121-128 [PMID: 19003734 DOI: 10.1024/0300-9831.78.3.121]
 - 223 **Gori T**, Münzel T. Oxidative stress and endothelial dysfunction: therapeutic implications. *Ann Med* 2011; **43**: 259-272 [PMID: 21284528 DOI: 10.3109/07853890.2010.543920]
 - 224 **Klein EA**, Thompson IM, Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, Minasian LM, Ford LG, Parnes HL, Gaziano JM, Karp DD, Lieber MM, Walther PJ, Klotz L, Parsons JK, Chin JL, Darke AK, Lippman SM, Goodman GE, Meyskens FL, Baker LH. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2011; **306**: 1549-1556 [PMID: 21990298 DOI: 10.1001/jama.2011.1437]
 - 225 **Farbstein D**, Kozak-Blickstein A, Levy AP. Antioxidant vitamins and their use in preventing cardiovascular disease. *Molecules* 2010; **15**: 8098-8110 [PMID: 21063272 DOI: 10.3390/molecules15118098]
 - 226 **Duffield-Lillico AJ**, Reid ME, Turnbull BW, Combs GF, Slate EH, Fischbach LA, Marshall JR, Clark LC. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 630-639 [PMID: 12101110]
 - 227 **Pantuck AJ**, Leppert JT, Zomorodian N, Aronson W, Hong J, Barnard RJ, Seeram N, Liker H, Wang H, Elashoff R, Heber D, Aviram M, Ignarro L, Belldegrun A. Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer. *Clin*

- Cancer Res* 2006; **12**: 4018-4026 [PMID: 16818701]
- 228 **Khan N**, Afaq F, Mukhtar H. Cancer chemoprevention through dietary antioxidants: progress and promise. *Antioxid Redox Signal* 2008; **10**: 475-510 [PMID: 18154485]
- 229 **Jian L**, Xie LP, Lee AH, Binns CW. Protective effect of green tea against prostate cancer: a case-control study in southeast China. *Int J Cancer* 2004; **108**: 130-135 [PMID: 14618627]
- 230 **Chen L**, Stacewicz-Sapuntzakis M, Duncan C, Sharifi R, Ghosh L, van Breemen R, Ashton D, Bowen PE. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. *J Natl Cancer Inst* 2001; **93**: 1872-1879 [PMID: 11752012]
- 231 **Kim HS**, Bowen P, Chen L, Duncan C, Ghosh L, Sharifi R, Christov K. Effects of tomato sauce consumption on apoptotic cell death in prostate benign hyperplasia and carcinoma. *Nutr Cancer* 2003; **47**: 40-47 [PMID: 14769536]
- 232 **Kawamori T**, Lubet R, Steele VE, Kelloff GJ, Kaskey RB, Rao CV, Reddy BS. Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Res* 1999; **59**: 597-601 [PMID: 9973206]
- 233 **Anand P**, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 2008; **25**: 2097-2116 [PMID: 18626751 DOI: 10.1007/s11095-008-9661-9]
- P- Reviewer:** Hann HW, Hou WH, Koch TR, Morales-Gonzalez JA, Sanchez-Alcazar JA **S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Lu YJ





Recent advances of cluster of differentiation 74 in cancer

Yu-Huei Liu, Jung-Yaw Lin

Yu-Huei Liu, Department of Medical Genetics and Medical Research, China Medical University Hospital, Taichung 40402, Taiwan

Yu-Huei Liu, Graduate Institute of Integrated Medicine, China Medical University, Taichung 40402, Taiwan

Jung-Yaw Lin, Department of Life Science, National Taiwan Normal University, Taipei 11677, Taiwan

Jung-Yaw Lin, Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, Taipei 11051, Taiwan

Author contributions: Liu YH and Lin JY solely contributed to this paper.

Supported by National Science Council of Taiwan, No. NSC 98-2320-B-002-050-MY2 and No. NSC 102-2320-B-039-032-MY3

Correspondence to: Jung-Yaw Lin, PhD, Institute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, Room 942, No.1 Jen-Ai Road, Taipei 11051, Taiwan. linjy@ntu.edu.tw

Telephone: +886-2-23123456-88206 Fax: +886-2-23954776

Received: March 8, 2014

Revised: June 3, 2014

Accepted: July 25, 2014

Published online: November 27, 2014

Abstract

Cluster of differentiation 74 (CD74) performs multiple roles in B cells, T cells, and antigen-presenting cells within the immune system; it also participates in major histocompatibility complex class II-restricted antigen presentation and inflammation. Recently, a role for CD74 in carcinogenesis has been described. CD74 promotes cell proliferation and motility and prevents cell death in a macrophage migration inhibitory factor-dependent manner. Its roles as an accessory signal receptor on the cell surface and the ability to interact with other signaling molecules make CD74 an attractive therapeutic target for the treatment of cancer. This review focuses on the original role of CD74 in the immune system and its emerging tumor-related functions. First, the structure of CD74 will be summarized. Second, the current understandings about the expression, cellular localization, molecular mechanisms and signaling pathways of CD74 in immunity and cancer

will be reviewed. Third, the examples that suggest CD74 is a promising molecular therapeutic target are reviewed and discussed. Although the safety and efficacy of CD74-targeted strategies are under development, deeply understanding of the regulation of CD74 will hold promise for the use of CD74 as a therapeutic target and may develop the CD74-targeted therapeutic agents such as neutralized antibody and compounds.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Cluster of differentiation 74; Invariant chain; Immune; Inflammation; Tumorigenesis; Cancer metastasis

Core tip: There are several structural and functional variants of cluster of differentiation 74 (CD74), each with their own expression pattern. Although this diversity may be required for normal homeostasis, it can lead to aberrant proliferation when dysregulated. This review focuses on the primary role of CD74 in the immune system and how the activity of this evolutionarily conserved molecule is subverted during tumorigenesis.

Liu YH, Lin JY. Recent advances of cluster of differentiation 74 in cancer. *World J Immunol* 2014; 4(3): 174-184 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i3/174.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i3.174>

STRUCTURE AND GENOMIC ORGANIZATION OF CLUSTER OF DIFFERENTIATION 74 AND ASSOCIATED VARIANTS

The cluster of differentiation 74 (CD74) gene, which is located on chromosome 5q32, encodes the type II integral membrane glycoprotein CD74; there are four major isoforms of this protein in humans^[1]. This evolutionarily

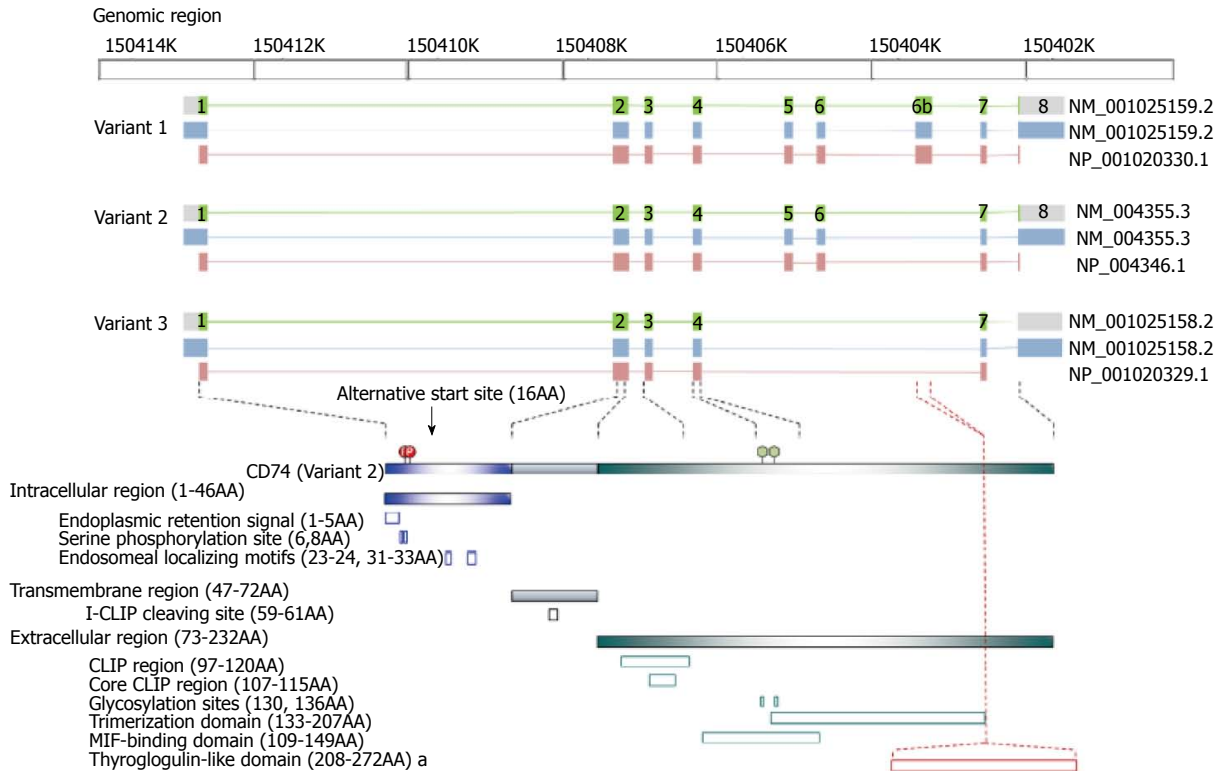


Figure 1 The variants and the corresponding protein structures of cluster of differentiation 74. The upper panel illustrates the corresponding position among genomic region, NCBI reference sequence number and reference protein accession, exon (green box) and intron (green line) localization of DNA, transcripts (light blue box), and protein (pink box) of the three cluster of differentiation 74 (CD74) variants. The lower panel, CD74 variants contains three regions including intracellular, transmembrane and extracellular regions with the indicated functional domains and identified residues for post-translational modification. Variant 2 transcripts two isoforms, p33 and p35 caused by the alternative start site. Variant 1 transcripts two isoforms, p41 and p43, with an exon 6b-encoded thyroglobulin type I domain to interact with cathepsin. Variant 3 lacks exon 5, 6, and 6b, which translates truncated trimerization domain and truncated macrophage migration inhibitory factor (MIF)-binding domain and remains only the CLIP region to function as major histocompatibility complex class II mask. The amino acid residues refer to human variant 2 (p35).

conserved molecule is the membrane form of the major histocompatibility complex class II (MHC class II) invariant chain (Ii) because none of the original isolates harbored polymorphisms^[2]. The most common isoform of CD74 is the p33 isoform (with a molecular weight of 33 kDa), which has a 29-residue N-terminal intracellular region, a 26-residue hydrophobic transmembrane region, and a 160-residue C-terminal extracellular region containing two N-linked glycosylation sites^[3,4]. The p35 isoform is also produced because of differential initiation of translation^[5], whereas p41 and p43 isoforms arise because of alternative splicing of the exon 6b transcription products that encode a thyroglobulin type I cathepsin-binding domain^[6-8]. Both the p33 and p35 isoforms regulate MHC class II antigen presentation through rapid internalization from the cell surface to endosomes (half-life under 10 min) when MHC class II-CD74 complexes are formed. However, approximately 2%-5% of these cell surface isoforms are not found in MHC class II complexes. Although the role(s) for the membrane-localized CD74 on some parenchymal epithelial cells remain largely unclear, the finding that it is involved in proliferative responses associated with intramembrane proteolysis (RIP)-processed led researchers to investigate its role in cancer^[9]. Domains, motifs, and active residues as well as the corresponding functions within the intracellular^[10-13],

transmembrane^[14], and extracellular region^[7,9,15-21] of CD74 have been identified. Figure 1 illustrates the CD74 variants and their corresponding protein structures.

THE PHYSIOLOGICAL ROLE OF CD74 IN THE IMMUNE SYSTEM

CD74 has several functions related to MHC class II-restricted antigen presentation, including the prevention of MHC class II to bind non-processed peptide and self-antigen^[22]. CD74 was originally reported to be a molecular chaperone for regulating MHC class II folding in the rough endoplasmic reticulum (ER), where it was thought to play a major role in processing and transporting of MHC class II molecules in the immune system, and in particular in antigen-presenting cells. Once synthesized, CD74 self-assembles into a trimer and serves as a scaffold onto which nascent MHC class II molecules assemble. After trafficking to the late endosome, CD74 is cleaved by cathepsin S (cathepsin L in thymic epithelial cells), leaving a small peptide, CLIP, to block the peptide binding cleft of MHC class II and in turn to prevent premature binding of antigenic peptides to MHC class II. The CLIP-MHC class II complex will then transport through the endosomal pathway^[5]. Upon binding of

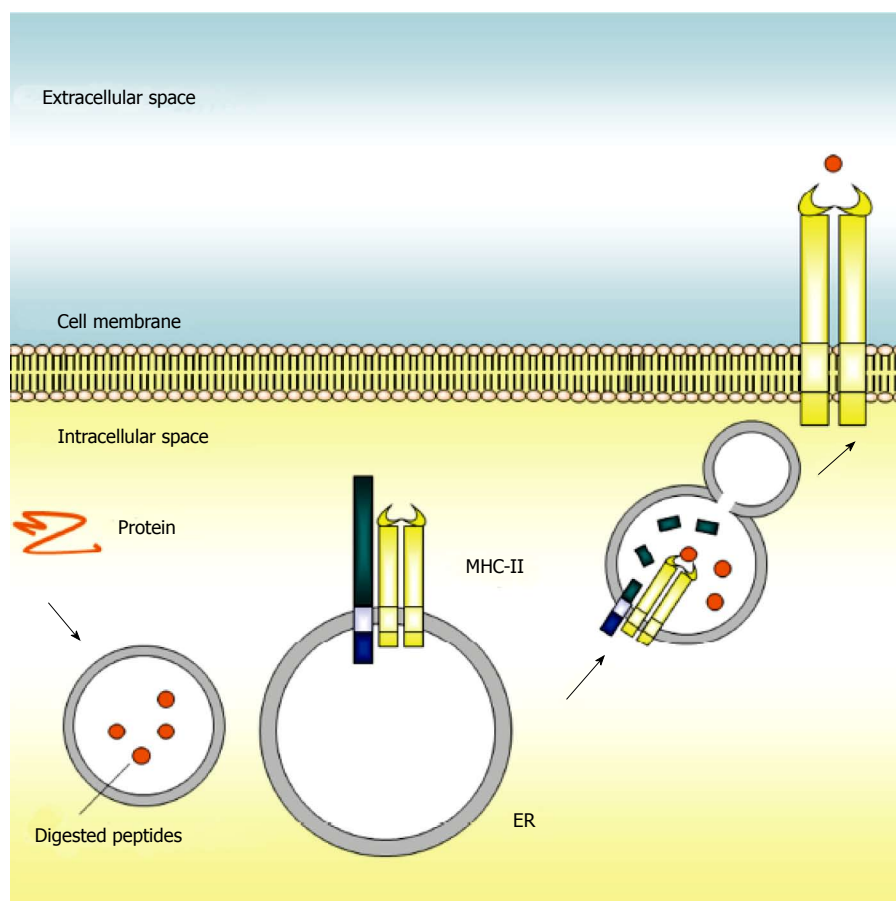


Figure 2 The canonical function of cluster of differentiation 74 in the immune system. Cluster of differentiation 74 (CD74) is present on endoplasmic reticulum (ER) where it can interact with major histocompatibility complex class II (MHC class II) and contribute to antigen presentation. Once synthesized, CD74 self-assembles as a trimer and serves as a scaffold onto which nascent MHC class II assemble. After trafficking to the late endosome, CD74 is cleaved into a small peptide, CLIP, to block the peptide binding cleft of MHC class II, prevent premature binding of antigenic peptides, and direct the MHC class II complex to the endosomal pathway. The MHC class II molecules with bound antigenic peptides are then exported to the surface of the antigen presenting cell for presentation of foreign peptides to CD4⁺ T cells.

HLA-DM to MHC class II, CLIP is released, which allows the peptide-binding cleft of MHC class II to open and bind further antigenic peptides. The MHC class II molecules with bound antigenic peptides are then exported to the surface of the antigen-presenting cell for presentation of foreign peptides to CD4⁺ T cells^[23,24]. Meanwhile, CLIP peptide is degraded by proteasomes, and newly synthesized CD74 is then generated.

Absence of CD74 results aberrant MHC class II-dependent antigen processing and perturbs host defenses. Deficiency of CD74 in mice is associated with aberrant MHC class II synthesis^[25], delayed MHC class II presentation by antigen-presenting cells^[26], and impaired maturation of CD4⁺ T cells^[27]. However, knockout of CD74 in mice was able to mount an efficient response against viral infection^[28]. Although how this efficient response for viral infection could work required further elucidated, an event that the function of CD4⁺ Th2 cells in CD74-null mice is compromised by CD4⁺ Th1 cells could in part explain the current observation^[29]. In addition, this compromise emphasizes the crucial role of CD74 in immune regulation. An alternative strategy would be to specifically inhibit the antigen presentation mechanism

and allow the pathogen to co-exist with the host during the initial phase of pathogen entry without promoting an immune reaction. This approach would be based on the observation that blockade of CD74 reduces migration inhibitory factor (MIF)-dependent monocyte arrest, chemokine expression, and neutrophil recruitment^[30]. In other words, although initial inflammatory mediators are required for recruitment of neutrophils and to resolve infection-induced innate immunity, an over-robust response would generate excessive inflammatory mediators and trigger a hypersensitivity response, which could ultimately cause tissue damage and pathology. As further support of the hypothesis that attenuating CD74 function may be of benefit in some cases, mice that lack CD74 are known to be protected against bacterial infection^[31]. However, a fine balance must be struck between modulating the host immune response and preventing the deleterious effects of pathogen exposure. All of these observations have generated considerable interest in CD74, as they suggest that the protein-binding ability of MHC class II molecules could be enhanced by modifying the expression and function of endoplasmic reticulum CD74. Figure 2 illustrates the canonical antigen presentation function of

CD74 in the immune system.

ASSOCIATION OF CD74 WITH CANCER

New functions and novel interactions associated with the evolutionarily conserved CD74 protein are continually being revealed. Rare single-nucleotide polymorphisms (SNPs) in the *CD74* gene have been reported, but SNPs in molecules that interact with CD74, such as MIF^[9], CD44^[32] and MHC class II^[15] are more frequent and are associated with the development of cancer^[33-37]. The imbalance in the regulation of inflammation that occurs in many cancers can induce cellular damage. This stimulates interaction between immune cells and the damaged cells, which then proliferate, invade, and subsequently develop into tumors^[38]. Together with its role in several immunological processes, these findings indicate that CD74 is a potential therapeutic target.

CD74 as a cell surface receptor

A decade ago, CD74 was reported as an accessory signaling molecule in cancers because of its localization on the plasma membrane in certain cell types, and its role as a surface-binding receptor for MIF, a pro-inflammatory cytokine^[9]. Indeed, it is now generally accepted that the oncogenic role of CD74 is MIF-dependent. In B cells, MIF induces NF- κ B activation, cell proliferation, and survival^[39]. MIF also induces upregulation of anti-apoptotic proteins Bcl-2 and Bcl-XL^[14]. These findings suggest that CD74 stimulation initiates a pro-survival signal. Genomic and immunohistochemical studies have revealed upregulation of CD74 in various cancers, suggesting that it may have some relationship with tumorigenesis. Table 1 summarizes the current information regarding expression and clinical significance of CD74 in human cancers. One interpretation of these observations is that persistent overexpression of CD74 in the intracellular space and on the cell surface could impair MHC class II antigen presentation by tumor cells, thereby contributing to immune escape and facilitating tumor metastasis^[40]. The underlying reasons for CD74 overexpression in cancer have remained largely unclear. However, the *CD74* locus is a common insertion site for viruses in murine B lymphomas^[11]; by inference, similar virus-mediated upregulation may occur in human tumors.

