ISSN 2219-2824 (online)

World Journal of *Immunology*

World J Immunol 2014 November 27; 4(3): 130-198

Published by Baishideng Publishing Group Inc

WJI

World Journal of Immunology

A peer-reviewed, online, open-access journal of immunology

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).

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 Passàli, *Siena*
Carlo Perricone, *Rome*
Alessandro Poggi, *Genoa*
Antonella Prisco, *Naples*
Frncesco 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*
Mª 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*
Jessy 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*

WJI

World Journal of Immunology

Contents

Four-monthly Volume 4 Number 3 November 27, 2014

REVIEW

130 Subcutaneous and sublingual immunotherapy: Where do we stand?
Yukselen A, Kendirli SG

141 Transgenic plants for allergen-specific immunotherapy
Nishimura T, Saeki M, Kaminuma O, 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

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

NAME OF JOURNAL
World Journal of Immunology

ISSN
ISSN 2219-2824 (online)

LAUNCH DATE
December 27, 2011

FREQUENCY
Four-monthly

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

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/

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v4.i3.130

World J Immunol 2014 November 27; 4(3): 130-140
ISSN 2219-2824 (online)

REVIEW

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, Osmangazi 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

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 cessassion of the treatment. SIT brings on regulatory T cells with the capacity to generate interleukin-10 and transforming growth factor-b, 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 be faced during this long-term treatment, recent investigations have been focused on the implementation of allergens in quite efficacious and safer ways.

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.

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-b (TGF-b) 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-b 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 contribute 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-g 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-b 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 hyperresponsiveness 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 heterogenity 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 monosensitised 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 different 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 occured 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 ocur 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 significant 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 pharmacotharapy 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 IgG_4 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 *et al*[84]	1996	Grass pollen	Double-blind, double-dummy	SCIT (n = 10) SLIT (n = 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 *et al*[83]	1999	Dust mite	Single-blind, placebo controlled	SCIT (n = 10) SLIT (n = 15) Placebo (n = 11)	↓ Rhinitis symptoms with SLIT ↓ Skin reactivity with SCIT ↑ Specific IgG4 with SCIT
Khinchi *et al*[82]	2004	Birch pollen	Randomized, double-blind, double-dummy, placebo-controlled	SCIT (n = 21) SLIT (n = 18) Placebo (n = 19)	Significant reduction in symptoms and medications for SCIT *vs* placebo and SLIT *vs* placebo No difference between SCIT and SLIT groups
Mauro *et al*[86]	2007	Birch pollen	Randomized, double-blind, double-dummy	SCIT (n = 19) SLIT (n = 15)	No difference in mean symptom and medication score between SCIT and SLIT Specific IgG4 with SCIT
Tahamiler *et al*[88]	2008	Dust mite	Open label, randomized	SCIT (n = 96) SLIT (n = 97)	↓ Rhinitis and conjunctivitis symptoms scores and nasal provocation score with SCIT and SLIT (greater improvement with SCIT)
Eifan *et al*[85]	2010	Dust mite	Open label, randomized, controlled	SCIT (n = 16) SLIT (n = 16) Pharmacotherapy (n = 16)	↓ Rhinitis and asthma symptom score, total medication score ↓ Skin reactivitiy with SCIT and SLIT ↓ Specific IgE with SCIT and SLIT
Keles *et al*[87]	2011	Dust mite	Open label, randomized, controlled	SCIT (n = 11) SLIT (n = 13) SCIT plus SLIT (n = 14) Pharmacotherapy (n = 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 *et al*[89]	2012	Dust mite	Randomized, double-blind, double-dummy, placebo-controlled	SCIT (n = 10) SLIT (n = 11) Placebo (n = 10)	Significant reduction in rhinitis and astma 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 intracellulary 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 ultrasonograpy 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, Karakaş T, Anlar Y, Söğüt A, Altintaş D, Canitez Y, Büyükdereli 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 seaso-

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 **Mascarell 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, Wrzyszcz 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, Løwenstein 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, Grønager 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**, Malling 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, Olaguíbel 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 **Blumberga 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, 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. 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 **Olaguíbel 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 **Pradalier 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**, Braidi 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, Echechipía S, García BE, Martin 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 Investig 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**, Ambriz-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 **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 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, Ozcora 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-Qutob 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: 10.2174/1872213x07666131113111159]
93 **Crameri 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**, Crameri 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

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v4.i3.141

World J Immunol 2014 November 27; 4(3): 141-148
ISSN 2219-2824 (online)

REVIEW

Transgenic plants for allergen-specific immunotherapy

Tomoe Nishimura, Mayumi Saeki, Osamu Kaminuma, Fumio Takaiwa, Takachika Hiroi

Tomoe Nishimura, Mayumi Saeki, Osamu Kaminuma, 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. hiroi-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

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 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.

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.

