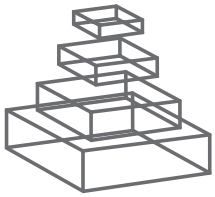
frontiers
RESEARCH TOPICS

THE MOLECULAR MECHANISMS OF CHRONIC INFLAMMATION DEVELOPMENT

Topic Editors
Masaaki Murakami and Toshio Hirano

frontiers in
IMMUNOLOGY



frontiers

Cover image provided by Ibbl sarl, Lausanne CH

ISSN 1664-8714
ISBN 978-2-88919-091-1
DOI 10.3389/978-2-88919-091-1

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.
All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!
Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

THE MOLECULAR MECHANISMS OF CHRONIC INFLAMMATION DEVELOPMENT

Topic Editors:
Masaaki Murakami, Osaka University, Japan
Toshio Hirano, Osaka University, Japan

Inflammation is critical for the development of many complex diseases and disorders including autoimmune diseases, metabolic syndrome, neurodegenerative diseases, cancers, and cardiovascular diseases.

Inflammation comes as two types: chronic inflammation, which can be defined as a dysregulated form of inflammation, and acute inflammation, which can defined as a regulated form. Because of its special role in the aforementioned diseases, establishing methods to control chronic inflammation is important for developing cures and treatments. One challenge for this purpose has been the ability to distinguish chronic and acute inflammation based on molecular biology diagnostics. Thus, this Research Topic is focused on articles that can shed some new light on the molecular mechanisms responsible for the development of chronic inflammation and its related conditions.

Table of Contents

frontiers in IMMUNOLOGY

EDITORIAL
published: 15 November 2012
doi: 10.3389/fimmu.2012.00323

The molecular mechanisms of chronic inflammation development

Masaaki Murakami*[1]* *and Toshio Hirano*[2]

[1] *Section of Developmental Immunology, JST-CREST, Graduate School of Frontier Biosciences, Graduate School of Medicine, iFReC, Osaka University, Japan*
[2] *JST-CREST, Osaka University, Japan*
**Correspondence: murakami@molonc.med.osaka-u.ac.jp; hirano@molonc.med.osaka-u.ac.jp*

Edited by:
Kendall A. Smith, Weill Medical College of Cornell University, USA

Reviewed by:
Kendall A. Smith, Weill Medical College of Cornell University, USA

Inflammation is critical for the development of many complex diseases and disorders including autoimmune diseases, metabolic syndrome, neurodegenerative diseases, cancers, and cardiovascular diseases. Inflammation comes in two types: chronic inflammation, which can be defined as a dysregulated form of inflammation, and acute inflammation, which can defined as a regulated form. Because of its special role in the aforementioned diseases, establishing methods to control chronic inflammation is important for developing cures and treatments against various diseases and disorders. One challenge to achieve this has been the ability to distinguish chronic and acute inflammation based on molecular biology diagnostics. Thus, this Research Topic is focused on articles that can shed new light on the molecular mechanisms responsible for the development of chronic inflammation and its related conditions.

This volume brings together 13 articles that are intended to provide a summary of some of the current thinking regarding the "molecular mechanism of chronic inflammation development." The 13 articles are briefly described below.

The first (Chilton et al., 2012) and second articles (Shichita et al., 2012) present the roles of TLR-mediated signals in chronic inflammation. The authors focus a TLR4 ligand, lipid A, produced by Gram-negative bacteria and its variants to investigate how these compounds induce acute and chronic inflammation (Chilton et al., 2012) and post-ischemic inflammation in the brain induced by endogenous TLR ligands, high mobility group box 1 (HMGB1), and peroxiredoxin family proteins (Shichita et al., 2012). Both these exogenous and endogenous TLR ligands should be triggering factors for both acute as well as chronic inflammation.

The next three articles focus on T cells in the process of chronic inflammation. Huseby et al. (2012) review pathogenic CD8+ T cells in multiple sclerosis (MS), while many researchers including us paid much attention to pathogenic CD4+ T cells. Because it is known that lymphocytes in MS plaques are biased toward the CD8 lineage, the authors emphasize that understanding how CNS-reactive CD8 T cells escape tolerance induction and induce CNS autoimmunity is critical to our ability to propose and test new therapies for MS. It is possible, for example, that pathogenic CD8+ T cells might play a major role in chronic inflammation during the course of MS development. Mauro and Marelli-Berg (2012) report T cell immunity in cardiovascular metabolic disorders. They proposed that an altered metabolism in the cardiovascular system initially induced by macrophages and innate immunity can fuel chronic inflammation and subsequent migration of antigen-non-specific activated T cells to the affected site. Komatsu and Takayanagi (2012) address the synergistic activity of immune cells, particularly activated helper T cells and mesenchymal cells such as synovial fibroblasts in joints. They propose that mesenchymal cells, which interact with the activated T cells, are an important determinant in the development of chronic inflammation in the joints.

The next two articles show events in the later phase of chronic inflammation. Ueha et al. (2012) present cellular and molecular mechanisms of organ fibrosis and Rubin et al. (2012) address the relationship between IBD and colon cancer, which develops in the affected sites during chronic inflammation. The authors stress the formation of myofibroblasts mediated by activated helper T cells as well as cytokines such as TGFb for the development of chronic inflammation-mediated fibrosis (Ueha et al., 2012) and review the genetic basis of IBD, the genetic and cellular alterations associated with colitis-associated colon cancer, and the emerging role of the intestinal microbiota and other environmental factors.

The next three articles address the negative regulation of chronic inflammation by DHA metabolome, IL-10, and regulatory T cells (Tregs). Shinohara et al. (2012) present an overview of functional metabolomics that identified a new bioactive metabolome of docosahexaenoic acid (DHA) including their recent *in vivo* and *in vitro* data. They suggest that the metabolome of DHA might have protective and anti-inflammatory actions relevant even in humans. Kubo and Motomura (2012) review the transcriptional regulation of the anti-inflammatory cytokine, IL-10, particularly in adaptive immune cells such as activated helper T cells. Because IL-10 has a strong immune suppressive effect, it is possible that a reduced IL-10 transcription in the affected tissues can be a triggering factor for chronic inflammation. Fujio et al. (2012) discuss Tregs, which strongly inhibit chronic inflammation, by paying special attention to autoantibody production and autoantibody-mediated autoimmune diseases. They conclude that several kinds of Tregs have the potential to control autoimmune inflammation by suppressing both autoantibody production and the local chronic inflammatory responses that are induced by autoantibodies.

The next two articles focus on current ambiguities in immunology. Barnaba et al. (2012) review the various cell populations in the immune system. They hypothesize that they maintain a state of chronic low-level inflammation during persisting

infections, and ultimately favor the species survival. Thus, dysregulation of low-level inflammation by enhancing harmful responses and/or reducing negative regulatory responses could develop into chronic inflammation. Prinz and Knobeloch (2012) present the good and bad of I-IFN with regards to immune responses and inflammation induction. I-IFN is associated with detrimental effects in Aicardi–Goutières syndrome (AGS), but at the same time is used as a standard therapeutic for the treatment of relapsing–remitting MS. The authors show mainly their own data for AGS, a severe disabling autoimmune inflammatory encephalopathy, and experimental autoimmune encephalomyelitis (EAE), a murine model of MS, to describe the roles of I-IFN during the development of chronic inflammation in the CNS.

The final report is ours (Murakami and Hirano, 2011). We have been studying the role of non-immune cells in the development of helper T cell-mediated autoimmune diseases. Although the NFκB-triggered positive-feedback-loop for IL-6-signaling (IL-6-amplifier) was originally discovered in mice to be a synergistic-activation signal that is activated following IL-17A and IL-6 stimulation in non-immune cells, results from disease models such as a rheumatoid arthritis, F759 mice, and EAE have shown that the IL-6-amplifier in fibroblasts, a type of endothelial cell, is activated by simultaneous stimulation of NFkB and STAT3 and locally induces chemokines and chronic inflammation. Indeed, we have proposed a four-step model for MHC class II-associated autoimmune diseases: (1) T cell activation regardless of antigen specificity; (2) local events inducing a tissue-specific accumulation of activated T cells; (3) transient activation of the IL-6 amplifier; and (4) enhanced sensitivity to cytokines in the target tissue. The interaction of these events results in chronic activation of the IL-6 amplifier and subsequent manifestation of autoimmune diseases. Thus, the IL-6 amplifier, which is chronically activated by these four events, is a critical regulator of chronic inflammations in tissue-specific MHC class II-associated autoimmune diseases.

As summarized above, the 13 articles present a range of data and issues that are under active investigation for understanding the molecular mechanism(s) of chronic inflammation, which is a general cause of various human diseases and disorders.

REFERENCES

Barnaba, V., Paroli, M., and Piconese, S. (2012). The ambiguity in immunology. *Front. Immun.* 3:18. doi: 10.3389/fimmu.2012.00018

Chilton, P. M., Embry, C. A., and Mitchell, T. C. (2012). Effects of differences in lipid A structure on TLR4 pro-inflammatory signaling and inflammasome activation. *Front. Immun.* 3:154. doi: 10.3389/fimmu.2012.00154

Fujio, K., Okamura, T., Sumitomo, S., and Yamamoto, K. (2012). Regulatory T cell-mediated control of autoantibody-induced inflammation. *Front. Immun.* 3:28. doi: 10.3389/fimmu.2012.00028

Huseby, E. S., Huseby, P. G., Shah, S., Smith, R., and Stadinski, B. D. (2012). Pathogenic CD8 T cells in multiple sclerosis and its experimental models. *Front. Immun.* 3:64. doi: 10.3389/fimmu.2012.00064

Komatsu, N., and Takayanagi, H. (2012). Inflammation and bone destruction in arthritis: synergistic activity of immune and mesenchymal cells in joints. *Front. Immun.* 3:77. doi: 10.3389/fimmu.2012.00077

Kubo, M., and Motomura, Y. (2012). Transcriptional regulation of the anti-inflammatory cytokine IL-10 in acquired immune cells. *Front. Immun.* 3:275. doi: 10.3389/fimmu.2012.00275

Mauro, C., and Marelli-Berg, F. M. (2012). T cell immunity and cardiovascular metabolic disorders: does metabolism fuel inflammation? *Front. Immun.* 3:173. doi: 10.3389/fimmu.2012.00173

Murakami, M., and Hirano, T. (2011). A four step model for the IL-6 amplifier, a regulator of chromic inflammations in tissue specific MHC class II-associated autoimmune diseases. *Front. Immun.* 2:22. doi: 10.3389/fimmu.2011.00022

Prinz, M., and Knobeloch, K.-P. (2012). Type I interferons as ambiguous modulators of chronic inflammation in the central nervous system. *Front. Immun.* 3:67. doi: 10.3389/fimmu.2012.00067

Rubin, D. C., Shaker, A., and Levin, M. S. (2012). Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. *Front. Immun.* 3:107. doi: 10.3389/fimmu.2012.00107

Shichita, T., Sakaguchi, R., Suzuki, M., and Yoshimura, A. (2012). Post-ischemic inflammation in the brain. *Front. Immun.* 3:132. doi: 10.3389/fimmu.2012.00132

Shinohara, M., Mirakaj, V., and Serhan, C. N. (2012). Functional metabolomics reveals novel active products in the DHA metabolome. *Front. Immun.* 3:81. doi: 10.3389/fimmu.2012.00081

Ueha, S., Shand, F. H. W., and Matsushima, K. (2012). Cellular and molecular mechanisms of chronic inflammation-associated organ fibrosis. *Front. Immun.* 3:71. doi: 10.3389/fimmu.2012.00071

Received: 02 October 2012; accepted: 07 October 2012; published online: 15 November 2012.

*Citation: Murakami M and Hirano T (2012) The molecular mechanisms of chronic inflammation development. Front. Immun. **3**:323. doi: 10.3389/fimmu.2012.00323*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 13 June 2012
doi: 10.3389/fimmu.2012.00154

Effects of differences in lipid A structure on TLR4 pro-inflammatory signaling and inflammasome activation

Paula M. Chilton[1], Chelsea A. Embry[2] and Thomas C. Mitchell[1]*

[1] *Department of Microbiology and Immunology, Institute for Cellular Therapeutics, School of Medicine, University of Louisville, Louisville, KY, USA*
[2] *Department of Integrative Health Science, Stetson University, Deland, FL, USA*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Daisuke Kamimura, Osaka University, Japan
Yasunobu Arima, Osaka University, Japan

****Correspondence:***
Thomas C. Mitchell, Department of Microbiology and Immunology, Institute for Cellular Therapeutics, School of Medicine, University of Louisville, 570 South Preston Street, Donald Baxter Building, Room 404, Louisville, KY 40202, USA.
e-mail: tom.mitchell@louisville.edu

The vertebrate immune system exists in equilibrium with the microbial world. The innate immune system recognizes pathogen-associated molecular patterns via a family of Toll-like receptors (TLR) that activate cells upon detection of potential pathogens. Because some microbes benefit their hosts, mobilizing the appropriate response, and then controlling that response is critical in the maintenance of health. TLR4 recognizes the various forms of lipid A produced by Gram-negative bacteria. Depending on the structural form of the eliciting lipid A molecule, TLR4 responses range from a highly inflammatory endotoxic response involving inflammasome and other pro-inflammatory mediators, to an inhibitory, protective response. Mounting the correct response against an offending microbe is key to maintaining health when exposed to various bacterial species. Further study of lipid A variants may pave the way to understanding how TLR4 responses are generally able to avoid chronic inflammatory damage.

Keywords: NLRP3, inflammasome, monophosphoryl lipid A, LPS, TLR4

INTRODUCTION

The innate immune response results from a highly regulated set of reactions that initiates and orchestrates the entire response to infection, injury, and tumorogenesis (Starczynowski and Karsan, 2010; Arslan et al., 2011; Osawa et al., 2011; Rock et al., 2011). However, dysregulation of the innate immune response leads to poor homeostasis that can initiate pathologic conditions including arthritis, autoimmunity, and cancer (Grivennikov et al., 2010; Bettini and Vignali, 2011; Delogu et al., 2011; Sikora and Grom, 2011; Davalos and Akassoglou, 2012).

Inflammasomes are intracellular staging areas organized to allow maturation of the powerful inflammatory cytokines interleukin (IL-) 1β, IL-18, and IL-33 from inactive precursors (Arend et al., 2008; Dunne, 2011). These cytokines are produced early upon exposure to infectious agents, often triggering receptors specific for chemistries conserved in microbial life (Janeway and Medzhitov, 2002; Kawai and Akira, 2009). The inflammatory environment produced by inflammasomes is important for the clearance of offending microbes, a process that involves the induction of other inflammatory cytokines (TNFα, IL-1β, and IL-6), chemokines (Schilling et al., 2002), and other factors that lead to the activation and differentiation of adaptive immune cells (Foell et al., 2007; Blanco et al., 2008). Dysregulation or over-activation of these cytokines can lead to massive inflammatory states so acute that they result in the death of the host after sterile clearance of infection.

Toll-like receptors (TLR) are innate immune cell receptors that recognize various conserved pathogen-associated molecular patterns (PAMP) and host-derived damage-activated molecular patterns (DAMP; Janeway and Medzhitov, 2002; Foell et al., 2007; Kawai and Akira, 2009). There are at least 13 distinct mammalian TLR that function as either homo- or hetero-dimers (Roach et al., 2005). TLR4 requires MD2 to bind its canonical ligand, lipopolysaccharide (LPS; Poltorak et al., 1998; Kennedy et al., 2004). LPS is a major cell wall component of all Gram-negative bacteria; however, its molecular structure differs depending on the bacterial species. In general, LPS molecules are made up of an O-antigen of varying lengths, the polysaccharide core, and the lipid A moiety (Raetz and Whitfield, 2002). The lipid A region is the endotoxin component of the molecule, and is the portion of the molecule that binds TLR4/MD2 (Carpenter and O'Neill, 2009).

Variations in the structure of lipid A determine inflammatory and immunostimulatory effects of TLR4 binding. The lipid A structure consists of a phosphorylated diglucosamine head group with a number of fatty acid side chains. The fatty acid (acyl) side chains are bound within the MD2 co-receptor via a large hydrophobic pocket, and the resultant complex then associates with a TLR4 monomer (Park et al., 2009). In addition, the lipid A diglucosamine head group, through phosphorylation of its 1- and 4′ carbons, associates with both TLR4 molecules in the signaling-competent dimer (Park et al., 2009). Structure-function studies of lipid A or structural mimetics demonstrate that differences in the number and the length of the acyl chain side groups are important in signaling strength of TLR4 (Coats et al., 2003; Stover et al., 2004; Reife et al., 2006). For example, Stover et al. (2004) showed that there is an optimal acyl chain length for TLR4 recognition of synthetic hexa-acyl lipid A mimetics. Analysis of the crystal structure of the ecto-domain of TLR4-associated with MD2 and lipid A suggests there is critical numbers or arrangement of the acyl chains of lipid A that are required both to bind within the MD2 pocket and associate with the monomer TLR4 molecule (Park et al., 2009).

The phosphorylation state of lipid A also affects its function through changes in the ability to engage TLR4. The prototypic lipid A structure is di-phosphorylated, with a phosphate on each of the glucosamine moieties. Unphosphorylated lipid A does not signal through TLR4, and thus acts as an inhibitor of lipid A (Coats et al., 2011). Monophosphoryl lipid A (MPLA) from *Salmonella minnesota* rough mutant Re 595 has been shown to be 0.1–1% as toxic (Ribi, 1984; Baldrick et al., 2002), but nearly as immunostimulatory as its parental LPS form (Thompson et al., 2005; Mata-Haro et al., 2007; Didierlaurent et al., 2009). The loss of a single phosphate from a synthetic form of lipid A also decreases the production of pro-inflammatory cytokines (Cekic et al., 2009). Recently, MPLA has been approved by the USDA as MPL® adjuvant as the first TLR agonist to be approved for use as a vaccine adjuvant based on its ability to direct adaptive immune responses with little toxicity (Didierlaurent et al., 2009).

Chronic inflammation can lead to serious conditions, including atherosclerosis, arthritis, and cancer (Coussens and Werb, 2002; Duewell et al., 2010). Various Gram-negative bacteria infect chronically and are associated with inflammatory disease and cancer. Chronic *Helicobacter pylori* infection causes peptic ulcers which are associated with stomach cancer (Suerbaum and Michetti, 2002). Lyme disease-associated arthritis occurs in individuals chronically infected with the Gram-negative spirochete, *Borrelia burgdorferi* (Murray and Shapiro, 2010). Chronic inflammation due to improper or lack of control over inflammasome function has also been linked to a variety of immune system disorders, including various forms of arthritis as well as Crohn's disease and ulcerative colitis.

In this Review, we will discuss the role of TLR4 on inflammasome activation, with a focus on the NLRP3 form of the inflammasome. In addition, the phenomenon of decreased TLR4 inflammatory signals when the lipid A agonist structure is changed in various ways will be addressed.

NLRP3 INFLAMMASOME ACTIVATION

Inflammasomes are multi protein structures formed in the cytoplasm of activated innate immune cells that lead to the maturation of IL-1β and IL-18 from inactive pro-proteins to their active, mature forms. The NLRP3 [nucleotide-binding oligomerization domain (NOD)-like receptor family, pryin domain containing 3]-inflammasome is most often associated with TLR4 activation. The other proteins of the NLRP3 inflammasome are ASC (apoptosis-associated speck-like protein containing a CARD domain), the pro-inflammatory caspase, caspase-1, and the precursor forms of IL-1β or IL-18 or both (Martinon et al., 2002; Schroder and Tschopp, 2010).

Two distinct signals are required for the production and secretion of mature IL-1β or IL-18 via the NLRP3 inflammasome (**Figure 1**). First, a priming signal occurs either through TLR4 or IL-1 receptor. Priming leads to transcription and translation of the inactive pro-forms of IL-1β or IL-18. We and others have shown that an increase in NLRP3 protein abundance occurs during this priming step, and that this increase plays a key role in the ultimate maturation of IL-1β (Bauernfeind et al., 2009; Embry et al., 2011). Priming alone does not lead to secretion of mature IL-1β because primed cells simply harbor immature pro-IL-1β; an activation signal that leads to inflammasome assembly and the proteolytic activity of caspase-1 is also needed. Known activation signals include extracellular ATP, taken up through the $P2X_7$ purinergic receptor (Pelegrin et al., 2008), and phagocytosed crystalline (Martinon et al., 2006) or particulate structures that lyse phagocytic vesicles via a cathepsin B-dependent mechanism (Niemi et al., 2011). After the activation signals, NLRP3 acts as the scaffolding protein that allows the spontaneous assembly of the accessory inflammasome components. In the presence of other inflammasome components, expression of NLRP3 leads to the production of one very large inflammasome or "speck" cluster per cell (Stutz et al., 2009); single specks are visible in primed/activated cells within 5 h (Embry et al., 2011). The assembled inflammasome includes enzymatically active caspase-1 that cleaves the precursor form of IL-1β, which can lead to secretion of both the cytokine and active caspase-1 from the cells (**Figure 1**a). Tight regulation of pro-IL-1β maturation and related pro-inflammatory proteins underscores the importance of avoiding potential damage to healthy tissue. This regulation is evidenced by the observation that two distinct steps are required to generate and secrete the active forms cytokine suggesting several different levels at which therapeutics may be applied to prevent or control inflammation.

NLRP3 MUTATIONS LEAD TO AUTO-INFLAMMATORY DISEASES

In humans as well as in mouse models, gain-of-function polymorphisms, or mutations in the gene encoding NLRP3 are associated with several similar inflammatory conditions that are episodic in nature, occurring in flares after periods of non-activity. These conditions include familial cold auto-inflammatory syndrome, Muckle–Wells syndrome, cryopyrin-associated periodic syndromes (CAPS), and neonatal onset multisystem inflammatory disease (NOMID; Akazawa et al., 2004; Koike et al., 2007; Kubota and Koike, 2010). Chronic inflammatory conditions are associated with an increased risk for development of colorectal cancer and inflammatory bowel diseases, such as ulcerative colitis, and Crohn's disease (McDermott and Aksentijevich, 2002). The auto-inflammatory episodes typical of these conditions make sense in the framework of inflammasome formation and regulation. Even if NLRP3 is expressed at inappropriate levels, other priming signals are required for the expression of pro-inflammatory cytokines. Therefore, therapeutic potential lies in gaining control of the NLRP3 inflammasome priming factors and inhibitory signals, or both.

Although increased function of NLRP3 is associated with increased inflammation, deletion mutants of NLRP3 do not result in the opposite phenotype. In mice, single deletion mutants of NLRP3 inflammasome components NLRP3, caspase-1, or ASC, are also more susceptible to both dextran sulfate sodium (DSS)-induced colitis and inflammation-associated colon cancer. However, NLRP3 deficient mice have a less pronounced association with cancer development than caspase-1 deficient mice. Further studies are required to determine if the difference is related to a regulatory function of the NLRP3 inflammasome in controlling cancer development caused by chronic inflammation or if it is possibly due to a decrease in apoptosis via reduced caspase-1 activity (Allen et al., 2010). Another study that found increased

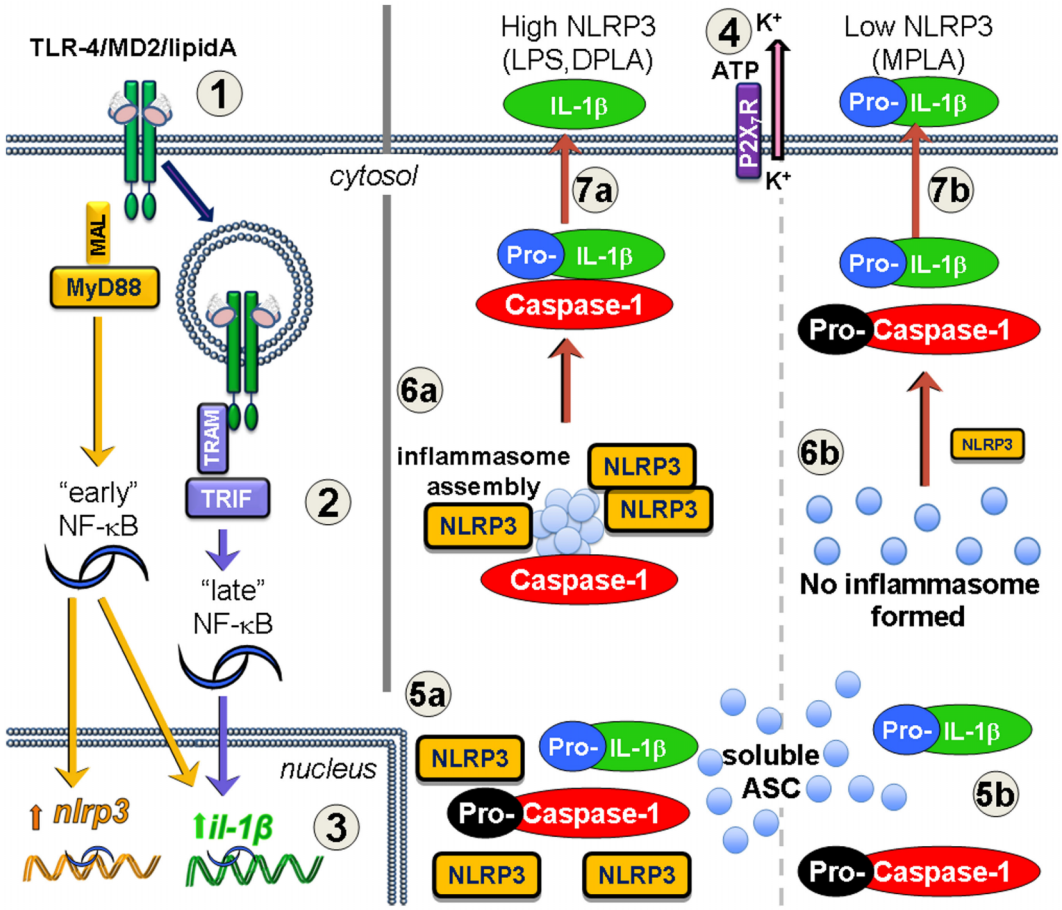


FIGURE 1 | Increased NLRP3 expression via MyD88 leads to inflammasome formation and processing of IL-1β after lipid A treatment. Priming (1–3) and activation (4–7) are required for mature IL-1β secretion. (1) TLR4/MD2 binds lipid A forming endotoxin receptor dimers that signal through either MyD88 or TRIF, or both (2) to activate NF-κB. The MyD88-dependent "early" phase of NF-κB activity results in increased transcription of both *nlrp3* and *il1b* genes. TRIF-dependent TLR4 signaling occurs after receptor-mediated endocytosis of TLR4 leading to the "late" phase of NF-κB activity, and increases *il1b* gene expression, but not *nlrp3*. Inflammasome activation occurs in response to such agonists as extracellular ATP which is sensed by the $P2X_7R$ and leads to K^+ loss through the pannexin-1 ion channel (4). If TLR4 signaling increases NLRP3 protein levels (5a), such as with MyD88-competent LPS or DPLA, then the activation event permits the newly expressed NLRP3 to aggregate with soluble ASC protein and pro-caspase-1, leading to caspase-1 processing to its active form and inflammasome assembly (6a). Pro-IL-1β that is associated with the inflammasome is processed to mature IL-1β and secreted into the extracellular space (7a). However, when TLR4 priming does not adequately increase NLRP3 expression (5b), as is the case with MPLA treatment, then there is little to no NLRP3 protein to direct inflammasome assembly, pro-caspase-1 does not get efficiently activated (6b), and any IL-1β that is secreted is generally in the inactive pro-IL-1β form (7b).

DSS-induced colitis in NLRP3 deficient mice showed poor regulation of intestinal homeostasis, including decreases in IL-10 levels, changes in the composition of intestinal microbiota, and decreases of β-defensin production (Hirota et al., 2011). Changes in gut microbiota in NLRP3 deficient mice include the presence of potentially pathogenic Enterobacteriaceae genera. Similar changes in the Enterobacteriaceae genera in the gut microbiota, including *Proteus* species, occur in the TRUC ($T\text{-bet}^{-/-}Rag1^{-/-}$ ulcerative colitis) mouse model of spontaneous inflammatory bowel disease (Garrett et al., 2010). These studies suggest a complex role for NLRP3 in the control of local inflammation, such as in the intestine. Therefore, the role of the NLRP3 inflammasome in determining the composition of a healthy gut microbiota needs to be determined before NLRP3 should be used as a therapeutic target to control of chronic inflammation that leads to colon cancers. A selective inhibitor of NLRP3 pro-inflammatory effects could be useful if capable of suppressing only the pro-inflammatory effects without affecting its beneficial roles, such as controlling the homeostasis of healthy microbiota.

MyD88-DEPENDENT SIGNALING IS REQUIRED TO PRIME THE NLRP3 INFLAMMASOME DOWNSTREAM OF TLR4 AND LIPID A

Preparations of MPLA from the Re595 rough mutant of *S. minnesota* have been shown to possess much lower toxicity than parental, di-phosphorylated lipid A preparations (Ribi, 1984; Thompson et al., 2005). *In vivo*, MPLA decreases inflammatory signaling from TLR4 with minimal impairment of its immunostimulatory adjuvant effect on the initial clonal expansion of T cell (Thompson et al., 2005; Mata-Haro et al., 2007). MPLA exerted

effective immunostimulatory effects at this stage of the T cell response since it retained to a significant amount of TLR4 TRIF-dependent signaling. In contrast, the TLR4 MyD88-dependent pro-inflammatory signaling was markedly reduced, but not completely absent (Mata-Haro et al., 2007). This, "TRIF-biased" signaling profile obtained with MPLA has been modeled *in vitro* using dendritic cell cultures and synthetic versions of the *E. coli* form of monophosphoryl lipid A (sMPLA) or diphosphoryl lipid A (sDPLA; Cekic et al., 2009, 2011). These molecules represent the detoxified or the toxic versions of lipid A, respectively. Following the work of Okemoto et al. (2006) showing decreased IL-1β production in response to MPLA, we hypothesized that the difference in toxicity would at least partially be caused by the reduced maturation of IL-1β using a TLR4 agonist that is "poor" in MyD88-dependent signaling. However, in our cultured dendritic cell system, both MyD88 and TRIF-signaling mutants increased the *Il1b* mRNA abundance to a similar extent when exposed to sDPLA. When using sDPLA or sMPLA to prime dendritic cell cultures with subsequent ATP activation, IL-1β protein levels were significantly higher after sDPLA treatment as compared to sMPLA treatment. Through Western Blot analysis of the culture supernatants, we showed that the IL-1β that was secreted after sMPLA treatment was actually the inactive, pro-IL-1β rather than mature IL-1β present in the supernatants from sDPLA-treated dendritic cells. We assessed the TLR4 signaling requirements for these priming effects and determined that although both TRIF and MyD88 adaptors contributed to the increase in IL-1β transcription, MyD88-signaling was required for the maturation of IL-1β from its inactive pro-form to its cleaved mature form (Embry et al., 2011). The MyD88-dependent component was then determined to be, at least in part, due to an increase in transcription and production of NLRP3 during priming (**Figure 1**a). Failure to generate sufficient MyD88-dependent signaling resulted in low expression of NLRP3, which limited the formation of inflammasomes when the activation signal, ATP, was provided (**Figure 1**b). Not only was decreased inflammasome formation assessed by decreased mature IL-1β secretion in response to ATP signals, we also observed a near absence of "specks" (using antibody against ASC), representing the fully formed NLRP3 inflammasome in confocal microscopy. In summary, these observations indicated a failure of inflammasome assembly in cells primed with sMPLA (decreased MyD88-signaling), as compared to sDPLA (intact MyD88-signaling; Embry et al., 2011).

The ability to activate innate immune system cells in a manner that limits the pro-inflammatory products produced is a goal for clinical treatment of inflammatory conditions. These results suggest that MPLA may prove safe and effective to use as a single-agent therapy for certain chronic inflammatory conditions. Indeed, MPLA promotes endotoxin receptor "tolerance" in the absence of inflammatory outcomes (Madonna et al., 1986; Cekic et al., 2011). The role of IL-1β in perpetuating pro-inflammatory reactions makes the control of its production and function a promising target in controlling inflammation and diseases associated with inflammatory states. Therefore, the ability to activate the immunostimulatory arm of TLR4 signaling without increasing production of mature IL-1β could be beneficial in controlling chronic inflammation in certain situations, especially where low-level infection is a suspected cause.

NATURALLY-OCCURRING DIFFERENCES IN LIPID A STRUCTURE CORRELATE WITH MICROBIAL VIRULENCE

For some time, we have been intrigued by the bifurcation of the TLR4 signaling system: why is it able to independently activate a largely pro-inflammatory program of events (MyD88-dependent) and a separate program that can be thought of as anti-viral and co-stimulatory (TRIF-dependent). This partial separation of signaling events has lead to speculation that responses to various lipid A agonists of TLR4 may be regulated separately, possibly through unique binding characteristics of different ligands. Naturally occurring forms of lipid A possess structural variations that elicit differential effects *in vivo* that are known to correlate with clinical presentations and microbial pathogenesis. For example, Jarvis and colleagues have shown that the structural heterogeneity of lipid oligosaccharide (LOS) produced by various clinical isolates of *Neisseria* correlates with their abilities to induce TNFα production by monocytes (John et al., 2009a,b). A pathogenic isolate of *N. meningitidis*, strain 89I, produces a more highly phosphorylated LOS structure that elicits higher responses from both sides of the TLR4 signaling branches in human monocytic cells. The LOS from that particular isolate induces higher levels of MyD88-dependent products, such as TNFα, IL-1β, and IL-12 p70 as well as TRIF-dependent products including IFNβ and CD80 (a co-stimulatory molecule), compared to the responses elicited by the LOS from two other *Neisseria* isolates (*N. gonorrhoeae*), which have lower phosphorylated states (Liu et al., 2010). Hence, the phosphorylation status of LOS affects both MyD88-dependent as well as TRIF-dependent signals (Liu et al., 2010).

Bordetella is another bacterial genus with species-specific differences in lipid A structure and TLR4 reactivity that can be correlated with infectious profiles. The easily cleared *B. bronchiseptica* highly stimulates TLR4, and this stimulation is required for clearance of infection. The more pathogenic species, *B. pertussis* and *B. parapertussis* are less reactive with TLR4, and indeed TLR4 deficiency does not change susceptibility to infection (Mann et al., 2004, 2005). The structural differences between these LPS forms do not seem to be based on phosphorylation status but rather which form of saccharide group is present: *B. bronchiseptica* and *B. parapertussis* species have smooth LPS (long-chain polysaccharide), whereas *B. pertussis* have rough LPS (short-chain polysaccharide; Di Fabio et al., 1992) and exhibits very different effects on cultured human dendritic cells than the smooth forms (Fedele et al., 2008).

Another example of structural changes in lipid A affecting virulence of Gram-negative bacterial species is in *Yersinia pestis*. *Y. pestis* expresses a TLR4-inhibiting, tetra-acylated form of LPS when grown at 37°C, the temperature of mammalian hosts. However, a TLR4-activating, hexa-acylated LPS form is expressed when *Y. pestis* is grown at 26°C, the temperature of insect hosts. Recombinant *Y. pestis* that have been forced to express only hexa-acylated LPS regardless of temperature cannot sustain a lethal infection in mice. Lethality of infection with this recombinant *Y. pestis* is restored in hosts that are deficient in TLR4, CD14, or MyD88, but not TRIF or TRAM deficient mice, indicating that MyD88-associated TLR4 outcomes are required to control *Y. pestis*

infection *in vivo* (Montminy et al., 2006), and that the bacteria has evolved a way to evade TLR4 signaling that allows infection in mammalian hosts.

NATURALLY OCCURRING FORMS OF MONOPHOSPHORYL LIPID A AND THEIR EFFECTS

Darveau and colleagues have worked extensively with structurally defined forms of lipid A isolated from various organisms, in order to study their inherent differences in inflammatory signaling. Several of the lipid As are naturally monophosphorylated. Two such forms have been isolated from the oral bacterium *Porphyromonas gingivalis* grown in different hemin concentrations, which regulate the expression of bacteria-produced phosphatases. *P. gingivalis* grown in high hemin concentrations leads primarily to the production of antagonistic, dephosphorylated, or monophosphorylated, tetra-acylated lipid A forms within the Gram-negative cell wall (Reife et al., 2006). These modifications provide the organism with a unique evasion mechanism that prevents detection by TLR4 while also decreasing the effectiveness of cationic anti-microbial peptides due to the neutralization of the negative charge of the bacterial cell wall (Coats et al., 2009).

The lipid A produced by the human gut symbiant, *Bacteroides thetaiotaomicron* is structurally very similar to that from *P. gingivalis* grown in low hemin concentrations. Although both these lipid A forms are penta-acylated and monophosphorylated, they differ in the placement of the phosphate group on the diglucosamine head group. *B. thetaiotaomicron* LPS, has its single phosphate on the 1-carbon, whereas the phosphate on the *P. gingivalis* LPS is on the 4′-carbon. This seemingly minor structural change results in differences in the ability to stimulate NF-κB in human TLR4/human MD2-expressing HEK-293 cells *in vitro* (Coats et al., 2011). However, NF-κB signaling was used as a readout in these *in vitro* studies, which is downstream of both MyD88- and TRIF-dependent signaling pathways. Further differences may be appreciated if other signaling readouts were to be examined.

Overall, the differences in lipid A structure described above have marked effects on TLR4 signaling, resulting in important effects on bacterial pathogenesis. Changes that result in decreased inflammatory responses increase the ability of the bacteria to establish infection, thus demonstrating lipid A modifications as an underappreciated mechanism for microbial evasion of the immune system. Both pathogenic (*P. gingivalis*) and commensal (*B. thetaiotaomicron*) bacteria appear to have taken advantage of monophosphorylation to evade sterilizing responses. More direct assessment is needed to determine the relative usage of the MyD88- and TRIF-dependent pathways after TLR4 engagement when using structurally distinct forms of lipid A, and whether or not such usage is correlated with the potential for immediate inflammatory damage (MyD88-associated innate response) or long-term protective immunity (TRIF-associated adaptive immunity).

Why has the mammalian LPS recognition system not evolved to counter this threat? One can imagine, for example, that mutations in TLR4 or MD2, or both, could permit much more efficient recognition of hypophosphorylated forms of LPS, which would lead to MyD88-dependent innate responses that conceivably could be of tremendous benefit to a mammalian host in responding to *P. gingivalis* or other monophosphorylated pathogens. We propose, however, that poor activation of MyD88-signaling by monophosphorylated lipid A is a critically important concession to prevent highly inflammatory responses from damaging host tissue, such as oral or gut epithelium, while also tolerating the preponderance of microbes that are beneficial in terms of enhancing nutrition and preventing colonization by truly pathogenic bacteria. Because even monophosphorylated commensal bacteria can be dangerous if they escape their niche in the gut or oral cavity (Teng et al., 2004; Goldstein et al., 2006), TRIF-dependent responses that are particularly relevant to adaptive immunity may provide "insurance" in the form of rapid responses in the event of niche escape.

DETOXIFICATION OF LIPID A BY TISSUE ALKALINE PHOSPHATASES: INFLAMMATORY IMPLICATIONS

Because lipid A-mediated inflammation can lead to septic shock and death if left unchecked, the ability to detoxify lipid A molecules is critical for host survival. In the late 1990s, Poelstra et al. showed that tissue-specific alkaline phosphatases (AP) decrease the phosphorylation state of various lipid A molecules *in vitro*. From this, they hypothesized that AP work *in vivo* to detoxify endotoxin and decrease its systemic effects (Poelstra et al., 1997a,b). It was interesting, however, that the same group also showed that MPLA is not completely dephosphorylated by AP (Bentala et al., 2002). Although that study did not discuss the possible biologic implications of failing to dephosphorylate MPLA fully, the results suggest that the monophosphorylated form of lipid A is less of a threat in locations with high levels of commensal bacteria (i.e., mucosal sites), than the di- or tri-phosphate forms. Additionally, by failing to form the non-phosphorylated lipid A versions of MPLA, non-signaling TLR4 antagonists that could have served as inhibitors of TLR4 binding, and activity are not produced.

Tissue AP belong to a family of proteins that are expressed by most tissues, but their activities are particularly high in the intestine, liver, and placenta. The intestines harbor large numbers of Gram-negative bacteria with an abundance of lipid A. Although intestinal alkaline phosphatase (IAP) is known to help in the uptake of nutrients such as pyridoxal phosphate and pyridoxamine phosphate (two forms of B_6 vitamins; Waymire et al., 1995) as well as phosphate, its ability to dephosphorylate LPS in the gastrointestinal tract arguably helps in the maintenance of commensal or mutual Gram-negative bacterial species in the gut without causing either continual stimulation or endotoxin tolerance to pathologic Gram-negative bacteria.

Bates et al. demonstrated in a zebra fish model of vertebrate intestine that the presence of innocuous or beneficial Gram-negative bacterial species drives the expression of IAP in a MyD88-dependent manner. Furthermore, IAP-deficient zebrafish are hypersensitive to their own microbiota (Bates et al., 2007). Oral co-administration of IAP inhibitors and fully phosphorylated LPS to rats leads to increased levels of LPS in the serum (Koyama et al., 2002). IAP-deficient mice exhibit a local gut endotoxin tolerance, but are fully responsive to LPS systemically (Chen et al., 2011). Although IAP deletion mutants are also more sensitive to DSS-induced colitis than wild-type mice, feeding them calf IAP abrogates this effect of the deletion (Ramasamy et al., 2011). In humans, Crohn's disease and ulcerative colitis are known

to be associated with lower IAP expression (Tuin et al., 2009). Whitehead has proposed that rosacea, a chronic inflammatory condition of the face, is caused by overactive immune responses to commensal species of the skin, and has correlated rosacea with decreases in IAP expression (Whitehead, 2009). Interestingly, dietary habits that increase IAP expression can help to decrease flairs in other inflammatory diseases (Blanchard and Cousins, 2000; Tuin et al., 2006; Kaur et al., 2007). In addition, expression of the TLR4-associated co-receptor, CD14, was shown to be co-localized with AP activity in the intestine, liver, and kidneys (Tuin et al., 2006). Together, these studies suggest that detoxification of LPS by tissue AP is a natural mechanism that helps control chronic inflammation in the gut. Maintenance of monophosphorylated forms of LPS, which are detoxified but remain immunostimulatory via TRIF-biased signaling by TLR4, may be essential for the peaceful co-existence of the vertebrate host with its microbiota.

CONCLUSION

The signaling of TLR4 and subsequent priming and activation of the NLRP3 inflammasome provides several potential avenues to control chronic inflammation, especially in localized sites such as the intestine. Therefore, developing therapeutics that can signal through TLR4 without the danger of inflammasome activation could not only safely activate the innate immune response, but could also open new therapeutic avenues to chronic inflammatory conditions in which either IL-1β or IL-18 plays an amplifying role. The studies discussed here emphasize the importance of controlling microbiogenic inflammation to microbiota to maintain tissue homeostasis, while retaining the ability to tolerate monophosphorylated forms of lipid A. This scenario is of utmost importance in the intestine. Linking these studies with the control of NLRP3 inflammasome will increase our understanding of inflammatory disease progression and help in determining potential for therapeutic interventions.

REFERENCES

Akazawa, T., Masuda, H., Saeki, Y., Matsumoto, M., Takeda, K., Tsujimura, K., Kuzushima, K., Takahashi, T., Azuma, I., Akira, S., Toyoshima, K., and Seya, T. (2004). Adjuvant-mediated tumor regression and tumor-specific cytotoxic response are impaired in MyD88-deficient mice. *Cancer Res.* 64, 757–764.

Allen, I. C., TeKippe, E. M., Woodford, R. M., Uronis, J. M., Holl, E. K., Rogers, A. B., Herfarth, H. H., Jobin, C., and Ting, J. P. (2010). The NLRP3 inflammasome functions as a negative regulator of tumorigenesis during colitis-associated cancer. *J. Exp. Med.* 207, 1045–1056.

Arend, W. P., Palmer, G., and Gabay, C. (2008). IL-1, IL-18, and IL-33 families of cytokines. *Immunol. Rev.* 223, 20–38.

Arslan, F., de Kleijn, D. P., and Pasterkamp, G. (2011). Innate immune signaling in cardiac ischemia. *Nat. Rev. Cardiol.* 8, 292–300.

Baldrick, P., Richardson, D., Elliott, G., and Wheeler, A. W. (2002). Safety evaluation of monophosphoryl lipid A (MPL): an immunostimulatory adjuvant. *Regul. Toxicol. Pharmacol.* 35, 398–413.

Bates, J. M., Akerlund, J., Mittge, E., and Guillemin, K. (2007). Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host Microbe* 2, 371–382.

Bauernfeind, F. G., Horvath, G., Stutz, A., Alnemri, E. S., MacDonald, K., Speert, D., Fernandes-Alnemri, T., Wu, J., Monks, B. G., Fitzgerald, K. A., Hornung, V., and Latz, E. (2009). Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. *J. Immunol.* 183, 787–791.

Bentala, H., Verweij, W. R., Huizinga-van der Vlag, A., van Loenen-Weemaes, A. M., Meijer, D. K., and Poelstra, K. (2002). Removal of phosphate from lipid A as a strategy to detoxify lipopolysaccharide. *Shock* 18, 561–566.

Bettini, M., and Vignali, D. A. (2011). T cell-driven initiation and propagation of autoimmune diabetes. *Curr. Opin. Immunol.* 23, 754–760.

Blanchard, R. K., and Cousins, R. J. (2000). Regulation of intestinal gene expression by dietary zinc: induction of uroguanylin mRNA by zinc deficiency. *J. Nutr.* 130, 1393S–1398S.

Blanco, P., Palucka, A. K., Pascual, V., and Banchereau, J. (2008). Dendritic cells and cytokines in human inflammatory and autoimmune diseases. *Cytokine Growth Factor Rev.* 19, 41–52.

Carpenter, S., and O'Neill, L. A. (2009). Recent insights into the structure of Toll-like receptors and post-translational modifications of their associated signalling proteins. *Biochem. J.* 422, 1–10.

Cekic, C., Casella, C. R., Eaves, C. A., Matsuzawa, A., Ichijo, H., and Mitchell, T. C. (2009). Selective activation of the p38 MAPK pathway by synthetic monophosphoryl lipid A. *J. Biol. Chem.* 284, 31982–31991.

Cekic, C., Casella, C. R., Sag, D., Antignano, F., Kolb, J., Suttles, J., Hughes, M. R., Krystal, G., and Mitchell, T. C. (2011). MyD88-dependent SHIP1 regulates proinflammatory signaling pathways in dendritic cells after monophosphoryl lipid A stimulation of TLR4. *J. Immunol.* 186, 3858–3865.

Chen, K. T., Malo, M. S., Beasley-Topliffe, L. K., Poelstra, K., Millan, J. L., Mostafa, G., Alam, S. N., Ramasamy, S., Warren, H. S., Hohmann, E. L., and Hodin, R. A. (2011). A role for intestinal alkaline phosphatase in the maintenance of local gut immunity. *Dig. Dis. Sci.* 56, 1020–1027.

Coats, S. R., Berezow, A. B., To, T. T., Jain, S., Bainbridge, B. W., Banani, K. P., and Darveau, R. P. (2011). The lipid A phosphate position determines differential host Toll-like receptor 4 responses to phylogenetically related symbiotic and pathogenic bacteria. *Infect. Immun.* 79, 203–210.

Coats, S. R., Reife, R. A., Bainbridge, B. W., Pham, T. T., and Darveau, R. P. (2003). Porphyromonas gingivalis lipopolysaccharide antagonizes *Escherichia coli* lipopolysaccharide at toll-like receptor 4 in human endothelial cells. *Infect. Immun.* 71, 6799–6807.

Coats, S. R., To, T. T., Jain, S., Braham, P. H., and Darveau, R. P. (2009). Porphyromonas gingivalis resistance to polymyxin B is determined by the lipid A 4'-phosphatase, PGN_0524. *Int. J. Oral Sci.* 1, 126–135.

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. *Nature* 420, 860–867.

Davalos, D., and Akassoglou, K. (2012). Fibrinogen as a key regulator of inflammation in disease. *Semin. Immunopathol.* 34, 43–62.

Delogu, L. G., Deidda, S., Delitala, G., and Manetti, R. (2011). Infectious diseases and autoimmunity. *J. Infect. Dev. Ctries.* 5, 679–687.

Di Fabio, J. L., Caroff, M., Karibian, D., Richards, J. C., and Perry, M. B. (1992). Characterization of the common antigenic lipopolysaccharide O-chains produced by *Bordetella bronchiseptica* and *Bordetella parapertussis*. *FEMS Microbiol. Lett.* 76, 275–281.

Didierlaurent, A. M., Morel, S., Lockman, L., Giannini, S. L., Bisteau, M., Carlsen, H., Kielland, A., Vosters, O., Vanderheyde, N., Schiavetti, F., Larocque, D., Van, M. M., and Garcon, N. (2009). AS04, an aluminum salt- and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. *J. Immunol.* 183, 6186–6197.

Duewell, P., Kono, H., Rayner, K. J., Sirois, C. M., Vladimer, G., Bauernfeind, F. G., Abela, G. S., Franchi, L., Nunez, G., Schnurr, M., Espevik, T., Lien, E., Fitzgerald, K. A., Rock, K. L., Moore, K. J., Wright, S. D., Hornung, V., and Latz, E. (2010). NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 464, 1357–1361.

Dunne, A. (2011). Inflammasome activation: from inflammatory disease to infection. *Biochem. Soc. Trans.* 39, 669–673.

Embry, C. A., Franchi, L., Nunez, G., and Mitchell, T. C. (2011). Mechanism of impaired NLRP3 inflammasome priming by monophosphoryl lipid A. *Sci. Signal.* 4, ra28.

Fedele, G., Nasso, M., Spensieri, F., Palazzo, R., Frasca, L., Watanabe, M., and Ausiello, C. M. (2008). Lipopolysaccharides from *Bordetella pertussis* and *Bordetella parapertussis* differently modulate human dendritic cell functions resulting in divergent prevalence of

Th17-polarized responses. *J. Immunol.* 181, 208–216.

Foell, D., Wittkowski, H., and Roth, J. (2007). Mechanisms of disease: a "DAMP" view of inflammatory arthritis. *Nat. Clin. Pract. Rheumatol.* 3, 382–390.

Garrett, W. S., Gallini, C. A., Yatsunenko, T., Michaud, M., DuBois, A., Delaney, M. L., Punit, S., Karlsson, M., Bry, L., Glickman, J. N., Gordon, J. I., Onderdonk, A. B., and Glimcher, L. H. (2010). Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* 8, 292–300.

Goldstein, E. J., Citron, D. M., Vaidya, S. A., Warren, Y. A., Tyrrell, K. L., Vreni, M. C., and Fernandez, H. (2006). In vitro activity of 11 antibiotics against 74 anaerobes isolated from pediatric intra-abdominal infections. *Anaerobe* 12, 63–66.

Grivennikov, S. I., Greten, F. R., and Karin, M. (2010). Immunity, inflammation, and cancer. *Cell* 140, 883–899.

Hirota, S. A., Ng, J., Lueng, A., Khajah, M., Parhar, K., Li, Y., Lam, V., Potentier, M. S., Ng, K., Bawa, M., McCafferty, D. M., Rioux, K. P., Ghosh, S., Xavier, R. J., Colgan, S. P., Tschopp, J., Muruve, D., Macdonald, J. A., and Beck, P. L. (2011). NLRP3 inflammasome plays a key role in the regulation of intestinal homeostasis. *Inflamm. Bowel Dis.* 17, 1359–1372.

Janeway, C. A. Jr., and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol.* 20, 197–216.

John, C. M., Liu, M., and Jarvis, G. A. (2009a). Natural phosphoryl and acyl variants of lipid A from *Neisseria meningitidis* strain 89I differentially induce tumor necrosis factor-alpha in human monocytes. *J. Biol. Chem.* 284, 21515–21525.

John, C. M., Liu, M., and Jarvis, G. A. (2009b). Profiles of structural heterogeneity in native lipooligosaccharides of *Neisseria* and cytokine induction. *J. Lipid Res.* 50, 424–438.

Kaur, J., Madan, S., Hamid, A., Singla, A., and Mahmood, A. (2007). Intestinal alkaline phosphatase secretion in oil-fed rats. *Dig. Dis. Sci.* 52, 665–670.

Kawai, T., and Akira, S. (2009). The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int. Immunol.* 21, 317–337.

Kennedy, M. N., Mullen, G. E., Leifer, C. A., Lee, C., Mazzoni, A., Dileepan, K. N., and Segal, D. M. (2004). A complex of soluble MD-2 and lipopolysaccharide serves as an activating ligand for Toll-like receptor 4. *J. Biol. Chem.* 279, 34698–34704.

Koike, R., Kubota, T., Hara, Y., Ito, S., Suzuki, K., Yanagisawa, K., Uchibori, K., and Miyasaka, N. (2007). A case of Muckle-Wells syndrome caused by a novel H312P mutation in NALP3 (cryopyrin). *Mod. Rheumatol.* 17, 496–499.

Koyama, I., Matsunaga, T., Harada, T., Hokari, S., and Komoda, T. (2002). Alkaline phosphatases reduce toxicity of lipopolysaccharides in vivo and in vitro through dephosphorylation. *Clin. Biochem.* 35, 455–461.

Kubota, T., and Koike, R. (2010). Cryopyrin-associated periodic syndromes: background and therapeutics. *Mod. Rheumatol.* 20, 213–221.

Liu, M., John, C. M., and Jarvis, G. A. (2010). Phosphoryl moieties of lipid A from *Neisseria meningitidis* and *N. gonorrhoeae* lipooligosaccharides play an important role in activation of both MyD88- and TRIF-dependent TLR4-MD-2 signaling pathways. *J. Immunol.* 185, 6974–6984.

Madonna, G. S., Peterson, J. E., Ribi, E. E., and Vogel, S. N. (1986). Early-phase endotoxin tolerance: induction by a detoxified lipid A derivative, monophosphoryl lipid A. *Infect. Immun.* 52, 6–11.

Mann, P. B., Elder, K. D., Kennett, M. J., and Harvill, E. T. (2004). Toll-like receptor 4-dependent early elicited tumor necrosis factor alpha expression is critical for innate host defense against *Bordetella bronchiseptica*. *Infect. Immun.* 72, 6650–6658.

Mann, P. B., Wolfe, D., Latz, E., Golenbock, D., Preston, A., and Harvill, E. T. (2005). Comparative toll-like receptor 4-mediated innate host defense to *Bordetella* infection. *Infect. Immun.* 73, 8144–8152.

Martinon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. *Mol. Cell* 10, 417–426.

Martinon, F., Petrilli, V., Mayor, A., Tardivel, A., and Tschopp, J. (2006). Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature* 440, 237–241.

Mata-Haro, V., Cekic, C., Martin, M., Chilton, P. M., Casella, C. R., and Mitchell, T. C. (2007). The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. *Science* 316, 1628–1632.

McDermott, M. F., and Aksentijevich, I. (2002). The autoinflammatory syndromes. *Curr. Opin. Allergy Clin. Immunol.* 2, 511–516.

Montminy, S. W., Khan, N., McGrath, S., Walkowicz, M. J., Sharp, F., Conlon, J. E., Fukase, K., Kusumoto, S., Sweet, C., Miyake, K., Akira, S., Cotter, R. J., Goguen, J. D., and Lien, E. (2006). Virulence factors of *Yersinia pestis* are overcome by a strong lipopolysaccharide response. *Nat. Immunol.* 7, 1066–1073.

Murray, T. S., and Shapiro, E. D. (2010). Lyme disease. *Clin. Lab. Med.* 30, 311–328.

Niemi, K., Teirila, L., Lappalainen, J., Rajamaki, K., Baumann, M. H., Oorni, K., Wolff, H., Kovanen, P. T., Matikainen, S., and Eklund, K. K. (2011). Serum amyloid A activates the NLRP3 inflammasome via P2×7 receptor and a cathepsin B-sensitive pathway. *J. Immunol.* 186, 6119–6128.

Okemoto, K., Kawasaki, K., Hanada, K., Miura, M., and Nishijima, M. (2006). A potent adjuvant monophosphoryl lipid A triggers various immune responses, but not secretion of IL-1beta or activation of caspase-1. *J. Immunol.* 176, 1203–1208.

Osawa, R., Williams, K. L., and Singh, N. (2011). The inflammasome regulatory pathway and infections: role in pathophysiology and clinical implications. *J. Infect.* 62, 119–129.

Park, B. S., Song, D. H., Kim, H. M., Choi, B. S., Lee, H., and Lee, J. O. (2009). The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 458, 1191–1195.

Pelegrin, P., Barroso-Gutierrez, C., and Surprenant, A. (2008). P2×7 receptor differentially couples to distinct release pathways for IL-1beta in mouse macrophage. *J. Immunol.* 180, 7147–7157.

Poelstra, K., Bakker, W. W., Klok, P. A., Hardonk, M. J., and Meijer, D. K. (1997a). A physiologic function for alkaline phosphatase: endotoxin detoxification. *Lab. Invest.* 76, 319–327.

Poelstra, K., Bakker, W. W., Klok, P. A., Kamps, J. A., Hardonk, M. J., and Meijer, D. K. (1997b). Dephosphorylation of endotoxin by alkaline phosphatase in vivo. *Am. J. Pathol.* 151, 1163–1169.

Poltorak, A., Smirnova, I., He, X., Liu, M. Y., Van, H. C., McNally, O., Birdwell, D., Alejos, E., Silva, M., Du, X., Thompson, P., Chan, E. K., Ledesma, J., Roe, B., Clifton, S., Vogel, S. N., and Beutler, B. (1998). Genetic and physical mapping of the Lps locus: identification of the toll-4 receptor as a candidate gene in the critical region. *Blood Cells Mol. Dis.* 24, 340–355.

Raetz, C. R., and Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annu. Rev. Biochem.* 71, 635–700.

Ramasamy, S., Nguyen, D. D., Eston, M. A., Alam, S. N., Moss, A. K., Ebrahimi, F., Biswas, B., Mostafa, G., Chen, K. T., Kaliannan, K., Yammine, H., Narisawa, S., Millan, J. L., Warren, H. S., Hohmann, E. L., Mizoguchi, E., Reinecker, H. C., Bhan, A. K., Snapper, S. B., Malo, M. S., and Hodin, R. A. (2011). Intestinal alkaline phosphatase has beneficial effects in mouse models of chronic colitis. *Inflamm. Bowel. Dis.* 17, 532–542.

Reife, R. A., Coats, S. R., Al-Qutub, M., Dixon, D. M., Braham, P. A., Billharz, R. J., Howald, W. N., and Darveau, R. P. (2006). Porphyromonas gingivalis lipopolysaccharide lipid A heterogeneity: differential activities of tetra- and penta-acylated lipid A structures on E-selectin expression and TLR4 recognition. *Cell. Microbiol.* 8, 857–868.

Ribi, E. (1984). Beneficial modification of the endotoxin molecule. *J. Biol. Response Mod.* 3, 1–9.

Roach, J. C., Glusman, G., Rowen, L., Kaur, A., Purcell, M. K., Smith, K. D., Hood, L. E., and Aderem, A. (2005). The evolution of vertebrate Toll-like receptors. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9577–9582.

Rock, K. L., Lai, J. J., and Kono, H. (2011). Innate and adaptive immune responses to cell death. *Immunol. Rev.* 243, 191–205.

Schilling, D., Thomas, K., Nixdorff, K., Vogel, S. N., and Fenton, M. J. (2002). Toll-like receptor 4 and Toll-IL-1 receptor domain-containing adapter protein (TIRAP)/myeloid differentiation protein 88 adapter-like (Mal) contribute to maximal IL-6 expression in macrophages. *J. Immunol.* 169, 5874–5880.

Schroder, K., and Tschopp, J. (2010). The inflammasomes. *Cell* 140, 821–832.

Sikora, K. A., and Grom, A. A. (2011). Update on the pathogenesis and treatment of systemic idiopathic arthritis. *Curr. Opin. Pediatr.* 23, 640–646.

Starczynowski, D. T., and Karsan, A. (2010). Innate immune signaling in the myelodysplastic syndromes.

Hematol. Oncol. Clin. North Am. 24, 343–359.

Stover, A. G., Da Silva, C. J., Evans, J. T., Cluff, C. W., Elliott, M. W., Jeffery, E. W., Johnson, D. A., Lacy, M. J., Baldridge, J. R., Probst, P., Ulevitch, R. J., Persing, D. H., and Hershberg, R. M. (2004). Structure-activity relationship of synthetic toll-like receptor 4 agonists. *J. Biol. Chem.* 279, 4440–4449.

Stutz, A., Golenbock, D. T., and Latz, E. (2009). Inflammasomes: too big to miss. *J. Clin. Invest.* 119, 3502–3511.

Suerbaum, S., and Michetti, P. (2002). *Helicobacter pylori* infection. *N. Engl. J. Med.* 347, 1175–1186.

Teng, L. J., Hsueh, P. R., Huang, Y. H., and Tsai, J. C. (2004). Identification of bacteroides thetaiotaomicron on the basis of an unexpected specific amplicon of universal 16S ribosomal DNA PCR. *J. Clin. Microbiol.* 42, 1727–1730.

Thompson, B. S., Chilton, P. M., Ward, J. R., Evans, J. T., and Mitchell, T. C. (2005). The low-toxicity versions of LPS, MPL adjuvant and RC529, are efficient adjuvants for CD4+ T cells. *J. Leukoc. Biol.* 78, 1273–1280.

Tuin, A., Huizinga-van der Vlag, A., van Loenen-Weemaes, A. M., Meijer, D. K., and Poelstra, K. (2006). On the role and fate of LPS-dephosphorylating activity in the rat liver. *Am. J. Physiol. Gastrointest. Liver Physiol.* 290, G377–G385.

Tuin, A., Poelstra, K., de Jager-Krikken, A., Bok, L., Raaben, W., Velders, M. P., and Dijkstra, G. (2009). Role of alkaline phosphatase in colitis in man and rats. *Gut* 58, 379–387.

Waymire, K. G., Mahuren, J. D., Jaje, J. M., Guilarte, T. R., Coburn, S. P., and MacGregor, G. R. (1995). Mice lacking tissue non-specific alkaline phosphatase die from seizures due to defective metabolism of vitamin B-6. *Nat. Genet.* 11, 45–51.

Whitehead, J. (2009). Intestinal alkaline phosphatase: the molecular link between rosacea and gastrointestinal disease? *Med. Hypotheses* 73, 1019–1022.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 19 March 2012; paper pending published: 03 April 2012; accepted: 25 May 2012; published online: 13 June 2012.

*Citation: Chilton PM, Embry CA and Mitchell TC (2012) Effects of differences in lipid A structure on TLR4 proinflammatory signaling and inflammasome activation. Front. Immun. **3**:154. doi: 10.3389/fimmu.2012.00154*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 31 May 2012
doi: 10.3389/fimmu.2012.00132

Post-ischemic inflammation in the brain

Takashi Shichita[1,2], Ryota Sakaguchi[1,3], Mayu Suzuki[1,3] and Akihiko Yoshimura[1,3]*

[1] *Department of Microbiology and Immunology, School of Medicine, Keio University, Tokyo, Japan*
[2] *Department of Research Promotion, Precursory Research for Embryonic Science and Technology, Tokyo, Japan*
[3] *Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo, Japan*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Daisuke Kamimura, Osaka University, Japan
Yasunobu Arima, Osaka University, Japan

****Correspondence:***
Akihiko Yoshimura, Department of Microbiology and Immunology, School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
e-mail: yoshimura@a6.keio.jp

Post-ischemic inflammation is an essential step in the progression of brain ischemia-reperfusion injury. In this review, we focus on the post-ischemic inflammation triggered by infiltrating immune cells, macrophages, and T lymphocytes. Brain ischemia is a sterile organ, but injury-induced inflammation is mostly dependent on Toll-like receptor (TLR) 2 and TLR4. Some endogenous TLR ligands, high mobility group box 1 (HMGB1) and peroxiredoxin family proteins, in particular, are implicated in the activation and inflammatory cytokine expression in infiltrating macrophages. Following macrophage activation, T lymphocytes infiltrate the ischemic brain and regulate the delayed phase inflammation. IL-17-producing γδT lymphocytes induced by IL-23 from macrophages promote ischemic brain injury, whereas regulatory T lymphocytes suppress the function of inflammatory mediators. A deeper understanding of the inflammatory mechanisms of infiltrating immune cells may lead to the development of novel neuroprotective therapies.

Keywords: cytokine, inflammation, ischemia, brain, stroke, T cells, macrophages, DAMPs

INTRODUCTION

Stroke is a leading cause of death and disability worldwide. The most common type of stroke is ischemic stroke (e.g., approximately 70% of strokes in Japan are ischemic). However, intravenous administration of tissue plasminogen activator (tPA) is the only globally approved treatment for ischemic stroke, and it is a time-dependent therapy that must be provided within 4.5 h after stroke onset. Thus, there is a need for an efficacious therapy that can be administered beyond this time window, one that targets neuroprotection rather than clot dissolution (Lo, 2010; Moskowitz et al., 2010).

Brain infarction is tissue death caused by ischemia due to severe stenosis or occlusion of a cerebral artery. Ischemic brain tissue is deprived of oxygen, glucose, and lipids, and eventually becomes necrotic. Brain inflammation occurs in this necrotic brain tissue, following the breakdown of the blood-brain barrier (BBB) and infiltration of blood immune cells. Infiltrating immune cells promote ischemic brain inflammation by producing various inflammatory mediators, and also clear away necrotic debris. After the demolition of necrotic debris has been completed, brain inflammation subsides.

Despite intensive studies, the complexity of the brain inflammation mechanism has thus far prevented sufficient clarification (Eltzschig and Eckle, 2011; Iadecola and Anrather, 2011; Macrez et al., 2011). Macrophages and neutrophils are pivotal players in the various processes of brain inflammation, but the mechanism of their activation is still unknown. In addition, T or B lymphocytes have been also reported to participate in delayed brain inflammation. This review focuses on the mechanism of ischemic brain inflammation triggered and sustained by infiltrating immune cells.

POST-ISCHEMIC INFLAMMATION IN THE EARLY PHASE OF BRAIN ISCHEMIA

Severe ischemia induces hypoxia and glucose deprivation in brain tissue. Calcium and sodium ions are stored within brain cells and glutamate is released into the extracellular compartment. The production of reactive oxygen species (ROS) activates platelets and endothelial cells, leading to microvascular occlusion. Oxidative stress reduces the beneficial effects of nitric oxide (NO), a potent vasodilator and inhibitor of platelet aggregation and leukocyte adhesion, in endothelial cells. Oxidative stress and the inflammatory cascade alter the permeability of the BBB. The activation of matrix metalloproteinases (MMPs) and the expression of various other proteases lead to BBB breakdown which exacerbates leukocyte extravasation. Intravascular leukocytes firmly adhere to activated endothelium by the interaction of endothelial expression of intercellular adhesion molecule-1 (ICAM-1) and leukocyte β2 integrins (Iadecola and Anrather, 2011). The infiltration of leukocytes is enhanced by BBB breakdown and by chemokines.

Brain cells, including astrocytes, oligodendrocytes, endothelium, and pericytes, constitute a neurovascular network, which is essential for metabolic requirement of neurons (Iadecola, 2004; Fraser, 2011). These brain cells also contribute to triggering post-ischemic inflammation by producing inflammatory mediators. TNF-α, IL-1β, NOS (nitric oxide synthetase), and MMPs which enhance cerebrovascular permeability and exaggerate brain edema (Takano et al., 2009; Morancho et al., 2010). Thus, Infiltrating leukocytes and injured brain cells produce various inflammatory mediators, leading to the beginning of post-ischemic inflammation (Barone and Feuerstein, 1999; **Figure 1**).

INNATE INFLAMMATORY CYTOKINES AND INFLAMMATORY MEDIATORS

Various cytokines and mediators are produced from infiltrating immune cells and brain cells as a result of ischemic changes of brain. IL-1β is expressed in ischemic brain tissue within 30 min after stroke onset. IL-1β directly induces apoptosis of neuronal cells and enhances the expression of chemokine (RANTES, etc.) in microglia and astrocytes. IL-1β is considered to be a neurotoxic mediator, given that the loss of IL-1β function is reported to reduce

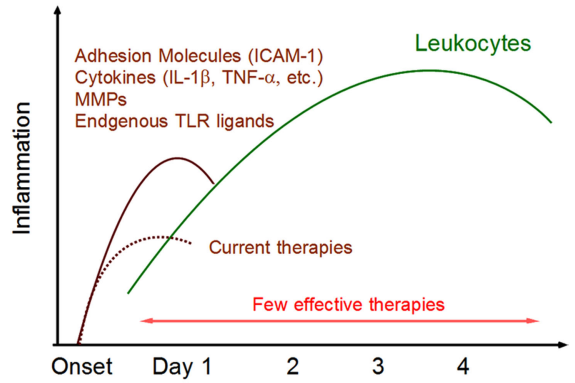


FIGURE 1 | Post-ischemic inflammation in the brain. Within 24 h after ischemic stroke onset, various inflammatory mediators are expressed in ischemic brain tissue. ICAM-1 promotes leukocytes infiltration. Cytokines activate infiltrating leukocytes and directly induce ischemic injury in brain cells. Matrix metalloproteinases (MMPs) alter the permeability of epithelial cells and are implicated in BBB breakdown. Endogenous TLR ligands (DAMPs) are released from necrotic brain cells and activate infiltrating immune cells. These inflammatory mediators trigger post-ischemic inflammation by infiltrating leukocytes. There are currently few effective therapies for this phase of leukocyte infiltration.

infarct size (Boutin et al., 2001). IL-1β is produced in an inactivate form, pro-IL-1β, which is cleaved by caspase-1 to become an active 17 kDa form. Recently, the mechanism of IL-1β production and caspase-1 activation mediated by inflammasome has been the subject of particular attention. Inflammasome has been shown to be present in neurons, astrocytes, microglia, and macrophages in the ischemic brain (Abulafia et al., 2009; Chakraborty et al., 2010). Several types of inflammasome have been discovered, denoted NALP1, NALP3, AIM, and so on, but the particular type most important in ischemic brain injury remains unknown. Hypoxia or ATP is reported to activate inflammasome, which then activates caspase-1 and induces IL-1β production (Martinon et al., 2002). In addition, IL-1β is mostly produced from monocytes which are activated by endogenous Toll-like receptor (TLR) ligands, given that IL-1β mRNA level in infiltrating mononuclear cells is drastically reduced in TLR2/4-double deficient mice after ischemic brain injury.

TNF-α is another important mediator implicated in the pathology of the ischemic brain. TNF-α is expressed in ischemic brain tissue within 1 h after stroke onset, and upregulation of the TNF receptors is observed thereafter. TNF-α exercises neurotoxic effects by inducing apoptotic neuronal cell death and enhancing MHC class II and ICAM-1 expression in astrocytes, leading to leukocyte infiltration and BBB breakdown. TNF-α gene knockout (KO) mice or anti-TNF-α neutralizing antibody administration has been shown to reduce infarct volume, compared with that in control mice. However, TNFR KO mice, which lack both p75 and p50 genes, exhibit enlargement of infarct volume on day 1 following ischemic brain injury, indicating that TNF-α can be considered to function as both a neurotoxic and a neuroprotective mediator (Hallenbeck, 2002). It appears that the opposing functions, toxic or protective, depends on the type of brain cell involved. TNF-α promotes post-ischemic inflammation but also participates in a negative feedback loop to suppress inflammatory signal cascades, and it controls the duration of post-ischemic inflammation by regulating these two functions.

IL-6 is also expressed in ischemic brain tissue, but ischemic brain damage is not attenuated in IL-6 KO mice or in anti-IL-6R antagonistic antibody-treated mice (Yamashita et al., 2005). However, it has been recently reported that IL-6 produced from brain cells contributes to neoangiogenesis and neuronal survival through STAT3 activation (Jung et al., 2011; Gertz et al., 2012). Consistent with this, the inhibition of JAK/STAT pathway or the enhanced role of SOCS3 (negative regulator of JAK/STAT pathway) has been reported to promote neuronal cell death (Yadav et al., 2005; Yamashita et al., 2005). Thus, it is possible that IL-6 protects neuron from cell death, although a significant role of IL-6 in brain ischemia has not yet been established.

Matrix metalloproteinases are essential neurotoxic mediators that promote BBB breakdown and post-ischemic inflammation. Functionally similar to IL-1β, MMPs induce apoptotic neuronal cell death by TNF-α and FasL processing. The neurotoxic function of MMP-9 is particularly established, given that infarct size is reduced in MMP-9-deficient mice compared to that in control mice (Asahi et al., 2000). Intercellular adhesion molecule-1 (ICAM-1) is another neurotoxic mediator. The increased expression of ICAM-1 observed in cerebrovascular endothelial cells is implicated in the promotion of leukocyte infiltration. ICAM-1-deficient mice reveal attenuated ischemic damage and the administration of anti-ICAM-1 antibody decreases the number of infiltrating immune cells (Connolly et al., 1996; Liesz et al., 2011).

Chemokines are also important enhancers of post-ischemic inflammation. Chemokines (RANTES, MCP-1, IL-8, etc.) have been reported to promote leukocyte infiltration into the ischemic brain (Terao et al., 2008, 2009; Strecker et al., 2011). Although the chemokines for lymphocyte infiltration remain unknown, CCL12, CCL20, and their receptor, CCR6, are essential for the exacerbation of experimental autoimmune encephalomyelitis (EAE; Martin et al., 2009; Reboldi et al., 2009). Whether these lymphocyte chemokines also function in acute organ injury, such as brain ischemia, should be elucidated in the future.

Sphingosine-1-phosphate (S1P) is a bioactive phospholipid. At sites of tissue injury, S1P is mainly released from platelets and mediates its effect via activation of cell-surface S1P receptors, which are ubiquitously expressed in brain cells (Dev et al., 2008). S1P receptors are also present on the surface of T lymphocytes; therefore, S1P has been thought to play an essential role in T lymphocyte infiltration of inflammatory tissue. Recently, the therapeutic effect of FTY720 (fingolimod), a functional S1P receptor antagonist, has attracted attention. The administration of FTY720 has been shown to attenuate ischemic brain damage and decrease the number of infiltrating T lymphocytes in the ischemic brain (Shichita et al., 2009; Hasegawa et al., 2010). Furthermore, S1P receptors are expressed in neurons, astrocytes, and microglial cells. S1P acts on these cells directly and exerts effects that include astrocyte proliferation and migration, oligodendrocyte differentiation and cell survival, and neurite outgrowth and neurogenesis

(Dev et al., 2008). Thus, S1P is considered to be an important inflammatory mediator in the ischemic brain.

ROLE OF TLR

Leukocyte infiltration is an essential step in the progression of post-ischemic inflammation. However, the mechanisms that activate these infiltrating immune cells, macrophages, and lymphocytes, are not yet fully clarified. TLRs are an essential type of receptor for innate and non-specific immune response to general pathogens such as bacteria, viruses, and so on. The demonstration that TLR2 and TLR4 are pivotal for sterile organ injury, including ischemic brain injury, has recently attracted attention (Chen et al., 2007; Tang et al., 2007).

Toll-like receptors are expressed on both leukocytes and brain cells, although whether the effect of TLRs on brain cells is neurotoxic or neuroprotective remains unclear. TLR stimulation in macrophages and lymphocytes induces strong and various inflammatory responses. In ischemic brain injury, post-ischemic inflammation and subsequent ischemic damage depend on TLR2 and TLR4, but not TLR9 (Tang et al., 2007; Hyakkoku et al., 2010). TLR2- or TLR4-deficient mice demonstrate both significant reduction of infarct volume and suppression of neurotoxic inflammatory responses. Analysis of bone marrow chimeric mice indicates that TLRs in infiltrated immune cells, but not in residential microglia, have a neurotoxic effect on ischemic brain injury (Yang et al., 2011). In addition, mice lacking MyD88, the adaptor protein under almost all TLR signaling cascades other than TLR3, are reported to show no improvement of ischemic brain injury (Famakin et al., 2011). These results indicate that the function of TLRs may be dependent on kind of cells in brain.

Toll-like receptor-2 or TLR4 deficiency suppresses inflammatory cytokine expression in infiltrating immune cells on day 1 after brain ischemia (Shichita et al., 2012). Although both TLR2 and TLR4 signaling cascades are essential triggers for post-ischemic inflammation, and activators of infiltrating immune cells, the particular molecules that activate TLR2 and TLR4 in the ischemic brain remain unclear. Because the brain is a sterile organ, pathogens derived from bacteria or viruses are completely lacking in the normal and ischemic brain. This indicates that certain endogenous molecules released from necrotic brain cells become TLR stimulators, and several endogenous molecules have indeed been reported to activate TLR signaling cascades. Such endogenous molecules are called danger associated molecular patterns (DAMPs), and are considered to be danger signals or alarm molecules that warn immune cells of tissue and cellular injury.

DAMPs IN INJURED BRAIN

Several molecules in the brain have been reported as DAMPs. Heat shock proteins (HSPs), β-amyloid (Aβ), hyaluronan, heparin sulfate, DNA or RNA immune complex, oxidized low-density lipoproteins (oxLDL), and others, can stimulate TLRs (Marsh et al., 2009; Rivest, 2009; Yanai et al., 2009; Stewart et al., 2010; Zhang et al., 2010). However, it remains unclear which molecule is the most important for triggering post-ischemic inflammation and inflammatory cytokine expression.

High mobility group box 1 (HMGB1) is a well-elucidated DAMP and is also implicated in ischemic brain injury (Kim et al., 2006; Liu et al., 2007; Hayakawa et al., 2010). HMGB1 increases vascular permeability and promotes BBB breakdown (Zhang et al., 2011). HMGB1, which is localized in cell nuclei in the normal brain, translocates into the cytosolic compartment and is released into the extracellular compartment in the ischemic condition. The administration of anti-HMGB1-neutralizing antibody protects the BBB and reduces infarct volume. Thus, HMGB1 is an essential DAMP in ischemic brain injury. Extracellular release of HMGB1 is observed within 6 h after stroke onset, but is diminished by 12 h after the onset (Qiu et al., 2008; Zhang et al., 2011). Thereafter, the infiltration of immune cells and the production of inflammatory cytokines become evident. This indicates that HMGB1 may not directly activate infiltrating immune cells in the ischemic brain.

We have identified the peroxiredoxin (Prx) family proteins as strong inducers of inflammatory cytokines in infiltrating immune cells (Shichita et al., 2012). Prx family proteins are known to exert a protective effect by catalyzing ROS. In the ischemic brain, the expression of Prx within brain cells is increased by ischemic stress, and such intracellular Prx is thought to be neuroprotective (Patenaude et al., 2005; Rashidian et al., 2009; **Figure 2**). However, as necrosis occurs, this Prx is released into the extracellular compartment where they induce inflammatory cytokine expression in infiltrating immune cells by stimulating TLR2 and TLR4 (**Figure 2**). It is interesting to note that the Prx family proteins have a common active region for TLR activation and are extracellularly released over 12 h after stroke onset, which coincides with the timing of leukocyte infiltration. Thus, Prx has two opposing functions, one inside, and one outside, brain cells. Furthermore, there is a time lag as well as functional differences between HMGB1 and Prx (**Figure 3**).

T LYMPHOCYTES IN ISCHEMIC BRAIN INJURY

It has been recently suggested that T lymphocytes play a role as mediators in the delayed phase of brain ischemia (Yilmaz et al., 2006). The number of infiltrating T lymphocytes in ischemic brain tissue increases over 24 h after stroke onset and reaches its peak in the delayed phase (around day 3; Schroeter et al., 1994; Jander et al., 1995). Infiltrating T lymphocytes appear to be localized

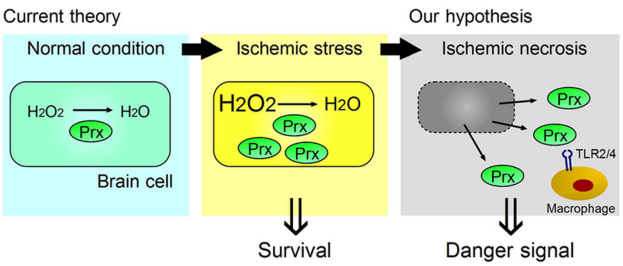


FIGURE 2 | Two opposing functions of Prx, one inside, and one outside, brain cells. Ischemic stress increases Prx expression within brain cells, which contributes to their survival by catabolizing reactive oxygen species (ROS). When ischemic phenomena finally result in necrosis, the Prx released from necrotic brain cells into the extracellular compartment then functions as a strong TLR2 and TLR4 stimulator (DAMP) for the infiltrating macrophages in ischemic brain tissue.

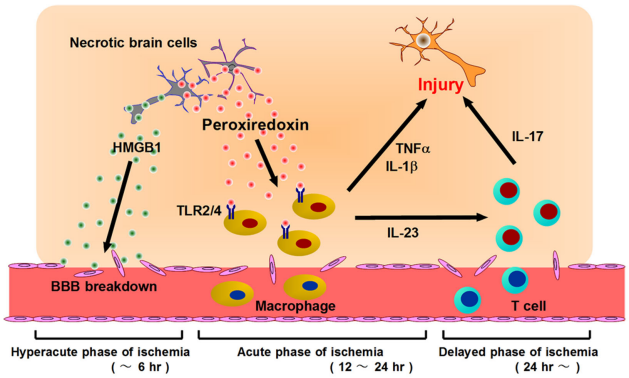


FIGURE 3 | Post-ischemic inflammation triggered by DAMPs and infiltrating immune cells. At the hyperacute phase of brain ischemia (within 6 h after stroke onset), HMGB1 is released from brain cells and induces BBB breakdown. Following this, blood cells begin to infiltrate into ischemic brain tissue via disrupted vessels during the acute phase of ischemia (12–24 h after stroke onset). Prx is extracellularly released from necrotic brain cells and activates infiltrating macrophages via TLR2 and TLR4. Activated macrophages produce inflammatory cytokines (IL-23, IL-1β, TNF-α, etc.) which promote ischemic brain injury. At the delayed phase of brain ischemia (more than 24 h after stroke onset), IL-23 induces IL-17 production from γδT lymphocytes, which further enhances ischemic damage. Thus, HMGB1 is a hyperacute DAMP, while Prx is secondarily active in post-ischemic inflammation, during the acute phase.

to the infarct boundary zones, typically close to blood vessels. By Percoll gradient centrifugation, infiltrating immune cells have been analyzed (Shichita et al., 2009). The number of infiltrating immune cells reaches the peak at day 3 after stroke onset and most of these are macrophages. Approximately 1 ~ 1.5% of immune cells are T lymphocytes which are consisted of 30 ~ 40% CD4+ helper T lymphocytes, 20 ~ 30% γδT lymphocytes, and 20 ~ 30% CD8+ cytotoxic T lymphocytes. Inflammatory cytokines, IL-1β, TNF-α, IL-23, and IL-12 have been shown to be produced from macrophage and play important roles in promoting brain injury. However, role of various cytokines from T lymphocytes, such as IFNγ and IL-17 in ischemic brain injury has not been clarified.

Although the function of these infiltrating T lymphocytes in the ischemic brain is not yet clear, T lymphocytes, on the whole, are considered to act as a neurotoxic effector. This is indicated by the fact that severe combined immunodeficient (SCID) mice, and recombination activating gene (RAG)-deficient mice, both of which lack T and B lymphocytes, reveal significant reduction of infarct volume (Yilmaz et al., 2006; Hurn et al., 2007). In addition, the depletion of CD4+ (helper) or CD8+ (cytotoxic) T lymphocytes, but not one of the B lymphocytes, is reported to attenuate ischemic brain damage. There is a report that regulatory B lymphocytes protect brain from ischemic damages; however, the neurotoxic effect of lymphocytes is considered to be majorly made by T lymphocytes (Ren et al., 2011). It will be important to elucidate which subtype of lymphocytes is implicated in ischemic brain injury (see T Lymphocyte Cytokines in Ischemic Brain Injury).

It is not yet clear whether a specific antigen in the brain is involved in the activation of these infiltrating T lymphocytes. Up to now, it has been thought likely that these T lymphocytes mediate antigen-independent, innate inflammatory responses, because post-ischemic inflammatory responses have been shown to be driven by the innate immune system. However, some reports suggest the importance of antigen recognition by T lymphocytes in ischemic brain injury. Treatment with T cell receptor (TCR) ligands, which are major histocompatibility complex (MHC) class II molecules bound to myelin peptides, is protective against ischemic brain injury (Subramanian et al., 2009). Infarct size in myelin basic protein (MBP) tolerized animals has been shown to be reduced compared to that in control mice. Thus, there is a possibility that some T lymphocyte subsets specifically tolerized to brain proteins could be protective to ischemic brain injury (Becker et al., 2003; Becker, 2009). This idea is supported by the recent finding that regulatory T lymphocytes are protective to ischemic brain injury (Liesz et al., 2009).

T LYMPHOCYTE CYTOKINES IN ISCHEMIC BRAIN INJURY

T lymphocytes are considered to mediate ischemic brain injury by producing various cytokines, and IFN-γ and IL-4 are well-known classical examples. In ischemic injury, IFN-γ is thought to be neurotoxic, as it acts on neurons directly and induces apoptotic neuronal cell death *in vitro* (Lambertsen et al., 2004). However, a protective effect by the IFN-γ deficiency has not been observed, and the role of IFN-γ in ischemic brain injury is controversial (Lambertsen et al., 2004; Yilmaz et al., 2006). IL-12 is produced from myeloid cells such as macrophages, dendritic cells, neutrophils, and so on, and is important for the differentiation of IFN-γ-producing helper T lymphocytes (Th1). IL-12 is expressed in ischemic brain tissue by infiltrating immune cells, but its function has not been fully elucidated.

IL-4 may have the potential to attenuate ischemic damage and promote tissue repair, since IL-4-deficient mice demonstrate exacerbated ischemic damage and neurological deficit (Xiong et al., 2011). Although the emerging recognition of the function of IL-4 for tissue repair has recently attracted attention, whether or not IL-4 is directly implicated in tissue repair in ischemic brain injury is still unclear (Chen et al., 2012).

IL-10 is an immunosuppressive cytokine and is thought to have a neuroprotective effect in ischemic brain injury. IL-10 is produced from regulatory T lymphocytes (Treg) and suppresses the neurotoxic function of TNF-α and IFN-γ (Liesz et al., 2009). The overexpression of IL-10 by an adenovirus vector protects hippocampal neurons against apoptotic cell death (Ooboshi et al., 2006).

IL-17 is an emerging therapeutic target for various organ injuries. IL-23 has been reported to be essential for IL-17 induction from T lymphocytes, and to play a critical role in EAE (Cua et al., 2003). In ischemic brain injury, IL-23 is produced by infiltrating macrophages on day 1, and it induces IL-17 production from γδT lymphocytes in the delayed phase (**Figure 3**). Both IL-23 KO mice and IL-17 KO mice show significantly attenuated ischemic brain damage on day 4. The IL-17 receptor is ubiquitously expressed in brain cells and modifies various inflammatory responses in the central nervous system. IL-17 has been reported to promote the expression of inflammatory cytokines and chemokines from macrophages (Fossiez et al., 1996). IL-17 also modulates the epithelial barrier function by promoting MMPs

and ICAM-1 expression (Kebir et al., 2007; Ifergan et al., 2008). Although it remains unknown whether IL-17 directly affects neurons, IL-17 is thought to be a promising therapeutic target for suppressing post-ischemic inflammation. Thus, IL-23-induced IL-17-producing γδT lymphocytes play a pivotal role in the delayed phase of brain ischemia (**Figure 4**). IL-17 production from γδT lymphocytes requires only IL-1β and IL-23 stimulation, but not specific TCR stimulation, and IL-6 and TGFβ stimulation are indispensable for IL-17-producing helper T lymphocyte (Th17) differentiation (Sutton et al., 2009). Thus, it is reasonable that γδT lymphocytes mediate ischemic brain injury, given that γδT lymphocytes produce IL-17 more rapidly than does Th17.

THE POSSIBILITY OF MEDICAL INTERVENTION IN POST-ISCHEMIC INFLAMMATION

Post-ischemic inflammation in the brain is considered to have two opposing functions in stroke patients; one beneficial, the other harmful. Post-ischemic inflammation promotes brain swelling (brain edema) which leads to the compression of normal brain tissue surrounding the ischemic core and the exacerbation of neurological deficits. This undesirable effect of post-ischemic brain inflammation should be suppressed by medical intervention when possible. However, post-ischemic inflammation is also thought to promote tissue repair in the recovery phase of ischemic stroke. Thus, suppression of all inflammatory responses in ischemic brain tissue is not always effective, due to their involvement in both ischemic injury and tissue repair processes. To create novel neuroprotective strategy, it may be possible to control the balance between the neurotoxic and neuroprotective effects of post-ischemic inflammation by targeting specific inflammatory mediators. For example, therapy which both suppresses the inflammatory subset of T lymphocytes (e.g., IL-17-producing γδT lymphocytes) and promotes Treg function may be desirable.

It is possible that administration of γδTCR-depleting antibody or anti-IL-12/IL-23p40 antibody [p40 is a common subunit of the IL-12 heterodimer (p35/p40) and IL-23 heterodimer (p19/p40)] will become a therapeutic tool for ischemic stroke (Shichita et al., 2009; Konoeda et al., 2010). Thus, neuroprotective therapies for ischemic brain injury may be developed by targeting the IL-23/IL-17 inflammatory pathway. Furthermore, one of the most advantageous points of therapy targeting IL-17-producing T lymphocytes is its long therapeutic time window. Since current globally approved therapy is limited to intravenous administration of tPA, which should be performed within 4.5 h after stroke onset, further elucidation of the inflammatory mechanisms of the T lymphocytes that infiltrate the ischemic brain during the 24-h-period after stroke onset is needed.

FTY720 is one of the most promising therapeutic tools for ischemic stroke at this time (Wei et al., 2011). FTY720 decreases the number of infiltrating T lymphocytes, including γδT lymphocytes. The most troublesome side effect of FTY720 administration is the increased incidence of bacterial pneumonia after stroke (Meisel and Meisel, 2011). It is possible that FTY720 interferes with peripheral lymphocyte distribution in the body after stroke and thus inhibits the protective function of peripheral T lymphocytes against bacterial infection. Although it has been reported that FTY720 does not promote spontaneous bacterial infection after experimental stroke in mice, future studies aimed at developing effective clinical interventions for stroke patients should seek to minimize this kind of detrimental effect of FTY720 (Pfeilschifter et al., 2011).

In conclusion, research has gradually shed light on the mechanisms of post-ischemic inflammation. A deeper understanding of these intricacies in the context of medical intervention should enable the development of novel neuroprotective strategies that are more effective and have a longer therapeutic time window (**Figure 5**).

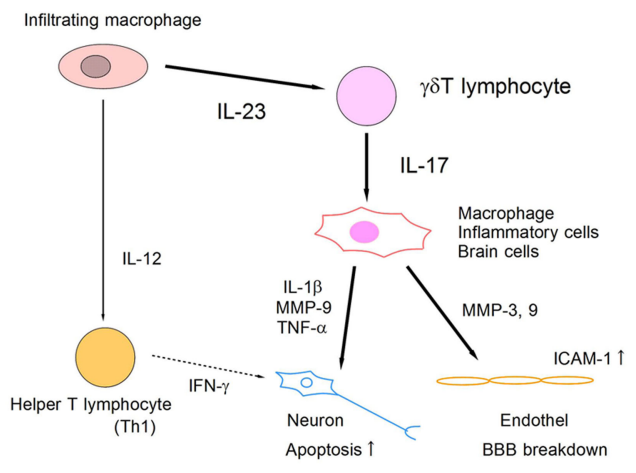


FIGURE 4 | Schematic model of IL-23/IL-17 inflammatory pathway in ischemic brain tissue. Infiltrating macrophages produce IL-23 and IL-12, which induce IL-17-producing γδT lymphocytes and IFN-γ-producing helper T lymphocytes (Th1), respectively. IL-17 from γδT lymphocytes acts on macrophages and brain cells directly, and promotes the expression of inflammatory mediators that enhance apoptotic neuronal cell death and BBB breakdown.