Oncogenic signaling through cell surface CD74

MIF is a multifunctional cytokine that is produced by several cell types, including epithelial cells and cells that participate in the innate and adaptive immune responses^[41-43]. CD74 is a receptor for extracellular MIF that is expressed in human B cells^[14], gastric epithelial cells^[44] and type II alveolar epithelial cells^[45]. Following MIF binding, CD74 is rapidly internalized, leading to downstream signaling cascades that trigger NF- κ B activation^[39], prostaglandin E2 production^[9,46], TAp63 upregulation^[47], and secretion of survival factors such as IL-8^[48] and VEGF-D^[49] *via* phosphorylation of ERK^[9,50] and AKT^[51]. The signaling

cascades trigger cell proliferation and migration, and prevent apoptosis^[14,49]. Overexpression of CD74 in HEK293 cells initiates MIF-dependent MEK/ERK and PI3K/AKT activation. This is followed by NF- κ B activation, which in turn triggers VEGF-D upregulation and VEGF-D-dependent cell proliferation and motility. The ultimate consequence is an increase in tumor mass, tumor-induced angiogenesis, and metastasis in xenograft-bearing mice^[49].

However, unlike other ligand-receptor axes, such as EGF/EGFR^[52] and VEGF-A/VEGFR2^[53], CD74 lacks intracellular signaling motifs for transducing downstream signals. Therefore, it must recruit other molecules in order to transduce signals in response to MIF stimulation. Indeed, the intracytoplasmic signaling domain of CD44, a transmembrane protein with kinase-activating properties, can relay signaling downstream of the MIF-CD74 interaction^[32]. CD74 forms a complex with CD44, which leads to PKA-dependent serine phosphorylation and Src activation; this eventually leads to p53 dephosphorylation, thereby stimulating cell proliferation and preventing apoptosis^[32]. Another transduction mechanism involves the functional interaction between CD74 and CXCR chemokine receptors during CD74-dependent cancer cell proliferation and invasion^[30,49,54]. There are also reports of fusions between *CD74* and the oncogenic receptor tyrosine kinase, *ROS1*; the resultant fusion protein activates a novel invasiveness pathway through the phosphorylation of the extended synaptotagmin-like protein, E-Syt1, in non-small cell lung cancer^[55-61]. Oncogenic CD74-ROS1 represents a tumor-specific target for drug therapy, against which next-generation kinase inhibitors can be developed. Whether CD74-ROS1 (or indeed, as yet unidentified CD74 fusion proteins) has additional substrates, and whether other coreceptors participate in CD74-dependent transformation, are important unresolved questions.

RIP-processed transcription factors

Most of the RIP-processed transcription factors are synthesized and maintained as inactive membrane-associated precursors that are activated after internal or environmental cues. Such stimuli include protease cleavage, which leads to release of intracellular fragments that translocate into the nucleus and drive transcription. This is exemplified by the functional interaction of CD74 with epithelial growth factor receptor (EGFR)^[62]. The Leu-Leu-Leu intramembrane proteases (I-CLIPs) cleavage site within the transmembrane domain is essential for the cleavage of CD74, as mutation of these residues abolishes the release of intracellular domain (ICD) of CD74^[14]. This cleavage occurs upon treatment with an activating anti-CD74 antibody, thereby liberating the CD74-ICD from the cell membrane into the cytoplasm. Following nuclear translocation, the CD74-ICD leads to the activation of the NF- κ B p65/RelA homodimer and its coactivator, TAF_{II}105, in CD74-overexpressed HEK293 cells and in mouse B lymphocytes^[14,39,63-67]. Subsequently, the signaling cascade is attenuated by ubiquitin-dependent proteasomal degra-

Table 1 Expression levels and clinical significance of cluster of differentiation 74 in human cancers

Cancer type	Event	Method	Ref
Renal cell cancer	CD74 was detected in 53 of 60 (88.3%) renal cell cancer tissues	IHC	[90,91]
	CD74 is a useful diagnostic marker for distinguishing clear cell RCC from chromophobe and oncocytoma RCC	IHC	[92]
	CD74 was upregulated in 34 of 40 (85.0%) of clear cell RCC tissues compared with the corresponding normal kidney tissues, and the expression level was positively correlated with VEGF-D (Pearson's correlation, $r = 0.65$, $P < 0.001$)	Quantitative real-time RT-PCR, IHC	[49]
Malignant fibrous histiocytoma	Differential expression of CD74 was found in atypical malignant fibrous histiocytoma (90% positive) and fibroxanthoma (10% positive), suggesting that CD74 may be a marker of tumor progression	IHC	[93]
Thymic epithelial neoplasm	CD74 was detected in 88% (15/17) of thymic carcinomas, 70% (14/20) of invasive thymomas, but only 33% (9/27) of benign thymomas (9/27), suggesting that CD74 is a useful marker for the classification of thymic epithelial neoplasms	IHC	[94]
Colorectal cancer	A linear increase of CD74 expression was found in the progression from low- to high-grade invasive cancer tissues	IHC	[95]
	High levels of CD74 were detected in 23 of 156 (15.0%) curatively resected colorectal cancer tissues	IHC	[96]
	CD74 was increased in dysplastic epithelial cells in 47 of 55 (85%) human colorectal adenomas, with CD74 and MIF protein levels together predicting increasing dysplasia in individual adenomas ($P = 0.003$)	IHC	[97]
Gastric cancer	CD74 was detected in 48 of 126 (38.1%) gastric cancer tissues, and the expression was negatively correlated with the depth of invasion and HLA-DR expression. The patients with detectable CD74 show poor surgical outcomes ($P < 0.05$)	IHC	[98]
	CD74 was detected in 39 of 58 (67.2%) gastric carcinoma tissues, showing significant correlation with the differentiation of gastric carcinoma ($P < 0.05$)	IHC	[99]
Breast cancer	The expression of CD74 was significantly more abundant in invasive or metastatic tumors than in SAGE ductal carcinoma in situ ($P = 0.02$ and $P = 0.05$, respectively)		[100]
	CD74 was detected in 468 of 580 (80.7%) breast cancer tissues, and was related to lymph node metastasis and triple-negative breast cancer ($P = 0.01$ and 0.001). In addition, CD74 expression had a linear correlation with lymph node metastasis and triple-negative breast cancer ($P = 0.02$ and 0.001)	IHC	[101]
	Stat1 and CD74 overexpression is co-dependent and linked to increased invasion and lymph node metastasis in triple-negative breast cancer	LC-MS/MS, IHC	[102]
Multiple myeloma	CD74 expression was increased in high-grade, invasive urothelial carcinoma of the bladder		[103]
Pancreatic cancer	CD74 was detected in 19 of 22 (86.4%) multiple myeloma tissues	IHC	[69]
	CD74 was identified as an overexpressed gene when compared with two SAGE libraries (6 pancreatic cancers vs 11 non-neoplastic tissues), and the expression of CD74 was detected in 15 of 18 (83%) pancreatic ductal adenocarcinoma tissues	SAGE, IHC	[104]
	CD74 was expressed in 52 of 67 (77.6%) pancreatic cancer tissues that was correlated with high perineural invasion ($P < 0.008$)	IHC	[105]
	Moreover, 47 of 68 (69.1%) and 21 of 68 (30.9%) pancreas tissues from patients receiving curative extended resection showed lower ($< 70\%$) and higher ($\geq 70\%$) CD74 expression, respectively. Patients with higher CD74 expression in pancreatic cancer tissues showed a higher rate of lymphatic permeation ($P = 0.04$), perineural invasion ($P = 0.01$), poor prognosis ($P = 0.006$), and poor survival ($P = 0.003$) compared with those with lower expression	IHC	[106]
	Fourteen of 46 (30.4%) and 32 of 46 (69.6%) pancreatic ductal adenocarcinoma tissues showed lower ($< 25\%$) and higher ($\geq 25\%$) CD74 expression, respectively. Patients with higher CD74 expression in pancreatic cancer tissues showed a higher rate of perineural invasion ($P = 0.007$) and poor 3- and 5-yr cumulative survival rates (41% and 62% vs 0% and 9%, $P = 0.000$) compared with those with lower expression	IHC	[107]
Cervical squamous cell carcinoma	CD74 expression was significantly higher in CIN than in the normal samples and higher in SCC than in CIN	IHC	[108]
Urothelial carcinoma of the bladder	CD74 was detected in 192 of 342 (56.1%) urothelial carcinoma of the bladder tissues, which is associated with older age at diagnosis (≥ 68 yr, $P = 0.048$), high World Health Organization grade ($P = 0.019$), advanced stages ($P = 0.001$), non-papillary growth pattern ($P = 0.040$), the absence of tumor-infiltrating inflammatory cells ($P < 0.001$), and the presence of tumor-associated inflammatory cells ($P = 0.017$). However, CD74 expression was not related to recurrence-free and overall survivals in primary and subgroup analyses	IHC	[103]
Non-small cell lung cancer	A case report found a mutation in CD74-ROS1 that is associated with acquired resistance to crizotinib.	FISH, RT-PCR	[109]
	CD74 was detected in 57 of 70 (81.4%) non-small cell lung cancer tissues	IHC	[110]
	CD74-ROS1 fusion transcript was detected in 5 of 1073 (0.5%) non-small cell lung cancer tissues	RT-PCR	[61]
	CD74-ROS1 fusion transcript was detected in 4 of 556 (0.7%) non-small cell lung cancer tissues	IHC	[111]
	CD74-ROS1 fusion transcript was detected in 1 of 114 (0.9%) non-small cell lung cancer tissues	RT-PCR	[56]
	CD74-ROS1 fusion transcript was detected in 2 of 208 (1.0%) never-smokers with lung adenocarcinoma tissues	RT-PCR	[112]
	CD74-ROS1 fusion transcript was detected in 2 of 447 (4.5%) never-smokers with lung adenocarcinoma tissues	Transcriptome sequencing	[113]
	Two CD74 polymorphisms, rs2748249 and rs1560661, are associated with hematologic toxicity in patients with non-small cell lung cancer after platinum-based chemotherapy	BeadChip	[114]

CD74: Cluster of differentiation 74; MIF: Migration inhibitory factor; RCC: Renal cell cancer; IHC: Immunohistochemistry; RT-PCR: Reverse-transcription polymerase chain reaction; SAGE: Serial analysis of gene expression; LC-MS/MS: Liquid chromatography-mass spectrometry/ mass spectrometry; FISH: Fluorescence *in situ* hybridization.

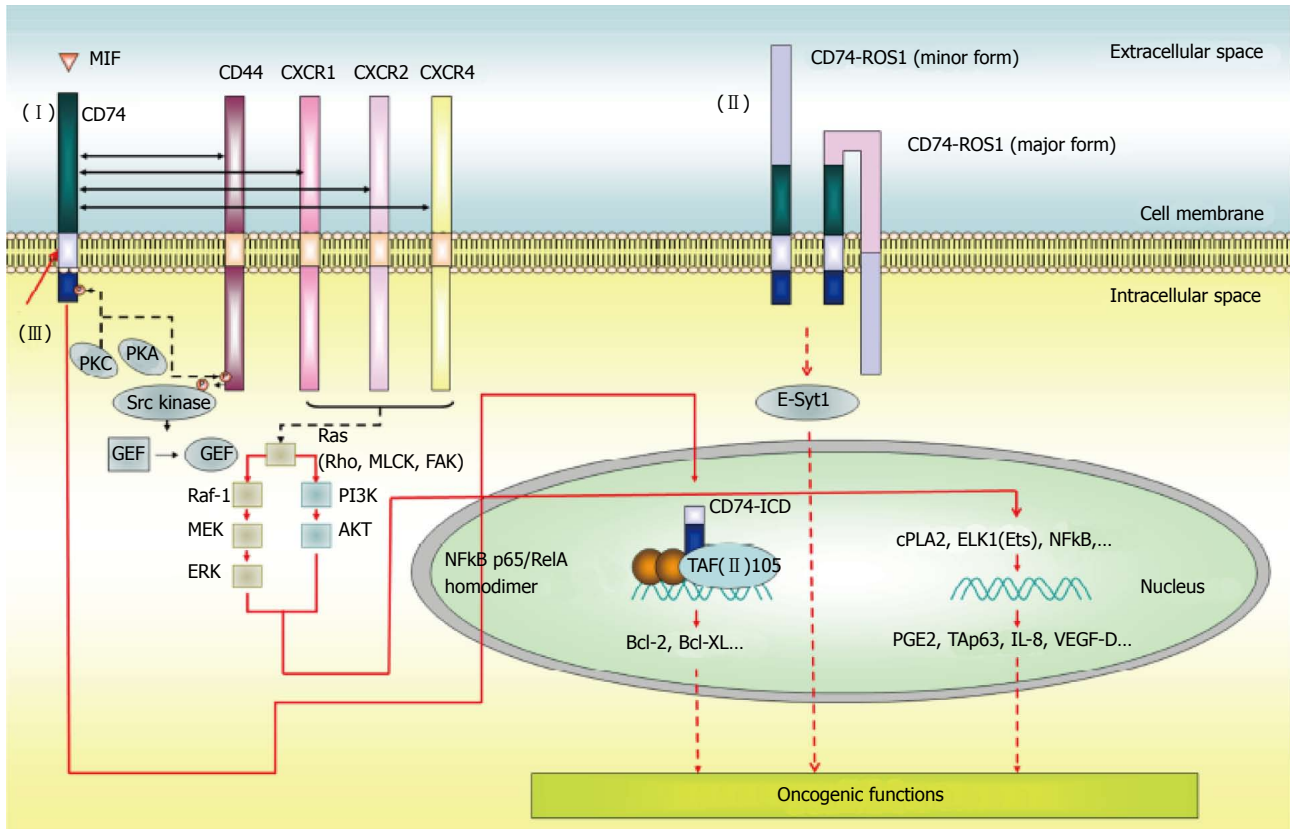


Figure 3 The function of cluster of differentiation 74 in the cancer development. (I) Membrane associated-CD74 is involved in modulating the expression of a variety of genes which involved cell proliferation, invasion and survival through interacting with CD44, CXCR1, CXCR2 or CXCR4 followed by activation of the signaling cascades in a MIF-dependent manner. (II) After MIF stimulation, CD74 releases its intracellular domain, CD74-ICD. The CD74-ICD translocates from cytoplasm into nucleus and functions as a transcription modulator. (III) The CD74-ROS1 fusion protein with one (minor form) or two (major form) transmembrane regions and one kinase domain, promotes novel invasiveness pathway through the phosphorylation of the extended synaptotagmin-like protein, E-Syt1. MIF: Macrophage migration inhibitory factor; CXCR: Chemokine (C-X-C motif) receptor; PKA: Protein kinase A; PKC: Protein kinase C; GEF: Guanine nucleotide exchange factor; Ras: Ras oncogene; Rho: Ras homolog family member; MLCK: Myosin light chain kinase; FAK: Focal adhesion kinase; Raf-1: RAF proto-oncogene serine/threonine-protein kinase; MEK: Mitogen-activated protein kinase kinase; ERK: Extracellular signal-regulated kinase; PI3K: Phosphoinositide 3-kinase; NF-κB: Nuclear factor-kappaB; TAF(II)105: Transcription initiation factor TFIID 105 kDa subunit; ROS-1: C-ros oncogene 1; E-Syt1: Extended synaptotagmin-like protein 1; Bcl-2: B-cell lymphoma 2; Bcl-XL: B-cell lymphoma-extra large; cPLA2: Cytosolic phospholipase A2; ELK1: Member of ETS oncogene family; Ets: V-ets erythroblastosis virus E26 oncogene; PGE2: Prostaglandin E2; Tap63: Tumor protein p63; IL-8: Interleukin 8; VEGF-D: Vascular endothelial growth factor D.

dation of CD74-ICD^[67]. Figure 3 illustrates the function of CD74 in cancer development.

CD74-TARGETED CANCER THERAPY

The high expression of CD74 in cancer cells in comparison with their normal counterparts provides a potential cancer-selective antitumor strategy. As mentioned above, oncogenic CD74-ROS1 represents a potential tumor-specific target against which next-generation kinase inhibitors might be developed. However, whether additional substrates co-exist with CD74-ROS1 or other unidentified CD74 fusion proteins, and whether other coreceptors participate in CD74-dependent transformation remains to be determined.

A monoclonal antibody, LL1, which binds to and rapidly internalizes cell surface CD74 into lysosomes^[68], increases the survival of mice bearing xenografts^[69]. Recent studies have also highlighted the efficacy of a humanized anti-CD74 monoclonal antibody derived from LL1, named milatuzumab, in the treatment of lymphoid

malignancies^[70,71], non-Hodgkin lymphoma^[72], chronic lymphocytic leukemia^[73], and mantle cell lymphoma^[74]. A phase I multicenter, dose-escalation trial of monotherapy with milatuzumab in advanced multiple myeloma has been evaluated^[75]. In addition, because the CD74 antibody enters lysosomes rapidly and at high concentration, it could be conjugated to a drug, and then used to target tumors expressing cell surface CD74. Successful preclinical examples include antibodies conjugated with radioisotopes^[76,77] and doxorubicin^[78,79], as well as combined therapy using milatuzumab and FTY720, a CD74 stimulator^[80]. However, further selectivity must be developed, since such antibodies could potentially bind to all antigen-presenting cells.

Targeted therapy using small molecules is another developing field. Some small molecules have demonstrated activity against other proteins that associate with CD74, and can thus indirectly block CD74 function^[30,81]. Examples are MIF activity modifiers, which prevent MIF binding to CD74. For instance, Ibudilast, a phosphodiesterase inhibitor, blocks MIF activity fol-

lowed by inhibited chemotactic activity of peripheral blood mononuclear cells^[82]. (S, R)-3(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (ISO-1) and 4-iodo-6-phenylpyrimidine (4-IPP) function as tautomerase inhibitors that also abolish MIF activity^[83]. Eb-selen disrupts the formation of MIF trimmers, thereby inactivating the complex^[84]. A second set of examples is the cathepsin S inhibitors that prevent antigen presentation and disease progression through inhibiting CD74 degradation. The accumulated CD74 binds to MHC class II molecules within endocytic compartments, which are targets for treatment of autoimmune diseases using molecules such as Clik60^[85], LHVS^[86], and SB-331750^[87], and RWJ-445380. Finally, there are CD74 expression modifiers such as Auraptene that suppresses CD74 expression and thus blocks *Helicobacter pylori* adhesion and pro-inflammatory mediator production in C57BL/6 mice^[88,89]. However, whether these small molecules will have anti-cancer activity remains to be determined. More specific targeted approaches will emerge from the ongoing screening efforts to find compounds that directly target CD74. Combined with an effective method to deliver the targeting agents efficiently to the tumor, this would be a critical breakthrough for the field.

CONCLUSION

Recent advances in our knowledge of CD74 functions have emerged through discovery of its natural ligand, additional interacting proteins, and elucidation of molecular mechanisms associated with CD74 signaling in immunity and cancer. Normal expression in antigen-presenting cells maintain proper MHC class II-restricted antigen presentation and an appropriate immune regulation. However, aberrant expression of CD74 in cells leads to an unbalanced immune system, and possibly also oncogenesis, in a MIF-dependent manner. Despite the natural protective actions of CD74 in the immune system, functional studies from several CD74-focused experimental models show that CD74 inhibition will also likely halt cancer progression and improve patient prognosis. There are ongoing clinical studies into the role of CD74 in diverse diseases, including various types of cancer. Further research into CD74 and its effect on cellular processes, including the complex interactions between CD74 and its binding partners, will undoubtedly translate into clinical benefit for patients.