Nishimura T, Saeki M, Kaminuma O, 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

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 restructured 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	*Cryptomeria Japonica* pollen (Japanese cedar pollen)	GluB-1	Fusion protein of mouse T-cel epitopes in Cry j 1 and Cry j 2, and glycinin A1aB1b	absence	presumaply PB-II	7 μg/ grain	IgE and IgG CD4+ T-cell proliferation Th2 cytokine Histamine Sneezing	↓ ↓ ↓ ↓ ↓	[74]
7Crp	Allergic rhinitis	*Cryptomeria Japonica* 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	*Cryptomeria Japonica* 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	*Dermatophagoides Pteronyssinus* (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	*Dermatophagoides Farinae* (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 an 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, Haahtela 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, Ramirez 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, Brewe 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**, Erekosima 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, 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]
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, Wrzyszcz 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: 0.1016/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, Kündig 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, 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]
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**, Crameri 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]
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, Björksten B, Dreborg S, Søborg 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 phisiology* 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.1365-313X.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, Matuhasi 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, Pouvelle-Moratille S, Närvänen A, Kinnunen T, Saarelainen S, Rytkönen-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 destructed 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:

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, Umezu-Goto 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

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v4.i3.149

World J Immunol 2014 November 27; 4(3): 149-157
ISSN 2219-2824 (online)

REVIEW

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

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.

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 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

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

DLII 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 gluconization, sulfonization, 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 trifluroacetyl 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

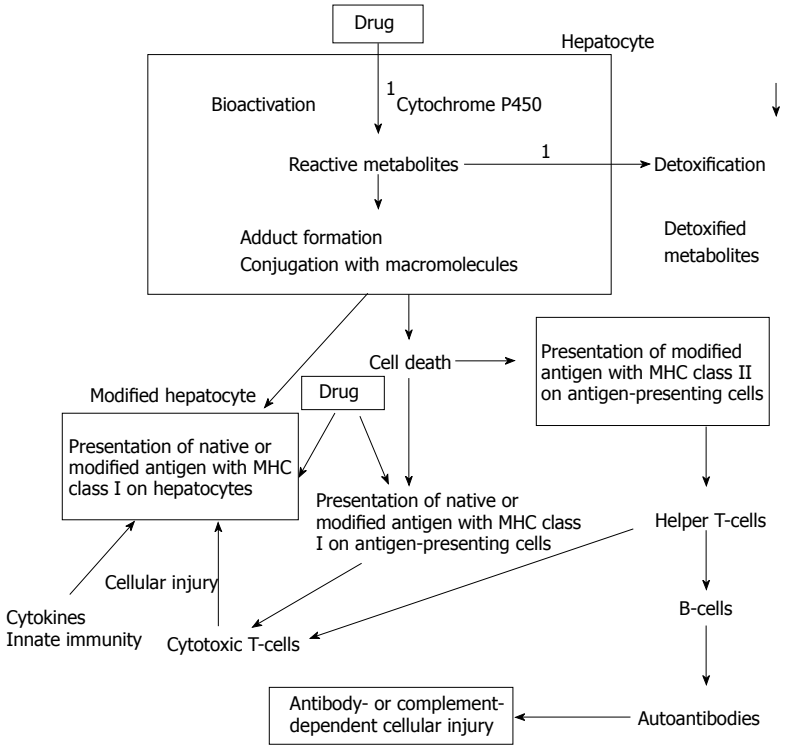

Figure 1 Hypothetical schema of the immunological pathogenesis of drug induced liver injury (ref. [5] with modifications). [1]Genetic polymorphism.

example, IL-4 was reported to mediate dicloxacillin-induced liver injury in a mouse model[26], and IL-17 and IL-1β 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-κB 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 patients 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-1β, 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

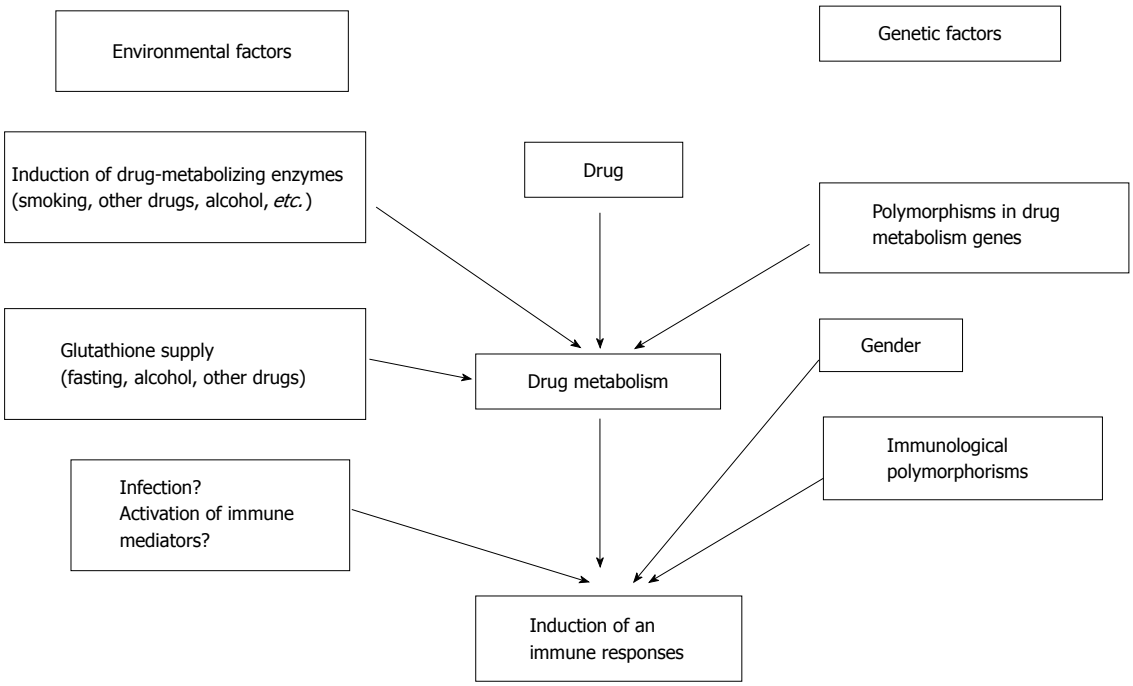

Figure 2 Contribution of environmental and genetic factors to drug metabolism and immune response in drug induced liver injury[30].