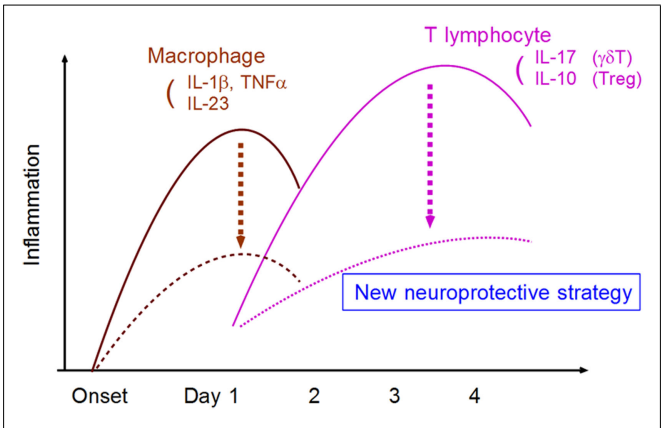


FIGURE 5 | Strategy for developing neuroprotective therapy by suppressing neurotoxic inflammatory response. The targeting of specific inflammatory mediators from macrophages and T lymphocytes can attenuate neurotoxic inflammatory reactions.

REFERENCES

Abulafia, D. P., de Rivero Vaccari, J. P., Lozano, J. D., Lotocki, G., Keane, R. W., and Dietrich, W. D. (2009). Inhibition of the inflammasome complex reduces the inflammatory response after thromboembolic stroke in mice. *J. Cereb. Blood Flow Metab.* 29, 534–544.

Asahi, M., Asahi, K., Jung, J. C., del Zoppo, G. J., Fini, M. E., and Lo, E. H. (2000). Role for matrix metalloproteinase 9 after cerebral ischemia: effects of gene knockout and enzyme inhibition with BB-94. *J. Cereb. Blood Flow Metab.* 20, 1681–1689.

Barone, F. C., and Feuerstein, G. Z. (1999). Inflammatory mediators and stroke: new opportunities for novel therapeutics. *J. Cereb. Blood Flow Metab.* 19, 819–834.

Becker, K., Kindrick, D., McCarron, R., Hallenbeck, J., and Winn, R. (2003). Adoptive transfer of myelin basic protein-tolerized splenocytes to naive animals reduces infarct size: a role for lymphocytes in ischemic brain injury? *Stroke* 34, 1809–1815.

Becker, K. J. (2009). Sensitization and tolerization to brain antigens in stroke. *Neuroscience* 158, 1090–1097.

Boutin, H., LeFeuvre, R. A., Horai, R., Asano, M., Iwakura, Y., and Rothwell, N. J. (2001). Role of IL-1alpha and IL-1beta in ischemic brain damage. *J. Neurosci.* 21, 5528–5534.

Chakraborty, S., Kaushik, D. K., Gupta, M., and Basu, A. (2010). Inflammasome signaling at the heart of central nervous system pathology. *J. Neurosci. Res.* 88, 1615–1631.

Chen, C. J., Kono, H., Golenbock, D., Reed, G., Akira, S., and Rock, K. L. (2007). Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. *Nat. Med.* 13, 851–856.

Chen, F., Liu, Z., Wu, W., Rozo, C., Bowdridge, S., Millman, A., Van Rooijen, N., Urban, J. F. Jr., Wynn, T. A., and Gause, W. C. (2012). An essential role for TH2-type responses in limiting acute tissue damage during experimental helminth infection. *Nat. Med.* 18, 260–266.

Connolly, E. S. Jr., Winfree, C. J., Springer, T. A., Naka, Y., Liao, H., Yan, S. D., Stern, D. M., Solomon, R. A., Gutierrez-Ramos, J. C., and Pinsky, D. J. (1996). Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion. Role of neutrophil adhesion in the pathogenesis of stroke. *J. Clin. Invest.* 97, 209–216.

Cua, D. J., Sherlock, J., Chen, Y., Murphy, C. A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T., Zurawski, S., Wiekowski, M., Lira, S. A., Gorman, D., Kastelein, R. A., and Sedgwick, J. D. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421, 744–748.

Dev, K. K., Mullershausen, F., Mattes, H., Kuhn, R. R., Bilbe, G., Hoyer, D., and Mir, A. (2008). Brain sphingosine-1-phosphate receptors: implication for FTY720 in the treatment of multiple sclerosis. *Pharmacol. Ther.* 117, 77–93.

Eltzschig, H. K., and Eckle, T. (2011). Ischemia and reperfusion – from mechanism to translation. *Nat. Med.* 17, 1391–1401.

Famakin, B. M., Mou, Y., Ruetzler, C. A., Bembry, J., Maric, D., and Hallenbeck, J. M. (2011). Disruption of downstream MyD88 or TRIF Toll-like receptor signaling does not protect against cerebral ischemia. *Brain Res.* 1388, 148–156.

Fossiez, F., Djossou, O., Chomarat, P., Flores-Romo, L., Ait-Yahia, S., Maat, C., Pin, J. J., Garrone, P., Garcia, E., Saeland, S., Blanchard, D., Gaillard, C., Das Mahapatra, B., Rouvier, E., Golstein, P., Banchereau, J., and Lebecque, S. (1996). T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *J. Exp. Med.* 183, 2593–2603.

Fraser, P. A. (2011). The role of radical generation in increasing cerebrovascular permeability. *Free Radic. Biol. Med.* 51, 967–977.

Gertz, K., Kronenberg, G., Kälin, R. E., Baldinger, T., Werner, C., Balkaya, M., Eom, G. D., Hellmann-Regen, J., Kröber, J., Miller, K. R., Lindauer, U., Laufs, U., Dirnagl, U., Heppner, F. L., and Endres, M. (2012). Essential role of interleukin-6 in post-stroke angiogenesis. *Brain.* doi: 10.1093/brain/aws075

Hallenbeck, J. M. (2002). The many faces of tumor necrosis factor in stroke. *Nat. Med.* 8, 1363–1368.

Hasegawa, Y., Suzuki, H., Sozen, T., Rolland, W., and Zhang, J. H. (2010). Activation of sphingosine 1-phosphate receptor-1 by FTY720 is neuroprotective after ischemic stroke in rats. *Stroke* 41, 368–374.

Hayakawa, K., Qiu, J., and Lo, E. H. (2010). Biphasic actions of HMGB1 signaling in inflammation and recovery after stroke. *Ann. N. Y. Acad. Sci.* 1207, 50–57.

Hurn, P. D., Subramanian, S., Parker, S. M., Afentoulis, M. E., Kaler, L. J., Vandenbark, A. A., and Offner, H. (2007). T- and B-cell-deficient mice with experimental stroke have reduced lesion size and inflammation. *J. Cereb. Blood Flow Metab.* 27, 1798–1805.

Hyakkoku, K., Hamanaka, J., Tsuruma, K., Shimazawa, M., Tanaka, H., Uematsu, S., Akira, S., Inagaki, N., Nagai, H., and Hara, H. (2010). Toll-like receptor 4 (TLR4), but not TLR3 or TLR9, knock-out mice have neuroprotective effects against focal cerebral ischemia. *Neuroscience* 171, 258–267.

Iadecola, C. (2004). Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat. Rev. Neurosci.* 5, 347–360.

Iadecola, C., and Anrather, J. (2011). The immunology of stroke: from mechanisms to translation. *Nat. Med.* 17, 796–808.

Ifergan, I., Kébir, H., Bernard, M., Wosik, K., Dodelet-Devillers, A., Cayrol, R., Arbour, N., and Prat, A. (2008). The blood-brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. *Brain* 131, 785–799.

Jander, S., Karemer, M., Schroeter, M., Witte, O. W., and Stoll, G. (1995). Lymphocytic infiltration and expression of intercellular adhesion molecule-1 in photochemically induced ischemia of the rat cortex. *J. Cereb. Blood Flow Metab.* 15, 42–51.

Jung, J. E., Kim, G. S., and Chan, P. H. (2011). Neuroprotection by interleukin-6 is mediated by signal transducer and activator of transcription 3 and antioxidative signaling in ischemic stroke. *Stroke* 42, 3574–3579.

Kebir, H., Kreymborg, K., Ifergan, I., Dodelet-Devillers, A., Cayrol, R., Bernard, M., Giuliani, F., Arbour, N., Becher, B., and Prat, A. (2007). Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat. Med.* 13, 1173–1175.

Kim, J. B., Sig Choi, J., Yu, Y. M., Nam, K., Piao, C. S., Kim, S. W., Lee, M. H., Han, P. L., Park, J. S., and Lee, J. K. (2006). HMGB1, a novel cytokine-like mediator linking acute neuronal death and delayed neuroinflammation in the postischemic brain. *J. Neurosci.* 26, 6413–6421.

Konoeda, F., Shichita, T., Yoshida, H., Sugiyama, Y., Muto, G., Hasegawa, E., Morita, R., Suzuki, N., and Yoshimura, A. (2010). Therapeutic effect of IL-12/23 and their signaling pathway blockade on brain ischemia model. *Biochem. Biophys. Res. Commun.* 402, 500–506.

Lambertsen, K. L., Gregersen, R., Meldgaard, M., Clausen, B. H., Heibøl, E. K., Ladeby, R., Knudsen, J., Frandsen, A., Owens, T., and Finsen, B. (2004). A role for interferon-gamma in focal cerebral ischemia in mice. *J. Neuropathol. Exp. Neurol.* 63, 942–955.

Liesz, A., Suri-Payer, E., Veltkamp, C., Doerr, H., Sommer, C., Rivest, S., Giese, T., and Veltkamp, R. (2009). Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat. Med.* 15, 192–199.

Liesz, A., Zhou, W., Mracskó, É., Karcher, S., Bauer, H., Schwarting, S., Sun, L., Bruder, D., Stegemann, S., Cerwenka, A., Sommer, C., Dalpke, A. H., and Veltkamp, R. (2011). Inhibition of lymphocyte trafficking shields the brain against deleterious neuroinflammation after stroke. *Brain* 134, 704–720.

Liu, K., Mori, S., Takahashi, H. K., Tomono, Y., Wake, H., Kanke, T., Sato, Y., Hiraga, N., Adachi, N., Yoshino, T., and Nishibori, M. (2007). Anti-high mobility group box 1 monoclonal antibody ameliorates brain infarction induced by transient ischemia in rats. *FASEB J.* 21, 3904–3916.

Lo, E. H. (2010). Degeneration and repair in central nervous system disease. *Nat. Med.* 16, 1205–1209.

Macrez, R., Ali, C., Toutirais, O., Le Mauff, B., Defer, G., Dirnagl, U., and Vivien, D. (2011). Stroke and the immune system: from pathophysiology to new therapeutic strategies. *Lancet Neurol.* 10, 471–480.

Marsh, B. J., Williams-Karnesky, R. L., and Stenzel-Poore, M. P. (2009). Toll-like receptor signaling in endogenous neuroprotection and stroke. *Neuroscience* 158, 1007–1020.

Martin, B., Hirota, K., Cua, D. J., Stockinger, B., and Veldhoen, M. (2009). Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. *Immunity* 31, 321–330.

Martinon, F., Burns, K., and Tschopp, J. (2002). The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1beta. *Mol. Cell* 10, 417–426.

Meisel, C., and Meisel, A. (2011). Suppressing immunosuppression after stroke. *N. Engl. J. Med.* 365, 2134–2136.

Morancho, A., Rosell, A., Garcia-Bonilla, L., and Montaner, J. (2010). Metalloproteinase and stroke infarct size: role for anti-inflammatory

treatment? *Ann. N. Y. Acad. Sci.* 1207, 123–133.

Moskowitz, M. A., Lo, E. H., and Ladecola, C. (2010). The science of stroke: mechanisms in search of treatments. *Neuron* 67, 181–198.

Ooboshi, H., Ibayashi, S., Shichita, T., Kumai, Y., Takada, J., Ago, T., Arakawa, S., Sugimori, H., Kamouchi, M., Kitazono, T., and Iida, M. (2006). Postischemic gene transfer of interleukin-10 protects against both focal and global brain ischemia. *Circulation* 111, 913–919.

Patenaude, A., Murthy, M. R., and Mirault, M. E. (2005). Emerging roles of thioredoxin cycle enzymes in the central nervous system. *Cell. Mol. Life Sci.* 62, 1063–1080.

Pfeilschifter, W., Czech-Zechmeister, B., Sujak, M., Foerch, C., Wichelhaus, T. A., and Pfeilschifter, J. (2011). Treatment with the immunomodulator FTY720 does not promote spontaneous bacterial infections after experimental stroke in mice. *Exp. Transl. Stroke Med.* 3, 2.

Qiu, J., Nishimura, M., Wang, Y., Sims, J. R., Qiu, S., Savitz, S. I., Salomone, S., and Moskowitz, M. A. (2008). Early release of HMGB-1 from neurons after the onset of brain ischemia. *J. Cereb. Blood Flow Metab.* 28, 927–938.

Rashidian, J., Rousseaux, M. W., Venderova, K., Qu, D., Callaghan, S. M., Phillips, M., Bland, R. J., During, M. J., Mao, Z., Slack, R. S., and Park, D. S. (2009). Essential role of cytoplasmic cdk5 and Prx2 in multiple ischemic injury models, in vivo. *J. Neurosci.* 29, 12497–12505.

Reboldi, A., Coisne, C., Baumjohann, D., Benvenuto, F., Bottinelli, D., Lira, S., Uccelli, A., Lanzavecchia, A., Engelhardt, B., and Sallusto, F. (2009). C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat. Immunol.* 10, 514–523.

Ren, X., Akiyoshi, K., Dziennis, S., Vandenbark, A. A., Herson, P. S., Hurn, P. D., and Offner, H. (2011). Regulatory B cells limit CNS inflammation and neurologic deficits in murine experimental stroke. *J. Neurosci.* 31, 8556–8563.

Rivest, S. (2009). Regulation of innate immune responses in the brain. *Nat. Rev. Immunol.* 9, 429–439.

Schroeter, M., Jander, S., Witte, O. W., and Stoll, G. (1994). Local immune responses in the rat middle cerebral artery occlusion. *J. Neuroimmunol.* 55, 195–203.

Shichita, T., Hasegawa, E., Kimura, A., Morita, R., Sakaguchi, R., Takada, I., Sekiya, T., Ooboshi, H., Kitazono, T., Yanagawa, T., Ishii, T., Takahashi, H., Mori, S., Nishibori, M., Kuroda, K., Miyake, K., Akira, S., and Yoshimura, A. (2012). Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. *Nat. Med.* doi: 10.1038/nm.2749

Shichita, T., Sugiyama, Y., Ooboshi, H., Sugimori, H., Nakagawa, R., Takada, I., Iwaki, T., Okada, Y., Iida, M., Cua, D. J., Iwakura, Y., and Yoshimura, A. (2009). Pivotal role of cerebral interleukin-17-producing gammadeltaT cells in the delayed phase of ischemic brain injury. *Nat. Med.* 15, 946–950.

Stewart, C. R., Stuart, L. M., Wilkinson, K., van Gils, J. M., Deng, J., Halle, A., Rayner, K. J., Boyer, L., Zhong, R., Frazier, W. A., Lacy-Hulbert, A., El Khoury, J., Golenbock, D. T., and Moore, K. J. (2010). CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer. *Nat. Immunol.* 11, 155–161.

Strecker, J. K., Minnerup, J., Gess, B., Ringelstein, E. B., Schäbitz, W. R., and Schilling, M. (2011). Monocyte chemoattractant protein-1-deficiency impairs the expression of IL-6, IL-1b and G-CSF after transient focal ischemia in mice. *PLoS ONE* 6, e25863. doi:10.1371/journal.pone.0025863

Subramanian, S., Zhang, B., Kosaka, Y., Burrows, G. G., Grafe, M. R., Vandenbark, A. A., Hurn, P. D., and Offner, H. (2009). Recombinant T cell receptor ligand treats experimental stroke. *Stroke* 40, 2539–2545.

Sutton, C. E., Lalor, S. J., Sweeney, C. M., Brereton, C. F., Lavelle, E. C., and Mills, K. H. (2009). Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 31, 331–341.

Takano, T., Oberheim, N., Cotrina, M. L., and Nedergaard, M. (2009). Astrocytes and ischemic injury. *Stroke* 40, S8–S12.

Tang, S. C., Arumugam, T. V., Xu, X., Cheng, A., Mughal, M. R., Jo, D. G., Lathia, J. D., Siler, D. A., Chigurupati, S., Ouyang, X., Magnus, T., Camandola, S., and Mattson, M. P. (2007). Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. *Proc. Natl. Acad. Sci. U.S.A.* 104, 13798–13803.

Terao, S., Yilmaz, G., Stokes, K. Y., Russell, J., Ishikawa, M., Kawase, T., and Granger, D. N. (2008). Blood cell-derived RANTES mediates cerebral microvascular dysfunction, inflammation, and tissue injury after focal ischemia-reperfusion. *Stroke* 39, 2560–2570.

Terao, Y., Ohta, H., Oda, A., Nakagaito, Y., Kiyota, Y., and Shintani, Y. (2009). Macrophage inflammatory protein-3alpha plays a key role in the inflammatory cascade in rat focal cerebral ischemia. *Neurosci. Res.* 64, 75–82.

Wei, Y., Yemisci, M., Kim, H. H., Yung, L. M., Shin, H. K., Hwang, S. K., Guo, S., Qin, T., Alsharif, N., Brinkmann, V., Liao, J. K., Lo, E. H., and Waeber, C. (2011). Fingolimod provides long-term protection in rodent models of cerebral ischemia. *Ann. Neurol.* 69, 119–129.

Xiong, X., Barreto, G. E., Xu, L., Ouyang, Y. B., Xie, X., and Giffard, R. G. (2011). Increased brain injury and worsened neurological outcome in interleukin-4 knockout mice after transient focal cerebral ischemia. *Stroke* 42, 2026–2032.

Yadav, A., Kalita, A., Dhillon, S., and Banerjee, K. (2005). JAK/STAT3 pathway is involved in survival of neurons in response to insulin like growth factor and negatively regulated by suppressor of cytokine signal-3. *J. Biol. Chem.* 280, 31830–31840.

Yamashita, T., Sawamoto, K., Suzuki, S., Suzuki, N., Adachi, K., Kawase, T., Mihara, M., Ohsugi, Y., Abe, K., and Okano, H. (2005). Blockade of interleukin-6 signaling aggravates ischemic cerebral damage in mice: possible involvement of Stat3 activation in the protection of neurons. *J. Neurochem.* 94, 459–468.

Yanai, H., Ban, T., Wang, Z., Choi, M. K., Kawamura, T., Negishi, H., Nakasato, M., Lu, Y., Hangai, S., Koshiba, R., Savitsky, D., Ronfani, L., Akira, S., Bianchi, M. E., Honda, K., Tamura, T., Kodama, T., and Taniguchi, T. (2009). HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. *Nature* 462, 99–103.

Yang, Q. W., Lu, F. L., Zhou, Y., Wang, L., Zhong, Q., Lin, S., Xiang, J., Li, J. C., Fang, C. Q., and Wang, J. Z. (2011). HMBG1 mediates ischemia-reperfusion injury by TRIF-adaptor independent Toll-like receptor 4 signaling. *J. Cereb. Blood Flow Metab.* 31, 593–605.

Yilmaz, G., Arumugam, T. V., Stokes, K. Y., and Granger, D. N. (2006). Role of T lymphocytes and interferon-gamma in ischemic stroke. *Circulation* 113, 2105–2112.

Zhang, J., Takahashi, H. K., Liu, K., Wake, H., Liu, R., Maruo, T., Date, I., Yoshino, T., Ohtsuka, A., Mori, S., and Nishibori, M. (2011). Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats. *Stroke* 42, 1420–1428.

Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, K., Itagaki, K., and Hauser, C. J. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464, 104–107.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 April 2012; paper pending published: 30 April 2012; accepted: 08 May 2012; published online: 31 May 2012.

Citation: Shichita T, Sakaguchi R, Suzuki M and Yoshimura A (2012) Post-ischemic inflammation in the brain. Front. Immun. ***3****:132. doi: 10.3389/fimmu.2012.00132*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 29 March 2012
doi: 10.3389/fimmu.2012.00064

Pathogenic CD8 T cells in multiple sclerosis and its experimental models

Eric S. Huseby*, Priya G. Huseby, Shivanee Shah, Rebecca Smith and Brian D. Stadinski

Department of Pathology, University of Massachusetts Medical School, Worcester, MA, USA

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Marco Prinz, Universitätsklinikum Freiburg, Germany
Hideki Ogura, Osaka University, Japan

****Correspondence:***
Eric S. Huseby, Department of Pathology, University of Massachusetts Medical School, Worcester, MA 01655, USA.
e-mail: eric.huseby@umassmed.edu

A growing body of evidence suggests that autoreactive CD8 T cells contribute to the disease process in multiple sclerosis (MS). Lymphocytes in MS plaques are biased toward the CD8 lineage, and MS patients harbor CD8 T cells specific for multiple central nervous system (CNS) antigens. Currently, there are relatively few experimental model systems available to study these pathogenic CD8 T cells *in vivo*. However, the few studies that have been done characterizing the mechanisms used by CD8 T cells to induce CNS autoimmunity indicate that several of the paradigms of how CD4 T cells mediate CNS autoimmunity do not hold true for CD8 T cells or for patients with MS. Thus, myelin-specific CD4 T cells are likely to be one of several important mechanisms that drive CNS disease in MS patients. The focus of this review is to highlight the current models of pathogenic CNS-reactive CD8 T cells and the molecular mechanisms these lymphocytes use when causing CNS inflammation and damage. Understanding how CNS-reactive CD8 T cells escape tolerance induction and induce CNS autoimmunity is critical to our ability to propose and test new therapies for MS.

Keywords: multiple sclerosis, experimental autoimmune encephalomyelitis, T cells, tolerance, autoimmunity, central nervous system, MHC, TCR

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is the most common neurological disease of young adults with over one million individuals worldwide afflicted by the disease (Noseworthy et al., 2000; Hafler et al., 2005; McFarland and Martin, 2007; Steinman, 2009). MS is an inflammatory disease of the central nervous system (CNS) that causes the demyelination of nerve cells and destroys oligodendrocytes, neurons, and axons (Lucchinetti et al., 2000; Frohman et al., 2006; Dutta and Trapp, 2007). Traditionally it has been thought that MS is an inflammatory disease primarily localized to the white matter of the brain and spinal cord. However, more recent studies have identified gray matter lesions in MS patients that appear at the earliest stages, accumulate over time, and exceed white matter lesions in progressive MS patients (Peterson et al., 2001; Bo et al., 2003; Calabrese et al., 2007; Lassmann et al., 2007; Fisher et al., 2008; Ontaneda et al., 2011). Within active MS lesions, the inflammatory immune cells are predominantly T cells and activated macrophages and microglia, and often form focal demyelinating plaques (Frohman et al., 2006; Lassmann et al., 2007). The targeting of the CNS by immune cells and the ensuing death of neuronal tissue causes a wide spectrum of disease pathologies in MS patients (Noseworthy et al., 2000; Keegan and Noseworthy, 2002; Hafler et al., 2005; Frohman et al., 2006). The disease course can be progressive with steadily increasing neurological deficits, or manifest as a relapsing–remitting disease, with discrete attacks of disease symptoms followed by periods of clinical stability (Steinman, 2009). The clinical signs of MS are highly variable. MS patients often have symptoms of upper motor neuron disease that include hyperreflexia, ataxia, spasticity, and visual defects. In some cases there is evidence of lower motor neuron disease such as sensory defects and partial or complete paralysis (Keegan and Noseworthy, 2002).

It is hypothesized that MS is a T cell mediated autoimmune disease. This hypothesis is primarily based on the genetic susceptibility of individuals for the disease, the presence of immune cells within active MS plaques and animal models of CNS autoimmunity mediated by T cells (McFarland and Martin, 2007; Steinman, 2009). The genetic susceptibility for MS is polygenic; however, the most prominent predisposing genetic element is genes within the major histocompatibility complex (MHC) locus (Fogdell-Hahn et al., 2000; Sospedra and Martin, 2005; Hafler et al., 2007; Olsson and Hillert, 2008; Sawcer et al., 2011). The MHC locus is a large gene cluster consisting of over 200 expressed genes. Due to extensive linkage disequilibrium it has been difficult to parse out the precise predisposing genes. In MS, it is now clear that the HLA-DR15 and HLA-DQ6 alleles of MHC class II have strong disease susceptibility association with MS (Hafler et al., 2007; Olsson and Hillert, 2008; Sawcer et al., 2011). These particular MHC alleles may be displaying particular sets of peptide antigens to autoreactive T cells (Wucherpfennig, 2001). In addition to MHC genes, polymorphisms in multiple immunologically relevant genes, including IL-7R and IL-2R, have been associated with MS susceptibility (Hafler et al., 2007; Sawcer et al., 2011). Therefore, one hypothesis has been that predisposing MHC class II alleles present particular disease relevant epitopes to dysregulated, pathogenic T cells. However, other MHC class II alleles can confer some protection from disease even when the susceptible MHC class II allele is expressed (Olsson and Hillert, 2008; Ramagopalan et al., 2009). This suggests that the role of MHC in autoimmunity is

likely to be multi-layered, involving functions from multiple gene products.

CD4 T cells specific for many CNS proteins, including myelin associated glycoprotein (MAG), myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP), can be isolated from MS patients and healthy individuals (Sospedra and Martin, 2005). These data, combined with evidence that MHC class II alleles confer the strongest genetic susceptibility, has suggested that in patients with MS, CNS protein-specific CD4 T cells become activated, cross the blood/brain barrier, and induce CNS autoimmunity (Sospedra and Martin, 2005; Steinman, 2009). Further support for this hypothesis comes from the many animal models of CD4 T cell mediated experimental autoimmune encephalomyelitis (EAE), that manifest clinical and pathological symptoms with many similarities to MS (Kuchroo et al., 2002; Ercolini and Miller, 2006; Goverman, 2009).

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Animal models of CNS autoimmunity are primarily focused on the role of Th1 and Th17 phenotype CD4 T cell mediated pathology (Bettelli et al., 2007; Goverman, 2009). The majority of CNS proteins identified that can be targets of EAE-inducing CD4 T cells are components of compact myelin or proteins associated with myelin synthesis (Sospedra and Martin, 2005). The classical animal model of MS, CD4 T cell mediated EAE (CD4–EAE), is induced by immunizing animals with myelin proteins or peptides emulsified in complete Freund's adjuvant. This protocol predominantly induces Th1 and Th17 phenotype CD4 T cell responses, and is a poor elicitor of CD8 responses (Zamvil and Steinman, 1990; Cua et al., 2003). In addition, CD4–EAE is induced by transferring CNS-specific Th1 or Th17 CD4 T cells into naïve recipient animals (Zamvil and Steinman, 1990; Langrish et al., 2005; Lees et al., 2008; O'Connor et al., 2008; Jager et al., 2009). Similar to patients with MS, the disease course of CD4–EAE can be acute, relapsing/remitting, or chronic progressive. However, the disease pathologies associated with CD4–EAE are usually less diverse than the disease symptoms in MS patients (Steinman and Zamvil, 2005; Friese et al., 2006; Gold et al., 2006).

The major mouse models of EAE–MOG induced EAE in C57BL/6 mice, MBP induced EAE in B10.PL mice, and PLP mediated disease in SJL mice – present symptoms of ascending flaccid paralysis (Zamvil and Steinman, 1990; Vanderlugt and Miller, 2002). The disease begins with a loss of tail tone and can progress through an ascending paralysis affecting the rear legs, followed by the fore legs until the animals become morbid. These pathologies are reminiscent of MS patients with lower motor neuron disease (Keegan and Noseworthy, 2002). There are some models of CD4–EAE that present "atypical" axial-rotatory disease, characterized by mice that roll continuously or have severe deficiency in balance without paralysis (Greer et al., 1996; Sobel, 2000; Abromson-Leeman et al., 2007; Lees et al., 2008; Stromnes et al., 2008). Several studies have shown that the ratio of Th1 versus Th17, or the ability of CNS cells to respond to IFNγ signaling, contributes to the location of affected CNS area and/or the presentation of classical or atypical disease symptoms (Lees et al., 2008; Stromnes et al., 2008; Jager et al., 2009). The genetic background of mice that get CD4 T cell mediated atypical EAE include C57BL/6, Balb/c, and C3H mice, genetic backgrounds that also develop classical ascending paralytic disease when immunized with different CNS antigens (Sobel, 2000). Thus, there are indications that the immunizing antigen, T cell trafficking and/or the phenotype of the CD4 T cells, and not necessarily the genetic background, may be determining factors in the induction of non-classical disease symptoms of EAE. Overall, although there are heterogenic disease pathologies induced by CD4 T cells, CD4–EAE still likely mimics some, but not all, aspects of CNS inflammation in MS.

Autoreactive CD4 T cells alone are unlikely to fully account for all of the effector mechanisms that drive the disease process in MS. Disease inducing, myelin-specific CD4 T cells within the CNS release the signature Th1 and Th17 cytokines. The release of cytokines and chemokines causes an increase in MHC expression within the CNS, leads to the activation of microglia, and induces the recruitment of monocytes and non-specific T cells to the area of inflammation (Prineas, 1979; Raine et al., 1984; Carson et al., 1999; Brabb et al., 2000; Krakowski and Owens, 2000; Juedes and Ruddle, 2001; Becher et al., 2006; Mildner et al., 2008). Several of these cytokines, including IFNγ, TNFα, IL-6, IL-12, and IL-23, have been directly implicated in disease pathogenesis (Krakowski and Owens, 1996; Okuda et al., 1998; Sean Riminton et al., 1998; Steinman, 2001; Cua et al., 2003; Deshpande et al., 2006). Using the CD4–EAE disease model as a screen, several therapies that ameliorate CD4–EAE have been developed for clinical trials to reduce symptoms in MS patients. While some of the treatments validated in the CD4–EAE model have reduced disease symptoms in MS patients, other therapies have not affected clinical symptoms, or have been detrimental to patients (Steinman and Zamvil, 2005; Friese et al., 2006; Gold et al., 2006). For example, the removal of CD4 T cells or TNFα from MS patients had little effect on disease symptoms, while both abolish disease in CD4–EAE models (Waldor et al., 1985; Llewellyn-Smith et al., 1997; van Oosten et al., 1997; Sean Riminton et al., 1998; Lenercept, 1999). Thus, CD4–EAE probably mimics only a subset of the events that occur during MS. The discovery of other key events in the pathogenesis of MS disease will be facilitated by animal models that mimic events not already evoked by CD4 T cells.

CD8 T CELLS IN MULTIPLE SCLEROSIS

The lymphocytes in MS plaques are biased toward the CD8 lineage. This bias occurs regardless of the stage of activity or disease, and can be as prominent as 10:1, CD8 versus CD4 T cells (Booss et al., 1983; Traugott et al., 1983b; Hauser et al., 1986; Babbe et al., 2000; Lassmann et al., 2007). At the margin of chronic and active lesions, CD8 T cells have been identified interacting with antigen presenting cells (APC; Serafini et al., 2006). Through normally poorly expressed, MHC class I proteins are highly expressed within the MS lesion on astrocytes, oligodendrocytes, and neurons, implying that CD8 T cells could be directly engaging these cell types as well (Traugott et al., 1983a; Wong et al., 1984a; Neumann et al., 1995, 1997). CD8 T cells with an activated/memory phenotype have been observed to be enriched within the CNS tissue and cerebrospinal fluid (CSF) of MS patients (Jacobsen et al., 2002; Junker et al., 2007), and histological analysis of some cases of acute MS have shown granzyme B-expressing CD8 T cells in close proximity or attached to oligodendrocytes or demyelinated axons

(Neumann et al., 2002; Serafini et al., 2006; Lassmann et al., 2007). Interestingly, Fugger and colleagues have found that a large percentage of CD8 T cells in acute and chronic MS lesions express IL-17 (Tzartos et al., 2008), a highly pro-inflammatory cytokine associated with pathogenic Th17 CD4 T cells and Tc17 CD8 T cells (Cua et al., 2003; Bettelli et al., 2007). This activated/memory phenotypic skewing of CD8 T cells within the CSF occurs even in early diagnosed MS patients. Furthermore, Brück and colleagues demonstrated that the number of CD8 T cells and macrophages within the lesions correlates with the extent of acute axonal damage, as defined by the accumulation of amyloid precursor protein (APP; Bitsch et al., 2000). In addition, genetic association studies have identified some MHC class I alleles, including HLA-A3, as a genetic susceptibility allele (Friese and Fugger, 2005; Sawcer et al., 2011). In contrast, the MHC class I allele, HLA-A*0201, has been shown to reduce the risk of MS in individuals that express the MHC class II proteins, HLA-DRB1*1501, and DQB1*06 (Fogdell-Hahn et al., 2000; Brynedal et al., 2007). These observations support the idea that CD8 T cells are involved in pathogenesis of MS, as active contributors to the development of MS lesions, and/or as protective regulatory T cell populations that limit disease.

Several groups have isolated T cells from the blood, CSF, and MS lesions (via microdissection), and analyzed these lymphocytes by complementarity determining region 3 (CDR3) spectratyping to determine the diversity of T cell clones. Initial studies by Babbe et al. (2000) demonstrated that the majority of CD8 T cells within an MS plaque were progeny of a few initial clones. One particular clone accounted for 35% of the CD8 T cells within an MS plaque. Later studies analyzing larger numbers of MS patients demonstrated that CD8 T cells isolated from CSF fluid also contained clonal expansions, some of which persisted for 5 years (Jacobsen et al., 2002; Skulina et al., 2004). To identify the antigen specificity of these CD8 T cells, paired TCRα, and TCRβ chains have been cloned and re-expressed in T cell lines (Seitz et al., 2006). However, the antigen specificity of these CD8 T cells has yet to be determined. Although the specificity of the CD8 T cells in MS plaques is unknown, their clonal nature suggests that they are responding against selected antigens.

Central nervous system-reactive CD8 T cells may be pathogenic in MS patients. Pioneering studies by Biddison and colleagues and Hafler and colleagues identified and analyzed CD8 T cells specific for several CNS proteins, including MAG, MBP, and PLP, from MS patients (Tsuchida et al., 1994; Dressel et al., 1997). These studies clearly demonstrated that MS patients carry a population of CD8 T cells specific for myelin proteins. These MAG-, MBP-, and PLP-specific CD8 T cells are capable of killing neuronal cells *in vitro* and release TNFα and IFNγ, suggesting that they may contribute to CNS disease, although the precise role of these cells in the pathogenesis of MS has not been fully elucidated (Tsuchida et al., 1994; Jurewicz et al., 1998; Medana et al., 2001). More recent analysis of peripheral blood suggests that MS patients carry CD8 T cells able to express IFNγ and TNFα in response to a multitude of CNS protein epitopes. Several studies have demonstrated that these CNS-reactive CD8 T cells were found in greater frequency and bore an activated/memory phenotype in MS patients, suggesting they had been activated within the patient by CNS antigens (Crawford et al., 2004; Zang et al., 2004; Niland et al., 2005). In contrast, these same T cells isolated from control subjects displayed a naïve T cell phenotype. Differences in the frequency of CNS antigen-specific CD8 T cells between MS patients and control subjects have not always been observed, as myelin-reactive CD8 T cells have been identified in healthy subjects as well (Berthelot et al., 2008). This group of studies clearly indicates that individuals carry a population of CD8 T cells specific for a variety of CNS antigens. The presence of CNS-reactive T cells exhibiting an activated/memory phenotype in MS patients and the oligoclonal nature of CD8 T cells within MS plaques suggest that CD8 T cells are active participants within the destructive CNS immune response.

In addition to a pathogenic role for CD8 T cells in MS, regulatory CD8 T cells may contribute to limiting disease severity or occurrence in MS patients. Early experiments demonstrated that the suppressor function of CD8 T regulatory cells in MS patients may be defective as compared to healthy individuals (Antel et al., 1986). Consistent with these findings, a specific CD8 T cell clone has been shown to regulate MBP-specific CD4 T cells (Chou et al., 1992). CD8 T cells that can lyse myelin-specific CD4 T cells have been detected in MS patients, and vaccination of MS patients with irradiated myelin-specific CD4 T cells elicited CD8 T cells that could specifically kill these CD4 T cells (Zhang et al., 1993; Correale et al., 2000). More recently, longitudinal magnetic resonance imaging (MRI) analysis has shown a negative correlation between the percentage of Tc2 cytokine-producing CD8 T cells in the periphery of MS patients and the development of lesions (Killestein et al., 2003). Furthermore, the beneficial effects of treating MS patients with glatiramer acetate may in part be due to activating regulatory CD8 T cells (Tennakoon et al., 2006). In addition to these, and many other studies of human CD8 T regulatory cells, mouse models have also shown potent disease modifying effects of CD8 T regulatory cells through the secretion of IL-10 and other soluble mediators, the regulation of APC function, as well as by eliminating activated CD4 T cells by CD8 T cells via recognition of the non-classical Qa-1 MHC molecule (Jiang and Chess, 2006; Goverman, 2009).

ANIMAL MODELS OF CD8 T CELL MEDIATED CNS AUTOIMMUNITY

Several years ago we and others started developing CD8 T cell models of CNS autoimmunity. At the time, MS was generally believed to be primarily a CD4 T cell mediated autoimmune disease. However, because MS plaques contain a large excess of CD8 T cells relative to CD4 T cells, we were concerned that a major lymphocyte population within the inflamed site was being under studied. Because of the cytotoxic and pro-inflammatory nature of CD8 T cells, we hypothesized that myelin-specific CD8 T cells contribute to CNS autoimmunity. Our studies focused on whether MBP-specific CD8 T cells could induce CNS autoimmunity. Because MBP is a self protein, we were concerned that CD8 T cells specific for MBP might be subject to immune tolerance mechanisms. To avoid tolerance issues, we initially isolated MBP-reactive CD8 T cells from C3H MBP-deficient *shiverer* (C3H $MBP^{-/-}$) mice (Huseby et al., 1999). These studies demonstrated that MBP-specific CD8 T cells, similar to several CD4 T cell epitopes of MBP and PLP, are subject to immune tolerance (Goverman, 2011). However, some

MBP-specific CD8 T cells are present in the peripheral repertoire of wild-type C3H mice. Furthermore, the activation of MBP-specific CD8 T cells in animals induces severe, demyelinating CNS autoimmunity (Huseby et al., 2001a).

The pathology of MBP-specific CD8 T cell mediated disease had many similarities to some human MS patients (Lucchinetti et al., 2000), including the presence of lesions throughout the brain (Huseby et al., 2001a). Histological analysis of diseased brains indicated the lesions were vascular in nature, involving capillaries and venules, and consisted of perivascular cuffing. Vacuolation of the surrounding nervous tissue was common and consisted of demyelination and cytoplasmic swelling. In contrast to many typical CD4–EAE models, this MBP-specific CD8 T cell mediated disease model had a predominance of lesions in the brain instead of the spinal cord, displayed a general lack of inflammation (except that which was directly associated with vascular walls), and showed severe demyelination and perivascular cell death, suggesting a cytotoxic or ischemic injury. Antibody blocking experiments demonstrated a role for IFNγ in contributing to disease severity (Huseby et al., 2001a). Using TCR Tg mice that generate MBP-specific CD8 T cells, Goverman and colleagues have gone on to show how endogenous MBP influences this T cell repertoire (Perchellet et al., 2004, 2008), and have demonstrated that viruses can induce CD8 T cell-mediated autoimmunity by breaking peripheral tolerance mechanisms (Ji et al., 2010).

CD8 T cell lines reactive to the MOG derived peptide, $MOG_{35–55}$, can induce chronic CNS autoimmunity in C57BL/6 mice (Sun et al., 2001; Ford and Evavold, 2005). Unlike the disease symptoms induced by MBP-specific CD8 T cells, these MOG-reactive CD8 T cell lines induced ascending flaccid paralysis, and caused lesions in both the spinal cord and brain. The reason for the differences in disease symptoms between the MBP and MOG models of CD8–EAE is unknown. The differences could be due to the CD8 T cells targeting different proteins or due to differences between the C3H and C57BL/6 genetic backgrounds.

Humanized mouse models have been used to study CD8 T cell responses targeting myelin epitopes presented by human HLA-A molecules. CD8 T cells targeting $MOG_{181–189}$, presented by the MHC class I allele, HLA-A*0201, were observed to potentiate an autoreactive CD4 T cell response by accelerating and worsening the encephalitogenic process (Mars et al., 2007). Friese et al. (2008) have more recently generated a humanized mouse model of MS in which an MHC class I-restricted TCR specific for $PLP_{45–53}$ bound to HLA-A*0301, isolated from an MS patient, is expressed in mice which also express the MHC Class I molecule HLA-A*0301. These mice, constructed on a C57BL/6 genetic background, develop a low grade spontaneous MS-like disease which becomes more severe upon immunization with PLP peptides. Following immunization with PLP peptides, there are two phases of disease, an early infiltration that is dominated by CD8 T cells and late disease that requires MHC class II expression. Coincident with the late disease is the expansion within the CNS of CD4 T cells reactive to $MOG_{35–55}$. These experiments nicely demonstrate that T cells expressing a human MHC class I-restricted TCR specific for a CNS protein can be pathogenic and can induce epitope spreading to the CD4 T cell compartment. When the human TCR Tg mice were crossed onto mice expressing HLA-A 0301 and the protective MHC class I allele, HLA-A*0201, thymocytes expressing this PLP-specific TCR were subject to elimination by negative selection. T cells expressing the $PLP_{45–53}$-specific TCR did not recognize the $PLP_{45–53}$ epitope bound to HLA-A*0201, suggesting the elimination of these pathogenic T cells was not induced by recognizing endogenous PLP (Friese et al., 2008). The induction of negative selection in mice expressing HLA-A*0201 support the hypothesis that one of the protective effects of specific MHC class I alleles is the elimination of pathogenic T cells during development.

Complementing the active induction models are a set of mice which spontaneously succumb to CD8 T cell-dependent CNS autoimmunity. Fournier and colleagues observed that transgenic C57BL/6 mice, in which the co-stimulatory molecule CD86 is constitutively expressed on peripheral T cells and resident CNS microglia, succumb to a CD8 T cell-dependent CNS demyelinating disease (Brisebois et al., 2006). CNS infiltration by CD8 T cells results in mice which display a deterioration of hind-limb control and coordination, weak tail movement, and weight loss. The age at which these mice succumb to spontaneous disease is younger in both MHC class II-deficient mice as well as CD4-deficient mice, suggesting the pathogenic CD8 T cells are being regulated by a CD4 T cell populations. The specificity of these pathogenic CD8 T cells has yet to be determined. The non-paralytic disease symptoms and pathologies in CD86 transgenic mice are similar to the MBP-specific CD8 T disease model in C3H mice, while differing from the paralytic disease mediated by $MOG_{35–55}$ specific CD8 T cells. Two additional models have recently been observed in which pathogenic CD8 T cells induce both clinical signs of CNS disease as well as demyelination. Mice over-expressing PLP within oligodendrocytes of the CNS developed progressive clinical signs of neurological damage including ataxia, tremors, and seizures after 12–18 months of age that was dependent up CD8 T cells (Ip et al., 2006). CD8 T cells and macrophages accumulated within the brains of these mice, with a limited B cell and CD4 T cell component. Affected areas of the CNS included the optic nerve, cerebral white matter, and spinal cord. Mice in which the oligodendrocytes are deficient in peroxisome show a similar axonal loss and neuroinflammation as the PLP over-expressing mice (Kassmann et al., 2007). The antigen specificity of the CNS infiltrating CD8 T cells remains unknown in both models. These spontaneous models, along with the active induction models, further suggest that CD8 T cells can induce a range of different disease manifestations, and clearly demonstrate that CNS-specific CD8 T cells are present in the peripheral T cell repertoire and when activated, can induce CNS autoimmunity.

CELLULAR TARGETS OF PATHOGENIC CD8 T CELLS

The cellular target of pathogenic myelin-specific CD8 T cells *in vivo* is unknown. Normally, there is very little MHC class I expression in the CNS. Neurons and oligodendrocytes express only low levels of MHC class I, constitutively. Astrocytes, microglia, blood vessel endothelial cells, and bone marrow derived-APC (BM-APC) resident within the CNS do express MHC class I constitutively, though none of these cells synthesize myelin antigens (Traugott et al., 1983a; Wong et al., 1984a; Massa et al., 1993; Neumann et al., 1995, 1997; Hoftberger et al., 2004; Frohman et al., 2006). However, BM-APC, and to a lesser extent, blood vessel

endothelial cells, can cross-present exogenously synthesized proteins on MHC class I proteins (Limmer et al., 2000; Rock and Shen, 2005; Galea et al., 2007). Thus, the initial recognition of myelin peptides presented on MHC class I proteins is likely by BM-APC or blood vessel endothelial cells. Whether CD8 T cells can directly lyse oligodendrocytes *in vivo* is unknown. MHC expression by oligodendrocytes does increase after inflammation or in response to IFNγ, therefore it may be possible for CD8 T cells to directly target oligodendrocytes once inflammation begins (Wong et al., 1984a,b; Tsuchida et al., 1994; Neumann et al., 1997; Hoftberger et al., 2004). Thus, it is not surprising that when we and others neutralized IFNγ, the severity of CD8–EAE disease is drastically reduced (Huseby et al., 2001a; Brisebois et al., 2006).

To begin to identify which CNS cell types could be targets of CD8 T cells, several groups have created transgenic mice which express neo-self antigens in specific cell populations. The first of these models, created by the Oldstone group, expressed the lymphocytic choriomeningitis virus (LCMV) glycoprotein under the MBP promoter (Evans et al., 1996). These mice expressed very low levels of the LCMV protein exclusively in the brain, likely in oligodendrocytes. Following two subsequent viral infections, focal areas of myelin degradation were observed, and expression of both MHC class I and class II molecules was found on oligodendrocytes. These data suggested that CD8 and/or CD4 T cells may interact directly with oligodendrocytes and play a role in oligodendrocyte injury. More recently, two sets of mice expressing either ovalbumin (OVA) or the influenza virus hemagglutinin (HA) proteins selectively within oligodendrocytes, have been constructed and analyzed.

When mice expressing OVA selectively in oligodendrocytes (ODC–OVA) were genetically crossed with OT-1 mice, transgenic mice expressing a TCR specific for $OVA_{257–264}$ presented by H2-K^b, the double transgenic mice succumbed to an extremely aggressive, lethal fulminant demyelinating CNS autoimmunity (Na et al., 2008). Lesions within the cerebellum, brainstem, optic nerve, and spinal cord were observed. *In vitro*, OVA-transgenic oligodendrocytes were capable of activating naïve OT-I cells. The potency of OVA-expressing oligodendrocytes to activate OT-I CD8 T cells *in vitro* was further amplified by the addition of exogenous IFNγ to the cultures. The secretion of IFNγ from OT-1 T cells was also found to be required for the loss of oligodendrocytes, as well as axon damage within the CNS. In contrast to this model, Balb/c mice expressing the HA protein in oligodendrocytes (MOG–HA) did not develop spontaneous autoimmunity when crossed with transgenic mice expressing an HA-specific TCR, Cl4, that recognizes $HA_{512–520}$ presented by H2-K^d (Saxena et al., 2008). In these mice, the HA-specific CD8 T cells maintained ignorance to the neo-self protein. However, transfer of *in vitro* activated HA-specific CD8 T cells induced weight loss, demyelination, and reduced mobility, but not paralysis. The inflammatory lesions were primarily within the spinal cord and optic nerve. HA-specific CD8 T cells within the CNS were found in close apposition with oligodendrocytes and some had polarized Granzyme B containing granules toward the oligodendrocytes (Saxena et al., 2008). The loss of oligodendrocytes prior to demyelination suggests these CD8 T cells are directly killing these cells. Interestingly, when these same HA-specific CD8 T cells are transferred into mice expressing the HA protein selectively in astrocytes, the mice developed a monophasic brain inflammation with an absence of any clinical signs of disease (Cabarrocas et al., 2003). Combined, these data suggest that CD8 T cells, which have a strong reactivity for an epitope that is processed and presented well on MHC class I molecules, can directly target oligodendrocytes within the CNS. The differences in disease course and severity among the transgenic neo-self models of CD8 T cell mediated CNS autoimmunity further suggests that a multitude of factors, including target antigen expression level, CNS cell type expression, TCR–pMHC affinity, and genetics, likely contribute to whether a CD8 T cell response is pathogenic.

WHY ARE PATHOGENIC CNS-REACTIVE CD8 T CELLS PRESENT WITHIN THE MATURE T CELL REPERTOIRE?

The T cell mediated autoimmune nature of MS and EAE indicates that T cell tolerance of CNS proteins is incomplete. During T cell development, TCR undergo somatic recombination of their peptide and MHC binding CDR3 loops, as well as TCRα/β chain pairing (Davis and Bjorkman, 1988). The immense TCR diversity generated during T cell development allows the adaptive immune response to be able to generate specific T cell responses to unknown pathogens. This process, however, also creates self-reactive T cells. Many of these self-reactive T cells are purged during negative selection, due to the expression of the self protein within the thymus by dendritic cells or medullary thymic epithelial cells (Kappler et al., 1987; Kisielow et al., 1988; Mathis and Benoist, 2004; Kyewski and Klein, 2006). However, some self-reactive T cells that escape negative selection are then subject to peripheral tolerance mechanisms (Zheng and Rudensky, 2007). Autoimmune diseases arise when failures of thymic and peripheral tolerance mechanisms allow self-reactive T cells to be activated and attack healthy tissues. It has been clearly established that endogenous MBP induces some tolerance to the CD4 and CD8 T cell repertoire (Goverman, 2011). PLP also appears to induce some tolerance to the CD4 T cell repertoire specific for it (Kuchroo et al., 2002). Because MBP and PLP are expressed at extremely high levels within the CNS and some isoforms of these proteins are expressed within the thymus (albeit at extremely low levels by medullary thymic epithelial cells), it is not surprising some T cell tolerance occurs (Kyewski and Klein, 2006). Negative selection can also occur to T cells specific for CNS proteins not expressed in the thymus. Our studies of CD4 T cells specific for $MBP_{121–140}$, an epitope that is not expressed in the thymus, indicate that BM-APC can acquire MBP from exogenous sources and cause the elimination of developing T cells (Huseby et al., 2001b). The extremely low level of MOG (around 1,000–5,000 times less abundant than MBP and PLP within the CNS) does not appear to induce significant tolerance to the CD4 T cell repertoire (Delarasse et al., 2003). It has not been determined whether other myelin proteins induce CD4 T cell tolerance, nor is it known whether CNS proteins other than MBP induce tolerance to the CD8 T cell repertoire.

There are three main hypotheses for how pathogenic myelin-reactive T cells escape tolerance induction in the thymus, primarily derived from studies of CD4 T cell responses: (1) T cells specific for exons of myelin proteins neither expressed nor presented within the thymus fail to be deleted because the antigen is not presented to

developing thymocytes (Kuchroo et al., 2002; Kyewski and Klein, 2006). (2) For myelin proteins that are presented well in the thymus, central tolerance eliminates thymocytes expressing TCR that have a strong reactivity for these myelin antigens while allowing thymocytes expressing TCR which have a weak reactivity to develop (Goverman, 2011). Thymocytes that escape this form of central tolerance may express TCR with a low affinity for myelin epitopes bound to self-MHC proteins, or express TCR that bind myelin epitopes bound to self-MHC with unconventional binding modes (Wucherpfennig et al., 2009). (3) Autoreactive T cells specific for myelin proteins that are expressed in the thymus but are poorly presented (e.g., peptide sequences that bind MHC proteins poorly) may not be deleted because the amount of MHC + peptide complex in the thymus is very low (Goverman et al., 1993; Liu et al., 1995). Myelin-specific T cells expressing low affinity TCR, TCR that bind pMHC with unconventional bind modes, or TCR that target epitopes that are poorly presented may be pathogenic because the expression level of the myelin protein is much higher in the CNS than thymus, and thus APC within the CNS can present enough MHC + peptide complexes to activate these lymphocytes.

Do these models of T cell tolerance to myelin antigens hold true for CD8 T cells as well? Though little is currently known about how CNS-reactive CD8 T cells escape tolerance induction, it was very surprising that some CD8 T cells with a strong reactivity for $MBP_{79–87}$ were allowed to develop and seed the mature T cell repertoire (Huseby et al., 2001a). This myelin epitope is expressed within the thymus and most T cells with a strong reactivity for this epitope are eliminated. Using a completely novel mechanism to escape tolerance induction, Perchellet et al. (2004) found that $MBP_{79–87}$-specific CD8 T cells could avoid both central and peripheral deletion by removing $H2K^k$–MBP_{79-87} complexes from APC without proliferating. Thus, the major form of immune regulation that governs myelin-specific CD4 T cells may not fully explain the development and control of CNS-reactive CD8 T cells. To understand why CNS autoimmunity arises, it will be important to identify the pathways in which CNS-reactive CD4 and CD8 T cells are normally eliminated or regulated, and why these processes fail in patients with MS. Overall, how similar or different the contribution of CNS-reactive CD4 versus CD8 T cells to the disease pathologies of MS is just beginning to be understood.

ACKNOWLEDGMENTS

The authors would like to thank the grant support from the NIH, grant to Eric S. Huseby (RAI088495A), and a NIH training grant to Brian D. Stadinski (T32 AI 007349). Eric S. Huseby is a member of the UMass DERC (grant DK32520).

REFERENCES

Abromson-Leeman, S., Ladell, D. S., Bronson, R. T., and Dorf, M. E. (2007). Heterogeneity of EAE mediated by multiple distinct T-effector subsets. *J. Neuroimmunol.* 192, 3–12.

Antel, J. P., Bania, M. B., Reder, A., and Cashman, N. (1986). Activated suppressor cell dysfunction in progressive multiple sclerosis. *J. Immunol.* 137, 137–141.

Babbe, H., Roers, A., Waisman, A., Lassmann, H., Goebels, N., Hohlfeld, R., Friese, M., Schroder, R., Deckert, M., Schmidt, S., Ravid, R., and Rajewsky, K. (2000). Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J. Exp. Med.* 192, 393–404.

Becher, B., Bechmann, I., and Greter, M. (2006). Antigen presentation in autoimmunity and CNS inflammation: how T lymphocytes recognize the brain. *J. Mol. Med.* 84, 532–543.

Berthelot, L., Laplaud, D. A., Pettre, S., Ballet, C., Michel, L., Hillion, S., Braudeau, C., Connan, F., Lefrere, F., Wiertlewski, S., Guillet, J. G., Brouard, S., Choppin, J., and Soulillou, J. P. (2008). Blood CD8+ T cell responses against myelin determinants in multiple sclerosis and healthy individuals. *Eur. J. Immunol.* 38, 1889–1899.

Bettelli, E., Oukka, M., and Kuchroo, V. K. (2007). T(H)-17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* 8, 345–350.

Bitsch, A., Schuchardt, J., Bunkowski, S., Kuhlmann, T., and Bruck, W. (2000). Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. *Brain* 123(Pt 6), 1174–1183.

Bo, L., Vedeler, C. A., Nyland, H. I., Trapp, B. D., and Mork, S. J. (2003). Subpial demyelination in the cerebral cortex of multiple sclerosis patients. *J. Neuropathol. Exp. Neurol.* 62, 723–732.

Booss, J., Esiri, M. M., Tourtellotte, W. W., and Mason, D. Y. (1983). Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J. Neurol. Sci.* 62, 219–232.

Brabb, T., von Dassow, P., Ordonez, N., Schnabel, B., Duke, B., and Goverman, J. (2000). In situ tolerance within the central nervous system as a mechanism for preventing autoimmunity. *J. Exp. Med.* 192, 871–880.

Brisebois, M., Zehntner, S. P., Estrada, J., Owens, T., and Fournier, S. (2006). A pathogenic role for CD8+ T cells in a spontaneous model of demyelinating disease. *J. Immunol.* 177, 2403–2411.

Brynedal, B., Duvefelt, K., Jonasdottir, G., Roos, I. M., Akesson, E., Palmgren, J., and Hillert, J. (2007). HLA-A confers an HLA-DRB1 independent influence on the risk of multiple sclerosis. *PLoS ONE* 2, e664. doi:10.1371/journal.pone.0000664

Cabarrocas, J., Bauer, J., Piaggio, E., Liblau, R., and Lassmann, H. (2003). Effective and selective immune surveillance of the brain by MHC class I-restricted cytotoxic T lymphocytes. *Eur. J. Immunol.* 33, 1174–1182.

Calabrese, M., De Stefano, N., Atzori, M., Bernardi, V., Mattisi, I., Barachino, L., Morra, A., Rinaldi, L., Romualdi, C., Perini, P., Battistin, L., and Gallo, P. (2007). Detection of cortical inflammatory lesions by double inversion recovery magnetic resonance imaging in patients with multiple sclerosis. *Arch. Neurol.* 64, 1416–1422.

Carson, M. J., Reilly, C. R., Sutcliffe, J. G., and Lo, D. (1999). Disproportionate recruitment of CD8+ T cells into the central nervous system by professional antigen-presenting cells. *Am. J. Pathol.* 154, 481–494.

Chou, Y. K., Henderikx, P., Jones, R. E., Kotzin, B., Hashim, G. A., Offner, H., and Vandenbark, A. A. (1992). Human CD8+ T cell clone regulates autologous CD4+ myelin basic protein specific T cells. *Autoimmunity* 14, 111–119.

Correale, J., Lund, B., McMillan, M., Ko, D. Y., McCarthy, K., and Weiner, L. P. (2000). T cell vaccination in secondary progressive multiple sclerosis. *J. Neuroimmunol.* 107, 130–139.

Crawford, M. P., Yan, S. X., Ortega, S. B., Mehta, R. S., Hewitt, R. E., Price, D. A., Stastny, P., Douek, D. C., Koup, R. A., Racke, M. K., and Karandikar, N. J. (2004). High prevalence of autoreactive, neuroantigen-specific CD8+ T cells in multiple sclerosis revealed by novel flow cytometric assay. *Blood* 103, 4222–4231.

Cua, D. J., Sherlock, J., Chen, Y., Murphy, C. A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T., Zurawski, S., Wiekowski, M., Lira, S. A., Gorman, D., Kastelein, R. A., and Sedgwick, J. D. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421, 744–748.

Davis, M. M., and Bjorkman, P. J. (1988). T-cell antigen receptor genes and T-cell recognition. *Nature* 334, 395–402.

Delarasse, C., Daubas, P., Mars, L. T., Vizler, C., Litzenburger, T., Iglesias, A., Bauer, J., Della Gaspera, B., Schubart, A., Decker, L., Dimitri, D., Roussel, G., Dierich, A., Amor, S., Dautigny, A., Liblau, R., and Pham-Dinh, D. (2003). Myelin/oligodendrocyte glycoprotein-deficient (MOG-deficient) mice reveal lack of immune tolerance to MOG in wild-type mice. *J. Clin. Invest.* 112, 544–553.

Deshpande, P., King, I. L., and Segal, B. M. (2006). IL-12 driven upregulation of P-selectin ligand on myelin-specific T cells is a critical step in an animal model of autoimmune demyelination. *J. Neuroimmunol.* 173, 35–44.

Dressel, A., Chin, J. L., Sette, A., Gausling, R., Hollsberg, P., and Hafler, D. A. (1997). Autoantigen recognition by human CD8 T cell clones: enhanced agonist response induced by altered peptide ligands. *J. Immunol.* 159, 4943–4951.

Dutta, R., and Trapp, B. D. (2007). Pathogenesis of axonal and neuronal damage in multiple sclerosis. *Neurology* 68, S22–S31; discussion S43–S54.

Ercolini, A. M., and Miller, S. D. (2006). Mechanisms of immunopathology in murine models of central nervous system demyelinating disease. *J. Immunol.* 176, 3293–3298.

Evans, C. F., Horwitz, M. S., Hobbs, M. V., and Oldstone, M. B. (1996). Viral infection of transgenic mice expressing a viral protein in oligodendrocytes leads to chronic central nervous system autoimmune disease. *J. Exp. Med.* 184, 2371–2384.

Fisher, E., Lee, J. C., Nakamura, K., and Rudick, R. A. (2008). Gray matter atrophy in multiple sclerosis: a longitudinal study. *Ann. Neurol.* 64, 255–265.

Fogdell-Hahn, A., Ligers, A., Gronning, M., Hillert, J., and Olerup, O. (2000). Multiple sclerosis: a modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. *Tissue Antigens* 55, 140–148.

Ford, M. L., and Evavold, B. D. (2005). Specificity, magnitude, and kinetics of MOG-specific CD8+ T cell responses during experimental autoimmune encephalomyelitis. *Eur. J. Immunol.* 35, 76–85.

Friese, M. A., and Fugger, L. (2005). Autoreactive CD8+ T cells in multiple sclerosis: a new target for therapy? *Brain* 128, 1747–1763.

Friese, M. A., Jakobsen, K. B., Friis, L., Etzensperger, R., Craner, M. J., McMahon, R. M., Jensen, L. T., Huygelen, V., Jones, E. Y., Bell, J. I., and Fugger, L. (2008). Opposing effects of HLA class I molecules in tuning autoreactive CD8(+) T cells in multiple sclerosis. *Nat. Med.* 1227–1235.

Friese, M. A., Montalban, X., Willcox, N., Bell, J. I., Martin, R., and Fugger, L. (2006). The value of animal models for drug development in multiple sclerosis. *Brain* 129, 1940–1952.

Frohman, E. M., Racke, M. K., and Raine, C. S. (2006). Multiple sclerosis – the plaque and its pathogenesis. *N. Engl. J. Med.* 354, 942–955.

Galea, I., Bernardes-Silva, M., Forse, P. A., van Rooijen, N., Liblau, R. S., and Perry, V. H. (2007). An antigen-specific pathway for CD8 T cells across the blood-brain barrier. *J. Exp. Med.* 204, 2023–2030.

Gold, R., Linington, C., and Lassmann, H. (2006). Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 129, 1953–1971.

Goverman, J. (2009). Autoimmune T cell responses in the central nervous system. *Nat. Rev. Immunol.* 9, 393–407.

Goverman, J., Woods, A., Larson, L., Weiner, L. P., Hood, L., and Zaller, D. M. (1993). Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity. *Cell* 72, 551–560.

Goverman, J. M. (2011). Immune tolerance in multiple sclerosis. *Immunol. Rev.* 241, 228–240.

Greer, J. M., Sobel, R. A., Sette, A., Southwood, S., Lees, M. B., and Kuchroo, V. K. (1996). Immunogenic and encephalitogenic epitope clusters of myelin proteolipid protein. *J. Immunol.* 156, 371–379.

Hafler, D. A., Compston, A., Sawcer, S., Lander, E. S., Daly, M. J., De Jager, P. L., de Bakker, P. I., Gabriel, S. B., Mirel, D. B., Ivinson, A. J., Pericak-Vance, M. A., Gregory, S. G., Rioux, J. D., McCauley, J. L., Haines, J. L., Barcellos, L. F., Cree, B., Oksenberg, J. R., and Hauser, S. L. (2007). Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med.* 357, 851–862.

Hafler, D. A., Slavik, J. M., Anderson, D. E., O'Connor, K. C., De Jager, P., and Baecher-Allan, C. (2005). Multiple sclerosis. *Immunol. Rev.* 204, 208–231.

Hauser, S. L., Bhan, A. K., Gilles, F., Kemp, M., Kerr, C., and Weiner, H. L. (1986). Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann. Neurol.* 19, 578–587.

Hoftberger, R., Aboul-Enein, F., Brueck, W., Lucchinetti, C., Rodriguez, M., Schmidbauer, M., Jellinger, K., and Lassmann, H. (2004). Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. *Brain Pathol.* 14, 43–50.

Huseby, E. S., Liggitt, D., Brabb, T., Schnabel, B., Ohlen, C., and Goverman, J. (2001a). A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. *J. Exp. Med.* 194, 669–676.

Huseby, E. S., Sather, B., Huseby, P. G., and Goverman, J. (2001b). Age-dependent T cell tolerance and autoimmunity to myelin basic protein. *Immunity* 14, 471–481.

Huseby, E. S., Ohlen, C., and Goverman, J. (1999). Cutting edge: myelin basic protein-specific cytotoxic T cell tolerance is maintained in vivo by a single dominant epitope in H-2k mice. *J. Immunol.* 163, 1115–1118.

Ip, C. W., Kroner, A., Bendszus, M., Leder, C., Kobsar, I., Fischer, S., Wiendl, H., Nave, K. A., and Martini, R. (2006). Immune cells contribute to myelin degeneration and axonopathic changes in mice overexpressing proteolipid protein in oligodendrocytes. *J. Neurosci.* 26, 8206–8216.

Jacobsen, M., Cepok, S., Quak, E., Happel, M., Gaber, R., Ziegler, A., Schock, S., Oertel, W. H., Sommer, N., and Hemmer, B. (2002). Oligoclonal expansion of memory CD8+ T cells in cerebrospinal fluid from multiple sclerosis patients. *Brain* 125, 538–550.

Jager, A., Dardalhon, V., Sobel, R. A., Bettelli, E., and Kuchroo, V. K. (2009). Th1, Th17, and Th9 effector cells induce experimental autoimmune encephalomyelitis with different pathological phenotypes. *J. Immunol.* 183, 7169–7177.

Ji, Q., Perchellet, A., and Goverman, J. M. (2010). Viral infection triggers central nervous system autoimmunity via activation of CD8+ T cells expressing dual TCRs. *Nat. Immunol.* 11, 628–634.

Jiang, H., and Chess, L. (2006). Regulation of immune responses by T cells. *N. Engl. J. Med.* 354, 1166–1176.

Juedes, A. E., and Ruddle, N. H. (2001). Resident and infiltrating central nervous system APCs regulate the emergence and resolution of experimental autoimmune encephalomyelitis. *J. Immunol.* 166, 5168–5175.

Junker, A., Ivanidze, J., Malotka, J., Eiglmeier, I., Lassmann, H., Wekerle, H., Meinl, E., Hohlfeld, R., and Dornmair, K. (2007). Multiple sclerosis: T-cell receptor expression in distinct brain regions. *Brain* 130, 2789–2799.

Jurewicz, A., Biddison, W. E., and Antel, J. P. (1998). MHC class I-restricted lysis of human oligodendrocytes by myelin basic protein peptide-specific CD8 T lymphocytes. *J. Immunol.* 160, 3056–3059.

Kappler, J. W., Roehm, N., and Marrack, P. (1987). T cell tolerance by clonal elimination in the thymus. *Cell* 49, 273–280.

Kassmann, C. M., Lappe-Siefke, C., Baes, M., Brugger, B., Mildner, A., Werner, H. B., Natt, O., Michaelis, T., Prinz, M., Frahm, J., and Nave, K. A. (2007). Axonal loss and neuroinflammation caused by peroxisome-deficient oligodendrocytes. *Nat. Genet.* 39, 969–976.

Keegan, B. M., and Noseworthy, J. H. (2002). Multiple sclerosis. *Annu. Rev. Med.* 53, 285–302.

Killestein, J., Eikelenboom, M. J., Izeboud, T., Kalkers, N. F., Ader, H. J., Barkhof, F., Van Lier, R. A., Uitdehaag, B. M., and Polman, C. H. (2003). Cytokine producing CD8+ T cells are correlated to MRI features of tissue destruction in MS. *J. Neuroimmunol.* 142, 141–148.

Kisielow, P., Bluthmann, H., Staerz, U. D., Steinmetz, M., and von Boehmer, H. (1988). Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4+8+ thymocytes. *Nature* 333, 742–746.

Krakowski, M., and Owens, T. (1996). Interferon-gamma confers resistance to experimental allergic encephalomyelitis. *Eur. J. Immunol.* 26, 1641–1646.

Krakowski, M. L., and Owens, T. (2000). Naive T lymphocytes traffic to inflamed central nervous system, but require antigen recognition for activation. *Eur. J. Immunol.* 30, 1002–1009.

Kuchroo, V. K., Anderson, A. C., Waldner, H., Munder, M., Bettelli, E., and Nicholson, L. B. (2002). T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. *Annu. Rev. Immunol.* 20, 101–123.

Kyewski, B., and Klein, L. (2006). A central role for central tolerance. *Annu. Rev. Immunol.* 24, 571–606.

Langrish, C. L., Chen, Y., Blumenschein, W. M., Mattson, J., Basham, B., Sedgwick, J. D., McClanahan, T., Kastelein, R. A., and Cua, D. J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201, 233–240.

Lassmann, H., Bruck, W., and Lucchinetti, C. F. (2007). The immunopathology of multiple sclerosis: an overview. *Brain Pathol.* 17, 210–218.

Lees, J. R., Golumbek, P. T., Sim, J., Dorsey, D., and Russell, J. H. (2008).

Regional CNS responses to IFN-gamma determine lesion localization patterns during EAE pathogenesis. *J. Exp. Med.* 205, 2633–2642.

Lenercept. (1999). TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. *Neurology* 53, 457–465.

Limmer, A., Ohl, J., Kurts, C., Ljunggren, H. G., Reiss, Y., Groettrup, M., Momburg, F., Arnold, B., and Knolle, P. A. (2000). Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. *Nat. Med.* 6, 1348–1354.

Liu, G. Y., Fairchild, P. J., Smith, R. M., Prowle, J. R., Kioussis, D., and Wraith, D. C. (1995). Low avidity recognition of self-antigen by T cells permits escape from central tolerance. *Immunity* 3, 407–415.

Llewellyn-Smith, N., Lai, M., Miller, D. H., Rudge, P., Thompson, A. J., and Cuzner, M. L. (1997). Effects of anti-CD4 antibody treatment on lymphocyte subsets and stimulated tumor necrosis factor alpha production: a study of 29 multiple sclerosis patients entered into a clinical trial of cM-T412. *Neurology* 48, 810–816.

Lucchinetti, C., Bruck, W., Parisi, J., Scheithauer, B., Rodriguez, M., and Lassmann, H. (2000). Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol.* 47, 707–717.

Mars, L. T., Bauer, J., Gross, D. A., Bucciarelli, F., Firat, H., Hudrisier, D., Lemonnier, F., Kosmatopoulos, K., and Liblau, R. S. (2007). CD8 T cell responses to myelin oligodendrocyte glycoprotein-derived peptides in humanized HLA-A*0201-transgenic mice. *J. Immunol.* 179, 5090–5098.

Massa, P. T., Ozato, K., and McFarlin, D. E. (1993). Cell type-specific regulation of major histocompatibility complex (MHC) class I gene expression in astrocytes, oligodendrocytes, and neurons. *Glia* 8, 201–207.

Mathis, D., and Benoist, C. (2004). Back to central tolerance. *Immunity* 20, 509–516.

McFarland, H. F., and Martin, R. (2007). Multiple sclerosis: a complicated picture of autoimmunity. *Nat. Immunol.* 8, 913–919.

Medana, I., Martinic, M. A., Wekerle, H., and Neumann, H. (2001). Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. *Am. J. Pathol.* 159, 809–815.

Mildner, A., Djukic, M., Garbe, D., Wellmer, A., Kuziel, W. A., Mack, M., Nau, R., and Prinz, M. (2008). Ly-6G+CCR2- myeloid cells rather than Ly-6ChighCCR2+ monocytes are required for the control of bacterial infection in the central nervous system. *J. Immunol.* 181, 2713–2722.

Na, S. Y., Cao, Y., Toben, C., Nitschke, L., Stadelmann, C., Gold, R., Schimpl, A., and Hunig, T. (2008). Naive CD8 T-cells initiate spontaneous autoimmunity to a sequestered model antigen of the central nervous system. *Brain* 131, 2353–2365.

Neumann, H., Cavalie, A., Jenne, D. E., and Wekerle, H. (1995). Induction of MHC class I genes in neurons. *Science* 269, 549–552.

Neumann, H., Medana, I. M., Bauer, J., and Lassmann, H. (2002). Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. *Trends Neurosci.* 25, 313–319.

Neumann, H., Schmidt, H., Cavalie, A., Jenne, D., and Wekerle, H. (1997). Major histocompatibility complex (MHC) class I gene expression in single neurons of the central nervous system: differential regulation by interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha. *J. Exp. Med.* 185, 305–316.

Niland, B., Banki, K., Biddison, W. E., and Perl, A. (2005). CD8+ T cell-mediated HLA-A*0201-restricted cytotoxicity to transaldolase peptide 168-176 in patients with multiple sclerosis. *J. Immunol.* 175, 8365–8378.

Noseworthy, J. H., Lucchinetti, C., Rodriguez, M., and Weinshenker, B. G. (2000). Multiple sclerosis. *N. Engl. J. Med.* 343, 938–952.

O'Connor, R. A., Prendergast, C. T., Sabatos, C. A., Lau, C. W., Leech, M. D., Wraith, D. C., and Anderton, S. M. (2008). Cutting edge: Th1 cells facilitate the entry of Th17 cells to the central nervous system during experimental autoimmune encephalomyelitis. *J. Immunol.* 181, 3750–3754.

Okuda, Y., Sakoda, S., Bernard, C. C., Fujimura, H., Saeki, Y., Kishimoto, T., and Yanagihara, T. (1998). IL-6-deficient mice are resistant to the induction of experimental autoimmune encephalomyelitis provoked by myelin oligodendrocyte glycoprotein. *Int. Immunol.* 10, 703–708.

Olsson, T., and Hillert, J. (2008). The genetics of multiple sclerosis and its experimental models. *Curr. Opin. Neurol.* 21, 255–260.

Ontaneda, D., Hyland, M., and Cohen, J. A. (2011). Multiple sclerosis: new insights in pathogenesis and novel therapeutics. *Annu. Rev. Med.* 63, 389–404.

Perchellet, A., Brabb, T., and Goverman, J. M. (2008). Crosspresentation by nonhematopoietic and direct presentation by hematopoietic cells induce central tolerance to myelin basic protein. *Proc. Natl. Acad. Sci. U.S.A.* 105, 14040–14045.

Perchellet, A., Stromnes, I., Pang, J. M., and Goverman, J. (2004). CD8+ T cells maintain tolerance to myelin basic protein by 'epitope theft.' *Nat. Immunol.* 5, 606–614.

Peterson, J. W., Bo, L., Mork, S., Chang, A., and Trapp, B. D. (2001). Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. *Ann. Neurol.* 50, 389–400.

Prineas, J. W. (1979). Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. *Science* 203, 1123–1125.

Raine, C. S., Mokhtarian, F., and McFarlin, D. E. (1984). Adoptively transferred chronic relapsing experimental autoimmune encephalomyelitis in the mouse. Neuropathologic analysis. *Lab. Invest.* 51, 534–546.

Ramagopalan, S. V., Knight, J. C., and Ebers, G. C. (2009). Multiple sclerosis and the major histocompatibility complex. *Curr. Opin. Neurol.* 22, 219–225.

Rock, K. L., and Shen, L. (2005). Cross-presentation: underlying mechanisms and role in immune surveillance. *Immunol. Rev.* 207, 166–183.

Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C., Patsopoulos, N. A., Moutsianas, L., Dilthey, A., Su, Z., Freeman, C., Hunt, S. E., Edkins, S., Gray, E., Booth, D. R., Potter, S. C., Goris, A., Band, G., Oturai, A. B., Strange, A., Saarela, J., Bellenguez, C., Fontaine, B., Gillman, M., Hemmer, B., Gwilliam, R., Zipp, F., Jayakumar, A., Martin, R., Leslie, S., Hawkins, S., Giannoulatou, E., D'Alfonso, S., Blackburn, H., Martinelli Boneschi, F., Liddle, J., Harbo, H. F., Perez, M. L., Spurkland, A., Waller, M. J., Mycko, M. P., Ricketts, M., Comabella, M., Hammond, N., Kockum, I., McCann, O. T., Ban, M., Whittaker, P., Kemppinen, A., Weston, P., Hawkins, C., Widaa, S., Zajicek, J., Dronov, S., Robertson, N., Bumpstead, S. J., Barcellos, L. F., Ravindrarajah, R., Abraham, R., Alfredsson, L., Ardlie, K., Aubin, C., Baker, A., Baker, K., Baranzini, S. E., Bergamaschi, L., Bergamaschi, R., Bernstein, A., Berthele, A., Boggild, M., Bradfield, J. P., Brassat, D., Broadley, S. A., Buck, D., Butzkueven, H., Capra, R., Carroll, W. M., Cavalla, P., Celius, E. G., Cepok, S., Chiavacci, R., Clerget-Darpoux, F., Clysters, K., Comi, G., Cossburn, M., Cournu-Rebeix, I., Cox, M. B., Cozen, W., Cree, B. A., Cross, A. H., Cusi, D., Daly, M. J., Davis, E., de Bakker, P. I., Debouverie, M., D'Hooghe, M. B., Dixon, K., Dobosi, R., Dubois, B., Ellinghaus, D., Elovaara, I., Esposito, F., Fontenille, C., Foote, S., Franke, A., Galimberti, D., Ghezzi, A., Glessner, J., Gomez, R., Gout, O., Graham, C., Grant, S. F., Guerini, F. R., Hakonarson, H., Hall, P., Hamsten, A., Hartung, H. P., Heard, R. N., Heath, S., Hobart, J., Hoshi, M., Infante-Duarte, C., Ingram, G., Ingram, W., Islam, T., Jagodic, M., Kabesch, M., Kermode, A. G., Kilpatrick, T. J., Kim, C., Klopp, N., Koivisto, K., Larsson, M., Lathrop, M., Lechner-Scott, J. S., Leone, M. A., Leppä, V., Liljedahl, U., Bomfim, I. L., Lincoln, R. R., Link, J., Liu, J., Lorentzen, A. R., Lupoli, S., Macciardi, F., Mack, T., Marriott, M., Martinelli, V., Mason, D., McCauley, J. L., Mentch, F., Mero, I. L., Mihalova, T., Montalban, X., Mottershead, J., Myhr, K. M., Naldi, P., Ollier, W., Page, A., Palotie, A., Pelletier, J., Piccio, L., Pickersgill, T., Piehl, F., Pobywajlo, S., Quach, H. L., Ramsay, P. P., Reunanen, M., Reynolds, R., Rioux, J. D., Rodegher, M., Roesner, S., Rubio, J. P., Rückert, I. M., Salvetti, M., Salvi, E., Santaniello, A., Schaefer, C. A., Schreiber, S., Schulze, C., Scott, R. J., Sellebjerg, F., Selmaj, K. W., Sexton, D., Shen, L., Simms-Acuna, B., Skidmore, S., Sleiman, P. M., Smestad, C., Sørensen, P. S., Søndergaard, H. B., Stankovich, J., Strange, R. C., Sulonen, A. M., Sundqvist, E., Syvänen, A. C., Taddeo, F., Taylor, B., Blackwell, J. M., Tienari, P., Bramon, E., Tourbah, A., Brown, M. A., Tronczynska, E., Casas, J. P., Tubridy, N., Corvin, A., Vickery, J., Jankowski, J., Villoslada, P., Markus, H. S., Wang, K., Mathew, C. G., Wason, J., Palmer, C. N., Wichmann, H. E., Plomin, R., Willoughby, E., Rautanen, A., Winkelmann, J., Wittig, M., Trembath, R. C., Yaouanq, J., Viswanathan, A. C., Zhang, H., Wood, N. W., Zuvich, R., Deloukas, P., Langford, C., Duncanson, A., Oksenberg, J. R., Pericak-Vance, M. A., Haines, J. L., Olsson, T., Hillert,

J., Ivinson, A. J., De Jager, P. L., Peltonen, L., Stewart, G. J., Hafler, D. A., Hauser, S. L., McVean, G., Donnelly, P., and Compston, A. (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 476, 214–219.

Saxena, A., Bauer, J., Scheikl, T., Zappulla, J., Audebert, M., Desbois, S., Waisman, A., Lassmann, H., Liblau, R. S., and Mars, L. T. (2008). Cutting edge: multiple sclerosis-like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. *J. Immunol.* 181, 1617–1621.

Sean Riminton, D., Korner, H., Strickland, D. H., Lemckert, F. A., Pollard, J. D., and Sedgwick, J. D. (1998). Challenging cytokine redundancy: inflammatory cell movement and clinical course of experimental autoimmune encephalomyelitis are normal in lymphotoxin-deficient, but not tumor necrosis factor-deficient, mice. *J. Exp. Med.* 187, 1517–1528.

Seitz, S., Schneider, C. K., Malotka, J., Nong, X., Engel, A. G., Wekerle, H., Hohlfeld, R., and Dornmair, K. (2006). Reconstitution of paired T cell receptor alpha- and beta-chains from microdissected single cells of human inflammatory tissues. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12057–12062.

Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E., Capello, E., Mancardi, G. L., and Aloisi, F. (2006). Dendritic cells in multiple sclerosis lesions: maturation stage, myelin uptake, and interaction with proliferating T cells. *J. Neuropathol. Exp. Neurol.* 65, 124–141.

Skulina, C., Schmidt, S., Dornmair, K., Babbe, H., Roers, A., Rajewsky, K., Wekerle, H., Hohlfeld, R., and Goebels, N. (2004). Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonal expansions in the cerebrospinal fluid and blood. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2428–2433.

Sobel, R. A. (2000). Genetic and epigenetic influence on EAE phenotypes induced with different encephalitogenic peptides. *J. Neuroimmunol.* 108, 45–52.

Sospedra, M., and Martin, R. (2005). Immunology of multiple sclerosis. *Annu. Rev. Immunol.* 23, 683–747.

Steinman, L. (2001). Myelin-specific CD8 T cells in the pathogenesis of experimental allergic encephalitis and multiple sclerosis. *J. Exp. Med.* 194, F27–F30.

Steinman, L. (2009). A molecular trio in relapse and remission in multiple sclerosis. *Nat. Rev. Immunol.* 9, 440–447.

Steinman, L., and Zamvil, S. S. (2005). Virtues and pitfalls of EAE for the development of therapies for multiple sclerosis. *Trends Immunol.* 26, 565–571.

Stromnes, I. M., Cerretti, L. M., Liggitt, D., Harris, R. A., and Goverman, J. M. (2008). Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. *Nat. Med.* 14, 337–342.

Sun, D., Whitaker, J. N., Huang, Z., Liu, D., Coleclough, C., Wekerle, H., and Raine, C. S. (2001). Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J. Immunol.* 166, 7579–7587.

Tennakoon, D. K., Mehta, R. S., Ortega, S. B., Bhoj, V., Racke, M. K., and Karandikar, N. J. (2006). Therapeutic induction of regulatory, cytotoxic CD8+ T cells in multiple sclerosis. *J. Immunol.* 176, 7119–7129.

Traugott, U., Reinherz, E. L., and Raine, C. S. (1983a). Multiple sclerosis. Distribution of T cells, T cell subsets and Ia-positive macrophages in lesions of different ages. *J. Neuroimmunol.* 4, 201–221.

Traugott, U., Reinherz, E. L., and Raine, C. S. (1983b). Multiple sclerosis: distribution of T cell subsets within active chronic lesions. *Science* 219, 308–310.

Tsuchida, T., Parker, K. C., Turner, R. V., McFarland, H. F., Coligan, J. E., and Biddison, W. E. (1994). Autoreactive CD8+ T-cell responses to human myelin protein-derived peptides. *Proc. Natl. Acad. Sci. U.S.A.* 91, 10859–10863.

Tzartos, J. S., Friese, M. A., Craner, M. J., Palace, J., Newcombe, J., Esiri, M. M., and Fugger, L. (2008). Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. *Am. J. Pathol.* 172, 146–155.

van Oosten, B. W., Lai, M., Hodgkinson, S., Barkhof, F., Miller, D. H., Moseley, I. F., Thompson, A. J., Rudge, P., McDougall, A., McLeod, J. G., Adèr, H. J., and Polman, C. H. (1997). Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology* 49, 351–357.

Vanderlugt, C. L., and Miller, S. D. (2002). Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat. Rev. Immunol.* 2, 85–95.

Waldor, M. K., Sriram, S., Hardy, R., Herzenberg, L. A., Lanier, L., Lim, M., and Steinman, L. (1985). Reversal of experimental allergic encephalomyelitis with monoclonal antibody to a T-cell subset marker. *Science* 227, 415–417.

Wong, G. H., Bartlett, P. F., Clark-Lewis, I., Battye, F., and Schrader, J. W. (1984a). Inducible expression of H-2 and Ia antigens on brain cells. *Nature* 310, 688–691.

Wong, G. H., Clark-Lewis, I., Harris, A. W., and Schrader, J. W. (1984b). Effect of cloned interferon-gamma on expression of H-2 and Ia antigens on cell lines of hemopoietic, lymphoid, epithelial, fibroblastic and neuronal origin. *Eur. J. Immunol.* 14, 52–56.

Wucherpfennig, K. W. (2001). Insights into autoimmunity gained from structural analysis of MHC-peptide complexes. *Curr. Opin. Immunol.* 13, 650–656.

Wucherpfennig, K. W., Call, M. J., Deng, L., and Mariuzza, R. (2009). Structural alterations in peptide-MHC recognition by self-reactive T cell receptors. *Curr. Opin. Immunol.* 21, 590–595.

Zamvil, S. S., and Steinman, L. (1990). The T lymphocyte in experimental allergic encephalomyelitis. *Annu. Rev. Immunol.* 8, 579–621.

Zang, Y. C., Li, S., Rivera, V. M., Hong, J., Robinson, R. R., Breitbach, W. T., Killian, J., and Zhang, J. Z. (2004). Increased CD8+ cytotoxic T cell responses to myelin basic protein in multiple sclerosis. *J. Immunol.* 172, 5120–5127.

Zhang, J., Medaer, R., Stinissen, P., Hafler, D., and Raus, J. (1993). MHC-restricted depletion of human myelin basic protein-reactive T cells by T cell vaccination. *Science* 261, 1451–1454.

Zheng, Y., and Rudensky, A. Y. (2007). Foxp3 in control of the regulatory T cell lineage. *Nat. Immunol.* 8, 457–462.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 11 January 2012; paper pending published: 04 February 2012; accepted: 15 March 2012; published online: 29 March 2012.

Citation: Huseby ES, Huseby PG, Shah S, Smith R and Stadinski BD (2012) Pathogenic CD8 T cells in multiple sclerosis and its experimental models. Front. Immun. ***3****:64. doi: 10.3389/fimmu.2012.00064*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

OPINION ARTICLE
published: 26 June 2012
doi: 10.3389/fimmu.2012.00173

T cell immunity and cardiovascular metabolic disorders: does metabolism fuel inflammation?

Claudio Mauro* and Federica M. Marelli-Berg

Heart Centre, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK
**Correspondence: c.mauro@qmul.ac.uk*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Daisuke Kamimura, Osaka University, Japan

Metabolic disorders of the cardiovascular system, including atherosclerosis, obesity, diabetes, and hypertension, are the leading cause of morbidity and mortality in the western world. Metabolic syndrome is associated with a combination of risk factors, including nutrient excess, hyperglycemia, hyperlipidemia, insulin-resistance, and obesity that, when occurring together, increase the risk of developing cardiovascular metabolic disorders (CVMDs). In individuals with this syndrome, different CVMDs often develop simultaneously, so that obese patients present with an increased risk of suffering also from type II diabetes and insulin-resistance, these being frequently associated with atherosclerosis and hypertension, suggesting the sharing of similar pathogenic mechanisms.

ADAPTIVE T CELL IMMUNITY IN CVMDs

Chronic low-grade inflammation is a key feature of CVMDs. Macrophages and innate immunity have been classically implicated in the pathogenesis of atherosclerosis, and more recently of hypertension, as well as other metabolic disorders associated with cardiovascular disease such as obesity and type II diabetes (Hotamisligil, 2006; Rocha and Libby, 2009; Harrison et al., 2010; Matarese et al., 2010; Schiffrin, 2010; Baker et al., 2011; Donath and Shoelson, 2011; Hansson and Hermansson, 2011; Lahoute et al., 2011).

Evidence for the contribution of T cell-mediated immunity to CVMDs has only recently emerged. T cells infiltrate atherosclerotic plaques, obese adipose tissue, pancreatic islets, and the perivascular fat and kidneys in hypertensive individuals. In these sites, proinflammatory immune responses mediated by $CD4^+$ T-helper type 1 (T_H1) and $CD8^+$ cytotoxic T lymphocytes are preponderant over immunomodulatory (T_H2) and immunosuppressive (T_{reg}) immunity, and drive disease progression (Rocha and Libby, 2009; Baker et al., 2011). Interferon (IFN)-γ, the signature T_H1 cytokine, is found in human atherosclerotic plaques and obese adipose tissue, where it exerts pathogenic effects by promoting the recruitment and activation of macrophages and the release of proinflammatory cytokines such as Tumor Necrosis Factor (TNF), all of which perpetuate the local inflammation (Gupta et al., 1997; Ranjbaran et al., 2007; Rocha and Libby, 2009; Hansson and Hermansson, 2011). In contrast, Interleukin (IL)-4, the prototypical anti-inflammatory cytokine of the T_H2 lineage, is not frequently observed in human plaques (Rocha and Libby, 2009; Hansson and Hermansson, 2011). T_H1-mediated proinflammatory mechanisms have also been involved in the pathogenesis of hypertension (Mahmoud et al., 2003; Shao et al., 2003; Seaberg et al., 2005; Guzik et al., 2007; Harrison et al., 2010; Schiffrin, 2010). $RAG1^{-/-}$ mice, which lack both T and B cells, develop neither angiotensin II- nor deoxycorticosterone acetate (DOCA) salt-induced hypertension, and adoptive transfer of T cells, but not B cells, restores the hypertensive phenotype induced by these stimuli (Guzik et al., 2007). Immunosuppressive T_{reg} lymphocytes appear to be reduced in number in human atherosclerotic plaques (de Boer et al., 2007; Lahoute et al., 2011) and in the adipose tissue of insulin-resistant obese mice (Feuerer et al., 2009; Matarese et al., 2010). Blockade of IL-10 and Transforming Growth Factor (TGF)-β, the two cytokines responsible for most of the immunosuppressive effects mediated by T_{reg} cells, accelerates lesion development (Lahoute et al., 2011). Adoptive transfer of T_{reg} cells reduces atherosclerosis in $Apo^{-/-}$ mice (Ait-Oufella et al., 2006; Lahoute et al., 2011) and insulin-resistance in obese mice (Feuerer et al., 2009; Matarese et al., 2010). How proinflammatory T cells accumulate in the inflamed sites is an open question.

THE METABOLIC MACHINERY AND T CELL FUNCTION

The regulation of energy metabolism is crucial to T cell-mediated immunity, including activation, proliferation, and differentiation toward effector versus regulatory T cells, and, as we discuss here, migration. Naïve T cells rely upon a catabolic type of metabolism whereby ATP is mainly generated via oxidative phosphorylation (OXPHOS). This slow metabolism is sufficient to support their requirements for survival, maintain housekeeping functions, such as ion transport and membrane integrity, and keep them away from engaging into cell proliferation (Rathmell et al., 2000; Frauwirth and Thompson, 2004). Upon TCR engagement by cognate antigen, T cells switch from catabolism to anabolism, with phosphatidylinositol 3′-kinase (PI3K) leading to the activation of the serine-threonine kinase AKT. This step promotes glucose metabolism by stimulating the localization of the glucose transporter Glut1 to the plasma membrane and the activity of hexokinase and phosphofructokinase, two rate-limiting enzymes of the glycolytic pathway. Increased glycolytic flux enables activated T cells to generate ATP and, at the same time, efficiently utilize carbon sources in the form of amino acids and lipids for the biosynthesis of proteins and membranes necessary for the expansion phase that characterizes the immune response. Downstream of TCR, AKT also controls the activation state of the mammalian target of rapamycin (mTOR), a sensor of nutritional and energetic status in cells that promotes protein synthesis. mTOR is a key regulator of T cell differ-

entiation toward proinflammatory subsets, and its inhibition with rapamycin promotes immunosuppression via the induction of anergic and T_{reg} cells (Jones and Thompson, 2007; Zheng et al., 2009; Peter et al., 2010; Powell and Delgoffe, 2010; Marelli-Berg et al., 2012; Mauro et al., 2012).