REFERENCES

- 1 **Pyrz M**, Wang B, Wabl M, Pedersen FS. A retroviral mutagenesis screen identifies Cd74 as a common insertion site in murine B-lymphomas and reveals the existence of a novel IFN γ -inducible Cd74 isoform. *Mol Cancer* 2010; **9**: 86 [PMID: 20416035 DOI: 10.1186/1476-4598-9-86]
- 2 **Long EO**, Strubin M, Wake CT, Gross N, Carrel S, Goodfellow P, Accolla RS, Mach B. Isolation of cDNA clones for the p33 invariant chain associated with HLA-DR antigens. *Proc Natl Acad Sci USA* 1983; **80**: 5714-5718 [PMID: 6412232 DOI: 10.1073/pnas.80.18.5714]
- 3 **Claesson L**, Larhammar D, Rask L, Peterson PA. cDNA clone for the human invariant gamma chain of class II histocompatibility antigens and its implications for the protein structure. *Proc Natl Acad Sci USA* 1983; **80**: 7395-7399 [PMID: 6324166 DOI: 10.1073/pnas.80.24.7395]
- 4 **Strubin M**, Mach B, Long EO. The complete sequence of the mRNA for the HLA-DR-associated invariant chain reveals a polypeptide with an unusual transmembrane polarity. *EMBO J* 1984; **3**: 869-872 [PMID: 6586420]
- 5 **Arunachalam B**, Lamb CA, Cresswell P. Transport properties of free and MHC class II-associated oligomers containing different isoforms of human invariant chain. *Int Immunol* 1994; **6**: 439-451 [PMID: 8186195 DOI: 10.1093/intimm/6.3.439]
- 6 **Warmerdam PA**, Long EO, Roche PA. Isoforms of the invariant chain regulate transport of MHC class II molecules to antigen processing compartments. *J Cell Biol* 1996; **133**: 281-291 [PMID: 8609162 DOI: 10.1083/jcb.133.2.281]
- 7 **Koch N**, Lauer W, Habicht J, Dobberstein B. Primary structure of the gene for the murine Ia antigen-associated invariant chains (Ii). An alternatively spliced exon encodes a cysteine-rich domain highly homologous to a repetitive sequence of thyroglobulin. *EMBO J* 1987; **6**: 1677-1683 [PMID: 3038530]
- 8 **Fineschi B**, Arneson LS, Naujokas MF, Miller J. Proteolysis of major histocompatibility complex class II-associated invariant chain is regulated by the alternatively spliced gene product, p41. *Proc Natl Acad Sci USA* 1995; **92**: 10257-10261 [PMID: 7479763 DOI: 10.1073/pnas.92.22.10257]
- 9 **Leng L**, Metz CN, Fang Y, Xu J, Donnelly S, Baugh J, Delohery T, Chen Y, Mitchell RA, Bucala R. MIF signal transduction initiated by binding to CD74. *J Exp Med* 2003; **197**: 1467-1476 [PMID: 12782713 DOI: 10.1084/jem.20030286]
- 10 **Anderson HA**, Roche PA. Phosphorylation regulates the delivery of MHC class II invariant chain complexes to antigen processing compartments. *J Immunol* 1998; **160**: 4850-4858 [PMID: 9590232]
- 11 **Hofmann MW**, Höning S, Rodionov D, Dobberstein B, von Figura K, Bakke O. The leucine-based sorting motifs in the cytoplasmic domain of the invariant chain are recognized by the clathrin adaptors AP1 and AP2 and their medium chains. *J Biol Chem* 1999; **274**: 36153-36158 [PMID: 10593899 DOI: 10.1074/jbc.274.51.36153]
- 12 **Motta A**, Bremnes B, Morelli MA, Frank RW, Saviano G, Bakke O. Structure-activity relationship of the leucine-based sorting motifs in the cytosolic tail of the major histocompatibility complex-associated invariant chain. *J Biol Chem* 1995; **270**: 27165-27171 [PMID: 7592972 DOI: 10.1074/jbc.270.45.27165]
- 13 **Schutze MP**, Peterson PA, Jackson MR. An N-terminal double-arginine motif maintains type II membrane proteins in the endoplasmic reticulum. *EMBO J* 1994; **13**: 1696-1705 [PMID: 8157008]
- 14 **Starlets D**, Gore Y, Binsky I, Haran M, Harpaz N, Shvidel L, Becker-Herman S, Berrebi A, Shachar I. Cell-surface CD74 initiates a signaling cascade leading to cell proliferation and survival. *Blood* 2006; **107**: 4807-4816 [PMID: 16484589 DOI: 10.1182/blood-2005-11-4334]
- 15 **Ericson ML**, Sundström M, Sansom DM, Charron DJ. Mutually exclusive binding of peptide and invariant chain to major histocompatibility complex class II antigens. *J Biol Chem* 1994; **269**: 26531-26538 [PMID: 7929377]
- 16 **Roche PA**, Cresswell P. Proteolysis of the class II-associated invariant chain generates a peptide binding site in intracellular HLA-DR molecules. *Proc Natl Acad Sci USA* 1991; **88**: 3150-3154 [PMID: 2014234 DOI: 10.1073/pnas.88.8.3150]
- 17 **Ghosh P**, Amaya M, Mellins E, Wiley DC. The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3. *Nature* 1995; **378**: 457-462 [PMID: 7477400 DOI: 10.1038/378457a0]

- 18 **Bijlmakers MJ**, Benaroch P, Ploegh HL. Mapping functional regions in the luminal domain of the class II-associated invariant chain. *J Exp Med* 1994; **180**: 623-629 [PMID: 7519244 DOI: 10.1084/jem.180.2.623]
- 19 **Ou WJ**, Cameron PH, Thomas DY, Bergeron JJ. Association of folding intermediates of glycoproteins with calnexin during protein maturation. *Nature* 1993; **364**: 771-776 [PMID: 8102790 DOI: 10.1038/364771a0]
- 20 **Sant AJ**, Schwartz BD, Cullen SE. Cellular distribution of the Ia-associated chondroitin sulfate proteoglycan. *J Immunol* 1985; **135**: 408-415 [PMID: 3923108]
- 21 **Sant AJ**, Cullen SE, Giacometto KS, Schwartz BD. Invariant chain is the core protein of the Ia-associated chondroitin sulfate proteoglycan. *J Exp Med* 1985; **162**: 1916-1934 [PMID: 3864916 DOI: 10.1084/jem.162.6.1916]
- 22 **Bertolino P**, Rabourdin-Combe C. The MHC class II-associated invariant chain: a molecule with multiple roles in MHC class II biosynthesis and antigen presentation to CD4⁺ T cells. *Crit Rev Immunol* 1996; **16**: 359-379 [PMID: 8954255]
- 23 **Jensen PE**, Weber DA, Thayer WP, Westerman LE, Dao CT. Peptide exchange in MHC molecules. *Immunol Rev* 1999; **172**: 229-238 [PMID: 10631949 DOI: 10.1111/j.1600-065X.1999.tb01368.x]
- 24 **Busch R**, Rinderknecht CH, Roh S, Lee AW, Harding JJ, Burster T, Hornell TM, Mellins ED. Achieving stability through editing and chaperoning: regulation of MHC class II peptide binding and expression. *Immunol Rev* 2005; **207**: 242-260 [PMID: 16181341 DOI: 10.1111/j.0105-2896.2005.00306.x]
- 25 **Bikoff EK**, Huang LY, Episkopou V, van Meerwijk J, Germain RN, Robertson EJ. Defective major histocompatibility complex class II assembly, transport, peptide acquisition, and CD4⁺ T cell selection in mice lacking invariant chain expression. *J Exp Med* 1993; **177**: 1699-1712 [PMID: 8098731]
- 26 **Viville S**, Neefjes J, Lotteau V, Dierich A, Lemeur M, Ploegh H, Benoist C, Mathis D. Mice lacking the MHC class II-associated invariant chain. *Cell* 1993; **72**: 635-648 [PMID: 7679955]
- 27 **Wong P**, Rudensky AY. Phenotype and function of CD4⁺ T cells in mice lacking invariant chain. *J Immunol* 1996; **156**: 2133-2142 [PMID: 8690902]
- 28 **Battegay M**, Bachmann MF, Burkhart C, Viville S, Benoist C, Mathis D, Hengartner H, Zinkernagel RM. Antiviral immune responses of mice lacking MHC class II or its associated invariant chain. *Cell Immunol* 1996; **167**: 115-121 [PMID: 8548834]
- 29 **Topilski I**, Harmelin A, Flavell RA, Levo Y, Shachar I. Preferential Th1 immune response in invariant chain-deficient mice. *J Immunol* 2002; **168**: 1610-1617 [PMID: 11823488 DOI: 10.4049/jimmunol.168.4.1610]
- 30 **Schwartz V**, Lue H, Kraemer S, Korbiel J, Krohn R, Ohl K, Bucala R, Weber C, Bernhagen J. A functional heteromeric MIF receptor formed by CD74 and CXCR4. *FEBS Lett* 2009; **583**: 2749-2757 [PMID: 19665027 DOI: 10.1016/j.febslet.2009.07.058]
- 31 **Zaidi T**, Reidy T, D'Ortona S, Fichorova R, Pier G, Gadjeva M. CD74 deficiency ameliorates *Pseudomonas aeruginosa*-induced ocular infection. *Sci Rep* 2011; **1**: 58 [PMID: 22355577 DOI: 10.1038/srep00058]
- 32 **Shi X**, Leng L, Wang T, Wang W, Du X, Li J, McDonald C, Chen Z, Murphy JW, Lolis E, Noble P, Knudson W, Bucala R. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. *Immunity* 2006; **25**: 595-606 [PMID: 17045821 DOI: 10.1016/j.immuni.2006.08.020]
- 33 **Yuan Q**, Wang M, Wang M, Zhang Z, Zhang W. Macrophage migration inhibitory factor gene -173G > C polymorphism and risk of bladder cancer in southeast China: a case-control analysis. *Mol Biol Rep* 2012; **39**: 3109-3115 [PMID: 21750916 DOI: 10.1007/s11033-011-1075-9]
- 34 **Li H**, Zang J, Wang P, Dai L, Zhang J, Wang K. Gastric cancer susceptibility in gastric cancer relatives: attributable risks of Macrophage migration inhibitory factor promoter polymorphism and *Helicobacter pylori*. *Cytokine* 2012; **60**: 346-351 [PMID: 22892326 DOI: 10.1016/j.cyto.2012.07.015]
- 35 **Wu S**, Lian J, Tao H, Shang H, Zhang L. Correlation of macrophage migration inhibitory factor gene polymorphism with the risk of early-stage cervical cancer and lymphatic metastasis. *Oncol Lett* 2011; **2**: 1261-1267 [PMID: 22848299 DOI: 10.3892/ol.2011.409]
- 36 **Vera PL**, Meyer-Siegler KL. Association between macrophage migration inhibitory factor promoter region polymorphism (-173 G/C) and cancer: a meta-analysis. *BMC Res Notes* 2011; **4**: 395 [PMID: 22168770 DOI: 10.1186/1756-0500-4-395]
- 37 **Jiang L**, Deng J, Zhu X, Zheng J, You Y, Li N, Wu H, Lu J, Zhou Y. CD44 rs13347 C > G; T polymorphism predicts breast cancer risk and prognosis in Chinese populations. *Breast Cancer Res* 2012; **14**: R105 [PMID: 22788972 DOI: 10.1186/bcr3225]
- 38 **Cordon-Cardo C**, Prives C. At the crossroads of inflammation and tumorigenesis. *J Exp Med* 1999; **190**: 1367-1370 [PMID: 10562311]
- 39 **Matza D**, Wolstein O, Dikstein R, Shachar I. Invariant chain induces B cell maturation by activating a TAF(II)105-NF-kappaB-dependent transcription program. *J Biol Chem* 2001; **276**: 27203-27206 [DOI: 10.1074/jbc.M104684200]
- 40 **Xu M**, Qiu G, Jiang Z, von Hofe E, Humphreys RE. Genetic modulation of tumor antigen presentation. *Trends Biotechnol* 2000; **18**: 167-172 [PMID: 10740263 DOI: S0167-7799(00)01421-9]
- 41 **Bloom BR**, Bennett B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* 1966; **153**: 80-82 [PMID: 5938421 DOI: 10.1126/science.153.3731.80]
- 42 **Calandra T**, Bernhagen J, Mitchell RA, Bucala R. The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J Exp Med* 1994; **179**: 1895-1902 [PMID: 8195715 DOI: 10.1084/jem.179.6.1895]
- 43 **Bacher M**, Meinhardt A, Lan HY, Mu W, Metz CN, Chesney JA, Calandra T, Gerns D, Donnelly T, Atkins RC, Bucala R. Migration inhibitory factor expression in experimentally induced endotoxemia. *Am J Pathol* 1997; **150**: 235-246 [PMID: 9006339]
- 44 **Beswick EJ**, Pinchuk IV, Suarez G, Sierra JC, Reyes VE. *Helicobacter pylori* CagA-dependent macrophage migration inhibitory factor produced by gastric epithelial cells binds to CD74 and stimulates procarcinogenic events. *J Immunol* 2006; **176**: 6794-6801 [PMID: 16709839 DOI: 10.4049/jimmunol.176.11.6794]
- 45 **Marsh LM**, Cakarova L, Kwapiszewska G, von Wulffen W, Herold S, Seeger W, Lohmeyer J. Surface expression of CD74 by type II alveolar epithelial cells: a potential mechanism for macrophage migration inhibitory factor-induced epithelial repair. *Am J Physiol Lung Cell Mol Physiol* 2009; **296**: L442-L452 [PMID: 19136583 DOI: 10.1152/ajplung.00525.2007]
- 46 **Mitchell RA**, Liao H, Chesney J, Fingerle-Rowson G, Baugh J, David J, Bucala R. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. *Proc Natl Acad Sci USA* 2002; **99**: 345-350 [PMID: 11756671 DOI: 10.1073/pnas.012511599]
- 47 **Lantner F**, Starlets D, Gore Y, Flaishon L, Yamit-Hezi A, Dikstein R, Leng L, Bucala R, Machluf Y, Oren M, Shachar I. CD74 induces TAP63 expression leading to B-cell survival. *Blood* 2007; **110**: 4303-4311 [PMID: 17846227 DOI: 10.1182/blood-2007-04-087486]
- 48 **Binsky I**, Haran M, Starlets D, Gore Y, Lantner F, Harpaz N, Leng L, Goldenberg DM, Shvidel L, Berrebi A, Bucala R, Shachar I. IL-8 secreted in a macrophage migration-inhibitory factor- and CD74-dependent manner regulates B cell chronic lymphocytic leukemia survival. *Proc Natl Acad Sci USA* 2007; **104**: 13408-13413 [PMID: 17686984 DOI: 10.1073/pnas.0701553104]