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 supersomes[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-

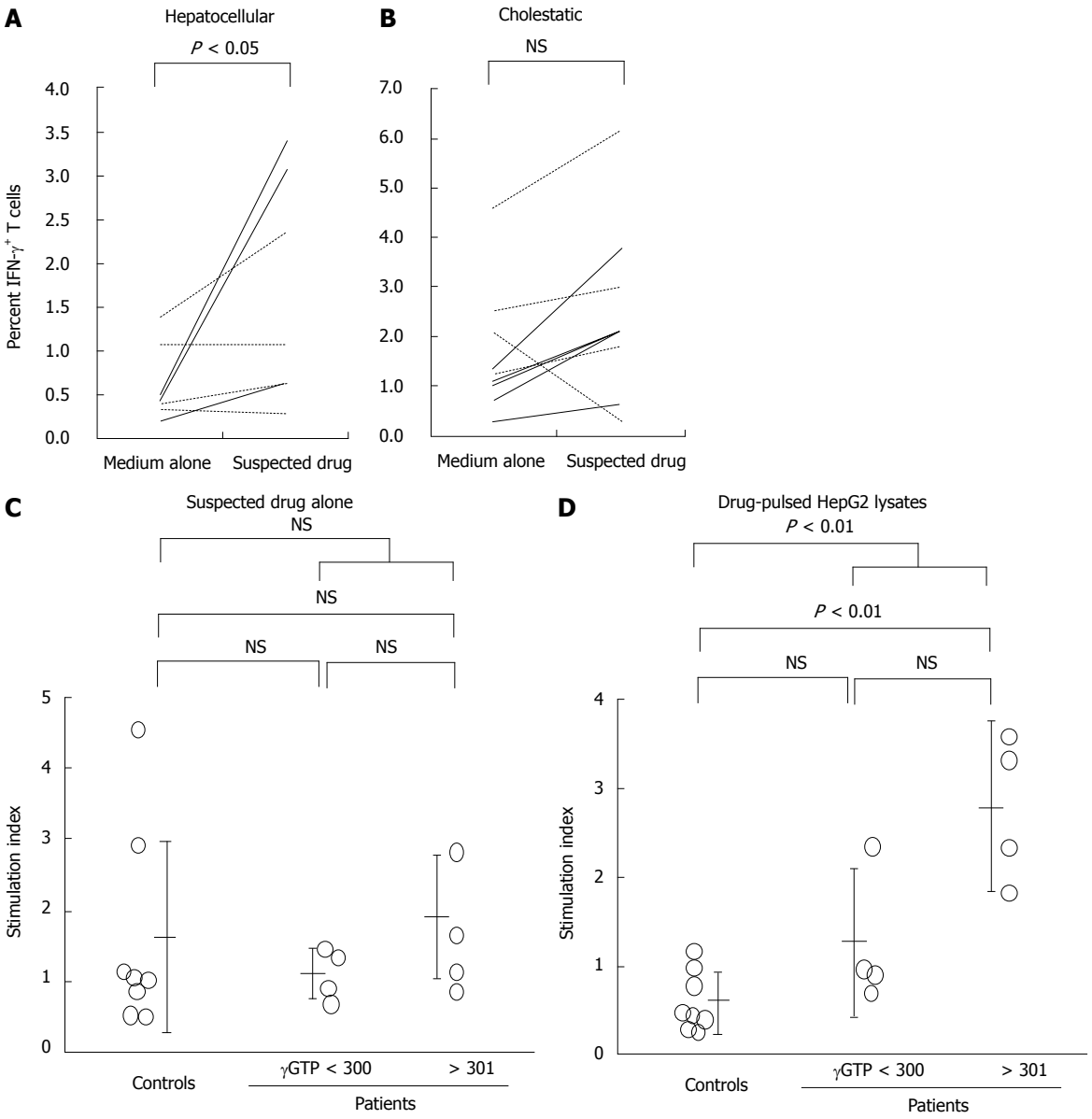

Figure 3 Cytokine production by immune cells after stimulation with suspected drug alone or suspected drug-pulsed HepG2 lysates in patients with drug induced liver injury[36]. A, B: The percentages of $CD8^+$ cells that are IFN-γ^+ after stimulation with the suspected drug alone in patients with hepatocellular 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. Flucloxacillin (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 pharmacologically, 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 M and V 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 ^{3}H-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 produced 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-alpha 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-alpha 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-gamma 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-κB 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: Kaplowittz 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**, Antoniades 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, Lyerly 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, Eddleston 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**, Tinel 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, Benitez 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, Alfirevic 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, Janecek 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 sparstolonin 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. *Hepatogastroenterology* 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

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v4.i3.158

World J Immunol 2014 November 27; 4(3): 158-173
ISSN 2219-2824 (online)

REVIEW

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.