Indirect evidence suggests that metabolic status can influence T cell homing patterns. Expression of the adhesion molecule CD62L (also known as L-selectin) and the chemokine receptors CC-chemokine receptor 7 (CCR7) and sphingosine-1-phosphate receptor 1 ($S1P_1$) on the surface of naïve T cells facilitates their trafficking to secondary lymphoid organs (SLOs). Upon TCR engagement, the PI3K-AKT-mTOR axis promotes the downregulation of CD62L, CCR7, and $S1P_1$, and prompts effector T cells expressing adhesion molecules [such as VLA4 (very late antigen 4) and ligands for P-selectin and E-selectin] and chemokine receptors (such as CXCR3 and CCR5) to traffic to the sites of inflammation (Sinclair et al., 2008).

Upon resolution of the immune response, the number of antigen specific T cells contracts, as effector cells die and only the memory subset survives. This step is characterized by a metabolic transition from anabolism to catabolism, from high mTOR activity to low mTOR activity, and from effector to memory cells (Sinclair et al., 2008; Finlay and Cantrell, 2011). This transition is associated with the re-expression of CD62L and CCR7, thus allowing the newly formed memory cells to continue surveillance by trafficking in and out of SLOs. Consistently, rapamycin-mediated inhibition of mTOR causes effector T cells to re-express CD62L and CCR7, and home to SLOs where they are trapped away from the target sites in the periphery (Sinclair et al., 2008; Finlay and Cantrell, 2011). Therefore, rapamycin can promote immunosuppression by redirecting effector T cells from peripheral tissues to SLOs.

NF-κB, INFLAMMATION, AND CVMDs

Nuclear Factor (NF)-κB is a family of transcription factors that promote immunity by controlling the expression of genes involved in inflammation (Baker et al., 2011). A number of recent studies have demonstrated a key role for NF-κB signaling pathways in the development of inflammation-driven metabolic disorders in adipose tissue, pancreatic β-cells, arterial walls, and the central nervous system (Yuan et al., 2001; Gareus et al., 2008; Baker et al., 2011; Purkayastha et al., 2011).

An important role for NF-κB in the organization of energy metabolism networks in the cell through the control of the balance between the utilization of glycolysis and OXPHOS has recently been demonstrated. NF-κB acts as a physiological regulator of mitochondrial respiration and via this function suppresses the metabolic reprogramming to aerobic glycolysis in cells and prevents necrosis upon nutrient starvation. This metabolic function of NF-κB involves the p53-dependent upregulation of mitochondrial Synthesis of Cytochrome *c* Oxidase 2 (SCO2), a crucial component of the electron transfer chain Complex IV or Cytochrome *c* Oxidase, which increases OXPHOS and reduces glycolytic flux in cells (Mauro et al., 2011).

Due to its ability to control both inflammatory and metabolic responses, NF-κB represents a major target, in addition to the mTOR pathway, for the development of novel therapeutic strategies in CVMDs (**Figure 1**).

METABOLIC STRESS AND T CELL MIGRATION

The control of T cell migration by TCR engagement and co-stimuli (Mirenda et al., 2007; Jarmin et al., 2008) implies that metabolic changes downstream of these receptors can influence both efficiency and topography of T cell trafficking. The metabolic machinery is also likely to directly affect and be affected by T cell migratory events, as T cells continuously recirculate between different microenvironments (e.g., blood, lymphoid tissues, and peripheral tissues) in which they are exposed to different nutrient availability and oxygen (O_2) tension, and must adapt their metabolic pathways to effectively mediate immune responses. The direct effect of metabolism on the trafficking ability of T cells, however, has not been investigated.

Memory lymphocyte trafficking is regulated by a number of complex mechanisms, which involve both pro-migratory and pro-static events. Following priming in the lymph nodes, T cells migrate to antigen-rich sites to exert their effector function. Effector T cells are retained in the tissue for a time sufficient to achieve effective immunity. Homeostatic mechanisms prescribe that long-lived memory T cells leave the tissue once the immune response is complete, and inflammation then subsides. A failure of T cells to be released from the tissue leads to persistent inflammation and progressive tissue damage (Burman et al., 2005). T cells are exposed to different metabolic environments during these migratory events, which are likely to affect their motility and ability to respond to migratory cues from the

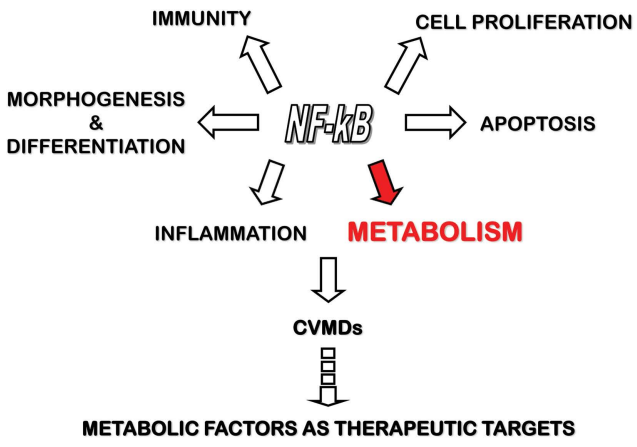


FIGURE 1 | The control of inflammation and metabolism by NF-κB and its relevance to CVMDs. NF-κB transcription factors control several physiologic functions, including morphogenesis and differentiation, cell proliferation and apoptosis, immunity and inflammation. Recent work has shed light in the control of cell energy metabolism by NF-κB. By regulating both inflammatory and metabolic pathways, NF-κB has been involved in the pathogenesis of CVMDs, thereby opening the way to the identification of metabolic factors under NF-κB control as target for therapy.

surrounding microenvironment. Thus, it is conceivable that altered migration might result from an altered metabolic microenvironment (blood, tissues, or the inflammatory milieu itself) and that this might in turn initiate or sustain chronic inflammation, as it happens in CVMDs. Specifically, metabolic alterations that characterize the metabolic syndrome, including nutrient excess, hyperglycemia and hyperlipidemia, might promote T cells infiltration of inflammatory sites by modifying their intracellular metabolism. Once inside the inflamed tissue, T cells could become immobile due to a drop in O_2 tension, nutrient availability, or pH that affects their metabolic status. The inability of differentiated T cells to exit inflamed tissues would favor the persistence of chronic inflammation.

CONCLUDING REMARKS

Immunosuppressive therapies have been proposed in CVMDs. TNF blockade reduced the incidence of cardiovascular events in patients with rheumatoid arthritis (RA), an autoimmune disease associated with a strong inflammatory component and with accelerated atherosclerosis (Jacobsson et al., 2005; Greenberg et al., 2011). Sirolimus (rapamycin), an inhibitor of the mTOR approved for use in the prevention of transplant rejection, is being tested in pre-clinical studies of cardiovascular disease in animal models and has been used with some success in the clinic for the local treatment of restenosis (Adelman, 2010).

In addition to conventional immunosuppression, compounds targeting metabolic pathways have been shown to exert anti-inflammatory properties. For instance, essential amino acid depletion has been shown to contribute to tolerance induction in experimental heart transplantation (Sucher et al., 2012). Similarly, *n*–3 polyunsaturated fatty acids (PUFAs) from fish oils, EPA, and DHA, popularly referred to as omega-3 fatty acids, with hypotriglyceridemic, hypotensive, and antithrombotic properties, have been shown to inhibit inflammatory responses associated with alteration of lipid metabolism (Cottin et al., 2011). The inhibition of immune-inflammation by correcting altered metabolism is an attractive therapeutic strategy which would specifically target CVMDs without the severe side-effects of conventional immunosuppression.

Investigation of the mechanisms for metabolic control of T cell trafficking represents a fascinating area – mostly unexplored – of research for the forthcoming years. The observations arising from such studies will provide key insights into both the physiology of T cell trafficking and the physiopathologic mechanisms of T cell infiltration in inflammatory sites, where T cells are retained and perpetuate the chronic inflammation that drives the disease progression. Importantly, while the antigen specificities promoting the activation of the adaptive immunity in humans are controversial, these diseases have been associated to non-antigen specific mechanisms involving altered T cell migration patterns (Burman et al., 2005; Full and Monaco, 2011). We propose that altered metabolism can fuel chronic inflammation in an antigen-non-specific manner, i.e., that otherwise harmless immune responses might fail to resolve leading to chronic inflammation and bystander damage when they occur in a metabolically altered environment. Similar mechanisms are likely to be important in other cardiovascular diseases (e.g., hypertension, stroke, heart transplantation, etc.) with a T cell-mediated inflammatory component. Additional associations are being drawn between CVMDs, such as obesity, and diseases that are less obviously linked to metabolic alterations, including asthma, arthritis, lupus, Alzheimer, and several forms of cancer (Mathis and Shoelson, 2011; Gerriets and Rathmell, 2012). Inflammation has been etiologically associated to the pathogenesis of each of these conditions, and the binomium metabolism-inflammation might provide new effective therapeutic protocols for a wide range of diseases that share similar pathogenic mechanisms.

ACKNOWLEDGMENTS

We thank E. Simpson for critical comments on the manuscript. Claudio Mauro is supported by the British Heart Foundation. Federica M. Marelli-Berg is supported by the British Heart Foundation, the Medical Research Council of the UK, and the Gates Foundation. This work was facilitated by National Institute for Health Research Cardiovascular Biomedical Research Unit at Barts and The London School of Medicine and Dentistry.

REFERENCES

Adelman, S. J. (2010). Sirolimus and its analogs and its effects on vascular diseases. *Curr. Pharm. Des.* 16, 4002–4011.

Ait-Oufella, H., Salomon, B. L., Potteaux, S., Robertson, A. K., Gourdy, P., Zoll, J., Merval, R., Esposito, B., Cohen, J. L., Fisson, S., Flavell, R. A., Hansson, G. K., Klatzmann, D., Tedgui, A., and Mallat, Z. (2006). Natural regulatory T cells control the development of atherosclerosis in mice. *Nat. Med.* 12, 178–180.

Baker, R. G., Hayden, M. S., and Ghosh, S. (2011). NF-kappaB, inflammation, and metabolic disease. *Cell Metab.* 13, 11–22.

Burman, A., Haworth, O., Hardie, D. L., Amft, E. N., Siewert, C., Jackson, D. G., Salmon, M., and Buckley, C. D. (2005). A chemokine-dependent stromal induction mechanism for aberrant lymphocyte accumulation and compromised lymphatic return in rheumatoid arthritis. *J. Immunol.* 174, 1693–1700.

Cottin, S. C., Sanders, T. A., and Hall, W. L. (2011). The differential effects of EPA and DHA on cardiovascular risk factors. *Proc. Nutr. Soc.* 70, 215–231.

de Boer, O. J., van der Meer, J. J., Teeling, P., van der Loos, C. M., and van der Wal, A. C. (2007). Low numbers of FOXP3 positive regulatory T cells are present in all developmental stages of human atherosclerotic lesions. *PLoS ONE* 2, e779. doi: 10.1371/journal.pone.0000779

Donath, M. Y., and Shoelson, S. E. (2011). Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.* 11, 98–107.

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A. B., Benoist, C., Shoelson, S., and Mathis, D. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* 15, 930–939.

Finlay, D., and Cantrell, D. A. (2011). Metabolism, migration and memory in cytotoxic T cells. *Nat. Rev. Immunol.* 11, 109–117.

Frauwirth, K. A., and Thompson, C. B. (2004). Regulation of T lymphocyte metabolism. *J. Immunol.* 172, 4661–4665.

Full, L. E., and Monaco, C. (2011). Targeting inflammation as a therapeutic strategy in accelerated atherosclerosis in rheumatoid arthritis. *Cardiovasc. Ther.* 29, 231–242.

Gareus, R., Kotsaki, E., Xanthoulea, S., van der Made, I., Gijbels, M. J., Kardakaris, R., Polykratis, A., Kollias, G., de Winther, M. P., and Pasparakis, M. (2008). Endothelial cell-specific NF-kappaB inhibition protects mice from atherosclerosis. *Cell Metab.* 8, 372–383.

Gerriets, V. A., and Rathmell, J. C. (2012). Metabolic pathways in T cell fate and function. *Trends Immunol.* 33, 168–173.

Greenberg, J. D., Furer, V., and Farkouh, M. E. (2011). Cardiovascular safety of biologic therapies for the treatment of RA. *Nat. Rev. Rheumatol.* 15, 13–21.

Gupta, S., Pablo, A. M., Jiang, X., Wang, N., Tall, A. R., and Schindler, C. (1997). IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. *J. Clin. Invest.* 99, 2752–2761.

Guzik, T. J., Hoch, N. E., Brown, K. A., McCann, L. A., Rahman, A., Dikalov, S., Goronzy, J., Weyand, C., and Harrison, D. G. (2007). Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J. Exp. Med.* 204, 2449–2460.

Hansson, G. K., and Hermansson, A. (2011). The immune system in atherosclerosis. *Nat. Immunol.* 12, 204–212.
Harrison, D. G., Vinh, A., Lob, H., and Madhur, M. S. (2010). Role of the adaptive immune system in hypertension. *Curr. Opin. Pharmacol.* 10, 203–207.
Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444, 860–867.
Jacobsson, L. T., Turesson, C., Gulfe, A., Kapetanovic, M. C., Petersson, I. F., Saxne, T., and Geborek, P. (2005). Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. *J. Rheumatol.* 32, 1213–1218.
Jarmin, S. J., David, R., Ma, L., Chai, J. G., Dewchand, H., Takesono, A., Ridley, A. J., Okkenhaug, K., and Marelli-Berg, F. M. (2008). T cell receptor-induced phosphoinositide-3-kinase p110 delta activity is required for T cell localization to antigenic tissue in mice. *J. Clin. Invest.* 118, 1154–1164.
Jones, R. G., and Thompson, C. B. (2007). Revving the engine: signal transduction fuels T cell activation. *Immunity* 27, 173–178.
Lahoute, C., Herbin, O., Mallat, Z., and Tedgui, A. (2011). Adaptive immunity in atherosclerosis: mechanisms and future therapeutic targets. *Nat. Rev. Cardiol.* 8, 348–358.
Mahmoud, F., Omu, A., Abul, H., El-Rayes, S., and Haines, D. (2003). Lymphocyte subpopulations in pregnancy complicated by hypertension. *J. Obstet. Gynaecol.* 23, 20–26.
Marelli-Berg, F. M., Fu, H., and Mauro, C. (2012). Molecular mechanisms of metabolic reprogramming in proliferating cells: implications for T cell-mediated immunity. *Immunology.* doi: 10.1111/j.1365-2567.2012.03583.x. [Epub ahead of print].
Matarese, G., Procaccini, C., De Rosa, V., Horvath, T. L., and La Cava, A. (2010). Regulatory T cells in obesity: the leptin connection. *Trends Mol. Med.* 16, 247–256.
Mathis, D., and Shoelson, S. E. (2011). Immunometabolism: an emerging frontier. *Nat. Rev. Immunol.* 11, 81.
Mauro, C., Fu, H., and Marelli-Berg, F. M. (2012). T cell trafficking and metabolism: novel mechanisms and targets for immunomodulation. *Curr. Opin. Pharmacol.* doi: 10.1016/j.coph.2012.02.018. [Epub ahead of print].
Mauro, C., Leow, S. C., Anso, E., Rocha, S., Thotakura, A. K., Tornatore, L., Moretti, M., De Smaele, E., Beg, A. A., Tergaonkar, V., Chandel, N. S., and Franzos, G. (2011). NF-kappaB controls energy homeostasis and metabolic adaptation by upregulating mitochondrial respiration. *Nat. Cell Biol.* 13, 1272–1279.
Mirenda, V., Jarmin, S. J., David, R., Dyson, J., Scott, D., Gu, Y., Lechler, R. I., Okkenhaug, K., and Marelli-Berg, F. M. (2007). Physiologic and aberrant regulation of memory T-cell trafficking by the costimulatory molecule CD28. *Blood* 109, 2968–2977.
Peter, C., Waldmann, H., and Cobbold, S. P. (2010). mTOR signalling and metabolic regulation of T cell differentiation. *Curr. Opin. Immunol.* 22, 655–661.
Powell, J. D., and Delgoffe, G. M. (2010). The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. *Immunity* 33, 301–311.
Purkayastha, S., Zhang, G., and Cai, D. (2011). Uncoupling the mechanisms of obesity and hypertension by targeting hypothalamic IKK-beta and NF-kappaB. *Nat. Med.* 17, 883–887.
Ranjbaran, H., Sokol, S. I., Gallo, A., Eid, R. E., Iakimov, A. O., D'Alessio, A., Kapoor, J. R., Akhtar, S., Howes, C. J., Aslan, M., Pfau, S., Pober, J. S., and Tellides, G. (2007). An inflammatory pathway of IFN-gamma production in coronary atherosclerosis. *J. Immunol.* 178, 592–604.
Rathmell, J. C., Vander Heiden, M. G., Harris, M. H., Frauwirth, K. A., and Thompson, C. B. (2000). In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. *Mol. Cell* 6, 683–692.
Rocha, V. Z., and Libby, P. (2009). Obesity, inflammation, and atherosclerosis. *Nat. Rev. Cardiol.* 6, 399–409.
Schiffrin, E. L. (2010). T lymphocytes: a role in hypertension? *Curr. Opin. Nephrol. Hypertens.* 19, 181–186.
Seaberg, E. C., Munoz, A., Lu, M., Detels, R., Margolick, J. B., Riddler, S. A., Williams, C. M., and Phair, J. P. (2005). Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003. *AIDS* 19, 953–960.
Shao, J., Nangaku, M., Miyata, T., Inagi, R., Yamada, K., Kurokawa, K., and Fujita, T. (2003). Imbalance of T-cell subsets in angiotensin II-infused hypertensive rats with kidney injury. *Hypertension* 42, 31–38.
Sinclair, L. V., Finlay, D., Feijoo, C., Cornish, G. H., Gray, A., Ager, A., Okkenhaug, K., Hagenbeek, T. J., Spits, H., and Cantrell, D. A. (2008). Phosphatidylinositol-3-OH kinase and nutrient-sensing mTOR pathways control T lymphocyte trafficking. *Nat. Immunol.* 9, 513–521.
Sucher, R., Fischler, K., Oberhuber, R., Kronberger, I., Margreiter, C., Ollinger, R., Schneeberger, S., Fuchs, D., Werner, E. R., Watschinger, K., Zelger, B., Tellides, G., Pilat, N., Pratschke, J., Margreiter, R., Wekerle, T., and Brandacher, G. (2012). IDO and regulatory T cell support are critical for cytotoxic T lymphocyte-associated Ag-4 Ig-mediated long-term solid organ allograft survival. *J. Immunol.* 188, 37–46.
Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z. W., Karin, M., and Shoelson, S. E. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science* 293, 1673–1677.
Zheng, Y., Delgoffe, G. M., Meyer, C. F., Chan, W., and Powell, J. D. (2009). Anergic T cells are metabolically anergic. *J. Immunol.* 183, 6095–6101.

Received: 08 May 2012; accepted: 08 June 2012; published online: 26 June 2012.
*Citation: Mauro C and Marelli-Berg FM (2012) T cell immunity and cardiovascular metabolic disorders: does metabolism fuel inflammation? Front. Immun. **3**:173. doi: 10.3389/fimmu.2012.00173*
This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 13 April 2012
doi: 10.3389/fimmu.2012.00077

Inflammation and bone destruction in arthritis: synergistic activity of immune and mesenchymal cells in joints

Noriko Komatsu [1,3] ***and Hiroshi Takayanagi*** [1,2,3] ******

[1] *Department of Cell Signaling, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan*
[2] *Takayanagi Osteonetwork Project, Exploratory Research for Advanced Technology, Japan Science and Technology Agency, Tokyo, Japan*
[3] *Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Rik Lories, Katholieke Universiteit Leuven, Belgium
Hideki Ogura, Osaka University, Japan

****Correspondence:***
Hiroshi Takayanagi, Department of Cell Signaling, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Yushima 1-5-45, Bunkyo-ku, Tokyo 113-8549, Japan.
e-mail: taka.csi@tmd.ac.jp

Rheumatoid arthritis (RA) is an immune-mediated disease of the joints that is characterized by chronic inflammation and synovial hyperplasia that eventually lead to cartilage and bone destruction. Synovial fibroblasts are mesenchymal cells recognized as a key cell population in RA due to their hyperproliferative and hypersensitive properties in the inflammatory milieu and hyperproduction of both inflammatory cytokines and matrix-degrading enzymes. On the immune cell side, a wealth of evidence has shown that $CD4^+$T-cells, especially IL-17 producing helper T (Th17) cells, play a prominent role, particularly in the initiation of systemic immune response in RA. However, it is still unclear how the local chronic inflammation in the joint is elicited by a systemic immune response. Recent studies have shed light on the importance of the interaction between immune and mesenchymal cells in joints including synovial fibroblasts. In particular, mesenchymal cells contribute to the Th17-mediated chronic inflammation in RA by promoting the migration of Th17 cells to the inflamed site and then the homeostatic proliferation and concomitant increase in IL-17 production. In addition, recent progress in osteoimmunology has provided new insight into the pathogenesis of the bone destruction which takes place in RA. Th17-related cytokines have been shown to enhance osteoclastogenesis, mainly via synovial fibroblasts. Thus, mesenchymal cells are a determinant of the development of RA that links the systemic immune response and the local disorder in the joints. In addition, the interaction of immune and mesenchymal cells plays a key role in both the chronic inflammation and bone destruction seen in RA. Elucidation of the precise events involved in this interaction will lead to a better understanding of the mechanisms by which chronic inflammation and bone destruction in joint results from a systemic immune response, and also will help provide a molecular basis for novel therapeutic strategies to treat RA.

Keywords: $CD4^+$T-cell, Th17 cell, synovial fibroblast, osteoclast, inflammation, bone destruction, rheumatoid arthritis

INTRODUCITON

Rheumatoid arthritis (RA) afflicts up to 1% of the general population worldwide. It is a chronic inflammatory disease characterized by synovial hyperplasia and bone destruction in multiple joints (Firestein, 2003). Three of the outstanding questions in RA pathogenesis are how the systemic immune response is elicited by genetic and/or environmental factors, how this in turn results in local joint inflammation and how inflammation causes bone destruction. In the affected joints, hyperplasia of the synovial membrane is a hallmark of RA pathology, which is characterized by both hyperproliferation of synovial fibroblasts and massive infiltration of inflammatory immune cells, including $CD4^+$T-cells and innate immune cells. Synovial fibroblasts have certain unique characteristics, such as hyperproliferative and hyperactive properties in response to an inflammatory environment, and are recognized as prominent determinants of the joint-specificity seen in RA. Therefore, it is important to establish how these pathogenetic immune cells migrate into joints and contribute to the chronic inflammation and bone destruction, especially via activation of the mesenchymal cells resident in joint, such as synovial fibroblasts. However, so far, despite the clearly evident importance and considerable effort expended, the interplay of immune and mesenchymal cells in joint is still not fully understood. Though significant roles of B-cells and antibody production are also widely appreciated in RA, here, we summarize recent findings on the RA pathogenesis by focusing on T-cells and synovial fibroblasts.

$CD4^+$T-CELLS ARE INDISPENSABLE FOR THE INITIATION OF RA

Although the etiology of RA remains unclear, the primary role of $CD4^+$T-cells in RA has been suggested by various findings: (1) the extensive infiltration of $CD4^+$T-cell into the inflammatory synovium, (2) the presence of autoantibodies, (3) the association of RA susceptibility with HLA-DRB1 alleles (Perricone et al., 2011) and genes related to T-cell function such as PTPN22 (Begovich et al., 2004; Lee et al., 2005) and CCR6 (Kochi et al., 2010; Stahl et al., 2010), and (4) the efficacy of a T-cell directed therapeutic drug, CTLA-4 Ig (Linsley and Nadler, 2009). Importantly, studies in a variety of animal models have further supported the importance of $CD4^+$T-cells in RA development.

Collagen-induced arthritis (CIA) and K/BxN mouse (Kouskoff et al., 1996) model that recapitulate the whole process of RA are the most widely used mouse models. In these models, arthritogenic antibodies contribute to the development of RA, since transfer of arthritogenic antibodies can induce arthritis. $CD4^+$T-cells are required for the full induction of CIA (Ranges et al., 1985) and K/BxN models (Kouskoff et al., 1996), as the administration of anti-CD4 depleting antibodies suppresses both the production of autoantibodies and disease severity (**Table 1**). In contrast, CAIA (Kagari et al., 2002) and K/BxN serum transfer arthritis (Korganow et al., 1999) do not require T-cells or B-cells, since this form of arthritis can be induced effectively in T and B-cell-deficient mice. Thus, it is indicated that $CD4^+$T-cells are required for the initiation phase of CIA and K/BxN arthritis partly by producing arthritogenic autoantibodies. $CD4^+$T-cells are not strictly necessary after the production of arthritogenic autoantibodies, although this does not mean $CD4^+$T-cells have no further role in the disease. Indeed, they are demonstrably capable of exacerbating arthritis, because adoptive transfer of a $CD4^+$T-cell subset augments the severity in the CAIA (Nandakumar et al., 2004) and K/BxN serum transfer models (Jacobs et al., 2009).

In this review, we divide a whole process of arthritis into the "initiation," "inflammatory," and "bone destruction" phases (**Figure 1**). We define the initiation phase as a phase when immune responses are triggered by an assumed antigen(s) and any apparent symptoms in joints are not yet observed. Inflammatory phase

Table 1 | The contribution of proinflammatory cytokines and lymphocytes to the development of mouse models of RA.

	IL-17A	IL-6	IL-1	TNF	$CD4^+$T-cell	B-cell
CIA	+	+	++	+	+	+
SKG	++	++	+	+	+	−
F759	++	++	NR	NR	+	−
IL-1Ra-deficient	++	−	NR	++	+	−
CAIA	NR	−	++	++	−	−
K/BxN serum transfer	NR	−	++	+	−	−
TNF-Tg	NR	−	++	++	−	−

−, Not required; +, partially required; ++, substantially required; NR, not reported.

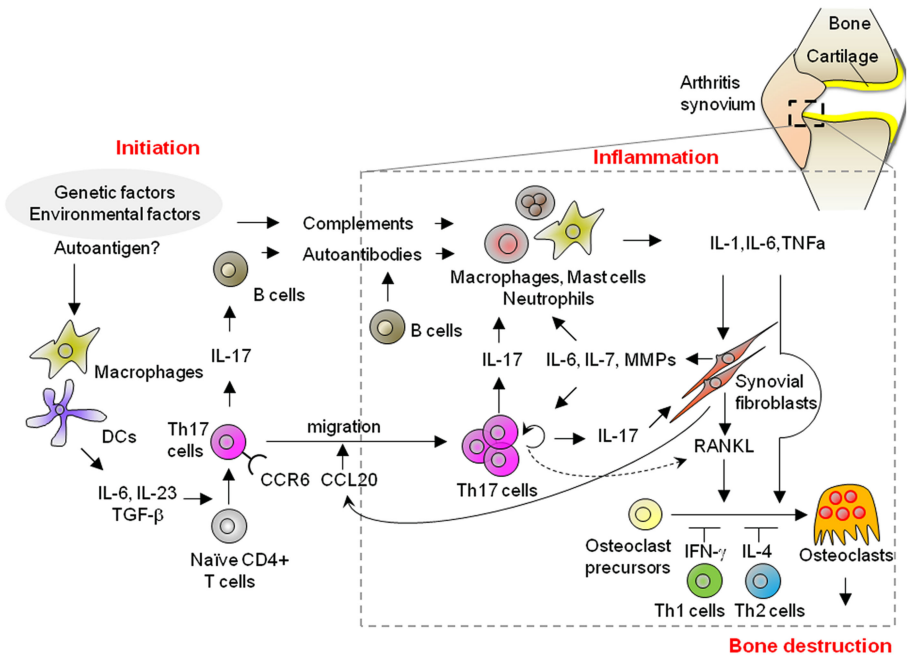


FIGURE 1 | Possible mechanisms of the initiation, inflammatory, and bone destruction phases in RA. A variety of different cell populations, including lymphocytes, innate immune cells, synovial fibroblasts, and osteoclasts, play a role in the development of RA. Th17 cells contribute to the development of arthritis in each of the initiation, inflammatory, and bone destructive phases through the production of autoantibodies as well as the activation of innate immunity and synovial fibroblasts, and then ultimately, bone destruction. Importantly, synovial fibroblasts contribute to Th17 immunity in the inflammatory phase of arthritis by promoting the migration of Th17 cells into the inflammatory joint, and then homeostatic proliferation with an increase in IL-17 production. Thus, the interaction of $CD4^+$T-cells and mesenchymal cells in joints plays a key role in the pathogenesis of RA in both the inflammation and bone destruction phases.

starts when any inflammatory symptoms such as swelling are recognized in joints and continues until any structural changes occur. Bone destruction phase is defined as a phase when structural damages in bone and cartilage are observed. Although the start point of initiation phase is difficult to tell in human, this phase must exist because it takes some time from the start to the point when clinical symptoms are observed. Thus, it is considered that both human RA and animal models of RA consist of all these phases.

The significance of $CD4^+$T-cells in RA development is also supported by T-cell-dependent models such as the SKG mouse, which has a mutation in ZAP70 (Sakaguchi et al., 2003), the F759 mouse with a mutation in the gp130 IL-6 receptor subunit (Atsumi et al., 2002), and the IL-1 receptor antagonist (IL-1Ra)-deficient mouse (Horai et al., 2000). These mice spontaneously develop arthritis due to a defect in TCR signaling or the altered sensitivity to inflammatory cytokines. The adoptive transfer of $CD4^+$T-cells from SKG mice into SCID mice induces arthritis, indicating that the arthritis in SKG mice is $CD4^+$T-cell-dependent (Sakaguchi et al., 2003). In addition, the arthritis which develops in F759 mice requires the presence of $CD4^+$T-cells, but not $CD8^+$T-cells or B-cells, in addition to the gp130 mutation in non-hematopoietic cells (Sawa et al., 2006). Furthermore, the arthritis in IL-1Ra-deficient mice is T-cell-dependent, as the T-cells from IL-1Ra-deficient mice induce disease in nude mice (Horai et al., 2004). Taken together, those T-cell-dependent mouse models indicate that RA can be provoked by $CD4^+$T-cells without the need of B-cell help, due to an intrinsic defect in TCR signaling or altered sensitivity to proinflammatory cytokines (**Table 1**).

In contrast, arthritis develops in human TNF-α transgenic (TNF-Tg) mice (Keffer et al., 1991) and mice with the myeloid-specific deletion of A20, a negative regulator of NF-κB signaling (Matmati et al., 2011). These arthritis are thought to recapitulate the inflammatory phase of RA, bypassing the initiation phase of RA. These mice develop arthritis even on the T, B-cell-deficient background (Douni et al., 1995). This suggested that hyperactivation of innate immune system is also able to induce RA (**Table 1**).

Considering the necessity of $CD4^+$T-cells for the initiation phase, one of the key questions is whether arthritogenic $CD4^+$T-cells recognize a specific antigen, and if so, a joint-specific antigen or not. In the form of arthritis in K/BxN and CIA, arthritogenic $CD4^+$T-cells recognize antigens that are abundant in the joints, although not exclusively joint-specific. In contrast, in the arthritis of F759 mice, the recognition of joint antigens by $CD4^+$T-cells may not be required, because F759 mice expressing a single TCR variant that recognizes a non-joint antigen do indeed develop arthritis (Murakami et al., 2011). Moreover, the antigen specificity of arthritogenic $CD4^+$T-cells in SKG mice remains unknown. Further studies are thus needed to elucidate the antigen specificity of arthritogenic $CD4^+$T-cells, the finding of which will provide new insight into how immunological tolerance is broken by the generation of arthritogenic $CD4^+$T-cells.

Taken together, $CD4^+$T-cells are necessary for at least the initiation phase of arthritis partly by producing arthritogenic antibodies. In contrast, $CD4^+$T-cells may not be required for the inflammatory phase of the disease, especially after arthritogenic autoantibodies are generated abundantly or innate immunity is hyper-activated. Nevertheless, $CD4^+$T-cells have been shown to at least augment the inflammatory phase of arthritis development.

Th17 CELLS: AN EMERGING PATHOGENIC SUBSET OF $CD4^+$T-CELLS

The $CD4^+$ helper T-cells (Th cells), that are differentiated from naïve $CD4^+$T-cells include Th1, Th2, and Th17 cell subsets. Th17 cells, via their production of IL-17, promote the development of autoimmune diseases while also protecting host against bacterial and fungal infection. IL-6 and TGF-β induce Th17 development and IL-23 promotes Th17 cell expansion (Miossec et al., 2009). In the past, Th1 cells, which predominantly produce IFN-γ, were thought to be the principal T-cell player in the pathogenesis of RA. However, accumulating evidence from animal models in fact indicates that Th17 immunity is crucially important.

In CIA, accelerated RA development is evident in IFN-γ receptor-deficient mice (Manoury-Schwartz et al., 1997; Vermeire et al., 1997). In contrast, disease development is markedly diminished in mice with IL-17A deficiency (Nakae et al., 2003a) or with antibody-mediated blockade of IL-17 (Lubberts et al., 2004). In the SKG model, RAG-deficient mice that received naïve SKG $CD4^+$T-cells exhibited arthritis, along with concomitant Th17 generation. This arthritis is Th17-dependent, as RAG mice which received a transfer of IL-17-deficient T-cells did not exhibit any sign of arthritis (Hirota et al., 2007a). Moreover, IL-1Ra-deficient mice with IL-17A deficiency display abrogated arthritis development (Nakae et al., 2003b). Furthermore, F759 mice with IL-17A deficiency (Ogura et al., 2008) and K/BxN mice treated with a neutralizing IL-17A antibody exhibited substantially diminished arthritis (Wu et al., 2010). Taken together, as shown in **Table 1**, regardless of whether the dependency was on IL-6, IL-1, or TNF-α, the development of arthritis was shown to be IL-17-dependent in most T-cell-dependent models, suggesting Th17 cell is a pathogenic subset of $CD4^+$T-cells.

As for the function of IL-17, it augments the production of proinflammatory cytokines, chemokines, and matrix-degrading enzymes of various kinds of cells such as macrophages, dendritic cells (DCs), endothelial cells, and fibroblasts (Miossec et al., 2009). Thus, Th17 cells exacerbate the inflammatory phase of arthritis through the activation of various kinds of cells in the inflamed joints. In addition, IL-17 is responsible for the production of autoantibodies in CIA (Nakae et al., 2003a) and K/BxN (Wu et al., 2010) mouse models. In particular, IL-17 has been shown to enhance germinal center (GC) formation in the K/BxN model. Thus, via IL-17 production, Th17 cells are able to exacerbate the initiation phase of arthritis through the production of autoantibodies. Moreover, IL-17 together with IL-6 amplifies the production of IL-6 by type 1 collagen$^+$fibroblasts, which in turn enhances IL-17 production in T-cells (Ogura et al., 2008) as discussed below. Taken together, Th17 cells can exacerbate arthritis both in the initiation and inflammatory phases.

Cells other than Th17 cells are also reported to produce IL-17 in arthritis affected joints. In the synovium of CIA, γδT-cells also produce IL-17, although few IL-17^+γδT-cells are in fact detected in the affected joints of SKG mice or RA patients (Ito et al., 2009). Mast cells also produce IL-17 in the inflamed joints of RA patients (Hueber et al., 2010a). Although the functional relevance of other IL-17

producing cells remains to be clarified, considering the wealth of evidence for the significance of $CD4^+$T-cells, it can be concluded that Th17 cells play a critical role in arthritis development.

Compared with the understanding of the function of Th17 cells, it remains largely unknown how Th17 cell are generated in the context of arthritis. Recently, several studies on this issue were reported. In SKG mice, Th17 cells are generated in the presence of the IL-6 produced by tissue-resident macrophages in response to C5a, because Th17 cell development is severely impaired in SKG mice having either a C5aR deficiency or a depletion of macrophages (Hashimoto et al., 2010). In addition, a deficiency of Toll-like receptor (TLR)-4 or administration of a TLR-4 antagonist suppresses the development of arthritis in IL-1Ra-deficient mice (Abdollahi-Roodsaz et al., 2008) and CIA (Abdollahi-Roodsaz et al., 2007) by decreasing the number of Th17 cells. This suggests that TLR-4 signaling is involved in Th17 generation. Moreover, in K/BxN mice, not only arthritis, but also Th17 generation and the production of arthritogenic autoantibodies cease under germ-free conditions, whereas the administration of even a single gut-residing species, segmented filamentous bacteria, can induce Th17 generation, GC formation, and the signs of arthritis. Thus, it is suggested that the gut environment affects the generation of $IL\text{-}17^+$ cells, presumably including Th17 cells, leading to the onset of arthritis (Wu et al., 2010).

Given the significant role of Th17 cells in arthritis in mouse models, Th17 is now recognized as a promising therapeutic target. Thus, it is important to clarify the transcriptional mechanisms regulating Th17 development. ROR nuclear receptors are essential for Th17 development (Miossec et al., 2009). IkBζ also regulates Th17 development by cooperating with RORs (Okamoto et al., 2010). Antagonizing ROR activity has been shown to be effective in suppressing Th17 differentiation and Th17-mediated autoimmunity in mice using a synthetic ligand for RORs (Solt et al., 2011) as well as digoxin and its derivatives (Huh et al., 2011). In addition, Abs against IL-17A, LY2439821 (Genovese et al., 2010), and AIN457 (Hueber et al., 2010b) have been shown to be beneficial for the treatment of RA in human, although they are unexpectedly less effective than anti-TNF Abs or anti-IL-6 Abs. This suggests that other IL-17 family members such as IL-17B or IL-17C may also contribute to RA pathogenesis (Yamaguchi et al., 2007). Alternatively, there may be a difference in the extent to which Th17 cells contribute to the pathogenesis of arthritis between mice and humans. Indeed, T-cells in the synovial fluid of patients with juvenile idiopathic arthritis (JIA) easily switch from a Th17 to Th1 phenotype via the intermediate step of a Th1/Th17 mixed phenotype (Cosmi et al., 2011), suggesting that human Th17 cells are more plastic than their mouse counterparts. In line with this, Ustekinumab, which is a human mAb against IL-12/23p40, significantly suppresses psoriatic arthritis in human (Gottlieb et al., 2009). In addition, a JAK inhibitor tofacitinib (CP690,550) which inhibits the established CIA presumably by suppressing pathogenic Th1 and Th17 cells (Ghoreschi et al., 2011), shows clinical benefit for RA (Kremer et al., 2009; Fleischmann et al., 2012). From this point of view, either an EP4 antagonist that blocks PGE2–EP4 signaling (Yao et al., 2009) or a depletion of anti-LT-α Abs (Chiang et al., 2009), which have been shown to suppress Th17-mediated autoimmune disease through the inhibition of both Th1 and Th17 immunity in mice, might be therapeutically beneficial in RA treatment.

Taken together, Th17 cells are crucial immune cells that are required for the initiation of arthritis and contribute to the augmentation of chronic inflammation in joints through the activation of both innate immunity and mesenchymal cells such as synovial fibroblasts in joints.

INNATE IMMUNE CELLS: AN ACCELERATOR OF ARTHRITIS

In addition to T-cell infiltration, RA exhibits a massive infiltration into affected joints innate immune cells, including macrophages, neutrophils, mast cells, and DCs. These cells react to complement or the Fc portion of IgG isotypes via receptors expressed on their surface. They also produce proinflammatory cytokines, chemokines, and matrix-degrading enzymes that drive chronic inflammation.

The importance of innate immunity in arthritis development has been shown in both T-cell-dependent and independent mouse models. In the T-cell-dependent models, SKG mice fail to develop arthritis when they are raised under a specific pathogen free (SPF) condition, whereas SKG mice raised under a conventional environment do develop arthritis. In addition, SKG mice under an SPF condition develop severe arthritis when administrated zymosan, a crude yeast cell wall extract. Proinflammatory cytokines, presumably including TNF-α, which are produced by Dectin-1 expressing DCs or macrophages in response to zymosan, are involved in this process (Yoshitomi et al., 2005). In addition, macrophages produce IL-6 in response to C5a, leading to the generation of Th17 cells in SKG mice (Hashimoto et al., 2010). These findings indicate that activation of adaptive immunity requires innate immunity in the initiation phase of arthritis.

Among the T-cell-independent models, the K/BxN serum transfer model has helped address the mechanisms by which activation of innate immune system triggered by autoantibodies leads to the development of arthritis. In the K/BxN model, the autoantigen is the glucose-6-phosphate isomerase (GPI) that is expressed in the joint, although it is not joint-specific. GPI-anti-GPI immune complexes bind to articular surfaces, leading to the local augmentation of immune effecter responses in the joint (Matsumoto et al., 2002). K/BxN serum transfer arthritis requires complement C5 and FcγRIII (Ji et al., 2002). Neutrophils and mast cells are also required, as mice depleted of neutrophils (Wipke and Allen, 2001) and mice lacking mast cells (Lee et al., 2002) are both resistant to the arthritis.

As I mentioned above, TNF-Tg mice and mice with selective deletion of A20 (TNFAIP3) in myeloid cells do not require either T-cells or B-cells for the development of arthritis (Douni et al., 1995; Matmati et al., 2011). These studies suggest that hyperactivation of innate immunity is sufficient to induce arthritis. In addition, these findings prompt a consideration of how it is that a systemic gene mutation results in local joint inflammation. There may be certain joint-specific factors, possibly expressed by synovial fibroblasts, which drive the preferential migration of activated innate immune cells and thus the amplification of chronic inflammation in the affected joints.

Taken together, adaptive immunity requires the activity of innate immunity for the development of full blown arthritis both

in the initiation and inflammatory phases. In addition, the hyperactivation of the innate immune response by itself is able to induce arthritis, presumably through an interaction with synovial fibroblasts, a unique mesenchymal cell population in joints.

SYNOVIAL FIBROBLASTS: MESENCHYMAL CELLS THAT DETERMINE JOINT-SPECIFICITY

In general, all RA patients and RA model mice exhibit proliferative and erosive synovitis in regions adjacent to cartilage and bone, regardless of differences in the initiating mechanisms. Synoviocytes are divided into synovial fibroblasts of mesenchymal origin and macrophage-like synoviocytes, depending on their surface markers. RA synovial fibroblasts are key cells in the chronic inflammation which occurs in RA.

Synovial fibroblasts express not only receptors for proinflammatory cytokines, but also TLRs (Bartok and Firestein, 2010). In synovitis, synovial fibroblasts exhibit high proliferative activity and produce large amounts of cytokines, chemokines, and matrix-degrading enzymes in response to proinflammatory cytokines and TLR ligands, which lead to the exacerbation of synovitis and joint destruction. For instance, Tenascin-C, an extracellular matrix glycoprotein specifically expressed in inflamed joints, was shown to be an endogenous activator of the TLR-4 expressed by synovial fibroblasts and macrophages, and is also essential for maintaining synovitis in K/BxN serum transfer arthritis (Midwood et al., 2009). Interestingly, the microparticles produced by activated platelets amplify inflammatory arthritis in the K/BxN serum transfer model via a collagen-receptor expressed on synovial fibroblasts (Boilard et al., 2010).

The invasive characteristics of synovial fibroblasts from RA synovium have been reported in following studies. Cultured synovial fibroblasts from human RA synovium were shown to invade and destroy cartilage when co-transplanted with cartilage into SCID mice (Muller-Ladner et al., 1996). These transplanted RA synovial fibroblasts specifically migrate into a distal cartilage even in the absence of other immune cells (Lefèvre et al., 2009). Thus, it is suggested that synovial fibroblasts appear to have intrinsically invasive properties and to be destined to localize specifically in the joint. In addition, the invasive characteristics of synovial fibroblasts have also been reported in synoviocyte clones obtained from TNF-Tg mice (Aidinis et al., 2003). These results suggest that the intrinsically invasive properties of synovial fibroblasts from inflamed joints are stably maintained even after several passages in culture and that epigenetic modification may be involved in this process. Indeed, the DNA of RA synovial fibroblasts is hypomethylated both in synovial tissues and *in vitro* (Karouzakis et al., 2009). In addition, the ratio of histone acetylase/deacetylase activity is higher in RA synovial tissue than that in normal synovial tissue (Huber et al., 2007). Furthermore, synovial fibroblasts preferentially express microRNA 146a and 155, among microRNAs which function as posttranscriptional repressors of gene expression (Stanczyk et al., 2008). Further studies are needed to clarify the mechanisms of epigenetic modification and their role in the maintenance of the activated phenotype of synovial fibroblasts in arthritic joints.

Given that the infiltration of $CD4^+$T-cells in inflamed joints is a hallmark of RA pathology, the interaction of synovial fibroblasts and $CD4^+$T-cells is assumed to play an important role. By *in vitro* co-culture experiments, it has been demonstrated that RA synovial fibroblasts and $CD4^+$T-cells activate each other through the ICAM-2 and LFA expressed on synovial fibroblasts and $CD4^+$T-cells, respectively (Singh et al., 2008). In addition, the IL-15 expressed on RA synovial fibroblasts augments the production of effecter cytokines from $CD4^+CD25^-$ cells, while also enhancing the proliferation of $CD4^+CD25^+$Treg cells (Benito-Miguel et al., 2009). Several reports suggest an antigen-presenting role for synovial fibroblasts. RA synovial fibroblasts in tissue express MHC class (Burmester et al., 1987) IFN-γ treated synovial fibroblasts *in vitro* stimulate T-cell activation in an MHC class II dependent manner (Tran et al., 2007). However, the capacity for MHC class II restricted antigen presentation in synovial fibroblasts and its role in RA development *in vivo* remain to be demonstrated.

Importantly, several recent reports have shed light on the relevance of the interaction of $CD4^+$T-cells and mesenchymal cells in the affected joint in the development of arthritis. In the SKG model, synovial fibroblasts produce CCL20 in response to proinflammatory cytokines such as TNF-α, leading to the recruitment of $CCR6^+$Th17 cells into the affected joint. This recruitment is essential, as the administration of a neutralizing anti-CCR6 antibody ameliorates the development of arthritis (Hirota et al., 2007b). Likewise, in F759 arthritis, type 1 collagen$^+$fibroblasts produce CCL20 in response to local stimuli such as microbleeding and preferentially recruit $CD4^+$T-cells into inflamed joints. The relevance of this recruitment has been demonstrated, because the inhibition of CCL20 diminished arthritis development (Murakami et al., 2011). In addition, non-hematopoietic cells, presumably synovial fibroblasts, produce elevated levels of IL-7 and IL-6, which enhances the homeostatic proliferation of $CD4^+$T-cells and the production of IL-17 in Th17 cells, respectively (Sawa et al., 2006; Ogura et al., 2008). Moreover, IL-6 together with IL-17 amplifies IL-6 production of synovial fibroblasts (Ogura et al., 2008). In line with this, by *in vitro* co-culture system, a JAK inhibitor, Tofacitinib suppress the production of IL-6 by RA synovial fibroblasts through the inhibition of IL-17 and IFN-γ by RA $CD4^+$T-cells (Maeshima et al., 2011).

Considering the important role of synovial fibroblasts, they may be a good therapeutic target for RA treatment. The induction of the cell senescence gene in synovial tissues successfully inhibits rat adjuvant-induced arthritis (Taniguchi et al., 1999). Yet only a few molecules have been identified as specific markers of synovial fibroblasts to date. Cadherin-11, a relatively specific marker, is required for the cellular connectivity of synovial fibroblasts. Cadherin-11-deficient mice exhibit a hypoplastic synovial lining of the synovium membrane and much less severe arthritis. Importantly, cadherin-11-directed therapeutics also markedly reduces synovial inflammation (Lee et al., 2007). Mechanistically, cadherin-11 contributes to the production of IL-6 in synovial fibroblasts (Chang et al., 2011). The identification of additional specific markers of synovial fibroblasts will ultimately lead to the establishment of "joint-preferential" therapeutic strategies.

These findings, taken together, indicate that synovial fibroblasts function as a unique "disease amplifier" in the inflammatory phase of RA through both the innate and acquired immunity pathways,

due to their intrinsically invasive, hypersensitive, and hyperproliferative properties. Studies on animal models of RA have revealed the role of synovial fibroblasts in Th17 immunity, i.e., promoting the migration of Th17 cells to the affected joints and then homeostatic proliferation with an accompanying increase in IL-17 production, ultimately leading to the augmentation of the chronic inflammation which characterizes RA (**Figure 1**).

PROINFLAMMATORY CYTOKINES MEDIATE THE INTERPLAY BETWEEN IMMUNE CELLS AND JOINTS

In RA synovium, elevated levels of the proinflammatory cytokines IL-1, IL-6, and TNF-α are produced by macrophages and synovial fibroblasts. These proinflammatory cytokines both directly and indirectly exert their effects through the production of additional proinflammatory cytokines and chemokines as well as matrix-degrading enzymes, resulting in a cytokine "storm" in the inflamed synovium. The relative contribution of IL-1, IL-6, and TNF-α to the development and progression of arthritis is different in the various mouse models (**Table 1**).

In CIA, the blockade of IL-1 prevents arthritis (Joosten et al., 1996). IL-6 deficiency suppresses disease development (Alonzi et al., 1998). However, administration of a neutralizing anti-IL-6 mAb suppresses arthritis development when given early, but the suppressive effect is not observed when given in the later phases (Fujimoto et al., 2008). Likewise, the blockade of TNF-α markedly decreases inflammation and joint destruction when given early (Williams et al., 1992; Joosten et al., 1996). Recently, the growth factor progranulin was shown to bind to TNF receptors and block TNF-α/TNFR signaling. Progranulin reverses inflammatory arthritis in TNF-Tg mice and prevents the development of both CIA and CAIA (Tang et al., 2011). In addition, inhibition of migration of pathogenic T-cells into the joints and the prevention of emigration out of draining lymph nodes are observed in CIA mice in which TNF/TNFR signaling has been blocked (Notley et al., 2008). In line with this, impaired migration of T-cells into the joints is also observed in human RA patients treated with an anti-TNFα mAb (Taylor et al., 2000).

Overall, it is clear that there is a substantial difference in the relative contribution of these inflammatory cytokines to the development of arthritis (**Table 1**). Dependency on IL-1 and TNFα in both the T-cell-dependent and independent arthritis models suggests that IL-1 and TNFα may be involved in the inflammatory phase of arthritis in mice. As for human RA, anti-TNF therapies achieved clinical remission while the IL-1 inhibitor IL-1Ra was less effective than would be expected from mouse studies, suggesting that IL-1 in RA may not be as important as it is in mouse arthritis (Buch et al., 2004). In contrast, the different pattern of dependency on IL-6 in the T-cell-dependent and T-cell-independent arthritis models suggests that IL-6 may be critically involved in T-cell mediated arthritis and affect pathogenesis of T-cells. Indeed, the protective effect of IL-6 blockade in CIA correlates with the inhibition of Th17 differentiation. In this model, IL-6 blockade was shown to be effective when administered at an early initiation phase (Fujimoto et al., 2008). However, a significant number of RA patients with the blockade of IL-6 signaling achieved clinical remission suggesting that IL-6 plays an important role even in the inflammatory phase in human.

Taken together, proinflammatory cytokines mediate the interplay between immune cells and joints, leading to the initiation and augmentation of chronic inflammation in RA. The substantial differences in cytokine-dependency in animal models may reflect the different effect of each cytokine in each phase of arthritis progression, in association with the triggering arthritogenic stimuli and type of the cells that constitute the inflammatory synovium.

OSTEOCLASTS; A KEY PLAYER IN THE BONE DESTRUCTION WHICH OCCURS IN RA

In the pathology of RA, chronic inflammation leads to bone destruction. The synovium is a site where the immune system interferes with normal bone homeostasis. Bone homeostasis is maintained by a balance between the continuous resorption activity of osteoclasts and formation by osteoblasts. In RA, the bone destruction which takes place is mainly due to the excessive bone resorption activity of osteoclasts.

Osteoimmunology is a cross-disciplinary research field that investigates the interplay of the bone and immune system at the molecular level (Takayanagi, 2009). The interaction of osteoclasts and immune cells is a major topic of interest in this field. Studies of the relationship of osteoclasts and macrophages have led to important mechanistic insights into osteoclast differentiation. In addition, studies of the interaction of osteoclasts and T-cells have contributed to an improved understanding of the mechanism of bone destruction in RA.

Historically, increased numbers of osteoclast-like giant cells had been identified in the synovium of RA joints by the early 1980s (Bromley and Woolley, 1984). Based on these pathological findings, it was therefore suggested that osteoclasts have an important role in bone resorption in arthritis. Importantly, osteoclast formation from cultured synovial cells was successfully performed without the need of any other cells, demonstrating that rheumatoid synovial cells contain both osteoclast precursor cells and osteoclastogenesis-supporting cells (Takayanagi et al., 1997). However, the molecular mechanism still remained unclear until the identification of RANKL as an osteoclast differentiation factor expressed on synovial cells (Gravallese et al., 2000; Takayanagi et al., 2000a).

Osteoclasts are formed when bone marrow cells are cultured in the presence of M-CSF and RANKL *in vitro*. Osteoclasts also are differentiated from bone marrow cells when co-cultured with mesenchymal cells, such as osteoblasts, in the presence of osteoclastogenic factors, including 1,25-dihydroxylvitamin D_3, which induce RANKL expression on mesenchymal cells. Recent studies indicate that osteocytes, which are embedded in bone, express a higher amount of RANKL than osteoblasts and are thus the major source of RANKL in bone remodeling *in vivo* (Nakashima et al., 2011; Xiong et al., 2011).

RANKL is essential for osteoclast differentiation, as RANKL-deficient mice exhibit an osteopetrotic phenotype (Theill et al., 2002). Of note, a critical role for both RANKL and osteoclasts in arthritic bone destruction was demonstrated in mouse models of RA (Pettit et al., 2001; Redlich et al., 2002). Bone destruction did not occur in the absence of osteoclasts in either of these models, but a level of inflammation similar to that in their wild-type counterparts was observed, indicating that RANKL and osteoclasts

are indispensable for bone destruction, but not for inflammation. There is a long-standing debate whether cells other than synovial fibroblasts express RANKL and thus contribute to osteoclastogenesis in arthritis. RANKL was originally identified as being expressed on activated T-cells (Wong et al., 1997). Histologically, in the RA synovium, RANKL is expressed by both synovial cells and T-cells (Kong et al., 1999; Gravallese et al., 2000; Takayanagi et al., 2000a). In addition, RANKL expression on B-cells in the arthritic joints of RA patients was reported (Yeo et al., 2012). However, it still remains unclear the extent to which lymphocytes, as a source of RANKL, contribute to the bone destruction in arthritis. Mice bearing a cell type-specific deletion of RANKL will be required to decide this issue. Given the important role of RANKL in osteoclastogenesis, RANKL is a promising pharmacological target for the prevention of joint destruction. Indeed, an anti-RANKL antibody was recently shown to inhibit joint destruction in human RA patients (Dore et al., 2010).

The discovery of RANKL shed light on the importance of understanding the molecular mechanisms that underlie osteoclast differentiation and function, which has led to the identification of NFATc1 as a master transcription regulator of osteoclastogenesis (Takayanagi et al., 2002) and other related signaling molecules. Notably, tyrosine kinases Btk and Tec regulate osteoclastogenesis and the inhibition of Tec kinase reduce inflammation-induced bone destruction (Shinohara et al., 2008). Further studies regarding precise mechanisms of osteoclast differentiation and function are required for a precise molecular basis for novel therapeutic strategies.

Table 2 | T-cell-related cytokines and osteoclastogenesis.

Th cell subsets	Associated cytokines	Main producer cells	Effects on osteoclastogenesis
Th1 cells	IFN-γ	Th1 cells and NK cells	Inhibition
	GM-CSF	Th1 cells	Inhibition
	IL-12	Macrophages and DCs	Inhibition
Th2 cells	IL-4	Th2 cells and Mast cells	Inhibition
	IL-10	Th2 cells and Treg cells	Inhibition
Th17 cells	IL-17	Th17 cells, γδT-cells, and Mast cells	Activation
	RANKL	Synoviocytes, Osteoblasts, and Th17 cells	Activation
	IL-1	Macrophages, Synoviocytes, and Mast cells	Activation
	IL-6	Macrophages, DCs, and Synoviocytes	Activation
	IL-21	Th17 cells, NKT cells	Activation
	IL-22	Th17 cells, NK cells	Activation
	IL-23	DCs and Macrophages	Activation
	TNFα	Macrophages and DCs	Activation
Treg cells	IL-10	Th2 cells and Treg cells	Inhibition
	TGF-β	Treg cells and DCs	Activation

The associated cytokines include those produced by T-cells as well as those that are important for T-cell induction.

SYNOVIAL FIBROBLASTS PROMOTE OSTEOCLASTOGENESIS VIA INTERACTION WITH IMMUNE CELLS

Activated T-cells express not only RANKL but also effector cytokines, including cytokines with either stimulatory or inhibitory effects on osteoclastogenesis, as shown in **Table 2** (Takayanagi et al., 2000b; Takayanagi, 2009). Thus, the osteoclastogenic capacity of T-cells is determined by both RANKL and cytokine expression.

IL-17 is known to enhance osteoclastogenesis *in vitro* by acting on osteoclastogenesis-supporting cells (Kotake et al., 1999). Of note, Th17 cells, but neither Th1 cell nor Th2 cells, comprise the osteoclastogenic helper T subset. Th17 cells do not produce either IFN-γ or IL-4, each of which inhibits osteoclastogenesis, but do produce IL-17, which stimulates osteoclastogenesis by its effect on osteoblasts that act as osteoclastogenesis-supporting mesenchymal cells (Sato et al., 2006).

Therefore, the presumable roles of IL-17 in the bone destruction which occurs in RA are as follows. First, IL-17 exerts its osteoclastogenic effect by stimulating RANKL expression by synovial fibroblasts. Furthermore, IL-17 up-regulates the expression of proinflammatory cytokines such as IL-1, IL-6, and TNF-α, which promote osteoclastogenesis through their effects on osteoclast precursor cells by enhancing RANK mediated signaling, or indirectly through upregulation of RANKL expression by synovial fibroblasts. These events synergistically promote osteoclastic bone resorption in the inflamed synovium.

Besides IL-17, IL-21(Kwok et al., 2012), and IL-22 (Kim et al., 2012), which are also produced by Th17 cells, stimulate osteoclastogenesis mainly by upregulating RANKL expression in synovial fibroblasts. Thus, it is plausible that synovial fibroblasts augment their capacity to induce osteoclastogenesis in the presence of Th17 cells.

An important role for Th17 in bone destruction is supported by studies in mouse models. In CIA, the neutralization of IL-17 after the onset of arthritis reduces the severity of joint destruction (Lubberts et al., 2004). Although both Th17 cells and γδT-cells produce IL-17 in the affected joints of CIA, Th17 cells, but not γδT-cells, have been shown by antibody-mediated depletion and adoptive transfer studies to reside adjacent to osteoclasts and to play a prominent role in bone destruction *in vivo* (Pollinger et al., 2011).

Osteoclast precursor cells express receptors for proinflammatory cytokines. Most of the proinflammatory cytokines which augment inflammation also promote osteoclastogenesis by augmenting RANK–RANKL signaling, with the exception that TNF-α and TGF-β together induce osteoclastogenesis even in the absence of RANK (Kim et al., 2005) This suggests that the inhibition of proinflammatory cytokines by neutralizing Abs would play a dual role in the suppression of inflammation and bone destruction in RA. Interestingly, the inhibition of cathepsin K, which was thought to be expressed exclusively by osteoclasts and to play an essential role in bone degradation, has been shown to play dual role in suppression of osteoclastic bone resorption and TLR-9 mediated-activation of DCs (Asagiri et al., 2008).

Taken together, synovial fibroblasts contribute not only to chronic inflammation but also to the bone destruction which occurs in RA by promoting RANKL-mediated osteoclastogenesis through the interaction of immune cells, mainly Th17 cells.

CONCLUDING REMARKS

Rheumatoid arthritis is an immune-mediated disease, characterized by local inflammation and bone destruction in joint as a result of alteration of systemic immune response. Recent studies have revealed that Th17 cells and synovial fibroblasts are the critical regulators. As shown in **Figure 1**, Th17 cells, differentiated in the presence of innate immunity, help B-cells produce arthritogenic autoantibodies in the initiation phase. In inflamed joints, Th17 cells activate innate immune cells and synovial fibroblasts by upregulating proinflammatory cytokines and matrix-degrading enzymes, thereby leading to an amplification of chronic inflammation. Moreover, Th17-related cytokines stimulate the differentiation of osteoclasts, mainly via the synovial fibroblasts in the joints, which eventually leads to bone destruction. Thus, Th17 cells are not only required for the initiation of the systemic immune response, they contribute to chronic inflammation and bone destruction. Importantly, synovial fibroblasts contribute to Th17 immunity in both the inflammatory and bone destruction phases of arthritis by promoting the migration of Th17 cells into the joint, inducing homeostatic proliferation with a concomitant increase in IL-17 production and promoting osteoclastogenesis by upregulation of RANKL expression. It is thus suggested that synovial fibroblasts connect the systemic immune response to local joint disorders by their intrinsic characteristics, including their "hyper-reactivity" and "hyper-chemoattractivity" in response to inflammatory stimuli.

Collectively, the interaction of immune cells and non-hematopoietic mesenchymal cells in the joints plays a key role in the pathogenesis of RA in both the inflammatory and bone destruction phases. Elucidation of the precise mechanisms involved in this interaction will lead to a better understanding of RA and provide a molecular basis for effective therapeutic strategies against this disease. Furthermore, the findings obtained from such investigation of RA will undoubtedly prove applicable to other diseases evoked through the interaction of immune and mesenchymal cells.

ACKNOWLEDGMENTS

We are grateful to all the members of our laboratory in the Graduate School of Medical and Dental Sciences. The work in our laboratory was supported in part by a grant for the ERATO, Takayanagi Osteonetwork Project from JST; Grant-in-Aid for Postdocs for from the Japan Society for the Promotion of Science (JSPS); a grant for the Global Center of Excellence Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT).

REFERENCES

Abdollahi-Roodsaz, S., Joosten, L. A., Koenders, M. I., Devesa, I., Roelofs, M. F., Radstake, T. R., Heuvelmans-Jacobs, M., Akira, S., Nicklin, M. J., Ribeiro-Dias, F., and van Den Berg, W. B. (2008). Stimulation of TLR2 and TLR4 differentially skews the balance of T cells in a mouse model of arthritis. *J. Clin. Invest.* 118, 205–216.

Abdollahi-Roodsaz, S., Joosten, L. A., Roelofs, M. F., Radstake, T. R., Matera, G., Popa, C., Van Der Meer, J. W., Netea, M. G., and Van Den Berg, W. B. (2007). Inhibition of Toll-like receptor 4 breaks the inflammatory loop in autoimmune destructive arthritis. *Arthritis Rheum.* 56, 2957–2967.

Aidinis, V., Plows, D., Haralambous, S., Armaka, M., Papadopoulos, P., Kanaki, M. Z., Koczan, D., Thiesen, H. J., and Kollias, G. (2003). Functional analysis of an arthritogenic synovial fibroblast. *Arthritis Res. Ther.* 5, R140–R157.

Alonzi, T., Fattori, E., Lazzaro, D., Costa, P., Probert, L., Kollias, G., De Benedetti, F., Poli, V., and Ciliberto, G. (1998). Interleukin 6 is required for the development of collagen-induced arthritis. *J. Exp. Med.* 187, 461–468.

Asagiri, M., Hirai, T., Kunigami, T., Kamano, S., Gober, H. J., Okamoto, K., Nishikawa, K., Latz, E., Golenbock, D. T., Aoki, K., Ohya, K., Imai, Y., Morishita, Y., Miyazono, K., Kato, S., Saftig, P., and Takayanagi, H. (2008). Cathepsin K-dependent toll-like receptor 9 signaling revealed in experimental arthritis. *Science* 319, 624–627.

Atsumi, T., Ishihara, K., Kamimura, D., Ikushima, H., Ohtani, T., Hirota, S., Kobayashi, H., Park, S. J., Saeki, Y., Kitamura, Y., and Hirano, T. (2002). A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. *J. Exp. Med.* 196, 979–990.

Bartok, B., and Firestein, G. S. (2010). Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol. Rev.* 233, 233–255.

Begovich, A. B., Carlton, V. E., Honigberg, L. A., Schrodi, S. J., Chokkalingam, A. P., Alexander, H. C., Ardlie, K. G., Huang, Q., Smith, A. M., Spoerke, J. M., Conn, M. T., Chang, M., Chang, S. Y., Saiki, R. K., Catanese, J. J., Leong, D. U., Garcia, V. E., Mcallister, L. B., Jeffery, D. A., Lee, A. T., Batliwalla, F., Remmers, E., Criswell, L. A., Seldin, M. F., Kastner, D. L., Amos, C. I., Sninsky, J. J., and Gregersen, P. K. (2004). A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am. J. Hum. Genet.* 75, 330–337.

Benito-Miguel, M., Garcia-Carmona, Y., Balsa, A., Perez De Ayala, C., Cobo-Ibanez, T., Martin-Mola, E., and Miranda-Carus, M. E. (2009). A dual action of rheumatoid arthritis synovial fibroblast IL-15 expression on the equilibrium between CD4+CD25+ regulatory T cells and CD4+CD25- responder T cells. *J. Immunol.* 183, 8268–8279.

Boilard, E., Nigrovic, P. A., Larabee, K., Watts, G. F., Coblyn, J. S., Weinblatt, M. E., Massarotti, E. M., Remold-O'Donnell, E., Farndale, R. W., Ware, J., and Lee, D. M. (2010). Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* 327, 580–583.

Bromley, M., and Woolley, D. E. (1984). Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. *Arthritis Rheum.* 27, 968–975.

Buch, M. H., Bingham, S. J., Seto, Y., Mcgonagle, D., Bejarano, V., White, J., and Emery, P. (2004). Lack of response to anakinra in rheumatoid arthritis following failure of tumor necrosis factor alpha blockade. *Arthritis Rheum.* 50, 725–728.

Burmester, G. R., Jahn, B., Rohwer, P., Zacher, J., Winchester, R. J., and Kalden, J. R. (1987). Differential expression of Ia antigens by rheumatoid synovial lining cells. *J. Clin. Invest.* 80, 595–604.

Chang, S. K., Noss, E. H., Chen, M., Gu, Z., Townsend, K., Grenha, R., Leon, L., Lee, S. Y., Lee, D. M., and Brenner, M. B. (2011). Cadherin-11 regulates fibroblast inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8402–8407.

Chiang, E. Y., Kolumam, G. A., Yu, X., Francesco, M., Ivelja, S., Peng, I., Gribling, P., Shu, J., Lee, W. P., Refino, C. J., Balazs, M., Paler-Martinez, A., Nguyen, A., Young, J., Barck, K. H., Carano, R. A., Ferrando, R., Diehl, L., Chatterjea, D., and Grogan, J. L. (2009). Targeted depletion of lymphotoxin-alpha-expressing TH1 and TH17 cells inhibits autoimmune disease. *Nat. Med.* 15, 766–773.

Cosmi, L., Cimaz, R., Maggi, L., Santarlasci, V., Capone, M., Borriello, F., Frosali, F., Querci, V., Simonini, G., Barra, G., Piccinni, M. P., Liotta, F., De Palma, R., Maggi, E., Romagnani, S., and Annunziato, F. (2011). Evidence of the transient nature of the Th17 phenotype of CD4+CD161+ T cells in the synovial fluid of patients with juvenile idiopathic arthritis. *Arthritis Rheum.* 63, 2504–2515.

Dore, R. K., Cohen, S. B., Lane, N. E., Palmer, W., Shergy, W., Zhou, L., Wang, H., Tsuji, W., and Newmark, R. (2010). Effects of denosumab on bone mineral density and bone turnover in patients with rheumatoid arthritis receiving concurrent glucocorticoids or bisphosphonates. *Ann. Rheum. Dis.* 69, 872–875.

Douni, E., Akassoglou, K., Alexopoulou, L., Georgopoulos, S., Haralambous, S., Hill, S., Kassiotis, G., Kontoyiannis, D., Pasparakis, M., Plows, D., Probert, L., and Kollias, G. (1995). Transgenic and knockout analyses of the role of TNF in immune regulation and disease pathogenesis. *J. Inflamm.* 47, 27–38.

Firestein, G. S. (2003). Evolving concepts of rheumatoid arthritis. *Nature* 423, 356–361.

Fleischmann, R., Cutolo, M., Genovese, M. C., Lee, E. B., Kanik, K. S., Sadis, S., Connell, C. A., Gruben, D., Krishnaswami, S., Wallenstein, G., Wilkinson, B. E., and Zwillich, S. H. (2012). Phase 2B dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) or adalimumab monotherapy versus placebo in patients with active rheumatoid arthritis with an inadequate response to DMARDs. *Arthritis Rheum.* 64, 617–629.

Fujimoto, M., Serada, S., Mihara, M., Uchiyama, Y., Yoshida, H., Koike, N., Ohsugi, Y., Nishikawa, T., Ripley, B., Kimura, A., Kishimoto, T., and Naka, T. (2008). Interleukin-6 blockade suppresses autoimmune arthritis in mice by the inhibition of inflammatory Th17 responses. *Arthritis Rheum.* 58, 3710–3719.

Genovese, M. C., Van Den Bosch, F., Roberson, S. A., Bojin, S., Biagini, I. M., Ryan, P., and Sloan-Lancaster, J. (2010). LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: a phase I randomized, double-blind, placebo-controlled, proof-of-concept study. *Arthritis Rheum.* 62, 929–939.

Ghoreschi, K., Jesson, M. I., Li, X., Lee, J. L., Ghosh, S., Alsup, J. W., Warner, J. D., Tanaka, M., Steward-Tharp, S. M., Gadina, M., Thomas, C. J., Minnerly, J. C., Storer, C. E., Labranche, T. P., Radi, Z. A., Dowty, M. E., Head, R. D., Meyer, D. M., Kishore, N., and O'Shea, J. J. (2011). Modulation of innate and adaptive immune responses by tofacitinib (CP-690,550). *J. Immunol.* 186, 4234–4243.

Gottlieb, A., Menter, A., Mendelsohn, A., Shen, Y. K., Li, S., Guzzo, C., Fretzin, S., Kunynetz, R., and Kavanaugh, A. (2009). Ustekinumab, a human interleukin 12/23 monoclonal antibody, for psoriatic arthritis: randomised, double-blind, placebo-controlled, crossover trial. *Lancet* 373, 633–640.

Gravallese, E. M., Manning, C., Tsay, A., Naito, A., Pan, C., Amento, E., and Goldring, S. R. (2000). Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. *Arthritis Rheum.* 43, 250–258.

Hashimoto, M., Hirota, K., Yoshitomi, H., Maeda, S., Teradaira, S., Akizuki, S., Prieto-Martin, P., Nomura, T., Sakaguchi, N., Kohl, J., Heyman, B., Takahashi, M., Fujita, T., Mimori, T., and Sakaguchi, S. (2010). Complement drives Th17 cell differentiation and triggers autoimmune arthritis. *J. Exp. Med.* 207, 1135–1143.

Hirota, K., Hashimoto, M., Yoshitomi, H., Tanaka, S., Nomura, T., Yamaguchi, T., Iwakura, Y., Sakaguchi, N., and Sakaguchi, S. (2007a). T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis. *J. Exp. Med.* 204, 41–47.

Hirota, K., Yoshitomi, H., Hashimoto, M., Maeda, S., Teradaira, S., Sugimoto, N., Yamaguchi, T., Nomura, T., Ito, H., Nakamura, T., Sakaguchi, N., and Sakaguchi, S. (2007b). Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J. Exp. Med.* 204, 2803–2812.

Horai, R., Nakajima, A., Habiro, K., Kotani, M., Nakae, S., Matsuki, T., Nambu, A., Saijo, S., Kotaki, H., Sudo, K., Okahara, A., Tanioka, H., Ikuse, T., Ishii, N., Schwartzberg, P. L., Abe, R., and Iwakura, Y. (2004). TNF-alpha is crucial for the development of autoimmune arthritis in IL-1 receptor antagonist-deficient mice. *J. Clin. Invest.* 114, 1603–1611.

Horai, R., Saijo, S., Tanioka, H., Nakae, S., Sudo, K., Okahara, A., Ikuse, T., Asano, M., and Iwakura, Y. (2000). Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J. Exp. Med.* 191, 313–320.

Huber, L. C., Brock, M., Hemmatazad, H., Giger, O. T., Moritz, F., Trenkmann, M., Distler, J. H., Gay, R. E., Kolling, C., Moch, H., Michel, B. A., Gay, S., Distler, O., and Jungel, A. (2007). Histone deacetylase/acetylase activity in total synovial tissue derived from rheumatoid arthritis and osteoarthritis patients. *Arthritis Rheum.* 56, 1087–1093.

Hueber, A. J., Asquith, D. L., Miller, A. M., Reilly, J., Kerr, S., Leipe, J., Melendez, A. J., and Mcinnes, I. B. (2010a). Mast cells express IL-17A in rheumatoid arthritis synovium. *J. Immunol.* 184, 3336–3340.

Hueber, W., Patel, D. D., Dryja, T., Wright, A. M., Koroleva, I., Bruin, G., Antoni, C., Draelos, Z., Gold, M. H., Durez, P., Tak, P. P., Gomez-Reino, J. J., Foster, C. S., Kim, R. Y., Samson, C. M., Falk, N. S., Chu, D. S., Callanan, D., Nguyen, Q. D., Rose, K., Haider, A., and Di Padova, F. (2010b). Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. *Sci. Transl. Med.* 2, 52ra72.

Huh, J. R., Leung, M. W., Huang, P., Ryan, D. A., Krout, M. R., Malapaka, R. R., Chow, J., Manel, N., Ciofani, M., Kim, S. V., Cuesta, A., Santori, F. R., Lafaille, J. J., Xu, H. E., Gin, D. Y., Rastinejad, F., and Littman, D. R. (2011). Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORgammat activity. *Nature* 472, 486–490.

Ito, Y., Usui, T., Kobayashi, S., Iguchi-Hashimoto, M., Ito, H., Yoshitomi, H., Nakamura, T., Shimizu, M., Kawabata, D., Yukawa, N., Hashimoto, M., Sakaguchi, N., Sakaguchi, S., Yoshifuji, H., Nojima, T., Ohmura, K., Fujii, T., and Mimori, T. (2009). Gamma/delta T cells are the predominant source of interleukin-17 in affected joints in collagen-induced arthritis, but not in rheumatoid arthritis. *Arthritis Rheum.* 60, 2294–2303.

Jacobs, J. P., Wu, H. J., Benoist, C., and Mathis, D. (2009). IL-17-producing T cells can augment autoantibody-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21789–21794.

Ji, H., Ohmura, K., Mahmood, U., Lee, D. M., Hofhuis, F. M., Boackle, S. A., Takahashi, K., Holers, V. M., Walport, M., Gerard, C., Ezekowitz, A., Carroll, M. C., Brenner, M., Weissleder, R., Verbeek, J. S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (2002). Arthritis critically dependent on innate immune system players. *Immunity* 16, 157–168.

Joosten, L. A., Helsen, M. M., Van De Loo, F. A., and Van Den Berg, W. B. (1996). Anticytokine treatment of established type II collagen-induced arthritis in DBA/1 mice. A comparative study using anti-TNF alpha, anti-IL-1 alpha/beta, and IL-1Ra. *Arthritis Rheum.* 39, 797–809.

Kagari, T., Doi, H., and Shimozato, T. (2002). The importance of IL-1 beta and TNF-alpha, and the non-involvement of IL-6, in the development of monoclonal antibody-induced arthritis. *J. Immunol.* 169, 1459–1466.

Karouzakis, E., Gay, R. E., Michel, B. A., Gay, S., and Neidhart, M. (2009). DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum.* 60, 3613–3622.

Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., and Kollias, G. (1991). Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J.* 10, 4025–4031.

Kim, K. W., Kim, H. R., Park, J. Y., Park, J. S., Oh, H. J., Woo, Y. J., Park, M. K., Cho, M. L., and Lee, S. H. (2012). IL-22 promotes osteoclastogenesis in rheumatoid arthritis through induction of RANKL in human synovial fibroblasts. *Arthritis Rheum.* 64, 1015–1023.

Kim, N., Kadono, Y., Takami, M., Lee, J., Lee, S. H., Okada, F., Kim, J. H., Kobayashi, T., Odgren, P. R., Nakano, H., Yeh, W. C., Lee, S. K., Lorenzo, J. A., and Choi, Y. (2005). Osteoclast differentiation independent of the TRANCE-RANK-TRAF6 axis. *J. Exp. Med.* 202, 589–595.

Kochi, Y., Okada, Y., Suzuki, A., Ikari, K., Terao, C., Takahashi, A., Yamazaki, K., Hosono, N., Myouzen, K., Tsunoda, T., Kamatani, N., Furuichi, T., Ikegawa, S., Ohmura, K., Mimori, T., Matsuda, F., Iwamoto, T., Momohara, S., Yamanaka, H., Yamada, R., Kubo, M., Nakamura, Y., and Yamamoto, K. (2010). A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. *Nat. Genet.* 42, 515–519.

Kong, Y. Y., Feige, U., Sarosi, I., Bolon, B., Tafuri, A., Morony, S., Capparelli, C., Li, J., Elliott, R., Mccabe, S., Wong, T., Campagnuolo, G., Moran, E., Bogoch, E. R., Van, G., Nguyen, L. T., Ohashi, P. S., Lacey, D. L., Fish, E., Boyle, W. J., and Penninger, J. M. (1999). Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* 402, 304–309.

Korganow, A. S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, H., Rajewsky, K., Pasquali, J. L., Benoist, C., and Mathis, D. (1999). From systemic T cell self-reactivity to organ-specific autoimmune disease

via immunoglobulins. *Immunity* 10, 451–461.

Kotake, S., Udagawa, N., Takahashi, N., Matsuzaki, K., Itoh, K., Ishiyama, S., Saito, S., Inoue, K., Kamatani, N., Gillespie, M. T., Martin, T. J., and Suda, T. (1999). IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest.* 103, 1345–1352.

Kouskoff, V., Korganow, A. S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (1996). Organ-specific disease provoked by systemic autoimmunity. *Cell* 87, 811–822.

Kremer, J. M., Bloom, B. J., Breedveld, F. C., Coombs, J. H., Fletcher, M. P., Gruben, D., Krishnaswami, S., Burgos-Vargas, R., Wilkinson, B., Zerbini, C. A., and Zwillich, S. H. (2009). The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: results of a double-blind, placebo-controlled phase IIa trial of three dosage levels of CP-690,550 versus placebo. *Arthritis Rheum.* 60, 1895–1905.

Kwok, S. K., Cho, M. L., Park, M. K., Oh, H. J., Park, J. S., Her, Y. M., Lee, S. Y., Youn, J., Ju, J. H., Park, K. S., Kim, S. I., Kim, H. Y., and Park, S. H. (2012). Interleukin-21 promotes osteoclastogenesis in rheumatoid arthritis in humans and mice. *Arthritis Rheum.* 64, 740–751.

Lee, A. T., Li, W., Liew, A., Bombardier, C., Weisman, M., Massarotti, E. M., Kent, J., Wolfe, F., Begovich, A. B., and Gregersen, P. K. (2005). The PTPN22 R620W polymorphism associates with RF positive rheumatoid arthritis in a dose-dependent manner but not with HLA-SE status. *Genes Immun.* 6, 129–133.

Lee, D. M., Friend, D. S., Gurish, M. F., Benoist, C., Mathis, D., and Brenner, M. B. (2002). Mast cells: a cellular link between autoantibodies and inflammatory arthritis. *Science* 297, 1689–1692.

Lee, D. M., Kiener, H. P., Agarwal, S. K., Noss, E. H., Watts, G. F., Chisaka, O., Takeichi, M., and Brenner, M. B. (2007). Cadherin-11 in synovial lining formation and pathology in arthritis. *Science* 315, 1006–1010.

Lefèvre, S., Knedla, A., Tennie, C., Kampmann, A., Wunrau, C., Dinser, R., Korb, A., Schnaker, E. M., Tarner, I. H., Robbins, P. D., Evans, C. H., Sturz, H., Steinmeyer, J., Gay, S., Scholmerich, J., Pap, T., Muller-Ladner, U., and Neumann, E. (2009). Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. *Nat. Med.* 15, 1414–1420.

Linsley, P. S., and Nadler, S. G. (2009). The clinical utility of inhibiting CD28-mediated costimulation. *Immunol. Rev.* 229, 307–321.

Lubberts, E., Koenders, M. I., Oppers-Walgreen, B., Van Den Bersselaar, L., Coenen-De Roo, C. J., Joosten, L. A., and Van Den Berg, W. B. (2004). Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. *Arthritis Rheum.* 50, 650–659.

Maeshima, K., Yamaoka, K., Kubo, S., Nakano, K., Iwata, S., Saito, K., Ohishi, M., Miyahara, H., Tanaka, S., Ishii, K., Yoshimatsu, H., and Tanaka, Y. (2011). A JAK inhibitor tofacitinib regulates synovitis through inhibition of IFN-gamma and IL-17 production by human CD4(+) T cells. *Arthritis Rheum.* doi: 10.1002/art.34329

Manoury-Schwartz, B., Chiocchia, G., Bessis, N., Abehsira-Amar, O., Batteux, F., Muller, S., Huang, S., Boissier, M. C., and Fournier, C. (1997). High susceptibility to collagen-induced arthritis in mice lacking IFN-gamma receptors. *J. Immunol.* 158, 5501–5506.

Matmati, M., Jacques, P., Maelfait, J., Verheugen, E., Kool, M., Sze, M., Geboes, L., Louagie, E., Guire, C. M., Vereecke, L., Chu, Y., Boon, L., Staelens, S., Matthys, P., Lambrecht, B. N., Schmidt-Supprian, M., Pasparakis, M., Elewaut, D., Beyaert, R., and Van Loo, G. (2011). A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat. Genet.* 43, 908–912.

Matsumoto, I., Maccioni, M., Lee, D. M., Maurice, M., Simmons, B., Brenner, M., Mathis, D., and Benoist, C. (2002). How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat. Immunol.* 3, 360–365.

Midwood, K., Sacre, S., Piccinini, A. M., Inglis, J., Trebaul, A., Chan, E., Drexler, S., Sofat, N., Kashiwagi, M., Orend, G., Brennan, F., and Foxwell, B. (2009). Tenascin-C is an endogenous activator of Toll-like receptor 4 that is essential for maintaining inflammation in arthritic joint disease. *Nat. Med.* 15, 774–780.

Miossec, P., Korn, T., and Kuchroo, V. K. (2009). Interleukin-17 and type 17 helper T cells. *N. Engl. J. Med.* 361, 888–898.

Muller-Ladner, U., Kriegsmann, J., Franklin, B. N., Matsumoto, S., Geiler, T., Gay, R. E., and Gay, S. (1996). Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice. *Am. J. Pathol.* 149, 1607–1615.

Murakami, M., Okuyama, Y., Ogura, H., Asano, S., Arima, Y., Tsuruoka, M., Harada, M., Kanamoto, M., Sawa, Y., Iwakura, Y., Takatsu, K., Kamimura, D., and Hirano, T. (2011). Local microbleeding facilitates IL-6- and IL-17-dependent arthritis in the absence of tissue antigen recognition by activated T cells. *J. Exp. Med.* 208, 103–114.

Nakae, S., Nambu, A., Sudo, K., and Iwakura, Y. (2003a). Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. *J. Immunol.* 171, 6173–6177.

Nakae, S., Saijo, S., Horai, R., Sudo, K., Mori, S., and Iwakura, Y. (2003b). IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proc. Natl. Acad. Sci. U.S.A.* 100, 5986–5990.

Nakashima, T., Hayashi, M., Fukunaga, T., Kurata, K., Oh-Hora, M., Feng, J. Q., Bonewald, L. F., Kodama, T., Wutz, A., Wagner, E. F., Penninger, J. M., and Takayanagi, H. (2011). Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat. Med.* 17, 1231–1234.

Nandakumar, K. S., Backlund, J., Vestberg, M., and Holmdahl, R. (2004). Collagen type II (CII)-specific antibodies induce arthritis in the absence of T or B cells but the arthritis progression is enhanced by CII-reactive T cells. *Arthritis Res. Ther.* 6, R544–R550.

Notley, C. A., Inglis, J. J., Alzabin, S., Mccann, F. E., Mcnamee, K. E., and Williams, R. O. (2008). Blockade of tumor necrosis factor in collagen-induced arthritis reveals a novel immunoregulatory pathway for Th1 and Th17 cells. *J. Exp. Med.* 205, 2491–2497.

Ogura, H., Murakami, M., Okuyama, Y., Tsuruoka, M., Kitabayashi, C., Kanamoto, M., Nishihara, M., Iwakura, Y., and Hirano, T. (2008). Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity* 29, 628–636.

Okamoto, K., Iwai, Y., Oh-Hora, M., Yamamoto, M., Morio, T., Aoki, K., Ohya, K., Jetten, A. M., Akira, S., Muta, T., and Takayanagi, H. (2010). IkappaBzeta regulates T(H)17 development by cooperating with ROR nuclear receptors. *Nature* 464, 1381–1385.

Perricone, C., Ceccarelli, F., and Valesini, G. (2011). An overview on the genetic of rheumatoid arthritis: a never-ending story. *Autoimmun. Rev.* 10, 599–608.

Pettit, A. R., Ji, H., Von Stechow, D., Muller, R., Goldring, S. R., Choi, Y., Benoist, C., and Gravallese, E. M. (2001). TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. *Am. J. Pathol.* 159, 1689–1699.

Pollinger, B., Junt, T., Metzler, B., Walker, U. A., Tyndall, A., Allard, C., Bay, S., Keller, R., Raulf, F., Di Padova, F., O'Reilly, T., Horwood, N. J., Patel, D. D., and Littlewood-Evans, A. (2011). Th17 cells, not IL-17+ gammadelta T cells, drive arthritic bone destruction in mice and humans. *J. Immunol.* 186, 2602–2612.

Ranges, G. E., Sriram, S., and Cooper, S. M. (1985). Prevention of type II collagen-induced arthritis by in vivo treatment with anti-L3T4. *J. Exp. Med.* 162, 1105–1110.

Redlich, K., Hayer, S., Ricci, R., David, J. P., Tohidast-Akrad, M., Kollias, G., Steiner, G., Smolen, J. S., Wagner, E. F., and Schett, G. (2002). Osteoclasts are essential for TNF-alpha-mediated joint destruction. *J. Clin. Invest.* 110, 1419–1427.

Sakaguchi, N., Takahashi, T., Hata, H., Nomura, T., Tagami, T., Yamazaki, S., Sakihama, T., Matsutani, T., Negishi, I., Nakatsuru, S., and Sakaguchi, S. (2003). Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* 426, 454–460.

Sato, K., Suematsu, A., Okamoto, K., Yamaguchi, A., Morishita, Y., Kadono, Y., Tanaka, S., Kodama, T., Akira, S., Iwakura, Y., Cua, D. J., and Takayanagi, H. (2006). Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J. Exp. Med.* 203, 2673–2682.

Sawa, S., Kamimura, D., Jin, G. H., Morikawa, H., Kamon, H., Nishihara, M., Ishihara, K., Murakami, M., and Hirano, T. (2006). Autoimmune arthritis associated with mutated interleukin (IL)-6 receptor gp130 is driven by STAT3/IL-7-dependent homeostatic proliferation of CD4+ T cells. *J. Exp. Med.* 203, 1459–1470.

Shinohara, M., Koga, T., Okamoto, K., Sakaguchi, S., Arai, K., Yasuda, H., Takai, T., Kodama, T., Morio, T.,

Geha, R. S., Kitamura, D., Kurosaki, T., Ellmeier, W., and Takayanagi, H. (2008). Tyrosine kinases Btk and Tec regulate osteoclast differentiation by linking RANK and ITAM signals. *Cell* 132, 794–806.

Singh, K., Colmegna, I., He, X., Weyand, C. M., and Goronzy, J. J. (2008). Synoviocyte stimulation by the LFA-1-intercellular adhesion molecule-2-Ezrin-Akt pathway in rheumatoid arthritis. *J. Immunol.* 180, 1971–1978.

Solt, L. A., Kumar, N., Nuhant, P., Wang, Y., Lauer, J. L., Liu, J., Istrate, M. A., Kamenecka, T. M., Roush, W. R., Vidovic, D., Schurer, S. C., Xu, J., Wagoner, G., Drew, P. D., Griffin, P. R., and Burris, T. P. (2011). Suppression of TH17 differentiation and autoimmunity by a synthetic ROR ligand. *Nature* 472, 491–494.

Stahl, E. A., Raychaudhuri, S., Remmers, E. F., Xie, G., Eyre, S., Thomson, B. P., Li, Y., Kurreeman, F. A., Zhernakova, A., Hinks, A., Guiducci, C., Chen, R., Alfredsson, L., Amos, C. I., Ardlie, K. G., Barton, A., Bowes, J., Brouwer, E., Burtt, N. P., Catanese, J. J., Coblyn, J., Coenen, M. J., Costenbader, K. H., Criswell, L. A., Crusius, J. B., Cui, J., De Bakker, P. I., De Jager, P. L., Ding, B., Emery, P., Flynn, E., Harrison, P., Hocking, L. J., Huizinga, T. W., Kastner, D. L., Ke, X., Lee, A. T., Liu, X., Martin, P., Morgan, A. W., Padyukov, L., Posthumus, M. D., Radstake, T. R., Reid, D. M., Seielstad, M., Seldin, M. F., Shadick, N. A., Steer, S., Tak, P. P., Thomson, W., Van Der Helm-Van Mil, A. H., Van Der Horst-Bruinsma, I. E., Van Der Schoot, C. E., Van Riel, P. L., Weinblatt, M. E., Wilson, A. G., Wolbink, G. J., Wordsworth, B. P., Wijmenga, C., Karlson, E. W., Toes, R. E., De Vries, N., Begovich, A. B., Worthington, J., Siminovitch, K. A., Gregersen, P. K., Klareskog, L., and Plenge, R. M. (2010). Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat. Genet.* 42, 508–514.

Stanczyk, J., Pedrioli, D. M., Brentano, F., Sanchez-Pernaute, O., Kolling, C., Gay, R. E., Detmar, M., Gay, S., and Kyburz, D. (2008). Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. *Arthritis Rheum.* 58, 1001–1009.

Takayanagi, H. (2009). Osteoimmunology and the effects of the immune system on bone. *Nat. Rev. Rheumatol.* 5, 667–676.

Takayanagi, H., Iizuka, H., Juji, T., Nakagawa, T., Yamamoto, A., Miyazaki, T., Koshihara, Y., Oda, H., Nakamura, K., and Tanaka, S. (2000a). Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. *Arthritis Rheum.* 43, 259–269.

Takayanagi, H., Ogasawara, K., Hida, S., Chiba, T., Murata, S., Sato, K., Takaoka, A., Yokochi, T., Oda, H., Tanaka, K., Nakamura, K., and Taniguchi, T. (2000b). T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma. *Nature* 408, 600–605.