- 49 **Liu YH**, Lin CY, Lin WC, Tang SW, Lai MK, Lin JY. Up-regulation of vascular endothelial growth factor-D expression in clear cell renal cell carcinoma by CD74: a critical role in cancer cell tumorigenesis. *J Immunol* 2008; **181**: 6584-6594 [PMID: 18941249 DOI: 10.4049/jimmunol.181.9.6584]
- 50 **Lue H**, Kapurniotu A, Fingerle-Rowson G, Roger T, Leng L, Thiele M, Calandra T, Bucala R, Bernhagen J. Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. *Cell Signal* 2006; **18**: 688-703 [PMID: 16122907 DOI: 10.1016/j.cellsig.2005.06.013]
- 51 **Lue H**, Thiele M, Franz J, Dahl E, Speckgens S, Leng L, Fingerle-Rowson G, Bucala R, Lüscher B, Bernhagen J. Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. *Oncogene* 2007; **26**: 5046-5059 [PMID: 17310986 DOI: 10.1038/sj.onc.1210318]
- 52 **Normanno N**, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, Carotenuto A, De Feo G, Caponigro F, Salomon DS. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* 2006; **366**: 2-16 [PMID: 16377102 DOI: 10.1016/j.gene.2005.10.018]
- 53 **Turner N**, Grose R. Fibroblast growth factor signalling: from development to cancer. *Nat Rev Cancer* 2010; **10**: 116-129 [PMID: 20094046 DOI: 10.1038/nrc2780]
- 54 **Tarnowski M**, Grymula K, Liu R, Tarnowska J, Drukala J, Ratajczak J, Mitchell RA, Ratajczak MZ, Kucia M. Macrophage migration inhibitory factor is secreted by rhabdomyosarcoma cells, modulates tumor metastasis by binding to CXCR4 and CXCR7 receptors and inhibits recruitment of cancer-associated fibroblasts. *Mol Cancer Res* 2010; **8**: 1328-1343 [PMID: 20861157 DOI: 10.1158/1541-7786.MCR-10-0288]
- 55 **Jun HJ**, Johnson H, Bronson RT, de Feraudy S, White F, Charest A. The oncogenic lung cancer fusion kinase CD74-ROS1 activates a novel invasiveness pathway through E-Syt1 phosphorylation. *Cancer Res* 2012; **72**: 3764-3774 [PMID: 22659450 DOI: 10.1158/0008-5472.CAN-11-3990]
- 56 **Matsuura S**, Shinmura K, Kamo T, Igarashi H, Maruyama K, Tajima M, Ogawa H, Tanahashi M, Niwa H, Funai K, Kohno T, Suda T, Sugimura H. CD74-ROS1 fusion transcripts in resected non-small cell lung carcinoma. *Oncol Rep* 2013; **30**: 1675-1680 [PMID: 23877438 DOI: 10.3892/or.2013.2630]
- 57 **Kim MH**, Shim HS, Kang DR, Jung JY, Lee CY, Kim DJ, Lee JG, Bae MK, Kim HR, Lim SM, Kim EY, Park JS, Chung KY, Kim HJ, Kim JH, Cho BC. Clinical and prognostic implications of ALK and ROS1 rearrangements in never-smokers with surgically resected lung adenocarcinoma. *Lung Cancer* 2014; **83**: 389-395 [PMID: 24462463 DOI: 10.1016/j.lungcan.2014.01.003]
- 58 **Xu L**, Zhao R, Dong Z, Zhu T. [Clinical significance of ROS1 rearrangements in non-small cell lung cancer]. *Zhongguo Fei Ai Za Zhi* 2013; **16**: 663-670 [PMID: 24345493 DOI: 10.3779/j.issn.1009-3419.2013.12.09]
- 59 **Yoshida A**, Tsuta K, Wakai S, Arai Y, Asamura H, Shibata T, Furuta K, Kohno T, Kushima R. Immunohistochemical detection of ROS1 is useful for identifying ROS1 rearrangements in lung cancers. *Mod Pathol* 2014; **27**: 711-720 [PMID: 24186139 DOI: 10.1038/modpathol.2013.192]
- 60 **Stumpfova M**, Jänne PA. Zeroing in on ROS1 rearrangements in non-small cell lung cancer. *Clin Cancer Res* 2012; **18**: 4222-4224 [PMID: 22859716 DOI: 10.1158/1078-0432.CCR-12-1812]
- 61 **Bergethson K**, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, Massion PP, Siwak-Tapp C, Gonzalez A, Fang R, Mark EJ, Batten JM, Chen H, Wilner KD, Kwak EL, Clark JW, Carbone DP, Ji H, Engelman JA, Mino-Kenudson M, Pao W, Iafrate AJ. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol* 2012; **30**: 863-870 [PMID: 22215748 DOI: 10.1200/JCO.2011.35.6345]
- 62 **Lin SY**, Makino K, Xia W, Martin A, Wen Y, Kwong KY, Bourguignon L, Hung MC. Nuclear localization of EGF receptor and its potential new role as a transcription factor. *Nat Cell Biol* 2001; **3**: 802-808 [PMID: 11533659 DOI: 10.1038/ncb0901-802]
- 63 **Calandra T**, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* 2003; **3**: 791-800 [PMID: 14502271 DOI: 10.1038/nri1200]
- 64 **Matza D**, Lantner F, Bogoch Y, Flaishon L, Hershkovich R, Shachar I. Invariant chain induces B cell maturation in a process that is independent of its chaperonic activity. *Proc Natl Acad Sci USA* 2002; **99**: 3018-3023 [PMID: 11867743 DOI: 10.1073/pnas.052703299]
- 65 **Matza D**, Kerem A, Medvedovsky H, Lantner F, Shachar I. Invariant chain-induced B cell differentiation requires intramembrane proteolytic release of the cytosolic domain. *Immunity* 2002; **17**: 549-560 [PMID: 12433362 DOI: 10.1016/S1074-7613(02)00455-7]
- 66 **Matza D**, Kerem A, Shachar I. Invariant chain, a chain of command. *Trends Immunol* 2003; **24**: 264-268 [PMID: 12738421 DOI: 10.1016/S1471-4906(03)00073-5]
- 67 **Becker-Herman S**, Arie G, Medvedovsky H, Kerem A, Shachar I. CD74 is a member of the regulated intramembrane proteolysis-processed protein family. *Mol Biol Cell* 2005; **16**: 5061-5069 [PMID: 16107560 DOI: 10.1091/mbc.E05-04-0327]
- 68 **Hansen HJ**, Ong GL, Diril H, Valdez A, Roche PA, Griffiths GL, Goldenberg DM, Mattes MJ. Internalization and catabolism of radiolabelled antibodies to the MHC class-II invariant chain by B-cell lymphomas. *Biochem J* 1996; **320** (Pt 1): 293-300 [PMID: 8947500]
- 69 **Burton JD**, Ely S, Reddy PK, Stein R, Gold DV, Cardillo TM, Goldenberg DM. CD74 is expressed by multiple myeloma and is a promising target for therapy. *Clin Cancer Res* 2004; **10**: 6606-6611 [PMID: 15475450]
- 70 **Stein R**, Mattes MJ, Cardillo TM, Hansen HJ, Chang CH, Burton J, Govindan S, Goldenberg DM. CD74: a new candidate target for the immunotherapy of B-cell neoplasms. *Clin Cancer Res* 2007; **13**: 5556s-5563s [PMID: 17875789 DOI: 10.1158/1078-0432.CCR-07-1167]
- 71 **Mark T**, Martin P, Leonard JP, Niesvizky R. Milatuzumab: a promising new agent for the treatment of lymphoid malignancies. *Expert Opin Investig Drugs* 2009; **18**: 99-104 [PMID: 19053886 DOI: 10.1517/13543780802636162]
- 72 **Sharkey RM**, Karacay H, Johnson CR, Litwin S, Rossi EA, McBride WJ, Chang CH, Goldenberg DM. Pretargeted versus directly targeted radioimmunotherapy combined with anti-CD20 antibody consolidation therapy of non-Hodgkin lymphoma. *J Nucl Med* 2009; **50**: 444-453 [PMID: 19223402 DOI: 10.2967/jnumed.108.058602]
- 73 **Hertlein E**, Triantafillou G, Sass EJ, Hessler JD, Zhang X, Jarjoura D, Lucas DM, Muthusamy N, Goldenberg DM, Lee RJ, Byrd JC. Milatuzumab immunoliposomes induce cell death in CLL by promoting accumulation of CD74 on the surface of B cells. *Blood* 2010; **116**: 2554-2558 [PMID: 20574049 DOI: 10.1182/blood-2009-11-253203]
- 74 **Alinari L**, Yu B, Christian BA, Yan F, Shin J, Lapalombella R, Hertlein E, Lustberg ME, Quinion C, Zhang X, Lozanski G, Muthusamy N, Prætorius-Ibba M, O'Connor OA, Goldenberg DM, Byrd JC, Blum KA, Baiocchi RA. Combination anti-CD74 (milatuzumab) and anti-CD20 (rituximab) monoclonal antibody therapy has in vitro and in vivo activity in mantle cell lymphoma. *Blood* 2011; **117**: 4530-4541 [PMID: 21228331 DOI: 10.1182/blood-2010-08-303354]
- 75 **Kaufman JL**, Niesvizky R, Stadtmauer EA, Chanan-Khan A, Siegel D, Horne H, Wegener WA, Goldenberg DM. Phase I, multicentre, dose-escalation trial of monotherapy with milatuzumab (humanized anti-CD74 monoclonal antibody) in relapsed or refractory multiple myeloma. *Br J Haematol*

- 2013; **163**: 478-486 [PMID: 24112026 DOI: 10.1111/bjh.12565]
- 76 **Ochakovskaya R**, Osorio L, Goldenberg DM, Mattes MJ. Therapy of disseminated B-cell lymphoma xenografts in severe combined immunodeficient mice with an anti-CD74 antibody conjugated with (111)indium, (67)gallium, or (90)yttrium. *Clin Cancer Res* 2001; **7**: 1505-1510 [PMID: 11410483]
 - 77 **Michel RB**, Rosario AV, Brechbiel MW, Jackson TJ, Goldenberg DM, Mattes MJ. Experimental therapy of disseminated B-Cell lymphoma xenografts with 213Bi-labeled anti-CD74. *Nucl Med Biol* 2003; **30**: 715-723 [PMID: 14499329 DOI: 10.1016/S0969-8051(03)00082-9]
 - 78 **Griffiths GL**, Mattes MJ, Stein R, Govindan SV, Horak ID, Hansen HJ, Goldenberg DM. Cure of SCID mice bearing human B-lymphoma xenografts by an anti-CD74 antibody-anthracycline drug conjugate. *Clin Cancer Res* 2003; **9**: 6567-6571 [PMID: 14695162]
 - 79 **Sapra P**, Stein R, Pickett J, Qu Z, Govindan SV, Cardillo TM, Hansen HJ, Horak ID, Griffiths GL, Goldenberg DM. Anti-CD74 antibody-doxorubicin conjugate, IMMU-110, in a human multiple myeloma xenograft and in monkeys. *Clin Cancer Res* 2005; **11**: 5257-5264 [PMID: 16033844]
 - 80 **Alinari L**, Mahoney E, Patton J, Zhang X, Huynh L, Earl CT, Mani R, Mao Y, Yu B, Quinion C, Towns WH, Chen CS, Goldenberg DM, Blum KA, Byrd JC, Muthusamy N, Praetorius-Ibba M, Baiocchi RA. FTY720 increases CD74 expression and sensitizes mantle cell lymphoma cells to milatuzumab-mediated cell death. *Blood* 2011; **118**: 6893-6903 [PMID: 22042694 DOI: 10.1182/blood-2011-06-363879]
 - 81 **Cho Y**, Crichlow GV, Vermeire JJ, Leng L, Du X, Hodsdon ME, Bucala R, Cappello M, Gross M, Gaeta F, Johnson K, Lolis EJ. Allosteric inhibition of macrophage migration inhibitory factor revealed by ibudilast. *Proc Natl Acad Sci USA* 2010; **107**: 11313-11318 [PMID: 20534506]
 - 82 **Saito T**, Tomita Y, Kimura M, Nishiyama T, Sato S. [Expression of HLA class II antigen-associated invariant chain on renal cell cancer]. *Nihon Hinyokika Gakkai Zasshi* 1993; **84**: 1036-1040
 - 83 **Winner M**, Meier J, Zierow S, Rendon BE, Crichlow GV, Riggs R, Bucala R, Leng L, Smith N, Lolis E, Trent JO, Mitchell RA. A novel, macrophage migration inhibitory factor suicide substrate inhibits motility and growth of lung cancer cells. *Cancer Res* 2008; **68**: 7253-7257 [PMID: 18794110 DOI: 10.1158/0008-5472.CAN-07-6227]
 - 84 **Ouertatani-Sakouhi H**, El-Turk F, Fauvet B, Cho MK, Pinar Karpinar D, Le Roy D, Dewor M, Roger T, Bernhagen J, Calandra T, Zweckstetter M, Lashuel HA. Identification and characterization of novel classes of macrophage migration inhibitory factor (MIF) inhibitors with distinct mechanisms of action. *J Biol Chem* 2010; **285**: 26581-26598 [PMID: 20516071 DOI: 10.1074/jbc.M110.113951]
 - 85 **Saegusa K**, Ishimaru N, Yanagi K, Arakaki R, Ogawa K, Saito I, Katunuma N, Hayashi Y. Cathepsin S inhibitor prevents autoantigen presentation and autoimmunity. *J Clin Invest* 2002; **110**: 361-369 [PMID: 12163455 DOI: 10.1172/JCI14682]
 - 86 **Riese RJ**, Mitchell RN, Villadangos JA, Shi GP, Palmer JT, Karp ER, De Sanctis GT, Ploegh HL, Chapman HA. Cathepsin S activity regulates antigen presentation and immunity. *J Clin Invest* 1998; **101**: 2351-2363 [PMID: 9616206 DOI: 10.1172/JCI1158]
 - 87 **Podolin PL**, Bolognese BJ, Carpenter DC, Davis TG, Johanson RA, Fox JH, Long E, Dong X, Marquis RW, Locastro SM, Terfloth GJ, Kurali E, Peterson JJ, Smith BR, McQueney MS, Yamashita DS, Capper-Spudich EA. Inhibition of invariant chain processing, antigen-induced proliferative responses, and the development of collagen-induced arthritis and experimental autoimmune encephalomyelitis by a small molecule cysteine protease inhibitor. *J Immunol* 2008; **180**: 7989-8003 [PMID: 18523262 DOI: 10.4049/jimmu-nol.180.12.7989]
 - 88 **Sekiguchi H**, Takabayashi F, Irie K, Murakami A. Auraptene attenuates gastritis via reduction of Helicobacter pylori colonization and pro-inflammatory mediator production in C57BL/6 mice. *J Med Food* 2012; **15**: 658-663 [PMID: 22471969 DOI: 10.1089/jmf.2011.1844]
 - 89 **Sekiguchi H**, Irie K, Murakami A. Suppression of CD74 expression and Helicobacter pylori adhesion by auraptene targeting serum starvation-activated ERK1/2 in NCI-N87 gastric carcinoma cells. *Biosci Biotechnol Biochem* 2010; **74**: 1018-1024 [PMID: 20460732 DOI: 10.1271/bbb.90910]
 - 90 **Saito T**, Tomita Y, Kimura M, Nishiyama T, Sato S. [Expression of HLA class II antigen-associated invariant chain on renal cell cancer]. *Nihon Hinyokika Gakkai Zasshi* 1993; **84**: 1036-1040 [PMID: 8345720]
 - 91 **Saito T**, Kimura M, Kawasaki T, Sato S, Tomita Y. MHC class II antigen-associated invariant chain on renal cell cancer may contribute to the anti-tumor immune response of the host. *Cancer Lett* 1997; **115**: 121-127 [PMID: 9097987 DOI: 10.1016/S0304-3835(97)04754-X]
 - 92 **Young AN**, Amin MB, Moreno CS, Lim SD, Cohen C, Petros JA, Marshall FF, Neish AS. Expression profiling of renal epithelial neoplasms: a method for tumor classification and discovery of diagnostic molecular markers. *Am J Pathol* 2001; **158**: 1639-1651 [PMID: 11337362 DOI: 10.1016/S0002-9440(10)64120-X]
 - 93 **Lazova R**, Moynes R, May D, Scott G. LN-2 (CD74). A marker to distinguish atypical fibroxanthoma from malignant fibrous histiocytoma. *Cancer* 1997; **79**: 2115-2124 [PMID: 9179057 DOI: 10.1002/(SICI)1097-0142(19970601)79:11<2115::AID-CNCR8>3.0.CO;2-N]
 - 94 **Datta MW**, Shahsafaei A, Nadler LM, Freeman GJ, Dorfman DM. Expression of MHC class II-associated invariant chain (Ii; CD74) in thymic epithelial neoplasms. *Appl Immunohistochem Mol Morphol* 2000; **8**: 210-215 [PMID: 10981873 DOI: 10.1097/00022744-200009000-00007]
 - 95 **Jiang Z**, Xu M, Savas L, LeClair P, Banner BF. Invariant chain expression in colon neoplasms. *Virchows Arch* 1999; **435**: 32-36 [PMID: 10431843]
 - 96 **Rossi HA**, Liu Q, Banner B, Hsieh CC, Savas L, Savarese D. The prognostic value of invariant chain (Ii) and Her-2/neu expression in curatively resected colorectal cancer. *Cancer J* 2002; **8**: 268-275 [PMID: 12074327 DOI: 10.1097/00130404-200205000-00011]
 - 97 **Cuthbert RJ**, Wilson JM, Scott N, Coletta PL, Hull MA. Differential CD74 (major histocompatibility complex Class II invariant chain) expression in mouse and human intestinal adenomas. *Eur J Cancer* 2009; **45**: 1654-1663 [PMID: 19269807 DOI: 10.1016/j.ejca.2009.02.005]
 - 98 **Ishigami S**, Natsugoe S, Tokuda K, Nakajo A, Iwashige H, Aridome K, Hokita S, Aikou T. Invariant chain expression in gastric cancer. *Cancer Lett* 2001; **168**: 87-91 [PMID: 11368882 DOI: 10.1016/S0304-3835(01)00503-1]
 - 99 **Tamori Y**, Tan X, Nakagawa K, Takai E, Akagi J, Kageshita T, Egami H, Ogawa M. Clinical significance of MHC class II-associated invariant chain expression in human gastric carcinoma. *Oncol Rep* 2005; **14**: 873-877 [PMID: 16142345]
 - 100 **Porter D**, Lahti-Domenici J, Keshaviah A, Bae YK, Argani P, Marks J, Richardson A, Cooper A, Strausberg R, Riggins GJ, Schnitt S, Gabrielson E, Gelman R, Polyak K. Molecular markers in ductal carcinoma in situ of the breast. *Mol Cancer Res* 2003; **1**: 362-375 [PMID: 12651909]
 - 101 **Tian B**, Zhang Y, Li N, Liu X, Dong J. CD74: a potential novel target for triple-negative breast cancer. *Tumour Biol* 2012; **33**: 2273-2277 [PMID: 22935920 DOI: 10.1007/s13277-012-0489-x]
 - 102 **Greenwood C**, Metodieva G, Al-Janabi K, Lausen B, Allridge L, Leng L, Bucala R, Fernandez N, Metodiev MV. Stat1 and CD74 overexpression is co-dependent and linked to increased invasion and lymph node metastasis in triple-

- negative breast cancer. *J Proteomics* 2012; **75**: 3031-3040 [PMID: 22178447 DOI: 10.1016/j.jprot.2011.11.033]
- 103 **Choi JW**, Kim Y, Lee JH, Kim YS. CD74 expression is increased in high-grade, invasive urothelial carcinoma of the bladder. *Int J Urol* 2013; **20**: 251-255 [PMID: 22905972 DOI: 10.1111/j.1442-2042.2012.03128.x]
 - 104 **Hustinx SR**, Cao D, Maitra A, Sato N, Martin ST, Sudhir D, Iacobuzio-Donahue C, Cameron JL, Yeo CJ, Kern SE, Goggins M, Mollenhauer J, Pandey A, Hruban RH. Differentially expressed genes in pancreatic ductal adenocarcinomas identified through serial analysis of gene expression. *Cancer Biol Ther* 2004; **3**: 1254-1261 [PMID: 15477757 DOI: 10.4161/cbt.3.12.1238]
 - 105 **Koide N**, Yamada T, Shibata R, Mori T, Fukuma M, Yamazaki K, Aiura K, Shimazu M, Hirohashi S, Nimura Y, Sakamoto M. Establishment of perineural invasion models and analysis of gene expression revealed an invariant chain (CD74) as a possible molecule involved in perineural invasion in pancreatic cancer. *Clin Cancer Res* 2006; **12**: 2419-2426 [PMID: 16638847 DOI: 10.1158/1078-0432.CCR-05-1852]
 - 106 **Nagata S**, Jin YF, Yoshizato K, Tomoeda M, Song M, Iizuka N, Kitamura M, Takahashi H, Eguchi H, Ohigashi H, Ishikawa O, Tomita Y. CD74 is a novel prognostic factor for patients with pancreatic cancer receiving multimodal therapy. *Ann Surg Oncol* 2009; **16**: 2531-2538 [PMID: 19499276 DOI: 10.1245/s10434-009-0532-3]
 - 107 **Zhang JF**, Hua R, Liu DJ, Liu W, Huo YM, Sun YW. Effect of CD74 on the prognosis of patients with resectable pancreatic cancer. *Hepatobiliary Pancreat Dis Int* 2014; **13**: 81-86 [PMID: 24463084]
 - 108 **Cheng RJ**, Deng WG, Niu CB, Li YY, Fu Y. Expression of macrophage migration inhibitory factor and CD74 in cervical squamous cell carcinoma. *Int J Gynecol Cancer* 2011; **21**: 1004-1012 [PMID: 21792010 DOI: 10.1097/IGC.0b013e31821c45b7]
 - 109 **Awad MM**, Katayama R, McTigue M, Liu W, Deng YL, Brown A, Friboulet L, Huang D, Falk MD, Timofeevski S, Wilner KD, Lockerman EL, Khan TM, Mahmood S, Gainor JF, Digumarthy SR, Stone JR, Mino-Kenudson M, Christensen JG, Iafrate AJ, Engelman JA, Shaw AT. Acquired resistance to crizotinib from a mutation in CD74-ROS1. *N Engl J Med* 2013; **368**: 2395-2401 [PMID: 23724914 DOI: 10.1056/NEJMoa1215530]
 - 110 **McClelland M**, Zhao L, Carskadon S, Arenberg D. Expression of CD74, the receptor for macrophage migration inhibitory factor, in non-small cell lung cancer. *Am J Pathol* 2009; **174**: 638-646 [PMID: 19131591]
 - 111 **Rimkunas VM**, Crosby KE, Li D, Hu Y, Kelly ME, Gu TL, Mack JS, Silver MR, Zhou X, Haack H. Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: identification of a FIG-ROS1 fusion. *Clin Cancer Res* 2012; **18**: 4449-4457 [PMID: 22661537 DOI: 10.1158/1078-0432.CCR-11-3351]
 - 112 **Kim HR**, Lim SM, Kim HJ, Hwang SK, Park JK, Shin E, Bae MK, Ou SH, Wang J, Jewell SS, Kang DR, Soo RA, Haack H, Kim JH, Shim HS, Cho BC. The frequency and impact of ROS1 rearrangement on clinical outcomes in never smokers with lung adenocarcinoma. *Ann Oncol* 2013; **24**: 2364-2370 [PMID: 23788756 DOI: 10.1093/annonc/mdt220]
 - 113 **Davies KD**, Le AT, Theodoro MF, Skokan MC, Aisner DL, Berge EM, Terracciano LM, Cappuzzo F, Incarbone M, Roncalli M, Alloisio M, Santoro A, Camidge DR, Varella-Garcia M, Doebele RC. Identifying and targeting ROS1 gene fusions in non-small cell lung cancer. *Clin Cancer Res* 2012; **18**: 4570-4579 [PMID: 22919003]
 - 114 **Tan X**, Wu Q, Cai Y, Zhao X, Wang S, Gao Z, Yang Y, Li X, Qian J, Wang J, Su B, Chen H, Han B, Jiang G, Lu D. Novel association between CD74 polymorphisms and hematologic toxicity in patients with NSCLC after platinum-based chemotherapy. *Clin Lung Cancer* 2014; **15**: 67-78.e12 [PMID: 24220096 DOI: 10.1016/j.clcc.2013.08.006]

P- Reviewer: Chowdhury P, Shah A **S- Editor:** Song XX

L- Editor: A **E- Editor:** Lu YJ



Modulation of monocyte subsets in infectious diseases

Mignane B Ka, Daniel Olive, Jean-Louis Mege

Mignane B Ka, Daniel Olive, Inserm Unité Mixte de Recherche (UMR) 1068, Centre de Recherche en Cancérologie de Marseille, 13009 Marseille, France

Mignane B Ka, Jean-Louis Mege, Department of Immunology and Infectious Diseases, Aix Marseille University, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, 13005 Marseille, France

Author contributions: Ka MB and Mege JL wrote the paper; Mege JL and Olive D corrected it.