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-κB) 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 ($\cdot O_2^-$) 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 ($NO\cdot$) is produced from arginine catalyzed by a nitric oxide synthase (NOS). Fast reaction between $\cdot O_2^-$ and $NO\cdot$ gives rise to peroxynitrite (ONOO-) and ONOO- is oxidizing molecule that connected to cancer. $NO\cdot$ 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-κB, 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 $\cdot O_2^-$ 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-κB 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-κB, activating protein-1 (AP-1), p53, and hypoxia inducible factor-1α (HIF-1α). 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-κB predisposes murine skin to squamous cell carcinoma. RelA subunit of NF-κB has tumor suppressing activity under some circumstances[67-71]. Because NF-κB 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. H_2O_2 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 oncocytomas, 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 oncocytoma. Further studies are needed to understand whether ROS may influence the proliferative potential and accumulation of mutations in oncocytoma. 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 oncocytic 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 H_2O_2, 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 IκB kinase/NF-κB 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-κB 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 (FOXM1) is expressed at low levels in normal cells, but its expression is markedly elevated in cancer cells[111]. FOXM1 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-κB, 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 H_2O_2 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]. TNFα, 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-κB facilitate TNF-induced necrotic cell death, suggesting that NF-κB 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-κB 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 H_2O_2 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 H_2O_2 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-1α and VEGF expression[157]. Antioxidant N-acetyl-l-aspartate (NAC) decreased vessels number *via* suppressing HIF-1α 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 $NRF\text{-}2^{-/-}$ mice, depleted for key anti-oxidant transcription factors) are converted into myofibroblasts[172]. Cancer cells themselves produce H_2O_2, which is a highly diffusible species. NOX enzymes, located at the plasma membrane in various carcinomas, might contribute to the production of H_2O_2 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-1α 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-1α protein are suppressed in human breast carcinoma cells. This suggests that superoxide may contribute to accumulation of HIF-1α[179].

HIF-1α 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-1α 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-1α can work with SIRT3 to regulate CAF metabolism, driving to metabolic reprogramming towards glycolysis[183]. In addition, hydrogen peroxide stabilizes HIF-1α 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-1α 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-κB 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 quiescent 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-κB[190]. Niclosamide is synergistic with the frontline chemotherapeutic agents, such as cytarabine, etoposide, and daunorubicin[190]. The peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-dependent transcription factor belonging to the nuclear hormone receptor superfamily[191]. PPARγ 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 H_2O_2 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