Takayanagi, H., Kim, S., Koga, T., Nishina, H., Isshiki, M., Yoshida, H., Saiura, A., Isobe, M., Yokochi, T., Inoue, J., Wagner, E. F., Mak, T. W., Kodama, T., and Taniguchi, T. (2002). Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. *Dev. Cell* 3, 889–901.

Takayanagi, H., Oda, H., Yamamoto, S., Kawaguchi, H., Tanaka, S., Nishikawa, T., and Koshihara, Y. (1997). A new mechanism of bone destruction in rheumatoid arthritis: synovial fibroblasts induce osteoclastogenesis. *Biochem. Biophys. Res. Commun.* 240, 279–286.

Tang, W., Lu, Y., Tian, Q. Y., Zhang, Y., Guo, F. J., Liu, G. Y., Syed, N. M., Lai, Y., Lin, E. A., and Kong, L., Su, J., Yin, F., Ding, A. H., Zanin-Zhorov, A., Dustin, M. L., Tao, J., Craft, J., Yin, Z., Feng, J. Q., Abramson, S. B., Yu, X. P., and Liu, C. J. (2011). The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science* 332, 478–484.

Taniguchi, K., Kohsaka, H., Inoue, N., Terada, Y., Ito, H., Hirokawa, K., and Miyasaka, N. (1999). Induction of the p16INK4a senescence gene as a new therapeutic strategy for the treatment of rheumatoid arthritis. *Nat. Med.* 5, 760–767.

Taylor, P. C., Peters, A. M., Paleolog, E., Chapman, P. T., Elliott, M. J., Mccloskey, R., Feldmann, M., and Maini, R. N. (2000). Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. *Arthritis Rheum.* 43, 38–47.

Theill, L. E., Boyle, W. J., and Penninger, J. M. (2002). RANK-L and RANK: T cells, bone loss, and mammalian evolution. *Annu. Rev. Immunol.* 20, 795–823.

Tran, C. N., Davis, M. J., Tesmer, L. A., Endres, J. L., Motyl, C. D., Smuda, C., Somers, E. C., Chung, K. C., Urquhart, A. G., Lundy, S. K., Kovats, S., and Fox, D. A. (2007). Presentation of arthritogenic peptide to antigen-specific T cells by fibroblast-like synoviocytes. *Arthritis Rheum.* 56, 1497–1506.

Vermeire, K., Heremans, H., Vandeputte, M., Huang, S., Billiau, A., and Matthys, P. (1997). Accelerated collagen-induced arthritis in IFN-gamma receptor-deficient mice. *J. Immunol.* 158, 5507–5513.

Williams, R. O., Feldmann, M., and Maini, R. N. (1992). Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 89, 9784–9788.

Wipke, B. T., and Allen, P. M. (2001). Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. *J. Immunol.* 167, 1601–1608.

Wong, B. R., Josien, R., Lee, S. Y., Sauter, B., Li, H. L., Steinman, R. M., and Choi, Y. (1997). TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J. Exp. Med.* 186, 2075–2080.

Wu, H. J., Ivanov, I. I., Darce, J., Hattori, K., Shima, T., Umesaki, Y., Littman, D. R., Benoist, C., and Mathis, D. (2010). Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 32, 815–827.

Xiong, J., Onal, M., Jilka, R. L., Weinstein, R. S., Manolagas, S. C., O'Brien, C. A. (2011). Matrix-embedded cells control osteoclast formation. *Nat. Med.* 17, 1235–1241.

Yamaguchi, Y., Fujio, K., Shoda, H., Okamoto, A., Tsuno, N. H., Takahashi, K., and Yamamoto, K. (2007). IL-17B and IL-17C are associated with TNF-alpha production and contribute to the exacerbation of inflammatory arthritis. *J. Immunol.* 179, 7128–7136.

Yao, C., Sakata, D., Esaki, Y., Li, Y., Matsuoka, T., Kuroiwa, K., Sugimoto, Y., and Narumiya, S. (2009). Prostaglandin E2-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. *Nat. Med.* 15, 633–640.

Yeo, L., Toellner, K. M., Salmon, M., Filer, A., Buckley, C. D., Raza, K., and Scheel-Toellner, D. (2012). Cytokine mRNA profiling identifies B cells as a major source of RANKL in rheumatoid arthritis. *Ann. Rheum. Dis.* 70, 2022–2028.

Yoshitomi, H., Sakaguchi, N., Kobayashi, K., Brown, G. D., Tagami, T., Sakihama, T., Hirota, K., Tanaka, S., Nomura, T., Miki, I., Gordon, S., Akira, S., Nakamura, T., and Sakaguchi, S. (2005). A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J. Exp. Med.* 201, 949–960.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 January 2012; paper pending published: 03 February 2012; accepted: 27 March 2012; published online: 13 April 2012.

Citation: Komatsu N and Takayanagi H (2012) Inflammation and bone destruction in arthritis: synergistic activity of immune and mesenchymal cells in joints. Front. Immun. ***3****:77. doi: 10.3389/fimmu.2012.00077*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

MINI REVIEW ARTICLE
published: 10 April 2012
doi: 10.3389/fimmu.2012.00071

Cellular and molecular mechanisms of chronic inflammation-associated organ fibrosis

Satoshi Ueha[1,2], Francis H. W. Shand[1,3] and Kouji Matsushima[1,2]*

[1] *Department of Molecular Preventive Medicine, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan*
[2] *Japan Science and Technology Agency, Core Research for Evolutional Science and Technology, Tokyo, Japan*
[3] *Department of Pharmacology, University of Melbourne, Melbourne, VIC, Australia*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Daisuke Kamimura, Osaka University, Japan
Hideki Ogura, Osaka University, Japan

***Correspondence:**
Kouji Matsushima, Department of Molecular Preventive Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
e-mail: koujim@m.u-tokyo.ac.jp

Organ fibrosis is a pathological condition associated with chronic inflammatory diseases. In fibrosis, excessive deposition of extracellular matrix (ECM) severely impairs tissue architecture and function, eventually resulting in organ failure. This process is mediated primarily by the induction of myofibroblasts, which produce large amounts of collagen I, the main component of the ECM. Accordingly, the origin, developmental pathways, and mechanisms of myofibroblast regulation are attracting increasing attention as potential therapeutic targets. The fibrotic cascade, from initial epithelial damage to eventual myofibroblast induction, is mediated by complex biological processes such as macrophage infiltration, a shift from Th1 to Th2 phenotype, and by inflammatory mediators such as transforming growth factor-β. Here, we review the current understanding of the cellular and molecular mechanisms underlying organ fibrosis.

Keywords: fibrosis, myofibroblast, fibroblast, chemokine, TGFb, mesenchymal stem cell, collagen I, pericyte

INTRODUCTION

Organ fibrosis is an intractable, progressive condition that arises in multi-factorial chronic inflammatory diseases in which excessive deposition of extracellular matrix (ECM), mainly composed of collagen I (Col I), severely impairs tissue architecture and function, eventually resulting in organ failure (Kis et al., 2011). Fibrosis affects various organs following tissue injury, including the lungs, liver, and kidneys, and has become a major cause of death in the developed world.

Lung fibrosis occurs mainly in idiopathic interstitial pneumonia (IIPs), a general term describing multi-factorial conditions such as idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP), and cryptogenic organizing pneumonia (COP). IPF is a chronic and progressive disease with an estimated prevalence of 20 cases per 100,000. The prognosis for patients with IPF is poor, and 50% die within 3 years of diagnosis.

Hepatic fibrosis (fibrosis of the liver) can be triggered by the hepatitis virus or alcohol. There are an estimated 350 million and 180 million carriers of the Hepatitis B (HBV) and C (HCV) viruses worldwide, respectively. In Japan, deaths from hepatic cirrhosis total around 15,000 per year (HCV, 50%; HBV, 12%; non B/non C, 4%; alcoholic hepatitis, 13%). In addition, hepatic cirrhosis is associated with hepatic cancer, which causes over 30,000 deaths annually. The prevalence of non-alcoholic steatohepatitis (NASH) ranges from 9 to 37% of the population depending on the country, and a subset of NASH patients eventually develops hepatitis and hepatic cancer.

Kidney fibrosis commonly occurs in glomerulonephritis and diabetic nephropathy. While the number of patients requiring dialysis due to chronic glomerulonephritis has decreased in recent years, the number of those with diabetic nephropathy continues to increase year by year. The cost of dialysis represents a considerable medical expense in advanced countries. In addition, organ fibrosis is associated with autoimmune diseases. About 15–30% of rheumatoid arthritis patients develop IPF, and about 30% of IIP cases are associated with autoimmune diseases.

Given the prevalence and severity of diseases involving tissue fibrosis, the prevention, and treatment of this condition remains a major medical challenge. This review focuses on the cellular and molecular bases for the accumulation of Col I producing fibroblasts and myofibroblasts, which are responsible for the excessive deposition of ECM during the fibrotic process.

THE ORIGIN OF Col I PRODUCING FIBROBLASTS AND MYOFIBROBLASTS

Fibroblasts are non-hematopoietic, non-epithelial, non-endothelial cells that widely distribute throughout the mesenchyme where they synthesize ECM proteins that form a structural framework to support tissue architecture and function in steady-state conditions. Fibroblasts also play an important role in tissue repair following multi-factorial tissue damage by forming a provisional ECM, a process preceding re-epithelialization in successful repair. Unfortunately, dysregulated activation, proliferation, and survival of fibroblasts often results in the excessive deposition of ECM proteins and the inhibition of re-epithelialization, leading to tissue fibrosis (Gabbiani, 2003). Therefore, control of the

Abbreviations: smooth muscle actin; Ang II, angiotensin II; BMP, bone morphogenic protein; Col I, collagen I; CTGF, connective tissue growth factor; EMT, epithelial–mesenchymal transition; FSP-1, fibroblast specific protein-1; HBV/HCV, hepatitis B/C virus; IIP, idiopathic interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; LPA, lysophosphatidic acid; LT, leukotriene; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; PDGF, platelet-derived growth factor; PGE_2, prostaglandin E_2; S1P, sphingosine-1-phosphate; TGFβ, transforming growth factor-β; TIMP, tissue inhibitor of matrix metalloproteinases.

activation, proliferation, and survival of fibroblasts is critical for the prevention and treatment of tissue fibrosis.

Fibroblasts form clusters within fibrotic tissues that are known as fibrotic foci (Visscher and Myers, 2006). These fibroblasts include α-smooth muscle actin (αSMA) expressing myofibroblasts that have the potential to produce large amounts of Col I, which has resulted in this cell population being widely considered to be the key effector cells in organ fibrosis (Gabbiani et al., 1971; Gabbiani, 2003; Sandbo and Dulin, 2011). Results in some models of organ fibrosis have suggested that there may be therapeutic benefit in targeting myofibroblasts, although the experimental approaches in these models leave-questions remaining about the selectivity of the interventions for myofibroblasts (Douglass et al., 2008). As mentioned above, fibroblasts are immunophenotypically identified as cells negative for hematopoietic, epithelial, and endothelial makers. The lack of specific markers for fibroblasts or possible subpopulations, including myofibroblasts, complicates the cellular and molecular understanding of these cells. Thus, the establishment of specific markers to identify fibroblasts and myofibroblasts remains a major challenge in this field.

Myofibroblasts have classically been considered to differentiate from tissue-resident fibroblasts. However, recent studies have suggested alternative sources of myofibroblasts (Hinz et al., 2007). Bone marrow-derived fibrocytes express both hematopoietic markers (CD45, CD11b, and HLADR) and ECM proteins (Col I and vimentin). These cells have been shown to be recruited from the circulation to inflamed tissues via chemokine receptors CXCR4 and CCR1, 2, 5, and 7, after which they differentiate into myofibroblasts (Phillips et al., 2004; Keeley et al., 2011). Epithelial cells are reported to trans-differentiate into myofibroblasts via chronic inflammation-induced epithelial–mesenchymal transition (EMT) in several fibrosis models (Kalluri and Neilson, 2003). In addition, blood vessel wall smooth muscle cells have been proposed as myofibroblast progenitors. Meanwhile, stellate cells (Ito cells), a type of hepatic pericyte, have attracted interest as a major precursor of Col I producing fibroblasts and myofibroblasts in the liver (Atzori et al., 2009). Despite these studies, overall understanding of the origin and differentiation pathways of Col I producing fibroblasts and myofibroblasts remains poor. Identification of the major developmental pathway of these cells will be an essential step toward the development of therapeutic interventions for organ fibrosis.

CHALLENGING THE EMT HYPOTHESIS

Epithelial–mesenchymal transition is a process that was originally characterized in the context of embryonic development, in which epithelial cells lose their original phenotypic and functional features, including cell–cell adhesion and cell polarity, while acquiring migratory and invasive properties (Thiery et al., 2009). *In vitro* cell culture studies have shown clearly and reproducibly that transforming growth factor-β (TGFβ) treatment of epithelial cells induces expression of mesenchymal markers and morphology with a concomitant loss of epithelial markers (Qi et al., 2005; Venkov et al., 2007). Over the past 15 years, numerous studies have proposed that EMT also contributes to the activated fibroblast pool in various regenerative and pathogenic processes. For example, transition from epithelial tumor cells to mesenchymal cells occurs at the invasive front of many tumors, driving tumor progression and metastasis. In addition, inflammation-induced epithelial cell damage in parenchymal organs such as the liver, lungs, and kidneys recapitulates part of the EMT process in that epithelial cells acquire mesenchymal cell-like properties and migrate beyond the basal membrane to the interstitium, where they differentiate into Col I producing fibroblasts and myofibroblasts. However, the inflammation-associated EMT hypothesis has been challenged by an increasing number of studies, and lacks convincing evidence (Wells, 2010; Kriz et al., 2011).

For example, the EMT hypothesis for kidney fibrosis was first reported by Strutz et al. (1995), when the authors used FSP-1 (fibroblast specific protein-1/S100A4) as a marker of mesenchymal lineage. However, subsequent characterization revealed that FSP-1 is not a mesenchymal cell specific marker, and is expressed on leukocytes and endothelial cells as well. Similarly, expression of vimentin, another marker commonly used in EMT studies, is not enough on its own to identify mesenchymal cells, because a subset of epithelial cells express vimentin in both resting and inflammatory-states (Grone et al., 1987; Witzgall et al., 1994). Moreover, recent extensive and well designed cell-fate tracing studies have not provided any evidence for inflammation-associated EMT (Humphreys et al., 2010; Scholten et al., 2010). Unless the inflammation-induced conversion of epithelial cells into Col I producing fibroblasts and myofibroblasts *in vivo* can be demonstrated more convincingly, the role of EMT in organ fibrosis should be reconsidered.

FIBROCYTES MAKE ONLY A MINIMAL CONTRIBUTION TO ORGAN FIBROSIS

The existence of bone marrow-derived fibrocytes was originally reported by Bucala et al. (1994). Later, Strieter and colleagues reported that fibrocytes express several chemokine receptors and are recruited to inflamed tissues in a CXCR4 dependent manner, where they contribute to the Col I producing myofibroblast pool after bleomycin-induced epithelial injury in the lungs (Phillips et al., 2004). We have also demonstrated that blocking chemokine receptors CCR1, 2, 5, and 7 in mouse lung or kidney fibrosis models reduces the number of myofibroblasts detected and ameliorates organ fibrosis (Sakai et al., 2006; Ishida et al., 2007). However, it remains unclear whether the cognate chemokines regulate organ fibrosis through the recruitment of fibrocytes to the inflamed tissues, by influencing the activation or differentiation of fibroblasts, or through the recruitment of inflammatory cells such as macrophages and neutrophils that subsequently influence the tissue microenvironment. While many studies have confirmed the presence of fibrocytes in fibrotic disease, accumulating experimental evidence suggests that the contribution of bone marrow-derived cells to the Col I producing fibroblast/myofibroblast pool is limited (Higashiyama et al., 2009, 2011).

ORIGIN OF CAPILLARY PERICYTES AND THEIR SIMILARITY WITH TISSUE FIBROBLASTS

Recently, a novel role for pericytes as precursors of pro-fibrotic Col I producing cells has been described. Studies using Col 1α2–GFP transgenic mice have demonstrated that $CD73^{+}PDGFR\beta^{+}$ pericytes/fibroblasts migrate from capillaries to the interstitial space

and differentiate to Col 1 producing myofibroblasts in kidney and liver fibrosis models (Lin et al., 2008; Higashiyama et al., 2009). In addition, Goritz et al. (2011) recently demonstrated that a specific pericyte subtype gives rise to scar-forming stromal cells in the injured spinal cord. However, because fibroblasts in the interstitial space not only provide a scaffold for micro-tissue architecture such as nephrons and renal tubules (in the case of the kidneys), but also come into direct contact with microvessels, it is often difficult to distinguish between pericytes and tissue fibroblasts under steady-state conditions (Kriz et al., 2011). The similarities, differences, and lineage relationship between pericytes and tissue fibroblasts remain to be elucidated.

THE ROLE OF INFLAMMATORY CELLS IN FIBROTIC TISSUE

Macrophage infiltration into inflamed tissues has been implicated in chronic inflammation-induced organ fibrosis (Wynn and Barron, 2010). Inflamed tissue-infiltrating macrophages are derived from $CCR2^+$ inflammatory monocytes or CX_3CR1^{hi} resident monocytes (Ricardo et al., 2008). The phenotype of these macrophages is generally reported to match that of alternatively activated cells (M2) rather than classically activated cells (M1). M2 macrophages express immunosuppressive molecules such as IL-10 and arginase I, which suppress the induction of Th1 cells that produce the anti-fibrotic cytokine IFNγ. On the other hand, M1 macrophages express IL-1, IL-12, IL-23, and induce Th1 cell infiltration and activation. However, it remains to be established whether a particular macrophage subset with M2-type properties preferentially infiltrates into fibrotic tissues, or whether it is the pro-fibrotic microenvironment that drives macrophage polarization toward an M2 phenotype. In addition to their roles in immune regulation, macrophages play a pivotal role in matrix regression during the recovery phase of fibrosis (Duffield et al., 2005) and in the regulation of stellate cell proliferation (Olaso et al., 2011). In the future, conditional and lineage specific depletion or gene targeting approaches may help to reveal the specific function and overall role of each macrophage subset in tissue fibrosis.

The contribution of T lymphocytes to organ fibrosis seems to be context dependent. While a number of studies suggest an exacerbating role of T cells in fibrosis, T cells also appear to be dispensable because T cell-deficient mice develop fibrosis in some models (Luzina et al., 2008). The general concept is that prolonged inflammation induces a shift from a Th1 to Th2 phenotype, and the resulting production of Th2 cytokines induces the infiltration of pro-fibrotic eosinophils via cognate chemokine (e.g., eotaxin) production. On the other hand, a role for recently identified functional T cell subsets such as Th17 and regulatory T cells in tissue fibrosis has also begun to emerge. For example, adoptive transfer of CD4 T cells restored bacterial-induced lung inflammatory and fibrotic responses in TCRβ deficient mice with an accompanying increase in lung IL-17A protein levels, and IL-17 receptor α deficient mice develop less severe inflammation and fibrosis than wild type counterparts (Simonian et al., 2009). Recently, platelet-derived growth factor (PDGF)-producing $CD4^+Foxp3^+$Tregs have been shown to promote lung fibrosis by activating fibroblasts (Lo Re et al., 2011). A better understanding of the roles that inflammatory cells play in the fibrotic process may reveal new points of therapeutic intervention, which may be able to induce a shift from a pro-fibrotic microenvironment to an anti-fibrotic microenvironment.

REGULATION OF FIBROSIS BY INFLAMMATORY MEDIATORS

The fibrotic signaling cascade that occurs during chronic inflammation, which is initiated by epithelial injury and results in irreversible organ damage, is regulated by various inflammatory mediators. The pro-fibrotic roles of plasma components, platelet-derived soluble factors, and cytokines produced by activated tissue cells and infiltrating leukocytes, have been demonstrated in animal models. These mediators include factors induced as a part of an inflammatory cascade, regulatory molecules that provide feedback during the inflammatory response, and factors constitutively expressed in the body.

Transforming growth factor-β plays a central role in fibroblast activation and fibroblast-to-myofibroblast differentiation, and induces the expression of genes for ECM components including *Col 1*. However, despite its great potential as a therapeutic target for fibrosis, inhibition of TGFβ signaling has unacceptable side effects due to the critical role of this cytokine in the maintenance of homeostasis (Leask, 2010).

Bone morphogenic proteins (BMPs) belong to the TGFβ family and regulate proliferation and differentiation of both mesenchymal cells and epithelial cells (Rider and Mulloy, 2010). Recent studies have revealed that BMP7 prevents fibrosis by promoting epithelial regeneration, while BMP antagonists such as gremlin and ectodin drive organ fibrosis by inhibiting BMP7 signaling. Interestingly, there is a direct Smad-dependent counteraction of the TGFβ pathway by BMP7 signaling, and vice versa (Zeisberg et al., 2003).

G-protein coupled receptor ligands also regulate chronic inflammation and the fibrotic cascade. Angiotensin II (Ang II) induces the expression of pro-fibrotic factors such as connective tissue growth factor (CTGF; Ruperez et al., 2003; Esteban et al., 2004), and recent studies have revealed that there is an intracellular cross-talk between Ang II signaling and TGFβ signaling that cooperatively promotes fibrosis (Campbell and Katwa, 1997; Schultz Jel et al., 2002; Gao et al., 2009). Leukotrienes (LTs) not only induce fibroblast migration, proliferation, and matrix protein synthesis, but also promote fibrosis through the stimulation and activation of TGFβ (Shim et al., 2006). On the contrary, prostaglandin E_2 (PGE_2), which has well established anti-inflammatory activities, may suppress fibrosis by inhibiting the proliferation, migration, and differentiation of myofibroblasts (Kohyama et al., 2001; Lama et al., 2002; Thomas et al., 2007). Recent studies have demonstrated that PGF2a receptor deficient mice are resistant against bleomycin-induced lung fibrosis (Oga et al., 2009), and that LTB4 receptor inhibitors and LPA1 inhibitors suppress bleomycin-induced lung fibrosis (Tager et al., 2008). Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are liberated from stored lipid precursors through enzymatic activation and provide migration, proliferation, and differentiation signals to a variety of cells through the LPA receptors ($LPA_{1–8}$) and S1P receptors ($S1P_{1–5}$), respectively (Pattanaik and Postlethwaite, 2010). LPA_1 deficient mice are protected from bleomycin-induced lung fibrosis and unilateral ureteral ligation induced-renal fibrosis (Tager et al., 2008). The pro-fibrotic role of LPA is reportedly mediated in part by

the induction of fibroblast-to-myofibroblast differentiation (Yin et al., 2008). S1P plays a critical role in the circulation of lymphocytes, and accordingly, inhibition of the S1P–$S1P_1$ axis results in strong immunosuppressive effects. In addition, S1P also regulates the migration and activation of fibroblasts, and recent studies have revealed cross-talk between the $S1P_3$ and TGFβ – Smad signaling pathways that promote cardiac fibrosis (Takuwa et al., 2010).

Plasma coagulation cascade proteases are also involved in fibrosis (Chambers and Laurent, 2002); thrombin, factor VII, and factor Xa activate protease-activated receptor-1 (PAR-1) on fibroblasts and induce their proliferation. In addition, these proteases promote fibrosis through the induction of pro-fibrotic molecules such as platelet-derived growth factors and CTGF. CTGF mediates mesenchymal stem cell (MSC)-to-fibroblast differentiation as well as fibroblast activation (Ponticos et al., 2009; Lee et al., 2010), while PDGFs induce the proliferation and activation of fibroblasts leading to vascular diseases and fibrosis. Ijichi et al. (2011) have demonstrated that CXC chemokines induce CTGF expression in fibroblasts, and that the inhibition of CXCR2 in tumor-bearing mice impairs tumor progression.

Matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of MMPs (TIMPs), play an important role in the regulation of ECM turnover in fibrotic tissues. While the degradation of pathological fibrillar collagen by MMPs is a key event in the resolution of fibrosis, the degradation of normal ECM components in the early stages of fibrosis promotes deposition of newly synthesized collagen (Hemmann et al., 2007).

ATP released from damaged epithelial cells serves as a danger signal to alert the immune system of tissue damage, and may also trigger a fibrotic cascade (Mortaz et al., 2010). Activation of the Wnt/β-catenin signaling pathway, which regulates epithelial and mesenchymal proliferation and activation, has been demonstrated in lung epithelial cells of IPF patients. Overall, this activation drives fibrosis rather than epithelial repair, possibly due to cross-talk with other pro-fibrotic factors such as TGFβ and CTGF (Konigshoff and Eickelberg, 2010). Furthermore, inhibition of Wnt signaling (Henderson et al., 2010) and the BMP binding protein ectodin (Tanaka et al., 2010) ameliorates renal fibrosis. A better understanding of the role of each inflammatory mediator in the fibrotic cascade is likely to reveal novel molecular targets for the early diagnosis, prevention, and treatment of fibrotic disease.

CONCLUSION AND FUTURE PERSPECTIVES

In recent years, confusion has surrounded the major source of myofibroblasts in fibrosis, with attention centering on tissue-resident fibroblasts and pericytes (**Figure 1**). However, the relative

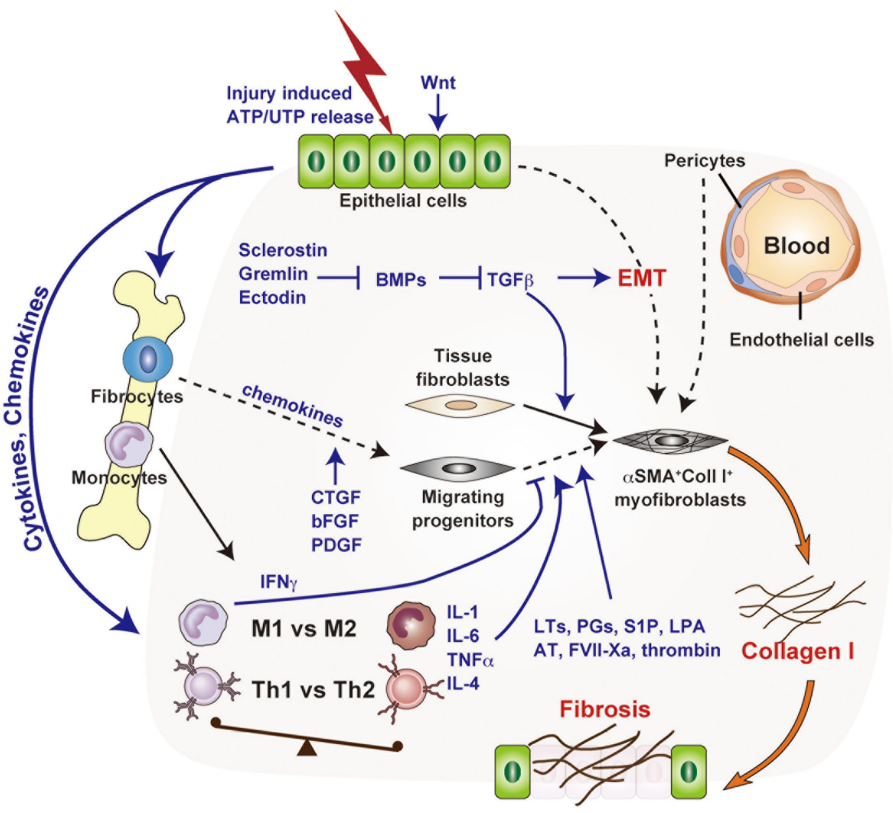


FIGURE 1 | Molecular and cellular mechanisms of chronic inflammation-associated organ fibrosis. Organ fibrosis is mediated primarily by the induction of myofibroblasts, which produce large amounts of collagen I. Tissue fibroblasts, transdifferentiated epithelial cells (EMT), bone marrow-derived fibrocytes, and pericytes have attracted interest as potential myofibroblast precursors. The fibrotic cascade, from initial epithelial damage to eventual myofibroblast induction, is mediated by complex biological processes such as macrophage infiltration, a shift from Th1 to Th2 phenotype, and by inflammatory mediators such as transforming growth factor-β.

importance of the various developmental pathways of Col I producing fibroblasts and myofibroblasts needs to be re-examined by lineage tracing approaches, utilizing cell-type specific promoters, and inducible systems in a range of fibrosis models. It will also be important to further elucidate the mechanisms underlying the maintenance of myofibroblasts during chronic inflammation. It is possible that precursor cells provide a continuous supply of myofibroblasts, that myofibroblasts have proliferative potential, or that the myofibroblast lifespan is relatively long. A deeper understanding of the population dynamics of myofibroblasts and their precursors may reveal new points of therapeutic intervention with the potential to halt myofibroblast accumulation in fibrotic tissue.

Although removal of the cause of chronic inflammation is essential and effective for the prevention and treatment of tissue fibrosis (for example, virus clearance by interferon effectively prevents viral hepatitis-associated fibrosis), this can be challenging as the precise cause of the inflammation is often unclear. Given that in most cases steroids are largely ineffective against fibrosis, currently there is no effective drug available for patients with clinically significant organ fibrosis. Further elucidation of the molecular and cellular bases for chronic inflammation-associated organ fibrosis is imperative for the development of effective anti-fibrotic therapies.

ACKNOWLEDGMENTS

This work was in part supported by The Ministry of Education, Culture, Sports, Science and Technology (MEXT) KAKENHI Grants-in-Aid for Young Scientists (B) 23790432 (S. Ueha) and Grants-in-Aid for Scientific Research (B) 22390095, and Grants-in-Aid for Scientific Research on Priority Areas 19059004 (K. Matsushima).

REFERENCES

Atzori, L., Poli, G., and Perra, A. (2009). Hepatic stellate cell: a star cell in the liver. *Int. J. Biochem. Cell Biol.* 41, 1639–1642.

Bucala, R., Spiegel, L. A., Chesney, J., Hogan, M., and Cerami, A. (1994). Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol. Med.* 1, 71–81.

Campbell, S. E., and Katwa, L. C. (1997). Angiotensin II stimulated expression of transforming growth factor-beta1 in cardiac fibroblasts and myofibroblasts. *J. Mol. Cell. Cardiol.* 29, 1947–1958.

Chambers, R. C., and Laurent, G. J. (2002). Coagulation cascade proteases and tissue fibrosis. *Biochem. Soc. Trans.* 30, 194–200.

Douglass, A., Wallace, K., Parr, R., Park, J., Durward, E., Broadbent, I., Barelle, C., Porter, A. J., and Wright, M. C. (2008). Antibody-targeted myofibroblast apoptosis reduces fibrosis during sustained liver injury. *J. Hepatol.* 49, 88–98.

Duffield, J. S., Forbes, S. J., Constandinou, C. M., Clay, S., Partolina, M., Vuthoori, S., Wu, S., Lang, R., and Iredale, J. P. (2005). Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J. Clin. Invest.* 115, 56–65.

Esteban, V., Lorenzo, O., Ruperez, M., Suzuki, Y., Mezzano, S., Blanco, J., Kretzler, M., Sugaya, T., Egido, J., and Ruiz-Ortega, M. (2004). Angiotensin II, via AT1 and AT2 receptors and NF-kappaB pathway, regulates the inflammatory response in unilateral ureteral obstruction. *J. Am. Soc. Nephrol.* 15, 1514–1529.

Gabbiani, G. (2003). The myofibroblast in wound healing and fibrocontractive diseases. *J. Pathol.* 200, 500–503.

Gabbiani, G., Ryan, G. B., and Majne, G. (1971). Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. *Experientia* 27, 549–550.

Gao, X., He, X., Luo, B., Peng, L., Lin, J., and Zuo, Z. (2009). Angiotensin II increases collagen I expression via transforming growth factor-beta1 and extracellular signal-regulated kinase in cardiac fibroblasts. *Eur. J. Pharmacol.* 606, 115–120.

Goritz, C., Dias, D. O., Tomilin, N., Barbacid, M., Shupliakov, O., and Frisen, J. (2011). A pericyte origin of spinal cord scar tissue. *Science* 333, 238–242.

Grone, H. J., Weber, K., Grone, E., Helmchen, U., and Osborn, M. (1987). Coexpression of keratin and vimentin in damaged and regenerating tubular epithelia of the kidney. *Am. J. Pathol.* 129, 1–8.

Hemmann, S., Graf, J., Roderfeld, M., and Roeb, E. (2007). Expression of MMPs and TIMPs in liver fibrosis – a systematic review with special emphasis on anti-fibrotic strategies. *J. Hepatol.* 46, 955–975.

Henderson, W. R. Jr., Chi, E. Y., Ye, X., Nguyen, C., Tien, Y. T., Zhou, B., Borok, Z., Knight, D. A., and Kahn, M. (2010). Inhibition of Wnt/beta-catenin/CREB binding protein (CBP) signaling reverses pulmonary fibrosis. *Proc. Natl. Acad. Sci. U.S.A.* 107, 14309–14314.

Higashiyama, R., Moro, T., Nakao, S., Mikami, K., Fukumitsu, H., Ueda, Y., Ikeda, K., Adachi, E., Bou-Gharios, G., Okazaki, I., and Inagaki, Y. (2009). Negligible contribution of bone marrow-derived cells to collagen production during hepatic fibrogenesis in mice. *Gastroenterology* 137, 1459–1466 e1451.

Higashiyama, R., Nakao, S., Shibusawa, Y., Ishikawa, O., Moro, T., Mikami, K., Fukumitsu, H., Ueda, Y., Minakawa, K., Tabata, Y., Bou-Gharios, G., and Inagaki, Y. (2011). Differential contribution of dermal resident and bone marrow-derived cells to collagen production during wound healing and fibrogenesis in mice. *J. Invest. Dermatol.* 131, 529–536.

Hinz, B., Phan, S. H., Thannickal, V. J., Galli, A., Bochaton-Piallat, M. L., and Gabbiani, G. (2007). The myofibroblast: one function, multiple origins. *Am. J. Pathol.* 170, 1807–1816.

Humphreys, B. D., Lin, S. L., Kobayashi, A., Hudson, T. E., Nowlin, B. T., Bonventre, J. V., Valerius, M. T., McMahon, A. P., and Duffield, J. S. (2010). Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am. J. Pathol.* 176, 85–97.

Ijichi, H., Chytil, A., Gorska, A. E., Aakre, M. E., Bierie, B., Tada, M., Mohri, D., Miyabayashi, K., Asaoka, Y., Maeda, S., Ikenoue, T., Tateishi, K., Wright, C. V., Koike, K., Omata, M., and Moses, H. L. (2011). Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. *J. Clin. Invest.* 121, 4106–4117.

Ishida, Y., Kimura, A., Kondo, T., Hayashi, T., Ueno, M., Takakura, N., Matsushima, K., and Mukaida, N. (2007). Essential roles of the CC chemokine ligand 3-CC chemokine receptor 5 axis in bleomycin-induced pulmonary fibrosis through regulation of macrophage and fibrocyte infiltration. *Am. J. Pathol.* 170, 843–854.

Kalluri, R., and Neilson, E. G. (2003). Epithelial-mesenchymal transition and its implications for fibrosis. *J. Clin. Invest.* 112, 1776–1784.

Keeley, E. C., Mehrad, B., and Strieter, R. M. (2011). The role of fibrocytes in fibrotic diseases of the lungs and heart. *Fibrogenesis Tissue Repair* 4, 2.

Kis, K., Liu, X., and Hagood, J. S. (2011). Myofibroblast differentiation and survival in fibrotic disease. *Expert Rev. Mol. Med.* 13, e27.

Kohyama, T., Ertl, R. F., Valenti, V., Spurzem, J., Kawamoto, M., Nakamura, Y., Veys, T., Allegra, L., Romberger, D., and Rennard, S. I. (2001). Prostaglandin E(2) inhibits fibroblast chemotaxis. *Am. J. Physiol. Lung Cell Mol. Physiol.* 281, L1257–L1263.

Konigshoff, M., and Eickelberg, O. (2010). WNT signaling in lung disease: a failure or a regeneration signal? *Am. J. Respir. Cell Mol. Biol.* 42, 21–31.

Kriz, W., Kaissling, B., and Le Hir, M. (2011). Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? *J. Clin. Invest.* 121, 468–474.

Lama, V., Moore, B. B., Christensen, P., Toews, G. B., and Peters-Golden, M. (2002). Prostaglandin E2 synthesis and suppression of fibroblast proliferation by alveolar epithelial cells is cyclooxygenase-2-dependent. *Am. J. Respir. Cell Mol. Biol.* 27, 752–758.

Leask, A. (2010). Potential therapeutic targets for cardiac fibrosis: TGFbeta, angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation. *Circ. Res.* 106, 1675–1680.

Lee, C. H., Shah, B., Moioli, E. K., and Mao, J. J. (2010). CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model. *J. Clin. Invest.* 120, 3340–3349.

Lin, S. L., Kisseleva, T., Brenner, D. A., and Duffield, J. S. (2008). Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. *Am. J. Pathol.* 173, 1617–1627.

Lo Re, S., Lecocq, M., Uwambayinema, F., Yakoub, Y., Delos, M., Demoulin, J. B., Lucas, S., Sparwasser, T., Renauld, J. C., Lison, D., and Huaux, F. (2011). PDGF-producing CD4+ Foxp3+ regulatory T lymphocytes promote lung fibrosis. *Am. J. Respir. Crit. Care Med.* 184, 1270–1281.

Luzina, I. G., Todd, N. W., Iacono, A. T., and Atamas, S. P. (2008). Roles of T lymphocytes in pulmonary fibrosis. *J. Leukoc. Biol.* 83, 237–244.

Mortaz, E., Folkerts, G., Nijkamp, F. P., and Henricks, P. A. (2010). ATP and the pathogenesis of COPD. *Eur. J. Pharmacol.* 638, 1–4.

Oga, T., Matsuoka, T., Yao, C., Nonomura, K., Kitaoka, S., Sakata, D., Kita, Y., Tanizawa, K., Taguchi, Y., Chin, K., Mishima, M., Shimizu, T., and Narumiya, S. (2009). Prostaglandin F(2alpha) receptor signaling facilitates bleomycin-induced pulmonary fibrosis independently of transforming growth factor-beta. *Nat. Med.* 15, 1426–1430.

Olaso, E., Arteta, B., Benedicto, A., Crende, O., and Friedman, S. L. (2011). Loss of discoidin domain receptor 2 promotes hepatic fibrosis after chronic carbon tetrachloride through altered paracrine interactions between hepatic stellate cells and liver-associated macrophages. *Am. J. Pathol.* 179, 2894–2904.

Pattanaik, D., and Postlethwaite, A. E. (2010). A role for lysophosphatidic acid and sphingosine 1-phosphate in the pathogenesis of systemic sclerosis. *Discov. Med.* 10, 161–167.

Phillips, R. J., Burdick, M. D., Hong, K., Lutz, M. A., Murray, L. A., Xue, Y. Y., Belperio, J. A., Keane, M. P., and Strieter, R. M. (2004). Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J. Clin. Invest.* 114, 438–446.

Ponticos, M., Holmes, A. M., Shi-wen, X., Leoni, P., Khan, K., Rajkumar, V. S., Hoyles, R. K., Bou-Gharios, G., Black, C. M., Denton, C. P., Abraham, D. J., Leask, A., and Lindahl, G. E. (2009). Pivotal role of connective tissue growth factor in lung fibrosis: MAPK-dependent transcriptional activation of type I collagen. *Arthritis Rheum.* 60, 2142–2155.

Qi, W., Twigg, S., Chen, X., Polhill, T. S., Poronnik, P., Gilbert, R. E., and Pollock, C. A. (2005). Integrated actions of transforming growth factor-beta1 and connective tissue growth factor in renal fibrosis. *Am. J. Physiol. Renal Physiol.* 288, F800–F809.

Ricardo, S. D., van Goor, H., and Eddy, A. A. (2008). Macrophage diversity in renal injury and repair. *J. Clin. Invest.* 118, 3522–3530.

Rider, C. C., and Mulloy, B. (2010). Bone morphogenetic protein and growth differentiation factor cytokine families and their protein antagonists. *Biochem. J.* 429, 1–12.

Ruperez, M., Lorenzo, O., Blanco-Colio, L. M., Esteban, V., Egido, J., and Ruiz-Ortega, M. (2003). Connective tissue growth factor is a mediator of angiotensin II-induced fibrosis. *Circulation* 108, 1499–1505.

Sakai, N., Wada, T., Yokoyama, H., Lipp, M., Ueha, S., Matsushima, K., and Kaneko, S. (2006). Secondary lymphoid tissue chemokine (SLC/CCL21)/CCR7 signaling regulates fibrocytes in renal fibrosis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 14098–14103.

Sandbo, N., and Dulin, N. (2011). Actin cytoskeleton in myofibroblast differentiation: ultrastructure defining form and driving function. *Transl. Res.* 158, 181–196.

Scholten, D., Osterreicher, C. H., Scholten, A., Iwaisako, K., Gu, G., Brenner, D. A., and Kisseleva, T. (2010). Genetic labeling does not detect epithelial-to-mesenchymal transition of cholangiocytes in liver fibrosis in mice. *Gastroenterology* 139, 987–998.

Schultz Jel, J., Witt, S. A., Glascock, B. J., Nieman, M. L., Reiser, P. J., Nix, S. L., Kimball, T. R., and Doetschman, T. (2002). TGF-beta1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. *J. Clin. Invest.* 109, 787–796.

Shim, Y. M., Zhu, Z., Zheng, T., Lee, C. G., Homer, R. J., Ma, B., and Elias, J. A. (2006). Role of 5-lipoxygenase in IL-13-induced pulmonary inflammation and remodeling. *J. Immunol.* 177, 1918–1924.

Simonian, P. L., Roark, C. L., Wehrmann, F., Lanham, A. K., Diaz del Valle, F., Born, W. K., O'Brien, R. L., and Fontenot, A. P. (2009). Th17-polarized immune response in a murine model of hypersensitivity pneumonitis and lung fibrosis. *J. Immunol.* 182, 657–665.

Strutz, F., Okada, H., Lo, C. W., Danoff, T., Carone, R. L., Tomaszewski, J. E., and Neilson, E. G. (1995). Identification and characterization of a fibroblast marker: FSP1. *J. Cell Biol.* 130, 393–405.

Tager, A. M., LaCamera, P., Shea, B. S., Campanella, G. S., Selman, M., Zhao, Z., Polosukhin, V., Wain, J., Karimi-Shah, B. A., Kim, N. D., Hart, W. K., Pardo, A., Blackwell, T. S., Xu, Y., Chun, J., and Luster, A. D. (2008). The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nat. Med.* 14, 45–54.

Takuwa, N., Ohkura, S., Takashima, S., Ohtani, K., Okamoto, Y., Tanaka, T., Hirano, K., Usui, S., Wang, F., Du, W., Yoshioka, K., Banno, Y., Sasaki, M., Ichi, I., Okamura, M., Sugimoto, N., Mizugishi, K., Nakanuma, Y., Ishii, I., Takamura, M., Kaneko, S., Kojo, S., Satouchi, K., Mitumori, K., Chun, J., and Takuwa, Y. (2010). S1P3-mediated cardiac fibrosis in sphingosine kinase 1 transgenic mice involves reactive oxygen species. *Cardiovasc. Res.* 85, 484–493.

Tanaka, M., Asada, M., Higashi, A. Y., Nakamura, J., Oguchi, A., Tomita, M., Yamada, S., Asada, N., Takase, M., Okuda, T., Kawachi, H., Economides, A. N., Robertson, E., Takahashi, S., Sakurai, T., Goldschmeding, R., Muso, E., Fukatsu, A., Kita, T., and Yanagita, M. (2010). Loss of the BMP antagonist USAG-1 ameliorates disease in a mouse model of the progressive hereditary kidney disease Alport syndrome. *J. Clin. Invest.* 120, 768–777.

Thiery, J. P., Acloque, H., Huang, R. Y., and Nieto, M. A. (2009). Epithelial-mesenchymal transitions in development and disease. *Cell* 139, 871–890.

Thomas, P. E., Peters-Golden, M., White, E. S., Thannickal, V. J., and Moore, B. B. (2007). PGE(2) inhibition of TGF-beta1-induced myofibroblast differentiation is Smad-independent but involves cell shape and adhesion-dependent signaling. *Am. J. Physiol. Lung Cell Mol. Physiol.* 293, L417–L428.

Venkov, C. D., Link, A. J., Jennings, J. L., Plieth, D., Inoue, T., Nagai, K., Xu, C., Dimitrova, Y. N., Rauscher, F. J., and Neilson, E. G. (2007). A proximal activator of transcription in epithelial-mesenchymal transition. *J. Clin. Invest.* 117, 482–491.

Visscher, D. W., and Myers, J. L. (2006). Histologic spectrum of idiopathic interstitial pneumonias. *Proc. Am. Thorac. Soc.* 3, 322–329.

Wells, R. G. (2010). The epithelial-to-mesenchymal transition in liver fibrosis: here today, gone tomorrow? *Hepatology* 51, 737–740.

Witzgall, R., Brown, D., Schwarz, C., and Bonventre, J. V. (1994). Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J. Clin. Invest.* 93, 2175–2188.

Wynn, T. A., and Barron, L. (2010). Macrophages: master regulators of inflammation and fibrosis. *Semin. Liver Dis.* 30, 245–257.

Yin, Z., Tong, Y., Zhu, H., and Watsky, M. A. (2008). ClC-3 is required for LPA-activated Cl- current activity and fibroblast-to-myofibroblast differentiation. *Am. J. Physiol. Cell Physiol.* 294, C535–C542.

Zeisberg, M., Hanai, J., Sugimoto, H., Mammoto, T., Charytan, D., Strutz, F., and Kalluri, R. (2003). BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat. Med.* 9, 964–968.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 December 2011; accepted: 22 March 2012; published online: 10 April 2012.

*Citation: Ueha S, Shand FHW and Matsushima K (2012) Cellular and molecular mechanisms of chronic inflammation-associated organ fibrosis. Front. Immun. **3**:71. doi: 10.3389/fimmu.2012.00071*

This article was submitted to Frontiers in Molecular Innate Immunity, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 08 May 2012
doi: 10.3389/fimmu.2012.00107

Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer

Deborah C. Rubin[1,2]*, Anisa Shaker[1] and Marc S. Levin[1,3]

[1] *Division of Gastroenterology, Department of Internal Medicine, Washington University School of Medicine, Saint Louis, MO, USA*

[2] *Department of Developmental Biology, Washington University School of Medicine, Saint Louis, MO, USA*

[3] *Saint Louis Veterans Administration Medical Center, Saint Louis, MO, USA*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Daisuke Kamimura, Osaka University, Japan
Hisako Kayama, Osaka University, Japan

****Correspondence:***
Deborah C. Rubin, Division of Gastroenterology, Department of Internal Medicine, Washington University School of Medicine, 660 South Euclid Avenue, Box 8124, Saint Louis, MO 63110, USA.
e-mail: drubin@dom.wustl.edu

The inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the intestine. The prevalence in the United States is greater than 200 cases per 100,000, with the total number of IBD patients between 1 and 1.5 million. CD may affect all parts of the gastrointestinal tract, from mouth to anus, but most commonly involves the distal part of the small intestine or ileum, and colon. UC results in colonic inflammation that can affect the rectum only, or can progress proximally to involve part of or the entire colon. Clinical symptoms include diarrhea, abdominal pain, gastrointestinal bleeding, and weight loss. A serious long-term complication of chronic inflammation is the development of colorectal cancer. A genetic basis for IBD had long been recognized based on the increased familial risk. However, significant discordance for CD in twins, and a much less robust phenotypic concordance for UC, suggested additional factors play a role in disease pathogenesis, including environmental factors. In the past several years, progress in understanding the molecular basis of IBD has accelerated, beginning with the generation of animal models of colitis and progressing to the identification of specific genetic markers from candidate gene, gene linkage, and genome-wide association analyses. Genetic studies have also resulted in the recognition of the importance of environmental factors, particularly the crucial role of the gut microbiota in CD and UC. Altered immune responses to the normal intestinal flora are key factors in IBD pathogenesis. In this research topic, the genetic basis of IBD, the genetic and cellular alterations associated with colitis-associated colon cancer, and the emerging role of the intestinal microbiota and other environmental factors will be reviewed.

Keywords: inflammatory bowel disease, chronic intestinal inflammation, colitis-associated colon cancer, Crohn's disease, ulcerative colitis

INTRODUCTION

The inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the intestine. The prevalence in the United States is greater than 200 cases per 100,000 for each disorder, with the total number of IBD patients between 1 and 1.5 million (Kappelman et al., 2007; Loftus Jr., 2007). CD may affect all parts of the gastrointestinal tract, from mouth to anus, but most commonly involves the distal part of the small intestine or ileum, and colon. UC results in colonic inflammation that can affect the rectum only (proctitis) or can cause continuous disease from the rectum proximally, to involve part of or the entire colon. Clinical symptoms include diarrhea, abdominal pain, gastrointestinal bleeding, and weight loss. A serious long-term complication of chronic inflammation is the development of colorectal cancer (CRC).

A genetic basis for IBD had long been recognized based on the increased familial risk, with a 5–30% incidence in families of affected individuals (Duerr, 2002) as well as 50–75% phenotypic concordance in monozygotic twins with CD (Tysk et al., 1988). A family history of IBD is more commonly found in patients with CD compared to UC (Duerr, 2002). However, significant discordance for CD in twins, as well as a much less robust phenotypic concordance for UC in twins (Tysk et al., 1988; Orholm et al., 2000), also suggested additional factors play a role in disease pathogenesis, including a significant impact of environmental factors. In the past several years, progress in understanding the molecular basis of IBD has accelerated markedly, beginning with the generation of rodent transgenic and mouse knockout models of colitis and progressing to the identification of specific genetic markers from candidate gene approaches, gene linkage, and genome-wide association analyses (Tysk et al., 1988; Duerr et al., 2006; Barrett et al., 2008; Fisher et al., 2008; Anderson et al., 2009; Silverberg et al., 2009; Franke et al., 2010). It has become increasingly clear that IBD is a polygenic, complex disorder with region- and ethnic-specific differences in genetic risk factors (Abraham and Cho, 2009). In addition, genetic studies have resulted in the recognition of the importance of environmental factors, particularly focusing on the critical importance of the gut microbiota in CD and UC (Nell et al., 2010). Altered immune responses to the normal intestinal flora of the gut are key factors in CD pathogenesis.

Chronic inflammation is also associated with malignancy and has been proposed to be a major contributor to a multitude of cancers (Coussens and Werb, 2002; Kundu and Surh, 2008; Mantovani et al., 2008; Danese and Mantovani, 2010; Solinas et al., 2010). Chronic colonic inflammation from UC or CD results in a well-recognized increased risk of colon carcinogenesis (Bernstein et al., 2001; Eaden et al., 2001; Itzkowitz and Yio, 2004; Ullman and Itzkowitz, 2011). CD is also associated with an increased risk of small bowel adenocarcinoma, due to chronic inflammation of the small intestine. The cumulative probability of CRC in UC patients has been shown in meta-analysis to range from 2% after 10 years of disease, up to 18% after 30 years of disease (Eaden et al., 2001; Feagins et al., 2009; Westbrook et al., 2010). Patients with Crohn's colitis also have an increased cumulative risk for CRC, from 2.9% at 10 years to 8.3% after 30 years of disease (Canavan et al., 2006). The risk of carcinogenesis is related to severity, extent, and duration of disease (Rutter et al., 2004). Patients are advised to undergo colonoscopy with a specific biopsy protocol, performed every 1–2 years after 8–10 years of disease to detect dysplasia and rule out carcinogenesis. Unlike in sporadic colorectal carcinoma, in which the dysplastic lesion is an adenomatous polyp, dysplasia in IBD can be flat or polypoid. Flat lesions can be particularly difficult to detect endoscopically, and more sensitive markers of dysplasia are still lacking and represent a major focus of current research. Because of the frequency of IBD, the early onset of disease and the significantly increased risk for carcinogenesis, the health, emotional, and economic burden is quite high.

In this review, the genetic basis of IBD, the genetic and cellular alterations associated with chronic inflammation-induced colon cancer, and the emerging role of the intestinal microbiota and other environmental factors will be reviewed.

CLINICAL CHARACTERISTICS OF THE INFLAMMATORY BOWEL DISEASES

CLINICAL CHARACTERISTICS COMMON TO ULCERATIVE COLITIS AND CROHN'S DISEASE

The peak age of incidence for IBD is between 16 and 30 years (Kuster et al., 1989). Both UC and CD can affect the colon, and patients with either UC or CD have an increased risk of colitis-associated cancer (CAC) after 8–10 years from the time of diagnosis. Symptoms of active disease include diarrhea and abdominal pain. Although both CD and UC patients can experience gastrointestinal bleeding, in UC hematochezia or the presence of visible bleeding is more common than in CD, in which there is occult or microscopic blood loss. Extraintestinal manifestations of IBD include arthralgias and arthritis, skin diseases such as erythema nodosum and pyoderma gangrenosum, ocular disorders including uveitis and iritis, and sclerosing cholangitis, in which there is inflammation of the liver's bile ducts. Urinary excretion of oxalate may be elevated in patients with CD, resulting in kidney stones, in patients who have not had a colectomy. Both UC and CD patients may develop strictures in the colon (UC or CD) or small bowel (CD only).

CLINICAL CHARACTERISTICS SPECIFIC TO CROHN'S DISEASE

Patients with CD suffer the consequences of a transmural inflammatory process and thus are at risk for fistulizing disease. Fistulas, which are communications between the gastrointestinal tract and other organs, may form between the bowel and bladder or the vagina, (e.g., enterovesicular or recto-vaginal fistulas), from the intestines to the skin (enterocutaneous fistulas), or from intestine to intestine (enteroenteric fistulas). Perianal disease is common and can be debilitating and refractory to treatment. Due to the transmural nature of the inflammatory process and involvement of the small intestine which is responsible for nutrient absorption, patients with CD are more prone to weight loss, nutrient deficiencies, and in children, growth retardation, especially after glucocorticoid therapy. Other serious complications include perforation or microperforation of the small or large bowel which may result in abscess formation. Surgical resection of the colon is not curative because CD can affect all parts of the gastrointestinal tract from mouth to anus. Pathological features specific to CD include the presence of granulomas on biopsy of the small bowel or colon.

CLINICAL CHARACTERISTICS SPECIFIC TO ULCERATIVE COLITIS

Colonic inflammation in UC is continuous, beginning in the rectum. Gross gastrointestinal bleeding is much more common in UC. The development of toxic megacolon is a dreaded complication of active inflammation, which may lead to emergent colectomy. Unlike in CD, colectomy is curative in UC.

MOUSE MODELS OF INFLAMMATORY BOWEL DISEASE

CHEMICAL MODELS

Mouse models of colitis and CAC have proven in selected circumstances to be relevant to the pathogenesis of these disorders in humans, have led to the identification of critical genetic factors and have provided a means for understanding the role of specific genes identified by linkage or genome-wide association studies. Two of the most widely used, non-genetic colitis models are the dextran sodium sulfate (DSS)-induced chemical injury model, and the trinitrobenzene sulfonic acid (TNBS) hapten-induced model (Strober et al., 2002). These have been particularly useful in identifying and studying the role of genetic factors that modify colitis, because both DSS and TNBS can be administered to genetically altered (knockout or transgenic) mice to rapidly induce colonic inflammation and ulceration resembling UC (Strober et al., 2002). DSS in drinking water induces an acute colitis within 5 days of exposure, and can also be utilized to mimic chronic colitis after repeated exposures. DSS in combination with azoxymethane (AOM) can be utilized to generate a mouse model of CAC (Greten et al., 2004; Neurath and Finotto, 2009). TNBS is administered by enema and results in a hapten-induced, interleukin-12 (IL-12) driven colitis (Neurath and Finotto, 2009).

GENETIC MODELS

The earliest murine genetic models of IBD were generated in mice in which the T cell receptor was inactivated (Mombaerts et al., 1993), IL-10 (Kuhn et al., 1993) or IL-2 (Sadlack et al., 1993) was deleted, or tumor necrosis factor-alpha (TNF$\alpha^{\Delta ARE}$ mice) was over-produced. These mice develop colitis after variable lengths of time, and a seminal discovery was the observation that in almost every genetic model, the microbiota are required for induction

of colitis (reviewed in Nell et al., 2010). Mice raised in either a germ-free environment (e.g., IL-10 mice; Sellon et al., 1998) or treated with antibiotics were protected from colitis. Subsequently, a plethora of mouse IBD models have been described, resulting from gene knockout or transgenic overexpression. As summarized in Rosenstiel et al. (2009), some mouse models correlate well with human disease risk loci or associated pathways, whereas others do not yet have clear relevance to human clinical syndromes.

GENETICS OF INFLAMMATORY BOWEL DISEASE

Exciting advances in the understanding of the complex genetics of IBD have resulted from comprehensive genetic studies including linkage and genome-wide association analyses. These have led to the identification of several predicted as well as novel pathways involved in CD and UC pathogenesis. For example, the critical roles of innate immunity and autophagy as well as epithelial barrier function have been supported by the identification of risk alleles in genes from these pathways, by genome-wide association studies (e.g., Duerr et al., 2006; Barrett et al., 2008; Silverberg et al., 2009). A complete list of genetic loci linked to IBD susceptibility (Kaser et al., 2010) shows that some are specific to CD, some to UC, and some are linked to both diseases.

INNATE IMMUNITY

NOD2/CARD15

A seminal discovery in unraveling the complex genetics underlying CD was that mutations in the NOD2/CARD15 gene locus are associated with risk for CD in Caucasian populations of European ancestry (Hugot et al., 2001; Ogura et al., 2001), and particularly for ileal disease (Lesage et al., 2002). Mutations in *NOD2* are not sufficient for generating CD, as a significant proportion of the normal population has NOD2 mutations but are not affected by this disorder. However, individuals who are heterozygous for a *NOD2* polymorphism have an increased risk of CD by a factor of 1.7–4, and homozygosity confers a risk factor of 11–27 (Economou et al., 2004). NOD2/CARD15 is a member of a family of cytosolic receptors containing a central nucleotide binding and oligomerization domain (NOD), an N-terminal effector binding domain, and leucine-rich repeats. These and other pattern recognition receptors, expressed in the epithelium and in a variety of immune cells, have important functions in innate immunity, particularly in regulating responses to intracellular pathogens and other exogenous injury-inducing stimuli. NOD2 recognizes components of the bacterial cell wall and elicits an NF-κB response, and also mediates the release of defensins, which are antimicrobial peptides. Mutations which impair NOD2 function result in defective downregulation of pro-inflammatory cytokines that normally occurs during chronic NOD2 stimulation (Hedl et al., 2007). In addition, in macrophages, others have shown that NOD2 is a negative regulator of Toll-like receptor 2 (TLR2)-mediated activation of NF-κB-c-Rel (Watanabe et al., 2004). However, the mechanisms by which loss of function mutations result in CD are still under investigation (Abraham and Cho, 2009). The discovery of this association led to further recognition of the importance of the microbiome in CD pathogenesis.

Autophagy genes ATG16 autophagy related 16-like 1 (ATG16L1) and immunity related GTPase family M

Genome-wide association studies have shown that polymorphisms in the ATG16L1 gene (Hampe et al., 2007) and sequence variants in the IRGM gene (Parkes et al., 2007) are linked to CD. These genes encode proteins that are critical for autophagy, a process that mediates degradation of intracellular proteins via vesicle-mediated delivery to the lysosome (reviewed in Glick et al., 2010; Huett et al., 2010). Autophagy is particularly important for defense against intracellular pathogens. A mouse model of ATG16L1 deficiency showed Paneth cell dysfunction with aberrant exocytosis, as well as an altered transcriptional profile, characterized by increased expression of pro-inflammatory cytokines and lipid metabolism genes. Patients with CD have a similar Paneth cell phenotype (Cadwell et al., 2008). An important observation derived from the ATG16L1 mouse model was that viral infection with murine norovirus, as well as the presence of commensal bacteria, were required for generating these specific Paneth cell abnormalities (Miller et al., 2008). Germ-free mice have normal Paneth cells and viral infection in the presence of commensal bacteria induced the characteristic changes in Paneth cell function. IRGM functions to protect cells from mycobacteria (Singh et al., 2006). These data provide further support for the hypothesis that microbial/viral interactions with the intestinal mucosa are required for disease generation, and suggest that combinatorial models for IBD pathogenesis are most relevant for the study of human disease pathogenesis.

Intelectin 1 or ITLN1

Genome-wide association studies have also identified an association between intelectin 1 and CD. Intelectin 1 is a lectin that recognizes galactofuranosyl residues in bacterial cell walls and is identical to human lactoferrin receptor (Tsuji et al., 2001). It is a lipid raft protein that resides in the enterocytic brush border and is also expressed in Paneth and goblet cells of the small bowel (Wrackmeyer et al., 2006). Its presumed function is to protect from parasitic infection and offer cytoprotection from bacterial translocation.

INFLAMMATORY/CYTOKINE SIGNALING PATHWAYS

IL-23 RECEPTOR, INTERLEUKIN 12B, AND OTHER GENES INVOLVED IN IL-23 SIGNALING AND T HELPER CELL 17 FUNCTION

Genome-wide association studies have shown an association between *IL23R* and CD (Duerr et al., 2006). This gene encodes a subunit of the IL-23 receptor (IL-23R) complex, which consists of the IL-23R and the IL-12 receptor B1. IL-23 is a pro-inflammatory cytokine that results in activation of Janus-associated kinase (JAK) 2 and signal transducers and activators of transcription 3 (STAT3), which are important downstream mediators of inflammation. The likely relevance of IL-23R to CD is suggested by its known biological functions, e.g., IL-23 expression is required for murine colitis (Yen et al., 2006), and IL-23 is important for T helper cell 17 (Th17) cell function and production of IL-17. IL-17 expression is increased in colons from patients with UC and CD (Fujino et al., 2003), and other members of IL-23R regulated pathways are linked to both UC and CD, e.g., *STAT3, JAK 2*, and *IL12B* (Barrett et al., 2008). In addition, the chemokine receptor *CCR6* is also

implicated in CD, and is expressed by immature dendritic cells and memory T cells (Barrett et al., 2008).

Interleukin-10

Interleukin-10 is an anti-inflammatory cytokine that has long been postulated to play a role in IBD. One of the first mouse models of IBD resulted from the generation of the IL-10 knockout mouse, which develops spontaneous inflammation (Kuhn et al., 1993), and which is dependent upon the presence of gut bacteria. Regulatory T cells (Tregs) express IL-10; selective deletion of IL-10 expression in Tregs results in spontaneous colitis and inflammation at other epithelial surfaces including skin and lungs (Rubtsov et al., 2008). Genome-wide association studies for UC have shown SNPs flanking the IL-10 gene to be linked to UC (Franke et al., 2008b). In addition, patients with early onset IBD (at less than 1 year of age) had mutations in the IL10RA and IL10RB genes, which resulted in abrogated STAT3 phosphorylation from deficient IL-10 signaling (Glocker et al., 2009). Interestingly, the phenotype of these patients is closest to CD, yet genome-wide association studies have not identified IL-10 as a susceptibility locus. However, these data all support a key role for IL-10 in IBD and suggest that future therapeutic trials with IL-10 may be warranted.

NKX2.3

Genome-wide association studies have shown that this homeodomain transcription factor is associated with CD and UC (Barrett et al., 2008; Franke et al., 2008a). Mice that are null for *Nkx2.3* have defective splenic and intestinal development. Homozygous null mice exhibit a marked delay in villus formation with crypt hypoproliferation. A subset of mice survive to adulthood and show massive hyperproliferation of the gut with decreased Bmp2 and Bmp4 expression (Pabst et al., 1999). Splenic development is markedly abnormal, in these mice, resulting in either small or completely absent spleens. Although the phenotype associated with *NKX2.3* mutations in humans has not been defined, mouse models suggest gut epithelial or splenic functional defects.

T CELL-MEDIATED RESPONSES

T helper cells differentiate into two distinct subtypes, Th1 and Th2 cells. These cells produce characteristic sets of cytokines. Many years of investigation has shown that Th1 cytokines are expressed in CD, whereas UC is a Th2 cytokine-mediated disease. A review of this vast body of research is beyond the scope of this manuscript, and is discussed in detail in other reviews (e.g., Strober and Fuss, 2011).

ENVIRONMENTAL RISK FACTORS

SMOKING

Smoking has emerged as one of the critically important risk factors for IBD, with an interesting paradoxical relationship for UC vs. CD disease activity. Smoking clearly increases the risk of CD activity (Calkins, 1989) and increases risk of recurrence after surgery (Unkart et al., 2008), yet appears to be protective for UC (Harries et al., 1982; Calkins, 1989). Nicotine has been studied as a primary treatment for UC (Pullan et al., 1994; Sandborn et al., 1997). Carbon monoxide, an important component of cigarette smoke, has been shown to suppress colonic pro-inflammatory cytokine production, and increase IL-10 secretion, through heme oxygenase-1 dependent pathways (Sheikh et al., 2011).

THE MICROBIOME

Bacterial, mycobacterial, or viral infections have long been postulated to be important in IBD pathogenesis (Lidar et al., 2009). A common feature of almost all rodent models of IBD is that treatment with antibiotics or rederivation of knockout or transgenic mice into germ-free conditions markedly mitigates disease activity (e.g., Taurog et al., 1994; Strober et al., 2002). Antibiotics can ameliorate disease activity in humans, and for certain complications of CD such as fistulizing disease, metronidazole is an important therapeutic agent. Viral infection is required to generate the Paneth cell defects found in ATG16L1 mice (Cadwell et al., 2010) suggesting that in addition to human bacterial microbiota, viral or fungal commensals may play a role in IBD pathogenesis.

Associations with single microorganisms

Microbial association studies in mouse models and analysis of intestinal mucosa or blood from patients with CD have implicated single pathogenic bacterial species in IBD pathogenesis, although none have yet been proven to be causative (reviewed in Lidar et al., 2009; Reiff and Kelly, 2010). The microorganisms most frequently implicated include *Mycobacterium avium* subspecies *paratuberculosis*, *Saccharomyces cerevisiae*, *Candida albicans*, adherent enteroinvasive *Escherichia coli*, and *Chlamydia pneumoniae*.

The microbiome in normal intestine and in inflammatory bowel disease

Recent discoveries implicating genes such as *NOD2* in the pathogenesis of CD have led to the recognition that the pathogenesis of IBD involves loss of tolerance to commensal organisms and enhanced immune responses to bacterial antigens. The intestine is colonized by the largest bacterial burden in the body, containing approximately 100 trillion organisms (Gill et al., 2006). Bacteria belonging to the Firmicutes (Gram-positive bacteria) and Bacteroidetes (Gram-negative bacteria) phyla are the two major groups in mammalian intestine (Backhed et al., 2005; Turnbaugh et al., 2007). Proteobacteria (which include *Helicobacter* and *Escherichia*) and Actinobacteria are also significant contributors to the gut flora. Multiple studies have shown that the gut microbiota is altered in IBD patients. For example, biopsy samples from CD patients were used to prepare bacterial DNA which was amplified using universal bacterial 16S rRNA primers (Gophna et al., 2006). A significant increase in Proteobacteria and Bacteroidetes was found in CD patients compared to controls, with a decrease in Clostridia. Metagenomic approaches were used to analyze fecal samples from Crohn's patients and healthy donors, and revealed reduced complexity of the Firmicutes in affected patients (Manichanh et al., 2006). Evaluation of the microbial populations in surgically resected tissue samples of small bowel and colon from Crohn's, UC, and non-IBD controls, by rRNA sequence analysis, showed that specific flora were not enriched in small bowel or colon from IBD patients. However, a subset of IBD samples showed alterations

in the representation of the Bacteroidetes and Firmicutes (Eckburg and Relman, 2007; Frank et al., 2007, 2011). Analysis of fecal samples from IBD patients compared to healthy subjects (Qin et al., 2010.) showed reduced bacterial diversity and altered bacterial species abundance, using metagenomic sequencing methods

Role of Toll-like receptors and nucleotide-binding oligomerization domain protein-like receptors

Toll-like receptors and NLRs are innate receptors that play an important role in recognizing commensal bacteria. Recognition of commensals by TLRs and NLRs has been shown to be critical for maintaining intestinal epithelial integrity and homeostasis. For example, mice deficient in the adaptor protein, MyD88, develop severe colitis following DSS administration (Rakoff-Nahoum et al., 2004). Inflammasomes composed of NLR proteins sense damage-associated molecular patterns. NLRP6 inflammasome-deficient mice had more severe colitis, reduced IL-18 levels, and altered gut microbiota (Elinav et al., 2011).

COLITIS/INFLAMMATION-ASSOCIATED DYSPLASIA AND CANCER

RELATIONSHIP TO COLORECTAL CANCER

The pathogenetic mechanisms underlying CAC compared to familial or sporadic CRC have significant similarities, but major differences have also been recognized (Feagins et al., 2009; Terzic et al., 2010; Ullman and Itzkowitz, 2011). Whereas dysplasia in CRC is focal, multiple areas of the colon are often involved in CAC, indicating a broader "field effect." Linkage analyses of families with rare, inherited early-onset CRC led to the identification of gene mutations which are highly relevant to the much more common sporadic CRC. In many circumstances, mutations in these genes also occur in CAC, but at a different stage of the disease, and other gene mutations are specific to CRC only. A seminal discovery in CRC pathogenesis was that inherited mutations in the adenomatous polyposis coli (APC) gene result in familial adenomatous polyposis or FAP, in which affected patients develop hundreds of adenomatous polyps and are at high risk for early death from CRC (Groden et al., 1991). *APC* mutations occur in sporadic CRC and are one of the earliest events in CRC pathogenesis. *APC* mutations are also found in CAC, but generally occur much later in the disease course (Redston et al., 1995; Tarmin et al., 1995; Aust et al., 2002). On the other hand, *KRAS* and *DCC/DPC4* mutations occur in both CAC and CRC (Ullman and Itzkowitz, 2011). P53 mutations are commonly found in CACs. P53 mutation is an early event that precedes loss of heterozygosity and is highly associated with aneuploidy (Brentnall et al., 1994). Chronic inflammation associated with increased pro-inflammatory cytokine release and signaling plays a critical role in the initiation of CAC, but sporadic CRC tumors also exhibit inflammatory infiltrates and activated immune response pathways (Terzic et al., 2010). These observations have led to the postulation that inflammation promotes tumorigenesis both extrinsically (driven by chronic inflammatory conditions such as IBD) and intrinsically (driven by inflammation and inflammatory cells recruited to and contained within tumors; Mantovani et al., 2008; Danese and Mantovani, 2010).

MOUSE MODELS OF COLITIS-ASSOCIATED CANCER

One of the most widely utilized mouse models of CAC is the AOM/DSS model (Becker et al., 2004; Greten et al., 2004; Suzuki et al., 2006; Neufert et al., 2007; Tanaka et al., 2008); AOM is a chemical carcinogen that acts by alkylation of DNA. It is further metabolized by the liver after intraperitoneal injection and is excreted in the bile. Additional metabolism by the bacterial flora further activates its carcinogenicity (Neufert et al., 2007). Multiple injections of AOM result in distal colonic tumorigenesis with histologic characteristics similar to human CRC. To mimic CAC, mouse models were developed that use AOM in combination with DSS, which when included in the drinking water, induces colitis, as above. The first models used AOM injection followed by one cycle of DSS. However, to further mimic states of chronic inflammation, AOM injection was combined with three cycles of DSS, which induces a chronic colitis. This model accelerates tumor formation and results in larger tumor size. Of interest, there are differences in susceptibility among mouse strains (Suzuki et al., 2006) and the formation of tumors in the same strain may vary among mouse facilities, suggesting that tumorigenesis is affected by microflora.

PATHOGENESIS AND MOLECULAR BIOLOGY OF COLITIS-ASSOCIATED CANCER

Cancer associated with chronic inflammation, similar to other cancers, is characterized by a loss of normal growth regulation, resulting from a series of genetic mutations and epigenetic alterations in important cancer-related regulatory genes. The cancer stem cell model postulates that expansion of stem cells occurs in response to these mutations, resulting in tumor formation. The mechanisms by which inflammation results in carcinogenesis are presently the focus of intense research. Multiple pathways are likely to play a role, including production of reactive oxygen species and cytokine and chemokine expression by immune cells, which increase the risk of mutagenesis, and interactions between cancer stem cells and the local tumor microenvironment, including immune cells and myofibroblasts (Shaker et al., 2010; Vermeulen et al., 2010; Worthley et al., 2010; Quante et al., 2011; Shaker and Rubin, 2011). Inflammation also affects DNA methylation patterns and histone modification. Cyclo-oxygenase 2, which metabolizes arachidonic acid to prostaglandins, exhibits increased expression in inflamed tissues and affects cell proliferation, apoptosis, and angiogenesis. Genetic factors also play a role as IBD patients with a family history of CRC have an additional increase in risk for CAC, suggesting overlapping mechanisms.

ROLE OF THE MICROBIOME, TLRs, AND NLRs

In addition to playing an important role in IBD, TLRs and NLRs also contribute to the pathogenesis of CAC. For example, MyD88 signaling appears to be protective in the AOM/DSS model of CAC; *Myd88*$^{-/-}$ mice had increased polyp numbers compared to controls, and developed infiltrating carcinomas (Salcedo et al., 2010). In addition, derepression of the inflammasome in *Casp12*$^{-/-}$ mice, resulted in enhanced epithelial repair processes with increased proliferation, increased inflammation and increased susceptibility to AOM/DSS CAC (Dupaul-Chicoine et al., 2010).

ROLE OF OXIDATIVE STRESS, CYTOKINES, AND CHEMOKINES

Inflammatory cells produce a variety of reactive oxygen and nitrogen species which may generate gene mutations and DNA damage, contributing to carcinogenesis (Kawanishi et al., 2006; Kundu and Surh, 2008; Mantovani et al., 2008; Colotta et al., 2009). For example, mice which sustain DNA damage from inflammation and which are deficient in base excision repair enzymes have increased tumor multiplicity in the AOM/DSS mouse model of CAC, indicating that inflammation can induce DNA damage which in turn contributes to colon carcinogenesis (Liao et al., 2008; Meira et al., 2008). Direct genotoxicity was documented in mouse models of intestinal inflammation, which correlated with the degree of systemic and local inflammation, and was associated with evidence of reactive oxygen species-mediated oxidative stress and DNA damage (Westbrook et al., 2010). On the other hand, the role of nitric oxide is less clear. Mice which lack inducible nitric oxide synthase, the enzyme that generates nitric oxide ($iNOS^{-/-}$ mice), when bred to $IL10^{-/-}$ mice (which spontaneously develop colitis and adenocarcinoma with aging), had higher numbers of polyps compared to $IL\text{-}10^{-/-}$ mice alone, suggesting that nitric oxide may be protective (Zhang et al., 2007). However, increased production of reactive oxygen and nitrogen species may also result in oncogene activation or tumor suppressor inactivation by inducing mutations in critical regulatory genes. For example, p53 mutations in codons 247 and 248 were found in inflamed colons of UC patients, associated with increased iNOS expression (Hussain et al., 2000).

The major cytokines that play the best-described role in promoting inflammation in CAC include TNFα, IL-1, and IL-6 (Greten et al., 2004; Grivennikov et al., 2009; Shaker et al., 2010). TNFα signaling via NF-κB pathways mediated downstream by IL-6 and STAT3 appear to play an important role in this disorder (Ullman and Itzkowitz, 2011).

GENE SILENCING BY METHYLATION OR BY miRNA

An important mechanism of tumorigenesis is epigenetic silencing of selected genes such as tumor suppressor genes, by promoter methylation or by microRNAs (miRNA). These include DNA mismatch repair (MMR) genes; hypermethylation is thought to be the mechanism responsible for loss of MMR activity. Loss of DNA MMR gene activity results in microsatellite instability, which is characterized by increased frameshift mutation rates. MMR-deficient tumors account for approximately 15% of all CRCs and are characterized by a right sided location, have a lymphocytic infiltrate and have a poorly differentiated, mucinous, or signet cell histologic appearance.

The methylation status of normal, inflamed, and dysplastic colonic tissue has been studied intensively. It has been proposed that gene methylation may be an early event in inflammation-associated tumorigenesis, and thus can potentially be a sensitive marker for predicting dysplasia. The methylation status of multiple genes has been examined, and generally, DNA methylation appears to be more frequent and is seen more commonly in non-neoplastic mucosa from UC patients with CAC, compared to non-neoplastic mucosa from UC patients without cancer. For example, the methylation status of RUNX2 and MINT1 was higher in non-neoplastic tissue of UC patients with CAC compared to non-neoplastic tissue of UC patients without cancer. In contrast, COX-2 was more frequently methylated in colons from UC patients without cancer compared to UC patients with cancer (Garrity-Park et al., 2010). Aging is associated with methylation and silencing of a panel of genes (including the estrogen receptor, MyoD, p16 exon1, and CSPG2). In non-dysplastic tissues from UC patients with high grade dysplasia, these genes also showed significantly higher degrees of methylation, compared to UC patients without dysplasia (Issa et al., 2001). Methylation of the estrogen receptor in non-neoplastic epithelium of UC patients with CAC occurred in a higher percentage compared to UC patients without cancer (Fujii et al., 2005). These data suggest that methylation status can be used as a biomarker for early detection of dysplasia, and also may help identify patients who are at increased risk for neoplasia.

CONCLUSION

Great progress has been made towards identifying the genetic basis for the IBD, and for understanding the interactions between the gut luminal/microbial environment and its epithelium. Future research will focus on understanding the function of identified disease risk genes and developing new targeted therapies. The burden of CAC continues to be high, and current research is focused on developing more sensitive markers of dysplasia. Intensive efforts will be focused on further delving into the mechanisms underlying the initiation of chronic inflammation-associated cancer, including the role of stromal–epithelial interactions within the unique environment of the gastrointestinal tract. As our understanding of gastrointestinal cancer stem cells progresses, so will our ability to optimally target the interactions between tumor epithelium and its microenvironment in CAC.

ACKNOWLEDGMENTS

The authors are supported by NIH NIDDK R01 DK 61216, 46122, 50466, and P30 DK 52574.

REFERENCES

Abraham, C., and Cho, J. H. (2009). Inflammatory bowel disease. *N. Engl. J. Med.* 361, 2066–2078.

Anderson, C. A., Massey, D. C., Barrett, J. C., Prescott, N. J., Tremelling, M., Fisher, S. A., Gwilliam, R., Jacob, J., Nimmo, E. R., Drummond, H., Lees, C. W., Onnie, C. M., Hanson, C., Blaszczyk, K., Ravindrarajah, R., Hunt, S., Varma, D., Hammond, N., Lewis, G., Attlesey, H., Watkins, N., Ouwehand, W., Strachan, D., McArdle, W., Lewis, C. M.; Wellcome Trust Case Control Consortium, Lobo, A., Sanderson, J., Jewell, D. P., Deloukas, P., Mansfield, J. C., Mathew, C. G., Satsangi, J., and Parkes, M. (2009). Investigation of Crohn's disease risk loci in ulcerative colitis further defines their molecular relationship. *Gastroenterology* 136, 523–529.e3.

Aust, D. E., Terdiman, J. P., Willenbucher, R. F., Chang, C. G., Molinaro-Clark, A., Baretton, G. B., Loehrs, U., and Waldman, F. M. (2002). The APC/beta-catenin pathway in ulcerative colitis-related colorectal carcinomas: a mutational analysis. *Cancer* 94, 1421–1427.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., and Gordon, J. I. (2005). Host–bacterial mutualism in the human intestine. *Science* 307, 1915–1920.

Barrett, J. C., Hansoul, S., Nicolae, D. L., Cho, J. H., Duerr, R. H., Rioux, J. D., Brant, S. R., Silverberg, M. S., Taylor, K. D., Barmada, M. M., Bitton, A., Dassopoulos, T., Datta, L. W., Green, T., Griffiths, A. M., Kistner,

E. O., Murtha, M. T., Regueiro, M. D., Rotter, J. I., Schumm, L. P., Steinhart, A. H., Targan, S. R., Xavier, R. J.; NIDDK IBD Genetics Consortium, Libioulle, C., Sandor, C., Lathrop, M., Belaiche, J., Dewit, O., Gut, I., Heath, S., Laukens, D., Mni, M., Rutgeerts, P., Van Gossum, A., Zelenika, D., Franchimont, D., Hugot, J. P., de Vos, M., Vermeire, S., Louis, E.; Belgian-French IBD Consortium; Wellcome Trust Case Control Consortium, Cardon, L. R., Anderson, C. A., Drummond, H., Nimmo, E., Ahmad, T., Prescott, N. J., Onnie, C. M., Fisher, S. A., Marchini, J., Ghori, J., Bumpstead, S., Gwilliam, R., Tremelling, M., Deloukas, P., Mansfield, J., Jewell, D., Satsangi, J., Mathew, C. G., Parkes, M., Georges, M., and Daly, M. J. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat. Genet.* 40, 955–962.

Becker, C., M. Fantini, C., Schramm, C., Lehr, H. A., Wirtz, S., Nikolaev, A., Burg, J., Strand, S., Kiesslich, R., Huber, S., Ito, H., Nishimoto, N., Yoshizaki, K., Kishimoto, T., Galle, P. R., Blessing, M., Rose-John, S., and Neurath, M. F. (2004). TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. *Immunity* 21, 491–501.

Bernstein, C. N., Blanchard, J. F., Kliewer, E., and Wajda, A. (2001). Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* 91, 854–862.

Brentnall, T. A., Crispin, D. A., Rabinovitch, P. S., Haggitt, R. C., Rubin, C. E., Stevens, A. C., and Burmer, G. C. (1994). Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology* 107, 369–378.

Cadwell, K., Liu, J. Y., Brown, S. L., Miyoshi, H., Loh, J., Lennerz, J. K., Kishi, C., Kc, W., Carrero, J. A., Hunt, S., Stone, C. D., Brunt, E. M., Xavier, R. J., Sleckman, B. P., Li, E., Mizushima, N., Stappenbeck, T. S., and Virgin, H. W. IV. (2008). A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 456, 259–263.

Cadwell, K., Patel, K. K., Maloney, N. S., Liu, T. C., Ng, A. C., Storer, C. E., Head, R. D., Xavier, R., Stappenbeck, T. S., and Virgin, H. W. (2010). Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. *Cell* 141, 1135–1145.

Calkins, B. M. (1989). A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig. Dis. Sci.* 34, 1841–1854.

Canavan, C., Abrams, K. R., and Mayberry, J. (2006). Meta-analysis: colorectal and small bowel cancer risk in patients with Crohn's disease. *Aliment. Pharmacol. Ther.* 23, 1097–1104.

Colotta, F., Allavena, P., Sica, A., Garlanda, C., and Mantovani, A. (2009). Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* 30, 1073–1081.

Coussens, L. M., and Werb, Z. (2002). Inflammation and cancer. *Nature* 420, 860–867.

Danese, S., and Mantovani, A. (2010). Inflammatory bowel disease and intestinal cancer: a paradigm of the Yin-Yang interplay between inflammation and cancer. *Oncogene* 29, 3313–3323.

Duerr, R. H. (2002). The genetics of inflammatory bowel disease. *Gastroenterol. Clin. North Am.* 31, 63–76.

Duerr, R. H., Taylor, K. D., Brant, S. R., Rioux, J. D., Silverberg, M. S., Daly, M. J., Steinhart, A. H., Abraham, C., Regueiro, M., Griffiths, A., Dassopoulos, T., Bitton, A., Yang, H., Targan, S., Datta, L. W., Kistner, E. O., Schumm, L. P., Lee, A. T., Gregersen, P. K., Barmada, M. M., Rotter, J. I., Nicolae, D. L., and Cho, J. H. (2006). A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 314, 1461–1463.

Dupaul-Chicoine, J., Veretssian, G., Doiron, K., Bergstrom, K. S., McIntire, C. R., LeBlanc, P. M., Meunier, C., Turbide, C., Gros, P., Beauchemin, N., Vallance, B. A., and Saleh, M. (2010). Control of intestinal homeostasis, colitis, and colitis-associated colorectal cancer by the inflammatory caspases. *Immunity* 32, 367–378.

Eaden, J. A., Abrams, K. R., and Mayberry, J. F. (2001). The risk of colorectal cancer in ulcerative colitis: a meta-analysis. *Gut* 48, 526–535.

Eckburg, P. B., and Relman, D. A. (2007). The role of microbes in Crohn's disease. *Clin. Infect. Dis.* 44, 256–262.

Economou, M., Trikalinos, T. A., Loizou, K. T., Tsianos, E. V., and Ioannidis, J. P. (2004). Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. *Am. J. Gastroenterol.* 99, 2393–2404.

Elinav, E., Strowig, T., Kau, A. L., Henao-Mejia, J., Thaiss, C. A., Booth, C. J., Peaper, D. R., Bertin, J., Eisenbarth, S. C., Gordon, J. I., and Flavell, R. A. (2011). NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145, 745–757.

Feagins, L. A., Souza, R. F., and Spechler, S. J. (2009). Carcinogenesis in IBD: potential targets for the prevention of colorectal cancer. *Nat. Rev. Gastroenterol. Hepatol.* 6, 297–305.

Fisher, S. A., Tremelling, M., Anderson, C. A., Gwilliam, R., Bumpstead, S., Prescott, N. J., Nimmo, E. R., Massey, D., Berzuini, C., Johnson, C., Barrett, J. C., Cummings, F. R., Drummond, H., Lees, C. W., Onnie, C. M, Hanson, C. E., Blaszczyk, K., Inouye, M., Ewels, P., Ravindrarajah, R., Keniry, A., Hunt, S., Carter, M., Watkins, N., Ouwehand, W., Lewis, C. M., Cardon, L.; Wellcome Trust Case Control Consortium, Lobo, A., Forbes, A., Sanderson, J., Jewell, D. P., Mansfield, J. C., Deloukas, P., Mathew, C. G., Parkes, M., and Satsangi, J. (2008). Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nat. Genet.* 40, 710–712.

Frank, D. N., Robertson, C. E., Hamm, C. M., Kpadeh, Z., Zhang, T., Chen, H., Zhu, W., Sartor, R. B., Boedeker, E. C., Harpaz, N., Pace, N. R., and Li, E. (2011). Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. *Inflamm. Bowel Dis.* 17, 179–184.

Frank, D. N., St Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N., and Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl. Acad. Sci. U.S.A.* 104, 13780–13785.

Franke, A., Balschun, T., Karlsen, T. H., Hedderich, J., May, S., Lu, T., Schuldt, D., Nikolaus, S., Rosenstiel, P., Krawczak, M., and Schreiber, S. (2008a). Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. *Nat. Genet.* 40, 713–715.

Franke, A., Balschun, T., Karlsen, T. H., Sventoraityte, J., Nikolaus, S., Mayr, G., Domingues, F. S., Albrecht, M., Nothnagel, M., Ellinghaus, D., Sina, C., Onnie, C. M., Weersma, R. K., Stokkers, P. C., Wijmenga, C., Gazouli, M., Strachan, D., McArdle, W. L., Vermeire, S., Rutgeerts, P., Rosenstiel, P., Krawczak, M., Vatn, M. H.; IBSEN study group, Mathew, C. G., and Schreiber, S. (2008b). Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. *Nat. Genet.* 40, 1319–1323.

Franke, A., McGovern, D. P., Barrett, J. C., Wang, K., Radford-Smith, G. L., Ahmad, T., Lees, C. W., Balschun, T., Lee, J., Roberts, R., Anderson, C. A., Bis, J. C., Bumpstead, S., Ellinghaus, D., Festen, E. M., Georges, M., Green, T., Haritunians, T., Jostins, L., Latiano, A., Mathew, C. G., Montgomery, G. W., Prescott, N. J., Raychaudhuri, S., Rotter, J. I., Schumm, P., Sharma, Y., Simms, L. A., Taylor, K. D., Whiteman, D., Wijmenga, C., Baldassano, R. N., Barclay, M., Bayless, T. M., Brand, S., Büning, C., Cohen, A., Colombel, J. F., Cottone, M., Stronati, L., Denson, T., De Vos, M., D'Inca, R., Dubinsky, M., Edwards, C., Florin, T., Franchimont, D., Gearry, R., Glas, J., Van Gossum, A., Guthery, S. L., Halfvarson, J., Verspaget, H. W., Hugot, J. P., Karban, A., Laukens, D., Lawrance, I., Lemann, M., Levine, A., Libioulle, C., Louis, E., Mowat, C., Newman, W., Panés, J., Phillips, A., Proctor, D. D., Regueiro, M., Russell, R., Rutgeerts, P., Sanderson, J., Sans, M., Seibold, F., Steinhart, A. H., Stokkers, P. C., Torkvist, L., Kullak-Ublick, G., Wilson, D., Walters, T., Targan, S. R., Brant, S. R., Rioux, J. D., D'Amato, M., Weersma, R. K., Kugathasan, S., Griffiths, A. M., Mansfield, J. C., Vermeire, S., Duerr, R. H., Silverberg, M. S., Satsangi, J., Schreiber, S., Cho, J. H., Annese, V., Hakonarson, H., Daly, M. J., and Parkes, M. (2010). Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. *Nat. Genet.* 42, 1118–1125.

Fujii, S., Tominaga, K., Kitajima, K., Takeda, J., Kusaka, T., Fujita, M., Ichikawa, K., Tomita, S., Ohkura, Y., Ono, Y., Imura, J., Chiba, T., and Fujimori, T. (2005). Methylation of the oestrogen receptor gene in non-neoplastic epithelium as a marker of colorectal neoplasia risk in longstanding and extensive ulcerative colitis. *Gut* 54, 1287–1292.

Fujino, S., Andoh, A., Bamba, S., Ogawa, A., Hata, K., Araki, Y., Bamba, T., and Fujiyama, Y. (2003). Increased expression of interleukin 17 in inflammatory bowel disease. *Gut* 52, 65–70.

Garrity-Park, M. M., Loftus, E. V. Jr., Sandborn, W. J., Bryant, S. C., and Smyrk, T. C. (2010). Methylation status of genes in non-neoplastic mucosa from patients with ulcerative colitis-associated colorectal cancer. *Am. J. Gastroenterol.* 105, 1610–1619.

Gill, S. R., Pop, M., Deboy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel,

B. S., Gordon, J. I., Relman, D. A., Fraser-Liggett, C. M., and Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *Science* 312, 1355–1359.

Glick, D., Barth, S., and Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. *J. Pathol.* 221, 3–12.

Glocker, E. O., Kotlarz, D., Boztug, K., Gertz, E. M., Schäffer, A. A., Noyan, F., Perro, M., Diestelhorst, J., Allroth, A., Murugan, D., Hätscher, N., Pfeifer, D., Sykora, K. W., Sauer, M., Kreipe, H., Lacher, M., Nustede, R., Woellner, C., Baumann, U., Salzer, U., Koletzko, S., Shah, N., Segal, A. W., Sauerbrey, A., Buderus, S., Snapper, S. B., Grimbacher, B., and Klein, C. (2009). Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N. Engl. J. Med.* 361, 2033–2045.

Gophna, U., Sommerfeld, K., Gophna, S., Doolittle, W. F., and Veldhuyzen van Zanten, S. J. (2006). Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J. Clin. Microbiol.* 44, 4136–4141.

Greten, F. R., Eckmann, L., Greten, T. F., Park, J. M., Li, Z. W., Egan, L. J., Kagnoff, M. F., and Karin, M. (2004). IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 118, 285–296.

Grivennikov, S., Karin, E., Terzic, J., Mucida, D., Yu, G. Y., Vallabhapurapu, S., Scheller, J., Rose-John, S., Cheroutre, H., Eckmann, L., and Karin, M. (2009). IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 15, 103–113.

Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., Sargeant, L., Krapcho, K., Wolff, E., Burt, R., Hughes, J. P., Warrington, J., McPherson, J., Wasmuth, J., Le Paslier, D., Abderrahim, H., Cohen, D., Leppert, M., and White, R. (1991). Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 66, 589–600.