Correspondence to: Jean-Louis Mege, Professor, Department of Immunology and Infectious Diseases, URMITE, Faculté de Médecine, 27 BD Jean Moulin, 13005 Marseille, France. jean-louis.mege@univ-amu.fr

Telephone: +33-4-91324970 Fax: +33-4-91324306

Received: June 1, 2014 Revised: July 10, 2014

Accepted: August 27, 2014

Published online: November 27, 2014

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Monocytes subsets; Infection; Virus; Bacteria

Core tip: In this review of the literature we show that monocyte subsets are differently affected during viral, bacterial, parasitic and fungal infections. We observe that the CD16⁺ compartment (intermediate and non-classical monocytes) is typically increased in the majority of infectious diseases. The measurement of monocyte subsets would be useful in better understanding of the role of monocyte activation in the pathophysiology of infectious diseases.

Ka MB, Olive D, Mege JL. Modulation of monocyte subsets in infectious diseases. *World J Immunol* 2014; 4(3): 185-193 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i3/185.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i3.185>

Abstract

Monocytes are effector immune cells but a precise analysis of their role in immune response has been precluded by their heterogeneity. Indeed, human monocytes are composed of at least three different subsets with different phenotypic characteristics and functional properties, the so-called classical, intermediate and non-classical monocytes. A review of the literature shows that these monocyte subsets are differently affected during viral, bacterial, parasitic and fungal infections. The expansion of the CD16⁺ compartment (intermediate and non-classical monocytes) is typically observed in the majority of infectious diseases and the increased proportion of CD16⁺ monocytes is likely related to their activation through their direct interaction with the pathogen or the inflammatory context. In contrast, the number of non-classical and intermediate monocytes is decreased in Q fever endocarditis, suggesting that complex mechanisms govern the equilibrium among monocyte subsets. The measurement of monocyte subsets would be useful in better understanding of the role of monocyte activation in the pathophysiology of infectious diseases.

INTRODUCTION

Human monocytes arise from bone marrow progenitors with myeloid-restricted differentiation potential and then circulate in the blood for a few days before migrating into tissues^[1]. Monocytes differentiate into macrophages and dendritic cells (DCs) during inflammation and less efficiently in the steady state^[2].

Monocytes play a pivotal role in the immune response as effector cells. These cells are equipped with pattern recognition receptors (PRRs) and phagocytic receptors necessary for the ingestion and elimination of microbes and damaged cells^[3,4]. They express adhesion molecules and chemokine receptors, which are required to migrate toward inflamed or infected tissues^[5]. Monocytes also initiate the adaptive immune response through their ability to produce cytokines and to differentiate into DCs, the major antigen-presenting cells (APCs)^[6]. Finally, monocytes play critical roles in homeostasis and tissue repair^[7].

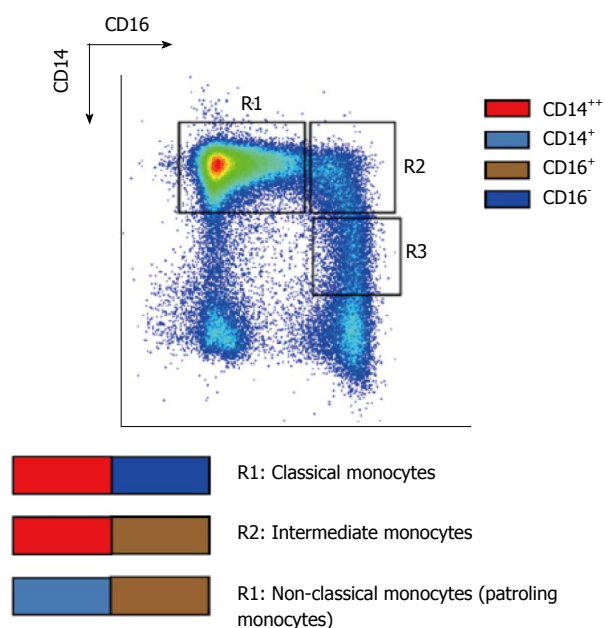


Figure 1 Expression of CD14⁺⁺ and CD16⁺ cells.

A fundamental property of monocytes consists of their high plasticity^[8]. They may adopt a biphasic response to a unique signal, first releasing inflammatory cytokines such as interleukin (IL)-6 and IL-1 β ^[9] and then releasing immunoregulatory cytokines such as IL-10 and transforming growth factor (TGF)- β , thus an avoiding excessive inflammatory response^[10]. We recently demonstrated that the gene expression program of human monocytes is determined by the time scale of the stimulation: although macrophage polarization genes are expressed in early stimulated monocytes, this expression is lost when the stimulation is sustained^[11].

Another difficulty in analyzing the precise role of monocytes in the immune response is related to their heterogeneity, as they are composed of at least three different subsets with different phenotypic characteristics and functional properties. The aim of this review is to summarize what is known regarding the functions of monocyte subsets and to describe the evolution of these monocyte subsets during infectious diseases.

DEFINITION OF MONOCYTE SUBSETS

Human monocytes were initially defined as an homogeneous population on the basis of morphology, cytochemistry (monocyte-specific esterase) and flow cytometry measurements, such as light scattering and the expression of CD14, the receptor of bacterial lipopolysaccharides (LPS)^[12]. Multi-color flow cytometry using antibodies against CD14 and CD16, the low affinity receptor for IgG, has revealed their heterogeneity, consisting of three subsets^[12,13]. The “classical monocytes” that represent approximately 90% of circulating monocytes highly express CD14 but not CD16 (CD14⁺⁺CD16⁻ cells). Other circulating monocytes express CD16: “non-classical

monocytes” representing approximately 5% of circulating monocytes, express low levels of CD14 but highly express CD16 (CD14⁺CD16⁺ cells) and “intermediate monocytes”, which highly express CD14 with the concomitant expression of CD16 (CD14⁺⁺CD16⁺ cells)^[14] (Figure 1). However, the notion of intermediate monocytes is still debated. For Ziegler-Heitbrock and Hofer, they are only a transition from^[14], conversely, for Hijdra *et al.*^[15], they consist of a true population of monocytes, as revealed by the expression of chemokine and Tumor Necrosis Factor (TNF) receptors. Because only the level of CD14 expression allows the distinction between non-classical monocytes and intermediate monocytes and many papers do not explicitly make this distinction, we propose referring to them collectively as CD16⁺ monocytes^[1] and precisely defining the type of monocyte subset when it is documented.

PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF MONOCYTE SUBSETS

The phenotypic properties of CD16⁻ (classical) and CD16⁺ (intermediate and non-classical) human monocytes are summarized in Table 1. CD16⁺ monocytes express lower levels of CD64 than CD16⁻ monocytes but highly express HLA-DR, CD86, and CD49d compared with CD16⁻ monocytes^[16,17], demonstrating an activated phenotype. The expression of PRRs and chemokine receptors varies according to the monocyte subset. The intermediate monocytes express higher levels of Toll-like receptor (TLR)-2 and TLR4 than classical and non-classical monocytes^[17,18]. The non-classical monocytes do not express CCR2, the membrane receptor of the chemokine CCL2, making them likely unable to migrate in response to CCL2. In contrast, classical and intermediate monocytes express CCR2 and migrate in response to CCL2^[19,20]. Intermediate monocytes, but not classical and non-classical monocytes, express CCR5^[19,21]. The responses to classical agonists of monocytes vary according to the monocyte subset. For instance Lipopolysaccharide (LPS) stimulation of classical monocytes, but not intermediate monocytes, decreases the membrane expression of CD163; hence, the majority of soluble CD163 found in plasma originates from classical monocytes^[22,23].

The functional properties of monocyte subsets are also different (Table 2). The phagocytosis of *Staphylococcus aureus* and *Escherichia coli* is lower in non-classical monocytes than in intermediate monocytes and classical monocytes, a property likely related to the expression of CD14^[18]. The non-classical monocytes produce less reactive oxygen species (ROS) in response to ligands of TLR4, TLR7 or TLR8 than the classical monocytes^[24,25]. In addition non-classical and intermediate monocytes produce lower levels of cytokines, including granulocyte colony-stimulating factor, IL-6, IL-10 and CCL2 in response to LPS stimulation than classical monocytes^[20].

Table 1 Major marker of monocyte subsets

Markers	Classical monocytes	Intermediate monocytes	Nonclassical-monocytes	Ref.
CD14	++	++	+	[1]
CD16	-	+	+	[1]
CD86	+	++	++	[17]
CD64	++	+	+	[17]
HLA-AB	+	++	+	[21]
HLA-DR	+	++	+	[21,25]
CCR1	++	+	-	[21]
CCR2	++	-	-	[15,20,21]
CCR5	+	++	-	[15,20,21]
CXCR1	++	-	-	[21]
CXCR2	++	-	-	[15,21]
CX3CR1	++	+	-	[15,20]
CD62L	++	-	-	[20,21]

++: High; +: Median; -: Low.

The monocyte subsets likely play different roles as APCs. CD16⁺ monocytes express higher levels of HLA-DR than classical monocytes^[12,26], suggesting that they are potent APCs. It has been shown that CD16⁺ monocytes are more efficient in presenting tetanus toxoid to CD4⁺ T cells than classical monocytes^[27]. Taken together, these results suggest that CD16⁺ monocytes are activated under homeostatic conditions but they are less responsive to monocyte stimuli than CD16⁻ monocytes.

Interestingly, monocytes are known to act as precursors of macrophages or DCs. It has been shown that the ability of monocyte subsets to differentiate into DCs is different according the monocyte subset. Indeed, non-classical monocytes are more prone to become DCs with a higher capacity to induce T cell proliferation and IL-4 production by CD4⁺ T cells^[28]. In addition, the functional properties of monocyte-derived macrophages are dependent on the type of monocyte subset. It has been recently shown that the macrophages derived from CD16⁺ monocytes are more phagocytic than those derived from classical monocytes; they also exhibit a specific gene expression program^[29].

The investigation of monocyte functions has benefited from the use of mouse models, though it remains unclear whether monocyte subsets are similar in humans and mice. Murine monocytes can be separated into at least two subpopulations, Gr1⁺ and Gr1⁻ monocytes. The major subset of murine monocytes is composed of “inflammatory” Gr1⁺ monocytes that produce high levels of TNF, ROS and nitric oxide (NO) but low levels of IL-10 upon *in vivo* infection with bacteria such as *Listeria monocytogenes* or parasites such as *Toxoplasma gondii*^[30]. Gr1⁺ monocytes also produce type I interferons (IFNs) in response to viral ligands^[31]. Murine Gr1⁺ monocytes resemble human classical monocytes based on surface marker expression, gene expression and a reduced ability to produce inflammatory cytokines^[32,33]. In contrast, the minor subset of murine monocytes does not express Gr1. These Gr1⁻ monocytes patrol the blood vasculature, differentiate into macrophages after extrava-

Table 2 Functional characteristics of monocyte subsets

Functions	Classical monocytes	Intermediate monocytes	Nonclassical-monocytes	Ref.
Phagocytosis	++	++	+	[25]
MHC II processing	+	++	+	[25]
Antigen presentation	+	++	+	[25]
CD4 ⁺ T cell proliferation	+	++	+	[25]
Transendothelial migration	-	-	++	[15]
Patrolling endothelium	-	-	++	[24]
Virus sensing	-	-	++	[24]
TNF production	+	-	++	[24]
IL-1 β production	+	++	++	[24]
CCL2 production	++	-	-	[24]
IL-10 production	++	-	-	[24]

++: High; +: Median; -: Low. IL: Interleukin.

sation into tissues and are likely associated with tissue repair^[34,35]. Murine Gr1⁻ monocytes, which resemble human CD16⁺ monocytes, are described as the main producers of inflammatory cytokines such as TNF and IL-1 β in response to LPS^[26].

The existence of different subsets of monocytes likely has pathophysiological consequences. An expansion of the CD16⁺ monocyte subsets inflammatory diseases including hemophagocytic lymphohistiocytosis^[36], asthma^[37], sarcoidosis^[38], peritonitis^[39], atopic eczema^[40], pancreatitis^[41] and alveolar proteinosis^[42] has been observed. Despite immunosuppressive therapy, the CD16⁺ monocyte compartment is also increased in kidney transplant patients, suggesting that this subset may be involved in the persistent, allograft-induced inflammatory reaction^[43]. In patients with colorectal cancer, the percentage of intermediate monocytes is mainly increased at the onset of the disease^[44], and this subset is also increased in adult survivors of childhood acute lymphoblastic leukemia^[13].

MONOCYTE SUBSETS AND VIRAL INFECTIONS

Human immunodeficiency virus

Human immunodeficiency virus (HIV) is a lentivirus that efficiently infects CD4⁺ T cells, leading to their apoptosis and a decreased number of circulating CD4⁺ T cells. The antiretroviral therapies to date restore the number of circulating CD4⁺ T cells but are unable to completely eliminate viral infection, as demonstrated by HIV persistence in tissues. Both *in vitro* and *in vivo* studies have clearly demonstrated that blood monocytes and tissue macrophages can be infected by HIV^[45,46]. During the early phase of HIV infection, the proportion of CD16⁺ monocytes is increased^[47], and this increase in CD16⁺ monocytes in treatment-naïve HIV-infected patients is correlated with high viral loads and low CD4⁺ cell counts^[48]. Convergent results have been obtained with the infection of non-human primates by simian immunodeficiency virus.

ciency virus (SIV), with SIV infecting both CD4⁺T cells and monocytes. Following the first description of CD16⁺ monocytes in cynomolgus monkeys (*Macaca fascicularis*) nearly two decades ago, an increase in CD16⁺ monocytes ten days after SIV infection has been observed. Note that increased levels of CD16⁺ monocytes have also been reported in rhesus monkeys (*Macaca mulatta*) with lentiviral encephalitis^[49]. The treatment of chronically infected macaques with high doses of corticosteroids decreased the proportion of CD16⁺ monocytes (intermediate monocytes), whereas the other subsets of monocytes were found to be unresponsive to corticosteroids^[50]. Highly active antiretroviral therapy (HAART) rescues the amount of intermediate monocytes^[51]. The viral efficiency of HAART is also associated with insulin resistance, and it has been reported that the abundance of classical monocytes predicts the risk of insulin resistance and metabolic syndrome during the chronic phase of HIV infection^[51].

HIV infection also affects the phenotype of monocyte subsets. The membrane expression of CD163, a receptor involved in the resolution of inflammation and M2 polarization^[52] by classical and intermediate monocytes is increased in HIV-1 infection, but HIV-infection does not induce the membrane expression of CD163 in non-classical monocytes^[53]. Note that plasma CD163 is not significantly altered by HIV-1 infection, demonstrating that CD163 shedding is not associated with the alteration of the membrane expression of CD163^[53]. The exposure of whole blood to HIV enhances the expression of tissue factor (TF) on non-classical monocytes, whereas LPS-activated TLR-4 increases TF expression on all monocyte subsets^[47]. The acquisition of such activated phenotypes by non-classical monocytes is reminiscent of the observation in acute coronary syndrome and suggests a potential role of non-classical monocytes in the cardiovascular risk of HIV infection. A recent study reported a decrease in the proportion of non-classical monocytes expressing TF in patients treated with rosuvastatin though anti-retroviral therapy has no effect on monocyte activation^[54]. The functional alteration of monocyte subsets is associated with that of the programmed death-1 (PD-1) pathway known to limit the functions of virus-specific T cells during chronic infections such as HIV infection^[55]. The expression of PD-1 by monocytes is increased in viremic subjects compared with healthy subjects, but the expression of PD-1 by CD16⁺ monocytes is twofold higher than that of classical monocytes. The relationship between HIV infection and PD-1 expression likely involves an indirect mechanism in which inflammatory cytokines play a major role^[56]. First, the expression of PD-1 by monocyte subsets is not related to viral load in patients with HIV infection. *In vitro*, viral material such as HIV single-stranded RNA (RNA40) fails to increase PD-1 expression by monocytes. Second, inflammatory cytokines such as TNF, IL-1 β and IL-6 increase the expression of PD-1 by monocytes in a dose-dependent manner, and it has been largely demonstrated that the circulating levels of these cytokines are increased in HIV infection^[57,58]. Taken together, these

results suggest that HIV infection leads to the modulation of monocyte subsets.

Dengue virus

Dengue fever, a public health problem in tropical countries, is due to the dengue virus (DENV), a flavivirus that is transmitted to humans *via* the bite of an *Aedes* mosquito^[59,60]. Monocytes are implicated in protection against DENV infection^[61,62]. Indeed, monocytes infected *in vitro* with DENV produced IFN- α which is protective against viruses^[63]. This is confirmed by the increase in DENV titers in mice deficient in IFN receptors^[64]. Nevertheless, the role of monocytes is likely more complex. Monocytes are involved in dengue pathogenesis through virus propagation^[65], and DENV-specific antibodies promote the infection of monocytes and thus increase the viral burden of individual monocytes^[66].

It has been demonstrated that the number of CD16⁺ monocytes is twofold higher in dengue patients than in healthy controls^[67], but the relative role of monocyte subsets in dengue infection remain unclear. *In vitro* classical monocytes and CD16⁺ monocytes are susceptible to DENV and produce molecules associated with dengue protection, such as IFN- α , CXCL10 and TNF-related apoptosis-inducing ligand (TRAIL), a cytokine known to induce cell apoptosis^[68]. Taken together, these results suggest that classical monocytes and CD16⁺ monocytes may potentially contribute to anti-dengue responses, however only CD16⁺ monocytes appear to be affected by DENV infection *in vivo*.