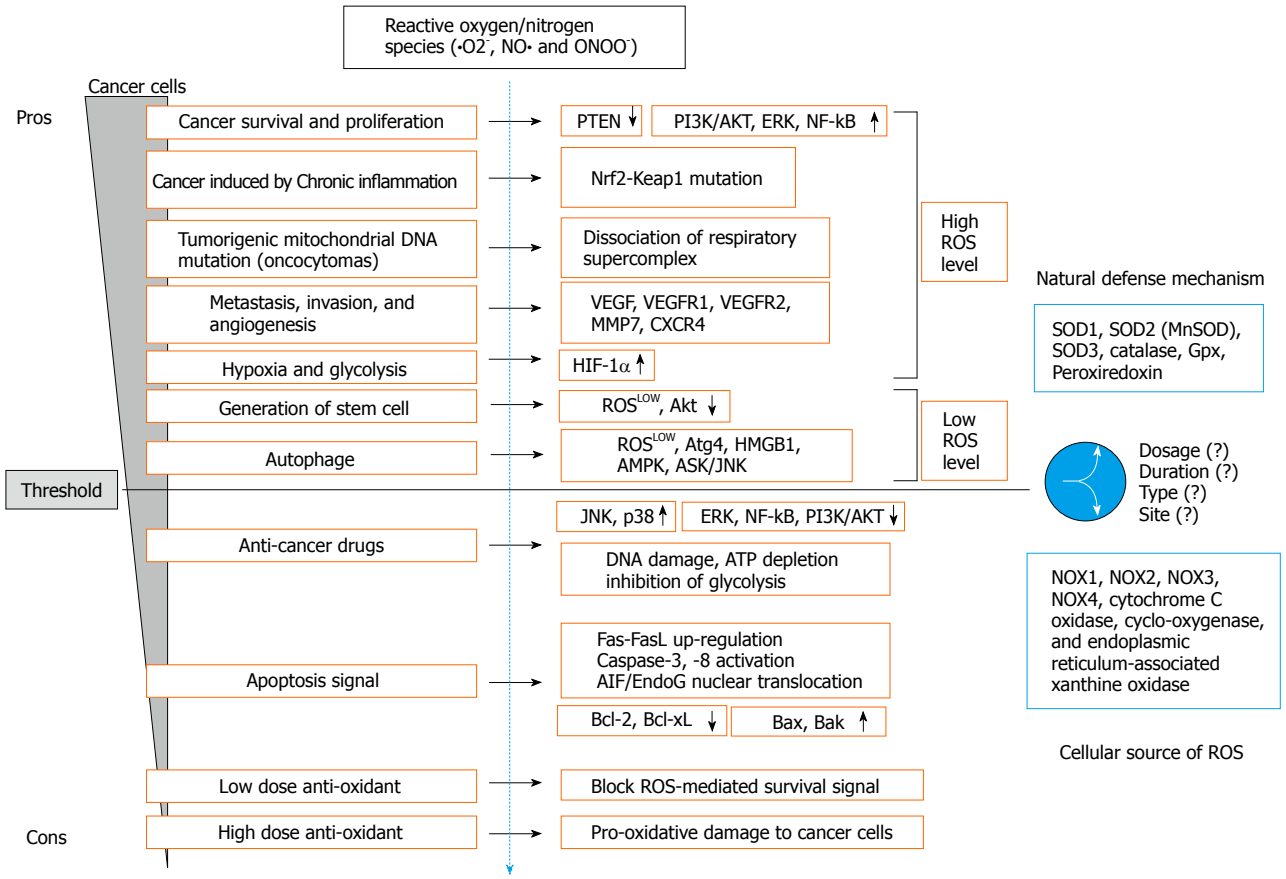

Figure 1 The role of reactive oxygen species on cancer cells to explain the different effects at each condition. Reactive oxygen species (ROS) level inside cancer cell appears varied even within a given type of cancer cells. A so-called "double-edged sword strategy" uses to manipulate the opposite role of ROS sequentially to kill cancer cells more effectively. VEGF: Vascular endothelial growth factor; PI3K: Phosphoinositide-3 kinase; PTEN: Phosphatase and tensin homolog; HIF-1α: Hypoxia inducible factor-1α; 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 $\cdot O_2^-$ induces autophagy and cell death. Exogenous H_2O_2 is effectively converted to intracellular $\cdot O_2^-$ 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 IC_{50}[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 G_2/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-κB, 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 μmol/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, McMullen JM, Davis PR, Murphy MP, Van Eldik LJ, St Clair D, Keller JN. Cognitive impairment in humanized APP×PS1 mice is linked to Aβ(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, Borzì 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, Griendling 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, Zagozdzon 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, Ouaaz 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.abb.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-Ruíz 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 nontoxic 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-κB 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 GI-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-kappaB 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-kappaB have a malignantly transformed phenotype. *Oncogene* 2002; **21**: 2484-2492 [PMID: 11971183]
69 **Ricca A**, Biroccio A, Trisciuoglio 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-kappaB function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF-kappaB. *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-kappaB 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-Cespedes 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 oncocytic 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 oncocytic 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-1alpha 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, Pandol 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, Pandol 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, Pandol 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, Sallese 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-alpha. *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, Vancompernolle 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-alpha. *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. Dimerumic acid inhibits SW620 cell invasion by attenuating H_2O_2-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 **Rungtabnapa 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, Gavriliuc 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, Rigaill 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-1alpha 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, Felsher 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 **Pouysségur 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, Schwitalla S, Kalna G, Ogg EL, Athineos D, Timpson P, Vidal M, Murray GI, Greten FR, Anderson KI, Sansom OJ. ROS production and NF-κB 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, Wulfkuhle 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-kappaB 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 PPARγ 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, Blommaart 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 **Kaminskyy 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-alpha 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 trancriptionally 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, Guillevin 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 H(2)O(2) accumulation in cancer cells by wogonin effectively blocks tumor necrosis factor-induced NF-κB 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

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v4.i3.174

World J Immunol 2014 November 27; 4(3): 174-184
ISSN 2219-2824 (online)

REVIEW

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.