Hampe, J., Franke, A., Rosenstiel, P., Till, A., Teuber, M., Huse, K., Albrecht, M., Mayr, G., De La Vega, F. M., Briggs, J., Günther, S., Prescott, N. J., Onnie, C. M., Häsler, R., Sipos, B., Fölsch, U. R., Lengauer, T., Platzer, M., Mathew, C. G., Krawczak, M., and Schreiber, S. (2007). A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat. Genet.* 39, 207–211.

Harries, A. D., Baird, A., and Rhodes, J. (1982). Non-smoking: a feature of ulcerative colitis. *Br. Med. J. (Clin. Res. Ed.)* 284, 706.

Hedl, M., Li, J., Cho, J. H., and Abraham, C. (2007). Chronic stimulation of Nod2 mediates tolerance to bacterial products. *Proc. Natl. Acad. Sci. U.S.A.* 104, 19440–19445.

Huett, A., Goel, G., and Xavier, R. J. (2010). A systems biology viewpoint on autophagy in health and disease. *Curr. Opin. Gastroenterol.* 26, 302–309.

Hugot, J. P., Chamaillard, M., Zouali, H., Lesage, S., Cézard, J. P., Belaiche, J., Almer, S., Tysk, C., O'Morain, C. A., Gassull, M., Binder, V., Finkel, Y., Cortot, A., Modigliani, R., Laurent-Puig, P., Gower-Rousseau, C., Macry, J., Colombel, J. F., Sahbatou, M., and Thomas, G. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411, 599–603.

Hussain, S. P., Amstad, P., Raja, K., Ambs, S., Nagashima, M., Bennett, W. P., Shields, P. G., Ham, A. J., Swenberg, J. A., Marrogi, A. J., and Harris, C. C. (2000). Increased p53 mutation load in noncancerous colon tissue from ulcerative colitis: a cancer-prone chronic inflammatory disease. *Cancer Res.* 60, 3333–3337.

Issa, J. P., Ahuja, N., Toyota, M., Bronner, M. P., and Brentnall, T. A. (2001). Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res.* 61, 3573–3577.

Itzkowitz, S. H., and Yio, X. (2004). Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287, G7–G17.

Kappelman, M. D., Rifas-Shiman, S. L., Kleinman, K., Ollendorf, D., Bousvaros, A., Grand, R. J., and Finkelstein, J. A. (2007). The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. *Clin. Gastroenterol. Hepatol.* 5, 1424–1429.

Kaser, A., Zeissig, S., and Blumberg, R. S. (2010). Inflammatory bowel disease. *Annu. Rev. Immunol.* 28, 573–621.

Kawanishi, S., Hiraku, Y., Pinlaor, S., and Ma, N. (2006). Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol. Chem.* 387, 365–372.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., and Müller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75, 263–274.

Kundu, J. K., and Surh, Y. J. (2008). Inflammation: gearing the journey to cancer. *Mutat. Res.* 659, 15–30.

Kuster, W., Pascoe, L., Purrmann, J., Funk, S., and Majewski, F. (1989). The genetics of Crohn disease: complex segregation analysis of a family study with 265 patients with Crohn disease and 5,387 relatives. *Am. J. Med. Genet.* 32, 105–108.

Lesage, S., Zouali, H., Cézard, J. P., Colombel, J. F., Belaiche, J., Almer, S., Tysk, C., O'Morain, C., Gassull, M., Binder, V., Finkel, Y., Modigliani, R., Gower-Rousseau, C., Macry, J., Merlin, F., Chamaillard, M., Jannot, A. S., Thomas, G., Hugot, J. P.; EPWG-IBD Group; EPIMAD Group; and GETAID Group. (2002). CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am. J. Hum. Genet.* 70, 845–857.

Liao, J., Seril, D. N., Lu, G. G., Zhang, M., Toyokuni, S., Yang, A. L., and Yang, G. Y. (2008). Increased susceptibility of chronic ulcerative colitis-induced carcinoma development in DNA repair enzyme Ogg1 deficient mice. *Mol. Carcinog.* 47, 638–646.

Lidar, M., Langevitz, P., and Shoenfeld, Y. (2009). The role of infection in inflammatory bowel disease: initiation, exacerbation and protection. *Isr. Med. Assoc. J.* 11, 558–563.

Loftus, E. V. Jr. (2007). The burden of inflammatory bowel disease in the United States: a moving target? *Clin. Gastroenterol. Hepatol.* 5, 1383–1384.

Manichanh, C., Rigottier-Gois, L., Bonnaud, E., Gloux, K., Pelletier, E., Frangeul, L., Nalin, R., Jarrin, C., Chardon, P., Marteau, P., Roca, J., and Dore, J. (2006). Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 55, 205–211.

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008). Cancer-related inflammation. *Nature* 454, 436–444.

Meira, L. B., Bugni, J. M., Green, S. L., Lee, C.-W., Pang, B., Borenshtein, D., Rickman, B. H., Rogers, A. B., Moroski-Erkul, C. A., McFaline, J. L., Schauer, D. B., Dedon, P. C., Fox, J. G., and Samson, L. D. (2008). DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. *J. Clin. Invest.* 118, 2516–2525.

Miller, B. C., Zhao, Z., Stephenson, L. M., Cadwell, K., Pua, H. H., Lee, H. K., Mizushima, N. N., Iwasaki, A., He, Y. W., Swat, W., and Virgin, H. W. IV. (2008). The autophagy gene ATG5 plays an essential role in B lymphocyte development. *Autophagy* 4, 309–314.

Mombaerts, P., Mizoguchi, E., Grusby, M. J., Glimcher, L. H., Bhan, A. K., and Tonegawa, S. (1993). Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell* 75, 274–282.

Nell, S., Suerbaum, S., and Josenhans, C. (2010). The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. *Nat. Rev. Microbiol.* 8, 564–577.

Neufert, C., Becker, C., and Neurath, M. F. (2007). An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat. Protoc.* 2, 1998–2004.

Neurath, M. F., and Finotto, S. (2009). Translating inflammatory bowel disease research into clinical medicine. *Immunity* 31, 357–361.

Ogura, Y., Bonen, D. K., Inohara, N., Nicolae, D. L., Chen, F. F., Ramos, R., Britton, H., Moran, T., Karaliuskas, R., Duerr, R. H., Achkar, J. P., Brant, S. R., Bayless, T. M., Kirschner, B. S., Hanauer, S. B., Nuñez, G., and Cho, J. H. (2001). A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411, 603–606.

Orholm, M., Binder, V., Sørensen, T. I., Rasmussen, L. P., and Kyvik, K. O. (2000). Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand. J. Gastroenterol.* 35, 1075–1081.

Pabst, O., Zweigerdt, R., and Arnold, H. H. (1999). Targeted disruption of the homeobox transcription factor Nkx2-3 in mice results in postnatal lethality and abnormal development of small intestine and spleen. *Development* 126, 2215–2225.

Parkes, M., Barrett, J. C., Prescott, N. J., Tremelling, M., Anderson, C. A., Fisher, S. A., Roberts, R. G., Nimmo, E. R., Cummings, F. R., Soars, D., Drummond, H., Lees, C. W., Khawaja, S. A., Bagnall, R., Burke, D. A., Todhunter, C. E., Ahmad, T., Onnie, C. M., McArdle, W., Strachan, D., Bethel, G., Bryan, C., Lewis, C. M., Deloukas, P., Forbes, A., Sanderson, J., Jewell, D. P., Satsangi, J., Mansfield, J. C.; Wellcome Trust Case Control Consortium, Cardon, L., and Mathew, C. G. (2007). Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat. Genet.* 39, 830–832.

Pullan, R. D., Rhodes, J., Ganesh, S., Mani, V., Morris, J. S., Williams, G. T., Newcombe, R. G., Russell, M., Feyerabend, C., Thomas, G., and Sawe, U. (1994). Transdermal nicotine for active ulcerative colitis. *N. Engl. J. Med.* 330, 811–815.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., Nielsen, T., Pons, N., Levenez, F., Yamada, T., Mende, D. R., Li, J., Xu, J., Li, S., Li, D., Cao, J., Wang, B., Liang, H., Zheng, H., Xie, Y., Tap, J., Lepage, P., Bertalan, M., Batto, J. M., Hansen, T., Le Paslier, D., Linneberg, A., Nielsen, H. B., Pelletier, E., Renault, P., Sicheritz-Ponten, T., Turner, K., Zhu, H., Yu, C., Li, S., Jian, M., Zhou, Y., Li, Y., Zhang, X., Li, S., Qin, N., Yang, H., Wang, J., Brunak, S., Doré, J., Guarner, F., Kristiansen, K., Pedersen, O., Parkhill, J., Weissenbach, J.; MetaHIT Consortium, Bork, P., Ehrlich, S. D., and Wang, J. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65.

Quante, M., Tu, S. P., Tomita, H., Gonda, T., Wang, S. S., Takashi, S., Baik, G. H., Shibata, W., Diprete, B., Betz, K. S., Friedman, R., Varro, A., Tycko, B., and Wang, T. C. (2011). Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell* 19, 257–272.

Rakoff-Nahoum, S., Paglino, J., Eslami-Varzaneh, F., Edberg, S., and Medzhitov, R. (2004). Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 118, 229–241.

Redston, M. S., Papadopoulos, N., Caldas, C., Kinzler, K. W., and Kern, S. E. (1995). Common occurrence of APC and K-ras gene mutations in the spectrum of colitis-associated neoplasias. *Gastroenterology* 108, 383–392.

Reiff, C., and Kelly, D. (2010). Inflammatory bowel disease, gut bacteria and probiotic therapy. *Int. J. Med. Microbiol.* 300, 25–33.

Rosenstiel, P., Sina, C., Franke, A., and Schreiber, S. (2009). Towards a molecular risk map–recent advances on the etiology of inflammatory bowel disease. *Semin. Immunol.* 21, 334–345.

Rubtsov, Y., Rasmussen, J. P., Chi, E. Y., Fontenot, J., Castelli, L., Ye, X., Treuting, P., Siewe, L., Roers, A., Henderson, W. R. Jr., Muller, W., and Rudensky, A. Y. (2008). Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 28, 546–558.

Rutter, M., Saunders, B., Wilkinson, K., Rumbles, S., Schofield, G., Kamm, M., Williams, C., Price, A., Talbot, I., and Forbes, A. (2004). Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 126, 451–459.

Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A. C., and Horak, I. (1993). Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 75, 253–261.

Salcedo, R., Worschech, A., Cardone, M., Jones, Y., Gyulai, Z., Dai, R. M., Wang, E., Ma, W., Haines, D., O'hUigin, C., Marincola, F. M., and Trinchieri, G. (2010). MyD88-mediated signaling prevents development of adenocarcinomas of the colon: role of interleukin 18. *J. Exp. Med.* 207, 1625–1636.

Sandborn, W. J., Tremaine, W. J., Offord, K. P., Lawson, G. M., Petersen, B. T., Batts, K. P., Croghan, I. T., Dale, L. C., Schroeder, D. R., and Hurt, R. D. (1997). Transdermal nicotine for mildly to moderately active ulcerative colitis. A randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med.* 126, 364–371.

Sellon, R. K., Tonkonogy, S., Schultz, M., Dieleman, L. A., Grenther, W., Balish, E., Rennick, D. M., and Sartor, R. B. (1998). Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* 66, 5224–5231.

Shaker, A., and Rubin, D. C. (2011). Intestinal stem cells and epithelial-mesenchymal interactions in the crypt and stem cell niche. *Transl. Res.* 156, 180–187.

Shaker, A., Swietlicki, E. A., Wang, L., Jiang, S., Onal, B., Bala, S., DeSchryver, K., Newberry, R., Levin, M. S., and Rubin, D. C. (2010). Epimorphin deletion protects mice from inflammation-induced colon carcinogenesis and alters stem cell niche myofibroblast secretion. *J. Clin. Invest.* 120, 2081–2093.

Sheikh, S. Z., Hegazi, R. A., Kobayashi, T., Onyiah, J. C., Russo, S. M., Matsuoka, K., Sepulveda, A. R., Li, F., Otterbein, L. E., and Plevy, S. E. (2011). An anti-inflammatory role for carbon monoxide and heme oxygenase-1 in chronic Th2-mediated murine colitis. *J. Immunol.* 186, 5506–5513.

Silverberg, M. S., Cho, J. H., Rioux, J. D., McGovern, D. P., Wu, J., Annese, V., Achkar, J. P., Goyette, P., Scott, R., Xu, W., Barmada, M. M., Klei, L., Daly, M. J., Abraham, C., Bayless, T. M., Bossa, F., Griffiths, A. M., Ippoliti, A. F., Lahaie, R. G., Latiano, A., Paré, P., Proctor, D. D., Regueiro, M. D., Steinhart, A. H., Targan, S. R., Schumm, L. P., Kistner, E. O., Lee, A. T., Gregersen, P. K., Rotter, J. I., Brant, S. R., Taylor, K. D., Roeder, K., and Duerr, R. H. (2009). Ulcerative colitis-risk loci on chromosomes 1p36 and 12q15 found by genome-wide association study. *Nat. Genet.* 41, 216–220.

Singh, S. B., Davis, A. S., Taylor, G. A., and Deretic, V. (2006). Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 313, 1438–1441.

Solinas, G., Marchesi, F., Garlanda, C., Mantovani, A., and Allavena, P. (2010). Inflammation-mediated promotion of invasion and metastasis. *Cancer Metastasis Rev.* 29, 243–248.

Strober, W., and Fuss, I. J. (2011). Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 140, 1756–1767.

Strober, W., Fuss, I. J., and Blumberg, R. S. (2002). The immunology of mucosal models of inflammation. *Annu. Rev. Immunol.* 20, 495–549.

Suzuki, R., Kohno, H., Sugie, S., Nakagama, H., and Tanaka, T. (2006). Strain differences in the susceptibility to azoxymethane and dextran sodium sulfate-induced colon carcinogenesis in mice. *Carcinogenesis* 27, 162–169.

Tanaka, F., Tominaga, K., Ochi, M., Tanigawa, T., Watanabe, T., Fujiwara, Y., Ohta, K., Oshitani, N., Higuchi, K., and Arakawa, T. (2008). Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. *Life Sci.* 83, 771–779.

Tarmin, L., Yin, J., Harpaz, N., Kozam, M., Noordzij, J., Antonio, L. B., Jiang, H. Y., Chan, O., Cymes, K., and Meltzer, S. J. (1995). Adenomatous polyposis coli gene mutations in ulcerative colitis-associated dysplasias and cancers versus sporadic colon neoplasms. *Cancer Res.* 55, 2035–2038.

Taurog, J. D., Richardson, J. A., Croft, J. T., Simmons, W. A., Zhou, M., Fernández-Sueiro, J. L., Balish, E., and Hammer, R. E. (1994). The germfree state prevents development of gut and joint inflammatory disease in HLA-B27 transgenic rats. *J. Exp. Med.* 180, 2359–2364.

Terzic, J., Grivennikov, S., Karin, E., and Karin, M. (2010). Inflammation and colon cancer. *Gastroenterology* 138, 2101–2114.e5.

Tsuji, S., Uehori, J., Matsumoto, M., Suzuki, Y., Matsuhisa, A., Toyoshima, K., and Seya, T. (2001). Human intelectin is a novel soluble lectin that recognizes galactofuranose in carbohydrate chains of bacterial cell wall. *J. Biol. Chem.* 276, 23456–23463.

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., and Gordon, J. I. (2007). The human microbiome project. *Nature* 449, 804–810.

Tysk, C., Lindberg, E., Järnerot, G., and Flodérus-Myrhed, B. (1988). Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 29, 990–996.

Ullman, T. A., and Itzkowitz, S. H. (2011). Intestinal inflammation and cancer. *Gastroenterology* 140, 1807–1816.

Unkart, J. T., Anderson, L., Li, E., Miller, C., Yan, Y., Gu, C. C., Chen, J., Stone, C. D., Hunt, S., and Dietz, D. W. (2008). Risk factors for surgical recurrence after ileocolic resection of Crohn's disease. *Dis. Colon Rectum* 51, 1211–1216.

Vermeulen, L., De Sousa, E. M. F., van der Heijden, M., Cameron, K., de Jong, J. H., Borovski, T., Tuynman, J. B., Todaro, M., Merz, C., Rodermond, H., Sprick, M. R., Kemper, K., Richel, D. J., Stassi, G., and Medema, J. P. (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat. Cell Biol.* 12, 468–476.

Watanabe, T., Kitani, A., Murray, P. J., and Strober, W. (2004). NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat. Immunol.* 5, 800–808.

Westbrook, A. M., Szakmary, A., and Schiestl, R. H. (2010). Mechanisms of intestinal inflammation and development of associated cancers: lessons learned from mouse models. *Mutat. Res.* 705, 40–59.

Worthley, D. L., Giraud, A. S., and Wang, T. C. (2010). Stromal fibroblasts in digestive cancer. *Cancer Microenviron.* 3, 117–125.

Wrackmeyer, U., Hansen, G. H., Seya, T., and Danielsen, E. M. (2006). Intelectin: a novel lipid raft-associated protein in the enterocyte brush border. *Biochemistry* 45, 9188–9197.

Yen, D., Cheung, J., Scheerens, H., Poulet, F., McClanahan, T., McKenzie, B., Kleinschek, M. A., Owyang, A., Mattson, J., Blumenschein, W., Murphy, E., Sathe, M., Cua, D. J., Kastelein, R. A., and Rennick, D. (2006). IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J. Clin. Invest.* 116, 1310–1316.

Zhang, R., Ma, A., Urbanski, S. J., and McCafferty, D. M. (2007). Induction of inducible nitric oxide synthase: a protective mechanism in colitis-induced adenocarcinoma. *Carcinogenesis* 28, 1122–1130.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 18 January 2012; paper pending published: 31 January 2012; accepted: 17 April 2012; published online: 08 May 2012.

Citation: Rubin DC, Shaker A and Levin MS (2012). Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. Front. Immun. ***3****:107. doi: 10.3389/fimmu.2012.00107*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 17 April 2012
doi: 10.3389/fimmu.2012.00081

Functional metabolomics reveals novel active products in the DHA metabolome

Masakazu Shinohara, Valbona Mirakaj and Charles N. Serhan*

Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Takayuki Yoshimoto, Tokyo Medical University, Japan
Hiroki Yoshida, Saga University Faculty of Medicine, Japan
Daisuke Kamimura, Osaka University, Japan

***Correspondence:**
Charles N. Serhan, Center for Experimental Therapeutics, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, HIM 829, Boston, MA 02115-5727, USA.
e-mail: cnserhan@zeus.bwh.harvard.edu

Endogenous mechanisms for successful resolution of an acute inflammatory response and the local return to homeostasis are of interest because excessive inflammation underlies many human diseases. In this review, we provide an update and overview of functional metabolomics that identified a new bioactive metabolome of docosahexaenoic acid (DHA). Systematic studies revealed that DHA was converted to DHEA-derived novel bioactive products as well as aspirin-triggered forms of protectins (AT-PD1). The new oxygenated DHEA-derived products blocked PMN chemotaxis, reduced P-selectin expression and platelet-leukocyte adhesion, and showed organ protection in ischemia/reperfusion injury. These products activated cannabinoid receptor (CB2 receptor) and not CB1 receptors. The AT-PD1 reduced neutrophil (PMN) recruitment in murine peritonitis. With human cells, AT-PD1 decreased transendothelial PMN migration as well as enhanced efferocytosis of apoptotic human PMN by macrophages. The recent findings reviewed here indicate that DHEA oxidative metabolism and aspirin-triggered conversion of DHA produce potent novel molecules with anti-inflammatory and organ-protective properties, opening the DHA metabolome functional roles.

Keywords: resolvins, protectins, specialized pro-resolving mediator, DHEA, neutrophil, aspirin

THE NEUTROPHIL (PMN) IN RESOLUTION AND THE PROGRAMMED OUTCOME OF ACUTE INFLAMMATION

The acute inflammatory response has several potential outcomes that include progression to chronic inflammation, scarring, and fibrosis or ideally complete resolution, see Kumar et al. (2005). Acute inflammation is a response that eliminates or neutralizes foreign bodies or organisms and is protective against injury or infection (Kumar et al., 2005; Serhan et al., 2010). This process is characterized by vascular dilation, enhanced permeability of capillaries, increased blood flow, and the production of fibrin-rich exudates (Majno and Joris, 2004). Vascular permeability and increased blood flow permit leukocyte recruitment from the circulation postcapillary venules (**Figure 1**) to form the PMN-rich exudate (Ryan and Majno, 1977). Leukocyte recruitment is classically defined by the initial trafficking of polymorphonuclear (PMN) granulocytes followed by monocytes that differentiate locally into macrophages (**Figure 1**). These events in pro-resolving responses are coupled with release of factors that prevent further or excessive trafficking of leukocytes in self-limited inflammation allowing for resolution (Serhan et al., 2000, 2002; Serhan and Savill, 2005). Pro-inflammatory mediators such as prostaglandins and leukotrienes play an important role (Borgeat and Samuelsson, 1979; Samuelsson, 1983; Samuelsson et al., 1987) early in the response, stimulating vascular changes as well as PMN recruitment. These events involve LTB_4 and IL-8, potent PMN chemoattractants, as well as many other endogenous and exogenous chemoattractants.

The progression from acute to chronic inflammation as in many widely occurring human diseases such as arthritis, periodontal disease, and cardiovascular disease, is generally viewed as an excess of pro-inflammatory mediators, but may also arise via a failure in mechanisms to resolve (Serhan, 2011). Although mononuclear cells can in many settings contribute to pro-inflammatory responses, they are also critical in wound healing, tissue repair, and remodeling in a non-inflammatory, non-phlogistic manner (Stables et al., 2011; Ariel and Serhan, 2012). Most successful inflammatory processes are self-limiting (Majno and Joris, 2004), which implies the existence of endogenous anti-inflammation pathways (Serhan et al., 2004; Serhan and Savill, 2005).

Complete or successful resolution of an acute inflammatory response and the local return to homeostasis is necessary for ongoing health. The ideal outcome is the removal of leukocytes from infected or inflamed tissues without leaving remnants of the host's combat between leukocytes, invading microbes, and/or other initiators of inflammation. The authors' research efforts have focused on identification of mediators involved in the successful resolution process (Serhan, 2007). It was widely believed and argued that simple dilution of pro-inflammatory mediators was enough to "turn off" or "burn out" inflammation, with the subsequent responses ending naturally or passively (Kumar et al., 2005). Now, a body of evidence indicates that resolution of acute inflammation and the return to homeostasis is not simply passive, but an active and highly regulated process that can be considered programmed resolution at the tissue level (Serhan, 2007; Morris et al., 2010). In this regard, the specialized pro-resolving lipid mediators (SPMs) comprise a genus (chemically distinct families) of endogenous mediators, including lipoxins, resolvins, protectins, and maresins (Serhan et al., 2009; **Figure 2**; **Table 1**). They

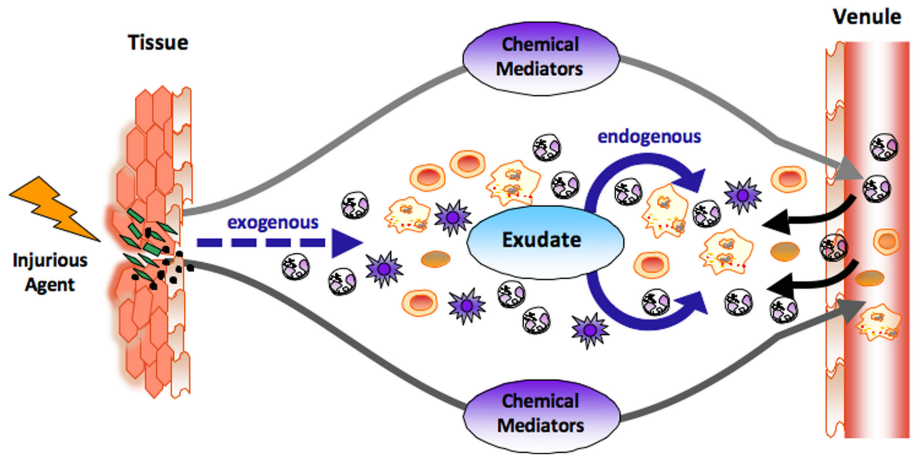


FIGURE 1 | The role of chemical mediators in the acute inflammatory response. At the site of tissue injury or bacterial invasion, both exogenous and endogenous chemical mediators are liberated. Classic endogenous mediators such as prostaglandins and leukotrienes dilate vasculatures, enhance permeability of capillaries, increase blood flow, and stimulate the recruitment of neutrophils (PMNs) to form inflammatory exudate. Novel chemical mediators are produced in the evolution and resolution of the exudate that regulate tissue responses (see text for details). The black arrow denotes leukocyte traffic from venules and the dashed arrow denotes exogenous, i.e., bacterial components and chemoattractants.

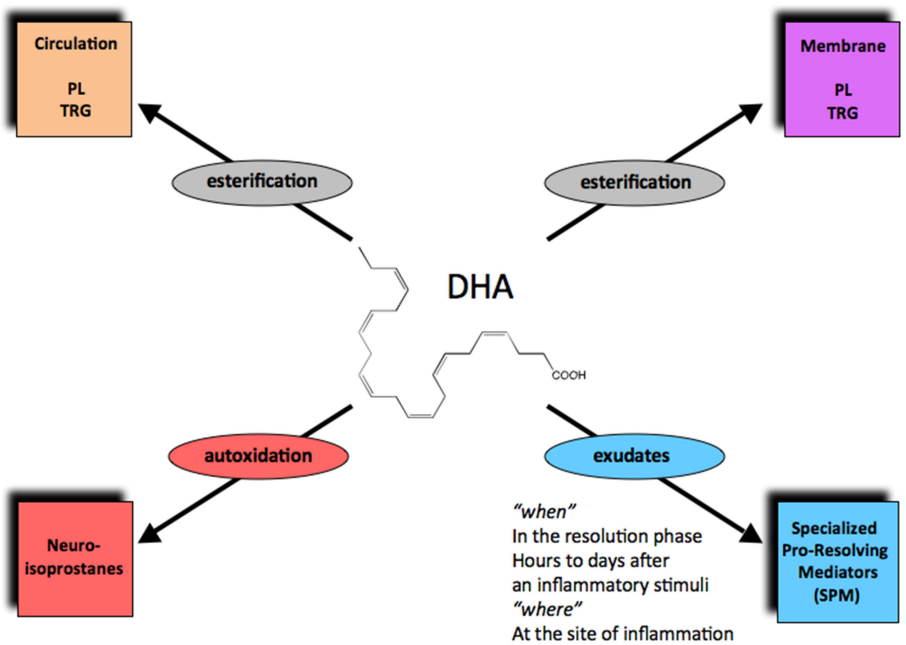


FIGURE 2 | The fate of DHA in its new metabolome *in vivo*. Via esterification, DHA enters phospholipids and triglycerides that can circulate or reside in cell membranes. Autooxidation of DHA can produce neuroisoprostanes, the DHA form of isoprostanes. Enzymatic conversion in local inflammatory exudates can generate the specialized pro-resolving lipid mediators (SPMs), including D-series resolvins, protectins, and maresins.

are actively biosynthesized during the resolution phase of acute inflammation and are potent agonists that control the magnitude and duration of inflammation. During resolution of inflammation, ω-3 fatty acids are rapidly imported (Kasuga et al., 2008) to the resolving exudate from circulation along with albumin and trafficking leukocytes that are then used as substrates to produce the local-acting E-series resolvins (Oh et al., 2011), D-series resolvins and protectins as well as maresins (Serhan et al., 2009). Each of these new families of potent local mediators carries both anti-inflammatory as well as pro-resolving actions in that they stop PMN (limit their further influx) to the site of inflammation and stimulate efferocytosis (Spite and Serhan, 2010; Serhan, 2011). Docosahexaenoic acid (DHA) is unique in this regard because it has several fates in its metabolome (**Figure 2**). DHA undergoes

Table 1 | Key structures of the SPM: resolvins, protectins, and maresins

RvE1	Resolvin E1 (5*S*,12*R*,18*R*-trihydroxy-eicosa-6*Z*,8*E*,10*E*,14*Z*,16*E*-pentaenoic acid)
RvE2	Resolvin E2 (5*S*,18*R*-dihydroxy-eicosapentaenoic acid)
PD1/NPD1	Protectin D1/neuroprotectin D1 (10*R*,17*S*-dihydroxy-docosa-4*Z*,7*Z*,11*E*,13*E*,15*Z*,19*Z*-hexaenoic acid)
RvD1	Resolvin D1 (7*S*,8*R*,17*S*-trihydroxy-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid)
RvD2	Resolvin D2 (7*S*,16*R*,17*S*-trihydroxydocosa-4*Z*,8*E*,10*Z*,12*E*,14*E*,19*Z*-hexaenoic acid)
RvD3	Resolvin D3 (4*S*,11,17*S*-trihydroxydocosa-5,7*E*,9*E*,13*Z*,15*E*,19*Z*-hexaenoic acid)
RvD4	Resolvin D4 (4*S*,5,17*S*-trihydroxydocosa-6*E*,8*E*,10*Z*,13*Z*,15*E*,19*Z*-hexaenoic acid)
RvD5	Resolvin D5 (7*S*,17*S*-dihydroxy-docosa-4*Z*,8*E*,10*Z*,13*Z*,15*E*,19*Z*-hexaenoic acid)
MaR1	Maresin 1 (7,14-dihydroxy-docosa-4*Z*,8,10,12,16*Z*,19*Z*-hexaenoic acid)

Complete structures of the key SPM are reported and total organic synthesis was recently reviewed in Serhan and Petasis (2011).

esterification to form complex lipids that circulate or reside in phospholipid membranes of cells and tissues (Bazan et al., 2010), or can undergo autooxidation of the products such as neuroisoprostanes that are potentially pro-inflammatory (Roberts et al., 1998; Roberts and Morrow, 2002), or undergo conversion in inflammatory exudates to potent pro-resolving mediators such as the SPMs. Hence, the fate of DHA is governed, in part, locally in its microenvironment and hence "*when and where*" determines its action and impact *in vivo* (**Figures 2** and **3**) in addition to DHA as a precursor to resolvins and their aspirin-triggered (AT) forms (Serhan et al., 2002). Systematic studies reveal that DHA is converted to DHEA-derived novel products (Yang et al., 2011) as well as AT forms of protectins (Serhan et al., 2011; see **Figure 3**). These are the main subjects of this review and are detailed in the next sections.

FUNCTIONAL METABOLOMICS AND THE NEW DHA METABOLOME

LC–UV–MS–MS IDENTIFICATION OF 17-HDHEA

To investigate the potential endogenous generation of DHEA-derived bioactive products in mice, brain was harvested and subject to solid phase extraction (Yang et al., 2011). Next, the methyl formate fractions were taken for LC–UV–MS–MS-based metabolomics. Tandem mass fragmentations and online UV spectrum with characteristic λ_{max} at 237 nm were consistent with the structure as shown in **Figure 4** and Yang et al. (2011). Because of the lack of suitable functional groups for direct efficient ionization and analysis of 17-hydroxy-4*Z*, 7*Z*, 10*Z*, 13*Z*, 15*Z*, 19*Z*-docosahexaenoylethanolamide (17-HDHEA) for LC–MS–MS, its acetate adduct *m*/*z* 446 = [M + CH_3COOH-H] was targeted. The major tandem mass ions were assigned for: *m*/*z* 386 = [M−H], 368 = [M−H − H_2O], 281 = [299 − H_2O]. The *m*/*z* 288 is consistent with fragmentation at position 17 (**Figure 4** and Yang et al., 2011).

Because of the limited quantities of endogenous 17-HDHEA produced in brain tissue, further studies and *in vitro* enzymatic preparation were carried out by incubating DHEA with 15-LOX followed by reduction with $NaBH_4$. Endogenous 17-HDHEA and the enzymatically prepared compound *in vitro* gave essentially the same LC retention times and tandem mass fragmentations. To assess their production by human and mouse tissues, DHEA was also incubated with isolated human PMN or whole mouse brain because DHEA is enriched in this tissue. LC–MS–MS-based targeted lipidomics indicated the production of a novel series of oxygenated products (see **Figure 4**).

DECODING

As second approach in parallel to structure elucidation, screening of HPLC-isolated DHEA metabolites obtained from mouse brain was carried out utilizing a microfluidic chamber (Irimia et al., 2006; Kasuga et al., 2008). After a gradient of IL-8 was introduced to the main channel of the device, P-selectin tethered leukocytes rapidly migrated in the IL-8 gradient at an average rate of 2.3 μm/min. Next, a mixture of isolated metabolites was infused into the main channel. Human PMN chemotaxis is dramatically reduced upon the addition of the brain metabolite mixture. For example, the average human PMN chemotaxis velocity dropped from 2.3 to ∼ 0.7 μm/min (Yang et al., 2011). These results indicated that the brain fraction of metabolites contained products that stopped PMN chemotaxis. Results from this screening uncovered that at least one bioactive product was present among the mixture of DHEA metabolites. Hence, we next pursued the metabolic fates of DHEA and 17-HpDHEA/17-HDHEA identified in mouse brain (Yang et al., 2011) using LC/UV/MS/MS-based lipidomics. As with 17-HDHEA, acetate adducts of potential DHEA-derived novel metabolites [M + CH_3COOH-H] were targeted for tandem mass analysis. From these studies we identified the presence and production of novel products in the DHEA metabolome (**Figure 4**).

Human PMN incubated with either DHEA or 17-HpDHEA led to the generation of 17-HDHEA, 4,17-diHDHEA, 10,17-diHDHEA, and 15-HEDPEA (see **Figure 4** and Yang et al., 2011). Human hemoglobin, which can be liberated upon tissue damage (Kumar et al., 2005), was next incubated with 17-HpDHEA. This gave 13-HEDPEA and 15-HEDPEA as prominent products, as well as 17-HDHEA (see **Figure 4**). Mouse brain homogenates with DHEA also produced 17-HDHEA and 4,17-diHDHEA as major products with smaller amounts of 7,17-diHDHEA, 10,17-diHDHEA, and 15-HEDPEA. The adduct parent ion, analyte parent ion and the ions resulted from neutral loss are *m*/*z* 462 = [M + CH_3COOH-H], 402 = [M−H], 384 = [M−H − H_2O], 366 = [M−H − 2H_2O], which are common signature ions for all dihydroxy-containing DHEA products. The ions *m*/*z* 333, 315 = [333 − H_2O], 304, 286 = [304-H_2O] were assigned as diagnostic ions for fragmentations at position 17. Fragmentations at position 4 can lead to *m*/*z* 144, 257 and 239 = [257-H_2O]. Its UV spectrum displayed characteristic maximum absorbance at 238 nm, which was consistent with the presence of two separated conjugated diene structures in this compound. Diagnostic ions *m*/*z* 304, 286 = [304-H_2O], 184, 156, corresponded to the fragmentations at positions 7 and 17 of 7,17-diHDHEA respectively

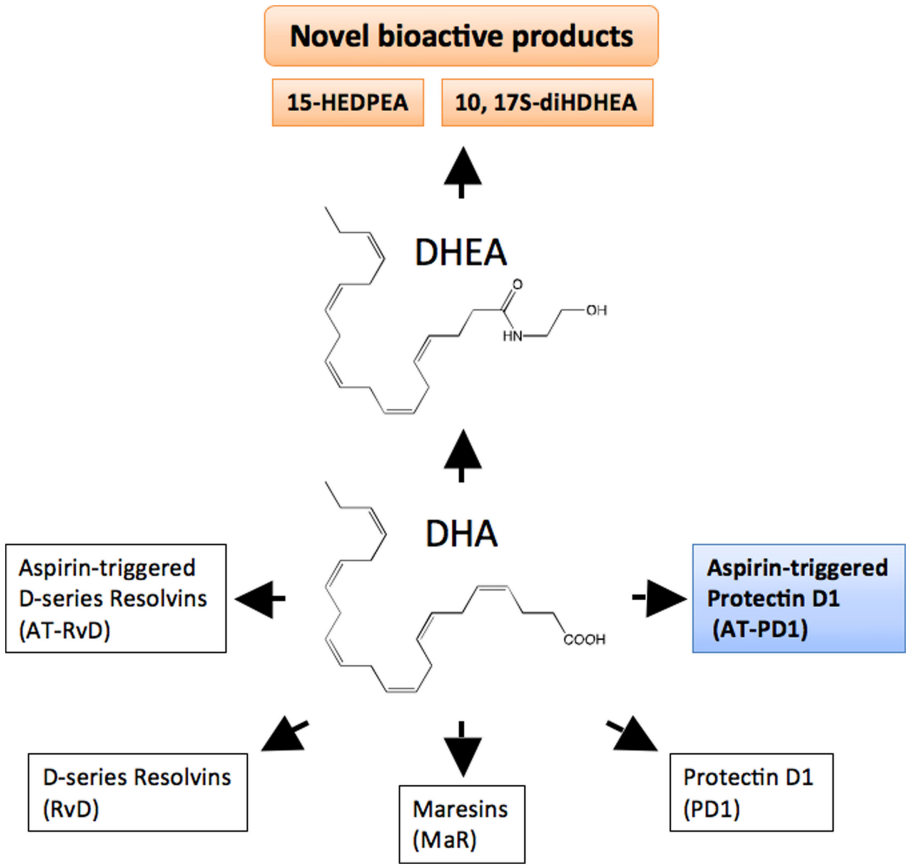


FIGURE 3 | DHA bioactive metabolome. DHA is the precursor to D-series resolvin (RvD), aspirin-triggered resolvin D (AT-RvD), maresins (MaR), protectin D1 (PD1), and aspirin-triggered protectin D1 (AT-PD1). Another metabolic fate of DHA is conversion to docosahexaenoyl ethanolamide (DHEA). See text for details on the recently identified novel series of bioactive oxygenated DHEA-derived products including 15-HEDPEA and 10,17S-diHDHEA.

(Yang et al., 2011). The UV spectrum of the compound displayed maximum absorbance, λ_{max}, at 246 nm (Tjonahen et al., 2006), consistent with the presence of two diene structures separated by a methylene group. For 10,17-diHDHEA, *m/z* 333, 315 = [333-H_2O], 304, 286 = [304-H_2O], 196 came from fragmentations at positions 10 and 17 (Yang et al., 2011). The presence of a conjugated triene structure in 10,17-diHDHEA was confirmed by the characteristic UV spectrum with λ_{max} at 270 nm. Tandem mass spectrum of 13-HEDPEA (Yang et al., 2011) gave signature fragmentation ions *m/z* 320, 304, 286 = [304-H_2O], 236. GC/MS was also utilized for additional structural analysis with 13-HEDPEA and 15-HEDPEA that helped to confirm the tandem MS assignments.

To determine concentrations, as well as to further confirm structures, HPLC-isolated 13-HEDPEA and 15-HEDPEA were characterized using proton NMR (^{1}H NMR). In the chemical shift assignments (Yang et al., 2011) for 15-HEDPEA, the proton at position 15 (H-15) displayed two distinct chemical shifts. Because of limited amounts of materials and the lack of informative UV chromophores present in these compounds, NMR spectroscopy was also used for quantitation using 17-HDHA as an internal standard with known concentrations. Each NMR quantitated compound was then used for HPLC calibration and quantitation (Yang et al., 2011).

Each of these HPLC-isolated dioxygenated DHEA products were screened for direct PMN actions using microfluidic chambers and freshly isolated PMN (Yang et al., 2011). Infusion of isolated 15-HEDPEA at 10 nM to the main channel stimulated changes in morphology and chemotaxis of human PMN in the IL-8 gradient and stopped further PMN migration (**Figure 4**). For direct comparison, PMN chemotaxis velocity did not change with time with the IL-8 gradient. Also, at 10 nM, 4,17-diHDHEA, 7,17-diHDHEA, or 10,17-diHDHEA did not significantly regulate chemotaxis, whereas at higher concentrations, e.g., 10 μM, 10,17-diHDHEA rapidly stopped PMN. Together, these results indicated that 15-HEDPEA is the most potent of this series in regulating human PMN shape change and motility.

CANNABINOID RECEPTORS AND NOVEL DHEA PRODUCTS

Because AEA exerts a wide range of bioactions via activating cannabinoid receptor(s) (Devane et al., 1992; Felder et al., 1993), we therefore tested whether DHEA, 10,17-diHDHEA or 15-HEDPEA also activated CB receptors. For these experiments, we used recombinant human CB receptors over-expressed in a

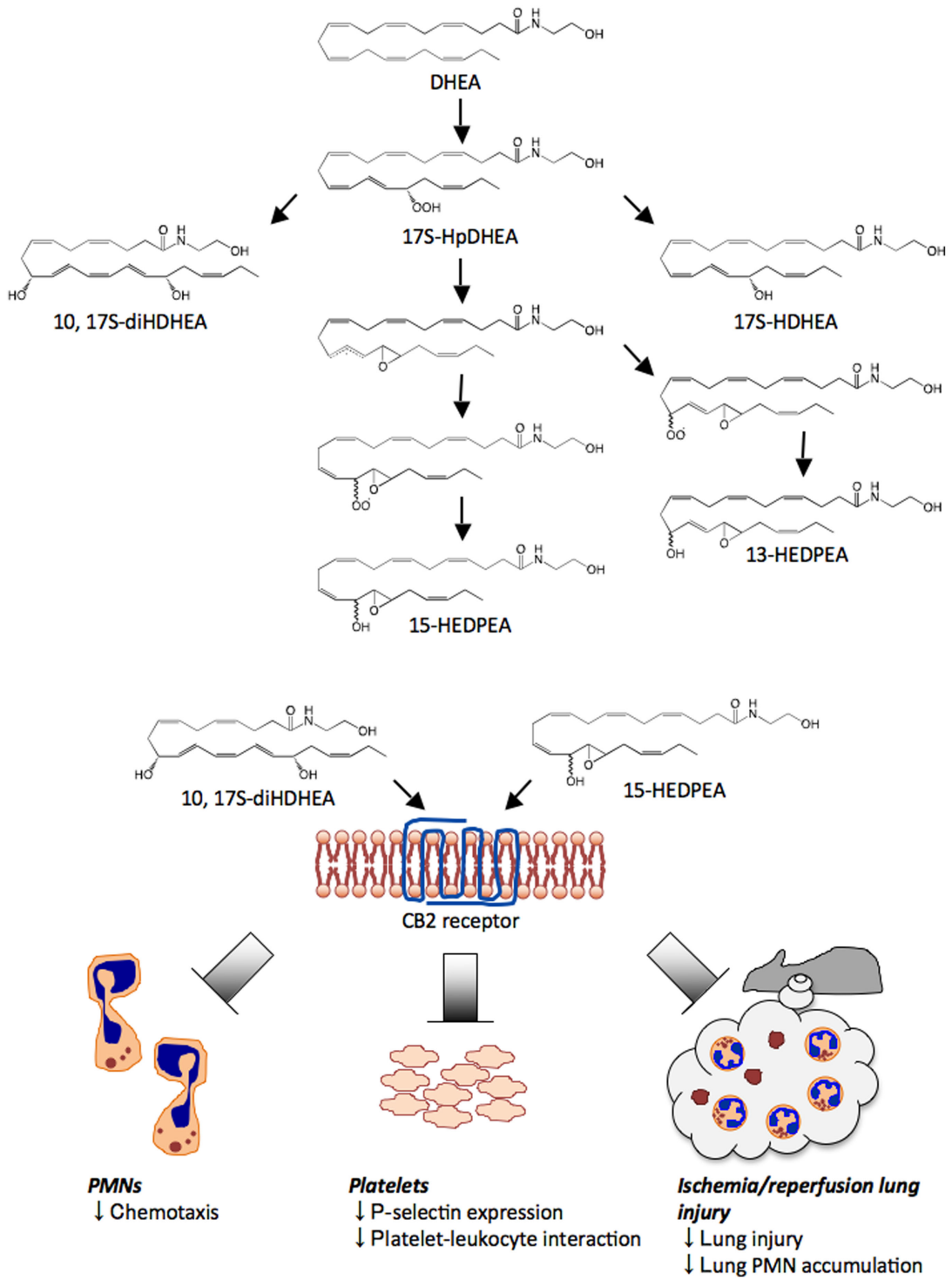


FIGURE 4 | Novel pathways and oxygenation products from DHEA. DHEA is first converted to 17-HpDHEA by 15-LOX. Next, 17-HpDHEA is partially reduced to the oxide radical, which reacts with the vicinal double bond at the 16-position to yield the 16(17)-epoxide radical (see text and Yang et al., 2011). Non- or low stereospecific addition of oxygen to the intermediate leads to formation of two types of peroxide radical diastereomers; their further reduction produces 13-HEDPEA and 15-HEDPEA. Via a LOX-related mechanism, 17-HpDHEA is converted to 10, 17S-diHDHEA or directly reduced to 17S-HDHEA. 10, 17S-diHDHEA, and 15-HEDPEA block PMN chemotaxis, reduce P-selectin expression and aggregation of platelets and leukocytes, and show organ protection in ischemia/reperfusion injury. Although these new products directly act on recombinant CB2 receptors *in vitro*, the activation of CB2 *in vivo* and/or additional receptors *in vivo* by 10,17*S*-diHDHEA and 15-HEDPEA remains of interest.

beta-arrestin system. HPLC purified AEA was used for direct comparison as a known receptor agonist of CB1 (Janero and Makriyannis, 2007; Vemuri et al., 2008). In these experiments, activation of CB2 by AEA gave $EC_{50} \sim 1.1 \times 10^{10}$ M and DHEA 9.8×10^{-9} M. For comparison, EC_{50} for metabolically oxygenated products, 10,17-diHDHEA and 15-HEDPEA, were 3.9×10^{-10} M and 1.0×10^{-10} M (Yang et al., 2011). These results demonstrated that the novel enzymatic oxidation products from DHEA are activators of CB2 receptors, and that 10,17-diHDHEA and 15-HEDPEA also activated CB1 receptors but required much higher concentrations. In this system, 15(*S*)-HETE ethanolamide (oxygenated product of AEA) did not stimulate CB2 receptors in this dose range. CB2 receptor-ligand interactions were confirmed with dose response of CB2 specific antagonist AM630. When incubated with GPCR CB2 over-expressed cells, AM630 inhibited activation stimulated with 15-HEDPEA (10 nM) and AEA (10 nM). AM630 also inhibited GPCR CB2 interaction with 10,17-diHDHEA at higher concentration (Yang et al., 2011).

NOVEL DHEA PRODUCTS REDUCED PLATELET-LEUKOCYTE AGGREGATE

Platelet-leukocyte interactions play important roles in hemostasis, thrombosis, and inflammation (for a recent review see van Gils et al., 2009 and references within). At concentrations as low as 10 pM, 10,17S-diHDHEA or 15-HEDPEA decreased PAF- (100 nM) stimulated platelet-monocyte aggregate formation in human whole blood by ∼30% (Yang et al., 2011). The inhibitory action of 10,17-diHDHEA displayed a bell-shaped dose response and reached maximum reduction at ∼40% with 100 pM. The formation of PMN-platelet aggregates with PAF (100 nM) was also inhibited by 10,17-diHDEA at concentrations as low as 10 pM, as was the surface expression of P-selectin on platelets in human whole blood (**Figure 4**). By comparison, the precursor DHEA was not active in this dose range.

NOVEL DHEA PRODUCTS ARE ORGAN PROTECTIVE IN ISCHEMIA/REPERFUSION INJURY

15-HEDPEA displayed potent bioactions with human PMN at single-cell level (**Figure 4** and Yang et al., 2011) and in human whole blood; hence, we questioned whether it had protective actions *in vivo* in murine hind limb ischemia (1 h) and second organ reperfusion (2 h) injury (Qiu et al., 2000). Indeed, following reperfusion, 15-HEDPEA significantly reduced lung PMN accumulation in mice and associated lung injury at 1 μg/mouse (**Figure 4** and Yang et al., 2011; ∼50% reduction directly compared with vehicle).

Lipidomic investigation of DHEA functional metabolome uncovered a series of novel oxygenated products that (1) are potent CB2 agonists, (2) regulate single-cell PMN chemotactic responses, (3) modulate platelet-leukocyte interaction in whole blood, and (4) are organ protective. In view of the role of lipid mediators in inflammation and its resolution as well as homeostasis (Serhan et al., 2008), the present new DHEA metabolome documented herein may serve as a counter-regulatory system in neural tissues and those rich in DHEA as well as in administration of DHA (Calder, 2010) to regulate leukocyte-mediated tissue damage. The link between CB2 receptor activation *in vitro* and *in vivo* actions of these novel products remains to be established. Also, it is possible that additional receptors *in vivo* may be functionally regulated by these new DHEA products.

ASPIRIN AND DHA: UNCOVERING THE AT-(NPD1/PD1) PATHWAY

Aspirin is well appreciated for its ability to inhibit COX-1 and inactivate this enzyme. On the other hand, the catalytic region of COX-2 is larger than that of COX-1, and when it is acetylated by aspirin, acetylated COX-2 remains active, producing lipoxygenase-like products, demonstrated *in vivo* in humans (Chiang et al., 2004; Chiang and Serhan, 2004). Aspirin is unique in that it can jump-start resolution by triggering production of E-series and D-series resolvins and AT-lipoxins (Serhan et al., 2000, 2002; Schwab et al., 2007; Morris et al., 2009). The previously unrecognized mediators and mechanisms involve the biosynthesis of AT lipid mediators. With Dr. Bazan (LSU) and Dr. Petasis (USC), we established DHA conversion to the AT-NPD1 pathway (Serhan et al., 2011).

To determine the complete stereochemical assignment and bioactions of AT-(NPD1/PD1), we directly compared the physical and biological properties of DHA-derived AT-(NPD1/PD1) and related 10,17 dihydroxy-docosatriene stereoisomers produced by leukocytes to those prepared in stereochemically pure form by total organic synthesis (Serhan et al., 2011). Since AT-(NPD1/PD1) was identified in resolving murine exudates treated with aspirin (Serhan et al., 2002), biologic AT-(NPD1/PD1) was obtained from resolving murine exudates *in vivo*. To this end, peritonitis was initiated via intra-peritoneal (i.p.) administration of zymosan A (1 mg/mouse), and exudates were harvested 24 h post injection (i.e., within the resolution phase; Bannenberg et al., 2005). Exudates were subject to solid phase extraction and analyzed using LC–UV–MS–MS-based mediator lipidomics. AT-(NPD1/PD1) was also isolated and identified from activated human leukocytes, namely, aspirin-treated human PMN. Collectively, by matching biologic materials from both human and murine exudate AT-(NPD1/PD1) with synthetic candidates, these results established the complete stereochemistry of endogenous AT-(NPD1/PD1) as 10R,17R-dihydroxydocosa-4*Z*, 7*Z*, 11*E*, 13*E*, 15*Z*, 19*Z*-hexaenoic acid (Serhan et al., 2011).

ANTI-INFLAMMATORY ACTIONS OF AT-NPD1/PD1

Earlier results indicated that NPD1/PD1 exerted potent anti-inflammatory actions regulating leukocyte trafficking in murine systems (Serhan et al., 2002; Hong et al., 2003). We compared the bioactions of NPD1/PD1 to those of AT-(NPD1/PD1) carried out in parallel with the physical matching experiments (Serhan et al., 2011). Synthetic AT-(NPD1/PD1) limited PMN infiltration into the peritoneum in TNFα-stimulated peritonitis (**Figure 5**). Both NPD1/PD1 (0.1–10 ng) and AT-(NPD1/PD1; 0.01–10.0 ng) proved to be significant regulators of TNFα-stimulated leukocyte infiltration into the peritoneum. AT-(NPD1/PD1) reduced total leukocyte population of the exudate including PMN, monocyte and lymphocyte infiltrates, reaching a maximal reduction at 1 ng/mouse by as much as $50.4 \pm 8.8\%$. The PMN population was also reduced with AT-(NPD1/PD1) reaching a maximal PMN reduction at 1 ng/mouse ($62.2 \pm 7.8\%$; Serhan et al., 2011). The flow cytometry results obtained with murine exudates indicated

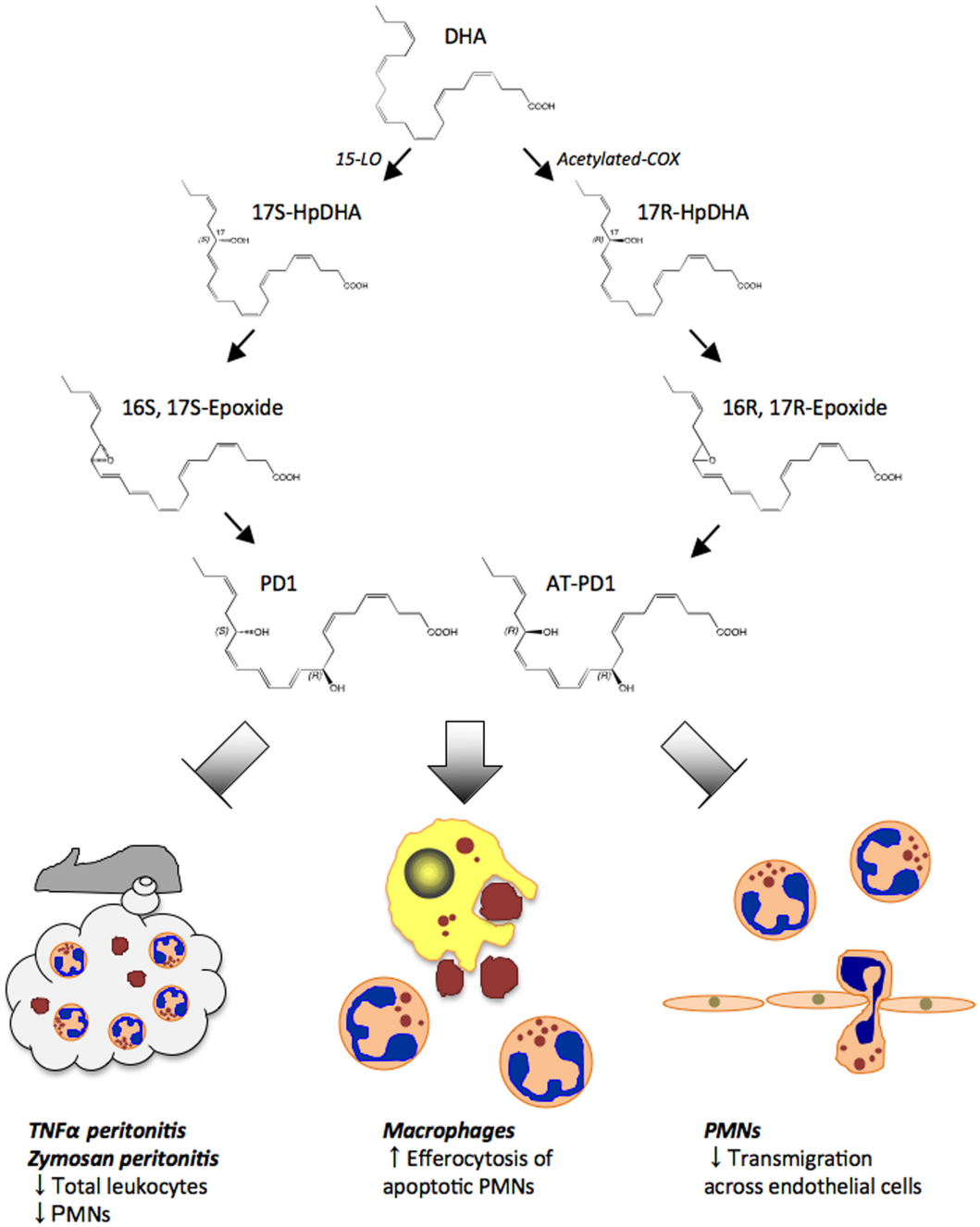


FIGURE 5 | Proposed biosynthesis scheme for protectin D1 (PD1) and aspirin-triggered protectin D1 (AT-PD1). DHA is first converted to 17S-HpDHA intermediate by 15-LOX. Then PD1 is generated through 16S, 17S-epoxide. In the aspirin-triggered pathway, 17R-HpDHA is generated from DHA via acetylated-COX, and converted to AT-PD1 via a 16R, 17R-containing epoxide intermediate. Both PD1 and AT-PD1 reduce leukocytes and PMN infiltration in peritonitis stimulated by either TNF-α or zymosan A. PD1 and AT-PD1 each enhance human macrophage efferocytosis of apoptotic human PMNs and limit human PMN transmigration across human endothelial cells, the defining pro-resolving bioactions.

a reduction in the $Ly6G^{+}CD11b^{+}$ population as compared to TNFα alone. Of note, the Δ^{15}-trans-AT-(NPD1/PD1) isomer did not reduce either the total exudate leukocyte population or PMN infiltration. Since NPD1/PD1 limited PMN infiltration in zymosan A-initiated peritonitis (Serhan et al., 2006), we determined whether AT-(NPD1/PD1) also reduced PMN infiltration in zymosan A-stimulated peritonitis. AT-(NPD1/PD1; 0.1–100.0 ng) significantly reduced total leukocytes, as well as PMN infiltration, reaching a maximal reduction of $47.8 \pm 10.0\%$ and $49.1 \pm 11.9\%$. In comparison to AT-(NPD1/PD1; 1.0 and 10.0 ng), equal doses of either the precursor DHA or Δ15-trans-AT-NPD1/PD1 did not reduce either total leukocyte infiltration or PMN

infiltration (Serhan et al., 2011). These findings indicated that AT-(NPD1/PD1) regulates inflammatory responses induced by the pro-inflammatory cytokine TNFα and the TLR ligand, zymosan A.

PMN-TRANSENDOTHELIAL MIGRATION AND ENHANCED EFFEROCYTOSIS

PMN-transendothelial migration is the first committed step of leukocytes in acute inflammation (Kumar et al., 2005). AT-(NPD1/PD1) and NPD1/PD1 (0.1–10.0 nM) reduced (∼30 and ∼50%) PMN-transendothelial migration stimulated by LTB_4. Equal concentrations of the Δ^{15}-trans isomer of AT-(NPD1/PD1) where the conjugated triene portion of the molecule was in the *trans* rather than *cis* configuration did not reduce PMN-transendothelial migration. Again, the precursor DHA (Kasuga et al., 2008) did not reduce LTB_4-stimulated PMN-transendothelial migration (Serhan et al., 2011). To corroborate these, we also used an electric cell-substrate impedance sensing system (ECIS) that sensitively quantitates cellular responses in two cell systems by real-time monitoring of barrier impedance (Tsikitis et al., 2004). Both AT-(NPD1/PD1) and NPD1/PD1 (1nM) decreased LTB_4-stimulated PMN-transendothelial migration, and AT-(NPD1/PD1) also enhanced the uptake of apoptotic human PMN by human macrophages at concentrations as low as 0.1 nM, as did NPD1/PD1 when compared directly. The response was bell-shaped and consistent with the dose response relationship observed for efferocytosis and pro-resolving lipid mediators such as RvE1 (Hong et al., 2008).

SOME CONSIDERATIONS FOR THE FUTURE

In summation, the novel products and pathway metabolome reviewed here might have protective and anti-inflammatory actions relevant in humans. This important level of evidence is of interest and remains to be established in human trials. For example, since RvE1 has a direct protective effect on cardiomyocytes against ischemia-reperfusion injury limiting infarct size when administered intravenously just before reperfusion (Keyes et al., 2010), the novel bioactive DHEA products discussed in this review may carry potential clinical applications. These new DHEA products (**Figure 4**) and/or related structures might be useful in the treatment of acute myocardial infarction, because platelet and neutrophil interactions have a key regulatory role in the site of inflammation (Marcus et al., 1982; Phillipson and Kubes, 2011). Along these lines, Pillai et al. (2012) from this laboratory recently reported on the time course and identification of inflammatory mediators (both cytokine and lipid mediators) after abdominal aortic aneurysm (AAA) surgery. Chemical mediator profiles from patients that had undergone AAA in that study assembled into two groups. The temporal profiles for local chemical mediators from these patients were either those with a pro-inflammatory profile of local mediators or a second group with a potential resolving profile of local mediators. These recent reports and results reviewed herein suggest that the temporal biosynthesis of local chemical mediators following inflammation or tissue injury can have an acute local impact as well as long-term effects following surgical intervention and tissue remodeling. Hence, knowledge of these new mediators and pathways as well as their potential roles may serve as a basis for new and more effective therapeutics that could improve the outcome of diseases, surgery and post-operative events.

ACKNOWLEDGMENTS

We thank Mary H. Small for skillful manuscript preparation.

REFERENCES

Ariel, A., and Serhan, C. N. (2012). New lives given by cell death: macrophage differentiation following their encounter with apoptotic leukocytes during the resolution of inflammation. *Front. Immun.* 3, 4. doi: 10.3389/fimmu.2012.00004

Bannenberg, G. L., Chiang, N., Ariel, A., Arita, M., Tjonahen, E., Gotlinger, K. H., Hong, S., and Serhan, C. N. (2005). Molecular circuits of resolution: formation and actions of resolvins and protectins. *J. Immunol.* 174, 4345–4355.

Bazan, N. G., Calandria, J. M., and Serhan, C. N. (2010). Rescue and repair during photoreceptor cell renewal mediated by docosahexaenoic acid-derived neuroprotectin D1. *J. Lipid Res.* 51, 2018–2031.

Borgeat, P., and Samuelsson, B. (1979). Arachidonic acid metabolism in polymorphonuclear leukocytes: effects of ionophore A23187. *Proc. Natl. Acad. Sci. U.S.A.* 76, 2148–2152.

Calder, P. C. (2010). Rationale and uses of n-3 fatty acids in artificial nutrition. *Proc. Nutr. Soc.* 69, 565–573.

Chiang, N., Bermudez, E. A., Ridker, P. M., Hurwitz, S., and Serhan, C. N. (2004). Aspirin triggers anti-inflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. *Proc. Natl. Acad. Sci. U.S.A.* 101, 15178–15183.

Chiang, N., and Serhan, C. N. (2004). Aspirin triggers formation of anti-inflammatory mediators: new mechanism for an old drug. *Discov. Med.* 4, 470–475.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., and Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258, 1946–1949.

Felder, C. C., Briley, E. M., Axelrod, J., Simpson, J. T., Mackie, K., and Devane, W. A. (1993). Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc. Natl. Acad. Sci. U.S.A.* 90, 7656–7660.

Hong, S., Gronert, K., Devchand, P., Moussignac, R. -L., and Serhan, C. N. (2003). Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glial cells: autacoids in anti-inflammation. *J. Biol. Chem.* 278, 14677–14687.

Hong, S., Porter, T. F., Lu, Y., Oh, S. F., Pillai, P. S., and Serhan, C. N. (2008). Resolvin E1 metabolome in local inactivation during inflammation-resolution. *J. Immunol.* 180, 3512–3519.

Irimia, D., Liu, S. -Y., Tharp, W. G., Samadani, A., Toner, M., and Poznansky, M. C. (2006). Microfluidic system for measuring neutrophil migratory responses to fast switches of chemical gradients. *Lab. Chip* 6, 191–198.

Janero, D. R., and Makriyannis, A. (2007). Targeted modulators of the endogenous cannabinoid system: future medications to treat addiction disorders and obesity. *Curr. Psychiatry Rep.* 9, 365–373.

Kasuga, K., Yang, R., Porter, T. F., Agrawal, N., Petasis, N. A., Irimia, D., Toner, M., and Serhan, C. N. (2008). Rapid appearance of resolvin precursors in inflammatory exudates: novel mechanisms in resolution. *J. Immunol.* 181, 8677–8687.

Keyes, K. T., Ye, Y., Lin, Y., Zhang, C., Perez-Polo, J. R., Gjorstrup, P., and Birnbaum, Y. (2010). Resolvin E1 protects the rat heart against reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol.* 299, H153–H164.

Kumar, V., Abbas, A. K., Fausto, N., Robbins, S. L., and Cotran, R. S. (2005). *Robbins and Cotran Pathologic Basis of Disease*. Philadelphia: Elsevier/Saunders.

Majno, G., and Joris, I. (2004). *Cells, Tissues, and Disease: Principles of General Pathology*. New York: Oxford University Press.

Marcus, A. J., Broekman, M. J., Safier, L. B., Ullman, H. L., Islam, N., Serhan, C. N., Rutherford, L. E., Korchak, H. M., and Weissmann, G. (1982). Formation of leukotrienes and other hydroxy acids during platelet-neutrophil interactions in vitro. *Biochem. Biophys. Res. Commun.* 109, 130–137.

Morris, T., Stables, M., Colville-Nash, P., Newson, J., Bellingan, G., De Souza, P. M., and Gilroy, D. W. (2010). Dichotomy in duration and severity of acute inflammatory responses in humans arising from differentially expressed proresolution pathways. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8842–8847.

Morris, T., Stables, M., Hobbs, A., De Souza, P., Colville-Nash, P., Warner, T., Newson, J., Bellingan, G., and Gilroy, D. W. (2009). Effects of low-dose aspirin on acute inflammatory responses in humans. *J. Immunol.* 183, 2089–2096.

Oh, S. F., Pillai, P. S., Recchiuti, A., Yang, R., and Serhan, C. N. (2011). Pro-resolving actions and stereoselective biosynthesis of 18S E-series resolvins in human leukocytes and murine inflammation. *J. Clin. Invest.* 121, 569–581.

Phillipson, M., and Kubes, P. (2011). The neutrophil in vascular inflammation. *Nat. Med.* 17, 1381–1390.

Pillai, P. S., Leeson, S., Porter, T. F., Owens, C. D., Kim, J. M., Conte, M. S., Serhan, C. N., and Gelman, S. (2012). Chemical mediators of inflammation and resolution in post-operative abdominal aortic aneurysm patients. *Inflammation* 35, 98–113.

Qiu, F.-H., Wada, K., Stahl, G. L., and Serhan, C. N. (2000). IMP and AMP deaminase in reperfusion injury down-regulates neutrophil recruitment. *Proc. Natl. Acad. Sci. U.S.A.* 97, 4267–4272.

Roberts, L. J. II, Montine, T. J., Markesbery, W. R., Tapper, A. R., Hardy, P., Chemtob, S., Dettbarn, W. D., and Morrow, J. D. (1998). Formation of isoprostane-like compounds (neuroprostanes) in vivo from docosahexaenoic acid. *J. Biol. Chem.* 273, 13605–13612.

Roberts, L. J., II, and Morrow, J. D. (2002). Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. *Cell. Mol. Life Sci.* 59, 808–820.

Ryan, G. B., and Majno, G. (1977). Acute inflammation. A review. *Am. J. Pathol.* 86, 183–276.

Samuelsson, B. (1983). Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 220, 568–575.

Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A., and Serhan, C. N. (1987). Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science* 237, 1171–1176.

Schwab, J. M., Chiang, N., Arita, M., and Serhan, C. N. (2007). Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* 447, 869–874.

Serhan, C. N. (2007). Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu. Rev. Immunol.* 25, 101–137.

Serhan, C. N. (2011). The resolution of inflammation: the devil in the flask and in the details. *FASEB J.* 25, 1441–1448.

Serhan, C. N., Chiang, N., and Van Dyke, T. E. (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* 8, 349–361.

Serhan, C. N., Clish, C. B., Brannon, J., Colgan, S. P., Chiang, N., and Gronert, K. (2000). Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J. Exp. Med.* 192, 1197–1204.

Serhan, C. N., Fredman, G., Yang, R., Karamnov, S., Belayev, L. S., Bazan, N. G., Zhu, M., Winkler, J. W., and Petasis, N. A. (2011). Novel proresolving aspirin-triggered DHA pathway. *Chem. Biol.* 18, 976–987.

Serhan, C. N., Gotlinger, K., Hong, S., and Arita, M. (2004). Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostaglandins Other Lipid Mediat.* 73, 155–172.

Serhan, C. N., Gotlinger, K., Hong, S., Lu, Y., Siegelman, J., Baer, T., Yang, R., Colgan, S. P., and Petasis, N. A. (2006). Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. *J. Immunol.* 176, 1848–1859.

Serhan, C. N., Hong, S., Gronert, K., Colgan, S. P., Devchand, P. R., Mirick, G., and Moussignac, R.-L. (2002). Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J. Exp. Med.* 196, 1025–1037.

Serhan, C. N., and Petasis, N. A. (2011). Resolvins and protectins in inflammation-resolution. *Chem. Rev.* 111, 5922–5943.

Serhan, C. N., and Savill, J. (2005). Resolution of inflammation: the beginning programs the end. *Nat. Immunol.* 6, 1191–1197.

Serhan, C. N., Ward, P. A., and Gilroy, D. W. (eds). (2010). *Fundamentals of Inflammation.* New York: Cambridge University Press.

Serhan, C. N., Yang, R., Martinod, K., Kasuga, K., Pillai, P. S., Porter, T. F., Oh, S. F., and Spite, M. (2009). Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J. Exp. Med.* 206, 15–23.

Spite, M., and Serhan, C. N. (2010). Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. *Circ. Res.* 107, 1170–1184.

Stables, M. J., Shah, S., Camon, E. B., Lovering, R. C., Newson, J., Bystrom, J., Farrow, S., and Gilroy, D. W. (2011). Transcriptomic analyses of murine resolution-phase macrophages. *Blood* 118, e192–e208.

Tjonahen, E., Oh, S. F., Siegelman, J., Elangovan, S., Percarpio, K. B., Hong, S., Arita, M., and Serhan, C. N. (2006). Resolvin E2: identification and anti-inflammatory actions: pivotal role of human 5-lipoxygenase in resolvin E series biosynthesis. *Chem. Biol.* 13, 1193–1202.

Tsikitis, V. L., Morin, N. A., Harrington, E. O., Albina, J. E., and Reichner, J. S. (2004). The lectin-like domain of complement receptor 3 protects endothelial barrier function from activated neutrophils. *J. Immunol.* 173, 1284–1291.

van Gils, J. M., Zwaginga, J. J., and Hordijk, P. L. (2009). Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. *J. Leukoc. Biol.* 85, 195–204.

Vemuri, V. K., Janero, D. R., and Makriyannis, A. (2008). Pharmacotherapeutic targeting of the endocannabinoid signaling system: drugs for obesity and the metabolic syndrome. *Physiol. Behav.* 93, 671–686.

Yang, R., Fredman, G., Krishnamoorthy, S., Agrawal, N., Irimia, D., Piomelli, D., and Serhan, C. N. (2011). Decoding functional metabolomics with docosahexaenoyl ethanolamide (DHEA) identifies novel bioactive signals. *J. Biol. Chem.* 286, 31532–31541.

Conflict of Interest Statement: The authors declare that this review was written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 07 February 2012; paper pending published: 21 February 2012; accepted: 29 March 2012; published online: 17 April 2012.

Citation: Shinohara M, Mirakaj V and Serhan CN (2012) Functional metabolomics reveals novel active products in the DHA metabolome. Front. Immun. ***3****:81. doi: 10.3389/fimmu.2012.00081*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in IMMUNOLOGY

REVIEW ARTICLE
published: 30 August 2012
doi: 10.3389/fimmu.2012.00275

Transcriptional regulation of the anti-inflammatory cytokine IL-10 in acquired immune cells

Masato Kubo*[1,2] *and* ***Yasutaka Motomura*[1]**

[1] *Division of Molecular Pathology, Research Institute for Biological Sciences, Tokyo University of Science, Noda, Japan*
[2] *Laboratory for Signal Network, Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama, Japan*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
David Hildeman, Cincinnati Children's Hospital, USA
Takayuki Yoshimoto, Tokyo Medical University, Japan

****Correspondence:***
Masato Kubo, Division of Molecular Pathology, Research Institute for Biological Sciences, Tokyo University of Science, 2669 Yamazaki, Noda, Chiba 278-0022, Japan.
e-mail: raysolfc@rcai.riken.jp

Although the major role of the immune response is host defense from a wide range of potentially pathogenic microorganisms, excess immune responses can result in severe host damage. The host thus requires anti-inflammatory mechanisms to prevent reactivity to self. Interleukin-10 (IL-10) is a cytokine with broad anti-inflammatory properties involved in the pathogenesis of various diseases. IL-10 was originally described as a T helper (T_H2) derived cytokine, but further studies indicated that IL-10 is expressed not only by many cells of the adaptive immune system, including T and B cells, but also by the innate immune cells, including dendritic cells (DCs), macrophages, mast cells, and natural killer (NK) cells. In addition, IL-10 can be induced in T_H1 and T_H17 cells by chronic inflammation as a system of feedback regulation. In this review, we focus on the molecular mechanisms underlying *IL10* gene expression in adaptive immune cells and summarize the recent progresses in epigenetic and transcriptional regulation of the *IL10* gene. Understanding the transcriptional regulatory events may help in the development of new strategies to control inflammatory diseases.

Keywords: epigenetics, interleukin-10, plasticity, regulatory B cells, transcriptional regulation

GENETIC ASSOCIATION OF IL10 WITH INFLAMMATORY DISEASES

Recent genome-wide association studies (GWAS) have demonstrated tight association of polymorphisms in the genes encoding *IL10* with systemic lupus erythematosis (SLE) (Gateva et al., 2009) and Bechet's disease (BD) (Mizuki et al., 2010; Remmers et al., 2010). BD is a genetically complex disease characterized by recurrent inflammation affecting urogenital mucosa, eye, and skin. Allelic imbalance of the rs158111 variant in pre-mRNA associates with expression of the *IL10* gene. The disease associated haplotype results in the reduction of the pre-mRNA transcript and Interleukin-10 (IL-10) production in mononuclear cells activated with lipopolysaccharide (LPS), suggesting that a genetic predisposition for low IL-10 production is a risk factor for BD (Remmers et al., 2010). Polymorphisms in the *IL10* gene region have been reported to be associated with ulcerative colitis (UC) (Franke et al., 2008), type I diabetes (Barrett et al., 2009), and severe juvenile rheumatoid arthritis (Crawley et al., 1999), and mutations in the genes encoding the subunits of the IL-10R were found in patients with inflammatory bowel disease (IBD) (Glocker et al., 2009). These observations strongly implicate IL-10 as an important regulator of the human immune system. A wide variety of cells are known to produce IL-10, but it remains unclear which cell type(s) is the major contributor to immune regulation. Therefore, it is important to better understand the source and the regulatory role of IL-10.

THE *In vivo* IDENTIFICATION OF IL-10 EXPRESSION BY USE OF REPORTER MICE

For identification of the cellular sources and the role of IL-10, several reporter mouse strains have been established as useful detection tools to track *in vivo* expression of IL-10 (Bouabe, 2012). These reporter strains often provide critical insight into the expression of IL-10 in various cell types and cell type-specific function. IL-10eYFP mice and IL10-IRES-EGFP mice are classical versions of such IL-10 reporter mice (Calado et al., 2006; Kamanaka et al., 2006; Neves et al., 2010; Bouabe et al., 2011), and reporter activity in these lines has been detected only in CD4 T cells after robust stimulation. Thus these lines have a relatively insensitive limit of detection of IL-10-driven expression of autofluorescent proteins. Improved versions of the reporter mice, IL10-IRES-eGFP-BGHpA mice, IL10Venus mice and IL10-Thy1.1-SV40pA BAC transgenic mice revealed steady expression of IL-10 in a large fraction of $CD4^+$ T cells, including Treg cells and NKT cells, $CD19^+B220^{low}$ B cells, $CD19^+CD138^+$plasma cells, and in a very small subset of $CD11b^+$ macrophages, $CD11c^+$ dendritic cells (DCs), and $NK1.1^+$ NK cells (Maynard et al., 2007; Madan et al., 2009; Atarashi et al., 2011). Accumulating evidence thus indicates that IL-10 is secreted by a wide variety of cells, such as helper and regulatory T cells, NKT cells, NK cells, regulatory B cells, macrophages, DCs, and monocytes, all of which may contribute to its immunoregulatory role (**Figure 1**).

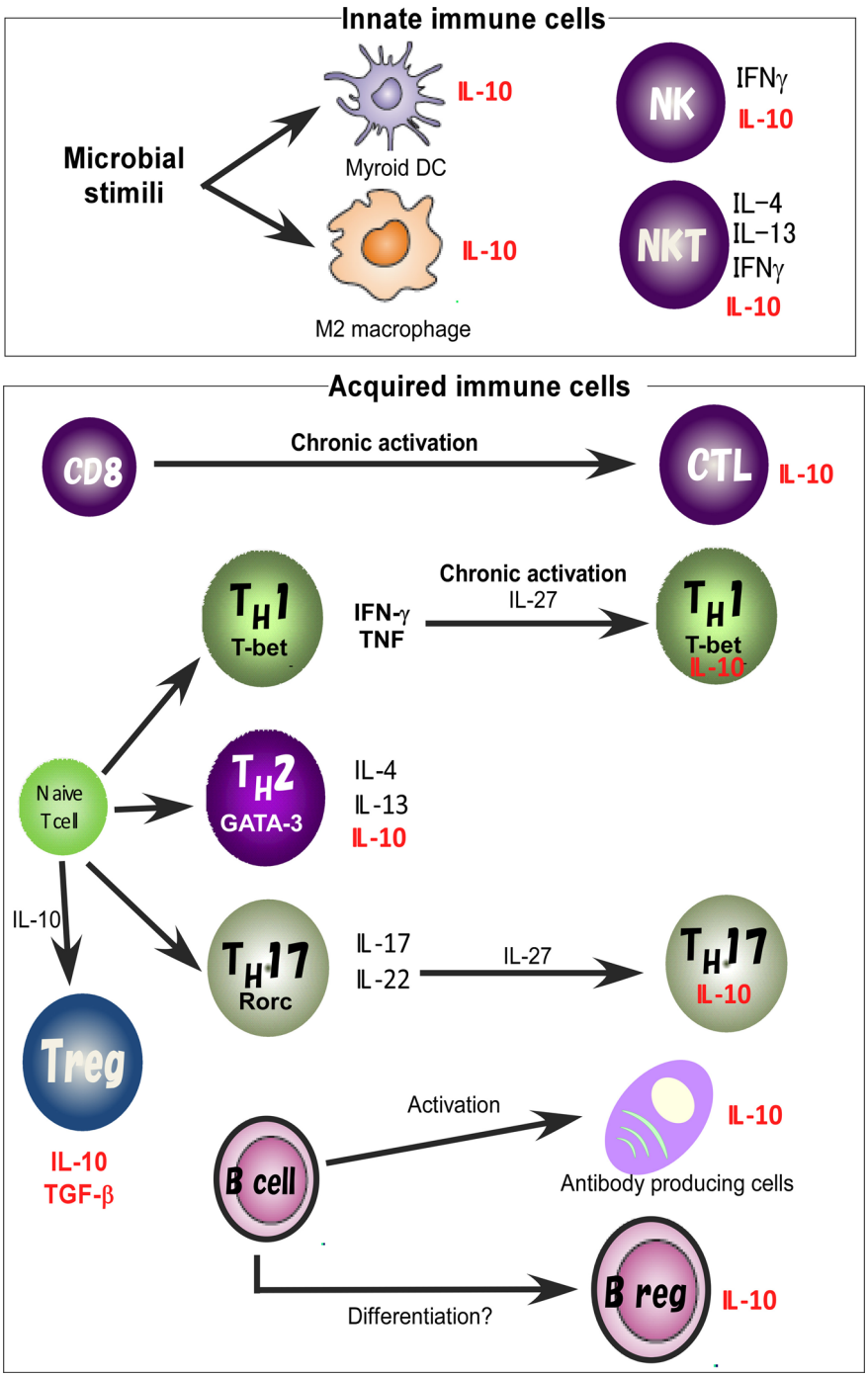


FIGURE 1 | IL-10 expression in the immune system. IL-10 is expressed by M2 macrophages and myeloid DCs. Treg, TH1, TH2, and TH17 cell subsets share the ability to produce IL-10. IL-10 regulates the function and/or IL-10 production of Treg cells. IL-10 production by TH1 cells is induced in chronically infected mice with parasites infection and in response to high-dose antigenic stimulation. IL-27 effectively blocks IL-17 production and induces the production of IL-10. Activated B cells and Bregs are also a key B cell subset responsible for IL-10 mediated regulatory function.

IL-10 PRODUCTION BY T CELLS

IL-10 is one of the key cytokines to down-regulate a variety of inflammatory responses mediated by lymphoid and myeloid cells (Berg et al., 2001). IL-10 was originally identified as cytokine synthesis inhibitory factor (CSIF). It was secreted from type 2 T-helper cells (T_H2 cells) and suppressed the differentiation and effector functions of Th1 cells (Fiorentino et al., 1989). It's inhibitory function was explained by its ability to suppress the production of pro-inflammatory cytokines such as IL-12 and TNF by DCs and macrophages, and to down regulate the

expression of MHC II and the costimulatory molecules CD80 and CD86 on antigen presenting cell (APCs), thereby resulting in the subsequent inhibition of T cell activation (Fiorentino et al., 1991; Berg et al., 2001). The *in vivo* importance of IL-10 in controlling inflammatory responses was originally recognized based on observations made in IL-10-deficient mice, which mount exaggerated T_H1 cell responses and develop spontaneous chronic enterocolitis in response to normal gut flora (Kuhn et al., 1993). IL-10-deficient mice die from spontaneous colitis (Kuhn et al., 1993), and this phenotype is partly retained even in mice lacking IL-10 only in T_{reg} cells (Rubtsov et al., 2008). In the colitis case, natural regulatory T (nT_{reg}) cells would be responsible for IL-10 production in response to the microflora. On the other hand, the type 1 regulatory T (Tr1) cell is a different type of T_{reg} cell. Tr1 cells are characterized by their low proliferative capacity and their high levels of IL-10 secretion (Groux et al., 1997). Consistent with IL-10's role in suppressing inflammation, immunization of IL-10 deficient mice with myelin antigens resulted in enhanced neuroinflammation with loss of recovery from experimental autoimmune encephalomyelitis (EAE), a mouse model for human multiple sclerosis (MS) (Bettelli et al., 1998). IL-10 therefore plays an important role in regulating overactive responses that would otherwise result in autoinflammatory disease.

PLASTICITY OF IL-10 PRODUCTION BY T CELLS

The differentiation of T_H1, T_H2, and T_H17 cells is regulated by distinct signaling pathways. Despite differences in their development, these T cell subsets share the ability to make IL-10, which can be involved in the regulation of immune responses (**Figure 1**). The mechanism of silencing IL-10 production in T_H1 cells was originally thought to be stable and immutable, but it is now clear that T_H1 cells can produce IL-10 under certain conditions. IL-10-producing T_H1 cells have been shown, on the one hand, to limit immunopathology during *Toxplasma gondii* infection (Shaw et al., 2006; Jankovic et al., 2007), but on the other hand, to attenuate protective immunity to *Leishmania major* (Anderson et al., 2007). IL-10 production by T_H1 cells has also been reported in animals chronically infected with these parasites and in response to high-dose antigenic stimulation. Such hyperactivation of T cell receptor (TCR)-mediated signaling leads to sustained phosphorylation of ERK1 and ERK2 MAP kinases, resulting in plasticity of IL-10 production in T_H1 cells (Saraiva et al., 2009). Continuous and excess antigen stimulation under T_H1 skewing conditions enhances the plasticity of IL-10 production (Motomura et al., 2011), and such IL-10 production from T_H1 cells may be an effective fail-safe mechanism to maintain immune homeostasis.

The presence of T_H17 subsets with regulatory functions correlates with their ability to produce IL-10 (Fitzgerald et al., 2007; McGeachy et al., 2007; Stumhofer et al., 2007; Saraiva et al., 2009). IL-27 added to the cultures under T_H17 skewing condition in the presence of TGF-β and IL-6 effectively blocks IL-17 production and induces the production of IL-10. The ability of IL-27 to induce IL-10 is important to suppress T_H17 cell–mediated autoimmunity (Fitzgerald et al., 2007; Diveu et al., 2009). Moreover, addition of IL-27 under T_H1 skewing conditions also induced the expression of IL-10. This activity was specific in that it had no suppressive effect on the ability of the cells to secrete IFN-γ (Batten et al., 2008). On the other hand, *in vitro* restimulation of T cells from mice with EAE in the presence of IL-23 generates cells able to efficiently transfer the disease, whereas restimulation in the presence of TGF-β and IL-6, with or without IL-23, generates nonpathogenic IL-10-producing cells (Fitzgerald et al., 2007). IL-23-induced T_H17 cells are pathogenic and probably more responsive to IL-27-induced upregulation of IL-10. Therefore, IL-23 and IL-27, which may derive from different subsets of DC (Gafa et al., 2006; Kinnebrew et al., 2012), control the direction of T cell responses to pathogenesis or resistance. In the case of humans, IL-1β can modify the capacity of pathogen-induced human T_H17 cells to produce either IFN-γ or IL-10 (Zielinski et al., 2012). Recently, DC and innate cell-derived IL-27 was reported to induce IL-10 production by $CD8^+$ cytotoxic lymphocytes (CTLs) during viral infection (Sun et al., 2011).

In many cases, DCs possess unique functions to control the plasticity of T cell function, and the lectin-like receptors (LLR) on DCs contribute to alteration of the quality of the T cell response. Indeed, activation of DCs with Dectin-1 results in polarization into a T_H17 response (Gringhuis et al., 2009), but Dectin-1 has also been reported to induce IL-10 production by DCs (Rogers et al., 2005). Dectin-1 in conjunction with TLR2 elicits DCs capable of inducing T_{reg} responses (Dillon et al., 2006). Another LLR, DC-SIGN, leads to not only a T_H2 response (Geurtsen et al., 2009) and but also to T_{reg} differentiation (Zhou et al., 2010). The scavenger receptor, DC-asialoglycoprotein receptor (DC-ASGPR) leads to generation of antigen specific suppressive IL-10-producing T cells via the induction of IL-10 production by DCs (Li et al., 2012). These results and others make it abundantly clear that the regulation of T cell subset differentiation and cytokine production is complex and occurs at multiple levels.

TRANSCRIPTIONAL REGULATION OF THE *IL10* GENE IN T CELLS

In previous studies, involvement of various transcription factors in activation of the *IL10* gene has been proposed (**Figure 2**). The T_H2 master regulator, GATA3 controls IL-10 expression in T_H2 cells through initiating changes of the chromatin structure at the *IL10* locus (Shoemaker et al., 2006; Chang et al., 2007). Indeed, Gata3 deficiency cause a loss of IL-10 expression in T_H2 cells (unpublished data). GATA3 binds to the *IL10* promoter, but GATA3 alone does not activate the promoter activity (Shoemaker et al., 2006), supporting the idea that GATA3 may determine the transcriptional permissibility of the chromatin structure in the *IL10* locus. However, in the case of other T cell types such as T_H1 and T_H17 that express a quite low level of GATA3, other factors would be necessary to replace its function to induce high levels of IL-10 expression (Jankovic et al., 2007; Saraiva et al., 2009).

We recently reported that E4BP4 has multiple functions in cytokine gene regulation and is an essential transcriptional factor to regulate IL-10 production not only in T_H2, NKT, and Treg cells, but also to allow plasticity of IL-10 production in T_H1 and T_H17 cells (Motomura et al., 2011). E4BP4 was originally identified

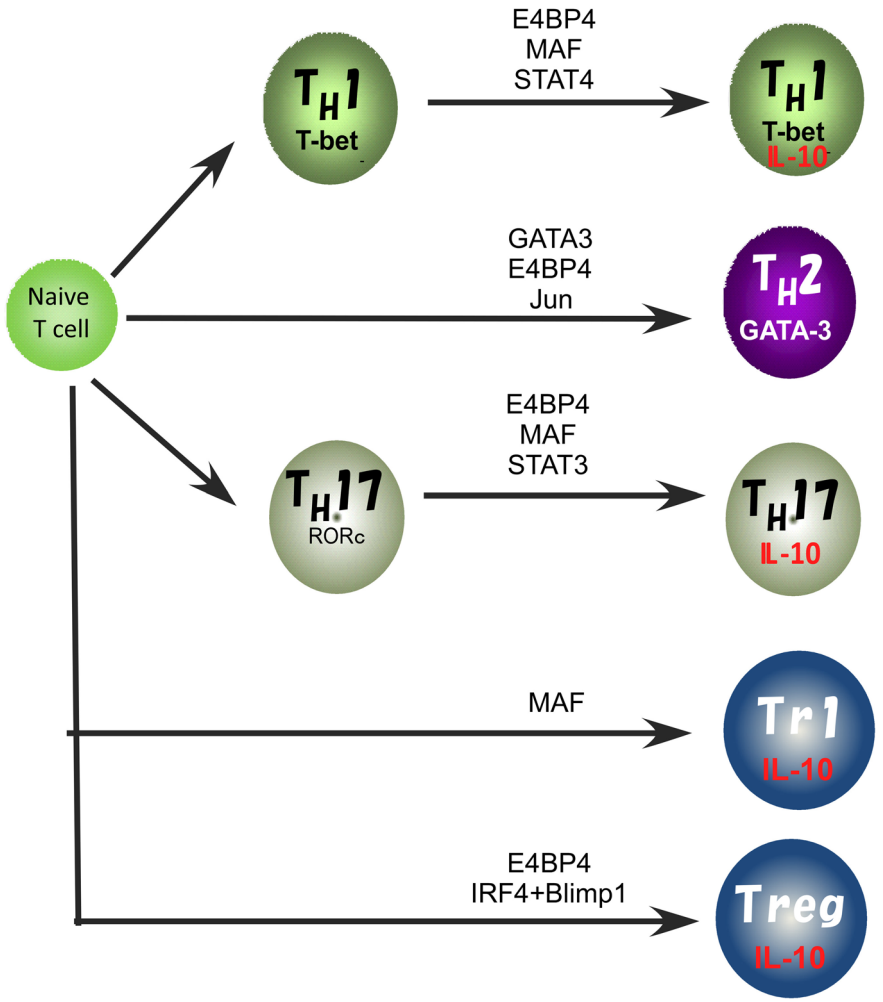


FIGURE 2 | Transcriptional factors regulating the IL10 gene expression in Treg, TH1, TH2, and TH17 cell subsets.

as a negative regulator of the mammalian circadian oscillatory system by virtue of its antagonistic binding to the same DNA regulatory sequences as a member of the PAR family of bZIP transcription factors (Cowell, 2002). The deletion of the *E4bp4* gene preferentially affected NK cell development (Gascoyne et al., 2009; Kamizono et al., 2009). By contrast, the proportion of conventional helper T cell lineages that developed in the culture conditions for T_H1, T_H2, iT_{reg}, and T_H17 cell induction was not affected in the *E4bp4* deficient mice. E4BP4 can induce IL-10 production under Gata3 deficient T_H1 conditions, suggesting that GATA-3 is dispensable for plasticity of IL-10 production by T_H1 cells. E4BP4 bound to intron 4 and the 3′ non-coding region of the *IL10* locus and activated the histone code in the *IL10* locus, as indicated by the finding that H3K9 methylation and H3K14 acetylation were completely abolished in *E4bp4*-deficient T_H2 cells. These findings indicate that E4BP4 is an epigenetic regulator to control the permissive status of the *IL10* locus (Motomura et al., 2011).

Another T_H2 cell-dominant transcription factor, MAF, can bind to the *IL10* promoter and plays a role in the regulation of IL-10 production in mouse macrophages stimulated with LPS and IL-4 (Cao et al., 2005). MAF is detectable in T_H1, T_H2 and T_H17 cells, where its expression coincides with IL-10 production. IL-27 induces the expression of MAF along with IL-21 and the costimulatory receptor ICOS, and these ultimately act coordinately to promote differentiation of IL-10-producing Tr1 cells (Pot et al., 2009). Moreover, induction of MAF expression in T_H1 and T_H17 cells depends on ERK activation, as does IL-10 expression (Saraiva et al., 2009). These reports suggest that MAF may be a common regulator for IL-10 production in both the innate and the adaptive immune systems. However, MAF alone is not sufficient to induce *IL10* expression in macrophages and T cells (Cao et al., 2005; Motomura et al., 2011), thus the role of MAF in IL-10 production is still controversial.