Hepatitis C virus

Hepatitis C is due to an RNA virus (HCV) that affects 160 million individuals worldwide and is responsible for chronic hepatitis and hepatocellular carcinoma^[69,70]. It has been recently demonstrated that HCV infects CD16⁺ monocytes but not classical monocytes in individuals infected with HCV. This specific tropism is related to the expression of CD81, the receptor considered to be necessary for HCV entry into target cells. Hence, CD81 is highly expressed on CD16⁺ monocytes but not on classical monocytes^[71]. These results also suggest that the expression of CD81 by monocyte subsets is associated with the expression of CD16. Furthermore, we can suppose that the monocyte subsets that express CD16 may serve as HCV reservoirs. In hemodialyzed patients with chronic hepatitis, the CD16⁺ monocyte subset is increased threefold compared with healthy donors^[72], suggesting an impact of the viral infection on monocyte distribution. The frequency of CD16⁺ monocytes is decreased and negatively correlated with viral load in chronic HCV infection. Furthermore the expression of PD-L1 allows the discrimination between chronic HCV infection and spontaneous HCV resolvers^[73].

Cytomegalovirus

Cytomegalovirus (CMV) is a herpes virus of medical importance in immune-compromised individuals. CMV has

a tropism for immune and non-immune cells *in vivo* and *in vitro*, yet peripheral blood leukocytes are involved in viremia and latency, regardless of the immune status of the patient^[74]. Monocytes are likely latent reservoirs and support viral dissemination by benefiting from the maturation of monocytes into permissive macrophages and dendritic cells. CMV encodes inflammatory viral chemokines required for viral dissemination. A recent study proposed that patrolling monocytes acquire the virus from the initial site of infection and deliver to the spleen and salivary glands where CMV can persist. Analysis of the recruitment of patrolling monocytes reveals two phases: the first phase is necessary for the activation of natural killer (NK) response; and the second phase, involving viral chemokine and CX3CR1, the marker of patrolling monocytes, is required for the amplification of monocyte recruitment. Although this study revealed a previously undescribed role for this minority monocyte subset as a latent reservoir, it is not clear whether this finding can be extrapolated to human disease^[75].

MONOCYTE SUBSETS AND BACTERIAL INFECTIONS

The study of monocyte subsets in bacterial infections is in its infancy. In patients with severe bacterial sepsis, the number of CD16⁺ monocytes is dramatically increased^[76]. Another report shows that the proportion of intermediate and non-classical monocytes increases during sepsis. CD16⁺ monocytes show a reduced ability to engulf a bacterium such as *E. coli*, express low levels of CD86 and HLA-DR, and poorly presents antigen to T cells^[77]. The hemolytic uremic syndrome observed in children is due to bacterial toxins. The acute period of this disease is characterized by an increased proportion of CD16⁺ monocytes that express higher levels of CD16 and lower levels of CD14 compared with those of healthy age-matched children. In addition, HLA-DR expression by classical monocytes is decreased in this patients, and this lower expression of HLA-DR is related to the severity of the disease^[78]. In patients with tuberculosis, the percentage and absolute numbers of CD16⁺ monocytes are increased^[79]; nevertheless, some authors did not find changes in the proportion of CD16⁺ and CD16⁻ monocytes during tuberculosis^[80]. When expanded, these monocytes exhibit decreased expression of markers associated with maturation and differentiation and also functional alterations. These alteration include a decrease in phagocytosis potential, a tendency toward cell death and an increased production of TNF after stimulation with live *M. tuberculosis*^[79]. In addition, CD16⁺ monocytes differentiate into cells that poorly express CD1a and CD209 (DC-SIN) and with a low capacity for presenting mycobacterial antigens. It is likely that this differentiated cell populations contributes to the impairment of DC maturation during tuberculosis^[81]. The expansion of these monocytes is amplified in patients with HIV co-infection^[82]. Q fever is an acute infectious disease caused by *Coxiella burnetii*, an

obligate intracellular bacterium that targets monocytes and macrophages^[83], in patients with valvular damage and in immunocompromised patients, the primo-infection may lead to a chronic disease that essentially manifests as endocarditis^[83]. We recently found that the distribution of monocyte subsets is altered in patients with Q fever endocarditis, with a decreased number of CD16⁺ monocytes (non-classical and intermediate monocytes) (submitted manuscript), which to our knowledge, is the first demonstration that minor monocyte subsets are decreased in an infectious disease.

MONOCYTE SUBSETS AND PARASITIC INFECTIONS

Only a few papers report the modulation of monocyte subsets in parasitic infections. It has been demonstrated that, the proportion of CD16⁺ monocytes is increased in pregnant women infected with *Plasmodium falciparum*, the agent of malaria. These CD16⁺ monocytes express higher levels of CCR5 than classical monocytes^[84]. CD16⁺ monocytes may play a major in the pathogenesis of maternal malaria because placental plasma concentrations of chemokines such as CCL3 and IL-8 are increased and are associated with placental monocyte infiltration^[84,85]. Nevertheless, classical monocytes appear to be critical for the control of *Toxoplasma gondii* infection in mice^[86] and *Leishmania brasiliensis* in humans *via* the generation of reactive oxygen species^[87].

MONOCYTE SUBSETS AND FUNGAL INFECTIONS

Aspergillus fumigatus

Aspergillus fumigatus (*A. fumigatus*) is an environmental fungus that causes life-threatening infections in neutropenic patients. Inhaled *A. fumigatus* spores (conidia) germinate in the lung and form hyphae that invade blood vessels and disseminate to other tissues^[88]. It has been clearly demonstrated that monocyte subsets contribute differently to the defense against *A. fumigatus* infection. Indeed, classical monocytes are efficient at restricting conidial germination *in vitro* whereas CD16⁺ monocytes fail to suppress the germination of conidia. The efficiency of monocyte subsets in controlling *A. fumigatus* germination is likely dependent on inflammatory cytokines. Although classical monocytes do not secrete TNF following infection, CD16⁺ monocytes produce high levels of TNF and IL-1β^[89]. These results are rather surprising because CD16⁺ monocytes are thought to be more mature and share features with tissue macrophages and, thus, might be expected to have stronger antimicrobial properties^[26]. These data suggest that CD16⁺ monocytes are the subset that is the most efficient in the control of *A. fumigatus* infection.

Candida albicans

Candida albicans (*C. albicans*) is responsible of the major

ity of fungal infections. In 30% of healthy subjects, *C. albicans* is present as commensal yeast. However when host defense mechanisms are impaired, *C. albicans* can cause mucocutaneous infections, or disseminate into the bloodstream, thereby infecting multiple organs^[90]. Monocytes are associated with systemic candidosis. While the uptake and killing of *C. albicans* by classical monocytes and CD16⁺ monocytes are similar, classical monocytes stimulated with heat-killed yeasts produce higher levels of IL-1 β and prostaglandin E2 (PGE2) than CD16⁺ monocytes^[91]. It has also been demonstrated that the production of IL-1 β by classical monocytes favors the production of IL-17A by CD4⁺ T lymphocytes and that PGE2 regulates inflammation^[92,94]. In addition, the higher production of IL-1 β and PGE2 by classical monocytes is associated with increased membrane expression of the mannose receptor (MR)^[92,95], suggesting that classical monocytes instead play an immunoregulatory role. These results suggest that only classical monocytes are able to initiate antifungal Th17 responses in human CD4⁺ T lymphocytes.

CONCLUSION

Circulating monocytes has been classically considered a homogeneous cell population, but in recent years it has become clear that they are composed of different subsets. A review of the literature shows that monocyte subsets are differently affected in infectious diseases caused by varied pathogens including virus, bacteria, parasites and fungi. In the majority of cases, an expansion of the CD16⁺ compartment is observed, and the increase in CD16⁺ monocytes is likely related to their activation through their direct interaction with the pathogen or through cytokines. More surprisingly, it has also been found that the relative number of non-classical and intermediate monocytes is decreased in Q fever endocarditis, suggesting that complex mechanisms govern the equilibrium between monocyte subsets. The measurement of monocyte subsets would be useful in better understanding of the role of monocyte activation in the pathophysiology of infectious diseases.

REFERENCES

- 1 Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJ, Liu YJ, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB. Nomenclature of monocytes and dendritic cells in blood. *Blood* 2010; **116**: e74-e80 [PMID: 20628149 DOI: 10.1182/blood-2010-02-258558]
- 2 Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. *Science* 2010; **327**: 656-661 [PMID: 20133564 DOI: 10.1126/science.1178331]
- 3 Wong-Baeza I, Alcántara-Hernández M, Mancilla-Herrera I, Ramírez-Saldivar I, Arriaga-Pizano L, Ferat-Orsorio E, López-Macías C, Isibasi A. The role of lipopeptidophosphoglycan in the immune response to *Entamoeba histolytica*. *J Biomed Biotechnol* 2010; **2010**: 254521 [PMID: 20145703 DOI: 10.1155/2010/254521]
- 4 Ishii KJ, Koyama S, Nakagawa A, Coban C, Akira S. Host innate immune receptors and beyond: making sense of microbial infections. *Cell Host Microbe* 2008; **3**: 352-363 [PMID: 18541212 DOI: 10.1016/j.chom.2008.05.003]
- 5 Williams DW, Eugenin EA, Calderon TM, Berman JW. Monocyte maturation, HIV susceptibility, and transmigration across the blood brain barrier are critical in HIV neuropathogenesis. *J Leukoc Biol* 2012; **91**: 401-415 [PMID: 22227964 DOI: 10.1189/jlb.0811394]
- 6 Rossol M, Heine H, Meusch U, Quandt D, Klein C, Sweet MJ, Hauschildt S. LPS-induced cytokine production in human monocytes and macrophages. *Crit Rev Immunol* 2011; **31**: 379-446 [PMID: 22142165]
- 7 Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. *Nat Rev Immunol* 2011; **11**: 762-774 [PMID: 21984070 DOI: 10.1038/nri3070]
- 8 Karlmark KR, Tacke F, Dunay IR. Monocytes in health and disease - Minireview. *Eur J Microbiol Immunol (Bp)* 2012; **2**: 97-102 [PMID: 24672677 DOI: 10.1556/EuJMI.2.2012.2.1]
- 9 Zimmermann HW, Trautwein C, Tacke F. Functional role of monocytes and macrophages for the inflammatory response in acute liver injury. *Front Physiol* 2012; **3**: 56 [PMID: 23091461 DOI: 10.3389/fphys.2012.00056]
- 10 Possamai LA, Antoniadis CG, Anstee QM, Quaglia A, Vergani D, Thursz M, Wendon J. Role of monocytes and macrophages in experimental and human acute liver failure. *World J Gastroenterol* 2010; **16**: 1811-1819 [PMID: 20397256]
- 11 Mehraj V, Textoris J, Ben Amara A, Ghigo E, Raoult D, Capo C, Mege JL. Monocyte responses in the context of Q fever: from a static polarized model to a kinetic model of activation. *J Infect Dis* 2013; **208**: 942-951 [PMID: 23801603 DOI: 10.1093/infdis/jit266]
- 12 Passlick B, Flieger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* 1989; **74**: 2527-2534 [PMID: 2478233]
- 13 Ziegler-Heitbrock L, Hofer TP. Toward a refined definition of monocyte subsets. *Front Immunol* 2013; **4**: 23 [PMID: 23382732 DOI: 10.3389/fimmu.2013.00023]
- 14 Ancuta P, Rao R, Moses A, Mehle A, Shaw SK, Luscinskas FW, Gabuzda D. Fractalkine preferentially mediates arrest and migration of CD16⁺ monocytes. *J Exp Med* 2003; **197**: 1701-1707 [PMID: 12810688]
- 15 Hijdra D, Vorselaars AD, Grutters JC, Claessen AM, Rijkers GT. Phenotypic characterization of human intermediate monocytes. *Front Immunol* 2013; **4**: 339 [PMID: 24155746 DOI: 10.3389/fimmu.2013.00339]
- 16 Strauss-Ayali D, Conrad SM, Mosser DM. Monocyte subpopulations and their differentiation patterns during infection. *J Leukoc Biol* 2007; **82**: 244-252 [PMID: 17475785]
- 17 Urrea X, Villamor N, Amaro S, Gómez-Choco M, Obach V, Oleaga L, Planas AM, Chamorro A. Monocyte subtypes predict clinical course and prognosis in human stroke. *J Cereb Blood Flow Metab* 2009; **29**: 994-1002 [PMID: 19293821 DOI: 10.1038/jcbfm.2009.25]
- 18 Skrzeczyńska-Moncznik J, Bzowska M, Loseke S, Grage-Griebenow E, Zembala M, Pryjma J. Peripheral blood CD14^{high} CD16⁺ monocytes are main producers of IL-10. *Scand J Immunol* 2008; **67**: 152-159 [PMID: 18201370 DOI: 10.1111/j.1365-3083.2007.02051.x]
- 19 Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, Ulrich C, Fliser D, Heine GH. CD14⁺⁺CD16⁺ monocytes and cardiovascular outcome in patients with chronic kidney disease. *Eur Heart J* 2011; **32**: 84-92 [PMID: 20943670 DOI: 10.1093/eurheartj/ehq371]
- 20 Wong KL, Tai JJ, Wong WC, Han H, Sem X, Yeap WH, Kourilsky P, Wong SC. Gene expression profiling reveals the defining features of the classical, intermediate, and non-classical human monocyte subsets. *Blood* 2011; **118**: e16-e31 [PMID: 21653326 DOI: 10.1182/blood-2010-12-326355]

- 21 **Weber C**, Belge KU, von Hundelshausen P, Draude G, Steppich B, Mack M, Frankenberger M, Weber KS, Ziegler-Heitbrock HW. Differential chemokine receptor expression and function in human monocyte subpopulations. *J Leukoc Biol* 2000; **67**: 699-704 [PMID: 10811011]
- 22 **Buechler C**, Ritter M, Orsó E, Langmann T, Klucken J, Schmitz G. Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and anti-inflammatory stimuli. *J Leukoc Biol* 2000; **67**: 97-103 [PMID: 10648003]
- 23 **Sulahian TH**, Högger P, Wahner AE, Wardwell K, Goulding NJ, Sorg C, Droste A, Stehling M, Wallace PK, Morganelli PM, Guyre PM. Human monocytes express CD163, which is upregulated by IL-10 and identical to p155. *Cytokine* 2000; **12**: 1312-1321 [PMID: 10975989]
- 24 **Cros J**, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, Puel A, Biswas SK, Moshous D, Picard C, Jais JP, D'Cruz D, Casanova JL, Trouillet C, Geissmann F. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity* 2010; **33**: 375-386 [PMID: 20832340 DOI: 10.1016/j.immuni.2010.08.012]
- 25 **Zawada AM**, Rogacev KS, Rotter B, Winter P, Marell RR, Fliser D, Heine GH. SuperSAGE evidence for CD14++CD16+ monocytes as a third monocyte subset. *Blood* 2011; **118**: e50-e61 [PMID: 21803849 DOI: 10.1182/blood-2011-01-326827]
- 26 **Belge KU**, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, Espevik T, Ziegler-Heitbrock L. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. *J Immunol* 2002; **168**: 3536-3542 [PMID: 11907116]
- 27 **Thomas R**, Lipsky PE. Human peripheral blood dendritic cell subsets. Isolation and characterization of precursor and mature antigen-presenting cells. *J Immunol* 1994; **153**: 4016-4028 [PMID: 7523513]
- 28 **Randolph GJ**, Sanchez-Schmitz G, Liebman RM, Schäkel K. The CD16(+) (FcγRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. *J Exp Med* 2002; **196**: 517-527 [PMID: 12186843]
- 29 **Frankenberger M**, Hofer TP, Marei A, Dayyani F, Schewe S, Strasser C, Aldraihi A, Stanzel F, Lang R, Hoffmann R, Prazeres da Costa O, Buch T, Ziegler-Heitbrock L. Transcript profiling of CD16-positive monocytes reveals a unique molecular fingerprint. *Eur J Immunol* 2012; **42**: 957-974 [PMID: 22531920 DOI: 10.1002/eji.201141907]
- 30 **Serbina NV**, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 2006; **7**: 311-317 [PMID: 16462739]
- 31 **Barbalat R**, Lau L, Locksley RM, Barton GM. Toll-like receptor 2 on inflammatory monocytes induces type I interferon in response to viral but not bacterial ligands. *Nat Immunol* 2009; **10**: 1200-1207 [PMID: 19801985 DOI: 10.1038/ni.1792]
- 32 **Ingersoll MA**, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, Lang R, Haniffa M, Collin M, Tacke F, Habenicht AJ, Ziegler-Heitbrock L, Randolph GJ. Comparison of gene expression profiles between human and mouse monocyte subsets. *Blood* 2010; **115**: e10-e19 [PMID: 19965649 DOI: 10.1182/blood-2009-07-235028]
- 33 **Geissmann F**, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity* 2003; **19**: 71-82 [PMID: 12871640]
- 34 **Auffray C**, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, Sarnacki S, Cumano A, Lauvau G, Geissmann F. Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science* 2007; **317**: 666-670 [PMID: 17673663]
- 35 **Nahrendorf M**, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, Libby P, Weissleder R, Pittet MJ. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med* 2007; **204**: 3037-3047 [PMID: 18025128]
- 36 **Emminger W**, Zlabinger GJ, Fritsch G, Urbanek R. CD14(dim)/CD16(bright) monocytes in hemophagocytic lymphohistiocytosis. *Eur J Immunol* 2001; **31**: 1716-1719 [PMID: 11385615]
- 37 **Rivier A**, Pène J, Rabesandratana H, Chanez P, Bousquet J, Campbell AM. Blood monocytes of untreated asthmatics exhibit some features of tissue macrophages. *Clin Exp Immunol* 1995; **100**: 314-318 [PMID: 7538057]
- 38 **Okamoto H**, Mizuno K, Horio T. Circulating CD14+ CD16+ monocytes are expanded in sarcoidosis patients. *J Dermatol* 2003; **30**: 503-509 [PMID: 12928539]
- 39 **Nagasawa T**, Kobayashi H, Aramaki M, Kiji M, Oda S, Izumi Y. Expression of CD14, CD16 and CD45RA on monocytes from periodontitis patients. *J Periodontol Res* 2004; **39**: 72-78 [PMID: 14687231]
- 40 **Novak N**, Allam P, Geiger E, Bieber T. Characterization of monocyte subtypes in the allergic form of atopic eczema/dermatitis syndrome. *Allergy* 2002; **57**: 931-935 [PMID: 12269940]
- 41 **Rahman SH**, Salter G, Holmfild JH, Larvin M, McMahon MJ. Soluble CD14 receptor expression and monocyte heterogeneity but not the C-260T CD14 genotype are associated with severe acute pancreatitis. *Crit Care Med* 2004; **32**: 2457-2463 [PMID: 15599151]
- 42 **Yoshioka Y**, Ohwada A, Harada N, Satoh N, Sakuraba S, Dambara T, Fukuchi Y. Increased circulating CD16+ CD14dim monocytes in a patient with pulmonary alveolar proteinosis. *Respirology* 2002; **7**: 273-279 [PMID: 12153694]
- 43 **Scherberich JE**, Estner H, Segerer W. Impact of different immunosuppressive regimens on antigen-presenting blood cells in kidney transplant patients. *Kidney Blood Press Res* 2004; **27**: 177-180 [PMID: 15256814]
- 44 **Schauer D**, Starlinger P, Reiter C, Jahn N, Zajc P, Buchberger E, Bachleitner-Hofmann T, Bergmann M, Stift A, Gruenberger T, Brostjan C. Intermediate monocytes but not TIE2-expressing monocytes are a sensitive diagnostic indicator for colorectal cancer. *PLoS One* 2012; **7**: e44450 [PMID: 22973451 DOI: 10.1371/journal.pone.0044450]
- 45 **Ho DD**, Rota TR, Hirsch MS. Infection of monocyte/macrophages by human T lymphotropic virus type III. *J Clin Invest* 1986; **77**: 1712-1715 [PMID: 2422213]
- 46 **Gartner S**, Markovits P, Markovitz DM, Kaplan MH, Gallo RC, Popovic M. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* 1986; **233**: 215-219 [PMID: 3014648]
- 47 **Funderburg NT**, Zidar DA, Shive C, Lioi A, Mudd J, Muschelwhite LW, Simon DI, Costa MA, Rodriguez B, Sieg SF, Lederman MM. Shared monocyte subset phenotypes in HIV-1 infection and in uninfected subjects with acute coronary syndrome. *Blood* 2012; **120**: 4599-4608 [PMID: 23065151 DOI: 10.1182/blood-2012-05-433946]
- 48 **Han J**, Wang B, Han N, Zhao Y, Song C, Feng X, Mao Y, Zhang F, Zhao H, Zeng H. CD14(high)CD16(+) rather than CD14(low)CD16(+) monocytes correlate with disease progression in chronic HIV-infected patients. *J Acquir Immune Defic Syndr* 2009; **52**: 553-559 [PMID: 19950429]
- 49 **Williams K**, Westmoreland S, Greco J, Ratai E, Lentz M, Kim WK, Fuller RA, Kim JP, Autissier P, Sehgal PK, Schinazi RF, Bischofberger N, Piatak M, Lifson JD, Masliah E, González RG. Magnetic resonance spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV neuroAIDS. *J Clin Invest* 2005; **115**: 2534-2545 [PMID: 16110325]
- 50 **Moniuszko M**, Liyanage NP, Doster MN, Parks RW, Grubczak K, Lipinska D, McKinnon K, Brown C, Hirsch V, Vaccari M, Gordon S, Pegu P, Fenizia C, Flisiak R, Grzeszczuk A, Dabrowska M, Robert-Guroff M, Silvestri G, Stevenson M, McCune J, Franchini G. Glucocorticoid Treatment at Moderate Doses of SIVmac251-Infected Rhesus Macaques Decreases the Frequency of Circulating CD14(+)CD16(++) Monocytes But Does Not Alter the Tissue Virus Reservoir.