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

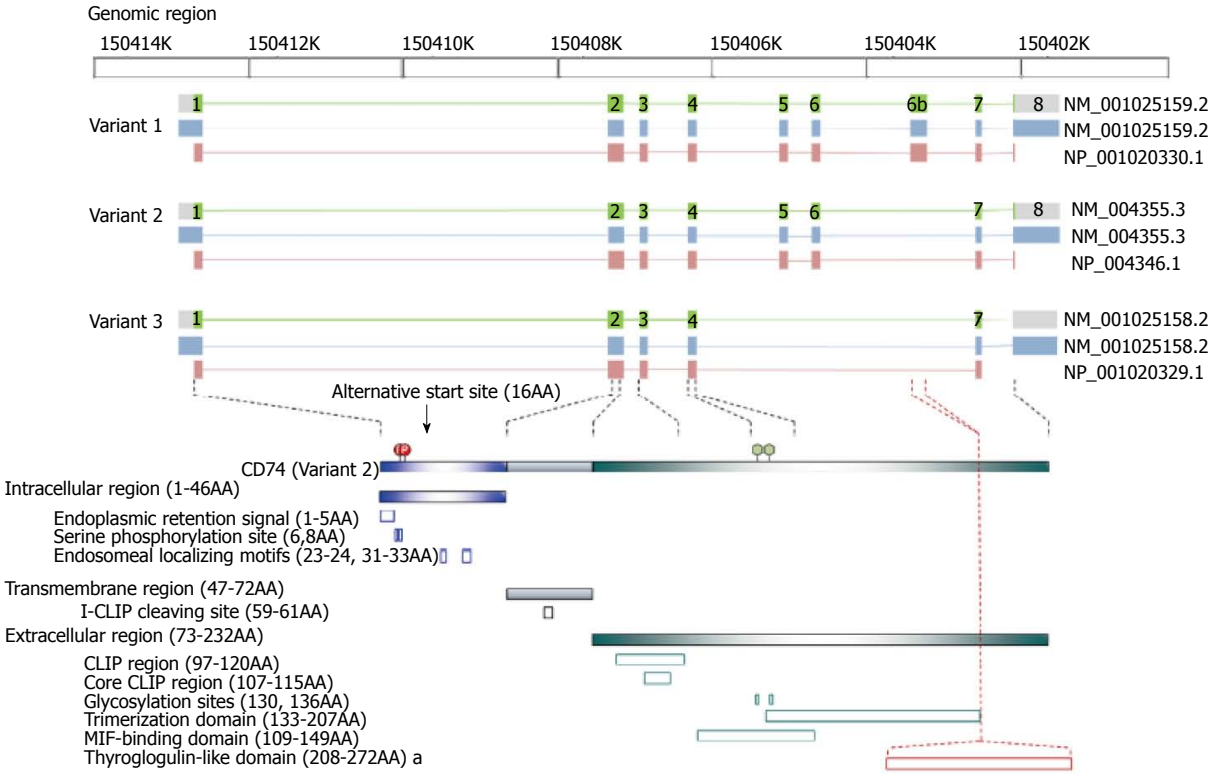

Figure 1 The variants and the corresponding protein structures of cluster of differentiation 74. The upper panel illustrates the corresponding position among genomic region, NCBI reference sequence number and reference protein accession, exon (green box) and intron (green line) localization of DNA, transcripts (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

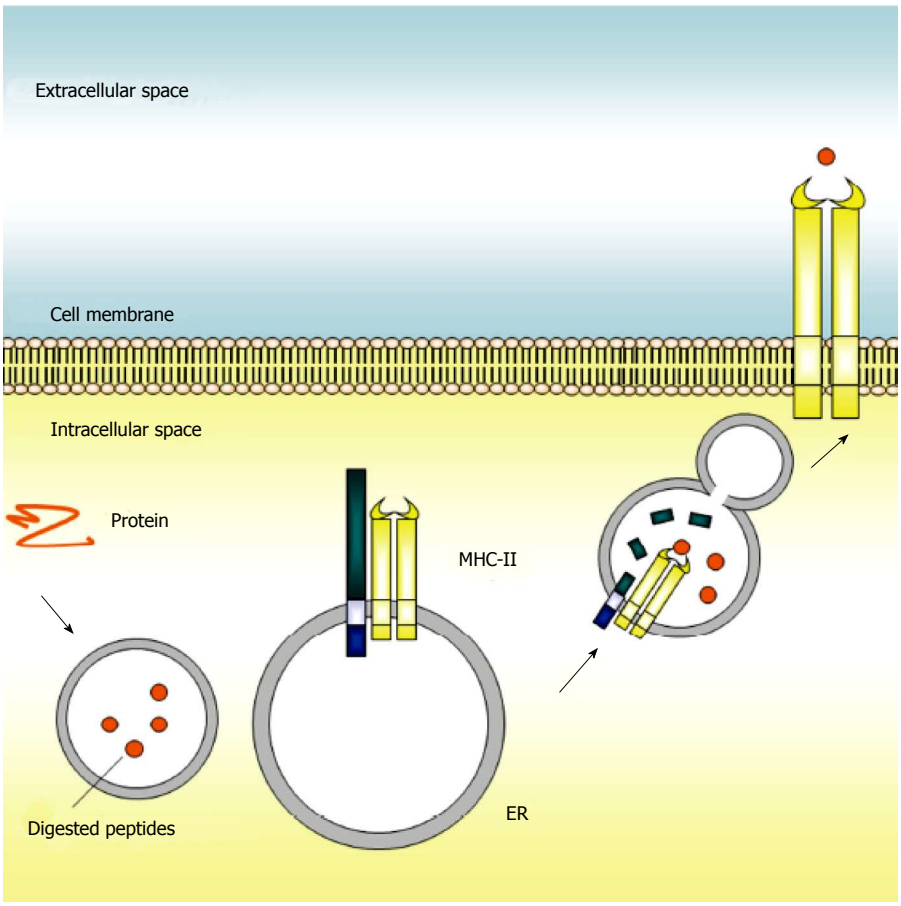

Figure 2 The canonical function of cluster of differentiation 74 in the immune system. Cluster of differentiation 74 (CD74) is present on endoplasmic reticulum (ER) where it can interact with major histocompatibility complex class Ⅱ (MHC class Ⅱ) and contribute to antigen presentation. Once synthesized, 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 $CD4^+$ T_H2 cells in CD74-null mice is compromised by $CD4^+$ T_H1 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-κB activation, cell proliferation, and survival[39]. MIF also induces upregulation of anti-apoptotic proteins Bcl-2 and Bcl-X_L[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[1]; 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-κB 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 initials MIF-dependent MEK/ERK and PI3K/AKT activation. This is followed by NF-κB 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-κB p65/RelA homodimer and its coactivator, TAFII105, 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]
		IHC	[99]
	CD74 was detected in 39 of 58 (67.2%) gastric carcinoma tissues, showing significant correlation with the differentiation of gastric carcinoma ($P < 0.05$)		
Breast cancer	The expression of CD74 was significantly more abundant in invasive or metastatic tumors than in ductal carcinoma in situ ($P = 0.02$ and $P = 0.05$, respectively)	SAGE	[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]
	CD74 expression was increased in high-grade, invasive urothelial carcinoma of the bladder		[103]
Multiple myeloma	CD74 was detected in 19 of 22 (86.4%) multiple myeloma tissues	IHC	[69]
Pancreatic cancer	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 (≥ 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 (≥ 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.