Distinct mechanisms seem to regulate the expression of *IL10* in the innate and acquired immune systems. NF-κB activation is

a major contributor to IL-10 production in macrophages, and the NF-κB p65 subunit binds to the *IL10* locus 4.5 kb upstream of the transcription start site (Saraiva et al., 2005). This finding is consistent with the report that IKK2-deficient mice show a defect in IL-10 production by LPS-stimulated macrophages. On the other hand, ERK signaling is required for optimal IL-10 induction in innate immune cells as well as T cells (Agrawal et al., 2003; Kaiser et al., 2009; Saraiva et al., 2009). ERK activation leads to the binding of AP-1 to the *IL10* locus through the cooperative function of Fos/Jun family proteins. Studies have suggested a role for Jun proteins in regulating *IL10 expression* in T_H2 cells, but not in T_H1 cells, and this is explained by their binding to a regulatory element located 6.45 kb downstream of the *IL10* transcription start site (Jones and Flavell, 2005; Wang et al., 2005). This report addresses the idea that ERK and MAF are required for *IL10* induction as common regulators in various cell types (Saraiva and O'Garra, 2010).

STAT proteins are reported to be another mechanism regulating IL-10 expression by both macrophages and T cells. In T cells, the induction of IL-10 by IL-27 seems to depend on both STAT1 and STAT3 (Stumhofer et al., 2007; Batten et al., 2008; Xu et al., 2009). STAT3 is also involved in IL-6-induced IL-10 expression (Stumhofer et al., 2007). Moreover, STAT3 is responsible for the IL-27-mediated IL-10 induction instead of the inhibition of T_H17 cell differentiation. The binding of STAT3 to the *IL10* promoter in human cell lines induced *IL10* transcription though STAT3-dependent promoter activation in conjunction with IFN-α induced IRF1 activation (Ziegler-Heitbrock et al., 2003). STAT4, which is important for the expression of IFN-γ, also regulates IL-10 production in T_H1 cells (Saraiva et al., 2009), and STAT4 was also reported to have a role in inducing its expression in NK cells (Grant et al., 2008). However, the STATs are also important in the differentiation process of T_H cell subsets, thus it remains unclear whether the function of STATs in IL-10 production is direct or indirect.

Recently, the coordinated activity of IRF4 and Blimp-1 has been reported to be critical for regulation of IL-10 production by Treg cells (Cretney et al., 2011). Blimp-1 is a transcriptional repressor well known for its role in promoting the differentiation of plasma cells (Nutt et al., 2008) but is also required for the maintenance of T cell homeostasis. Mice lacking Blimp-1 specifically in T cells accumulate activated T cells and develop immune pathology, including colitis and lung inflammation (Kallies et al., 2006; Martins et al., 2006), suggesting that Blimp-1 has a critical role in Treg cell function. Indeed, Blimp-1 is required for IL-10 production and high ICOS expression in mature effector Treg cells. Blimp-1 is known to be preferentially expressed in T_H2 cells that produce high levels of IL-10, and IL-10 production by CTLs is regulated by a Blimp-1 dependent mechanism (Sun et al., 2011). Therefore, Blimp-1 is an important regulator of IL10 expression.

IL-10 PRODUCTION BY B CELLS

IL-10- producing B cells play an important role in controlling autoimmunity, such as in EAE, an animal model of MS, and in a systemic lupus erythematosus (SLE)-like disease that develops in the Lyn-deficient mouse and during murine cytomegalovirus (MCMV4) infection (Fillatreau et al., 2002; Madan et al., 2009; Scapini et al., 2011). This idea has its origins in the discovery that B cell deficient μMT mice cannot recover from EAE (Wolf et al., 1996), and the identification of a regulatory role of IL-10 producing B cells in EAE lead to the realization of the importance of this type of B cell (Fillatreau et al., 2002). Furthermore, the importance of IL-10–producing B cells in controlling chronic inflammatory diseases has also been demonstrated in collagen-induced arthritis and chronic intestinal inflammation (Mizoguchi et al., 2002; Mauri et al., 2003). On the other hand, IL-10 production by B cells has been shown to prevent protective immunity to infection with *Salmonella* Typhimurium (Neves et al., 2010) and to decrease MCMV4-specific $CD8^+$ T cell responses (Madan et al., 2009).

IL-10 is secreted from several B cell subsets that can be distinguished by cell surface phenotype. Regulatory B cells (Bregs) are considered as a key B cell subset responsible for IL-10 mediated regulatory function (Fillatreau et al., 2002; Mizoguchi et al., 2002; Mauri et al., 2003). However, there is no precise marker that exclusively defines the Breg (Mauri and Bosma, 2012). Transitional 2 marginal zone precursor (T2-MZP) B cells ($CD19^+CD21^{hi}CD23^{hi}CD24^{hi}IgD^{hi}IgM^{hi}CD1^{hi}$) appear to be the most likely candidate for being Breg cells. T2-MZP B cells isolated from arthritic mice produced copious amount of IL-10 after stimulation with collagen type II antigen in conjunction with anti-CD40 antibody. Transfer of T2-MZP B cells suppressed the development of CIA (Evans et al., 2007). T2-MZP B cells also suppress other autoimmune diseases including antigen-induced arthritis (AIA) and lupus (Inoue et al., 2006; Carter et al., 2011), and Schistosoma mansoni infection generates IL-10 producing T2-MZP B cells with regulatory function (Amu et al., 2010). However, T2-MZP B cells do not fully satisfy of the complete phenotype of Breg cells, because T2-MZP B cells also contain the largest fraction of transitional immature B cells.

$CD5^+$ B1 B cells are also known to be distinct source of IL-10 (O'Garra et al., 1992), and they also have an immunoregulatory function by killing of CD4 T cells by FasL/Fas-dependent mechanisms (Lundy and Fox, 2009). B1 B cells also play a role in protection from colitis, but their protective role is not due to the production of IL-10, instead IgM and IgA are responsible for the protection (Shimomura et al., 2008).

IL10 REGULATION IN BREG CELLS

In studies to understand the mechanisms underlying control of IL10 expression in B cells, the importance of TLRs has been emphasized. *In vitro* stimulation with LPS, together with PMA and ionomycin, promotes the development of IL-10 producing B cells. There is a rare population expressing CD5 and $CD1d^{hi}$ termed IL-10 producing B cells (B10 cells) that suppresses oxazolone-induced contact hypersensitivity (Yanaba et al., 2008). Stimulation of B cells *in vitro* with LPS induces the expression of IL10 in plasma cells ($CD19^+CD138^+$), but only very low levels of IL-10 in the B10 population (Neves et al., 2010). On the other hand, TLR2 and TLR4 signaling predominantly controls IL-10 production in MZ B cells, but not in follicular B cells (Gray et al., 2007). The importance of IL-10 from B cells is confirmed in mice lacking TLR2, TLR4 or

Myd88, a signaling molecule downstream of TLRs, which develop a chronic form of EAE (Lampropoulou et al., 2008), and expression of MyD88 is required for IL-10 production and inhibitory function of B cells. The importance of TLR9, which is a receptor for CpG, has also been proposed in regulating IL-10 production by B cells (Barr et al., 2007). However, TLR9- deficient B cells can still inhibit EAE, suggesting a redundant role of TLR9 in Breg cells (Lampropoulou et al., 2008).

Multiple studies have indicated an essential function of the CD40-CD154 interaction for the activation of Breg cells. Mice lacking CD40 on B cells develop severe EAE, with increased induction of encephalitogenic T_H1 and T_H17 responses, and have a profound decrease in IL-10 production (Mizoguchi et al., 2000). Furthermore, administration of an agonistic antibody against CD40 improves arthritis by the provision of IL-10 (Mauri et al., 2003; Evans et al., 2007) and IL-10 mediated T_H1 inhibition (Mauri et al., 2000).

Several previous studies have suggested that Bregs require signaling through the B cell receptor (BCR) for their activity. Indeed, mice lacking the BCR co-receptor molecule CD19 develop a severe EAE, suggesting the importance of the BCR in the generation of Bregs (Sato et al., 1996; Yanaba et al., 2008). A major component downstream of BCR signaling is intracellular Ca^{2+} (Feske, 2007). Mice lacking the Ca^{2+} channel molecules, STIM1 and STIM2, develop an elaborated EAE and low numbers of IL-10 producing Bregs (Matsumoto et al., 2011). These mice showed normal B cell development and antibody responses, however, they have a defect in the activation of nuclear factor of activated T cells (NFAT). Inconsistently, mice with an NFATc1 deficiency display an increased number of IL-10 producing B cells and development of milder EAE (Bhattacharyya et al., 2011). Therefore, at present, it remains unclear which signaling pathway is the major one.

CONCLUSION

Recent GWAS have demonstrated tight association of polymorphisms in the gene encoding *IL10* with several inflammatory diseases, indicating importance of understanding the source and the regulation of IL-10. IL-10 is expressed by many acquired immune cells including, T_H1, T_H2 and T_H17 cells, Treg cells, $CD8^+$ T cells and B cells. In this review, we have summarized the current view of *IL10* regulation in these T cells and B cells. *IL10* expression in different T cell subsets is regulated by a complex of multiple transcriptional factors, such as GATA-3, E4BP4, MAF, Blimp1, and so on, and these multiple levels of regulation occur at independent differentiation stages in accordance with certain rules that are just beginning to be understood. Therefore, the expression of the *IL10 gene* has the flexibility and plasticity. This plasticity is sometimes controlled by continuous antigen stimulation or by a particular cytokine environment, such as DC derived IL-27, and selectively occurs in inflammatory type of helper T cells, T_H1 and T_H17 cells. On the other hand, the importance of IL-10 from B cells is proposed in several autoimmune and inflammatory disease models. Similar to T cell, a complex of multiple signaling pathways, including BCR and CD40-CD40L pathways, is required for the generation of Bregs and the induction of IL-10 in B cells. However, it remains virtually unknown at present which signaling pathway is the major one and how *IL10* is transcriptionally regulated. There may be critical transcriptional regulatory machinery that could be specific to certain cell types and this machinery may be turned on by specific signals in certain diseases. Therefore, it will be quite important to understand the meaning of the SNPs that associate with several inflammatory diseases and the molecular mechanisms underlying transcriptional regulation of the *IL10* gene. Moreover, this approach may lead to the development of innovative therapeutic strategies for controlling these diseases.

REFERENCES

Agrawal, S., Agrawal, A., Doughty, B., Gerwitz, A., Blenis, J., Van Dyke, T., and Pulendran, B. (2003). Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J. Immunol.* 171, 4984–4989.

Amu, S., Saunders, S. P., Kronenberg, M., Mangan, N. E., Atzberger, A., and Fallon, P. G. (2010). Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. *J. Allergy Clin. Immunol.* 125, 1114–1124.

Anderson, C. F., Oukka, M., Kuchroo, V. J., and Sacks, D. (2007). CD4(+)CD25(−)Foxp3(−) Th1 cells are the source of IL-10-mediated immune suppression in chronic cutaneous leishmaniasis. *J. Exp. Med.* 204, 285–297.

Atarashi, K., Tanoue, T., Shima, T., Imaoka, A., Kuwahara, T., Momose, Y., Cheng, G., Yamasaki, S., Saito, T., Ohba, Y., Taniguchi, T., Takeda, K., Hori, S., Ivanov, I. I., Umesaki, Y., Itoh, K., and Honda, K. (2011). Induction of colonic regulatory T cells by indigenous Clostridium species. *Science* 331, 337–341.

Barr, T. A., Brown, S., Ryan, G., Zhao, J., and Gray, D. (2007). TLR-mediated stimulation of APC: distinct cytokine responses of B cells and dendritic cells. *Eur. J. Immunol.* 37, 3040–3053.

Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., Julier, C., Morahan, G., Nerup, J., Nierras, C., Plagnol, V., Pociot, F., Schuilenburg, H., Smyth, D. J., Stevens, H., Todd, J. A., Walker, N. M., and Rich, S. S. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat. Genet.* 41, 703–707.

Batten, M., Kljavin, N. M., Li, J., Walter, M. J., de Sauvage, F. J., and Ghilardi, N. (2008). Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells. *J. Immunol.* 180, 2752–2756.

Berg, D. J., Zhang, J., Lauricella, D. M., and Moore, S. A. (2001). Il-10 is a central regulator of cyclooxygenase-2 expression and prostaglandin production. *J. Immunol.* 166, 2674–2680.

Bettelli, E., Das, M. P., Howard, E. D., Weiner, H. L., Sobel, R. A., and Kuchroo, V. K. (1998). IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. *J. Immunol.* 161, 3299–3306.

Bhattacharyya, S., Deb, J., Patra, A. K., Thuy Pham, D. A., Chen, W., Vaeth, M., Berberich-Siebelt, F., Klein-Hessling, S., Lamperti, E. D., Reifenberg, K., Jellusova, J., Schweizer, A., Nitschke, L., Leich, E., Rosenwald, A., Brunner, C., Engelmann, S., Bommhardt, U., Avots, A., Muller, M. R., Kondo, E., and Serfling, E. (2011). NFATc1 affects mouse splenic B cell function by controlling the calcineurin–NFAT signaling network. *J. Exp. Med.* 208, 823–839.

Bouabe, H. (2012). Cytokine reporter mice: the special case of IL-10 scand. *J. Immunol.* 75, 553–567.

Bouabe, H., Liu, Y., Moser, M., Bosl, M. R., and Heesemann, J. (2011). Novel highly sensitive IL-10-β-Lactamase reporter mouse reveals cells of the innate immune system as a substantial source of IL-10 *in vivo*. *J. Immunol.* 187, 3165–3176.

Calado, D. P., Paixao, T., Holmberg, D., and Haury, M. (2006). Stochastic monoallelic expression of IL-10 in T cells. *J. Immunol.* 177, 5358–5364.

Cao, S., Liu, J., Song, L., and Ma, X. (2005). The protooncogene c-Maf is an essential transcription factor for IL-10 gene expression in macrophages. *J. Immunol.* 174, 3484–3492.

Carter, N. A., Vasconcellos, R., Rosser, E. C., Tulone, C., Munoz-Suano, A., Kamanaka, M., Ehrenstein, M. R., Flavell, R. A., and Mauri, C. (2011). Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. *J. Immunol.* 186, 5569–5579.

Chang, H. D., Helbig, C., Tykocinski, L., Kreher, S., Koeck, J., Niesner, U., and Radbruch, A. (2007). Expression of IL-10 in Th memory lymphocytes is conditional on IL-12 or IL-4, unless the IL-10 gene is imprinted by GATA-3. *Eur. J. Immunol.* 37, 807–817.

Cowell, I. G. (2002). E4BP4/NFIL3, a PAR-related bZIP factor with many roles. *Bioessays* 24, 1023–1029.

Crawley, E., Kay, R., Sillibourne, J., Patel, P., Hutchinson, I., and Woo, P. (1999). Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum.* 42, 1101–1108.

Cretney, E., Xin, A., Shi, W., Minnich, M., Masson, F., Miasari, M., Belz, G. T., Smyth, G. K., Busslinger, M., Nutt, S. L., and Kallies, A. (2011). The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. *Nat. Immunol.* 12, 304–311.

Dillon, S., Agrawal, S., Banerjee, K., Letterio, J., Denning, T. L., Oswald-Richter, K., Kasprowicz, D. J., Kellar, K., Pare, J., van Dyke, T., Ziegler, S., Unutmaz, D., and Pulendran, B. (2006). Yeast zymosan, a stimulus for TLR2 and dectin-1, induces regulatory antigen-presenting cells and immunological tolerance. *J. Clin. Invest.* 116, 916–928.

Diveu, C., McGeachy, M. J., Boniface, K., Stumhofer, J. S., Sathe, M., Joyce-Shaikh, B., Chen, Y., Tato, C. M., McClanahan, T. K., de Waal Malefyt, R., Hunter, C. A., Cua, D. J., and Kastelein, R. A. (2009). IL-27 blocks RORc expression to inhibit lineage commitment of Th17 cells. *J. Immunol.* 182, 5748–5756.

Evans, J. G., Chavez-Rueda, K. A., Eddaoudi, A., Meyer-Bahlburg, A., Rawlings, D. J., Ehrenstein, M. R., and Mauri, C. (2007). Novel suppressive function of transitional 2 B cells in experimental arthritis. *J. Immunol.* 178, 7868–7878.

Feske, S. (2007). Calcium signalling in lymphocyte activation and disease. *Nat. Rev. Immunol.* 7, 690–702.

Fillatreau, S., Sweenie, C. H., McGeachy, M. J., Gray, D., and Anderton, S. M. (2002). B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol.* 3, 944–950.

Fiorentino, D. F., Bond, M. W., and Mosmann, T. R. (1989). Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* 170, 2081–2095.

Fiorentino, D. F., Zlotnik, A., Vieira, P., Mosmann, T. R., Howard, M., Moore, K. W., and O'Garra, A. (1991). IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol.* 146, 3444–3451.

Fitzgerald, D. C., Zhang, G. X., El-Behi, M., Fonseca-Kelly, Z., Li, H., Yu, S., Saris, C. J., Gran, B., Ciric, B., and Rostami, A. (2007). Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. *Nat. Immunol.* 8, 1372–1379.

Franke, A., Balschun, T., Karlsen, T. H., Sventoraityte, J., Nikolaus, S., Mayr, G., Domingues, F. S., Albrecht, M., Nothnagel, M., Ellinghaus, D., Sina, C., Onnie, C. M., Weersma, R. K., Stokkers, P. C., Wijmenga, C., Gazouli, M., Strachan, D., McArdle, W. L., Vermeire, S., Rutgeerts, P., Rosenstiel, P., Krawczak, M., Vatn, M. H., Mathew, C. G., and Schreiber, S. (2008). Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. *Nat. Genet.* 40, 1319–1323.

Gafa, V., Lande, R., Gagliardi, M. C., Severa, M., Giacomini, E., Remoli, M. E., Nisini, R., Ramoni, C., Di Francesco, P., Aldebert, D., Grillot, R., and Coccia, E. M. (2006). Human dendritic cells following *Aspergillus fumigatus* infection express the CCR7 receptor and a differential pattern of interleukin-12 (IL-12), IL-23, and IL-27 cytokines, which lead to a Th1 response. *Infect. Immun.* 74, 1480–1489.

Gascoyne, D. M., Long, E., Veiga-Fernandes, H., de Boer, J., Williams, O., Seddon, B., Coles, M., Kioussis, D., and Brady, H. J. (2009). The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat. Immunol.* 10, 1118–1124.

Gateva, V., Sandling, J. K., Hom, G., Taylor, K. E., Chung, S. A., Sun, X., Ortmann, W., Kosoy, R., Ferreira, R. C., Nordmark, G., Gunnarsson, I., Svenungsson, E., Padyukov, L., Sturfelt, G., Jonsen, A., Bengtsson, A. A., Rantapaa-Dahlqvist, S., Baechler, E. C., Brown, E. E., Alarcon, G. S., Edberg, J. C., Ramsey-Goldman, R., McGwin, G. Jr., Reveille, J. D., Vila, L. M., Kimberly, R. P., Manzi, S., Petri, M. A., Lee, A., Gregersen, P. K., Seldin, M. F., Ronnblom, L., Criswell, L. A., Syvanen, A. C., Behrens, T. W., and Graham, R. R. (2009). A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat. Genet.* 41, 1228–1233.

Geurtsen, J., Chedammi, S., Mesters, J., Cot, M., Driessen, N. N., Sambou, T., Kakutani, R., Ummels, R., Maaskant, J., Takata, H., Baba, O., Terashima, T., Bovin, N., Vandenbroucke-Grauls, C. M., Nigou, J., Puzo, G., Lemassu, A., Daffe, M., and Appelmelk, B. J. (2009). Identification of mycobacterial alpha-glucan as a novel ligand for DC-SIGN: involvement of mycobacterial capsular polysaccharides in host immune modulation. *J. Immunol.* 183, 5221–5231.

Glocker, E. O., Kotlarz, D., Boztug, K., Gertz, E. M., Schaffer, A. A., Noyan, F., Perro, M., Diestelhorst, J., Allroth, A., Murugan, D., Hatscher, N., Pfeifer, D., Sykora, K. W., Sauer, M., Kreipe, H., Lacher, M., Nustede, R., Woellner, C., Baumann, U., Salzer, U., Koletzko, S., Shah, N., Segal, A. W., Sauerbrey, A., Buderus, S., Snapper, S. B., Grimbacher, B., and Klein, C. (2009). Inflammatory bowel disease and mutations affecting the interleukin-10 receptor. *N. Engl. J. Med.* 361, 2033–2045.

Grant, L. R., Yao, Z. J., Hedrich, C. M., Wang, F., Moorthy, A., Wilson, K., Ranatunga, D., and Bream, J. H. (2008). Stat4-dependent, T-bet-independent regulation of IL-10 in NK cells. *Genes Immun.* 9, 316–327.

Gray, M., Miles, K., Salter, D., Gray, D., and Savill, J. (2007). Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. *Proc. Natl. Acad. Sci. U.S.A.* 104, 14080–14085.

Gringhuis, S. I., den Dunnen, J., Litjens, M., van der Vlist, M., Wevers, B., Bruijns, S. C., and Geijtenbeek, T. B. (2009). Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. *Nat. Immunol.* 10, 203–213.

Groux, H., O'Garra, A., Bigler, M., Rouleau, M., Antonenko, S., de Vries, J. E., and Roncarolo, M. G. (1997). A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389, 737–742.

Inoue, S., Leitner, W. W., Golding, B., and Scott, D. (2006). Inhibitory effects of B cells on antitumor immunity. *Cancer Res.* 66, 7741–7747.

Jankovic, D., Kullberg, M. C., Feng, C. G., Goldszmid, R. S., Collazo, C. M., Wilson, M., Wynn, T. A., Kamanaka, M., Flavell, R. A., and Sher, A. (2007). Conventional T-bet(+)Foxp3(-) Th1 cells are the major source of host-protective regulatory IL-10 during intracellular protozoan infection. *J. Exp. Med.* 204, 273–283.

Jones, E. A., and Flavell, R. A. (2005). Distal enhancer elements transcribe intergenic RNA in the IL-10 family gene cluster. *J. Immunol.* 175, 7437–7446.

Kaiser, F., Cook, D., Papoutsopoulou, S., Rajsbaum, R., Wu, X., Yang, H. T., Grant, S., Ricciardi-Castagnoli, P., Tsichlis, P. N., Ley, S. C., and O'Garra, A. (2009). TPL-2 negatively regulates interferon-beta production in macrophages and myeloid dendritic cells. *J. Exp. Med.* 206, 1863–1871.

Kallies, A., Hawkins, E. D., Belz, G. T., Metcalf, D., Hommel, M., Corcoran, L. M., Hodgkin, P. D., and Nutt, S. L. (2006). Transcriptional repressor Blimp-1 is essential for T cell homeostasis and self-tolerance. *Nat. Immunol.* 7, 466–474.

Kamanaka, M., Kim, S. T., Wan, Y. Y., Sutterwala, F. S., Lara-Tejero, M., Galán, J. E., Harhaj, E., and Flavell, R. A. (2006). Expression of interleukin-10 in intestinal lymphocytes detected by an interleukin-10 reporter knockin tiger mouse. *Immunity* 25, 941–952.

Kamizono, S., Duncan, G. S., Seidel, M. G., Morimoto, A., Hamada, K., Grosveld, G., Akashi, K., Lind, E. F., Haight, J. P., Ohashi, P. S., Look, A. T., and Mak, T. W. (2009). Nfil3/E4bp4 is required for the development and maturation of NK cells *in vivo*. *J. Exp. Med.* 206, 2977–2986.

Kinnebrew, M. A., Buffie, C. G., Diehl, G. E., Zenewicz, L. A., Leiner, I., Hohl, T. M., Flavell, R. A., Littman, D. R., and Pamer, E. G. (2012). Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal

innate immune defense. *Immunity* 36, 276–287.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., and Muller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75, 263–274.

Lampropoulou, V., Hoehlig, K., Roch, T., Neves, P., Calderon Gomez, E., Sweenie, C. H., Hao, Y., Freitas, A. A., Steinhoff, U., Anderton, S. M., and Fillatreau, S. (2008). TLR-activated B cells suppress T cell-mediated autoimmunity. *J. Immunol.* 180, 4763–4773.

Li, D., Romain, G., Flamar, A. L., Duluc, D., Dullaers, M., Li, X. H., Zurawski, S., Bosquet, N., Palucka, A. K., Le Grand, R., O'Garra, A., Zurawski, G., Banchereau, J., and Oh, S. (2012). Targeting self- and foreign antigens to dendritic cells via DC-ASGPR generates IL-10-producing suppressive CD4+ T cells. *J. Exp. Med.* 209, 109–121.

Lundy, S. K., and Fox, D. A. (2009). Reduced Fas ligand-expressing splenic CD5+ B lymphocytes in severe collagen-induced arthritis. *Arthritis Res. Ther.* 11, R128.

Madan, R., Demircik, F., Surianarayanan, S., Allen, J. L., Divanovic, S., Trompette, A., Yogev, N., Gu, Y., Khodoun, M., Hildeman, D., Boespflug, N., Fogolin, M. B., Gröbe, L., Greweling, M., Finkelman, F. D., Cardin, R., Mohrs, M., Müller, W., Waisman, A., Roers, A., and Karp, C. L. (2009). Nonredundant roles for B cell-derived IL-10 in immune counter-regulation. *J. Immunol.* 183, 2312–2320.

Martins, G. A., Cimmino, L., Shapiro-Shelef, M., Szabolcs, M., Herron, A., Magnusdottir, E., and Calame, K. (2006). Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. *Nat. Immunol.* 7, 457–465.

Matsumoto, M., Fujii, Y., Baba, A., Hikida, M., Kurosaki, T., and Baba, Y. (2011). The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. *Immunity* 34, 703–714.

Mauri, C., and Bosma, A. (2012). Immune regulatory function of B cells. *Annu. Rev. Immunol.* 30, 221–241.

Mauri, C., Gray, D., Mushtaq, N., and Londei, M. (2003). Prevention of arthritis by interleukin 10-producing B cells. *J. Exp. Med.* 197, 489–501.

Mauri, C., Mars, L. T., and Londei, M. (2000). Therapeutic activity of agonistic monoclonal antibodies against CD40 in a chronic autoimmune inflammatory process. *Nat. Med.* 6, 673–679.

Maynard, C. L., Harrington, L. E., Janowski, K. M., Oliver, J. R., Zindl, C. L., Rudensky, A. Y., and Weaver, C. T. (2007). Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. *Nat Immunol.* 8, 931–941.

McGeachy, M. J., Bak-Jensen, K. S., Chen, Y., Tato, C. M., Blumenschein, W., McClanahan, T., and Cua, D. J. (2007). TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat. Immunol.* 8, 1390–1397.

Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R. S., and Bhan, A. K. (2002). Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity* 16, 219–230.

Mizoguchi, E., Mizoguchi, A., Preffer, F. I., and Bhan, A. K. (2000). Regulatory role of mature B cells in a murine model of inflammatory bowel disease. *Int. Immunol.* 12, 597–605.

Mizuki, N., Meguro, A., Ota, M., Ohno, S., Shiota, T., Kawagoe, T., Ito, N., Kera, J., Okada, E., Yatsu, K., Song, Y. W., Lee, E. B., Kitaichi, N., Namba, K., Horie, Y., Takeno, M., Sugita, S., Mochizuki, M., Bahram, S., Ishigatsubo, Y., and Inoko, H. (2010). Genome-wide association studies identify IL23R-IL12RB2 and IL10 as Behcet's disease susceptibility loci. *Nat. Genet.* 42, 703–706.

Motomura, Y., Kitamura, H., Hijikata, A., Matsunaga, Y., Matsumoto, K., Inoue, H., Atarashi, K., Hori, S., Watarai, H., Zhu, J., Taniguchi, M., and Kubo, M. (2011). The transcription factor E4BP4 regulates the production of IL-10 and IL-13 in CD4+ T cells. *Nat. Immunol.* 12, 450–459.

Neves, P., Lampropoulou, V., Calderon-Gomez, E., Roch, T., Stervbo, U., Shen, P., Kuhl, A. A., Loddenkemper, C., Haury, M., Nedospasov, S. A., Kaufmann, S. H., Steinhoff, U., Calado, D. P., and Fillatreau, S. (2010). Signaling via the MyD88 adaptor protein in B cells suppresses protective immunity during *Salmonella typhimurium* infection. *Immunity* 33, 777–790.

Nutt, S. L., Kallies, A., and Belz, G. T. (2008). Blimp-1 connects the intrinsic and extrinsic regulation of T cell homeostasis. *J. Clin. Immunol.* 28, 97–106.

O'Garra, A., Chang, R., Go, N., Hastings, R., Haughton, G., and Howard, M. (1992). Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. *Eur. J. Immunol.* 22, 711–717.

Pot, C., Jin, H., Awasthi, A., Liu, S. M., Lai, C. Y., Madan, R., Sharpe, A. H., Karp, C. L., Miaw, S. C., Ho, I. C., and Kuchroo, V. K. (2009). Cutting edge: IL-27 induces the transcription factor c-Maf, cytokine IL-21, and the costimulatory receptor ICOS that coordinately act together to promote differentiation of IL-10-producing Tr1 cells. *J. Immunol.* 183, 797–801.

Remmers, E. F., Cosan, F., Kirino, Y., Ombrello, M. J., Abaci, N., Satorius, C., Le, J. M., Yang, B., Korman, B. D., Cakiris, A., Aglar, O., Emrence, Z., Azakli, H., Ustek, D., Tugal-Tutkun, I., Akman-Demir, G., Chen, W., Amos, C. I., Dizon, M. B., Kose, A. A., Azizlerli, G., Erer, B., Brand, O. J., Kaklamani, V. G., Kaklamanis, P., Ben-Chetrit, E., Stanford, M., Fortune, F., Ghabra, M., Ollier, W. E., Cho, Y. H., Bang, D., O'Shea, J., Wallace, G. R., Gadina, M., Kastner, D. L., and Gul, A. (2010). Genome-wide association study identifies variants in the MHC class I, IL10, and IL23R-IL12RB2 regions associated with Behcet's disease. *Nat. Genet.* 42, 698–702.

Rogers, N. C., Slack, E. C., Edwards, A. D., Nolte, M. A., Schulz, O., Schweighoffer, E., Williams, D. L., Gordon, S., Tybulewicz, V. L., Brown, G. D., and Reis e Sousa, C. (2005). Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* 22, 507–517.

Rubtsov, Y. P., Rasmussen, J. P., Chi, E. Y., Fontenot, J., Castelli, L., Ye, X., Treuting, P., Siewe, L., Roers, A., Henderson, W. R. Jr., Muller, W., and Rudensky, A. Y. (2008). Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 28, 546–558.

Saraiva, M., Christensen, J. R., Tsytsykova, A. V., Goldfeld, A. E., Ley, S. C., Kioussis, D., and O'Garra, A. (2005). Identification of a macrophage-specific chromatin signature in the IL-10 locus. *J. Immunol.* 175, 1041–1046.

Saraiva, M., Christensen, J. R., Veldhoen, M., Murphy, T. L., Murphy, K. M., and O'Garra, A. (2009). Interleukin-10 production by Th1 cells requires interleukin-12-induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dose. *Immunity* 31, 209–219.

Saraiva, M., and O'Garra, A. (2010). The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10, 170–181.

Sato, T., McCue, P., Masuoka, K., Salwen, S., Lattime, E. C., Mastrangelo, M. J., and Berd, D. (1996). Interleukin 10 production by human melanoma. *Clin. Cancer Res.* 2, 1383–1390.

Scapini, P., Lamagna, C., Hu, Y., Lee, K., Tang, Q., DeFranco, A. L., and Lowell, C. A. (2011). B cell-derived IL-10 suppresses inflammatory disease in Lyn-deficient mice. *Proc. Natl. Acad. Sci. U.S.A.* 108, E823–E832.

Shaw, M. H., Freeman, G. J., Scott, M. F., Fox, B. A., Bzik, D. J., Belkaid, Y., and Yap, G. S. (2006). Tyk2 negatively regulates adaptive Th1 immunity by mediating IL-10 signaling and promoting IFN-gamma-dependent IL-10 reactivation. *J. Immunol.* 176, 7263–7271.

Shimomura, Y., Mizoguchi, E., Sugimoto, K., Kibe, R., Benno, Y., Mizoguchi, A., and Bhan, A. K. (2008). Regulatory role of B-1 B cells in chronic colitis. *Int. Immunol.* 20, 729–737.

Shoemaker, J., Saraiva, M., and O'Garra, A. (2006). GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4+ T cells. *J. Immunol.* 176, 3470–3479.

Stumhofer, J. S., Silver, J. S., Laurence, A., Porrett, P. M., Harris, T. H., Turka, L. A., Ernst, M., Saris, C. J., O'Shea, J. J., and Hunter, C. A. (2007). Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat. Immunol.* 8, 1363–1371.

Sun, J., Dodd, H., Moser, E. K., Sharma, R., and Braciale, T. J. (2011). CD4+ T cell help and innate-derived IL-27 induce Blimp-1-dependent IL-10 production by antiviral CTLs. *Nat. Immunol.* 12, 327–334.

Wang, Z. Y., Sato, H., Kusam, S., Sehra, S., Toney, L. M., and Dent, A. L. (2005). Regulation of IL-10 gene expression in Th2 cells by Jun proteins. *J. Immunol.* 174, 2098–2105.

Wolf, S. D., Dittel, B. N., Hardardottir, F., and Janeway, C. A. Jr. (1996). Experimental autoimmune encephalomyelitis induction in

genetically B cell-deficient mice. *J. Exp. Med.* 184, 2271–2278.

Xu, J., Yang, Y., Qiu, G., Lal, G., Wu, Z., Levy, D. E., Ochando, J. C., Bromberg, J. S., and Ding, Y. (2009). c-Maf regulates IL-10 expression during Th17 polarization. *J. Immunol.* 182, 6226–6236.

Yanaba, K., Bouaziz, J. D., Haas, K. M., Poe, J. C., Fujimoto, M., and Tedder, T. F. (2008). A regulatory B cell subset with a unique CD1dhiCD5+ phenotype controls T cell-dependent inflammatory responses. *Immunity* 28, 639–650.

Zhou, Y., Kawasaki, H., Hsu, S. C., Lee, R. T., Yao, X., Plunkett, B., Fu, J., Yang, K., Lee, Y. C., and Huang, S. K. (2010). Oral tolerance to food-induced systemic anaphylaxis mediated by the C-type lectin SIGNR1. *Nat. Med.* 16, 1128–1133.

Ziegler-Heitbrock, L., Lotzerich, M., Schaefer, A., Werner, T., Frankenberger, M., and Benkhart, E. (2003). IFN-alpha induces the human IL-10 gene by recruiting both IFN regulatory factor 1 and Stat3. *J. Immunol.* 171, 285–290.

Zielinski, C. E., Mele, F., Aschenbrenner, D., Jarrossay, D., Ronchi, F., Gattorno, M., Monticelli, S., Lanzavecchia, A., and Sallusto, F. (2012). Pathogen-induced human TH17 cells produce IFN-gamma or IL-10 and are regulated by IL-1beta. *Nature* 484, 514–518.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 23 May 2012; paper pending published: 18 June 2012; accepted: 13 August 2012; published online: 30 August 2012.

*Citation: Kubo M and Motomura Y (2012) Transcriptional regulation of the anti-inflammatory cytokine IL-10 in acquired immune cells. Front. Immun. **3**:275. doi: 10.3389/fimmu.2012.00275*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 27 February 2012
doi: 10.3389/fimmu.2012.00028

Regulatory T cell-mediated control of autoantibody-induced inflammation

Keishi Fujio*, Tomohisa Okamura, Shuji Sumitomo and Kazuhiko Yamamoto

Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Shohei Hori, RIKEN, Japan
Yuko Okuyama, Osaka University, Japan

****Correspondence:***
Keishi Fujio, Department of Allergy and Rheumatology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan.
e-mail: kfujio-tky@umin.ac.jp

Autoimmune inflammation including autoantibody-induced inflammation is responsible for the lethal organ damage. Autoantibody-induced inflammation can be separated in two components, autoantibody production, and local inflammatory responses. Accumulating evidence has suggested that regulatory T cells (Treg) control both antibody production and the numbers and functions of effector cells such as innate cells and T helper cells. Autoantibodies are produced by both the follicular and extrafollicular pathways. Recently, follicular regulatory T cells (T_{FR}) and Qa-1 restricted $CD8^+$ Treg were identified as populations that are capable of suppressing follicular T helper cell (T_{FH})-mediated antibody production. In local inflammation, $CD4^+CD25^+Foxp3^+$ Treg have the capacity to control inflammation by suppressing cytokine production in T helper cells. Although complement proteins contribute to autoantibody-induced local inflammation by activating innate cells, Treg including $CD4^+CD25^+Foxp3^+$ Treg are able to suppress innate cells, chiefly via IL-10 production. IL-10-secreting T cells such as T regulatory type I (Tr1) and Tr1-like cells might also play roles in the control of Th17 and innate cells. Therefore, several kinds of Tregs have the potential to control autoimmune inflammation by suppressing both autoantibody production and the local inflammatory responses induced by autoantibodies.

Keywords: chronic inflammation, autoantibody, regulatory T cells, IL-10, Tr1 cells

INTRODUCTION

Autoimmune inflammation is responsible for the lethal organ damage, and autoantibodies play a pivotal role in triggering inflammation. Immune complexes are readily detectable in the articular tissues of rheumatoid arthritis (RA) patients and the glomeruli of systemic lupus erythematosus (SLE) patients. These immune complexes are regarded as important players in the pathogeneses of these diseases as they initiate and maintain the inflammatory cascade and tissue destruction. In addition, immune complex deposition in articular tissue has been reported to have harmful effects in many experimental models of arthritis. The passive transfer of antibodies to an autoantigen that is found in the joints is an established method for inducing arthritis. For example, antibodies to glucose-6-phosphate isomerase (GPI), a ubiquitous cytoplasmic enzyme, induce spontaneous arthritis upon injection into susceptible mice (Matsumoto et al., 2002), and the administration of a cocktail of anti-type II collagen antibodies to DBA/1 mice also invokes severe arthritis (Terato et al., 1992). However, it is particularly notable that these antibody-induced arthritis models are transient and fail to achieve chronicity (Myers et al., 1997), which suggests that a continuous supply of autoantibodies is required for the development of chronic joint inflammation.

In contrast to arthritis models, there are no lupus models that are invoked by the passive transfer of autoantibodies. However, plasma cell-depletion by a proteasome inhibitor clearly demonstrated the importance of a continuous supply of autoantibodies for systemic autoimmunity; i.e., treatment with bortezomib, a proteasome inhibitor, depleted the number of plasma cells producing antibodies to double stranded DNA; eliminated autoantibody production; ameliorated glomerulonephritis; and prolonged the survival of two lupus-prone mice strains, NZB/W F1, and MRL/*lpr* mice. Among five bortezomib-treated mice that displayed proteinuria of >100 mg/dl before treatment, four showed proteinuria of <100 mg/dl after treatment. These findings suggest that the suppression of autoantibody production leads to reduced organ inflammation in lupus (Neubert et al., 2008).

In addition to the production of autoantibodies by B cells, antibody-induced inflammation by itself is another target of therapeutic intervention. In mouse models of arthritis, the synthesized immune complexes bind to "inflammatory" Fc-receptors on intra-articular cells and then activate complement protein (Rowley et al., 2008). Complement fragments bound to immune complexes induce tissue injury, and FcR stimulation cumulatively activates mononuclear cells *in situ*, causing the activated cells to release pro-inflammatory cytokines. In turn, these responses attract neutrophils and macrophages, which can damage synoviocytes and chondrocytes. As mentioned above, immune complex-induced arthritis is a prototypic inflammatory process that is characterized by the release of pro-inflammatory cytokines and the activation of degradative enzymes. In a GPI-induced arthritis model, it was found that anti-GPI autoantibodies act through FcγRIII receptors and C5a (Ji et al., 2002). Micro-positron emission tomography studies revealed that the localization of anti-GPI antibodies is dependent on mast cells, neutrophils, Fc-receptors, and immune complexes (Mandik-Nayak and Allen, 2005). In anti-collagen antibody-induced arthritis (CAIA), complement activation, and

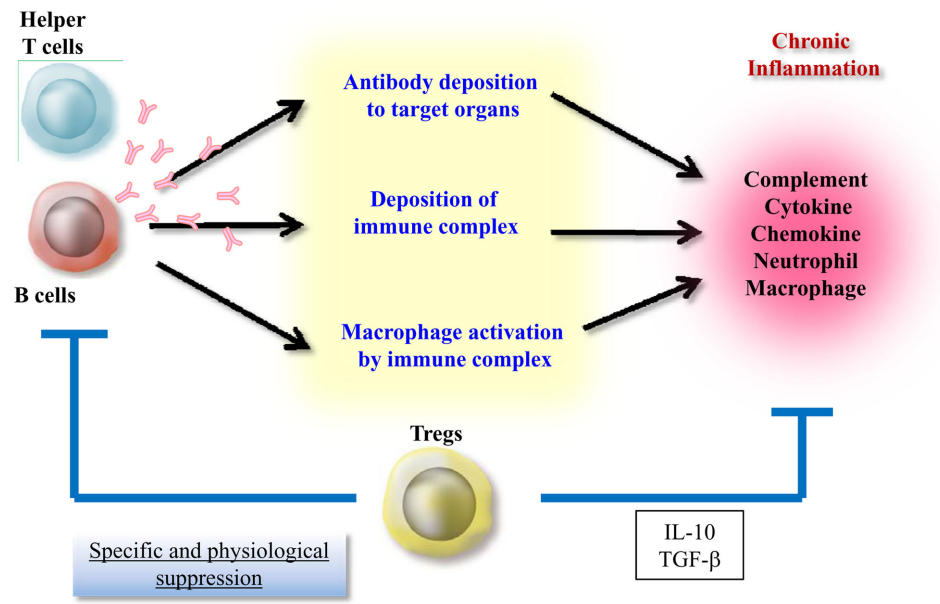


FIGURE 1 | Control of autoantibody-induced inflammation by regulatory T cells.

innate cells are also critical for the effector phase of arthritis (Hietala et al., 2004; Daha et al., 2011). With regard to systemic autoimmunity, MRL/*lpr* mice lacking factor B or factor D developed less severe nephritis than the control mice (Watanabe et al., 2000; Elliott et al., 2004). Factor B is cleaved by factor D and resulting catalytic subunit Bb forms C3 convertase. In addition, the anti-double stranded DNA antibody titer is not altered by factor D deficiency, indicating that complement activation is not required for the production of autoantibodies in MRL/*lpr* mice. Activated complement interacts with Fcγ receptors and complement receptors on innate effector cells (such as macrophages and monocytes) to induce local inflammation (Wasowska, 2010).

Therefore, autoantibody-induced inflammation can be separated into two components, autoantibody production and the local inflammatory response. Recently, accumulating evidence has shown that regulatory T cells (Treg) control both antibody production and the numbers and functions of effector cells such as innate cells and T helper cells. This article will discuss the Treg-mediated suppression of these two components during inflammation (**Figure 1**).

Treg-MEDIATED SUPPRESSION OF AUTOANTIBODY PRODUCTION

TWO MECHANISMS FOR AUTOANTIBODY PRODUCTION

In the course of thymus-dependent responses, B cells interact with T cells in the outer T cell zones of the lymphoid organs and differentiate along either the follicular or extrafollicular pathway (Lee et al., 2011). In the follicular pathway, activated B cells form germinal centers (GC) and undergo somatic hypermutation and selection. Subsequently, they exit GC as high-affinity long-lived plasma cells or memory B cells. In the extrafollicular pathway, B cells migrate to splenic bridging channels or junction zones and the borders between T cell zones and the red pulp or extramedullary lymph node cords. These migrated B cells form clusters of short-lived plasmablasts. Thus both the follicular and extrafollicular pathways contribute to autoantibody production in murine disease models.

Extrafollicular B cell response-mediated autoantibody production

William et al. (2002) observed that the splenic autoreactive B cells of autoimmune MRL/*lpr* mice proliferate and undergo active somatic hypermutation at the T zone-red pulp border rather than in GC. They examined the extrafollicular generation of plasmablasts in AM14 VH transgenic (Tg) mice, which possess rheumatoid factor (RF)-producing B cells with moderate affinity for IgG2a. Intriguingly, AM14 B cells on the MRL/*lpr* background spontaneously differentiate into extrafollicular plasmablasts and undergo somatic hypermutation at the T zone/red pulp border. In addition, they reported that the extrafollicular plasmablast response is induced by the administration of IgG2a anti-chromatin antibodies, which presumably form immune complexes *in vivo* with endogenous chromatin (Herlands et al., 2007). This response was found to be T cell independent, although it was totally dependent on MyD88 signaling downstream of Toll-like receptor 7 (TLR7) and TLR9 (Herlands et al., 2008). However, another study revealed that although AM14 B cells can be activated, differentiate, and undergo isotype-switching independent of antigen-specific T helper cells, T cells dramatically enhance the AM14 B cell response via CD40L and IL-21 signaling (Sweet et al., 2011).

GC-mediated autoantibody production

Because affinity-enhancing somatic hypermutations are prevalent in autoantibodies, it has long been hypothesized that these autoantibodies are derived from GC. Mouse strains that frequently develop autoimmune diseases (NZB/W F1, BXSB, MRL/*lpr*, sanroque, and NOD mice) spontaneously form GC-like structures in their spleens, and the onset of autoantibody production correlates with GC formation. Recently, several pieces of evidence have suggested that dysregulated T follicular helper (T_{FH}) cells significantly contribute to autoimmunity by inducing the aberrant selection of autoreactive B cells. The lupus-like disease that occurs in sanroque mice is caused by Roquin$^{san/san}$-induced accumulation of T_{FH} cells that maintain spontaneously formed GC (Vinuesa et al., 2005). The glomerulonephritis and pathogenic autoantibody production displayed by sanroque mice are ameliorated by *Bcl6* haploinsufficiency (Linterman et al., 2009). Moreover, SLAM-associated protein (SAP) deficiency experiments have highlighted the important roles played by T_{FH} cells in the conditions suffered by sanroque mice. SAP interacts with a conserved tyrosine-based motif that is found in the cytoplasmic tail of SLAM family members, and *Sh2d1a* (the gene for SAP) deficiency abrogates T_{FH} formation and GC responses, but not extrafollicular antibody responses. Since SAP deficiency ameliorates the lupus-like phenotype of sanroque mice, it can be assumed that aberrant T_{FH} cell activation is responsible for the autoimmunity that they display. BXSB mice develop a severe form of lupus caused by the *yaa* locus, which induces the overexpression of a cluster of X-linked genes that includes *Tlr7* gene. Although B6.*yaa* mice are not overtly autoimmune, the introduction of *Sle1*, which contains the autoimmune-predisposing *Slam/Cd2* haplotype, into their genome causes them to develop fetal lupus (Subramanian et al., 2006). Intriguingly, $CD4^+$ T cells from B6.*Sle1.yaa* mice develop the molecular signature of T_{FH} cells and also show altered expression levels of various cytokines and chemokines.

The source of human autoantibodies revealed by B cell-depletion therapy

As discussed above, dysregulation of the follicular or extrafollicular pathway can cause systemic autoimmune disease in mice. However, the contributions of follicular and extrafollicular checkpoints to the production of disease-associated autoantibodies are more difficult to evaluate in humans than in mice. Levels of autoantibodies do not always correlate with disease activity and response to treatment. For example, the serum concentrations of some autoantibodies correlate with disease activity (i.e., anti-double stranded DNA antibodies and anti-PR3 antibodies), while the titers of other autoantibodies [i.e., anti-ribonucleoprotein (RNP) antibodies and anti-Ro and La antibodies] remain stable irrespective of disease status. The heterogeneous autoantibody effects have also been observed in patients treated with anti-CD20 monoclonal antibody, which depletes B cells and plasmablasts but not long-lived plasma cells (Cambridge et al., 2003, 2006; Lu et al., 2009). In lupus patients, the levels of anti-nucleosome and anti-double stranded DNA antibodies are significantly decreased at 6–8 months after the administration of anti-CD20 monoclonal antibody. In contrast, the same treatment does not significantly alter the levels of anti-Ro, Sm, or RNP antibodies (Cambridge et al., 2006). This suggests that anti-nucleosome and anti-double stranded DNA antibodies are produced through extrafollicular responses, which usually generate short-lived plasma cells, while antibodies to nucleic acid-associated antigens (Ro, Sm, and RNP) are derived from follicular responses, which generate long-lived plasma cells. In RA patients, the levels of IgA-RF, IgG-RF, and IgG anti-cyclic citrullinated peptide (CCP) antibodies are decreased at 6 months after the administration of anti-CD20 monoclonal antibody, and the decreases are proportionately greater than the decreases in their respective total immunoglobulin classes (Cambridge et al., 2003). Plasmablasts and short-lived plasma cells originating from the extrafollicular response might be the major source of RF and anti-CCP antibodies (Looney et al., 2008). Therefore, both extrafollicular- and follicular-mediated antibody productions should be controlled in the treatment of human autoimmune inflammation.

APPROACHES TO AUTOANTIBODY SUPPRESSION

Antibody suppression with $CD4^+$ $CD25^+$ $Foxp3^+$ Treg

In general, T cells are indispensable sources of help signals, which promote B cell antibody production. Therefore, control of antibody production at the level of T cells is a rational approach to autoantibody suppression. Indeed, several T cell populations are able to suppress B cell antibody production. In humans, $CD4^+CD25^+CD69^-$ Treg that suppress antibody production *in vitro* have been found in GC. The fact that these $CD4^+CD25^+CD69^-$ Treg hardly express CXCR5 suggests that they mainly reside in the T cell-rich zones of secondary lymphoid tissues (Lim et al., 2004). However, T cell activation switches their chemokine receptor expression pattern from CCR7 to CXCR5 and switches their chemotactic responses from CCL19 to CXCL13. Thus, activation might change the migratory behavior of $CD4^+CD25^+CD69^-$ Treg so that they can migrate to GC. After migrating to GC, $CD4^+CD25^+CD69^-$ Treg negatively regulate T cell-dependent B cell responses through their suppressive activity toward T cells.

The preferential killing of antigen-presenting B cells by $CD4^+CD25^+$ Treg was reported in C57BL/6 mice (Zhao et al., 2006). B cell death is not mediated by the Fas–Fas ligand pathway, but instead is mediated by a granzyme-dependent, partially perforin-dependent pathway. Direct suppression of B cells by Treg was also reported in chronic systemic autoimmunity (Iikuni et al., 2009). For example, $CD4^+CD25^+$ Treg have been demonstrated to inhibit B cell antibody production in *in vitro* models of murine and human lupus. Treg use granule exocytosis pathways involving perforin and granzyme to induce contact-dependent apoptosis in B cells. However, in spite of the fact that $CD4^+CD25^+$ Treg from both young and old NZB/W F1 mice retain a capacity to suppress IgG production in B cells, autoantibodies continuously accumulate in these mice. Therefore, whether $CD4^+CD25^+$ Treg could be used to efficiently control autoantibody production in systemic autoimmunity needs to be examined further.

Recently, several groups simultaneously identified mice $CD4^+CD25^+Foxp3^+$ Treg subpopulations that are able to suppress B cell antibody production (Chung et al., 2011; Linterman et al., 2011; Wollenberg et al., 2011). Chung et al. (2011) identified a subset of Treg cells that express CXCR5 and Bcl6 and localize to GC in mice and humans. The expression of CXCR5 on Treg depends

on Bcl6, and CXCR5$^+$Bcl6$^+$ Treg are absent from the thymus but can be generated from CXCR5$^-$Foxp3$^+$ natural Treg precursors. A deficiency of CXCR5$^+$ Treg results in enhanced GC reactions involving B cells, affinity maturation of antibodies, and plasma cell differentiation. These results demonstrated that the Bcl6–CXCR5 axis of Treg is one mechanism by which GC responses are controlled. In addition, they observed that Foxp3-mutated scurfy mice display a moderate increase in their T_{FH} population but a markedly increased number of GL7$^+$CD95$^+$ B cells. Collectively, these observations suggest that Foxp3$^+$ follicular regulatory (T_{FR}) cells are more specialized for controlling the generation of GC B cells. Linterman et al. (2011) also described a population of Foxp3$^+$Blimp-1$^+$CD4$^+$ T cells that accounted for 10–25% of the CXCR5^{high}PD-1^{high}CD4$^+$ T cells found in immunized GC. In the absence of these T_{FR} cells, they noted outgrowths of non-antigen-specific B cells in GC and a decreased number of antigen-specific B cells. Therefore, both groups revealed that T_{FR} play a role in controlling GC reactions by inhibiting the selection of antigen-specific- and non-specific B cells. Because CXCR5-expressiong T_{FR} localize to GC, T_{FR} may suppress GC-mediated autoantibody production. However, whether T_{FR} actually suppress autoantibody production and whether T_{FR} deficiency results in autoimmunity remain to be addressed.

Antibody suppression with Qa-1 restricted CD8$^+$ Treg and other Treg subsets

A recent study reported that Qa-1 restricted CD8$^+$ Treg cells directly inhibit Qa-1$^+$ T_{FH} cells. Qa-1 is a non-classical MHC class Ib molecule presenting a peptide derived from the signal sequence of classical MHC class I proteins, named Qa-1 determinant modifier (Qdm), as well as peptides derived from proteins associated with infectious or inflammatory responses (Lu et al., 2006). Previously, a subpopulation of CD8$^+$ T cells was reported to suppress T cell help to B cells (Noble et al., 1998), and subsequent studies have shown that Qa-1 restricted CD8$^+$ T cells inhibit experimental autoimmune encephalomyelitis (EAE) by targeting autoreactive CD4$^+$ cells (Hu et al., 2004). Nevertheless, although Qa-1 deficient mice showed dysregulated immune responses to immunization with self and foreign antigens, Qa-1$^{-/-}$ mice do not develop spontaneous autoimmunity. Since Qa-1 interacts with both the T cell receptor (TCR) on CD8$^+$ T cells and the CD94/NKG2A receptor expressed by activated CD4$^+$ T cells, Qa-1 knock-in mice, B6 Qa-1(D227K) mice, were generated. B6 Qa-1 (D227K) mice harbor a Qa-1 amino acid exchange mutation that disrupts the binding of Qa-1 to the TCR/CD8 complex, but has no effect on its binding to the inhibitory NKG2A receptor. Intriguingly, the B6 Qa-1 (D227K) mice exhibit lupus-like systemic autoimmune disease and a five-fold to sixfold increase in their numbers of T_{FH} cells (Kim et al., 2010).

Analysis of the surface phenotype of Qa-1 restricted CD8$^+$ Treg indicated that they express CD44, ICOSL, and CXCR5 and the CD44$^+$ICOSL$^+$CD8$^+$ T cells inhibit the generation of high-affinity antibodies and Qa-1$^+$ T_{FH} cells. This observation provides a clue that might greatly increase our understanding of autoantibody production. However, the antigen-specificity of Qa-1 restricted CD8$^+$ Treg during T_{FH} cell suppression remains unclear because the repertoire of peptides presented by Qa-1 is substantially smaller than the repertoire of classical MHC molecules (Lu et al., 2006). Only a small number of peptides have been identified that bind to Qa-1 and stimulate CD8$^+$ T cells, including dominant Qdm as well as peptides from HSP60, insulin, *Salmonella* GroEL, and TCR Vβ chains. Thus, Qa-1 restricted CD8$^+$ Treg might suppress T_{FH} cells irrespective of the antigen-specificity of the TCR on T_{FH} cells. Because Qa-1 restricted CD8$^+$ Treg express CXCR5 and migrate to lymphoid follicles (Kim et al., 2010), Qa-1 restricted CD8$^+$ Treg may suppress GC-mediated autoantibody production.

T_{FR} and Qa-1 restricted CD8$^+$ Treg appear to be important checking mechanisms for antibody production. However, no Treg populations that control autoantibody production and autoimmunity in an antigen-specific manner have yet been identified. Although the importance of T regulatory type I (Tr1) cells for controlling immune responses has been described in a number of reports, anti-CD46-induced IL-10-secreting T cells even enhance antibody production by B cells (Fuchs et al., 2009). Recently, several CD4$^+$ T cell populations that possess regulatory activity have been identified (Fujio et al., 2010). CD4$^+$CD25$^-$LAP$^+$ T cells and CD4$^+$NKG2D$^+$ T cells produce both IL-10 and TGF-β (Oida et al., 2003; Dai et al., 2009), and CD4$^+$CD25$^-$IL-7R$^-$ T cells and CD4$^+$CD25$^-$LAG3$^+$ T cells produce large amounts of IL-10 (Haringer et al., 2009; Okamura et al., 2009). The association between these recently identified Treg and antigen-specific autoantibody suppression should be investigated. In particular, CD4$^+$CD25$^-$LAG3$^+$ T cells, which characteristically express the anergy-linked transcription factor Egr2, might be associated with autoantibody suppression, because T cell-specific Egr2-deficient mice exhibit lupus-like disease (Zhu et al., 2008) and polymorphisms in the EGR2 gene are associated with human SLE susceptibility (Myouzen et al., 2010). Although both T_{FR} cells and Qa-1 restricted CD8$^+$ Treg express CXCR5 and may suppress GC-mediated autoantibody production, Treg populations which suppress extrafollicular response are yet to be identified.

Treg-MEDIATED SUPPRESSION OF LOCAL INFLAMMATION

IL-10-MEDIATED SUPPRESSION OF INFLAMMATION

Nguyen et al. (2007) reported a role of CD4$^+$CD25$^+$Foxp3$^+$ Treg in antibody-induced arthritis at several levels. They examined the effect of the *scurfy* loss of function mutation of the *Foxp3* gene in K/BxN mouse model. These mice carry the KRN transgene, which encodes a TCR reactive against a peptide from GPI and the autoreactive T cells promote the production of vast quantities of anti-GPI antibodies, which are sufficient to induce arthritis after transfer into normal recipients (Korganow et al., 1999). The absence of CD4$^+$CD25$^+$Foxp3$^+$ Treg led to more accelerated aggressive arthritis with significantly earlier autoantibody production. However, the broadened spectrum of affected joints in *Foxp3*-mutated K/BxN mice was not due to the earlier appearance of autoantibodies and could not be reproduced by increasing anti-GPI antibody load. Therefore, CD4$^+$CD25$^+$Foxp3$^+$ Treg are supposed to play a role in effector phase manifestations. Their another observation that Foxp3$^+$ Treg accumulated in inflamed joint of K/BxN serum-transferred B6 mice suggested that Foxp3$^+$ Treg actively migrate to the site of antibody-induced inflammation and control

the local inflammatory process. Although the mechanism of this Foxp3$^+$ Treg-mediated suppression was not clarified, IL-10 was mentioned as a candidate mediator.

Furthermore, a single transfer of CD4$^+$CD25$^+$ Treg markedly slowed the progression of collagen-induced arthritis (CIA), which could not be attributed to the loss of systemic type II collagen-specific T and B cell responses (Morgan et al., 2005). The transferred CD4$^+$CD25$^+$ Treg were found in the inflamed synovium soon after the transfer, indicating that regulation occurs locally in the joints. It is unlikely that the transferred CD4$^+$CD25$^+$ Treg acted against CIA solely via the suppression of T cell immunity involving the Th17 cell response since the effector phase of CIA depends on T cell-independent immune responses (Ehinger et al., 2001). Thus, the transferred CD4$^+$CD25$^+$ Treg might have interacted with local innate cells as well as effector T cells.

CD4$^+$CD25$^+$ Treg-mediated control of innate cells was found to be IL-10 and TGF-β dependent in a colitis model (Maloy et al., 2003). Indeed, IL-10 production might be a key factor controlling local inflammation. Human IL-10 suppresses the expression of MHC class II, co-stimulatory, and adhesion molecules (De Waal Malefyt et al., 1991; Willems et al., 1994). IL-10 also inhibits the production of inflammatory cytokines and the T cell stimulating capacity of antigen-presenting cells (APC; Fiorentino et al., 1991; Allavena et al., 1998), and local IL-10 production has been shown to suppress TNF-α and IL-1α production (Lubberts et al., 2000). CD4$^+$CD25$^+$ Treg downregulate the expression of co-stimulatory molecules on APC (Cederbom et al., 2000) and restrain the maturation and antigen-presenting function of dendritic cells in an IL-10-dependent manner (Misra et al., 2004; Houot et al., 2006). Furthermore, IL-10 was recently reported to suppress Th17 cells (Huber et al., 2011). Interestingly, both CD4$^+$Foxp3$^+$ Treg and CD4$^+$Foxp3$^-$IL-10-producing cells (Tr1) are able to control Th17 cell numbers in an IL-10-dependent manner. Therefore, it was suggested that Tr1 cells can compensate for a paucity of Foxp3$^+$ Treg and vice versa during the suppression of innate and Th17 cells.

Tr1 cells are considered to be different from Th1, Th2, and Th17 cells based on their cytokine production profile; i.e., they secrete high levels of IL-10. Tr1 cells are inducible *in vitro* and *in vivo*, and they can also be isolated from humans and mice in steady state conditions (Roncarolo et al., 2011). Tr1 cells are able to suppress Th1-mediated colitis induced by the transfer of naïve CD4$^+$CD45RB^{hi} cells into SCID mice as well as EAE (Roncarolo et al., 2001). Although few reports have directly compared IL-10 production between CD4$^+$CD25$^+$ Treg and Tr1-like cells, CD4$^+$CD25$^-$LAG3$^+$ Treg secrete significantly higher amounts of IL-10 than CD4$^+$CD25$^+$ Treg (Okamura et al., 2009). Thus, Tr1 cells and Tr1-like cells might have the ability to control innate immune cells.

SUPPRESSION OF T CELL CYTOKINE PRODUCTION

In several antibody-induced autoimmune inflammations such as RA-synovitis and lupus nephritis, co-existence of antibody deposition and T cell infiltration is frequently observed. RA is a prototypic autoimmune disease characterized by chronic joint inflammation and the production of cytokines, including TNF-α, IL-6, IL-15, IL-17, and IL-1β. These cytokines are thought to be derived from both innate cells and effector T cells. In the K/BxN arthritis model, T cells can augment antibody-induced arthritis independently of their influence on antibody production (Jacobs et al., 2009). This enhancement was mediated by IL-17 producing CD4$^+$ T cells preferentially recruited to the environment of the arthritic joint. Therefore, Treg-mediated suppression of effector T cells may be also beneficial in controlling autoantibody-induced inflammation accompanied with T cell infiltration. In the past, IFN-γ producing Th1 cells were thought to be the principal mediators of autoimmune inflammation such as that observed in RA. However, IL-17 has emerged as a key driver of inflammation and is detectable in the RA synovium. IL-17 and IL-17F promote inflammation on several levels, as their receptors IL-17RA and IL-17RC are expressed on both hematopoietic and non-hematopoietic cells. IL-17 and IL-17F induce the production of pro-inflammatory cytokines like IL-6, IL-1β, and TNF-α, and pro-inflammatory chemokines such as CXCL1, GCP-2, and IL-8 and thus promote tissue inflammation and neutrophil recruitment at sites of inflammation (Bettelli et al., 2008).

CD4$^+$CD25$^+$ Treg not only suppress the proliferation of conventional T cells, but also their production of inflammatory cytokines, such as TNF-α and IFN-γ. In contrast, IL-17 production is not suppressed when human CD4$^+$CD25$^+$ Treg are added to responder T cells *in vitro* (Annunziato et al., 2008; Flores-Borja et al., 2008), and murine CD4$^+$CD25$^+$ Treg promote Th17 cell development both *in vitro* and *in vivo* (Chen et al., 2011; Pandiyan et al., 2011). As IL-17 is important for infection control, the resistance of Th17 cells to suppression by CD4$^+$CD25$^+$ Treg cells makes sense. However, in a previous study CD4$^+$CD25$^+$Foxp3$^+$ Treg-specific ablation of STAT3 resulted in the development of fetal intestinal inflammation due to the loss of Th17 cell suppression in mice (Chaudhry et al., 2009). Moreover, other studies have suggested that some subpopulations of CD4$^+$CD25$^+$ Treg cells are capable of regulating Th17 cell responses. For example, CD4$^+$CD25$^+$ Treg expressing CD39 (an ectonucleotidase that hydrolyzes ATP) were reported to be able to suppress Th17 cell responses (Fletcher et al., 2009). In addition, CD4$^+$CD25$^+$CD39$^+$ Treg numbers are reduced in patients with multiple sclerosis (MS), suggesting that an association exists between this Treg population and the suppression of pathogenic Th17 cells. Therefore, at least some CD4$^+$CD25$^+$ Treg are suspected to suppress the production of inflammatory cytokines in inflamed organs.

EAE is an animal model of MS that is induced by the injection of myelin components. Until recently, the pathogeneses of MS and EAE were thought to be initiated by myelin-specific Th1 cells. However, a number of lines of evidence have indicated that Th17 cells induce central nervous system (CNS) inflammation (Oukka, 2007). For example, it was reported that the Th17:Th1 ratio of infiltrating T cells in EAE determines where inflammation occurs in the CNS (Stromnes et al., 2008), and T cell infiltration and inflammation in the brain parenchyma only occur when Th17 cells outnumber Th1 cells and trigger a disproportionate increase in IL-17 expression in the brain. In contrast, T cells showing a wide range of Th17:Th1 ratios induce spinal cord parenchymal inflammation. Tg mice bearing

a TCR against the myelin basic protein (MBP) that had been crossed with recombination-activating gene 1 (*Rag1*)-deficient mice (Tg MBP/$Rag^{-/-}$) developed spontaneous EAE, whereas Tg MBP/$Rag^{+/+}$ mice did not (Lafaille et al., 1994). This discrepancy can be explained by the existence of Treg in the $Rag^{+/+}$ mice but not the $Rag^{-/-}$ mice because the adoptive transfer of $CD4^+CD25^+$ Treg from wild-type mice to Tg MBP/$Rag^{-/-}$ mice prevented the development of spontaneous EAE (Hori et al., 2002). Moreover, adoptive transfer experiments have revealed that transferring large numbers of $CD4^+CD25^+$ Treg purified from the peripheral lymph nodes of naive mice reduced the incidence and severity of EAE (Kohm et al., 2003). In a study conducted by Matsumoto et al. (2007), peripheral $CD4^+CD25^+$ Treg from mice with EAE suppressed the development of chronic EAE in the recipient rats. Therefore, $CD4^+CD25^+Foxp3^+$ Treg apparently have the capability to suppress T helper cell-mediated organ inflammation, and this effect may be beneficial in the control of the antibody-induced inflammation accompanied with effector T cell infiltration.

CONCLUSION

The current standard treatment for autoimmune disease is non-specific immunosuppression with steroids and immunosuppressants, which inevitably leads to opportunistic infections. As autoantibodies are key components in the development of autoimmune inflammation, targeting autoantibody-induced immunity is a rational approach to the treatment of autoimmune diseases. The modulation of Treg function is a promising physiological approach to suppressing both autoantibody production and autoantibody-induced local inflammation. Further examinations of $CD4^+CD25^+$ Treg and other Treg subsets are necessary in future.

ACKNOWLEDGMENTS

This work was supported by grants from the Japan Society for the Promotion of Science, Ministry of Health, Labor and Welfare, and the Ministry of Education, Culture, Sports, Science, and Technology (MEXT; in part by Global COE Program Chemical Biology of the Diseases, by the MEXT), Japan.

REFERENCES

Allavena, P., Piemonti, L., Longoni, D., Bernasconi, S., Stoppacciaro, A., Ruco, L., and Mantovani, A. (1998). IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. *Eur. J. Immunol.* 28, 359–369.

Annunziato, F., Cosmi, L., Liotta, F., Maggi, E., and Romagnani, S. (2008). The phenotype of human Th17 cells and their precursors, the cytokines that mediate their differentiation and the role of Th17 cells in inflammation. *Int. Immunol.* 20, 1361–1368.

Bettelli, E., Korn, T., Oukka, M., and Kuchroo, V. K. (2008). Induction and effector functions of T(H)17 cells. *Nature* 453, 1051–1057.

Cambridge, G., Leandro, M. J., Edwards, J. C., Ehrenstein, M. R., Salden, M., Bodman-Smith, M., and Webster, A. D. (2003). Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. *Arthritis Rheum.* 48, 2146–2154.

Cambridge, G., Leandro, M. J., Teodorescu, M., Manson, J., Rahman, A., Isenberg, D. A., and Edwards, J. C. (2006). B cell depletion therapy in systemic lupus erythematosus: effect on autoantibody and antimicrobial antibody profiles. *Arthritis Rheum.* 54, 3612–3622.

Cederbom, L., Hall, H., and Ivars, F. (2000). $CD4^+CD25^+$ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. *Eur. J. Immunol.* 30, 1538–1543.

Chaudhry, A., Rudra, D., Treuting, P., Samstein, R. M., Liang, Y., Kas, A., and Rudensky, A. Y. (2009). $CD4^+$ regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science* 326, 986–991.

Chen, Y., Haines, C. J., Gutcher, I., Hochweller, K., Blumenschein, W. M., Mcclanahan, T., Hammerling, G., Li, M. O., Cua, D. J., and Mcgeachy, M. J. (2011). Foxp3(+) regulatory T cells promote T helper 17 cell development in vivo through regulation of interleukin-2. *Immunity* 34, 409–421.

Chung, Y., Tanaka, S., Chu, F., Nurieva, R. I., Martinez, G. J., Rawal, S., Wang, Y. H., Lim, H., Reynolds, J. M., Zhou, X. H., Fan, H. M., Liu, Z. M., Neelapu, S. S., and Dong, C. (2011). Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat. Med.* 17, 983–988.

Daha, N. A., Banda, N. K., Roos, A., Beurskens, F. J., Bakker, J. M., Daha, M. R., and Trouw, L. A. (2011). Complement activation by (auto-) antibodies. *Mol. Immunol.* 48, 1656–1665.

Dai, Z., Turtle, C. J., Booth, G. C., Riddell, S. R., Gooley, T. A., Stevens, A. M., Spies, T., and Groh, V. (2009). Normally occurring $NKG2D^+CD4^+$ T cells are immunosuppressive and inversely correlated with disease activity in juvenile-onset lupus. *J. Exp. Med.* 206, 793–805.

De Waal Malefyt, R., Haanen, J., Spits, H., Roncarolo, M. G., Te Velde, A., Figdor, C., Johnson, K., Kastelein, R., Yssel, H., and De Vries, J. E. (1991). Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J. Exp. Med.* 174, 915–924.

Ehinger, M., Vestberg, M., Johansson, A. C., Johannesson, M., Svensson, A., and Holmdahl, R. (2001). Influence of CD4 or CD8 deficiency on collagen-induced arthritis. *Immunology* 103, 291–300.

Elliott, M. K., Jarmi, T., Ruiz, P., Xu, Y., Holers, V. M., and Gilkeson, G. S. (2004). Effects of complement factor D deficiency on the renal disease of MRL/lpr mice. *Kidney Int.* 65, 129–138.

Fiorentino, D. F., Zlotnik, A., Mosmann, T. R., Howard, M., and O'Garra, A. (1991). IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147, 3815–3822.

Fletcher, J. M., Lonergan, R., Costelloe, L., Kinsella, K., Moran, B., O'Farrelly, C., Tubridy, N., and Mills, K. H. (2009). $CD39^+Foxp3^+$ regulatory T cells suppress pathogenic Th17 cells and are impaired in multiple sclerosis. *J. Immunol.* 183, 7602–7610.

Flores-Borja, F., Jury, E. C., Mauri, C., and Ehrenstein, M. R. (2008). Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 105, 19396–19401.

Fuchs, A., Atkinson, J. P., Fremeaux-Bacchi, V., and Kemper, C. (2009). CD46-induced human Treg enhance B-cell responses. *Eur. J. Immunol.* 39, 3097–3109.

Fujio, K., Okamura, T., and Yamamoto, K. (2010). The family of IL-10-secreting $CD4^+$ T cells. *Adv. Immunol.* 105, 99–130.

Haringer, B., Lozza, L., Steckel, B., and Geginat, J. (2009). Identification and characterization of IL-10/IFN-gamma-producing effector-like T cells with regulatory function in human blood. *J. Exp. Med.* 206, 1009–1017.

Herlands, R. A., Christensen, S. R., Sweet, R. A., Hershberg, U., and Shlomchik, M. J. (2008). T cell-independent and toll-like receptor-dependent antigen-driven activation of autoreactive B cells. *Immunity* 29, 249–260.

Herlands, R. A., William, J., Hershberg, U., and Shlomchik, M. J. (2007). Anti-chromatin antibodies drive in vivo antigen-specific activation and somatic hypermutation of rheumatoid factor B cells at extrafollicular sites. *Eur. J. Immunol.* 37, 3339–3351.

Hietala, M. A., Nandakumar, K. S., Persson, L., Fahlen, S., Holmdahl, R., and Pekna, M. (2004). Complement activation by both classical and alternative pathways is critical for the effector phase of arthritis. *Eur. J. Immunol.* 34, 1208–1216.

Hori, S., Haury, M., Coutinho, A., and Demengeot, J. (2002). Specificity requirements for selection and effector functions of $CD25^+4^+$ regulatory T cells in anti-myelin basic protein T cell receptor transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* 99, 8213–8218.

Houot, R., Perrot, I., Garcia, E., Durand, I., and Lebecque, S. (2006). Human $CD4^+$CD25high regulatory T cells

modulate myeloid but not plasmacytoid dendritic cells activation. *J. Immunol.* 176, 5293–5298.

Hu, D., Ikizawa, K., Lu, L., Sanchirico, M. E., Shinohara, M. L., and Cantor, H. (2004). Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat. Immunol.* 5, 516–523.

Huber, S., Gagliani, N., Esplugues, E., O'Connor, W. Jr., Huber, F. J., Chaudhry, A., Kamanaka, M., Kobayashi, Y., Booth, C. J., Rudensky, A. Y., Roncarolo, M. G., Battaglia, M., and Flavell, R. A. (2011). Th17 cells express interleukin-10 receptor and are controlled by Foxp3 and Foxp3$^+$ regulatory CD4$^+$ T cells in an interleukin-10-dependent manner. *Immunity* 34, 554–565.

Iikuni, N., Lourenco, E. V., Hahn, B. H., and La Cava, A. (2009). Cutting edge: regulatory T cells directly suppress B cells in systemic lupus erythematosus. *J. Immunol.* 183, 1518–1522.

Jacobs, J. P., Wu, H. J., Benoist, C., and Mathis, D. (2009). IL-17-producing T cells can augment autoantibody-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21789–21794.

Ji, H., Ohmura, K., Mahmood, U., Lee, D. M., Hofhuis, F. M., Boackle, S. A., Takahashi, K., Holers, V. M., Walport, M., Gerard, C., Ezekowitz, A., Carroll, M. C., Brenner, M., Weissleder, R., Verbeek, J. S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (2002). Arthritis critically dependent on innate immune system players. *Immunity* 16, 157–168.

Kim, H. J., Verbinnen, B., Tang, X., Lu, L., and Cantor, H. (2010). Inhibition of follicular T-helper cells by CD8(+) regulatory T cells is essential for self tolerance. *Nature* 467, 328–332.

Kohm, A. P., Carpentier, P. A., and Miller, S. D. (2003). Regulation of experimental autoimmune encephalomyelitis (EAE) by CD4$^+$CD25$^+$ regulatory T cells. *Novartis Found. Symp.* 252, 45–52; discussion 52–54, 106–114.

Korganow, A. S., Ji, H., Mangialaio, S., Duchatelle, V., Pelanda, R., Martin, T., Degott, C., Kikutani, H., Rajewsky, K., Pasquali, J. L., Benoist, C., and Mathis, D. (1999). From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity* 10, 451–461.

Lafaille, J. J., Nagashima, K., Katsuki, M., and Tonegawa, S. (1994). High incidence of spontaneous autoimmune encephalomyelitis in immunodeficient anti-myelin basic protein T cell receptor transgenic mice. *Cell* 78, 399–408.

Lee, S. K., Rigby, R. J., Zotos, D., Tsai, L. M., Kawamoto, S., Marshall, J. L., Ramiscal, R. R., Chan, T. D., Gatto, D., Brink, R., Yu, D., Fagarasan, S., Tarlinton, D. M., Cunningham, A. F., and Vinuesa, C. G. (2011). B cell priming for extrafollicular antibody responses requires Bcl-6 expression by T cells. *J. Exp. Med.* 208, 1377–1388.

Lim, H. W., Hillsamer, P., and Kim, C. H. (2004). Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses. *J. Clin. Invest.* 114, 1640–1649.

Linterman, M. A., Pierson, W., Lee, S. K., Kallies, A., Kawamoto, S., Rayner, T. F., Srivastava, M., Divekar, D. P., Beaton, L., Hogan, J. J., Fagarasan, S., Liston, A., Smith, K. G., and Vinuesa, C. G. (2011). Foxp3(+) follicular regulatory T cells control the germinal center response. *Nat. Med.* 17, 975–982.

Linterman, M. A., Rigby, R. J., Wong, R. K., Yu, D., Brink, R., Cannons, J. L., Schwartzberg, P. L., Cook, M. C., Walters, G. D., and Vinuesa, C. G. (2009). Follicular helper T cells are required for systemic autoimmunity. *J. Exp. Med.* 206, 561–576.

Looney, R. J., Srinivasan, R., and Calabrese, L. H. (2008). The effects of rituximab on immunocompetency in patients with autoimmune disease. *Arthritis Rheum.* 58, 5–14.

Lu, L., Werneck, M. B., and Cantor, H. (2006). The immunoregulatory effects of Qa-1. *Immunol. Rev.* 212, 51–59.

Lu, T. Y., Ng, K. P., Cambridge, G., Leandro, M. J., Edwards, J. C., Ehrenstein, M., and Isenberg, D. A. (2009). A retrospective seven-year analysis of the use of B cell depletion therapy in systemic lupus erythematosus at University College London Hospital: the first fifty patients. *Arthritis Rheum.* 61, 482–487.

Lubberts, E., Joosten, L. A., Van Den Bersselaar, L., Helsen, M. M., Bakker, A. C., Xing, Z., Richards, C. D., and Van Den Berg, W. B. (2000). Intra-articular IL-10 gene transfer regulates the expression of collagen-induced arthritis (CIA) in the knee and ipsilateral paw. *Clin. Exp. Immunol.* 120, 375–383.

Maloy, K. J., Salaun, L., Cahill, R., Dougan, G., Saunders, N. J., and Powrie, F. (2003). CD4$^+$CD25$^+$ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *J. Exp. Med.* 197, 111–119.

Mandik-Nayak, L., and Allen, P. M. (2005). Initiation of an autoimmune response: insights from a transgenic model of rheumatoid arthritis. *Immunol. Res.* 32, 5–13.

Matsumoto, I., Maccioni, M., Lee, D. M., Maurice, M., Simmons, B., Brenner, M., Mathis, D., and Benoist, C. (2002). How antibodies to a ubiquitous cytoplasmic enzyme may provoke joint-specific autoimmune disease. *Nat. Immunol.* 3, 360–365.

Matsumoto, Y., Sakuma, H., Kohyama, K., and Park, I. K. (2007). Paralysis of CD4(+)CD25(+) regulatory T cell response in chronic autoimmune encephalomyelitis. *J. Neuroimmunol.* 187, 44–54.

Misra, N., Bayry, J., Lacroix-Desmazes, S., Kazatchkine, M. D., and Kaveri, S. V. (2004). Cutting edge: human CD4$^+$CD25$^+$ T cells restrain the maturation and antigen-presenting function of dendritic cells. *J. Immunol.* 172, 4676–4680.

Morgan, M. E., Flierman, R., Van Duivenvoorde, L. M., Witteveen, H. J., Van Ewijk, W., Van Laar, J. M., De Vries, R. R., and Toes, R. E. (2005). Effective treatment of collagen-induced arthritis by adoptive transfer of CD25$^+$ regulatory T cells. *Arthritis Rheum.* 52, 2212–2221.

Myers, L. K., Rosloniec, E. F., Cremer, M. A., and Kang, A. H. (1997). Collagen-induced arthritis, an animal model of autoimmunity. *Life Sci.* 61, 1861–1878.

Myouzen, K., Kochi, Y., Shimane, K., Fujio, K., Okamura, T., Okada, Y., Suzuki, A., Atsumi, T., Ito, S., Takada, K., Mimori, A., Ikegawa, S., Yamada, R., Nakamura, Y., and Yamamoto, K. (2010). Regulatory polymorphisms in EGR2 are associated with susceptibility to systemic lupus erythematosus. *Hum. Mol. Genet.* 19, 2313–2320.

Neubert, K., Meister, S., Moser, K., Weisel, F., Maseda, D., Amann, K., Wiethe, C., Winkler, T. H., Kalden, J. R., Manz, R. A., and Voll, R. E. (2008). The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. *Nat. Med.* 14, 748–755.

Nguyen, L. T., Jacobs, J., Mathis, D., and Benoist, C. (2007). Where FoxP3-dependent regulatory T cells impinge on the development of inflammatory arthritis. *Arthritis Rheum.* 56, 509–520.

Noble, A., Zhao, Z. S., and Cantor, H. (1998). Suppression of immune responses by CD8 cells. II. Qa-1 on activated B cells stimulates CD8 cell suppression of T helper 2 responses. *J. Immunol.* 160, 566–571.

Oida, T., Zhang, X., Goto, M., Hachimura, S., Totsuka, M., Kaminogawa, S., and Weiner, H. L. (2003). CD4$^+$CD25- T cells that express latency-associated peptide on the surface suppress CD4$^+$CD45RBhigh-induced colitis by a TGF-beta-dependent mechanism. *J. Immunol.* 170, 2516–2522.

Okamura, T., Fujio, K., Shibuya, M., Sumitomo, S., Shoda, H., Sakaguchi, S., and Yamamoto, K. (2009). CD4$^+$CD25-LAG3$^+$ regulatory T cells controlled by the transcription factor Egr-2. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13974–13979.

Oukka, M. (2007). Interplay between pathogenic Th17 and regulatory T cells. *Ann. Rheum. Dis.* 66(Suppl. 3), iii87–iii90.

Pandiyan, P., Conti, H. R., Zheng, L., Peterson, A. C., Mathern, D. R., Hernandez-Santos, N., Edgerton, M., Gaffen, S. L., and Lenardo, M. J. (2011). CD4(+)CD25(+)Foxp3(+) regulatory T cells promote Th17 cells in vitro and enhance host resistance in mouse *Candida albicans* Th17 cell infection model. *Immunity* 34, 422–434.

Roncarolo, M. G., Bacchetta, R., Bordignon, C., Narula, S., and Levings, M. K. (2001). Type 1 T regulatory cells. *Immunol. Rev.* 182, 68–79.

Roncarolo, M. G., Gregori, S., Lucarelli, B., Ciceri, F., and Bacchetta, R. (2011). Clinical tolerance in allogeneic hematopoietic stem cell transplantation. *Immunol. Rev.* 241, 145–163.

Rowley, M. J., Nandakumar, K. S., and Holmdahl, R. (2008). The role of collagen antibodies in mediating arthritis. *Mod. Rheumatol.* 18, 429–441.

Stromnes, I. M., Cerretti, L. M., Liggitt, D., Harris, R. A., and Goverman, J. M. (2008). Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. *Nat. Med.* 14, 337–342.

Subramanian, S., Tus, K., Li, Q. Z., Wang, A., Tian, X. H., Zhou, J., Liang, C., Bartov, G., Mcdaniel, L. D., Zhou, X. J., Schultz, R. A., and Wakeland, E. K. (2006). A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. *Proc. Natl. Acad. Sci. U.S.A.* 103, 9970–9975.

Sweet, R. A., Ols, M. L., Cullen, J. L., Milam, A. V., Yagita, H., and Shlomchik, M. J. (2011). Facultative role for T cells in extrafollicular Toll-like receptor-dependent autoreactive B-cell responses in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7932–7937.

Terato, K., Hasty, K. A., Reife, R. A., Cremer, M. A., Kang, A. H., and

Stuart, J. M. (1992). Induction of arthritis with monoclonal antibodies to collagen. *J. Immunol.* 148, 2103–2108.

Vinuesa, C. G., Cook, M. C., Angelucci, C., Athanasopoulos, V., Rui, L., Hill, K. M., Yu, D., Domaschenz, H., Whittle, B., Lambe, T., Roberts, I. S., Copley, R. R., Bell, J. I., Cornall, R. J., and Goodnow, C. C. (2005). A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 435, 452–458.

Wasowska, B. A. (2010). Mechanisms involved in antibody- and complement-mediated allograft rejection. *Immunol. Res.* 47, 25–44.

Watanabe, H., Garnier, G., Circolo, A., Wetsel, R. A., Ruiz, P., Holers, V. M., Boackle, S. A., Colten, H. R., and Gilkeson, G. S. (2000). Modulation of renal disease in MRL/lpr mice genetically deficient in the alternative complement pathway factor B. *J. Immunol.* 164, 786–794.

Willems, F., Marchant, A., Delville, J. P., Gerard, C., Delvaux, A., Velu, T., De Boer, M., and Goldman, M. (1994). Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur. J. Immunol.* 24, 1007–1009.

William, J., Euler, C., Christensen, S., and Shlomchik, M. J. (2002). Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. *Science* 297, 2066–2070.

Wollenberg, I., Agua-Doce, A., Hernandez, A., Almeida, C., Oliveira, V. G., Faro, J., and Graca, L. (2011). Regulation of the germinal center reaction by Foxp3$^+$ follicular regulatory T cells. *J. Immunol.* 187, 4553–4560.

Zhao, D. M., Thornton, A. M., Dipaolo, R. J., and Shevach, E. M. (2006). Activated CD4$^+$CD25$^+$ T cells selectively kill B lymphocytes. *Blood* 107, 3925–3932.

Zhu, B., Symonds, A. L., Martin, J. E., Kioussis, D., Wraith, D. C., Li, S., and Wang, P. (2008). Early growth response gene 2 (Egr-2) controls the self-tolerance of T cells and prevents the development of lupus like autoimmune disease. *J. Exp. Med.* 205, 2295–2307.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 December 2011; paper pending published: 04 January 2012; accepted: 10 February 2012; published online: 27 February 2012.

*Citation: Fujio K, Okamura T, Sumitomo S and Yamamoto K (2012) Regulatory T cell-mediated control of autoantibody-induced inflammation. Front. Immun. **3**:28. doi: 10.3389/fimmu.2012.00028*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in IMMUNOLOGY

HYPOTHESIS AND THEORY ARTICLE
published: 22 February 2012
doi: 10.3389/fimmu.2012.00018

The ambiguity in immunology

Vincenzo Barnaba[1]*, Marino Paroli[2] and Silvia Piconese[1]

[1] *Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma, Rome, Italy*
[2] *Dipartimento di Scienze e Biotecnologie Medico-Chirurgiche, Sapienza Università di Roma, Rome, Italy*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Silvano Sozzani, University of Brescia, Italy
Arnaud Hot, Hopital Edouard Herriot, France

***Correspondence:**
Vincenzo Barnaba, Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma, Viale del Policlinico 155, Rome 00161, Italy.
e-mail: vincenzo.barnaba@uniroma1.it

In the present article, we discuss the various ambiguous aspects of the immune system that render this complex biological network so highly flexible and able to defend the host from different external invaders. This ambiguity stems mainly from the property of the immune system to be both protective and harmful. Immunity cannot be fully protective without producing a certain degree of damage (immunopathology) to the host. The balance between protection and tissue damage is, therefore, critical for the establishment of immune homeostasis and protection. In this review, we will consider as ambiguous, various immunological tactics including: (a) the opposing functions driving immune responses, immune-regulation, and contra-regulation, as well as (b) the phenomenon of chronic immune activation as a result of a continuous cross-presentation of apoptotic T cells by dendritic cells. All these plans participate principally to maintain a state of chronic low-level inflammation during persisting infections, and ultimately to favor the species survival.

Keywords: ambiguity, homeostasis, inflammation, autoimmunity, cancer

INTRODUCTION

To better understand the mechanisms underlying the immune response, immunologists have attempted to establish some general rules of how pathogens are fought and possibly defeated. One of the predominant views has considered the immune response as a network where different types of specialized immune cells interact with each other to ensure host survival. This "cooperative" interpretation has been recently challenged by the observation that the immune system is committed not only to attacking "invaders," but also to suppressing the ongoing immune response, most likely to limit excessive immunopathology. The concept that the immune response might act to maintain a homeostatic balance between aggression and suppression has, therefore, emerged. It has been also hypothesized that the inhibitory activity of the immune system is the predominant one. For example, the immune system allows bacteria of the intestinal flora to survive and to produce substances (e.g., vitamin B12) that are essential for the well-being of the host (Barthlott et al., 2003; Coombes et al., 2005). However, even this hypothesis does not appear to be completely satisfying. The possible emergence of self-reactive immune responses, of useless or even harmful antibodies, of chronic inflammatory infectious diseases represents part of the "ambiguous" aspects of the immune system. We will, therefore, discuss how this ambiguity has paradoxically evolved to favor host survival, and how the immune system represents a valid biological model to highlight the evolutionistic value of the ambiguity itself.

The etymology of the term *ambiguity* is derived from Latin *ambagere*, which means the ability "to push something in different directions" or "to be understood in different ways." Semantics identifies ambiguity with the polysemy (from the Greek word: *polysemos*), which is the property of a word to express more than one meaning (Jakobson, 1995). In artwork, ambiguity is a critical element of esthetical information. Aristotle, the Greek philosopher, celebrated that no human work can be defined as "art" in the absence of a certain degree of ambiguity (Hintikka, 1959). In philosophy, ambiguity has been recently considered as "a productive element that is both against and beyond any metaphysical dogma, passing through the crisis of subjectivity" or, alternatively, "the action by which any event is made possible, by either direct creation or construction from pre-existing elements" (Pasqualotto, 1997). Thus, ambiguity can represent a prerequisite of the creativity that has, psychologically speaking, "the ability to produce ideas or elements providing novel and/or alternative solutions to a wide array of problems." From a biological point of view, all these definitions seem to be quite theoretical or even intangible. However, ambiguity and the resulting creativity could be concretely related to the capacity of a biological system to put into action different mechanisms simultaneously that are only apparently opposite but ultimately result in an evolutionary advantage for the host[1]. To better understand how ambiguity can be applied to the immune response, a short review of the fundamental aspects of immunity should be conducted.