- AIDS Res Hum Retroviruses 2014 Mar 3; Epub ahead of print [PMID: 24432835]
- 51 **Shikuma CM**, Chow DC, Gangcuangco LM, Zhang G, Keating SM, Norris PJ, Seto TB, Parikh N, Kallianpur KJ, Nakamoto BK, Nagamine LS, Ndhlovu LC, Barbour JD. Monocytes expand with immune dysregulation and is associated with insulin resistance in older individuals with chronic HIV. *PLoS One* 2014; **9**: e90330 [PMID: 24587328 DOI: 10.1371/journal.pone.0090330]
- 52 **Schaer DJ**, Alayash AI, Buehler PW. Gating the radical hemoglobin to macrophages: the anti-inflammatory role of CD163, a scavenger receptor. *Antioxid Redox Signal* 2007; **9**: 991-999 [PMID: 17508920]
- 53 **Tippett E**, Cheng WJ, Westhorpe C, Cameron PU, Brew BJ, Lewin SR, Jaworowski A, Crowe SM. Differential expression of CD163 on monocyte subsets in healthy and HIV-1 infected individuals. *PLoS One* 2011; **6**: e19968 [PMID: 21625498 DOI: 10.1371/journal.pone.0019968]
- 54 **Funderburg NT**, Jiang Y, Debanne SM, Storer N, Labbato D, Clagett B, Robinson J, Lederman MM, McComsey GA. Rosuvastatin treatment reduces markers of monocyte activation in HIV-infected subjects on antiretroviral therapy. *Clin Infect Dis* 2014; **58**: 588-595 [PMID: 24253250 DOI: 10.1093/cid/cit748]
- 55 **Palmer BE**, Neff CP, Lecureux J, Ehler A, Dsouza M, Remling-Mulder L, Korman AJ, Fontenot AP, Akkina R. In vivo blockade of the PD-1 receptor suppresses HIV-1 viral loads and improves CD4+ T cell levels in humanized mice. *J Immunol* 2013; **190**: 211-219 [PMID: 23209326 DOI: 10.4049/jimmunol.1201108]
- 56 **Said EA**, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, Hill BJ, Noto A, Ancuta P, Peretz Y, Fonseca SG, Van Grevenynghe J, Boulassel MR, Bruneau J, Shoukry NH, Routy JP, Douek DC, Haddad EK, Sekaly RP. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. *Nat Med* 2010; **16**: 452-459 [PMID: 20208540 DOI: 10.1038/nm.2106]
- 57 **Breen EC**, Rezai AR, Nakajima K, Beall GN, Mitsuyasu RT, Hirano T, Kishimoto T, Martinez-Maza O. Infection with HIV is associated with elevated IL-6 levels and production. *J Immunol* 1990; **144**: 480-484 [PMID: 2295799]
- 58 **Than S**, Hu R, Oyaizu N, Romano J, Wang X, Sheikh S, Pahwa S. Cytokine pattern in relation to disease progression in human immunodeficiency virus-infected children. *J Infect Dis* 1997; **175**: 47-56 [PMID: 8985195]
- 59 **Mackenzie JS**, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med* 2004; **10**: S98-109 [PMID: 15577938]
- 60 **Ishikawa T**, Konishi E. [Flaviviruses]. *Uirusu* 2011; **61**: 221-238 [PMID: 22916569]
- 61 **Durbin AP**, Vargas MJ, Wanionek K, Hammond SN, Gordon A, Rocha C, Balmaseda A, Harris E. Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. *Virology* 2008; **376**: 429-435 [PMID: 18452966 DOI: 10.1016/j.virol.2008.03.028]
- 62 **Jessie K**, Fong MY, Devi S, Lam SK, Wong KT. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in situ hybridization. *J Infect Dis* 2004; **189**: 1411-1418 [PMID: 15073678]
- 63 **Diamond MS**, Roberts TG, Edgil D, Lu B, Ernst J, Harris E. Modulation of Dengue virus infection in human cells by alpha, beta, and gamma interferons. *J Virol* 2000; **74**: 4957-4966 [PMID: 10799569]
- 64 **Fink K**, Ng C, Nkenfou C, Vasudevan SG, van Rooijen N, Schul W. Depletion of macrophages in mice results in higher dengue virus titers and highlights the role of macrophages for virus control. *Eur J Immunol* 2009; **39**: 2809-2821 [PMID: 19637226 DOI: 10.1002/eji.200939389]
- 65 **Chen LC**, Shyu HW, Lin HM, Lei HY, Lin YS, Liu HS, Yeh TM. Dengue virus induces thrombomodulin expression in human endothelial cells and monocytes in vitro. *J Infect* 2009; **58**: 368-374 [PMID: 19307023 DOI: 10.1016/j.jinf.2009.02.018]
- 66 **Bosch I**, Khaja K, Estevez L, Raines G, Melichar H, Warke RV, Fournier MV, Ennis FA, Rothman AL. Increased production of interleukin-8 in primary human monocytes and in human epithelial and endothelial cell lines after dengue virus challenge. *J Virol* 2002; **76**: 5588-5597 [PMID: 11991987]
- 67 **Azeredo EL**, Neves-Souza PC, Alvarenga AR, Reis SR, Torrentes-Carvalho A, Zagne SM, Nogueira RM, Oliveira-Pinto LM, Kubelka CF. Differential regulation of toll-like receptor-2, toll-like receptor-4, CD16 and human leucocyte antigen-DR on peripheral blood monocytes during mild and severe dengue fever. *Immunology* 2010; **130**: 202-216 [PMID: 20113369 DOI: 10.1111/j.1365-2567.2009.03224.x]
- 68 **Wong KL**, Chen W, Balakrishnan T, Toh YX, Fink K, Wong SC. Susceptibility and response of human blood monocyte subsets to primary dengue virus infection. *PLoS One* 2012; **7**: e36435 [PMID: 22574162 DOI: 10.1371/journal.pone.0036435]
- 69 **Ashfaq UA**, Javed T, Rehman S, Nawaz Z, Riazuddin S. An overview of HCV molecular biology, replication and immune responses. *Virol J* 2011; **8**: 161 [PMID: 21477382 DOI: 10.1186/1743-422X-8-161]
- 70 **Calland N**, Dubuisson J, Rouillé Y, Séron K. Hepatitis C virus and natural compounds: a new antiviral approach? *Viruses* 2012; **4**: 2197-2217 [PMID: 23202460 DOI: 10.3390/v4102197]
- 71 **Coquillard G**, Patterson BK. Determination of hepatitis C virus-infected, monocyte lineage reservoirs in individuals with or without HIV coinfection. *J Infect Dis* 2009; **200**: 947-954 [PMID: 19678757 DOI: 10.1086/605476]
- 72 **Nockher WA**, Scherberich JE. Expanded CD14+ CD16+ monocyte subpopulation in patients with acute and chronic infections undergoing hemodialysis. *Infect Immun* 1998; **66**: 2782-2790 [PMID: 9596748]
- 73 **Zheng J**, Liang H, Xu C, Xu Q, Zhang T, Shen T, Lu F. An unbalanced PD-L1/CD86 ratio in CD14(++)CD16(+) monocytes is correlated with HCV viremia during chronic HCV infection. *Cell Mol Immunol* 2014; **11**: 294-304 [PMID: 24531620 DOI: 10.1038/cmi.2013.70]
- 74 **Reeves M**, Sinclair J. Aspects of human cytomegalovirus latency and reactivation. *Curr Top Microbiol Immunol* 2008; **325**: 297-313 [PMID: 18637513]
- 75 **Daley-Bauer LP**, Roback LJ, Wynn GM, Mocarski ES. Cytomegalovirus hijacks CX3CR1(hi) patrolling monocytes as immune-privileged vehicles for dissemination in mice. *Cell Host Microbe* 2014; **15**: 351-362 [PMID: 24629341 DOI: 10.1016/j.chom.2014.02.002]
- 76 **Fingerle G**, Pforte A, Passlick B, Blumenstein M, Ströbel M, Ziegler-Heitbrock HW. The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. *Blood* 1993; **82**: 3170-3176 [PMID: 7693040]
- 77 **Skrzeczynska J**, Kobylarz K, Hartwich Z, Zembala M, Pryjma J. CD14+CD16+ monocytes in the course of sepsis in neonates and small children: monitoring and functional studies. *Scand J Immunol* 2002; **55**: 629-638 [PMID: 12028567]
- 78 **Fernández GC**, Ramos MV, Gómez SA, Dran GI, Exeni R, Alduncin M, Grimoldi I, Vallejo G, Elías-Costa C, Isturiz MA, Palermo MS. Differential expression of function-related antigens on blood monocytes in children with hemolytic uremic syndrome. *J Leukoc Biol* 2005; **78**: 853-861 [PMID: 16046554]
- 79 **Castañó D**, García LF, Rojas M. Increased frequency and cell death of CD16+ monocytes with Mycobacterium tuberculosis infection. *Tuberculosis (Edinb)* 2011; **91**: 348-360 [PMID: 21621464 DOI: 10.1016/j.tube.2011.04.002]

- 80 **Barcelos W**, Martins-Filho OA, Guimarães TM, Oliveira MH, Spíndola-de-Miranda S, Carvalho BN, Toledo Vde P. Peripheral blood mononuclear cells immunophenotyping in pulmonary tuberculosis patients before and after treatment. *Microbiol Immunol* 2006; **50**: 597-605 [PMID: 16924144]
- 81 **Balboa L**, Romero MM, Laborde E, Sabio Y, García CA, Basile JI, Schierloh P, Yokobori N, Musella RM, Castagnino J, de la Barrera S, Sasiain MC, Alemán M. Impaired dendritic cell differentiation of CD16-positive monocytes in tuberculosis: role of p38 MAPK. *Eur J Immunol* 2013; **43**: 335-347 [PMID: 23192690 DOI: 10.1002/eji.201242557]
- 82 **Vanham G**, Edmonds K, Qing L, Hom D, Toossi Z, Jones B, Daley CL, Huebner B, Kestens L, Gigase P, Ellner JJ. Generalized immune activation in pulmonary tuberculosis: coactivation with HIV infection. *Clin Exp Immunol* 1996; **103**: 30-34 [PMID: 8565282]
- 83 **Raoult D**, Marrie T, Mege J. Natural history and pathophysiology of Q fever. *Lancet Infect Dis* 2005; **5**: 219-226 [PMID: 15792739]
- 84 **Jaworowski A**, Kamwendo DD, Ellery P, Sonza S, Mwapasa V, Tadesse E, Molyneux ME, Rogerson SJ, Meshnick SR, Crowe SM. CD16+ monocyte subset preferentially harbors HIV-1 and is expanded in pregnant Malawian women with *Plasmodium falciparum* malaria and HIV-1 infection. *J Infect Dis* 2007; **196**: 38-42 [PMID: 17538881]
- 85 **Abrams ET**, Brown H, Chensue SW, Turner GD, Tadesse E, Lema VM, Molyneux ME, Rochford R, Meshnick SR, Rogerson SJ. Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. *J Immunol* 2003; **170**: 2759-2764 [PMID: 12594307]
- 86 **Dunay IR**, Fuchs A, Sibley LD. Inflammatory monocytes but not neutrophils are necessary to control infection with *Toxoplasma gondii* in mice. *Infect Immun* 2010; **78**: 1564-1570 [PMID: 20145099 DOI: 10.1128/IAI.00472-09]
- 87 **Novais FO**, Nguyen BT, Beiting DP, Carvalho LP, Glenzie ND, Passos S, Carvalho EM, Scott P. Human classical monocytes control the intracellular stage of *Leishmania braziliensis* by reactive oxygen species. *J Infect Dis* 2014; **209**: 1288-1296 [PMID: 24403561 DOI: 10.1093/infdis/jiu013]
- 88 **Brown JM**. Fungal infections in bone marrow transplant patients. *Curr Opin Infect Dis* 2004; **17**: 347-352 [PMID: 15241080]
- 89 **Serbina NV**, Cherny M, Shi C, Bleau SA, Collins NH, Young JW, Pamer EG. Distinct responses of human monocyte subsets to *Aspergillus fumigatus* conidia. *J Immunol* 2009; **183**: 2678-2687 [PMID: 19635902 DOI: 10.4049/jimmunol.0803398]
- 90 **Perlroth J**, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. *Med Mycol* 2007; **45**: 321-346 [PMID: 17510856]
- 91 **Smeekens SP**, van de Veerdonk FL, Joosten LA, Jacobs L, Jansen T, Williams DL, van der Meer JW, Kullberg BJ, Netea MG. The classical CD14⁺⁺ CD16⁻ monocytes, but not the patrolling CD14⁺ CD16⁺ monocytes, promote Th17 responses to *Candida albicans*. *Eur J Immunol* 2011; **41**: 2915-2924 [PMID: 21695694 DOI: 10.1002/eji.201141418]
- 92 **Chung Y**, Chang SH, Martinez GJ, Yang XO, Nurieva R, Kang HS, Ma L, Watowich SS, Jetten AM, Tian Q, Dong C. Critical regulation of early Th17 cell differentiation by interleukin-1 signaling. *Immunity* 2009; **30**: 576-587 [PMID: 19362022 DOI: 10.1016/j.immuni.2009.02.007]
- 93 **Boniface K**, Bak-Jensen KS, Li Y, Blumenschein WM, McGeachy MJ, McClanahan TK, McKenzie BS, Kastelein RA, Cua DJ, de Waal Malefyt R. Prostaglandin E2 regulates Th17 cell differentiation and function through cyclic AMP and EP2/EP4 receptor signaling. *J Exp Med* 2009; **206**: 535-548 [PMID: 19273625 DOI: 10.1084/jem.20082293]
- 94 **Napolitani G**, Acosta-Rodriguez EV, Lanzavecchia A, Salusto F. Prostaglandin E2 enhances Th17 responses via modulation of IL-17 and IFN-gamma production by memory CD4⁺ T cells. *Eur J Immunol* 2009; **39**: 1301-1312 [PMID: 19384872 DOI: 10.1002/eji.200838969]
- 95 **Ouyang W**, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008; **28**: 454-467 [PMID: 18400188 DOI: 10.1016/j.immuni.2008.03.004]

P- Reviewer: Vogt G, Zhang LL **S- Editor:** Ji FF **L- Editor:** A
E- Editor: Lu YJ





Autoimmune hepatitis in a patient infected by HIV-1 and under highly active antiretroviral treatment: Case report and literature review

Marta Casal Moura, Edite Pereira, Vítor Braz, Catarina Eloy, Joanne Lopes, Fátima Carneiro, José Paulo Araújo

Marta Casal Moura, Edite Pereira, Vítor Braz, Catarina Eloy, Joanne Lopes, Fátima Carneiro, José Paulo Araújo, Faculdade de Medicina da Universidade do Porto, 4200-319 Porto, Portugal
Marta Casal Moura, Edite Pereira, Vítor Braz, José Paulo Araújo, Serviço de Medicina Interna, Centro Hospitalar de São João, 4200-319 Porto, Portugal

Catarina Eloy, Joanne Lopes, Fátima Carneiro, Serviço de Anatomia Patológica, Centro Hospitalar de São João, 4200-319 Porto, Portugal

Author contributions: All authors contributed to this manuscript.

Correspondence to: Marta Casal Moura, MD, Serviço de Medicina Interna, Centro Hospitalar de São João, E.P.E. Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal. martacasalmoura@gmail.com

Telephone: +351-22-5512100 Fax: +351-22-502576

Received: July 6, 2014 Revised: October 12, 2014

Accepted: October 28, 2014

Published online: November 27, 2014

Abstract

Liver disease has recently been described as an important cause of morbidity and mortality in patients infected with human immunodeficiency virus (HIV). Liver test changes are useful surrogates of the burden of liver disease. Previous studies have shown that transaminase elevations are frequent among these patients. The cause of those changes is harder to establish in HIV-patients. We present a 61-year-old caucasian male, diagnosed with HIV type 1 infection since 1998, under highly active antiretroviral treatment (HAART), with virological suppression and immunological recovery. He presented in a follow-up laboratory workup high values of transaminases, arthralgia at the hip joints and hepatomegaly. Liver function tests were normal. The antibodies to hepatitis viruses were negative. However, autoimmune study and liver biopsy were compatible with autoimmune hepatitis (AIH). The AIH is a rare di-

agnosis in HIV-infected patients perhaps because the elevation of transaminases and changes in liver function tests are often associated to HAART or to other possible liver diseases, namely viral hepatitis and non-alcoholic steatohepatitis. The diagnosis may be underestimated. There are no specific recommendations available for the treatment of HIV-associated AIH although the immunosuppression with slower tapering seems the most reasonable approach.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Autoimmune hepatitis; Human immunodeficiency virus type 1; Highly active antiretroviral treatment; Liver tests; Liver biopsy

Core tip: Autoimmune hepatitis diagnosis is a rare diagnosis in human immunodeficiency virus (HIV)-infected patients perhaps because the elevation of transaminases and changes in liver function tests are often attributed to the Highly Active Antiretroviral Treatment or to other possible liver diseases, namely viral hepatitis and Non-Alcoholic Steatohepatitis. The diagnosis may be underestimated. There is no established treatment in those patients but it seems reasonable to consider immunosuppression also in HIV-infected patients.