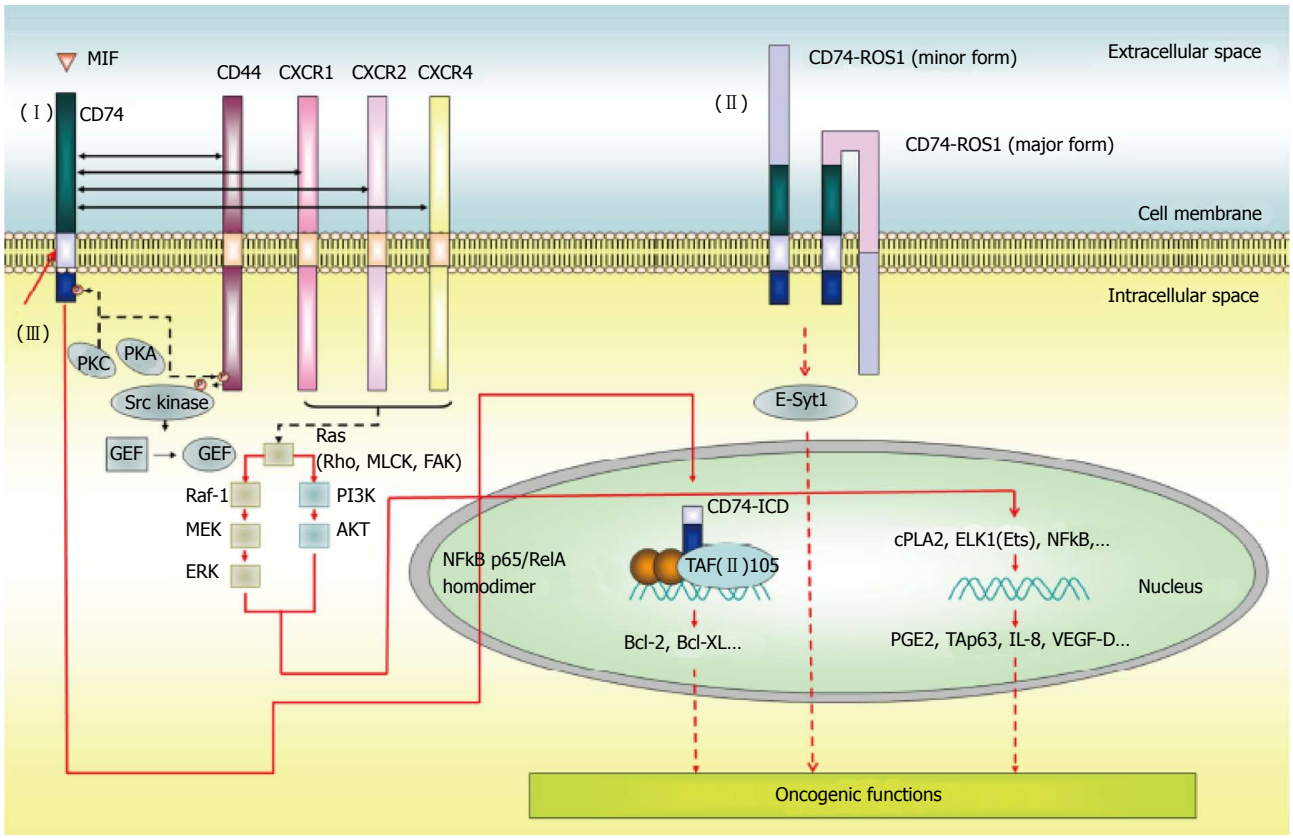

Figure 3 The function of cluster of differentiation 74 in the cancer development. (Ⅰ) Membrane associated-CD74 is involved in modulating the expression of a variety of genes which involved cell proliferation, invasion and survival through interacting with CD44, CXCR1, CXCR2 or CXCR4 followed by activation 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]. Ebselen 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 IFNgamma-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 lumenal 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, Giacoletto 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, Burhkart 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& gt; 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& gt; 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 CPrives C.** At the crossroads of inflammation and tumorigenesis. *J Exp Med* 1999; **190:** 1367-1370 [PMID: 10562311]

39 **Matza D,** Wolstein O, Dikstein RShachar 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, Gemsa 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.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-ROS 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 **Bergethon 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, Matin 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, Hershkoviz 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/jimmunol.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, Alldridge 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, Brooun 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 SArenberg 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.cllc.2013.08.006]

P- Reviewer: Chowdhury P, Shah A **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v4.i3.185

World J Immunol 2014 November 27; 4(3): 185-193
ISSN 2219-2824 (online)

MINIREVIEWS

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

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.

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

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].