AMBIGUITY AND B OR T CELL ONTOGENESIS

The adaptive immune response is mediated by the B and T cells, both recognizing antigens through highly specialized and clonally distributed B cell or T cell receptors (BCRs and TCRs). To allow the generation of a huge number of antigen specificity, the immune

[1]Here, we will not consider the definition of ambiguity generally attributed by the psychoanalysis, as the incapacity to discriminate between *ego* and *non-ego* potentially leading to severe social and psychic disorders Bleger (1967). Simbiosis y ambiguedad, estudio psiconalitico. *Editorial Paidos, Buenos Aires.* If ever, according to the etymologic, semantic, or philosophic definitions reported above, we will consider the biological ambiguities (such as those cellular and molecular largely described for the immunology in this review) as critical events participating to the general homeostasis of the individual that ultimately favors the species survival.

system has evolved a very effective molecular organization of gene expression for the production of BCRs and TCRs following the encounter with self antigens, which occurs at the level of central lymphoid tissues (the bone marrow for B cells and the thymus for T cells). In particular, the recombination mechanisms (including gene rearrangement, junctional diversity, and N-region addition) enable a limited number of variable (*V*), diversity (D), and joining (J) minigene elements to produce about 10^{13}–10^{18} different genes encoding for an equal multiplicity of antigen receptors (Tonegawa, 1983; Yoshikai et al., 1984; Pullen et al., 1989; Oettinger et al., 1990; Shinkai et al., 1992; Wayne et al., 1994; Agrawal et al., 1998; West et al., 2005; Murphy et al., 2007). This enormous BCR or TCR diversity confers the potential *immune identity* to each individual, because the lymphocyte repertoire – and, hence, the capacity to respond to antigens – is customized for each individual, being different even amongst monozygotic twins. However, such an extraordinarily elegant mechanism, the aim of which is to protect the host from any possible invader, also produces undesired effects. The main side effect of such a broad response is the generation of "unwanted" B or T lymphocytes ("first level of ambiguity"). Indeed, a multitude of developing lymphocytes will result (a) ignorant because they will never meet their specific antigens throughout the life of a single individual; (b) apparently useless such as those able to recognize selective pathogen-associated epitopes, but not critical for the pathogen neutralization; and (c) dangerous, such as those autoreactive. To limit the generation and/or the harmful activity of these cells, the host has evolved different checkpoints to render the immune response mostly effective in fighting dangerous microorganisms without damaging host tissues. With regard to the T cell development, the first checkpoint determines that only a tiny population of thymocytes likely recognizing ubiquitous self antigens that are presented by cortical thymic epithelial cells (cTECs) can survive (positive selection mechanism; Snodgrass et al., 1985; Marrack et al., 1989; Jameson and Bevan, 1998; McCaughtry et al., 2008). This mechanism allows the deletion of a huge number of useless or harmful T cells that otherwise would flood and destroy the "vital space" at the level of central or peripheral lymphoid tissues, or may potentially induce autoimmunity (McCaughtry et al., 2008). As a consequence, only the thymocytes that have been positively selected through the recognition of ubiquitous self-epitopes could progress and reach the thymus medulla, where they will be submitted to a second checkpoint (medullar negative selection). A fundamental role is played by the autoimmune regulator (AIRE) gene, which allows the expression of a wide array of peripheral tissue-specific proteins in medullary (m)TECs (Anderson et al., 2002; Liston et al., 2003; Kuroda et al., 2005; Zhu et al., 2006; Gillard et al., 2007). Thymocytes with high affinity/avidity to tissue-specific self-proteins presented by both mTEC or thymic dendritic cells (DCs; which have phagocytosed mTEC) are deleted, a process which protects the host from the generation of autoreactive T cells with a high potential to induce organ-specific autoimmunity (Killeen et al., 1998). Similarly, self-reactive B lymphocytes are deleted in the bone marrow (Neuberger, 1997; Pillai, 1999; Hardy and Hayakawa, 2001). Nevertheless, a number of autoreactive T or B cells with high affinity antigen receptors have a second chance to survive because of a mechanism known as *receptor editing* (Tiegs et al., 1993; McGargill et al., 2000). As a consequence, all mature B or T lymphocytes migrating into the periphery on the one hand can recognize self-epitopes with low affinity, but they can be potentially protective by recognition of microbial antigens with high affinity, on the other hand. This process is extremely advantageous for the economy of the immune system because it allows: (a) dangerous lymphocytes recognizing self-proteins with high affinity/avidity are deleted, thus minimizing the emergence of autoimmunity; (b) a restrict lymphocyte repertoire as compared with what is potentially generated upon somatic recombination harbors the peripheral lymphoid tissues to defend the host from possible harmful aliens; (c) this repertoire of mature T or B lymphocytes is however enough to recognize an almost limitless number of microbial determinants and to control the continuous emergence of mutations of immunodominant epitopes.

In some T cells, the ambiguity of antigen recognition may be further amplified by a dual TCR expression. Indeed, the inefficiency of allelic exclusion following the rearrangement of TCR *alpha* chains (Casanova et al., 1991; Borgulya et al., 1992) allows the generation of a significant proportion of mature T cells harboring two functional TCRs with distinct antigen specificities (Padovan et al., 1993). Controversy still exists about the significance of dual TCR expression in the responses to foreign (He et al., 2002; Dash et al., 2010) or self antigens (Elliott and Altmann, 1995), in the development of regulatory T (T_{reg}) cells (Tuovinen et al., 2006) or in microbial triggers of autoimmunity (Ji et al., 2010). Anyway, the availability of a second specificity by a stimulated T cell may be interpreted as a "reserve" of response, which may extend immunity to additional exogenous as well to endogenous antigens.

Therefore, the intrinsic ambiguity of this process [i.e., maturation of protective lymphocytes is dependent on (low affinity) self-antigen recognition] provides the host with a significant advantage, which outweighs the side effects (autoimmunity, chronic inflammatory diseases. . .) that are generally controlled by the mechanisms of peripheral tolerance.

Evolution may have selected those processes of T cell development aimed at maximizing repertoire width, even at the expenses of the single individual safeguard. A proof of this possibility comes from the hypothesis that vertebrates developed the thymus from gut-associated lymphoid tissue (GALT; Matsunaga and Rahman, 2001). Indeed, due to structural constraints, in GALT only negative but not positive selection of T cells can efficiently take place (Matsunaga and Rahman, 2001). This scenario may suggest interpreting ambiguity in antigen recognition as an advantageous feature that has been fixed by natural selection.

THE AMBIGUITY OF THE PERIPHERAL IMMUNE RESPONSE

INNATE IMMUNE CELLS AND SIGNALS

As described above, the development of the mature B- and T-cell repertoires is dependent on recognition of self antigens in the thymus and bone marrow, respectively: mature B and T cells can then fight invaders as a result of their capacity to cross-react with single pathogen-associated epitopes. This high level of ambiguity of the immune system has led to the hypothesis that the main function of the immune response is to discriminate the *dangerous/infectious* from the *non-dangerous/non-infectious* rather than the *foreign* from the *non-foreign* (Matzinger, 1994; Gallucci and Matzinger,

2001; Janeway and Medzhitov, 2002; Medzhitov and Janeway, 2002). According to these theories, innate immunity plays a key role through the presence of sensors, such as the toll-like receptors (TLRs) expressed mainly by innate immune cells (e.g., monocytes, neutrophils, DCs. . .) and B lymphocytes, or intracellular nuclear oligomerization domain (NOD)-like receptors (Matzinger, 2002; Inohara et al., 2005; Akira et al., 2006; Fritz et al., 2006; Meylan et al., 2006; Petrilli et al., 2007). These receptors can identify dangerous/infectious signals because they recognize molecular patterns common to different pathogens [pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), bacterial DNA, or viral RNA], or dangerous compounds, such as endogenous (e.g., uric acid causing the gout. . .) or exogenous (e.g., asbestos causing mesothelioma or asbestosis, silica dust causing silicosis. . .) crystals (Meylan et al., 2006; Otsuki et al., 2007; Petrilli et al., 2007; Pope and Tschopp, 2007). As regards B cells, PAMPs deliver via TLRs critical costimulatory signals required for the plasma cell differentiation and the generation of memory B cells (Lanzavecchia and Sallusto, 2007). Once activated via TLRs, innate immune cells do the following: (a) induce an adequate inflammatory response in order to respond very early to pathogens and to limit the microbial invasion and (b) generate an effective adaptive immune response through cooperation (Zarember and Godowski, 2002; Viglianti et al., 2003; Hoebe et al., 2004; Schulz et al., 2005; Kawai and Akira, 2006; Matzinger, 2007). In addition, the tissues undergoing inflammation critically interact with innate immune cells to influence the type of local adaptive immune responses (Cho, 2008; Coombes and Powrie, 2008). Matzinger and Kamala (2011) have proposed that tissue-derived, rather than pathogen-derived, signals mostly dictate which classes of effector cells and molecules will be induced to achieve the maximal protection with the minimal damage in a given organ. On the one hand, this complex network has evolved to determine a high defense/offense ratio at the level of the different types of inflamed tissues; on the other hand, it can produce tissue damage and develop immunopathology under certain conditions. The critical issue is if and how this ambiguous aspect of the immune system is biologically advantageous and/or necessary.

B CELL RESPONSES

As discussed above, the enormous BCR repertoire, which has evolved to recognize as many different pathogens as possible, has two main – and apparently contradictory – effects. The principal protective effect results in the production of neutralizing antibodies addressed to fight pathogens, and to control the continuous emergence of microbial mutants (**Figure 1**). In contrast, the "side effect" is the emergence of potentially harmful antibodies (i.e., the autoreactive), or of those apparently useless, such as the wide range of antimicrobial antibodies that are generated in response to a given pathogen, but do not appear to be critical

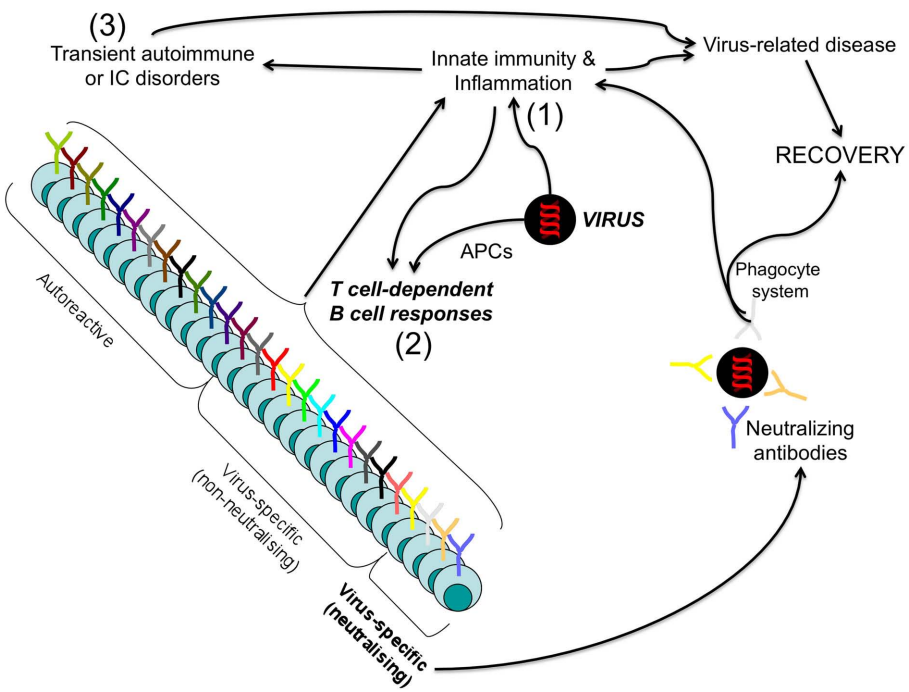


FIGURE 1 | Protective and harmful B cell responses leading to infection resolution. Upon infection by a given pathogen (i.e., a virus), innate immunity is firstly elicited (1) and acts both to promptly control the pathogen spread and to cooperate with the virus-related antigens (presented by professional APCs) for the priming of the adaptive immunity harm (2). Both pathogen-specific antibodies (particularly, the non-neutralizing, which represent the majority of antibodies generated in response to a given pathogen!) and autoantibodies (that can be generated via cross-reactivity or bystander mechanisms) can form immune complexes (ICs) and produce damage through the cooperation with the innate immune system (macrophages, complement factors, inflammatory cytokines. . .) (3). However, neutralizing, non-neutralizing virus-specific B cells, as well as the autoreactive, act in concert to fight the pathogen: the neutralizing in an antigen-specific manner, the non-neutralizing and autoreactive in a bystander manner via the activation of the phagocytic system by ICs resulting in the amplification of the inflammatory responses (acute disease). This inflammatory storm is self-limited, when the pathogen is cleared.

for pathogen clearance (non-neutralizing or non-protective antibodies; **Figure 1**). In particular, autoreactive antibodies can be elicited through the cross-reactivity between self and non-self antigens (Rose and Mackay, 2000; Benoist and Mathis, 2001), or generated in response to cryptic self-epitopes that have been unveiled from injured cells, during the course of a given infection or an inflammatory process (Salemi et al., 1995; Barnaba, 1996; Di Rosa and Barnaba, 1998; Rice et al., 2005). Moreover, both autoantibodies and pathogen-specific antibodies (particularly, the non-neutralizing, which represent the majority of antibodies generated in response to a given pathogen!) can produce damage via the formation of circulating immune complexes (ICs). The resulting systemic inflammatory processes can provoke damage in various cells (e.g., blood cells) or the formation of vasculitis phenomena, which affect several tissues (e.g., joints, skin, kidney, brain; Oates and Gilkeson, 2002; Rice et al., 2005; Alard et al., 2008; Klareskog et al., 2008). However, these injuries are generally self-limited and rarely cause organ or tissue failure. Indeed, they disappear in relation to the contraction of the protective immune responses that have promptly cleared the pathogen. Even under these conditions, ICs can participate to the quick amplification of the inflammatory cascade (via the activation of the complement system, the innate immune cells and the huge variety of inflammatory molecules) that is mandatory for both the control of the pathogen and the cooperation between the innate immunity and the adaptive T and B cell mediated responses, ultimately leading to recovery (Avrameas, 1991). The antibodies, already intrinsically ambiguous *per se* (because of the simultaneous generation of both protective and dangerous antibodies that, as previously described, is inevitable during the development of the lymphocyte repertoire), perform their potential functions through equally ambiguous phenomena – protective effects strictly related to prompt inflammatory reactions.

T CELL RESPONSES

Dendritic cells are essential to prime T cell responses, upon the processing and presentation of exogenous antigens, which are preferentially presented on major histocompatibility complex (MHC) class II molecules, or endogenous antigens, which vice-versa are preferentially presented on MHC class I molecules. However, the capacity of DCs to present exogenous antigens derived from other cells (usually necrotic or apoptotic cells) or soluble antigens on class I molecules is defined as cross-presentation (Guermonprez et al., 2003; Norbury et al., 2004; Accapezzato et al., 2005; Savina et al., 2006; Burgdorf et al., 2007; Dudziak et al., 2007).

Distinct DC subsets spread throughout the body, and although they share common features, they also have specialized functions. Humans and mice display two major DC types: myeloid DCs (myDCs, also called conventional DCs), and plasmacytoid DCs (pDCs; Palucka et al., 2010). In humans, myDCs are subdivided in two populations, on the basis of expression of BDCA-1 (CD1c) or BDCA-3 (CD141). $CD1c^+$ DCs represent the most abundant population of myDCs, express a wider repertoire of TLRs than the $CD141^+$ DCs, and hence they play a key role in sensing infectious/danger signals, in turn essential for their activation, migration, and T cell priming (see below). $CD141^+$ DCs represent the human counterpart of mouse lymphoid $CD8^+$ DCs. Indeed, both these subsets perform the cross-presentation mechanism with high efficiency, express the chemokine receptor XCR1 allowing them to migrate in response to the specific ligand (XCR1L) that is produced by NK and activated $CD8^+$ T cells (Bachem et al., 2010; Crozat et al., 2010), and express the adhesion molecule Necl2 binding to class-I-restricted T-cell-associated molecule (CRTAM), a cell surface protein primarily expressed by NK, NK-T, and activated $CD8^+$ T cells. Thus, mouse $CD8^+$ DCs and human $CD141^+$ DCs appear to be addressed for generation of $CD8^+$ T cell immunity (Shortman and Heath, 2010). BDCA-2^+ pDCs express high amounts of IL-3Rα chain (CD123) and ILT-7 and are considered the front line in antiviral immunity owing to their capacity to rapidly produce high amounts of type I interferon in response to viruses (Siegal et al., 1999; Cao et al., 2006). pDCs recognize viral components and self nucleic acids through TLR7 and TLR9, and possibly other as yet unidentified receptors (Matsui et al., 2009), allowing the secretion of two sequential cytokines: type I IFN is responsible for generation of non-Ig-secreting plasma blasts and IL-6 driving their differentiation into Ig-secreting plasma cells (Jego et al., 2003). Recently, it has been proposed that, in addition to their primary role to produce high levels of antiviral IFNs of type 1, pDCs are capable of performing efficient antigen-presenting cell (APC) functions (Guiducci et al., 2006; Di Pucchio et al., 2008). Other subsets, such as DCs in B cell follicular regions or dermal $CD14^+$ DCs in human skin, have specialized function in their selective districts. DCs in B cell follicular regions interact with B cells, inducing humoral immunity to unprocessed soluble antigen presented by these DCs (Wykes et al., 1998). Dermal $CD14^+$ DCs express a huge repertoire of surface C-type lectins and TLRs (van der Aar et al., 2007; Klechevsky et al., 2009), and induce naïve T cells to differentiate into cells with properties of T follicular helper (TFH) cells (Klechevsky et al., 2008), able to induce naïve B cells to produce large amounts of IgM and to induce B cells to switch isotypes toward IgG and IgA.

All tissues are "patrolled" by conventional myDCs that, like bifacial Janus (the most ambiguous Roman divinity), can perform opposing (tolerogenic or stimulatory) functions, according to the context in which they work (Lanzavecchia and Sallusto, 2001; Steinman et al., 2003). Depending on the signal myDCs will undergo activation/maturation, the quality of which will determine the type of elicited adaptive immunity. Under steady state conditions, myDCs phagocytose self antigens associated with dying (apoptotic) tissue cells (derived from the physiological cell turnover), process them, present the resulting peptides on MHC class II or class I molecules and migrate into the draining lymph nodes with very low efficiency, where they can induce tolerance or cross-tolerance of autoreactive $CD4^+$ T cells or $CD8^+$ T cells, respectively (peripheral tolerance; Lanzavecchia and Sallusto, 2001; Steinman et al., 2003). Peripheral tolerance is enforced by the AIRE expression in extrathymic stromally derived cells resident in peripheral lymphoid organs, which hence can present a wide array of tissue-specific antigens and are capable of interacting with and deleting naïve autoreactive T cells (Gardner et al., 2008). DCs that exist at the steady state, can also establish tolerance indirectly by inducing T_{reg} cells (Roncarolo et al., 2001; Yamazaki et al., 2006), the key cell population involved in the

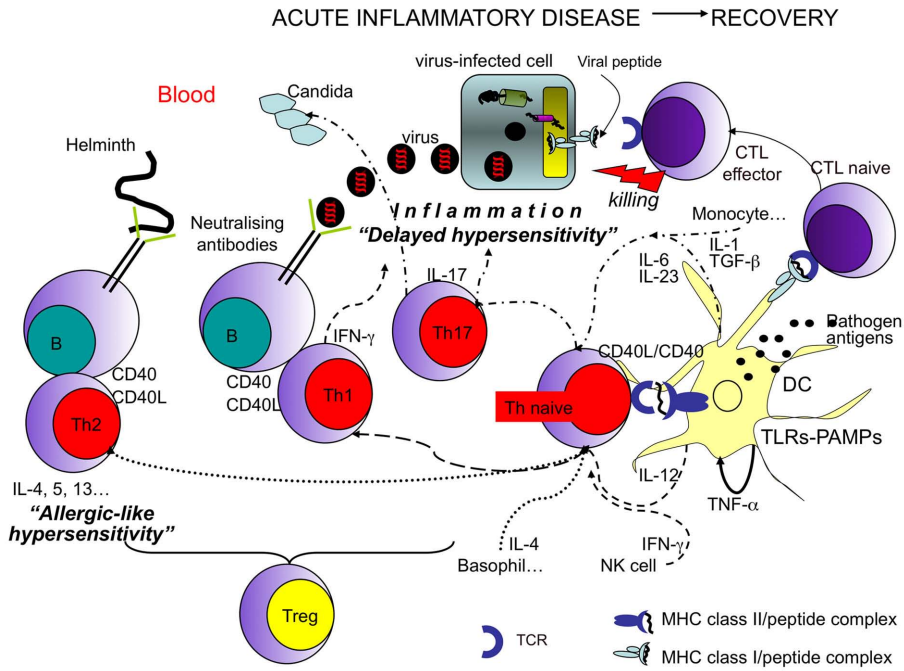


FIGURE 2 | Protective and harmful T cell responses leading to infection resolution. Recovery from infections is dependent on the efficient priming of T cells by professional APCs (DCs) working in concert with other innate immune cells. The efficiency of DCs in migrating from inflamed tissues to lymph nodes, in presenting antigens, and in T cell priming is in turn dependent on the engagement of different types of DC sensors by pathogens (such as TLRs engaged by PAMPs). In addition ($CD4^+$) Th1 cell polarization (dashed arrows) requires the presence of IFN-γ generally donated by NK cells, and IL-12, produced by DCs. ($CD4^+$) Th2 cell polarization (dotted arrows) requires the presence of IL-4 particularly produced by basophils. Whereas ($CD4^+$) Th17 cell polarization (arrows with dashes and dots) requires the presence of several cytokines, such as TGF-β, IL-6, IL-1 (particularly produced by monocytes and endothelial cells), and IL-23 (mainly produced by DCs). In all cases, the functional activity of DCs, T, or B cells is increased by the interaction between CD40, expressed by both DCs and B cells, and CD40 ligand (L), expressed by activated T cells. Then Th1 cells display protective activity against intracellular pathogens (i.e., viruses…), via both IFN-γ production (antiviral activity) and by cooperating with B cells producing IgG1 (in humans) or IgG2 (in mice) neutralizing antibodies. Th2 cells are protective against extracellular pathogens, such as helminthes, via the production of a series of cytokines (e.g., IL-4, IL-5, IL-13) addressed to provoke "allergic-like" hypersensitivity (obtained via the recruitment of various cells, including eosinophils and mast-cells) and production of protective IgE antibodies by B cells. Th17 cells seem to be important in fighting extracellular pathogens, such as fungal infections. ($CD8^+$) Cytotoxic T lymphocytes (CTLs) directly kill infected cells via the recognition of class I molecules expressing pathogen peptides. In any case, activated T cells can eliminate the pathogen via the establishment of a severe inflammatory disease. T_{reg} cells control exaggerated responses or the emergence of autoreactive lymphocytes.

regulation of immune responses and homeostasis (Sakaguchi et al., 2010; see The Control of the Immune Response). These DCs may not simply be unstimulated or immature. Activation of the Wnt and β-catenin signaling pathway in DCs has been shown to promote induced T_{reg} cell production, at least in the mouse (Jiang et al., 2007). Similarly, in the thymus, production of thymic stroma lymphopoietin (TSLP) is essential for selection of naturally occurring $CD4^+CD25^{hi}$ T_{reg} cells (Watanabe et al., 2005). By contrast, in an inflammatory context (mainly induced by infectious agents or tissue-derived signals), DCs are activated (for instance, through TLR engagement by PAMPs or necrotic cell products), increase for a short time (West et al., 2004) the ability to internalize and to process both microbial and self antigens, as well as they upregulate the expression of stimulatory, costimulatory, and pro-migratory molecules [i.e., the lymph node-specific chemokine receptors (Cys–Cys chemokine receptor 7, CCR7); **Figure 2**; Lanzavecchia and Sallusto, 2001; Steinman et al., 2003]. Then, they can reach the lymph nodes, where they prime or cross-prime both microbial- or self-antigen-specific naïve $CD4^+$ or $CD8^+$ T lymphocytes (Ridge et al., 1998; Schulz et al., 2005). Therefore, the generation of autoreactive T cell responses is potentially a common event occurring in relation to the protective responses against the "invaders" (ambiguity). As a consequence, both pathogen- and self-reactive effector T lymphocytes migrate to the inflamed tissues because of the newly acquired expression of tissue-specific chemokine receptors (Sprent and Surh, 2002; Masopust and Ahmed, 2004; Sallusto et al., 2004; Lang et al., 2005). According to the microenvironmental context, lymphocytes are polarized toward different types of effector capacities that can provide opposing protective and harmful effects (ambiguity; **Figure 2**). If DCs are conditioned by infectious or danger signals to produce adequate amounts of IL-12, in the presence of interferon (IFN)-γ, $CD4^+$, or $CD8^+$ T cell priming is skewed toward the polarization of either proinflammatory Th1 cells or $CD8^+$ T cells with high cytotoxic potential, respectively: these cells will simultaneously provide protective responses against intracellular pathogens and harmful responses via their immunopathological activities (Romagnani, 1997). In the presence of IL-4, naïve T

cells preferentially differentiate into Th2 (producing IL-4, IL-5, IL-13. . .) with protective responses against extracellular pathogens or harmful responses in the case of allergic reactions (Nelms et al., 1999; de Jong et al., 2005). Under conditions in which DCs produce IL-23 (another member of the IL-12 family), and in the presence of IL-6, transforming growth factor (TGF)-β, and IL-1β, T cells differentiate toward Th17 cells (producing IL-17), described to be responsible for causing severe immunopathological damages (i.e., in several models of autoimmunity) and for providing protection against some extra or intracellular pathogens (Acosta-Rodriguez et al., 2007; Bettelli et al., 2007; Dong, 2008; Luger et al., 2008; McGeachy and Cua, 2008; Curtis and Way, 2009). Sustained stimulation by DCs is critical for maintaining a large pool of memory T cells. Upon infection resolution, effector cells disappear, whereas memory cells remain numerically constant because of the expression of receptors specific for the homeostatic (IL-7 and IL-15) cytokines (Surh et al., 2006; Sabbagh et al., 2007). The homeostatic proliferation of memory cells, in the absence of antigen, is critical for prompt differentiation into effector cells should they re-encounter the original infecting pathogen.

An additional support for the idea that DCs carry out opposing functions according to their maturational stage and the microenvironment in which they work is provided by studies investigating the complex interplay amongst cross-presentation, apoptotic cells, DCs, and stimulatory signals. The current view suggests that, under steady state conditions, apoptotic cells are consistently captured by immature DCs that in turn induce tolerance (or cross-tolerance) of apoptotic cell-derived antigen-specific T cells (Albert, 2004; Kazama et al., 2008). Otherwise, DCs mature and cross-prime these T cells in the presence of sustained infectious/inflammatory mediators, necrotic cell products, or $CD4^+$ Th cells inducing DC maturation via the CD40/CD40L interaction (Inaba et al., 1998; Propato et al., 2001; Albert, 2004; Blachere et al., 2005; Rawson et al., 2007). The rationale behind this model is that apoptosis may be a physiological or a pathological event, depending upon the circumstances involved. Thus, apoptosis occurring physiologically during the development of a given tissue should cause tolerance, whereas apoptosis caused by a microbial infection or other inflammatory processes should result in T cell priming (Propato et al., 2001; Winau et al., 2006). In the steady state conditions, apoptotic cells have been proposed to induce T cell deletion, anergy, immune deviation from Th1 to Th2 responses, or T_{reg} cell induction (Kurts et al., 1998; Ferguson et al., 2002; Griffith et al., 2007), via a series of not completely clear mechanisms, including the capacity of apoptotic cells to release immunosuppressive cytokines (i.e., IL-10 or TGF-β), or to engage receptors (i.e., CD36 or phosphatidyl serine receptors) subverting the stimulatory DC functions (Gao et al., 1998; Chen et al., 2001; Serhan and Savill, 2005). In addition, it has been demonstrated that apoptotic cell-dependent tolerance can be determined by the caspase-induced production of a reactive oxygen species scavenger leading to oxidization and inactivation of the high mobility group protein B1, a powerful danger signal normally involved in the full DC activation and immune response initiation (Kazama et al., 2008). However, the presence of sustained infectious/inflammatory mediators, necrotic cell products or $CD4^+$ T cell help can bypass the tolerogenic effects of apoptotic cells and induce DCs to prime or cross-prime T cells (Inaba et al., 1998; Propato et al., 2001; Albert, 2004; Blachere et al., 2005; Rawson et al., 2007; Tesniere et al., 2008).

Our previous data proposed that an alternative mechanism of abrogation of the apoptosis-associated tolerance can take place via the expression of CD40L by apoptotic cells (**Figure 3**; Propato et al., 2001). $CD40L^+$ apoptotic cells can be derived from activated $CD40L^+$ T cells that undergo apoptosis once they have performed their effector function in a given inflamed tissue or upon infection with proapoptotic viruses, such as HIV. $CD40L^+$ apoptotic T cells directly induce DC maturation and condition them to induce cross-priming of $CD8^+$ T cells specific to apoptotic cell-associated self antigens, irrespective of additional exogenous signals (**Figure 3**). In contrast, if apoptotic T cells are $CD40L^-$ (such as those derived from resting T cells), the help of a third party activated T cell or surrogate CD40L molecule is needed for priming (**Figure 3**). The finding that $CD40L^+$ apoptotic T cells induce DC maturation and cross-priming without the addition of exogenous stimuli indicates that the surface phenotype and possibly the lineage of origin of apoptotic cells may ultimately dictate the outcome of cross-presentation. This notion may help reconcile several apparently contradictory findings. For example, it explains why apoptotic cells derived from epithelial or resting T cells that are $CD40L^-$ are unable to provide DC maturation stimuli and are tolerogenic in the absence of sustained infectious/inflammatory signals (De Vita et al., 1998; Propato et al., 2001; Albert, 2004; Kazama et al., 2008). Thus, the balance between $CD40L^+$ and $CD40L^-$ apoptotic cells during cross-presentation appears to dictate tolerance or induction of CD8 T cell responses against T-cell-associated epitopes, and to maintain or to stop the related responses in the course of an inflammatory process. These responses have a critical role in the amplification of chronic inflammation via the continuous bystander effects of inflammatory cytokines produced by T cells specific for the apoptotic T-cell-associated antigens (Propato et al., 2001; Rawson et al., 2007).

For all these reasons, adaptive immune responses clearly show a deeper level of ambiguity: (a) an effective T cell response depends on an adequate level of tissue inflammation, which in turn also induce tissue injury; (b) effector lymphocyte responses are both protective and harmful at the same time.

However, the ambiguity appears to be not only advantageous but also necessary. Indeed, the control of a given infectious agent (protection) is dependent on the immunopathological process at the level of the site of infection (damage), which generally results in an acute disease undergoing recovery when the anti-pathogen responses are prompt and efficient. In this situation, the contraction of both protective effector responses and the associated immunopathological process (e.g., delayed hypersensitivity Th1 or likely Th17 cell responses, including the autoreactive, "allergic-like" hypersensitivity Th2 cell responses) occurs in relation to the clearance of intra or extracellular pathogens (**Figure 2**). In addition, even the autoreactive and the apparently useless T or B responses that can arise together with the protective responses via the mechanisms described previously are beneficial in supporting the immunopathology required for the recovery: also, these responses generally disappear in relation with the pathogen

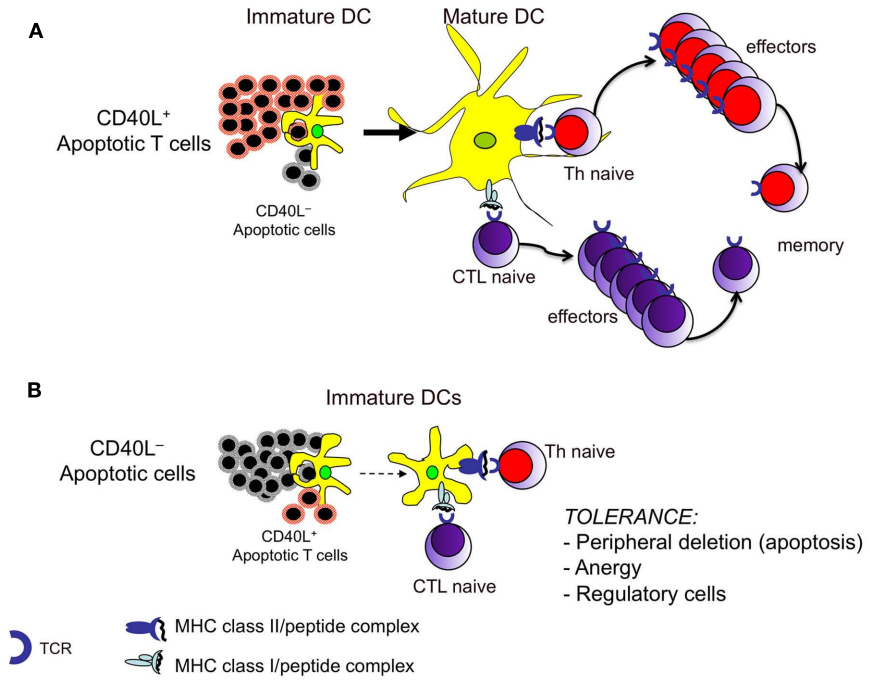


FIGURE 3 | The balance between CD40L$^+$ and CD40L$^-$ apoptotic cells during cross-presentation dictates induction or tolerance of T cell responses. (A) Apoptotic T cells expressing CD40L can directly provide to DCs both (apoptotic cell-derived) self antigens and the necessary maturation stimuli for T cell priming. **(B)** Under conditions in which the CD40L$^+$ apoptotic T cells are overwhelmed by the CD40L$^-$ apoptotic cells (i.e., under normal conditions or when an inflammatory process is terminated), the latter will be unable to provide the appropriate signals to DCs, which will deliver tolerance signals to autoreactive T cells, even though they carry apoptotic cell-derived peptides.

clearance (Di Rosa and Barnaba, 1998; Murali-Krishna et al., 1998).

Paradoxically, the possibility of recovering from a given infection is dependent on the high level of immunopathology (acute disease; **Figure 2**). Conversely, weak responses are generally associated with low-grade, long-lasting inflammation that, although causes minimal tissue injury, will be unable to clear the pathogen, which in turn will persist and, together with the persistent weak immune responses, will lead to irreversible chronic diseases.

THE CONTROL OF THE IMMUNE RESPONSE

The ambiguity of the immune response can be found at further other levels. One important aspect is the downregulation of the immune response itself. The mechanisms evolved to control the ongoing effector response may be achieved by multiple, non-mutually exclusive mechanisms of peripheral tolerance, including T cell anergy, apoptosis, or exhaustion (Lanzavecchia and Sallusto, 2001; Walker and Abbas, 2002; Steinman et al., 2003; Barron et al., 2008).

The extinction of effector responses often involves the secretion of immunosuppressive cytokines by T helper themselves, such as IL-10 and IL-22. These two molecules, belonging to the same family, operate to rescue immune and tissue homeostasis, respectively (Sanjabi et al., 2009). Indeed, IL-10R is mainly expressed by immune cells, mediating the inhibition of proinflammatory signals; conversely, IL-22R is present predominantly on the surface of epithelial and tissue cells. This cytokine has revealed some functional ambiguity: on the one hand, during acute inflammatory reactions in gut and liver, IL-22 protects the tissue from immune-mediated injury, facilitates regeneration, promotes secretion of antimicrobial substances and maintains barrier integrity against bacterial translocation (Zenewicz et al., 2008; Zheng et al., 2008); on the other hand, IL-22 becomes pathogenic in skin diseases, fostering keratinocyte hyperplasia, and local inflammation (Duhen et al., 2009; Eyerich et al., 2009). Thus, the activities of IL-22 may result beneficial or detrimental to the host depending on tissue type, cytokine milieu, and target cells.

A special emphasis has been recently placed on the PD-1, a death receptor over-expressed by chronically stimulated lymphocytes that induces peripheral T or B cell tolerance upon the simultaneous interaction of TCR or BCR with antigens and of PD-1 with its own ligands: PD-L1, which is virtually expressed on all somatic cells (particularly from inflamed tissues; Keir et al., 2006; Sharpe et al., 2007), and PD-L2, which is mainly expressed by DCs. However, this mechanism becomes detrimental when the PD-1/PD-L1 interaction takes place in tissues infected with persistent pathogens, via its capacity to induce exhaustion of antimicrobial T cell responses (Probst et al., 2005; Barber et al., 2006; Day et al., 2006; Sharpe et al., 2007). Through this mechanism, effector lymphocytes express the death receptor PD-1 and are blocked in their functional abilities when interact with cells expressing PD-L1, the counter-receptor, which is the case with epithelial cells when chronically infected by a virus or during an inflammatory process (Probst et al., 2005;

Barber et al., 2006; Day et al., 2006; Keir et al., 2006; Sharpe et al., 2007).

The extrinsic mechanisms of peripheral tolerance are put into action mainly by $CD4^+CD25^+Foxp3^+$ T_{reg} cells (Shevach, 2002; Bluestone and Abbas, 2003; von Boehmer, 2005). These cells develop either in the thymus (natural) or in the periphery from conventional $CD4^+$ T cells (induced), and express the transcription factor Foxp3 (Shevach, 2002; Bluestone and Abbas, 2003; Fontenot et al., 2003; Hori et al., 2003; Sakaguchi, 2004; von Boehmer, 2005; Ziegler, 2006). A lack of Foxp3 expression results in the complete absence of T_{reg} cells, which leads to the development of severe autoimmunity, as observed in immuno dysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome. T_{reg} cells induce suppression via several mechanisms, involving membrane molecules, such as CTLA-4 or adenosine receptors, suppressive cytokine production, such as TGF-β or IL-10, dominant absorption of IL-2 by high CD25 expression (Shevach et al., 2001; Shevach, 2002; von Boehmer, 2005; Belkaid, 2007; Deaglio et al., 2007; Pandiyan et al., 2007; Vignali et al., 2008). Because of the expression of the *Il2* gene-inhibitory Foxp3 transcription factor, T_{reg} cells do not produce IL-2 and are unable to respond to antigens (anergy; Schubert et al., 2001; Coffer and Burgering, 2004; Fontenot et al., 2005). However, they promptly proliferate in response to relevant antigens in the presence of paracrine IL-2, which is mainly produced by effector T lymphocytes but is dominantly absorbed by T_{reg} cells via the high expression of IL-2 receptors (CD25 high; de la Rosa et al., 2004; Barthlott et al., 2005; Kretschmer et al., 2005; Scheffold et al., 2005; Setoguchi et al., 2005). The main physiological functions of T_{reg} cells are as follows: (a) to participate in the establishment of peripheral tolerance by inhibiting autoreactive lymphocytes that escaped either thymus or bone marrow checkpoints (central tolerance), (b) to suppress ongoing protective immune responses once they are no longer necessary or become harmful after the elimination of the pathogen, and (c) to limit excessive immunopathology during chronic inflammatory diseases (Shevach, 2002; Fontenot et al., 2003, 2005; Hori et al., 2003; Sakaguchi, 2004; Kretschmer et al., 2005; Setoguchi et al., 2005; von Boehmer, 2005; Ziegler, 2006; Belkaid, 2007). The immune system, by using highly specialized molecular mechanisms, on the one hand attacks the invading agents, on the other hand it suppresses the same responses. Again, this ambiguous aspect results in an advantage for the host, because it is necessary to defend, but also to limit potentially harmful proinflammatory responses.

In the model of hepatitis C virus (HCV) infection, $CD4^+CD25^+Foxp3^+$ T_{reg} cells have been proposed to participate in the establishment of a fine equilibrium between immunopathology and immune protection, ultimately resulting in the long-lasting survival of the host during chronic infections (Shevach, 2002; Accapezzato et al., 2004; von Boehmer, 2005; Belkaid, 2007; Ward et al., 2007; Ebinuma et al., 2008; Heeg et al., 2009; **Figure 2**). This would be dependent on a compromise between a status of chronic low-level hepatic inflammation and the generation of antiviral responses that, although unable to clear HCV, are enough to limit excessive viral spread. It is unclear how T_{reg} cells control unwarranted inflammation without completely suppressing the protective immune responses. High CD25 expression by T_{reg} cells drives a positive feedback loop, as the dominant IL-2 capture increases STAT-5 phosphorylation (pSTAT-5) that in turn drives T_{reg} cell proliferation and function. We recently showed that PD-1 is over-expressed on $Foxp3^+$ T_{reg} cells and limits T_{reg} cell proliferation and function during chronic HCV infection. The expression of PD-1, upon the contact with its own ligands, inhibits pSTAT-5 via the activation of Src homology 2-containing tyrosine phosphatases (Franceschini et al., 2009; **Figure 4**). As a consequence, responder T cells can escape from excessive expansion of T_{reg} cells and render them available for responding to possible novel waves of infection. This negative feedback loop assumes a different significance during chronic infections, such as HCV. The incapacity to clear HCV by the immune system (due to the various mechanisms emphasized above) maintains a vicious spiral, whereby responder T cells are chronically stimulated to produce IL-2 that will be dominantly adsorbed by $CD25^{hi}$ T_{reg} cells that in turn will continuously suppress the effector responses. The PD-1 upregulation limits the excessive expansion of T_{reg} cells by controlling pSTAT-5 and fine-tunes the T_{reg} function in order to minimize the immunopathology without completely switch off those intended to limit excessive viral spread (**Figure 4**). This may represent a critical contra-suppression mechanism that has evolved to control that T_{reg} cells have a limited suppression. Homeostatic balance participates in establishing a status of chronic low-level liver inflammation that is in turn instrumental to ensure a long-lasting survival of the host.

A further level of T_{reg} contra-suppression may be achieved with T_{reg} trans-differentiation into alternative fates. Indeed, many recent observations point to an inherent plasticity of T_{reg} cells, particularly prone to acquire proinflammatory functions under adequate microenvironmental cues (Zhou et al., 2009a). For instance, an unexpected discovery is that human T_{reg} cells have considerable plasticity that allows them to produce the proinflammatory cytokine IL-17 under certain conditions particularly related to autoimmunity (Cvetanovich and Hafler, 2010). The T_{reg} plasticity has been related to a CpG-rich intronic enhancer region known as the T_{reg}-specific demethylated region (TSDR), present at the level of four conserved, non-coding regions in the human *Foxp3* locus, and containing the most strikingly T_{reg}-specific pattern of CpG methylation (Huehn et al., 2009; Lal and Bromberg, 2009). The methylated state of TSDR in activated conventional T cells and TGF-β-induced T_{reg} cells allows these cells to transiently express Foxp3. In contrast, the demethylated state of the TSDR in human T_{reg} cells allows them to be the only cells that generally exhibit long-term stability of Foxp3 expression. The Foxp3 instability may account for the capacity of conventional T cells and TGF-β-induced T_{reg} cells expressing Foxp3 to convert into Th17 cells, particularly when they are strongly activated in the presence of proinflammatory cytokines during different forms of autoimmune diseases (Dominguez-Villar et al., 2011). The participation of TGF-β in the differentiation of Th17 cells places the Th17 lineage in close relationship with T_{reg} cells, as TGF-β also induces differentiation of naive T cells into $Foxp3^+$ T_{reg} cells (Korn et al., 2009). Controversy still exists about the stability of Foxp3 expression along T_{reg} reprogramming (Zhou et al., 2009b; Rubtsov et al., 2010). In any case, T_{reg} cells have been recognized as the preferential precursors for follicular helper T

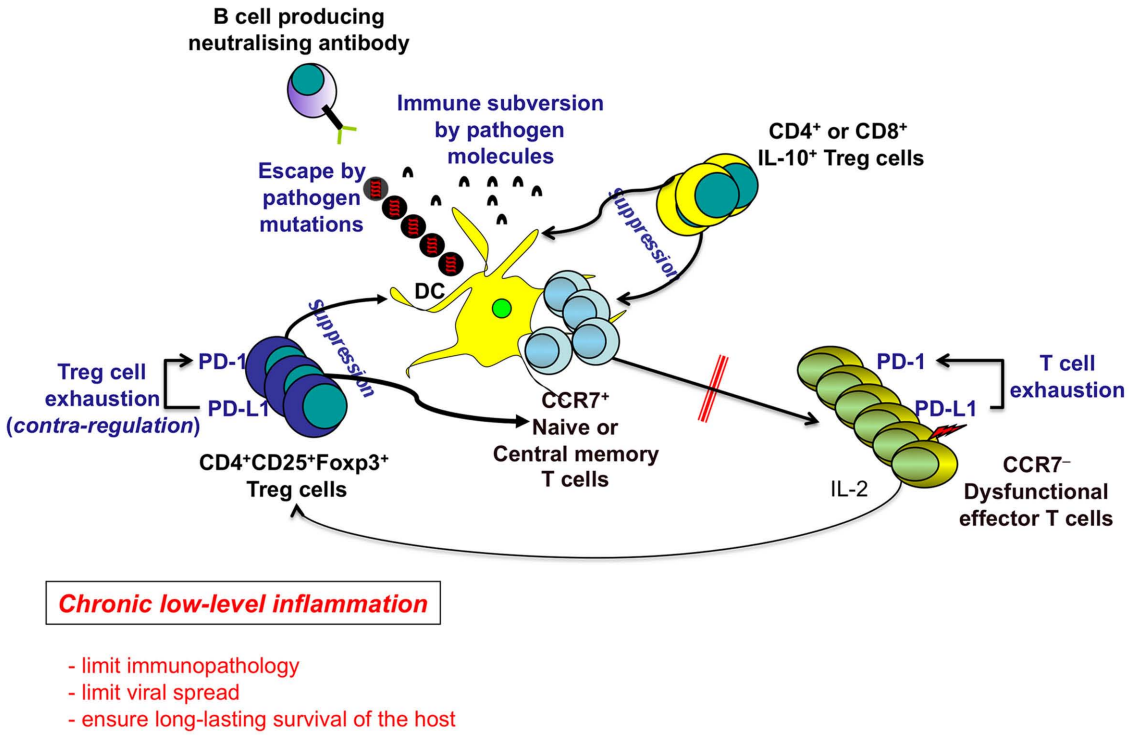


FIGURE 4 | Chronic low-level inflammation as an advantage for the long-term human survival. Several mechanisms induced by both persistent pathogens (e.g., escape by pathogen mutations, pathogen-related immune subversion) and the host [e.g., PD-1-dependent T cell exhaustion, Regulatory T (T_{reg}) cells] contribute to the maintenance of chronic inflammatory diseases, by down-modulating DC functions, T and B cell priming, generation of memory cells, and ultimately differentiation of terminal effector cells. T_{reg} cells can be represented by the following: (a) $CD4^+CD25^+Foxp3^+$ T_{reg} cells, which suppress via cell–cell contact, IL-2 capture to effector cells, TGF-β production, etc.; (b) T_{reg} cells producing immunosuppressive cytokines (e.g., IL-10, TGF-β). PD-1 is upregulated on both responder and T_{reg} cells and upon contact with PD-L1/2 limits both T effector memory (T_{EM}) cell responses and excessive T_{reg} cell function, respectively. The resulting contra-regulation of T_{reg} cells will have an important role to limit excessive suppression of immune responses allowing the control of the viral spread, at the cost of the maintenance of the chronic low-level liver immunopathology. This mechanism establishes a long-lasting survival of the host.

cells in gut Peyer's patches (Tsuji et al., 2009) and for CD40L-expressing helper T cells in the establishment of antitumor immunity (Sharma et al., 2010). Hence, T_{reg} cells may rapidly turn from immune-suppressive into immune-protective cells in an "innate-like" fashion (Zhou et al., 2009a). Accordingly, T_{reg} ablation impaired, rather than promoting, early antiviral local responses in a mouse model of infection (Lund et al., 2008). The extracellular signals driving T_{reg} plasticity are mostly unknown and may comprise not only proinflammatory cytokines but also costimulatory receptors. OX40 is a receptor belonging to the TNFR superfamily that is constitutively expressed by T_{reg} cells, supporting their homeostasis (Piconese et al., 2010) and regulating T_{reg} contra-suppression (Piconese et al., 2008). However, it is becoming increasingly clear that the outcome of OX40 signal is somehow modulated by the cytokine milieu and that, in turn, OX40 may affect T_{reg} responses to cytokines. Indeed, OX40 enhances T_{reg} susceptibility to IL-2 (Piconese et al., 2010), and different outcomes of OX40 stimulation depend on the cytokine context (Ruby et al., 2009). Therefore, we may envisage a role for OX40, and possibly for other costimulatory molecules showing comparable behaviors, in fine-tuning T_{reg} ambiguity in response to microenvironmental soluble signals.

IMMUNITY/IMMUNOPATHOLOGY BALANCE, AS A MODEL OF AMBIGUITY

As suggested above, we can assume that the contradictory aspect of the immune response is the result of the evolution of biological systems. This evolution proceeds through the selection of the most advantageous processes to ensure the survival of the species. Conversely, these selected processes may have a high cost for the single individual in terms of morbidity and/or mortality. Therefore, a fine line exists between immunity and immunopathology, because, as previously discussed, it is not possible to obtain an effective immune response without the onset of immunopathology. A problem arises when the immunopathologic reactions become persistent or chronic, producing irreversible damage to tissues and organs. Indeed, chronic inflammation may be interpreted as an adaptive response to challenges producing irreversible modifications of tissue homeostasis, often at the expenses of tissue functions (Medzhitov, 2010). A sustained inflammatory response can also be responsible for the development of tumors. In the next section, we will discuss the main mechanisms of chronic inflammation and the ambiguity of this process, which not only helps to control persistent infections but also may induce severe disease of the host (i.e., autoimmunity, tumors).

CHRONIC INFLAMMATION AS AN ADVANTAGE FOR SPECIES SURVIVAL

The induction of a state of chronic inflammation secondary to infections occurs for those pathogens that are able to evade the immune response and establish a status of persistence. Typical microorganisms capable of evading the immune system and of establishing chronic diseases in humans include HIV, hepatitis B virus (HBV), HCV, *Mycobacterium tuberculosis*, and *Leishmania* (Zinkernagel, 1996; Zinkernagel et al., 1999; Tortorella et al., 2000; Phillips, 2002; Klenerman and Hill, 2005).

The relationship between the host and the latent viruses, particularly the herpesviruses (i.e., herpes simplex virus, Epstein–Barr virus, cytomegalovirus), is completely different. These viruses generally establish latency in specific cell types or tissues, are continuously controlled by the immune system, and, in contrast to the persistent pathogens previously mentioned, remain lethargic with regard to their replication capacity (Nikolich-Zugich, 2008). Only occasionally, these viruses are reactivated and stimulate memory T cells, which are generally capable of returning the virus to a state of latency. In a very tiny number of healthy carriers, these viruses can degenerate in severe forms of tumors. However, the intermittent (but not continuous, as in the case of persistent pathogens) T cell stimulation does not result in the impairment or exhaustion of antigen-specific T cells and the development of chronic diseases. Latent viruses seem to play a critical role in expanding memory T cell populations and in maintaining constant their frequencies in old age. Therefore, we will not discuss latent viruses further in this review, which aims to define the role of chronic inflammation in long-term host survival during persistent infections.

Through the different (non-mutually exclusive) mechanisms illustrated above, the host could survive for a long time in parallel with both the "partially controlled" persistent agent and a low-grade inflammation. Therefore, chronic inflammatory processes are paradoxically useful, supporting the concept that the ambiguity is advantageous for the evolutionary process. If the immune responses were invariantly strong and aggressive during a persistent infection, they would be unable to eliminate the persistent pathogen, because of the acquired capacity by the persistent pathogen to escape or to subvert them. In such a situation, exuberant (but non-protective) responses would produce irreversible tissue damage in the host, leading to catastrophic epidemic infections. Considering this point of view, chronic (low-level) inflammatory diseases seem to represent a sort of safeguard for the human survival!

We can assume that chronic inflammation may be defined as the "*Yin and Yang*" of the immune system, because, via the sophisticated mechanisms mentioned previously, it guarantees the long-term survival of human hosts despite the pathogen persistence. However, the imbalance of the homeostatic mechanisms maintaining chronic inflammation may degenerate into severe "side effects" (i.e., the development of either autoimmune diseases or tumors) in some individuals (**Figure 5**).

FIGURE 5 | Chronic inflammatory diseases as the *"Yin and Yang"* of the immune system. Under conditions in which the immune system has been bypassed by persistent pathogens, chronic low-level inflammation guarantees long-term survival of the majority of infected individuals in spite of pathogen persistence. The imbalance of the homeostatic mechanisms maintaining chronic inflammation may degenerate into severe "side effects" (such as autoimmune disorders or tumors) in some individuals. However, these side effects are considered irrelevant in terms of the survival of the species.

AUTOIMMUNITY AND CANCER

From an evolutionary point of view, the onset of autoimmune diseases or of some tumors might be the price to pay following the establishment of chronic inflammation. It has been suggested that the intensity and nature of the inflammation might explain the apparent contradiction between the inducing and inhibiting effects on tumor survival. Indeed, vigorous acute inflammatory processes can favor an immune effector response potentially able to induce tumor regression (Mantovani et al., 2008a,b). Conversely, a status of pre-existing chronic inflammation can participate to the development of cancer, by the production of growth and angiogenic factors eventually promoting cancer-cell survival, implantation, and growth. In addition, chronic inflammation can affect the immune-surveillance directly via its own intrinsic mechanisms (i.e., expansion of T_{reg} cells, T cell exhaustion, etc.), and indirectly by the incapacity to limit the immunosuppressive effects by tumors. In these conditions, the suppression of the immune response, that is certainly useful to terminate the effector responses or to limit excessive immunopathology, can result detrimental. In fact, the chronic activity of T_{reg} cells is believed to play a crucial role in suppressing the immune-surveillance against tumors by $CD8^+$, Th1, and probably Th17 cells (Curiel et al., 2004; Zou, 2006; Colombo and Piconese, 2007; Curiel, 2007; Nair et al., 2007; Zhou and Levitsky, 2007; Piconese et al., 2008).

The production of soluble factors (i.e., proinflammatory or cell growth cytokines) that favor cell proliferation, generally needed to the immune system to defend the host efficaciously, can facilitate the mitotic cycle also of non-lymphoid cells. In the long run, this prolonged stimulation, which is characteristic of chronic inflammatory processes, can induce tissue damage, as in the case of liver cirrhosis by both HBV and HCV, where the phenomena of necrosis, cell renewal, and even neoplastic transformation might simultaneously occur (Tan et al., 2008). The sustained recognition of antigens that cannot be eliminated by the immune system can induce errors in VDJ rearrangement by B cells or cause chromosomal translocations. These conditions can generate the onset of lymphomas. A typical case is the mucosa-associated lymphoid tissue (MALT) lymphoma associated with the presence of several infectious agents including *Helicobacter pylori*, *Campylobacter jejuni*, *Borrelia burgdorferi*, *Chlamydia psittaci*, and HCV, inducing gastric lymphomas, immunoproliferative small intestinal disease, cutaneous lymphoma, ocular lymphoma, and spleen lymphoma,

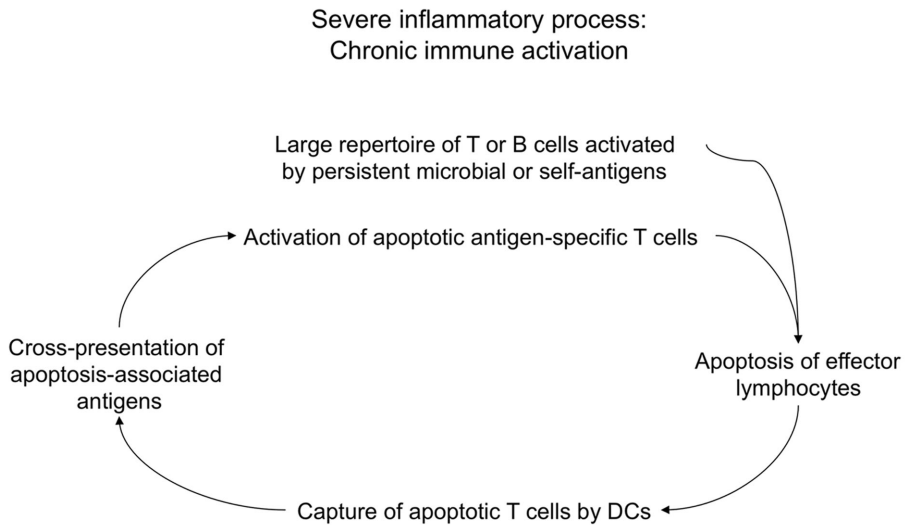


FIGURE 6 | Chronic immune activation as a result of cross-presentation of apoptotic T cells. During severe inflammatory processes, chronic immune activation may be in part maintained by a vicious spiral, which may be initiated by activated lymphocytes (e.g., specific for persistent microbial antigens or self antigens) undergoing apoptosis upon they performed their effector functions. These apoptotic cells are cross-presented by DCs, which cross-prime a huge repertoire of autoreactive T cells specific for a multitude of apoptotic cell-associated epitopes. Apoptotic antigen-specific T cells participate to the maintenance of chronic immune activation via their effector performances, then they will undergo apoptosis, and the resulting apoptotic cells will be cross-presented by DCs that will cross-prime new waves of apoptotic antigen-specific T cells, and so on.

respectively, following the chronic stimulation of local B cells (Suarez et al., 2006; Sagaert et al., 2007). In addition, the chronic stimulation of B lymphocytes by HCV infection can induce the monoclonal expansion of anti-IgG antibodies, which are responsible for the formation of cryoglobulins or even the establishment of follicular B cell lymphomas (Landau et al., 2007).

Another complex issue deserving discussion is how autoreactive T lymphocytes become pathogenetic and develop chronic autoimmune diseases. Under conditions in which the stimulatory capacities gain the upper hand over the tolerogenic capacities of DCs (i.e., during inflammatory or infectious processes), they phagocytose both microbial and self antigens and migrate with high efficiency into the draining lymph nodes, where they efficiently prime both pathogen-specific and autoreactive T cells (Lang et al., 2005; Marshak-Rothstein, 2006). Generally (as previously discussed in the B Cell Responses and T Cell Responses), these responses are switched off when the pathogen has been cleared, including the autoreactive responses that have participated in the amplification of the protective responses. Under certain conditions, several genetic and environmental factors (many of which are still unknown) can, however, allow the expansion of autoreactive clones, which escape the mechanisms of peripheral tolerance, and can induce autoimmune diseases.

In this context, it is of interest our recent observation indicating that the phenomenon of chronic immune activation, commonly observed during different viral or autoimmune diseases (Propato et al., 2001; Chernysheva et al., 2002; Bangs et al., 2006; Grossman et al., 2006), is in part caused by a vicious cycle, which is maintained by a continuous cross-presentation of apoptotic lymphoid cells that are derived from chronically activated lymphocytes (**Figure 6**; Rawson et al., 2007). The phagocytosis of these apoptotic cells by DCs leads to the generation of a huge number of apoptotic cell-derived self-epitopes. In a chronic inflammatory context and in virtue of the activatory signals provided by apoptotic T cells expressing CD40L, this activity also leads to the cross-priming of a large repertoire of apoptotic epitope-specific T cells, which in turn expand the inflammation and undergo apoptosis after they have performed their effector functions, and so on (Propato et al., 2001; Rawson et al., 2007). Thus, chronic immune activation can establish a milieu favoring the emergence and the maintenance of autoimmune responses, and ultimately contribute to the irreversible impairment of the immune system, such as in the case of HIV infection or in the final stages of several systemic autoimmune diseases (Propato et al., 2001; Rawson et al., 2007).

Finally, functional defects of T_{reg} cells seem to play a key role in the induction of autoimmune processes. Defects of these cells have been clearly documented in several autoimmune diseases (e.g., rheumatoid arthritis, type I diabetes, multiple sclerosis; Chatenoud et al., 2001; Shevach et al., 2001; Bluestone and Abbas, 2003; von Herrath and Harrison, 2003; Ehrenstein et al., 2004; Hsieh et al., 2004; Lerman et al., 2004; Tang et al., 2004; Viglietta et al., 2004; Lindley et al., 2005; Sakaguchi, 2005) as well as in allergic diseases (Medoff et al., 2008). Similar mechanisms play a crucial role in the uncontrolled generation of autoantibodies recognizing apoptotic cell-associated self-epitopes, as in the case of several systemic autoimmune diseases (i.e., systemic lupus erythematosus, progressive systemic sclerosis, Sjogren's diseases; Chatenoud et al., 2001; Shevach et al., 2001; Bluestone and Abbas, 2003; von Herrath and Harrison, 2003; Ehrenstein et al., 2004; Hsieh et al., 2004; Lerman et al., 2004; Tang et al., 2004; Viglietta et al., 2004; Lindley et al., 2005; Sakaguchi, 2005).

CONCLUSION

Based on the definition of ambiguity as "a symptom of the crisis of the subject, a productive function against and beyond the metaphysic (creativity)," we can consider this concept as a hallmark of biological systems. When the definition of ambiguity is applied to the immune system, the "crisis" should derive by its capacity to recognize everything and thus to risk to offend and not only to defend the host. Therefore, the "creativity" of the immune system consists of its capacity to express simultaneously different strategies that are only apparently opposite but eventually result in an evolutionary advantage. On the one hand, the immune response contributes to the species survival; on the other hand, it can lead to the sacrifice of single individuals. During the evolutionary process, the selective pressure led to the generation of multiple ambiguous mechanisms to better counteract the aggression of infectious agents. Although this is obtained at the cost of severe side effects (tumor development, autoimmune diseases) in several individuals, these side effects are considered irrelevant in terms of the survival of the species.

ACKNOWLEDGMENTS

This work was supported by: European Union grants (IMECS no. 201169, FP7-Health-2007-A and SPHYNX no. 261365, FP7-Health-2010); MinisterodellaSanità [Ricercafinalizzata (RFPS-2006-3-337923 and RFPS-2007-1-636647); IstitutoSuperiore di Sanità (Progetti AIDS-2006 and -2008)]; Ministerodell'Istruzione, dell'Università e dellaRicerca [MIUR; Programmi di ricerca di interessenazionale (PRIN)-2008/10 no. 7245/1; Fondo per gliinvestimenti di ricerca di base (FIRB)-2011/13 no. RBAP10TPXK]; FondazioneCariplo (progetti no. 13535 and 3603 2010/12); AssociazioneItaliana per la RicercasulCancro [AIRC; Investigator Grant (IG)-2010/13 no. 10756]. Silvia Piconese is supported by My First AIRC grant (no. 8726).

REFERENCES

Accapezzato, D., Francavilla, V., Paroli, M., Casciaro, M., Chircu, L. V., Cividini, A., Abrignani, S., Mondelli, M. U., and Barnaba, V. (2004). Hepatic expansion of a virus-specific regulatory CD8(+) T cell population in chronic hepatitis C virus infection. *J. Clin. Invest.* 113, 963–972.

Accapezzato, D., Visco, V., Francavilla, V., Molette, C., Donato, T., Paroli, M., Mondelli, M. U., Doria, M., Torrisi, M. R., and Barnaba, V. (2005). Chloroquine enhances human CD8+ T cell responses against soluble antigens in vivo. *J. Exp. Med.* 202, 817–828.

Acosta-Rodriguez, E. V., Napolitani, G., Lanzavecchia, A., and Sallusto, F. (2007). Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat. Immunol.* 8, 942–949.

Agrawal, A., Eastman, Q. M., and Schatz, D. G. (1998). Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system. *Nature* 394, 744–751.

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell* 124, 783–801.

Alard, J. E., Dueymes, M., Youinou, P., and Jamin, C. (2008). HSP60 and anti-HSP60 antibodies in vasculitis: they are two of a kind. *Clin. Rev. Allergy Immunol.* 35, 66–71.

Albert, M. L. (2004). Death-defying immunity: do apoptotic cells influence antigen processing and presentation? *Nat. Rev. Immunol.* 4, 223–231.

Anderson, M. S., Venanzi, E. S., Klein, L., Chen, Z., Berzins, S. P., Turley, S. J., von Boehmer, H., Bronson, R., Dierich, A., Benoist, C., and Mathis, D. (2002). Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298, 1395–1401.

Avrameas, S. (1991). Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton.' *Immunol. Today* 12, 154–159.

Bachem, A., Guttler, S., Hartung, E., Ebstein, F., Schaefer, M., Tannert, A., Salama, A., Movassaghi, K., Opitz, C., Mages, H. W., Henn, V., Kloetzel, P. M., Gurka, S., and Kroczek, R. A. (2010). Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J. Exp. Med.* 207, 1273–1281.

Bangs, S. C., McMichael, A. J., and Xu, X. N. (2006). Bystander T cell activation – implications for HIV infection and other diseases. *Trends Immunol.* 27, 518–524.

Barber, D. L., Wherry, E. J., Masopust, D., Zhu, B., Allison, J. P., Sharpe, A. H., Freeman, G. J., and Ahmed, R. (2006). Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439, 682–687.

Barnaba, V. (1996). Viruses, hidden self-epitopes and autoimmunity. *Immunol. Rev.* 152, 47–66.

Barron, L., Knoechel, B., Lohr, J., and Abbas, A. K. (2008). Cutting edge: contributions of apoptosis and anergy to systemic T cell tolerance. *J. Immunol.* 180, 2762–2766.

Barthlott, T., Kassiotis, G., and Stockinger, B. (2003). T cell regulation as a side effect of homeostasis and competition. *J. Exp. Med.* 197, 451–460.

Barthlott, T., Moncrieffe, H., Veldhoen, M., Atkins, C. J., Christensen, J., O'Garra, A., and Stockinger, B. (2005). CD25+ CD4+ T cells compete with naive CD4+ T cells for IL-2 and exploit it for the induction of IL-10 production. *Int. Immunol.* 17, 279–288.

Belkaid, Y. (2007). Regulatory T cells and infection: a dangerous necessity. *Nat. Rev. Immunol.* 7, 875–888.

Benoist, C., and Mathis, D. (2001). Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? *Nat. Immunol.* 2, 797–801.

Bettelli, E., Korn, T., and Kuchroo, V. K. (2007). Th17: the third member of the effector T cell trilogy. *Curr. Opin. Immunol.* 19, 652–657.

Blachere, N. E., Darnell, R. B., and Albert, M. L. (2005). Apoptotic cells deliver processed antigen to dendritic cells for cross-presentation. *PLoS Biol.* 3, e185. doi:10.1371/journal.pbio.0030185

Bleger, J. (1967). *Simbiosis y ambiguedad, estudio psiconalitico.* Buenos Aires: Editorial Paidos.

Bluestone, J. A., and Abbas, A. K. (2003). Natural versus adaptive regulatory T cells. *Nat. Rev. Immunol.* 3, 253–257.

Borgulya, P., Kishi, H., Uematsu, Y., and von Boehmer, H. (1992). Exclusion and inclusion of alpha and beta T cell receptor alleles. *Cell* 69, 529–537.

Burgdorf, S., Kautz, A., Bohnert, V., Knolle, P. A., and Kurts, C. (2007). Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. *Science* 316, 612–616.

Cao, W., Rosen, D. B., Ito, T., Bover, L., Bao, M., Watanabe, G., Yao, Z., Zhang, L., Lanier, L. L., and Liu, Y. J. (2006). Plasmacytoid dendritic cell-specific receptor ILT7-Fc epsilonRI gamma inhibits toll-like receptor-induced interferon production. *J. Exp. Med.* 203, 1399–1405.

Casanova, J. L., Romero, P., Widmann, C., Kourilsky, P., and Maryanski, J. L. (1991). T cell receptor genes in a series of class I major histocompatibility complex-restricted cytotoxic T lymphocyte clones specific for a *Plasmodium berghei* nonapeptide: implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* 174, 1371–1383.

Chatenoud, L., Salomon, B., and Bluestone, J. A. (2001). Suppressor T cells – they're back and critical for regulation of autoimmunity! *Immunol. Rev.* 182, 149–163.

Chen, W., Frank, M. E., Jin, W., and Wahl, S. M. (2001). TGF-beta released by apoptotic T cells contributes to an immunosuppressive milieu. *Immunity* 14, 715–725.

Chernysheva, A. D., Kirou, K. A., and Crow, M. K. (2002). T cell proliferation induced by autologous non-T cells is a response to apoptotic cells processed by dendritic cells. *J. Immunol.* 169, 1241–1250.

Cho, J. H. (2008). The genetics and immunopathogenesis of inflammatory bowel disease. *Nat. Rev. Immunol.* 8, 458–466.

Coffer, P. J., and Burgering, B. M. (2004). Forkhead-box transcription factors and their role in the immune system. *Nat. Rev. Immunol.* 4, 889–899.

Colombo, M. P., and Piconese, S. (2007). Regulatory-T-cell inhibition versus depletion: the right choice in cancer immunotherapy. *Nat. Rev. Cancer* 7, 880–887.

Coombes, J. L., and Powrie, F. (2008). Dendritic cells in intestinal immune regulation. *Nat. Rev. Immunol.* 8, 435–446.

Coombes, J. L., Robinson, N. J., Maloy, K. J., Uhlig, H. H., and Powrie,

F. (2005). Regulatory T cells and intestinal homeostasis. *Immunol. Rev.* 204, 184–194.

Crozat, K., Guiton, R., Contreras, V., Feuillet, V., Dutertre, C. A., Ventre, E., Vu Manh, T. P., Baranek, T., Storset, A. K., Marvel, J., Boudinot, P., Hosmalin, A., Schwartz-Cornil, I., and Dalod, M. (2010). The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J. Exp. Med.* 207, 1283–1292.

Curiel, T. J. (2007). Tregs and rethinking cancer immunotherapy. *J. Clin. Invest.* 117, 1167–1174.

Curiel, T. J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M., Conejo-Garcia, J. R., Zhang, L., Burow, M., Zhu, Y., Wei, S., Kryczek, I., Daniel, B., Gordon, A., Myers, L., Lackner, A., Disis, M. L., Knutson, K. L., Chen, L., and Zou, W. (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat. Med.* 10, 942–949.

Curtis, M. M., and Way, S. S. (2009). Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology* 126, 177–185.

Cvetanovich, G. L., and Hafler, D. A. (2010). Human regulatory T cells in autoimmune diseases. *Curr. Opin. Immunol.* 22, 753–760.

Dash, P., McClaren, J. L., Oguin, T. H. III, Rothwell, W., Todd, B., Morris, M. Y., Becksfort, J., Reynolds, C., Brown, S. A., Doherty, P. C., and Thomas, P. G. (2010). Paired analysis of TCRalpha and TCRbeta chains at the single-cell level in mice. *J. Clin. Invest.* 121, 288–295.

Day, C. L., Kaufmann, D. E., Kiepiela, P., Brown, J. A., Moodley, E. S., Reddy, S., Mackey, E. W., Miller, J. D., Leslie, A. J., DePierres, C., Mncube, Z., Duraiswamy, J., Zhu, B., Eichbaum, Q., Altfeld, M., Wherry, E. J., Coovadia, H. M., Goulder, P. J., Klenerman, P., Ahmed, R., Freeman, G. J., and Walker, B. D. (2006). PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 443, 350–354.

de Jong, E. C., Smits, H. H., and Kapsenberg, M. L. (2005). Dendritic cell-mediated T cell polarization. *Springer Semin. Immunopathol.* 26, 289–307.

de la Rosa, M., Rutz, S., Dorninger, H., and Scheffold, A. (2004). Interleukin-2 is essential for CD4+CD25+ regulatory T cell function. *Eur. J. Immunol.* 34, 2480–2488.

De Vita, L., Accapezzato, D., Mangino, G., Morrone, S., Santilio, I., Casciaro, M. A., Fava, D., Bruno, G., Del Prete, G., Santoni, A., and Barnaba, V. (1998). Defective Th1 and Th2 cytokine synthesis in the T-T cell presentation model for lack of CD40/CD40 ligand interaction. *Eur. J. Immunol.* 28, 3552–3563.

Deaglio, S., Dwyer, K. M., Gao, W., Friedman, D., Usheva, A., Erat, A., Chen, J. F., Enjyoji, K., Linden, J., Oukka, M., Kuchroo, V. K., Strom, T. B., and Robson, S. C. (2007). Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J. Exp. Med.* 204, 1257–1265.

Di Pucchio, T., Chatterjee, B., Smed-Sorensen, A., Clayton, S., Palazzo, A., Montes, M., Xue, Y., Mellman, I., Banchereau, J., and Connolly, J. E. (2008). Direct proteasome-independent cross-presentation of viral antigen by plasmacytoid dendritic cells on major histocompatibility complex class I. *Nat. Immunol.* 9, 551–557.

Di Rosa, F., and Barnaba, V. (1998). Persisting viruses and chronic inflammation: understanding their relation to autoimmunity. *Immunol. Rev.* 164, 17–27.

Dominguez-Villar, M., Baecher-Allan, C. M., and Hafler, D. A. (2011). Identification of T helper type 1-like, Foxp3+ regulatory T cells in human autoimmune disease. *Nat. Med.* 17, 673–675.

Dong, C. (2008). TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat. Rev. Immunol.* 8, 337–348.

Dudziak, D., Kamphorst, A. O., Heidkamp, G. F., Buchholz, V. R., Trumpfheller, C., Yamazaki, S., Cheong, C., Liu, K., Lee, H. W., Park, C. G., Steinman, R. M., and Nussenzweig, M. C. (2007). Differential antigen processing by dendritic cell subsets in vivo. *Science* 315, 107–111.

Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A., and Sallusto, F. (2009). Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat. Immunol.* 10, 857–863.

Ebinuma, H., Nakamoto, N., Li, Y., Price, D. A., Gostick, E., Levine, B. L., Tobias, J., Kwok, W. W., and Chang, K. M. (2008). Identification and in vitro expansion of functional antigen-specific CD25+ FoxP3+ regulatory T cells in hepatitis C virus infection. *J. Virol.* 82, 5043–5053.

Ehrenstein, M. R., Evans, J. G., Singh, A., Moore, S., Warnes, G., Isenberg, D. A., and Mauri, C. (2004). Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J. Exp. Med.* 200, 277–285.

Elliott, J. I., and Altmann, D. M. (1995). Dual T cell receptor alpha chain T cells in autoimmunity. *J. Exp. Med.* 182, 953–959.

Eyerich, S., Eyerich, K., Pennino, D., Carbone, T., Nasorri, F., Pallotta, S., Cianfarani, F., Odorisio, T., Traidl-Hoffmann, C., Behrendt, H., Durham, S. R., Schmidt-Weber, C. B., and Cavani, A. (2009). Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J. Clin. Invest.* 119, 3573–3585.

Ferguson, T. A., Green, D. R., and Griffith, T. S. (2002). Cell death and immune privilege. *Int. Rev. Immunol.* 21, 153–172.

Fontenot, J. D., Gavin, M. A., and Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4, 330–336.

Fontenot, J. D., Rasmussen, J. P., Gavin, M. A., and Rudensky, A. Y. (2005). A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nat. Immunol.* 6, 1142–1151.

Franceschini, D., Paroli, M., Francavilla, V., Videtta, M., Morrone, S., Labbadia, G., Cerino, A., Mondelli, M. U., and Barnaba, V. (2009). PD-L1 negatively regulates CD4+CD25+Foxp3+ Tregs by limiting STAT-5 phosphorylation in patients chronically infected with HCV. *J. Clin. Invest.* 119, 551–564.

Fritz, J. H., Ferrero, R. L., Philpott, D. J., and Girardin, S. E. (2006). Nod-like proteins in immunity, inflammation and disease. *Nat. Immunol.* 7, 1250–1257.

Gallucci, S., and Matzinger, P. (2001). Danger signals: SOS to the immune system. *Curr. Opin. Immunol.* 13, 114–119.

Gao, Y., Herndon, J. M., Zhang, H., Griffith, T. S., and Ferguson, T. A. (1998). Antiinflammatory effects of CD95 ligand (FasL)-induced apoptosis. *J. Exp. Med.* 188, 887–896.

Gardner, J. M., Devoss, J. J., Friedman, R. S., Wong, D. J., Tan, Y. X., Zhou, X., Johannes, K. P., Su, M. A., Chang, H. Y., Krummel, M. F., and Anderson, M. S. (2008). Deletional tolerance mediated by extrathymic Aire-expressing cells. *Science* 321, 843–847.

Gillard, G. O., Dooley, J., Erickson, M., Peltonen, L., and Farr, A. G. (2007). Aire-dependent alterations in medullary thymic epithelium indicate a role for Aire in thymic epithelial differentiation. *J. Immunol.* 178, 3007–3015.

Griffith, T. S., Kazama, H., VanOosten, R. L., Earle, J. K. Jr., Herndon, J. M., Green, D. R., and Ferguson, T. A. (2007). Apoptotic cells induce tolerance by generating helpless CD8+ T cells that produce TRAIL. *J. Immunol.* 178, 2679–2687.

Grossman, Z., Meier-Schellersheim, M., Paul, W. E., and Picker, L. J. (2006). Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat. Med.* 12, 289–295.

Guermonprez, P., Saveanu, L., Kleijmeer, M., Davoust, J., Van Endert, P., and Amigorena, S. (2003). ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature* 425, 397–402.

Guiducci, C., Ott, G., Chan, J. H., Damon, E., Calacsan, C., Matray, T., Lee, K. D., Coffman, R. L., and Barrat, F. J. (2006). Properties regulating the nature of the plasmacytoid dendritic cell response to toll-like receptor 9 activation. *J. Exp. Med.* 203, 1999–2008.

Hardy, R. R., and Hayakawa, K. (2001). B cell development pathways. *Annu. Rev. Immunol.* 19, 595–621.

He, X., Janeway, C. A. Jr., Levine, M., Robinson, E., Preston-Hurlburt, P., Viret, C., and Bottomly, K. (2002). Dual receptor T cells extend the immune repertoire for foreign antigens. *Nat. Immunol.* 3, 127–134.

Heeg, M. H., Ulsenheimer, A., Gruner, N. H., Zachoval, R., Jung, M. C., Gerlach, J. T., Raziorrouh, B., Schraut, W., Horster, S., Kauke, T., Spannagl, M., and Diepolder, H. M. (2009). FOXP3 expression in hepatitis C virus-specific CD4+ T cells during acute hepatitis C. *Gastroenterology* 137, 1280–1288. e1281–e1286.

Hintikka, J. (1959). Aristotle and the ambiguity of ambiguity. *Inquiry* 2, 137–151.

Hoebe, K., Janssen, E., and Beutler, B. (2004). The interface between innate and adaptive immunity. *Nat. Immunol.* 5, 971–974.

Hori, S., Nomura, T., and Sakaguchi, S. (2003). Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299, 1057–1061.

Hsieh, C. S., Liang, Y., Tyznik, A. J., Self, S. G., Liggitt, D., and Rudensky, A. Y. (2004). Recognition of the peripheral self by naturally arising

CD25+ CD4+ T cell receptors. *Immunity* 21, 267–277.

Huehn, J., Polansky, J. K., and Hamann, A. (2009). Epigenetic control of FOXP3 expression: the key to a stable regulatory T-cell lineage? *Nat. Rev. Immunol.* 9, 83–89.

Inaba, K., Turley, S., Yamaide, F., Iyoda, T., Mahnke, K., Inaba, M., Pack, M., Subklewe, M., Sauter, B., Sheff, D., Albert, M., Bhardwaj, N., Mellman, I., and Steinman, R. M. (1998). Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. *J. Exp. Med.* 188, 2163–2173.

Inohara, N., Chamaillard, M., McDonald, C., and Nunez, G. (2005). NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu. Rev. Biochem.* 74, 355–383.

Jakobson, R. (1995). "On realism in art," in *On Language*, Chap. I, eds. L. Waugh and M. Monville-Burston (Cambridge: Harvard University Press), 21–23.

Jameson, S. C., and Bevan, M. J. (1998). T-cell selection. *Curr. Opin. Immunol.* 10, 214–219.

Janeway, C. A. Jr., and Medzhitov, R. (2002). Innate immune recognition. *Annu. Rev. Immunol.* 20, 197–216.

Jego, G., Palucka, A. K., Blanck, J. P., Chalouni, C., Pascual, V., and Banchereau, J. (2003). Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. *Immunity* 19, 225–234.

Ji, Q., Perchellet, A., and Goverman, J. M. (2010). Viral infection triggers central nervous system autoimmunity via activation of CD8+ T cells expressing dual TCRs. *Nat. Immunol.* 11, 628–634.

Jiang, A., Bloom, O., Ono, S., Cui, W., Unternaehrer, J., Jiang, S., Whitney, J. A., Connolly, J., Banchereau, J., and Mellman, I. (2007). Disruption of E-cadherin-mediated adhesion induces a functionally distinct pathway of dendritic cell maturation. *Immunity* 27, 610–624.

Kawai, T., and Akira, S. (2006). Innate immune recognition of viral infection. *Nat. Immunol.* 7, 131–137.

Kazama, H., Ricci, J. E., Herndon, J. M., Hoppe, G., Green, D. R., and Ferguson, T. A. (2008). Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. *Immunity* 29, 21–32.

Keir, M. E., Liang, S. C., Guleria, I., Latchman, Y. E., Qipo, A., Albacker, L. A., Koulmanda, M., Freeman, G. J., Sayegh, M. H., and Sharpe, A. H. (2006). Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J. Exp. Med.* 203, 883–895.

Killeen, N., Irving, B. A., Pippig, S., and Zingler, K. (1998). Signaling checkpoints during the development of T lymphocytes. *Curr. Opin. Immunol.* 10, 360–367.

Klareskog, L., Widhe, M., Hermansson, M., and Ronnelid, J. (2008). Antibodies to citrullinated proteins in arthritis: pathology and promise. *Curr. Opin. Rheumatol.* 20, 300–305.

Klechevsky, E., Liu, M., Morita, R., Banchereau, R., Thompson-Snipes, L., Palucka, A. K., Ueno, H., and Banchereau, J. (2009). Understanding human myeloid dendritic cell subsets for the rational design of novel vaccines. *Hum. Immunol.* 70, 281–288.

Klechevsky, E., Morita, R., Liu, M., Cao, Y., Coquery, S., Thompson-Snipes, L., Briere, F., Chaussabel, D., Zurawski, G., Palucka, A. K., Reiter, Y., Banchereau, J., and Ueno, H. (2008). Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity* 29, 497–510.

Klenerman, P., and Hill, A. (2005). T cells and viral persistence: lessons from diverse infections. *Nat. Immunol.* 6, 873–879.

Korn, T., Bettelli, E., Oukka, M., and Kuchroo, V. K. (2009). IL-17 and Th17 Cells. *Annu. Rev. Immunol.* 27, 485–517.

Kretschmer, K., Apostolou, I., Hawiger, D., Khazaie, K., Nussenzweig, M. C., and von Boehmer, H. (2005). Inducing and expanding regulatory T cell populations by foreign antigen. *Nat. Immunol.* 6, 1219–1227.

Kuroda, N., Mitani, T., Takeda, N., Ishimaru, N., Arakaki, R., Hayashi, Y., Bando, Y., Izumi, K., Takahashi, T., Nomura, T., Sakaguchi, S., Ueno, T., Takahama, Y., Uchida, D., Sun, S., Kajiura, F., Mouri, Y., Han, H., Matsushima, A., Yamada, G., and Matsumoto, M. (2005). Development of autoimmunity against transcriptionally unrepressed target antigen in the thymus of Aire-deficient mice. *J. Immunol.* 174, 1862–1870.

Kurts, C., Heath, W. R., Kosaka, H., Miller, J. F., and Carbone, F. R. (1998). The peripheral deletion of autoreactive CD8+ T cells induced by cross-presentation of self-antigens involves signaling through CD95 (Fas, Apo-1). *J. Exp. Med.* 188, 415–420.

Lal, G., and Bromberg, J. S. (2009). Epigenetic mechanisms of regulation of Foxp3 expression. *Blood* 114, 3727–3735.

Landau, D. A., Saadoun, D., Calabrese, L. H., and Cacoub, P. (2007). The pathophysiology of HCV induced B-cell clonal disorders. *Autoimmun. Rev.* 6, 581–587.

Lang, K. S., Recher, M., Junt, T., Navarini, A. A., Harris, N. L., Freigang, S., Odermatt, B., Conrad, C., Ittner, L. M., Bauer, S., Luther, S. A., Uematsu, S., Akira, S., Hengartner, H., and Zinkernagel, R. M. (2005). Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nat. Med.* 11, 138–145.

Lanzavecchia, A., and Sallusto, F. (2001). Regulation of T cell immunity by dendritic cells. *Cell* 106, 263–266.

Lanzavecchia, A., and Sallusto, F. (2007). Toll-like receptors and innate immunity in B-cell activation and antibody responses. *Curr. Opin. Immunol.* 19, 268–274.

Lerman, M. A., Larkin, J. III, Cozzo, C., Jordan, M. S., and Caton, A. J. (2004). CD4+ CD25+ regulatory T cell repertoire formation in response to varying expression of a neo-self-antigen. *J. Immunol.* 173, 236–244.

Lindley, S., Dayan, C. M., Bishop, A., Roep, B. O., Peakman, M., and Tree, T. I. (2005). Defective suppressor function in CD4(+)CD25(+) T-cells from patients with type 1 diabetes. *Diabetes* 54, 92–99.

Liston, A., Lesage, S., Wilson, J., Peltonen, L., and Goodnow, C. C. (2003). Aire regulates negative selection of organ-specific T cells. *Nat. Immunol.* 4, 350–354.

Luger, D., Silver, P. B., Tang, J., Cua, D., Chen, Z., Iwakura, Y., Bowman, E. P., Sgambellone, N. M., Chan, C. C., and Caspi, R. R. (2008). Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J. Exp. Med.* 205, 799–810.

Lund, J. M., Hsing, L., Pham, T. T., and Rudensky, A. Y. (2008). Coordination of early protective immunity to viral infection by regulatory T cells. *Science* 320, 1220–1224.

Mantovani, A., Allavena, P., Sica, A., and Balkwill, F. (2008a). Cancer-related inflammation. *Nature* 454, 436–444.

Mantovani, A., Romero, P., Palucka, A. K., and Marincola, F. M. (2008b). Tumour immunity: effector response to tumour and role of the microenvironment. *Lancet* 371, 771–783.

Marrack, P., McCormack, J., and Kappler, J. (1989). Presentation of antigen, foreign major histocompatibility complex proteins and self by thymus cortical epithelium. *Nature* 338, 503–505.

Marshak-Rothstein, A. (2006). Toll-like receptors in systemic autoimmune disease. *Nat. Rev. Immunol.* 6, 823–835.

Masopust, D., and Ahmed, R. (2004). Reflections on CD8 T-cell activation and memory. *Immunol. Res.* 29, 151–160.

Matsui, T., Connolly, J. E., Michnevitz, M., Chaussabel, D., Yu, C. I., Glaser, C., Tindle, S., Pypaert, M., Freitas, H., Piqueras, B., Banchereau, J., and Palucka, A. K. (2009). CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions. *J. Immunol.* 182, 6815–6823.

Matsunaga, T., and Rahman, A. (2001). In search of the origin of the thymus: the thymus and GALT may be evolutionarily related. *Scand. J. Immunol.* 53, 1–6.

Matzinger, P. (1994). Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* 12, 991–1045.

Matzinger, P. (2002). An innate sense of danger. *Ann. N. Y. Acad. Sci.* 961, 341–342.

Matzinger, P. (2007). Friendly and dangerous signals: is the tissue in control? *Nat. Immunol.* 8, 11–13.

Matzinger, P., and Kamala, T. (2011). Tissue-based class control: the other side of tolerance. *Nat. Rev. Immunol.* 11, 221–230.

McCaughtry, T. M., Baldwin, T. A., Wilken, M. S., and Hogquist, K. A. (2008). Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla. *J. Exp. Med.* 205, 2575–2584.

McGargill, M. A., Derbinski, J. M., and Hogquist, K. A. (2000). Receptor editing in developing T cells. *Nat. Immunol.* 1, 336–341.

McGeachy, M. J., and Cua, D. J. (2008). Th17 cell differentiation: the long and winding road. *Immunity* 28, 445–453.

Medoff, B. D., Thomas, S. Y., and Luster, A. D. (2008). T cell trafficking in allergic asthma: the ins and outs. *Annu. Rev. Immunol.* 26, 205–232.

Medzhitov, R. (2010). Inflammation 2010: new adventures of an old flame. *Cell* 140, 771–776.

Medzhitov, R., and Janeway, C. A. Jr. (2002). Decoding the patterns of self and nonself by the innate immune system. *Science* 296, 298–300.

Meylan, E., Tschopp, J., and Karin, M. (2006). Intracellular pattern recognition receptors in the host response. *Nature* 442, 39–44.

Murali-Krishna, K., Altman, J. D., Suresh, M., Sourdive, D. J., Zajac, A. J., Miller, J. D., Slansky, J., and Ahmed, R. (1998). Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* 8, 177–187.

Murphy, K., Travers, P., and Walport, M. (2007). *Janeway's Immunobiology*, 7th Edn. New York: Garland Science.

Nair, S., Boczkowski, D., Fassnacht, M., Pisetsky, D., and Gilboa, E. (2007). Vaccination against the forkhead family transcription factor Foxp3 enhances tumor immunity. *Cancer Res.* 67, 371–380.

Nelms, K., Keegan, A. D., Zamorano, J., Ryan, J. J., and Paul, W. E. (1999). The IL-4 receptor: signaling mechanisms and biologic functions. *Annu. Rev. Immunol.* 17, 701–738.

Neuberger, M. S. (1997). Antigen receptor signaling gives lymphocytes a long life. *Cell* 90, 971–973.

Nikolich-Zugich, J. (2008). Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. *Nat. Rev. Immunol.* 8, 512–522.

Norbury, C. C., Basta, S., Donohue, K. B., Tscharke, D. C., Princiotta, M. F., Berglund, P., Gibbs, J., Bennink, J. R., and Yewdell, J. W. (2004). CD8+ T cell cross-priming via transfer of proteasome substrates. *Science* 304, 1318–1321.

Oates, J. C., and Gilkeson, G. S. (2002). Mediators of injury in lupus nephritis. *Curr. Opin. Rheumatol.* 14, 498–503.

Oettinger, M. A., Schatz, D. G., Gorka, C., and Baltimore, D. (1990). RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* 248, 1517–1523.

Otsuki, T., Maeda, M., Murakami, S., Hayashi, H., Miura, Y., Kusaka, M., Nakano, T., Fukuoka, K., Kishimoto, T., Hyodoh, F., Ueki, A., and Nishimura, Y. (2007). Immunological effects of silica and asbestos. *Cell. Mol. Immunol.* 4, 261–268.

Padovan, E., Casorati, G., Dellabona, P., Meyer, S., Brockhaus, M., and Lanzavecchia, A. (1993). Expression of two T cell receptor alpha chains: dual receptor T cells. *Science* 262, 422–424.

Palucka, K., Banchereau, J., and Mellman, I. (2010). Designing vaccines based on biology of human dendritic cell subsets. *Immunity* 33, 464–478.

Pandiyan, P., Zheng, L., Ishihara, S., Reed, J., and Lenardo, M. J. (2007). CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. *Nat. Immunol.* 8, 1353–1362.

Pasqualotto, G. (1997). *Illuminismo e illuminazione*. Rome: Donzelli.

Petrilli, V., Dostert, C., Muruve, D. A., and Tschopp, J. (2007). The inflammasome: a danger sensing complex triggering innate immunity. *Curr. Opin. Immunol.* 19, 615–622.

Phillips, R. E. (2002). Immunology taught by Darwin. *Nat. Immunol.* 3, 987–989.

Piconese, S., Pittoni, P., Burocchi, A., Gorzanelli, A., Care, A., Tripodo, C., and Colombo, M. P. (2010). A non-redundant role for OX40 in the competitive fitness of Treg in response to IL-2. *Eur. J. Immunol.* 40, 2902–2913.

Piconese, S., Valzasina, B., and Colombo, M. P. (2008). OX40 triggering blocks suppression by regulatory T cells and facilitates tumor rejection. *J. Exp. Med.* 205, 825–839.

Pillai, S. (1999). The chosen few? Positive selection and the generation of naive B lymphocytes. *Immunity* 10, 493–502.

Pope, R. M., and Tschopp, J. (2007). The role of interleukin-1 and the inflammasome in gout: implications for therapy. *Arthritis Rheum.* 56, 3183–3188.

Probst, H. C., McCoy, K., Okazaki, T., Honjo, T., and van den Broek, M. (2005). Resting dendritic cells induce peripheral CD8+ T cell tolerance through PD-1 and CTLA-4. *Nat. Immunol.* 6, 280–286.

Propato, A., Cutrona, G., Francavilla, V., Ulivi, M., Schiaffella, E., Landt, O., Dunbar, R., Cerundolo, V., Ferrarini, M., and Barnaba, V. (2001). Apoptotic cells overexpress vinculin and induce vinculin-specific cytotoxic T-cell cross-priming. *Nat. Med.* 7, 807–813.