Casal Moura M, Pereira E, Braz V, Eloy C, Lopes J, Carneiro F, Araújo JP. Autoimmune hepatitis in a patient infected by HIV-1 and under highly active antiretroviral treatment: Case report and literature review. *World J Immunol* 2014; 4(3): 194-198 Available from: URL: <http://www.wjgnet.com/2219-2824/full/v4/i3/194.htm> DOI: <http://dx.doi.org/10.5411/wji.v4.i3.194>

INTRODUCTION

Liver disease has recently been described as an important

Table 1 Features supporting the diagnosis of autoimmune hepatitis

Parameters	Diagnosis of autoimmune hepatitis
Clinical	Female gender Association with other autoimmune diseases
Autoantibodies	ANA or SMA > 1:40 (type 1) LKM > 1:40 (type 2) SLA + (type 3)
Immunoglobulin	IgG > upper normal limit
Biochemistry	Hepatic pattern (raised AST and ALT levels)
Histology	Plasma cell-rich mononuclear infiltrate Interface hepatitis with ballooning and rosetting of peri-portal hepatocyte; $\pm$ peri-portal fibrosis Lobular necroinflammatory activity No bile duct loss or chronic cholestasis
Radiology and ERCP	Normal
Exclusion of other etiology	Exclusion of viral, metabolic, drug and alcoholic etiology
Response to steroid	Good

ALT: Alanine aminotransferase; ANA: Antinuclear antibodies; AST: Aspartate aminotransferase; IgG: Immunoglobulin G; LKM: Liver kidney microsome antibodies; SLA: Soluble liver antigen antibodies; SMA: Smooth muscle antibodies.

morbidity and mortality cause in human immunodeficiency virus (HIV) patients and liver tests are typically part of routine care^[1,2]. Liver dysfunction in HIV patients, at the acquired immune deficiency syndrome (AIDS) era, mainly corresponded to opportunistic infections, like cytomegalovirus (CMV), mycobacteria and leishmaniasis; cholangitis related to AIDS caused by parasitic infections like cryptosporidiosis and microsporidiosis; tumors like lymphoma and Kaposi sarcoma; hepatitis related with medication caused by antibiotics like trimetoprim-sulfamethoxazol^[3]. Alcohol or drugs may also be considered^[1]. With the rising obesity epidemic, reports have suggested that non-alcoholic steatohepatitis (NASH) may be an important cause of liver disease in the general population, but data among HIV-infected patients are more limited^[1].

In AIH there is a loss of immune tolerance to antigens on hepatocytes with hepatic parenchyma is destruction by auto-reactive T cells^[4]. T cells CD4⁺ and CD8⁺ interaction towards effector responses mediated by NK cells and cdT cells plays a major role in immunopathogenesis^[4]. Many factors have been identified as possible triggers: viruses, xenobiotics, and drugs. This may suggest that regulatory T-cells (Treg) defects might be related to the pathogenesis of AIH^[4].

AIH diagnosis relies in several aspects: clinic, biochemistry, immunology and histology features (Table 1)^[4].

The International Autoimmune Hepatitis Working Group (IAHG), created a score in order to develop uniform diagnostic criteria. If left untreated, the prognosis of AIH is poor, the rates for 5 and 10-year survival are, respectively, 50% and 10%^[4,5]. The therapy with prednisolone leads to better survival^[4]. Cirrhosis at diagnosis is present on histology in up to 30% of adult patients^[4].

The two phases of standard therapy are: high-dose of corticosteroids for induction of remission and low-dose corticosteroids and azathioprine for maintenance^[4]. On prednisolone and azathioprine, more than 80% of the patients will achieve remission^[4].

There are fourteen case reports in the literature of AIH in HIV patients^[6-9].

CASE REPORT

The patient was a 61-year-old caucasian male, married, carpenter, emigrant in France. He was diagnosed with HIV type 1 infection since 1998, sexually acquired, under HAART - Zidovudine 300 mg tid, Lamivudine 150 mg bid and Atazanavir 200 mg bid, with virological suppression and immunological recovery (CD4⁺ cell count of 779/mm³). He was a smoker, he had no history of alcohol consumption or drug abuse. No new medications were introduced in the previous six months, neither over-the-counter medication. He was followed in Portugal in Infectious Diseases' consultations where he was observed twice a year.

In a follow-up laboratory workup, transaminases elevation was detected: ALT (437 U/L), AST (227 U/L) and GGT (220 U/L). These values decreased in three months and increased again after, reaching the highest values at six months: ALT (684 U/L, almost 20 times above the upper normal limit), AST (367 U/L, 10 times above the upper normal limit) and GGT (290 U/L). The alkaline phosphatase was normal. Bilirubin levels were elevated, both total (4.67 mg/dL) and conjugated (0.69 mg/dL). The total protein, albumin and coagulation parameters were normal. His CD4 count was 779/mm³.

At that time, he presented with severe arthralgia at the hip joints with no other symptoms. Jaundice, rash, petechiae, lymphadenopathy, lipodystrophy or parotid hypertrophy were absent. Arterial blood pressure tended to be low (95/65 mmHg). Signs of hypervolemia namely jugular venous distension, hepatojugular reflux, pulmonary stasis, or edema were not identified. The abdominal examination revealed hepatomegaly; the spleen was not palpable.

Some laboratory tests were performed in an attempt to elucidate the etiology of transaminase elevations. The cell blood count and platelets count were normal. Lipid profile was normal (Table 2). He was immune to hepatitis B and antibodies to hepatitis viruses A (HAV), B (HBV) and C (HCV) were negative. The copper metabolism was

Table 2 Laboratory workup results

Auto-immunity	11.2009	Reference values
ANA	> 1/1000 nucleolar	< 1/100
SMA	Slightly positive	Negative
Copper Metabolism		
Copper (mcg/dL)	92	50-140
Ceruloplasmin (mg/dL)	24.6	18-45
Iron Metabolism		
Iron (mcg/dL)	274	53-167
Transferrin (mg/dL)	233	206-360
Ferritin (ng/mL)	1274.8	16.4-293.9
Transferrin Saturation (%)	84	15-50 (males)
Lipid Profile		
Total cholesterol (mg/dL)	140	< 200
LDL cholesterol (mg/dL)	83	< 130
HDL cholesterol (mg/dL)	41	> 40
Triglycerides (mg/dL)	69	< 150

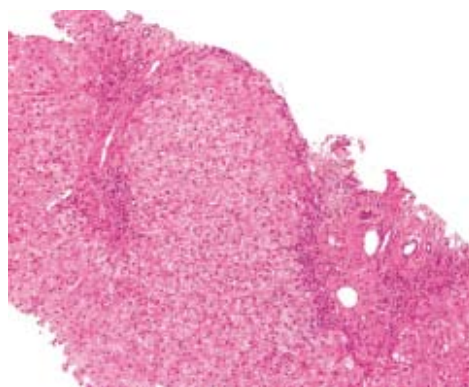
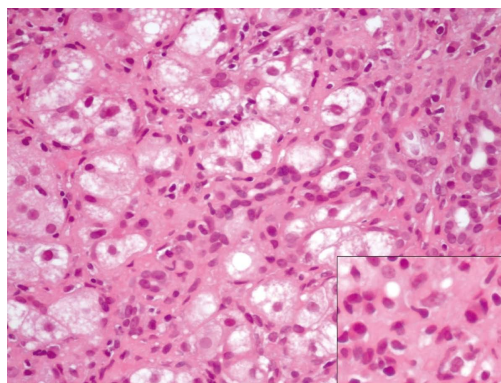
ANA: Antinuclear antibodies; HDL: High density lipoprotein; LDL: Low density lipoprotein; SMA: Smooth muscle antibodies.

normal (Table 2), excluding Wilson disease. There were alterations in iron metabolism (Table 2) with high values of transferrin saturation (84%). Hemochromatosis was excluded, he was heterozygous to the HFE gene (without mutation). The alpha 1 antitrypsin value was normal. The autoimmunity assays revealed anti-nuclear antibody (ANA) of 1/1000 with a nucleolar pattern and a slightly positive anti smooth-muscle antibody (SMA). The seric protein electrophoresis and IgG levels were normal. The alpha fetoprotein was normal. The abdominal ultrasound showed a normal sized liver with an heterogenous appearance but without focal lesions, normal biliary ducts, a portal vein with normal caliber and patent; the pancreas and spleen were normal.

A transthoracic percussion guided liver biopsy was performed after five months, without complications. The histological study showed portal fibrosis with porto-portal bridging (Figure 1). Density of inflammatory infiltrates was variable in portal ducts. Piece-meal necrosis was identified in the periphery of portal ducts and septa (interface hepatitis). Inflammatory infiltrates were also seen in the sinusoids were plasma cells (isolated or in aggregates) were easily identified (Figure 2). No signs of viral inclusions were identified. Immunostaining for CMV was negative. In the clinical and analytical setting of the patient, the diagnosis of AIH was strongly favoured. The definite diagnosis was type I AIH with a pre-treatment score of 16.

He started corticosteroids with prednisolone 60 mg per day and kept the HAART scheme he was doing. The clinical response to the treatment was good leading to the reduction of corticosteroid dose to 40 mg per day after two months of therapy. Then, corticosteroid dose was reduced to 30 mg per day after five months and azathioprine 25 mg per day was added at seven months of therapy. After eighteen months, the transaminases levels were normal.

There was no infectious complication. Also, he

**Figure 1 Portal fibrosis and porto-portal bridging (H and E).****Figure 2 Mononuclear inflammatory infiltrates in sinusoids with plasma cells (inset).**

showed sustained virological suppression and exhibited immunological recovery with CD4⁺ cell count of 1619/mm³ after starting the immunosuppressive treatment with azathioprine.

DISCUSSION

Liver test changes are being found increasingly on testing for other symptoms or diseases and HIV patients treated with HAART present them frequently^[1,2]. Those changes are influenced by hepatotoxicity to medication, co-infections by hepatotropic virus, other liver diseases (steatosis and metabolic diseases), alcohol and drugs mediated hepatotoxicity^[4]. More severe biochemical and pathological liver disease results from the interplay between liver lesions, leading to greater progression of fibrosis which may be triggered by greater sensitivity to toxic agents (alcohol and drug), HAART worsening underlying steatosis, HCV cytotoxicity especially in the cases of an increased HCV load in the liver. However, some studies show that the use of medications was not significantly associated with liver abnormalities in HIV-infected patients^[4]. Also, 51% of abnormal liver tests are unexplained^[1].

Clinical history was negative for risk factors such as alcohol, drugs and over-the-counter or prescribed medications, except for HAART. The latter was not discontinued or changed since it was established. In biochemical

study alterations concerning iron metabolism might be reactive in nature since hemochromatosis was excluded. The increased levels of autoantibodies (ANAs and anti-SMA) raised the probability of AIH and a liver biopsy was performed in the absence of contra-indications. Due to indolent changes on liver tests and to the patient only visited Portugal twice a year, liver biopsy was postponed a couple of months. The objectives were to confirm the diagnosis of AIH; to evaluate disease severity at the baseline for immunosuppressive therapy for better evaluation of response; for stratification of liver disease; for prognostic information (immunosuppression may improve interface hepatitis whereas fibrosis or cirrhosis usually occur from established bridging necrosis), which is associated with an adverse prognosis and to evaluate putative co-existent lesions^[4,5].

The diagnosis of AIH was confirmed according to the criteria of the IAHG and the patient scored 16. The score has high sensitivity and specificity but with concomitant diseases like non-alcoholic fatty liver, biliary disease or fulminant hepatitis, it does not perform so well^[4,5]. Importantly, there are no studies on the use of this score in patients infected with HIV.

The diagnosis of AIH in HIV-infected patients is hard to support because usually HIV-infection is considered has being protective against autoimmunity. However, there are many mechanisms proposed by which HIV-infection may predispose to autoimmunity.

It is thought that viral infections may generate a proper environment, pro-inflammatory, that overcomes regulatory networks resulting in the generation and self-perpetuating of autoimmune reactions^[4]. So, the virus itself may be a trigger.

AIH seems to be related with Treg defects in both the number and function. Regulatory T cells are a necessary component of the immune homeostasis^[4]. It is not known if their function is similar in HIV-infected patients. Clarification of the Treg function, molecular and cellular bases may help to AIH management^[4].

The Th17 T cells are crucial in autoimmunity^[4]. In the HIV-infected patient we don't know how this balance is achieved and perhaps there are some alterations in the proportion of CD4⁺ T-cell. It means that an unbalance towards the higher prevalence of Th17 T cells may be present and may be an explanation for triggering autoimmunity in HIV-infected patients. In our patient, this unbalance may be supported for the fact that he had immunological recovery in the period that the liver test abnormalities settled, with CD4⁺ T-cells always above 700/mm³, which means that some of these T-cells were maybe directed towards the production of Th17 T cells.

Another suggested mechanism is the role of immune restoration^[1,4,7-9], something that can't be an explanation in our patient because he already had immunological recovery. Also, is difficult to establish the link between immune restoration and liver deterioration^[4].

Uncontrolled viral replication was suggested as pos-

sible mechanism^[10]. Our patient always had virological suppression while he developed the liver test abnormalities.

Homeostasis in the liver is maintained by complex networks of effector and regulatory lymphocytes and its impairment may result in a proper local environment leads to disruption of autoimmunity and AIH^[4]. The case herein presented is different from those previously reported because the AIH results from a conjunction of factors independent of the virological suppression and immunological recovery.

Another interesting possibility is the genetic susceptibility. HLA-DR3 (A1-B8-DR3) and DR4 make more probable the recognition of self-antigens despite adequate thymic selection: increase susceptibility to type 1 AIH and DR7 associated with type 2 AIH and immune responses against hepatocyte enzyme CYP2D6^[4]. This hypothesis was not evaluated in our patient and it remains to be elucidated the role of this mechanism in HIV-infected patients.

According to the recommendations of the IAHG for AIH management, our patient fulfilled the criteria for treatment: AST > 10 fold, multiacinar necrosis in the liver biopsy and disabling symptoms.

No specific recommendations are available for the treatment of HIV-associated AIH. In the present case we decided for immunosuppression with corticosteroids at the dose generally used in other AIHs. However, corticosteroids dose reduction was slower than usual and azathioprine was introduced only seven months after the beginning of treatment and in a lower dose. The limited experience and small amount of studies considering the treatment of AIH in an HIV-infected patient and the fact that we only could evaluate this patient twice a year made us follow an individualized scheme of treatment in the case herein reported.

AIH is a rare diagnosis in HIV-infected patients perhaps because the elevation of transaminases and changes in liver function tests are often attributed to the HAART or to other possible liver diseases, namely viral hepatitis and NASH. So, the diagnosis may be underestimated. Also, the liver biopsy should be performed while evaluating hepatitis of undetermined etiology in HIV-infected patients. Many possible mechanisms were suggested to explain the pathogenesis of AIH in HIV-infected patients. The treatment has to be individualized after consideration of the risks and benefits but it seems reasonable to consider that immunosuppression should not be postponed in HIV patients.

ACKNOWLEDGMENTS

We acknowledge to the Serviço de Anatomia Patológica, Centro Hospitalar de São João, especially to Professor Dr^a Fátima Carneiro for her support, contribution to discussion and empowerment of my scientific and academic work. All authors report no conflict of interests.

COMMENTS

Case characteristics

A 61-year-old male Caucasian patient with human immunodeficiency virus (HIV)-1 infection under highly active antiretroviral treatment (HAART) presented with arthralgia, hepatomegaly and changes in liver tests.

Clinical diagnosis

Autoimmune hepatitis in an HIV-1 patient under HAART.

Differential diagnosis

Hepatotoxicity to medication, co-infections by hepatotropic virus and parasites, other liver diseases like non-alcoholic fatty liver disease and metabolic diseases, alcohol and drugs mediated hepatotoxicity.

Laboratory diagnosis

Elevated transaminase values, positive anti-nuclear antibody of 1/1000 with a nucleolar pattern and a slightly positive anti smooth-muscle antibody.

Imaging diagnosis

Trans-thoracic liver biopsy.

Pathologic diagnosis

Liver biopsy histology showed piece-meal necrosis identified in the periphery of portal ducts and septa (interface hepatitis) and inflammatory infiltrates were also seen in the sinusoids were plasma cells (isolated or in aggregates).

Treatment

Standard treatment adapted with corticosteroids and azathioprine in lower doses and slower tapering.

Related reports

Liver test changes etiology is hard to establish in patients with HIV-1 infection and sometimes lead to changes in HAART therapy doses because they are easily attributed to secondary effects of the medication.

Experiences and lessons

Liver test changes in HIV-1 patients should be looked up carefully: autoimmune hepatitis is a possible etiology (although the mechanism is not totally understood) and it should be treated in with an adapted standard treatment probably with lower doses and slower tapering.

Peer review

Nice case report.

REFERENCES

- 1 Crum-Cianflone N, Collins G, Medina S, Asher D, Campin R, Bavaro M, Hale B, Hames C. Prevalence and factors as-

- sociated with liver test abnormalities among human immunodeficiency virus-infected persons. *Clin Gastroenterol Hepatol* 2010; **8**: 183-191 [PMID: 19800985 DOI: 10.1016/j.cgh.2009.09.025]
- 2 Ioannou GN, Boyko EJ, Lee SP. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. *Am J Gastroenterol* 2006; **101**: 76-82 [PMID: 16405537]
- 3 Pol S, Lebray P, Vallet-Pichard A. HIV infection and hepatic enzyme abnormalities: intricacies of the pathogenic mechanisms. *Clin Infect Dis* 2004; **38** Suppl 2: S65-S72 [PMID: 14986277]
- 4 Oo YH, Hubscher SG, Adams DH. Autoimmune hepatitis: new paradigms in the pathogenesis, diagnosis, and management. *Hepatol Int* 2010; **4**: 475-493 [PMID: 20827405]
- 5 Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, Vierling JM. Diagnosis and management of autoimmune hepatitis. *Hepatology* 2010; **51**: 2193-2213 [PMID: 20513004 DOI: 10.1002/hep.23584]
- 6 Puius YA, Dove LM, Brust DG, Shah DP, Lefkowitz JH. Three cases of autoimmune hepatitis in HIV-infected patients. *J Clin Gastroenterol* 2008; **42**: 425-429 [PMID: 18277893 DOI: 10.1097/01.mcg.0000225591.08825.3e]
- 7 German V, Vassiloyanopoulos A, Sampaziotis D, Giannakos G. Autoimmune hepatitis in an HIV infected patient that responded to antiretroviral therapy. *Scand J Infect Dis* 2005; **37**: 148-151 [PMID: 15764206]
- 8 Daas H, Khatib R, Nasser H, Kamran F, Higgins M, Saravolatz L. Human immunodeficiency virus infection and autoimmune hepatitis during highly active anti-retroviral treatment: a case report and review of the literature. *J Med Case Rep* 2011; **5**: 233 [PMID: 21702972 DOI: 10.1186/1752-1947-5-233]
- 9 O'Leary JG, Zachary K, Misdradi J, Chung RT. De novo autoimmune hepatitis during immune reconstitution in an HIV-infected patient receiving highly active antiretroviral therapy. *Clin Infect Dis* 2008; **46**: e12-e14 [PMID: 18171203 DOI: 10.1086/524082]
- 10 Esposito A, Conti V, Cagliuso M, Pastori D, Fantauzzi A, Mezzaroma I. Management of HIV-1 associated hepatitis in patients with acquired immunodeficiency syndrome: role of a successful control of viral replication. *AIDS Res Ther* 2011; **8**: 9 [PMID: 21362160]

P- Reviewer: A-Kader HH, McQuillan GM, Teschke R

S- Editor: Ji FF L- Editor: A E- Editor: Lu YJ





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