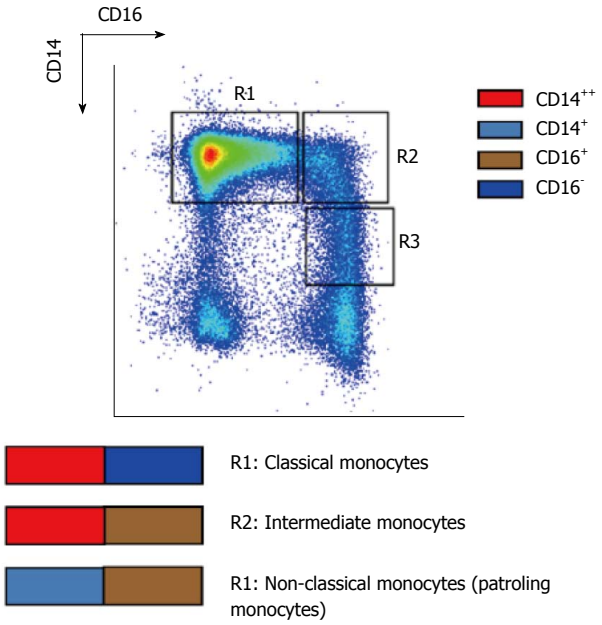

Figure 1 Expression of $CD14^{++}$ and $CD16^{+}$ cells.

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-1β[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.

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-1β production	+	++	++	[24]
CCL2 production	++	-	-	[24]
IL-10 production	++	-	-	[24]

++: High; +: Median; -: Low. IL: Interleukin.

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, nonclassical monocytes are more prone to becominge 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 extravasation 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-1β 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], peridontitis[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 immunodefi-

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-1β 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-1β and prostaglandin E2 (PGE2) than $CD16^+$ monocytes[91]. It has also been demonstrated that the production of IL-1β 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-1β 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-Saldívar I, Arriaga-Pizano L, Ferat-Osorio 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**, Antoniades 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 **Urra 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 CD14high 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 nonclassical 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 antiinflammatory 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(+) (FcgammaRIII(+)) 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, Aldraihim 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 Periodontal 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, Holmfield 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, Musselwhite 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**, Xhaja 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 **Skrzeczyńska 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, Alduncín 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 **Castaño 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: co-activation 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, Glennie 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, Sallusto 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

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5411/wji.v4.i3.194

World J Immunol 2014 November 27; 4(3): 194-198
ISSN 2219-2824 (online)

CASE REPORT

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, 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 diagnosis 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 immunosupression with slower tapering seems the most reasonable approach.

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 in favor of the diagnosis of alanine aminotransferase

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	Hepatitic pattern (raised AST and ALT levels)
Histology	Plasma cell-rich mononuclear infiltrate
	Interface hepatitis with ballooning and rosetting of peri-portal hepatocyte; ± 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/mm^3). 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/mm^3.

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 showed sustained virological suppression and exhibited immunological recovery with $CD4^+$ cell count of 1619/mm^3 after starting the immunosuppressive treatment with azathioprine.

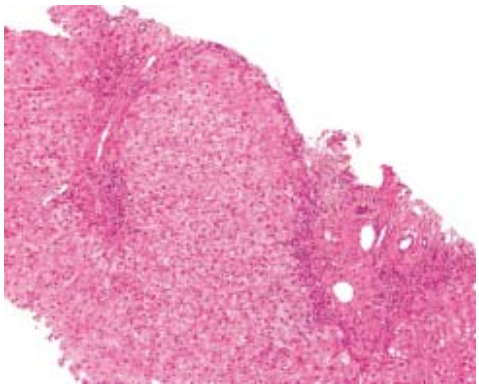

Figure 1 Portal fibrosis and porto-portal bridging (H and E).

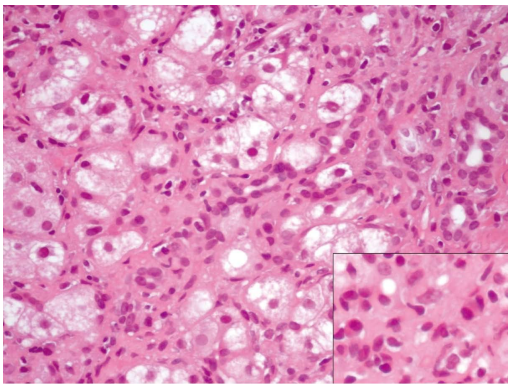

Figure 2 Mononuclear inflammatory infiltrates in sinusoids with plasma cells (inset).

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 $700/mm^3$, 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 possible 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 associated 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, Lefkowitch 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**, Vassiloyanakopoulos 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, Misdraji 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

World Journal of Immunology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
www.wjgnet.com

World J Immunol 2014 November 27; 4(3): I-V
ISSN 2219-2824 (online)

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 43 OA clinical medical journals, including 42 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, physical 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) Research Report: 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

ISSN
ISSN 2219-2824 (online)

Launch date
December 27, 2011

Frequency
Four-monthly

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastrointestinal Surgery
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

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 journals owned by the BPG represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

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, Ridit, 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 Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of BPG, 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. montgomery.bissell@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 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 ^{1}F, ^{2}F, ^{3}F; 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[1,2]". 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

Pleased 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-diarrhoea. *Shijie Huaren Xiaohua 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 PMCID: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. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary 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. Wieczorek 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 ± SD or mean ± 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 υ (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 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; 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 quantums 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: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* 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 BPG. 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: 698 USD per article. All invited articles are published free of charge.

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