Pullen, A. M., Kappler, J. W., and Marrack, P. (1989). Tolerance to self antigens shapes the T-cell repertoire. *Immunol. Rev.* 107, 125–139.

Rawson, P. M., Molette, C., Videtta, M., Altieri, L., Franceschini, D., Donato, T., Finocchi, L., Propato, A., Paroli, M., Meloni, F., Mastroianni, C. M., d'Ettorre, G., Sidney, J., Sette, A., and Barnaba, V. (2007). Cross-presentation of caspase-cleaved apoptotic self antigens in HIV infection. *Nat. Med.* 13, 1431–1439.

Rice, J. S., Kowal, C., Volpe, B. T., DeGiorgio, L. A., and Diamond, B. (2005). Molecular mimicry: anti-DNA antibodies bind microbial and nonnucleic acid self-antigens. *Curr. Top. Microbiol. Immunol.* 296, 137–151.

Ridge, J. P., Di Rosa, F., and Matzinger, P. (1998). A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* 393, 474–478.

Romagnani, S. (1997). The Th1/Th2 paradigm. *Immunol. Today* 18, 263–266.

Roncarolo, M. G., Levings, M. K., and Traversari, C. (2001). Differentiation of T regulatory cells by immature dendritic cells. *J. Exp. Med.* 193, F5–F9.

Rose, N. R., and Mackay, I. R. (2000). Molecular mimicry: a critical look at exemplary instances in human diseases. *Cell. Mol. Life Sci.* 57, 542–551.

Rubtsov, Y. P., Niec, R. E., Josefowicz, S., Li, L., Darce, J., Mathis, D., Benoist, C., and Rudensky, A. Y. (2010). Stability of the regulatory T cell lineage in vivo. *Science* 329, 1667–1671.

Ruby, C. E., Yates, M. A., Hirschhorn-Cymerman, D., Chlebeck, P., Wolchok, J. D., Houghton, A. N., Offner, H., and Weinberg, A. D. (2009). Cutting edge: OX40 agonists can drive regulatory T cell expansion if the cytokine milieu is right. *J. Immunol.* 183, 4853–4857.

Sabbagh, L., Snell, L. M., and Watts, T. H. (2007). TNF family ligands define niches for T cell memory. *Trends Immunol.* 28, 333–339.

Sagaert, X., De Wolf-Peeters, C., Noels, H., and Baens, M. (2007). The pathogenesis of MALT lymphomas: where do we stand? *Leukemia* 21, 389–396.

Sakaguchi, S. (2004). Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol.* 22, 531–562.

Sakaguchi, S. (2005). Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat. Immunol.* 6, 345–352.

Sakaguchi, S., Miyara, M., Costantino, C. M., and Hafler, D. A. (2010). FOXP3+ regulatory T cells in the human immune system. *Nat. Rev. Immunol.* 10, 490–500.

Salemi, S., Caporossi, A. P., Boffa, L., Longobardi, M. G., and Barnaba, V. (1995). HIVgp120 activates autoreactive CD4-specific T cell responses by unveiling of hidden CD4 peptides during processing. *J. Exp. Med.* 181, 2253–2257.

Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu. Rev. Immunol.* 22, 745–763.

Sanjabi, S., Zenewicz, L. A., Kamanaka, M., and Flavell, R. A. (2009). Anti-inflammatory and pro-inflammatory roles of TGF-beta, IL-10, and IL-22 in immunity and autoimmunity. *Curr. Opin. Pharmacol.* 9, 447–453.

Savina, A., Jancic, C., Hugues, S., Guermonprez, P., Vargas, P., Moura, I. C., Lennon-Dumenil, A. M., Seabra, M. C., Raposo, G., and Amigorena, S. (2006). NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. *Cell* 126, 205–218.

Scheffold, A., Huhn, J., and Hofer, T. (2005). Regulation of CD4+CD25+ regulatory T cell activity: it takes (IL-) two to tango. *Eur. J. Immunol.* 35, 1336–1341.

Schubert, L. A., Jeffery, E., Zhang, Y., Ramsdell, F., and Ziegler, S. F. (2001). Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J. Biol. Chem.* 276, 37672–37679.

Schulz, O., Diebold, S. S., Chen, M., Naslund, T. I., Nolte, M. A., Alexopoulou, L., Azuma, Y. T., Flavell, R. A., Liljestrom, P., and Reis e Sousa, C. (2005). Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature* 433, 887–892.

Serhan, C. N., and Savill, J. (2005). Resolution of inflammation: the beginning programs the end. *Nat. Immunol.* 6, 1191–1197.

Setoguchi, R., Hori, S., Takahashi, T., and Sakaguchi, S. (2005). Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization. *J. Exp. Med.* 201, 723–735.

Sharma, M. D., Hou, D. Y., Baban, B., Koni, P. A., He, Y., Chandler, P. R., Blazar, B. R., Mellor, A. L., and Munn, D. H. (2010). Reprogrammed foxp3(+) regulatory T cells provide essential help to support cross-presentation and CD8(+) T cell priming in naive mice. *Immunity* 33, 942–954.

Sharpe, A. H., Wherry, E. J., Ahmed, R., and Freeman, G. J. (2007). The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat. Immunol.* 8, 239–245.

Shevach, E. M. (2002). CD4+ CD25+ suppressor T cells: more questions than answers. *Nat. Rev. Immunol.* 2, 389–400.

Shevach, E. M., McHugh, R. S., Piccirillo, C. A., and Thornton, A. M.

(2001). Control of T-cell activation by CD4+ CD25+ suppressor T cells. *Immunol. Rev.* 182, 58–67.

Shinkai, Y., Rathbun, G., Lam, K. P., Oltz, E. M., Stewart, V., Mendelsohn, M., Charron, J., Datta, M., Young, F., Stall, A. M., and Alt, F. W. (1992). RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* 68, 855–867.

Shortman, K., and Heath, W. R. (2010). The CD8+ dendritic cell subset. *Immunol. Rev.* 234, 18–31.

Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., Antonenko, S., and Liu, Y. J. (1999). The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284, 1835–1837.

Snodgrass, H. R., Kisielow, P., Kiefer, M., Steinmetz, M., and von Boehmer, H. (1985). Ontogeny of the T-cell antigen receptor within the thymus. *Nature* 313, 592–595.

Sprent, J., and Surh, C. D. (2002). T cell memory. *Annu. Rev. Immunol.* 20, 551–579.

Steinman, R. M., Hawiger, D., and Nussenzweig, M. C. (2003). Tolerogenic dendritic cells. *Annu. Rev. Immunol.* 21, 685–711.

Suarez, F., Lortholary, O., Hermine, O., and Lecuit, M. (2006). Infection-associated lymphomas derived from marginal zone B cells: a model of antigen-driven lymphoproliferation. *Blood* 107, 3034–3044.

Surh, C. D., Boyman, O., Purton, J. F., and Sprent, J. (2006). Homeostasis of memory T cells. *Immunol. Rev.* 211, 154–163.

Tan, A., Yeh, S. H., Liu, C. J., Cheung, C., and Chen, P. J. (2008). Viral hepatocarcinogenesis: from infection to cancer. *Liver Int.* 28, 175–188.

Tang, Q., Henriksen, K. J., Bi, M., Finger, E. B., Szot, G., Ye, J., Masteller, E. L., McDevitt, H., Bonyhadi, M., and Bluestone, J. A. (2004). In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med.* 199, 1455–1465.

Tesniere, A., Apetoh, L., Ghiringhelli, F., Joza, N., Panaretakis, T., Kepp, O., Schlemmer, F., Zitvogel, L., and Kroemer, G. (2008). Immunogenic cancer cell death: a key-lock paradigm. *Curr. Opin. Immunol.* 20, 504–511.

Tiegs, S. L., Russell, D. M., and Nemazee, D. (1993). Receptor editing in self-reactive bone marrow B cells. *J. Exp. Med.* 177, 1009–1020.

Tonegawa, S. (1983). Somatic generation of antibody diversity. *Nature* 302, 575–581.

Tortorella, D., Gewurz, B. E., Furman, M. H., Schust, D. J., and Ploegh, H. L. (2000). Viral subversion of the immune system. *Annu. Rev. Immunol.* 18, 861–926.

Tsuji, M., Komatsu, N., Kawamoto, S., Suzuki, K., Kanagawa, O., Honjo, T., Hori, S., and Fagarasan, S. (2009). Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. *Science* 323, 1488–1492.

Tuovinen, H., Salminen, J. T., and Arstila, T. P. (2006). Most human thymic and peripheral-blood CD4+ CD25+ regulatory T cells express 2 T-cell receptors. *Blood* 108, 4063–4070.

van der Aar, A. M., Sylva-Steenland, R. M., Bos, J. D., Kapsenberg, M. L., de Jong, E. C., and Teunissen, M. B. (2007). Loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. *J. Immunol.* 178, 1986–1990.

Viglianti, G. A., Lau, C. M., Hanley, T. M., Miko, B. A., Shlomchik, M. J., and Marshak-Rothstein, A. (2003). Activation of autoreactive B cells by CpG dsDNA. *Immunity* 19, 837–847.

Viglietta, V., Baecher-Allan, C., Weiner, H. L., and Hafler, D. A. (2004). Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 199, 971–979.

Vignali, D. A., Collison, L. W., and Workman, C. J. (2008). How regulatory T cells work. *Nat. Rev. Immunol.* 8, 523–532.

von Boehmer, H. (2005). Mechanisms of suppression by suppressor T cells. *Nat. Immunol.* 6, 338–344.

von Herrath, M. G., and Harrison, L. C. (2003). Antigen-induced regulatory T cells in autoimmunity. *Nat. Rev. Immunol.* 3, 223–232.

Walker, L. S., and Abbas, A. K. (2002). The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat. Rev. Immunol.* 2, 11–19.

Ward, S. M., Fox, B. C., Brown, P. J., Worthington, J., Fox, S. B., Chapman, R. W., Fleming, K. A., Banham, A. H., and Klenerman, P. (2007). Quantification and localisation of FOXP3+ T lymphocytes and relation to hepatic inflammation during chronic HCV infection. *J. Hepatol.* 47, 316–324.

Watanabe, N., Wang, Y. H., Lee, H. K., Ito, T., Cao, W., and Liu, Y. J. (2005). Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature* 436, 1181–1185.

Wayne, J., Suh, H., Misulovin, Z., Sokol, K. A., Inaba, K., and Nussenzweig, M. C. (1994). A regulatory role for recombinase activating genes, RAG-1 and RAG-2, in T cell development. *Immunity* 1, 95–107.

West, K. L., Singha, N. C., De Ioannes, P., Lacomis, L., Erdjument-Bromage, H., Tempst, P., and Cortes, P. (2005). A direct interaction between the RAG2 C terminus and the core histones is required for efficient V(D)J recombination. *Immunity* 23, 203–212.

West, M. A., Wallin, R. P., Matthews, S. P., Svensson, H. G., Zaru, R., Ljunggren, H. G., Prescott, A. R., and Watts, C. (2004). Enhanced dendritic cell antigen capture via toll-like receptor-induced actin remodeling. *Science* 305, 1153–1157.

Winau, F., Weber, S., Sad, S., de Diego, J., Hoops, S. L., Breiden, B., Sandhoff, K., Brinkmann, V., Kaufmann, S. H., and Schaible, U. E. (2006). Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. *Immunity* 24, 105–117.

Wykes, M., Pombo, A., Jenkins, C., and MacPherson, G. G. (1998). Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. *J. Immunol.* 161, 1313–1319.

Yamazaki, S., Inaba, K., Tarbell, K. V., and Steinman, R. M. (2006). Dendritic cells expand antigen-specific Foxp3+ CD25+ CD4+ regulatory T cells including suppressors of alloreactivity. *Immunol. Rev.* 212, 314–329.

Yoshikai, Y., Anatoniou, D., Clark, S. P., Yanagi, Y., Sangster, R., Van den Elsen, P., Terhorst, C., and Mak, T. W. (1984). Sequence and expression of transcripts of the human T-cell receptor beta-chain genes. *Nature* 312, 521–524.

Zarember, K. A., and Godowski, P. J. (2002). Tissue expression of human toll-like receptors and differential regulation of toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *J. Immunol.* 168, 554–561.

Zenewicz, L. A., Yancopoulos, G. D., Valenzuela, D. M., Murphy, A. J., Stevens, S., and Flavell, R. A. (2008). Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 29, 947–957.

Zheng, Y., Valdez, P. A., Danilenko, D. M., Hu, Y., Sa, S. M., Gong, Q., Abbas, A. R., Modrusan, Z., Ghilardi, N., de Sauvage, F. J., and Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* 14, 282–289.

Zhou, G., and Levitsky, H. I. (2007). Natural regulatory T cells and de novo-induced regulatory T cells contribute independently to tumor-specific tolerance. *J. Immunol.* 178, 2155–2162.

Zhou, X., Bailey-Bucktrout, S., Jeker, L. T., and Bluestone, J. A. (2009a). Plasticity of CD4(+) FoxP3(+) T cells. *Curr. Opin. Immunol.* 21, 281–285.

Zhou, X., Bailey-Bucktrout, S. L., Jeker, L. T., Penaranda, C., Martinez-Llordella, M., Ashby, M., Nakayama, M., Rosenthal, W., and Bluestone, J. A. (2009b). Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat. Immunol.* 10, 1000–1007.

Zhu, M., Chin, R. K., Christiansen, P. A., Lo, J. C., Liu, X., Ware, C., Siebenlist, U., and Fu, Y. X. (2006). NF-kappaB2 is required for the establishment of central tolerance through an Aire-dependent pathway. *J. Clin. Invest.* 116, 2964–2971.

Ziegler, S. F. (2006). FOXP3: of mice and men. *Annu. Rev. Immunol.* 24, 209–226.

Zinkernagel, R. M. (1996). Immunology taught by viruses. *Science* 271, 173–178.

Zinkernagel, R. M., Planz, O., Ehl, S., Battegay, M., Odermatt, B., Klenerman, P., and Hengartner, H. (1999). General and specific immunosuppression caused by antiviral T-cell responses. *Immunol. Rev.* 168, 305–315.

Zou, W. (2006). Regulatory T cells, tumour immunity and immunotherapy. *Nat. Rev. Immunol.* 6, 295–307.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 30 October 2011; accepted: 02 February 2012; published online: 22 February 2012.

*Citation: Barnaba V, Paroli M and Piconese S (2012) The ambiguity in immunology. Front. Immun. **3**:18. doi: 10.3389/fimmu.2012.00018*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 09 April 2012
doi: 10.3389/fimmu.2012.00067

Type I interferons as ambiguous modulators of chronic inflammation in the central nervous system

***Marco Prinz*[1,2]* and *Klaus-Peter Knobeloch*[1]**

[1] *Department of Neuropathology, University Clinic Freiburg, Freiburg, Germany*
[2] *BIOSS Centre for Biological Signaling Studies, Freiburg im Breisgau, Germany*

Edited by:
Masaaki Murakami, Osaka University, Japan

Reviewed by:
Barbara Viviani, University of Milan, Italy
Eric Huseby, University of Massachusetts Medical School, USA

***Correspondence:**
Marco Prinz, Department of Neuropathology, University Clinic Freiburg, Breisacher Str. 64, 79106 Freiburg im Breisgau, Germany.
e-mail: marco.prinz@uniklinik-freiburg.de

Type I interferons (IFNs) were originally identified as antiviral effector molecules that exert pleiotropic physiological processes ranging from immune modulation, control of proliferation, apoptosis to antitumor activity. However, type I IFNs were recently also shown to apply both beneficial and detrimental effects to the central nervous system (CNS) and a tightly balanced equilibrium between cellular activation and inhibition seems to be essential to maintain homeostasis within the CNS. In inflammatory pathologies affecting the CNS, type I IFNs are in the center of attention not only because interferon beta (IFN-β) is used as a standard therapeutic in the treatment of relapsing–remitting multiple sclerosis (MS), but also as type I IFN expression is associated with distinct pathologies. Despite the great efficiency of IFN-β in reducing MS relapses and attenuation of novel inflammatory lesions is well documented, underlying molecular mechanisms and cellular target specificities are just beginning to emerge. In contrast to the curative effects, aberrant activation of the type I IFN response were also recently shown to be associated with detrimental effects exemplified by the Aicardi–Goutières syndrome (AGS), a severe disabling autoimmune inflammatory encephalopathy. This review will highlight the dual role of type I interferons during chronic CNS inflammation. Recently uncovered molecular and cellular mechanisms in the etiology of AGS and experimental autoimmune encephalomyelitis (EAE), the murine model of MS will be highlighted.

Keywords: interferon, experimental autoimmune encephalomyelitis, RIG-I, MDA5,TREX1, AGS, SAMHD1, RNASEH2

TYPE I INTERFERONS AND THEIR INDUCTION

Interferons (IFNs) represent a family of cytokines which were originally identified by their ability to mediate antiviral effects. Since their discovery more than 54 years ago (Lindenmann et al., 1957), this class of proteins now embraces around 30 members. Based on common structural, biochemical, and signaling properties as well as the source of cells producing these factors, IFNs can be classed into three distinct subfamilies namely type I, type II, and class III IFNs. While IFN-γ is the sole type II IFN and the three different IFN-λs constitute the type III IFNs, type I IFNs are a highly divergent group of cytokines encompassing 13 different IFN-α subtypes, IFN-β, IFN-κ, IFN-ε, IFN-ω, IFN-τ, IFN-δ and three different IFN-ζs (IL-28A/B and IL-29; Noppert et al., 2007).

Consistent with the functional role of type I IFNs in pathogen defense, induction of these cytokines is predominantly triggered by distinct pathogen-associated molecular patterns (PAMPs) which are recognized by specific pathogen recognition receptors (PRRs). As depicted in **Figure 1**, the surface toll-like receptor (TLR) 4 recognizing lipopolysaccharide from Gram-negative bacteria as well as TLRs 3, 7, 8, and 9, which recognize pathogen-derived nucleic acids, induce type I IFNs (Blasius and Beutler, 2010). TLR3 recognizes viral double-stranded RNA (dsRNA) while viral single-stranded RNA (ssRNA) is detected by TLR7 and TLR8. Viral or bacterial unmethylated DNA, commonly referred to as CpG DNA, is sensed by TLR 9 (Akira et al., 2006; Barber, 2011; Kawai and Akira, 2011).

The localization of nucleic acid sensing TLRs at the endoplasmic reticulum and endosomal membranes limits the detection of viruses by TLRs to this specific compartment. In general, signal transduction for type I IFN induction via the TLRs mentioned above starts with the recruitment of either Toll-IL-1 receptor (Tir) domain-containing factor (TRIF; for TLR4, TLR3) and/or myeloid differentiation primary response gene 88 (MyD88; for TLR7, TLR9) to the activated receptor. Subsequent signaling events involving the molecules interleukin-1 receptor-associated kinase (IRAK) 1, IRAK 4, tumor necrosis associated factor (TRAF) 6 and TRAF3 activate the kinases TGF-β activated kinase 1 (TAK1) and TANK-binding kinase 1 (TBK1). While TAK1 acts as a common activator of nuclear factor of kappa light polypeptide gene enhancer in B cells (NF-κB) signaling via the I kappa B kinase (IKK) complex which mediates phosphorylation and degradation of the inhibitory component IκBα, TBK1 phosphorylates interferon regulatory factor (IRF) 3 leading to its dimerization (Mori et al., 2004; Tamura et al., 2008). Finally, dimeric IRF3 and NF-κB translocate into the nucleus to induce type I IFN expression. In addition, engagement of distinct TLRs also causes IRF7 phosphorylation, which preferentially activates IFN-α but also leads to IFN-β induction (Honda and Taniguchi, 2006).

Alternative to the TLRs, the cell employs the retinoic acid-inducible gene-I (RIG-I) like helicases (RLHs) RIG-I and the melanoma differentiation-associated gene 5 (MDA5) to detect viral infection (Pichlmair and Reis e Sousa, 2007). RIG-I detects

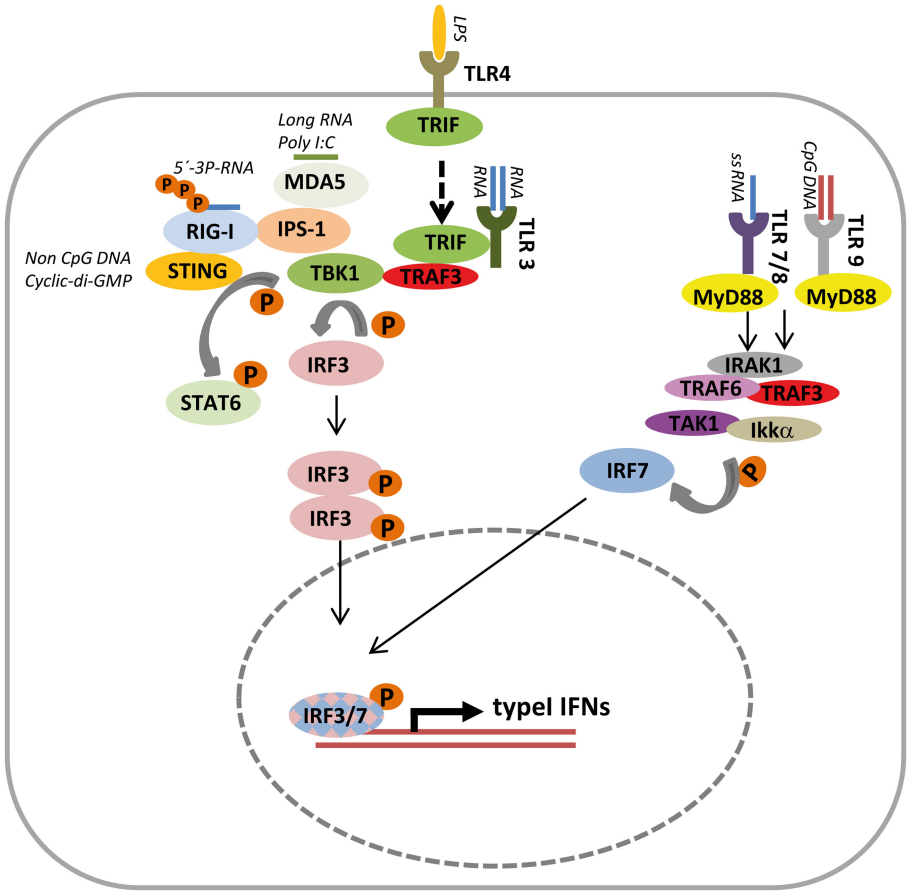


FIGURE 1 | Overview of typical signaling cascades inducing type I Interferon expression. Upon ligand engagement, several toll-like receptors (TLRs) and RIG-I like helicases (RLHs) induce transcription of type I interferons (IFN). TLR4 located at the cell surface is typically induced extracellular while TLR3, TLR 7/8, and TLR9 sense pathogen-derived single-stranded RNA (ssRNA), double-stranded RNA (dsRNA), and unmethylated DNA (CpG DNA) within the cell sequestered from the cytoplasmic compartment. Intracellular TLRs are localized, traffic, and initiate signaling cascades in membrane surrounded compartments like the endoplasmic reticulum, endosomes, lysosomes, and phagocytic vesicles. Upon ligand binding, TLR4 is endocytosed (indicated by dashed arrows). Downstream signaling inducing type I IFN is mediated by initial binding to either MyD88 (TLR7/8/9) or TRIF (TLR3/4), followed by recruitment of multicomponent protein complexes. Typically a complex with TLR3 or TLR4 together with TRIF and TRAF3 activates the kinase TBK1 mediating phosphorylation of IRF3, which subsequently forms homodimers, translocates to the nucleus, and initiates type I IFN gene expression. MyD88 recruited to TLR7/8/9 complexes with IRAK1, TRAF6, TRAF3, and the kinases TAK1 and IKKα, which phosphorylate and thus activate IRF7 to drive type I IFN expression. The cytoplasmic RLHs MDA5 and RIG-I recognize longer RNAs like poly I:C or 5′-3-P-RNA respectively and engage IPS at the mitochondrial membrane. Recruitment of a complex containing TBK1 induces phosphorylation and thus dimerization of IRF3 followed by type I IFN gene expression. Independent from TLR and RLH intracellular, non-CpG DNA, and cyclic-di-GMP are sensed in a STING dependent manner. STING interacts with RIG-I and activates type I IFN transcription via the IRF3 axis but is also capable to recruit STAT6 to the ER followed by TBK1 mediated STAT6 phosphorylation.

5′-triphosphate-containing RNA which for example is typical for vesicular stomatitis virus (VSV; Hornung et al., 2006; Schlee et al., 2009). Characteristic for MDA5 is the recognition of longer RNA molecules as exemplified by the synthetic dsRNA poly I:C or picornavirus genomes (Kato et al., 2006). RLHs are characterized by a helicase domain for RNA binding and a caspase recruitment domain (CARD) mediating protein interaction for downstream signaling. Binding to viral RNA induces homodimerization of these sensors resulting in the engagement of MAVS (IPS-1, VISA, CARDIF; Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005) located in the mitochondrial membrane followed by the recruitment of TRAF3, TRAF6, TBK1, and IKKα/β. As a result of these signaling events, IRF3, IRF7, and NF-κB are activated and together with cJUN/ATF2 and the coactivators CBP/p300 initiate the expression of type I IFNs (Kawai and Akira, 2011).

The observation that TLR9 deficient cells are still capable to efficiently mount type I IFN production when transfected with double-stranded DNA (dsDNA) led to the search of alternative DNA sensing molecules especially in non-plasmacytoid dendritic cells (pDCs; Kawai and Akira, 2009). These investigations led to the identification of the DNA sensor endoplasmic reticulum IFN stimulator (ERIS) now better known as stimulator of interferon genes (STING) which is located in the ER membrane and plays an important role in the induction of IFN by non-CpG intracellular DNA

species (Ishikawa and Barber, 2008; Zhong et al., 2008). Analysis of STING−/− mice revealed that the protein, which has no homology to other DNA sensors, is essential for host defense against DNA pathogens such as herpes simplex virus 1 (HSV-1; Ishikawa et al., 2009). Recent work also provided strong evidence that STING also acts as a direct sensor for the bacterial second messenger cyclic-di-GMP, which was shown previously to elicit a type I IFN response in host cells (McWhirter et al., 2009; Woodward et al., 2010; Burdette et al., 2011). It was recently shown that STING recruits STAT6 to the endoplasmatic reticulum (ER) followed by TBK-mediated phosphorylation. Remarkably, this STAT6 activation at the ER by STING differs from cytokine induced STAT6 phosphorylation at the plasma membrane as Janus kinases (JAKs) are not involved and phosphorylation is not restricted to distinct cell types. The virus-induced STAT6 activation also occurs in IFNAR2−/− or IRF3−/− cells as well as in cells lacking individual JAKs providing strong evidence that STING-triggered STAT6 activation represents a novel signaling module. While the canonical STAT6 activation is MAVS independent this non-canonical STAT6 activation required the presence of MAVS and STAT6 phosphorylation is mediated by TBK1. Consistent with a fundamental role of STAT6 in antiviral activity, STAT6 deficient mice exhibited exacerbated pathologies when challenged with either VSV, Sendai virus, or herpes simplex virus (Chen et al., 2011).

IFN SIGNALING

As a common feature all interferons activate the JAK– signal transducers of activation and transcription (STAT) signaling pathway. A hallmark of type I IFNs is that they all bind and signal via the interferon alpha receptor (IFNAR), which is a heterodimer composed of interferon receptor 1 (IFNAR1) and interferon receptor 2 (IFNAR2). It is a unique feature compared to other cytokine receptors that IFNAR can bind and mediate signaling of multiple ligands. Type I IFNs bind to the IFNAR and as a consequence they activate the kinases JAKs Tyk 2 and JAK 1 which are associated with IFNAR1 and IFNAR2 respectively and phosphorylate receptor tyrosine residues on the receptor (de Weerd et al., 2007). Via SH2 domains STAT bind to the phosphotyrosines on the receptor and become themselves phosphorylated. Phosphorylated STAT1 forms heterodimers with STAT2 and together with IRF9 constitutes a transcription factor (ISGF3γ) which binds to interferon-stimulated response elements (ISREs) of interferon-stimulated target genes (ISGs) inducing their expression. Further complexity is added to the system by the formation of STAT1 and STAT3 homo- and heterodimers which activate GAS elements controlling the expression of other ISGs which are also inducible by IFN-γ. In addition, also STAT independent pathways are activated (Boxel-Dezaire et al., 2006). Ultimately, hundreds of ISGs, which might vary depending on the cell type, are induced and translated into the effector proteins responsible to mediate biological effects like antiviral activity, control of cellular proliferation, apoptosis, and immune regulation.

INFLAMMATORY BRAIN PATHOLOGIES ASSOCIATED WITH ABERRANT TYPE I IFN INDUCTION

Interferon beta has proinflammatory properties and contributes to the pathology of autoimmune diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis, and psoriasis (Preble et al., 1982; Baechler et al., 2006). Within the central nervous system (CNS) proinflammatory detrimental effects of IFN-β were described for Aicardi–Goutières syndrome (AGS) and neuromyelitis optica (NMO).

Aicardi–Goutières syndrome represents a fatal genetic disease that manifests as an encephalopathy. The disease is characterized by increased lymphocyte numbers in the cerebrospinal fluid, demyelination of the white matter and calcification of basal ganglia thus mimicking pathological consequences of congenital infection which often leads to misdiagnoses (Rice et al., 2007). One hallmark of AGS are increased levels of IFN-α in serum and cerebrospinal fluid (Dussaix et al., 1985). Increased levels of IFN-α are also a key feature of SLE and indeed, molecular mechanisms underlying AGS and SLE exhibit striking parallels allocating AGS into the group of autoimmune diseases. Furthermore, some children with AGS also exhibit an early onset form of SLE (De Laet et al., 2005). AGS is inherited in an autosomal recessive trait and mutations in different genetic loci were identified. It was shown before that recessive mutations in the genes encoding either human 3′repair exonuclease 1 (TREX1), different components of the RNASEH2 complex or the SAM-domain HD-domain-containing protein 1 (SAMHD1) can cause this severe inflammatory disease (Crow et al., 2006a,b; Rice et al., 2009). TREX1 was shown to be a 3′–5′ DNA exonuclease preferentially binding and cleaving single-stranded DNA (ssDNA; Mazur and Perrino, 1999, 2001). Initially appearing paradox for a DNAse, TREX1 is predominantly localized in the cytoplasm where the protein is associated with the endoplasmic reticulum. TREX1 is part of the SET complex and in response to oxidative stress the protein can translocate to the nucleus and is involved in Granzyme A-mediated apoptosis (Martinvalet et al., 2005; Chowdhury et al., 2006). In concordance with an extranuclear function, absence of TREX in mice and humans results in the accumulation of cytoplasmic ssDNA (Yang et al., 2007; Stetson et al., 2008). It was reported that excessive and mislocalized ssDNA originates from excision mediated DNA repair (Yang et al., 2007). Work by Medzhitov's group identified TREX1 as an important negative regulator of the interferon stimulatory DNA (ISD) response. They provided compelling evidence that the ssDNA accumulating in the absence of TREX arises from endogenous retroelements which are no longer degraded properly by the TREX nuclease. As a consequence, the elevated levels of ssDNA which might mimic viral infection are sensed by an unidentified DNA sensor, initiate the ISD pathway and activate IRF3 resulting in the production of high levels of type I IFN and lymphocyte mobilization (Stetson et al., 2008). In line with an aberrant activation of the innate immune system TREX1−/− mice develop an inflammatory myocarditis (Morita et al., 2004). The observed cardiomyopathy in TREX1 deficient mice might be analogous to the encephalopathy in AGS patients associated with an autoinflammatory response which arises in the absence of an infection. The observation that the phenotype of TREX1−/− mice can be rescued in the absence of either IRF3, IFNAR, or the recombination activating gene (RAG) further underlines that the autoimmune mechanism is triggered by activation of IRF3 mediated type I IFN induction (Stetson et al., 2008) but also shows that secondary mechanisms like leukocyte recruitment are essential for disease manifestation. In line with the central

role of TREX in the maintenance of immune homeostasis is the observation that mutations in this gene are also associated with SLE (Lee-Kirsch et al., 2007).

The central role of TREX1 as a negative regulator of type I IFN induction is exploited by the human immunodeficiency virus (HIV) type 1. Recent work showed that TREX1 not only degrades DNA from endogenous retroelements to prevent autoimmune activation. The nuclease is also active against HIV DNA which arises during HIV infection after reverse transcription (RT; Geijtenbeek, 2010; Yan et al., 2010). Apparently, the virus hijacks TREX1's specificity for retrovirus-derived DNA to avoid activation of cytoplasmic nucleic acid sensors, which usually trigger the antiviral effector system. TREX1-mediated degradation of HIV DNA thus represents one mechanism how HIV escapes immune recognition and helps to explain why infection of T cells and macrophages do not elicit an effective antiviral interferon response (Geijtenbeek, 2010).

Interestingly, also another AGS susceptibility gene, SAMHD1, which is highly expressed in macrophages and dendritic cells and upregulated upon viral infection was proposed to act as a negative regulator of the innate immune response (Lafuse et al., 1995; Li et al., 2000; Rice et al., 2009). However, the molecular function until recently remained elusive. SAMHD1 function was now shown to be tightly linked to the control of retroviral infection (Hrecka et al., 2011; Laguette et al., 2011). HIV is incapable to transduce dendritic cells and impaired in the transduction of macrophages because efficient viral cDNA synthesis is inhibited in these cells. In contrast, other retroviruses encoding Vpx accessory proteins circumvent this inhibition (Ayinde et al., 2010). Two recent publications identified SAMHD1 as the factor restricting HIV infection in myeloid and dendritic cells and showed that Vpx proteins counteract this restriction by mediating proteasomal degradation of SAMHD1 (Hrecka et al., 2011; Laguette et al., 2011).

Recently, also the molecular function of SAMHD1 was identified. It was shown that the protein is an effective dGTP-stimulated deoxyguanosine triphosphate triphosphohydrolase, which catalyzes the conversion of dGTP to guanosine and inorganic triphosphate (Goldstone et al., 2011). SAMHD1 rapidly hydrolyzes dGTP but not dATP, dCTP, and dTTP. However, SAMHD1 is allosterically activated by dGTP which binds to SAMHD1 leading to a more promiscuous substrate specificity which is extended to dATP, dCTP, and dTTP. dGTP is thus both a substrate and an activator of the enzyme against the other dNTPs. The catalytic activity should strongly limit the dNTP pool within the cytoplasm and thus exert a role in nucleic acid metabolism. It is reasonable to hypothesize that SAMHD1 limits the dNTP pool and thus interferes with HIV replication by inhibiting endogenous RT. With respect to the etiology of AGS, loss of SAMHD1 activity would no longer counteract RT of endogenous retroviral elements as the dNTP pool is no longer restricted. As a consequence, the loss of RT restriction might promote the accumulation of cytoplasmic DNA similar to what is observed in TREX1 mutants. Although further experimental evidence needs to be added, this model would be a straight forward explanation for the similar phenotypic manifestations arising from human mutations in SAMHD1 and TREX genes. In summary, TREX1 and SAMHD1 provide excellent examples for the intricate relationship between the control of retroviral infection and the control of cell intrinsic nucleic acids harboring the risk to trigger autoimmune inflammation induced by type I IFNs.

In addition, also mutations in subunits of the RNase H2 complex were shown to cause AGS (Crow et al., 2006b). The RNase H2 is the dominant cytoplasmic RNase in human cells and composed of a heterotrimeric complex in which all three components are needed to constitute the active enzyme. RNase H2 enzymes recognize RNA:DNA hybrids, degrade the polyribonucleotide strand and thus also play a central role in nucleic acid metabolism (Eder and Walder, 1991; Rydberg and Game, 2002). Although direct experimental evidence is still missing, it is likely that RNase H2 mutations which impair degradation of RNA:DNA hybrids alter cytoplasmic nucleic acid homeostasis resulting in the activation of the ISD response.

As depicted in **Figure 2**, analysis of the molecular mechanisms underlying AGS revealed a role of all the susceptibility genes mentioned above in the control of cytoplasmic nucleic acid homeostasis which needs to be tightly controlled in order to prevent autoimmunity induced by type I IFNs.

NEUROMYELITIS OPTICA (DEVIC'S DISEASE) AND TYPE I IFNs

Neuromyelitis optica represents a neuroinflammatory disease previously considered to be a variant of relapsing–remitting multiple sclerosis (RRMS). However, in contrast to RRMS, NMO is characterized by demyelination in the spinal cord and optic nerve (Lucchinetti et al., 2002). Autoantibodies directed against the aquaporin 4 water channel are found in these patients and occurrence of anti-aquaporin antibodies also is the major parameter in the diagnosis of NMO (Paul et al., 2007). Driven by the supposed analogy to RRMS, IFN-β was tried as a therapeutic but it was quickly clear that IFN-β treatment even worsens the disease and induces severe relapses (Warabi et al., 2007; Palace et al., 2010; Shimizu et al., 2010; Uzawa et al., 2010). Notably, the dominant infiltrating cells are granulocytes which is in sharp contrast to classical multiple sclerosis (MS). This clearly different infiltratory make up might explain the different response upon IFN-β treatment. Interestingly, levels of IL-17 were found to be elevated in the cerebrospinal fluid of people suffering from NMO and it has been proposed that IL-17 mediated induction of IL-8, granulocyte stimulating factor (G-CSF) and Gro-alpha causes granulocyte recruitment (Axtell et al., 2011).

ANTI-INFLAMMATORY EFFECTS OF TYPE I IFNs IN RELAPSING–REMITTING MULTIPLE SCLEROSIS AND EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

As exemplified for SLE and AGS, type I IFNs were frequently causally associated with the development of autoimmune pathologies. In contrast, type I IFNs were also shown to exert anti-inflammatory effects and IFN-β is used as a routine therapeutic for the treatment of RRMS in patients (Jacobs et al., 2000; Filippi et al., 2004). MS is an autoimmune inflammatory disease of the CNS of unclear etiology characterized by demyelination of axons in brain and spinal cord. Characteristic for the disease is the infiltration of the CNS with inflammatory cells like monocytes, TH1

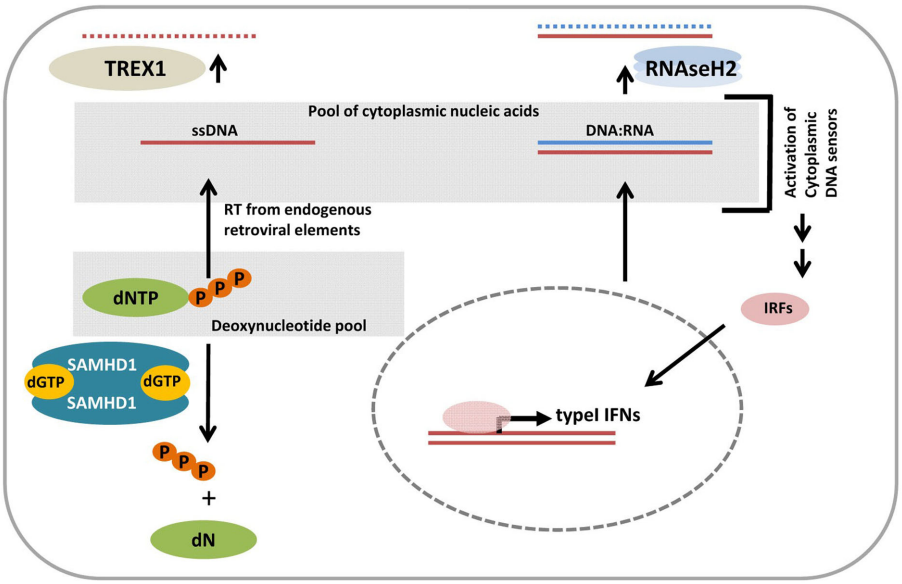


FIGURE 2 | Model for the role of cytoplasmic nucleic acid homeostasis in the etiology of Aicardi–Goutieres syndrome (AGS). TREX1 is an 5′–3′ Exonuclease which degrades cytoplasmic single-stranded DNA (ssDNA) originating from reverse transcription of endogenous retrotransposons. Lack of TREX1 activity results in the congestion of ssDNA thereby augmenting the pool of nucleic acids in the cytoplasm. SAMHD1 cleaves inorganic triphosphate from deoxynucleotides and thus restricts the cytoplasmic dNTP concentrations hence constraining reverse transcription by limiting the amount of "building blocks" for DNA synthesis. SAMHD1 mutations would eliminate this dNTP control mechanism, enhance the dNTP pool, and could thus fuel reverse transcription from endogenous retroelements resulting in increased levels of cytoplasmic ssDNA. RNASEH2 is an endonuclease composed of three subunits and required to cleave ribonucleotides from RNA:DNA duplexes which arise from intracellular processes. Lack of function mutations in RNASeH2 would thus also impair the control of nucleic acid homeostasis. Thus mutations in any kind of the genes mentioned above which were found in AGS patients would lead to the accumulation of nucleic acids in the cytoplasm. These aberrant levels are sensed by cytoplasmic DNA sensors originally designated to detect viral infections. Consequently the initiated signaling cascade activates interferon regulatory transcription factors (IRFs) for type I IFN induction and drive autoimmune disease onset.

and TH17 cells. In RRMS, phases with no or only minor disease progression are followed by unpredictable acute relapses causing deficits which might at least partially resolve in times of remission.

According to results from clinical trials, IFN-β treatment reduces relapse rates by about 30%, decreases the formation of inflammatory lesions in the CNS and extends remission periods (Schwid and Panitch, 2007). However, a major problem is that a high proportion of about 20% of the patients do not or only poorly respond to IFN-β treatment. Despite excessive attempts to define biomarkers for responders and non-responders it is still impossible to predict whether an individual patient will respond to IFN-β therapy. Mechanism underlying this variety include development of antibodies against IFN-β, polymorphisms in components of the IFN signaling pathway and IFN effector genes as well as variable MS pathomechanisms (Killestein and Polman, 2011). By further dissecting type I IFN signatures in MS patients, IFN-β non-responders were found to exhibit increased monocyte-specific type I IFN secretion upon innate immune stimuli via TLR 4, by increased endogenous production of type I interferon, and by an elevated activation status of myeloid dendritic cells (Comabella et al., 2009). These findings indicate that perturbations of the type I IFN signaling pathway in monocytes are related to a lack of response to IFN-β and type I IFN-regulated genes may be used as response markers in IFN-β treatment in MS. Consistent with the concept that genetic variants define the individual response is that induction of interferon response genes among different patients varies significantly but is remarkably stable within a long time period.

A frequently used system to gain insight into the molecular and cellular events underlying MS is the model of experimental autoimmune encephalomyelitis (EAE). In this well-established mouse model active immunization with myelin components together with a strong adjuvant induces T-cell mediated neuroinflammation and demyelination resembling multiple aspects of MS (Owens et al., 2001; Gold et al., 2006).

Consistent with the beneficial effect of IFN-β in human MS, EAE in rodents can be suppressed by injection of recombinant IFN-β. In addition, mice lacking IFN-β exhibit strongly exacerbated disease parameters in the EAE model (Teige et al., 2003). Furthermore, IFNβ is stronger induced in the CNS than in the periphery. To trace down the cell type mediating the "protective" immune regulation of type I IFNs via the type I receptor (IFNAR), mice lacking this receptor only on distinct cell types were used for EAE experiments and IFN-β treatment (Prinz et al., 2008). It could be shown that negative regulation of autoimmunity in the EAE model relies on the presence of IFNAR on cells of the myeloid lineage while absence of IFNAR on T cells, B cells, and cells of neuroectodermal origin does not enhance disease severity. These experiments suggest a scenario where locally produced IFN-β within the CNS acts on invading myeloid cells to

attenuate autoimmune damage especially in the effector phase of EAE. Another study which also employed EAE in IFNAR deficient mice provided evidence for a functional role of type I interferon in negatively regulating the development of TH17 cells. Numbers of encephalitogenic TH17 cells were found by this group to be elevated after EAE induction in IFNAR1 and TRIF deficient mice (Guo et al., 2008). TH17 cells develop from naive T cells which induced by TGFβ and IL-6 secrete IL-21. This cytokine subsequently induces the transcription factor RORγt in an autocrine manner which triggers the TH17 differentiation program (Veldhoen et al., 2006; Stockinger and Veldhoen, 2007). IFN-β induces expression of IL-27 which acts on naive CD4+ T cells as a negative regulator of TH17 development (Prinz and Kalinke, 2010). IL-27 also promotes IL-10 secretion by T cells which was shown to suppress autoimmune inflammation of the CNS (Fitzgerald et al., 2007). IFN-β was furthermore shown to inhibit IL-23 induced proliferation of TH17 cells (Harrington et al., 2005). Increased levels of TH17 cells in IFNAR−/− mice would thus be explained by the lack of these inhibitory effects of type I IFNs.

Disease symptoms and generation of antigen specific TH17 cells were also enhanced in TRIF deficient mice suggesting that type I IFN production counteracting autoimmunity in EAE is triggered by a TRIF dependent mechanism (Guo et al., 2008). Interestingly, type I IFN-related genes were found to be strongly induced in toxic mouse models of demyelination whereas the presence of IFNAR could neither modulate demyelination or myelin repair (Schmidt et al., 2009).

Just recently, also cytosolic RLHs were shown to act as negative regulators of sterile inflammation within the CNS (Dann et al., 2012). Mice lacking the RIG-I like helicase adaptor IPS-1 developed exacerbated disease symptoms. In a reciprocal experiment the same study showed that on the other hand activation of RLHs via IFN-inducing 5′-triphosphate RNA oligonucleotides ameliorated disease outcome providing compelling evidence that RLHs mediate signals counteracting encephalitogenic immune responses. While RLH stimulation did not affect T-cell differentiation, the maintenance and expansion of TH1 and TH17 cells was strongly suppressed upon RLH engagement (**Figure 3**). Repression of proinflammatory TH1 and TH17 cells could only be observed

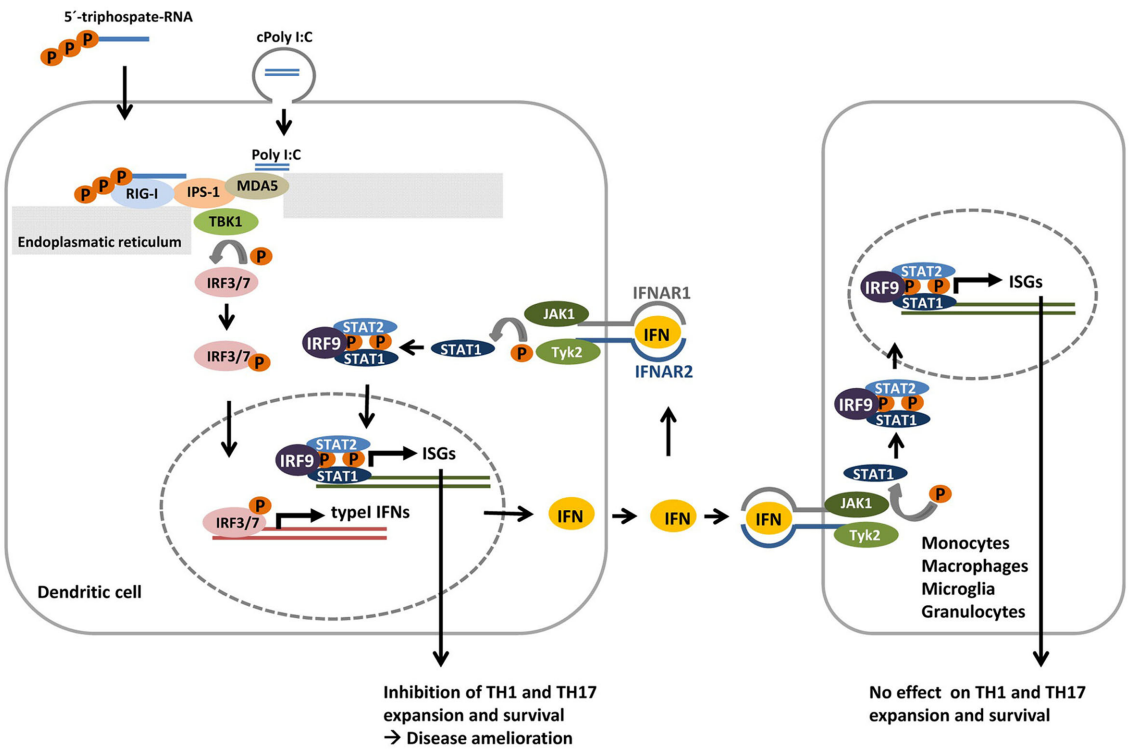


FIGURE 3 | Model for negative regulation of sterile CNS inflammation by RIG-I like helicases (RLHs). In dendritic cells activation of RLHs RIG-I or MDA5 by 5′triphosphate RNA or polyI:C complexed to liposomes (cPolyI:C) respectively, recruits IPS-1 to the RLH. This initiates a signaling cascade which involves TBK1 mediated phosphorylation of IRF3 and/or IRF7 which upon dimerization enter the nucleus and lead to the expression of type I IFN. Secreted type IFNs (IFN) can either bind to type I interferon receptor (IFNAR) in an autocrine or paracrine manner and stimulate the expression of interferon-stimulated target genes (ISGs). While lack of IPS-1 leads to exacerbated disease pathology in the EAE model of sterile inflammation, RLH stimulation improved clinical signs of disease and inhibits TH1 and TH17 expansion and survival. This regulation is type I IFN mediated as the "therapeutic effect" of RLH stimulation is abrogated in IFNAR deficient mice. However, mice specifically lacking IFNAR on monocytes, macrophages, microglia, or granulocytes like wildtype mice showed reduced symptoms upon RLH induction. IFNAR signaling on these cells is thus dispensable for the suppressive effect of RLH engagement. In contrast, when mice specifically lacking IFNAR on dendritic cells were used, RLH treatment did not ameliorate autoimmunity clearly showing that interferon stimulation in this particular celltype is essential for IFN mediated TH1 and TH17 inhibition and disease improvement.

when the type I interferon receptor was present on dendritic cells by using CD11cCre mice crossed with conditional IFNAR animals. Remarkably, absence of IFNAR1 on macrophages or microglia did not interfere with RLH mediated TH1 or TH17 repression (Dann et al., 2012). These results imply that engagement of RLHs might be a passable way to stimulate endogenous type I IFNs for therapeutic intervention. Replacing systemic administration with the endogenous induction of type I IFNs should prevent the development of neutralizing antibodies. It might also be more effective than systemic administration as type I IFNs are biologically designed to exert their effects in a spatially restricted area affecting cells in their direct neighborhood.

Recognition of triphosphate RNA by RIG-I like helicases followed by IFN induction is largely independent from the sequence used for the dsRNA (Hornung et al., 2006). Thus, a specific short interfering RNA (RNAi) suitable to suppress expression of a specific target protein can be used. This, at least in principle, offers therapeutic strategies where activation of the type I IFN response can be combined with the suppression of harmful gene products. As a proof of principle for the feasibility of such an approach it was shown in a melanoma model that BCL2 specific siRNA carrying 5′ triphosphate ends efficiently activated type I IFN via RIG-I and in parallel silenced Bcl2 expression (Poeck et al., 2008). As a consequence, the synergistic activation of the innate immune response combined with suppression of a pro survival signal caused massive apoptosis of tumor cells, prolonged survival of the animals, and reduced tumor size.

It might thus also be suitable to combine type I IFN induction with RNAi mediated inhibition of proinflammatory factors or proteins triggering the differentiation of deleterious cells. Such a strategy where for example type I IFN induction could be combined with RNAi mediated inhibition of RORγt or T-bet to simultaneously repress TH17 and TH1 differentiation might potentially open new ways to modulate the immune response and counteract autoimmunity within the CNS.

CONCLUSION AND FUTURE PERSPECTIVES

Despite extensively analyzed, the functions of type I IFNs within the CNS are still far from being well understood. One principal problem to generalize type I IFN effects is surely based on the fact that hundreds of genes, which even vary among different cell types, are controlled by this particular group of cytokines. In addition, the levels and kind of IFN target genes induced are differentially regulated depending on the situation, interaction and environment of the cell. Further complexity is added to the system as multiple negative regulators of the IFN signaling system modulate the outcome and refractory mechanisms blunt restimulation. A major task for the future will be to identify the particular interferon effector genes responsible for the anti-inflammatory and therapeutic properties which even might considerably differ between various cell types. With respect to the etiology of type I IFN associated autoimmune disease like AGS it will be interesting to see which particular molecules are involved in the pathways sensing enhanced levels of endogenous nucleic acids in the cytoplasm.

REFERENCES

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell* 124, 783–801.

Axtell, R. C., Raman, C., and Steinman, L. (2011). Interferon-beta exacerbates Th17-mediated inflammatory disease. *Trends Immunol.* 32, 272–277.

Ayinde, D., Maudet, C., Transy, C., and Margottin-Goguet, F. (2010). Limelight on two HIV/SIV accessory proteins in macrophage infection: is Vpx overshadowing Vpr? *Retrovirology* 7, 35.

Baechler, E. C., Batliwalla, F. M., Reed, A. M., Peterson, E. J., Gaffney, P. M., Moser, K. L., Gregersen, P. K., and Behrens, T. W. (2006). Gene expression profiling in human autoimmunity. *Immunol. Rev.* 210, 120–137.

Barber, G. N. (2011). Cytoplasmic DNA innate immune pathways. *Immunol. Rev.* 243, 99–108.

Blasius, A. L., and Beutler, B. (2010). Intracellular toll-like receptors. *Immunity* 32, 305–315.

Boxel-Dezaire, A. H., Rani, M. R., and Stark, G. R. (2006). Complex modulation of cell type-specific signaling in response to type I interferons. *Immunity* 25, 361–372.

Burdette, D. L., Monroe, K. M., Sotelo-Troha, K., Iwig, J. S., Eckert, B., Hyodo, M., Hayakawa, Y., and Vance, R. E. (2011). STING is a direct innate immune sensor of cyclic di-GMP. *Nature* 478, 515–518.

Chen, H., Sun, H., You, F., Sun, W., Zhou, X., Chen, L., Yang, J., Wang, Y., Tang, H., Guan, Y., Xia, W., Gu, J., Ishikawa, H., Gutman, D., Barber, G., Qin, Z., and Jiang, Z. (2011). Activation of STAT6 by STING is critical for antiviral innate immunity. *Cell* 147, 436–446.

Chowdhury, D., Beresford, P. J., Zhu, P., Zhang, D., Sung, J. S., Demple, B., Perrino, F. W., and Lieberman, J. (2006). The exonuclease TREX1 is in the SET complex and acts in concert with NM23-H1 to degrade DNA during granzyme A-mediated cell death. *Mol. Cell* 23, 133–142.

Comabella, M., Lunemann, J. D., Rio, J., Sanchez, A., Lopez, C., Julia, E., Fernandez, M., Nonell, L., Camina-Tato, M., Deisenhammer, F., Caballero, E., Tortola, M. T., Prinz, M., Montalban, X., and Martin, R. (2009). A type I interferon signature in monocytes is associated with poor response to interferon-beta in multiple sclerosis. *Brain* 132, 3353–3365.

Crow, Y. J., Hayward, B. E., Parmar, R., Robins, P., Leitch, A., Ali, M., Black, D. N., van Bokhoven, H., Brunner, H. G., Hamel, B. C., Corry, P. C., Cowan, F. M., Frints, S. G., Klepper, J., Livingston, J. H., Lynch, S. A., Massey, R. F., Meritet, J. F., Michaud, J. L., Ponsot, G., Voit, T., Lebon, P., Bonthron, D. T., Jackson, A. P., Barnes, D. E., and Lindahl, T. (2006a). Mutations in the gene encoding the 3′-5′ DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus. *Nat. Genet.* 38, 917–920.

Crow, Y. J., Leitch, A., Hayward, B. E., Garner, A., Parmar, R., Griffith, E., Ali, M., Semple, C., Aicardi, J., Babul-Hirji, R., Baumann, C., Baxter, P., Bertini, E., Chandler, K. E., Chitayat, D., Cau, D., Dery, C., Fazzi, E., Goizet, C., King, M. D., Klepper, J., Lacombe, D., Lanzi, G., Lyall, H., Martinez-Frias, M. L., Mathieu, M., McKeown, C., Monier, A., Oade, Y., Quarrell, O. W., Rittey, C. D., Rogers, R. C., Sanchis, A., Stephenson, J. B., Tacke, U., Till, M., Tolmie, J. L., Tomlin, P., Voit, T., Weschke, B., Woods, C. G., Lebon, P., Bonthron, D. T., Ponting, C. P., and Jackson, A. P. (2006b). Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutieres syndrome and mimic congenital viral brain infection. *Nat. Genet.* 38, 910–916.

Dann, A., Poeck, H., Croxford, A. L., Gaupp, S., Kierdorf, K., Knust, M., Pfeifer, D., Maihoefer, C., Endres, S., Kalinke, U., Meuth, S. G., Wiendl, H., Knobeloch, K. P., Akira, S., Waisman, A., Hartmann, G., and Prinz, M. (2012). Cytosolic RIG-I-like helicases act as negative regulators of sterile inflammation in the CNS. *Nat. Neurosci.* 15, 98–106.

De Laet, C., Goyens, P., Christophe, C., Ferster, A., Mascart, F., and Dan, B. (2005). Phenotypic overlap between infantile systemic lupus erythematosus and Aicardi-Goutieres syndrome. *Neuropediatrics* 36, 399–402.

de Weerd, N. A., Samarajiwa, S. A., and Hertzog, P. J. (2007). Type I interferon receptors: biochemistry and biological functions. *J. Biol. Chem.* 282, 20053–20057.

Dussaix, E., Lebon, P., Ponsot, G., Huault, G., and Tardieu, M. (1985). Intrathecal synthesis of different alpha-interferons in patients with various neurological diseases. *Acta Neurol. Scand.* 71, 504–509.

Eder, P. S., and Walder, J. A. (1991). Ribonuclease H from K562 human erythroleukemia cells. Purification, characterization, and substrate specificity. *J. Biol. Chem.* 266, 6472–6479.

Filippi, M., Rovaris, M., Inglese, M., Barkhof, F., De Stefano, N., Smith, S., and Comi, G. (2004). Interferon beta-1a for brain tissue loss in patients at presentation with syndromes suggestive of multiple sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet* 364, 1489–1496.

Fitzgerald, D. C., Zhang, G. X., El Behi, M., Fonseca-Kelly, Z., Li, H., Yu, S., Saris, C. J., Gran, B., Ciric, B., and Rostami, A. (2007). Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. *Nat. Immunol.* 8, 1372–1379.

Geijtenbeek, T. B. (2010). Host DNase TREX1 hides HIV from DNA sensors. *Nat. Immunol.* 11, 979–980.

Gold, R., Linington, C., and Lassmann, H. (2006). Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. *Brain* 129, 1953–1971.

Goldstone, D. C., Ennis-Adeniran, V., Hedden, J. J., Groom, H. C., Rice, G. I., Christodoulou, E., Walker, P. A., Kelly, G., Haire, L. F., Yap, M. W., de Carvalho, L. P., Stoye, J. P., Crow, Y. J., Taylor, I. A., and Webb, M. (2011). HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature* 480, 379–382.

Guo, B., Chang, E. Y., and Cheng, G. (2008). The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice. *J. Clin. Invest.* 118, 1680–1690.

Harrington, L. E., Hatton, R. D., Mangan, P. R., Turner, H., Murphy, T. L., Murphy, K. M., and Weaver, C. T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6, 1123–1132.

Honda, K., and Taniguchi, T. (2006). Toll-like receptor signaling and IRF transcription factors. *IUBMB Life* 58, 290–295.

Hornung, V., Ellegast, J., Kim, S., Brzozka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K. K., Schlee, M., Endres, S., and Hartmann, G. (2006). 5′-Triphosphate RNA is the ligand for RIG-I. *Science* 314, 994–997.

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S. K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M. P., and Skowronski, J. (2011). Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474, 658–661.

Ishikawa, H., and Barber, G. N. (2008). STING is an endoplasmic reticulum adaptor that facilitates innate immune signaling. *Nature* 455, 674–678.

Ishikawa, H., Ma, Z., and Barber, G. N. (2009). STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 461, 788–792.

Jacobs, L. D., Beck, R. W., Simon, J. H., Kinkel, R. P., Brownscheidle, C. M., Murray, T. J., Simonian, N. A., Slasor, P. J., and Sandrock, A. W. (2000). Intramuscular interferon beta-1a therapy initiated during a first demyelinating event in multiple sclerosis. CHAMPS Study Group. *N. Engl. J. Med.* 343, 898–904.

Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., Uematsu, S., Jung, A., Kawai, T., Ishii, K. J., Yamaguchi, O., Otsu, K., Tsujimura, T., Koh, C. S., Reis e Sousa, C., Matsuura, Y., Fujita, T., and Akira, S. (2006). Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature* 441, 101–105.

Kawai, T., and Akira, S. (2009). The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int. Immunol.* 21, 317–337.

Kawai, T., and Akira, S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650.

Kawai, T., Takahashi, K., Sato, S., Coban, C., Kumar, H., Kato, H., Ishii, K. J., Takeuchi, O., and Akira, S. (2005). IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat. Immunol.* 6, 981–988.

Killestein, J., and Polman, C. H. (2011). Determinants of interferon beta efficacy in patients with multiple sclerosis. *Nat. Rev. Neurol.* 7, 221–228.

Lafuse, W. P., Brown, D., Castle, L., and Zwilling, B. S. (1995). Cloning and characterization of a novel cDNA that is IFN-gamma-induced in mouse peritoneal macrophages and encodes a putative GTP-binding protein. *J. Leukoc. Biol.* 57, 477–483.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Segeral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474, 654–657.

Lee-Kirsch, M. A., Chowdhury, D., Harvey, S., Gong, M., Senenko, L., Engel, K., Pfeiffer, C., Hollis, T., Gahr, M., Perrino, F. W., Lieberman, J., and Hubner, N. (2007). A mutation in TREX1 that impairs susceptibility to granzyme A-mediated cell death underlies familial chilblain lupus. *J. Mol. Med. (Berl.)* 85, 531–537.

Li, N., Zhang, W., and Cao, X. (2000). Identification of human homologue of mouse IFN-gamma induced protein from human dendritic cells. *Immunol. Lett.* 74, 221–224.

Lindenmann, J., Burke, D. C., and Isaacs, A. (1957). Studies on the production, mode of action and properties of interferon. *Br. J. Exp. Pathol.* 38, 551–562.

Lucchinetti, C. F., Mandler, R. N., McGavern, D., Bruck, W., Gleich, G., Ransohoff, R. M., Trebst, C., Weinshenker, B., Wingerchuk, D., Parisi, J. E., and Lassmann, H. (2002). A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* 125, 1450–1461.

Martinvalet, D., Zhu, P., and Lieberman, J. (2005). Granzyme A induces caspase-independent mitochondrial damage, a required first step for apoptosis. *Immunity* 22, 355–370.

Mazur, D. J., and Perrino, F. W. (1999). Identification and expression of the TREX1 and TREX2 cDNA sequences encoding mammalian 3′ → 5′ exonucleases. *J. Biol. Chem.* 274, 19655–19660.

Mazur, D. J., and Perrino, F. W. (2001). Structure and expression of the TREX1 and TREX2 3′ → 5′ exonuclease genes. *J. Biol. Chem.* 276, 14718–14727.

McWhirter, S. M., Barbalat, R., Monroe, K. M., Fontana, M. F., Hyodo, M., Joncker, N. T., Ishii, K. J., Akira, S., Colonna, M., Chen, Z. J., Fitzgerald, K. A., Hayakawa, Y., and Vance, R. E. (2009). A host type I interferon response is induced by cytosolic sensing of the bacterial second messenger cyclic-di-GMP. *J. Exp. Med.* 206, 1899–1911.

Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R., and Tschopp, J. (2005). Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 437, 1167–1172.

Mori, M., Yoneyama, M., Ito, T., Takahashi, K., Inagaki, F., and Fujita, T. (2004). Identification of Ser-386 of interferon regulatory factor 3 as critical target for inducible phosphorylation that determines activation. *J. Biol. Chem.* 279, 9698–9702.

Morita, M., Stamp, G., Robins, P., Dulic, A., Rosewell, I., Hrivnak, G., Daly, G., Lindahl, T., and Barnes, D. E. (2004). Gene-targeted mice lacking the Trex1 (DNase III) 3′ → 5′ DNA exonuclease develop inflammatory myocarditis. *Mol. Cell Biol* 24, 6719–6727.

Noppert, S. J., Fitzgerald, K. A., and Hertzog, P. J. (2007). The role of type I interferons in TLR responses. *Immunol. Cell Biol.* 85, 446–457.

Owens, T., Wekerle, H., and Antel, J. (2001). Genetic models for CNS inflammation. *Nat. Med.* 7, 161–166.

Palace, J., Leite, M. I., Nairne, A., and Vincent, A. (2010). Interferon beta treatment in neuromyelitis optica: increase in relapses and aquaporin 4 antibody titers. *Arch. Neurol.* 67, 1016–1017.

Paul, F., Jarius, S., Aktas, O., Bluthner, M., Bauer, O., Appelhans, H., Franciotta, D., Bergamaschi, R., Littleton, E., Palace, J., Seelig, H. P., Hohlfeld, R., Vincent, A., and Zipp, F. (2007). Antibody to aquaporin 4 in the diagnosis of neuromyelitis optica. *PLoS. Med.* 4, e133. doi:10.1371/journal.pmed.0040133

Pichlmair, A., and Reis e Sousa, C. (2007). Innate recognition of viruses. *Immunity* 27, 370–383.

Poeck, H., Besch, R., Maihoefer, C., Renn, M., Tormo, D., Morskaya, S. S., Kirschnek, S., Gaffal, E., Landsberg, J., Hellmuth, J., Schmidt, A., Anz, D., Bscheider, M., Schwerd, T., Berking, C., Bourquin, C., Kalinke, U., Kremmer, E., Kato, H., Akira, S., Meyers, R., Hacker, G., Neuenhahn, M., Busch, D., Ruland, J., Rothenfusser, S., Prinz, M., Hornung, V., Endres, S., Tuting, T., and Hartmann, G. (2008). 5′-Triphosphate-siRNA: turning gene silencing and Rig-I activation against melanoma. *Nat. Med.* 14, 1256–1263.

Preble, O. T., Black, R. J., Friedman, R. M., Klippel, J. H., and Vilcek, J. (1982). Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. *Science* 216, 429–431.

Prinz, M., and Kalinke, U. (2010). New lessons about old molecules: how type I interferons shape Th1/Th17-mediated autoimmunity in the CNS. *Trends Mol. Med.* 16, 379–386.

Prinz, M., Schmidt, H., Mildner, A., Knobeloch, K. P., Hanisch, U. K., Raasch, J., Merkler, D., Detje, C., Gutcher, I., Mages, J., Lang, R., Martin, R., Gold, R., Becher, B., Bruck, W., and Kalinke, U. (2008). Distinct and nonredundant in vivo functions of IFNAR on myeloid cells limit autoimmunity in the central nervous system. *Immunity* 28, 675–686.

Rice, G., Patrick, T., Parmar, R., Taylor, C. F., Aeby, A., Aicardi, J., Artuch, R., Montalto, S. A., Bacino, C. A., Barroso, B., Baxter, P., Benko, W. S., Bergmann, C., Bertini, E., Biancheri, R., Blair, E. M., Blau, N., Bonthron, D. T., Briggs, T., Brueton, L. A., Brunner, H. G., Burke, C. J., Carr, I. M., Carvalho, D. R., Chandler, K. E., Christen, H. J., Corry, P. C., Cowan, F. M., Cox, H., D'Arrigo, S., Dean, J., De Laet, C., De Praeter, C., Dery, C., Ferrie, C. D., Flintoff, K., Frints, S. G., Garcia-Cazorla, A., Gener, B., Goizet, C., Goutieres, F., Green, A. J., Guet, A., Hamel, B. C., Hayward, B. E., Heiberg, A., Hennekam, R. C., Husson, M., Jackson, A. P., Jayatunga, R., Jiang, Y. H., Kant, S. G., Kao, A., King, M. D., Kingston, H. M., Klepper, J., van der Knaap, M. S., Kornberg, A. J., Kotzot, D., Kratzer, W., Lacombe, D., Lagae, L., Landrieu, P. G., Lanzi, G., Leitch, A., Lim, M. J., Livingston, J. H., Lourenco, C. M., Lyall, E. G., Lynch, S. A., Lyons, M. J., Marom, D., McClure, J. P., McWilliam, R., Melancon, S. B., Mewasingh, L. D., Moutard, M. L., Nischal, K. K., Ostergaard, J. R., Prendiville, J., Rasmussen, M., Rogers, R. C., Roland, D., Rosser, E. M., Rostasy, K., Roubertie, A., Sanchis, A., Schiffmann, R., Scholl-Burgi, S., Seal, S., Shalev, S. A., Corcoles, C. S., Sinha, G. P., Soler, D., Spiegel, R., Stephenson, J. B., Tacke, U., Tan, T. Y., Till, M., Tolmie, J. L., Tomlin, P., Vagnarelli, F., Valente, E. M., Van Coster, R. N., Van der, A. N., Vanderver, A., Vles, J. S., Voit, T., Wassmer, E., Weschke, B., Whiteford, M. L., Willemsen, M. A., Zankl, A., Zuberi, S. M., Orcesi, S., Fazzi, E., Lebon, P., and Crow, Y. J. (2007). Clinical and molecular phenotype of Aicardi-Goutieres syndrome. *Am. J. Hum. Genet.* 81, 713–725.

Rice, G. I., Bond, J., Asipu, A., Brunette, R. L., Manfield, I. W., Carr, I. M., Fuller, J. C., Jackson, R. M., Lamb, T., Briggs, T. A., Ali, M., Gornall, H., Couthard, L. R., Aeby, A., Attard-Montalto, S. P., Bertini, E., Bodemer, C., Brockmann, K., Brueton, L. A., Corry, P. C., Desguerre, I., Fazzi, E., Cazorla, A. G., Gener, B., Hamel, B. C., Heiberg, A., Hunter, M., van der Knaap, M. S., Kumar, R., Lagae, L., Landrieu, P. G., Lourenco, C. M., Marom, D., McDermott, M. F., van der, M. W., Orcesi, S., Prendiville, J. S., Rasmussen, M., Shalev, S. A., Soler, D. M., Shinawi, M., Spiegel, R., Tan, T. Y., Vanderver, A., Wakeling, E. L., Wassmer, E., Whittaker, E., Lebon, P., Stetson, D. B., Bonthron, D. T., and Crow, Y. J. (2009). Mutations involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate immune response. *Nat. Genet.* 41, 829–832.

Rydberg, B., and Game, J. (2002). Excision of misincorporated ribonucleotides in DNA by RNase H (type 2) and FEN-1 in cell-free extracts. *Proc. Natl. Acad. Sci. U.S.A.* 99, 16654–16659.

Schlee, M., Roth, A., Hornung, V., Hagmann, C. A., Wimmenauer, V., Barchet, W., Coch, C., Janke, M., Mihailovic, A., Wardle, G., Juranek, S., Kato, H., Kawai, T., Poeck, H., Fitzgerald, K. A., Takeuchi, O., Akira, S., Tuschl, T., Latz, E., Ludwig, J., and Hartmann, G. (2009). Recognition of 5′ triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. *Immunity* 31, 25–34.

Schmidt, H., Raasch, J., Merkler, D., Klinker, F., Krauss, S., Bruck, W., and Prinz, M. (2009). Type I interferon receptor signaling is induced during demyelination while its function for myelin damage and repair is redundant. *Exp. Neurol.* 216, 306–311.

Schwid, S. R., and Panitch, H. S. (2007). Full results of the evidence of interferon dose-response-European North American comparative efficacy (EVIDENCE) study: a multicenter, randomized, assessor-blinded comparison of low-dose weekly versus high-dose, high-frequency interferon beta-1a for relapsing multiple sclerosis. *Clin. Ther.* 29, 2031–2048.

Seth, R. B., Sun, L., Ea, C. K., and Chen, Z. J. (2005). Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell* 122, 669–682.

Shimizu, J., Hatanaka, Y., Hasegawa, M., Iwata, A., Sugimoto, I., Date, H., Goto, J., Shimizu, T., Takatsu, M., Sakurai, Y., Nakase, H., Uesaka, Y., Hashida, H., Hashimoto, K., Komiya, T., and Tsuji, S. (2010). IFNbeta-1b may severely exacerbate Japanese optic-spinal MS in neuromyelitis optica spectrum. *Neurology* 75, 1423–1427.

Stetson, D. B., Ko, J. S., Heidmann, T., and Medzhitov, R. (2008). Trex1 prevents cell-intrinsic initiation of autoimmunity. *Cell* 134, 587–598.

Stockinger, B., and Veldhoen, M. (2007). Differentiation and function of Th17 T cells. *Curr. Opin. Immunol.* 19, 281–286.

Tamura, T., Yanai, H., Savitsky, D., and Taniguchi, T. (2008). The IRF family transcription factors in immunity and oncogenesis. *Annu. Rev. Immunol.* 26, 535–584.

Teige, I., Treschow, A., Teige, A., Mattsson, R., Navikas, V., Leanderson, T., Holmdahl, R., and Issazadeh-Navikas, S. (2003). IFN-beta gene deletion leads to augmented and chronic demyelinating experimental autoimmune encephalomyelitis. *J. Immunol.* 170, 4776–4784.

Uzawa, A., Mori, M., Hayakawa, S., Masuda, S., and Kuwabara, S. (2010). Different responses to interferon beta-1b treatment in patients with neuromyelitis optica and multiple sclerosis. *Eur. J. Neurol.* 17, 672–676.

Veldhoen, M., Hocking, R. J., Atkins, C. J., Locksley, R. M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24, 179–189.

Warabi, Y., Matsumoto, Y., and Hayashi, H. (2007). Interferon beta-1b exacerbates multiple sclerosis with severe optic nerve and spinal cord demyelination. *J. Neurol. Sci.* 252, 57–61.

Woodward, J. J., Iavarone, A. T., and Portnoy, D. A. (2010). c-di-AMP secreted by intracellular *Listeria monocytogenes* activates a host type I interferon response. *Science* 328, 1703–1705.

Xu, L. G., Wang, Y. Y., Han, K. J., Li, L. Y., Zhai, Z., and Shu, H. B. (2005). VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol. Cell* 19, 727–740.

Yan, N., Regalado-Magdos, A. D., Stiggelbout, B., Lee-Kirsch, M. A., and Lieberman, J. (2010). The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1. *Nat. Immunol.* 11, 1005–1013.

Yang, Y. G., Lindahl, T., and Barnes, D. E. (2007). Trex1 exonuclease degrades ssDNA to prevent chronic checkpoint activation and autoimmune disease. *Cell* 131, 873–886.

Zhong, B., Yang, Y., Li, S., Wang, Y. Y., Li, Y., Diao, F., Lei, C., He, X., Zhang, L., Tien, P., and Shu, H. B. (2008). The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 29, 538–550.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 16 December 2011; paper pending published: 07 February 2012; accepted: 19 March 2012; published online: 09 April 2012.

*Citation: Prinz M and Knobeloch K-P (2012) Type I interferons as ambiguous modulators of chronic inflammation in the central nervous system. Front. Immun. **3**:67. doi: 10.3389/fimmu.2012.00067*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.

frontiers in
IMMUNOLOGY

MINI REVIEW ARTICLE
published: 16 June 2011
doi: 10.3389/fimmu.2011.00022

A four-step model for the IL-6 amplifier, a regulator of chronic inflammations in tissue-specific MHC class II-associated autoimmune diseases

Masaaki Murakami [1,2,3] ***and Toshio Hirano*** [1,2,3,4] *******

[1] *Laboratory of Developmental Immunology, JST-CREST, Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan*
[2] *Laboratory of Developmental Immunology, JST-CREST, Graduate School of Medicine, Osaka University, Osaka, Japan*
[3] *WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan*
[4] *Laboratory of Cytokine Signaling, RIKEN Research Center for Allergy and Immunology, Yokohama, Japan*

Edited by:
Anna Rubartelli, National Cancer Research Institute, Italy

Reviewed by:
Massimo Gadina, National Institute of Arthritis Musculoskeletal and Skin diseases-National Institutes of Health, USA
Marco Gattorno, G. Gaslini Institute for Children, Italy

****Correspondence:***
Toshio Hirano, Laboratory of Developmental Immunology, JST-CREST, Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan.
e-mail: hirano@molonc.med.osaka-u.ac.jp

It is commonly thought that autoimmune diseases are caused by the breakdown of self-tolerance, which suggests the recognition of specific antigens by autoreactive CD4+ T cells contribute to the specificity of autoimmune diseases (Marrack et al., 2001; Mathis and Benoist, 2004). In several cases, however, even for diseases associated with class II major histocompatibility complex (MHC) alleles, the causative tissue-specific antigens recognized by memory/activated CD4+ T cells have not been established (Mocci et al., 2000; Skapenko et al., 2005). Rheumatoid arthritis (RA) and arthritis in F759 knock-in mice (F759 mice) are such examples (Atsumi et al., 2002; Brennan et al., 2002; Falgarone et al., 2009). These include associations with class II MHC and CD4 molecules; increased numbers of memory/activated CD4+ T cells; and improved outcomes in response to suppressions and/or deficiencies in class II MHC molecules, CD4+ T cells, and the T cell survival cytokine IL-7. Regarding the development of arthritis in F759 mice, it is not only the immune system, but also non-immune tissue that are involved, indicating that the importance of their interactions (Sawa et al., 2006, 2009; Ogura et al., 2008; Hirano, 2010; Murakami et al., 2011). Furthermore, we have shown that local events such as microbleeding together with an accumulation of activated CD4+ T cells in a manner independent of tissue antigen-recognitions induces arthritis in the joints of F759 mice (Murakami et al., 2011). For example, local microbleeding-mediated CCL20 expression induce such an accumulation, causing arthritis development via chronic activation of an IL-17A-dependent IL-6 signaling amplification loop in type 1 collagen+ cells that is triggered by CD4+ T cell-derived cytokine(s) such as IL-17A, which leads to the synergistic activation of STAT3 and NFκB in non-hematopoietic cells in the joint (Murakami et al., 2011). We named this loop *the IL-6-mediated inflammation amplifier,* or IL-6 amplifier for short (Ogura et al., 2008; Hirano, 2010; Murakami et al., 2011). Thus, certain class II MHC-associated, tissue-specific autoimmune diseases, including some RA subtypes, may be induced by local events that cause an antigen-independent accumulation of effector CD4+ T cells followed by the induction of the IL-6 amplifier in the affected tissue. In other words, in certain cases, the target tissue itself may determine the specificity of the autoimmune disease via activation of the IL-6 amplifier. To explain this hypothesis, we have proposed a four-step model for MHC class II-associated autoimmune diseases (Murakami et al., 2011): (1) T cell activation regardless of antigen specificity; (2) local events inducing a tissue-specific accumulation of activated T cells; (3) transient activation of the IL-6 amplifier; and (4) enhanced sensitivity to cytokines in the target tissue. The interaction of these events results in chronic activation of the IL-6 amplifier and subsequent manifestation of autoimmune diseases. Thus, the IL-6 amplifier, which is chronically activated by these four events, is a critical regulator of chronic inflammations in tissue-specific MHC class II-associated autoimmune diseases.

Keywords: MHC class II association, autoimmune diseases, inflammation, IL-6-mediated inflammation amplifier, cytokines, chemokines, Th17 cells

TISSUE-SPECIFIC MHC CLASS II-ASSOCIATED AUTOIMMUNE DISEASES AND ANTIGEN-RECOGNITIONS BY CD4+ T CELLS

It has been proposed that autoimmune diseases are caused by a breakdown of self-tolerance due to multiple genetic and/or environmental factors (Marrack et al., 2001; Mathis and Benoist, 2004), suggesting the dysregulation of immune responses is fundamental to autoimmune diseases. This agrees with the theory that certain autoimmune diseases like rheumatoid arthritis (RA) develop in specific tissues as a result of cognate antigen-recognition by CD4+ T cells, particularly when these diseases are associated with class II major histocompatibility complex (MHC) alleles (Steinman, 2001; Zhang et al., 2008; Imboden, 2009). Consistent with this, joint-specific antigenic peptides such as derivatives of aggrecan, fibrillin, and collagen have been identified in humans (Polgár et al., 2003; Chapuy-Regaud et al., 2005; Takizawa et al., 2006; Van Steendam et al., 2010), while immunodominant MHC class II peptides in an animal model have been found to match those seen in human RA (Andersson et al., 2010). However, it is unclear whether these peptides are a result or cause of joint damage. Despite the evidence for antigen-specific T cell activation in some RA patients, tissue-specific self or non-self antigens recognized by activated CD4+ T cells in many class II MHC-associated diseases and even a majority of RA cases have not been well-established (Mocci et al., 2000; Skapenko et al., 2005). This raises the possibility that a breakdown in CD4+ T cell tolerance for a tissue-specific antigen is not always necessary for tissue-specific autoimmune diseases. Instead, it may be the consequence of local events that are initiated by inflammation triggered by certain genetic and/or environmental factors (Hirano, 1998, 2010; Matsumoto et al., 1999; Marrack et al., 2001; Sawa et al., 2006) such that the specificity of an autoimmune disease could be determined by the non-immune target tissue itself (Brennan et al., 2002; Hirano, 2002). In these cases, CD4+T cells may act as the source for a variety of inflammatory cytokines (Brennan et al., 2002). In fact, various subsets of effector CD4+ T cells – e.g., T helper 1 (Th1) cells, Th2 cells, and Th17 cells, which produce IFNγ, IL-4, and IL-17A, respectively (Mosmann and Coffman, 1989; Glimcher and Murphy, 2000; Cua et al., 2003; Harrington et al., 2005; Park et al., 2005; Veldhoen et al., 2006; Zhu et al., 2006; Bettelli et al., 2007; Nishihara et al., 2007) – may initiate and drive the progression of disease. This may help explain why RA is more common in older populations, as there exists an age-dependent increase in memory/activated CD4+ T cells resulting from a homeostatic proliferation that is mediated by a reduction in T cell input from the thymus (Surh and Sprent, 2000). Moreover, it has been reported that an age-dependent reduction in naive CD4+ T cells in peripheral second lymphoid organs increases the likelihood of (i) weak interactions between TCRs and peptides presented by self-class II MHC molecules including auto-antigenic peptides involved in positive selections in the thymus and (ii) cytokine consumption per CD4+ T cell including the T cell survival factor IL-7 (Surh and Sprent, 2000). This could help explain the occurrence of other diseases too, as the homeostatic proliferation of CD4+ T cells has been shown to be involved in the development of diabetes, arthritis, and Omenn syndrome (King et al., 2004; Jang et al., 2006; Sawa et al., 2006; Khiong et al., 2007). This process is also associated with a specific cytokine profile that includes the IL-17A and IFNγ produced by CD4+ T cells (Gudmundsdottir and Turka, 2001; Khiong et al., 2007; Nishihara et al., 2007).

IL-6 IN AUTOIMMUNE DISEASES

IL-6 is a pleiotropic cytokine that regulates multiple biological processes including the development of the nervous and hematopoietic systems, acute-phase responses, inflammation, and immune responses (Hirano, 1998). To date, 10 IL-6 family cytokines have been identified: IL-6, oncostatin M, LIF, CNTF, CT-1, NNT-1, neuropoietin, IL-11, IL-27, and IL-31 (Kamimura et al., 2003; Murakami et al., 2004; Suthaus et al., 2010). All of these share gp130 as the signal transducer in their receptor complexes. Upon IL-6 stimulation, gp130 transduces two major signaling pathways: the JAK–signal transducer and activator of transcription 3 (STAT3) pathway, which is mediated by the YxxQ motif of gp130, and the SHP2–Gab-Ras-Erk–MAPK pathway, which is regulated by Y759, a cytoplasmic SOCS3 binding residue in gp130 (Fukada et al., 1996; Ohtani et al., 2000; Kamimura et al., 2003). Additionally, a number of studies have suggested IL-6 has an important role in autoimmune diseases (Hirano, 1998, 2010; O'Shea et al., 2002; Sakaguchi and Sakaguchi, 2005; Awasthi and Kuchroo, 2009). The F759 knock-in mouse line (F759), for example, which expresses a mutant variant of gp130 where Y759 is substituted for phenylalanine (F), shows enhanced IL-6-mediated STAT3 activation due to a lack of SOCS3-mediated suppression (Ohtani et al., 2000). As these mice age, they spontaneously develop a RA-like tissue-specific disease, indicating that constitutive activation of IL-6 signaling is involved in the development of certain autoimmune diseases (Atsumi et al., 2002). Moreover, patients with RA show high synovial concentrations of IL-6 (Hirano et al., 1988), while anti-IL-6 receptor therapy is effective for some RA patients (Nakagawa et al., 2010). These observations support the use of the F759 mouse as a murine model for RA. Furthermore, we have previously shown that MHC II-restricted CD4+ T cells, but not $CD8^+$ T cells and B cells, are involved in the development of arthritis in F759 mice (Sawa et al., 2006), while a subset of $CD8^+$ T cells that express Foxp3 and are induced by IL-6 signaling suppress it (Nakagawa et al., 2010).

AN IL-17A-DEPENDENT IL-6 SIGNALING AMPLIFICATION LOOP IN TYPE 1 COLLAGEN+ CELLS, THE IL-6 AMPLIFIER, PLAYS A ROLE IN THE DEVELOPMENT OF MHC CLASS II-ASSOCIATED AUTOIMMUNE DISEASES

While CD4+T cells are required for F759 arthritis, bone marrow transplantation experiments have demonstrated the F759 mutation in non-hematopoietic cells is sufficient for F759 arthritis without any accompanying mutation in the CD4+ T cells (Sawa et al., 2006). This shows that an interaction between non-immune tissues/cells and the immune system plays a critical role this and most likely several other autoimmune and chronic inflammatory diseases (Hirano, 1998, 2010). Our detailed experiments utilizing bone marrow chimera mice and knock-out mice further demonstrate that excess IL-6 signaling in non-hematopoietic

cells, particularly type 1 collagen^{+} cells, due to the F759 mutation induces an enhanced production of the T cell survival factor IL-7, which increases memory/activated CD4+ T cells via an increase in homeostatic proliferation – a process that is critical for arthritis development in F759 mice (Sawa et al., 2006). Furthermore, IL-17A-expressing CD4+ T cells show a memory/activated phenotype *in vivo* in F759 mice, while IL-17A regulates their arthritis (Ogura et al., 2008; Murakami et al., 2011). Thus, it is plausible that the age-dependent increase in homeostatic proliferation via IL-6-mediated IL-7 expression plays a role in the accumulation of antigen-experienced, memory/activated CD4+ T cells expressing IL-17A. This is especially true for those F759 mice that show excess IL-6 signaling. We previously showed that IL-17A-triggerred positive feedback of IL-6 signaling, which results in synergistic hyper-expressions of chemokines and IL-6 itself in type 1 collagen^{+} cells, is enhanced in a manner dependent on NF-κB and STAT3, which our themselves stimulated by IL-17A in the presence of an IL-6 signal (Ogura et al., 2008; **Figure 1**). We named this IL-17A-dependent IL-6 signaling amplification loop in type 1 collagen+ cells *the IL-6-mediated inflammation amplifier*, or IL-6 amplifier for short (Ogura et al., 2008; Hirano, 2010). Furthermore, activation of the IL-6 amplifier is critical not only for the development of arthritis in F759 mice but also for MOG antigen-specific, T cell-mediated experimental autoimmune encephalomyelitis (EAE; Ogura et al., 2008). These results further support the idea that interactions between the immune system and non-immune tissue play roles in the development of autoimmune diseases and that the IL-6 amplifier makes a major contribution to this interaction.

A FOUR-STEP MODEL EXPLAINS THE CHRONIC ACTIVATION OF THE IL-6 AMPLIFIER THAT IS FOLLOWED BY THE DEVELOPMENT OF MHC CLASS II-ASSOCIATED AUTOIMMUNE DISEASES

Because CD4+ T cells bearing a single TCR that recognizes antigens not related to joint tissue induces arthritis in Rag2 deficient mice that have the F759 mutation, we concluded that cognate antigen-recognition by effector CD4+ T cells is not necessary for tissue specificity in F759 mice (Murakami et al., 2011). From this, we hypothesized that disease specificity may be determined by the tissue itself such that local events in the joint may determine and initiate the disease via the IL-6 amplifier. For example, intravenous transfer of *in vitro* polarized Th17 cells into young F759 mice does not develop arthritis within 3 months. This sharply contrasts with the case where MOG antigen-specific Th17 transfer induces EAE. However, if Th17 cell transfer is done before inducing experimental microbleeding in one leg of F759 mice, then this leg and only this leg will develop arthritis. Even Th17 cells derived from TCR transgenic mice induced arthritis in the microbleeding-induced leg of F759 mice. These findings are consistent with the idea that local events determine the disease specificity even if activation of tissue antigen-specific T cells does not occur. We further observed that T cells accumulate in the joint where arthritis occurs. This microbleeding-induced accumulation of Th17 cells is dependent on the production of CCL20, a target of the IL-6 amplifier, in

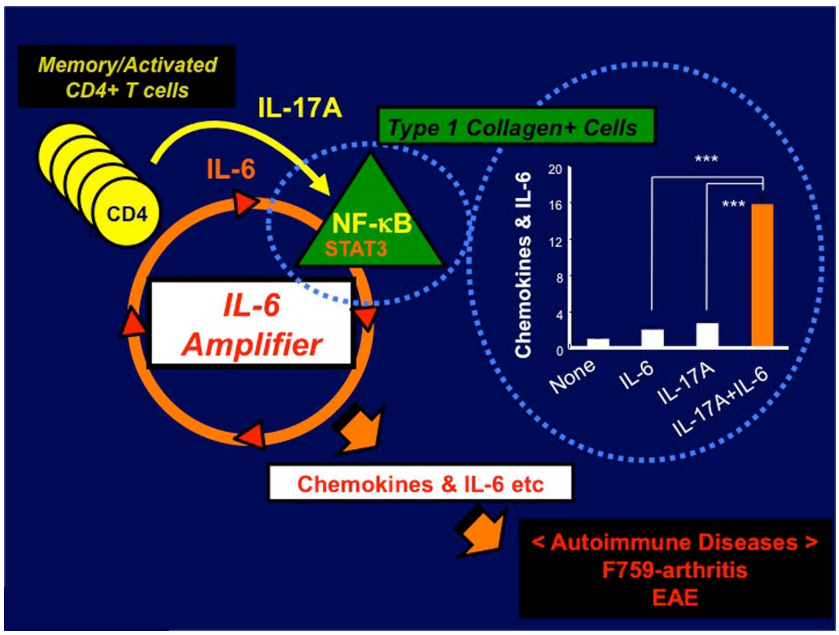


FIGURE 1 | IL-6 amplifier activation plays a role in the development of autoimmune diseases such as arthritis in F759 mice and EAE. IL-17A-triggerred positive feedback of IL-6 signaling, which results in synergistic hyper-expressions of chemokines and IL-6 itself in type 1 collagen+ cells, is enhanced in a manner dependent on NF-κB and STAT3, which our themselves stimulated by IL-17A in the presence of an IL-6 signal (bar graph). We named this IL-17A-dependent IL-6 signaling amplification loop in type 1 collagen+ cells *the IL-6-mediated inflammation amplifier*, or *IL-6 amplifier* for short. Importantly, activation of the IL-6 amplifier is critical not only for the development of arthritis in F759 mice but also for MOG antigen-specific, T cell-mediated experimental autoimmune encephalomyelitis (EAE; Ogura et al., 2008).

the joint. Disease induction requires T cell produced IL-17A, IL-6, and enhanced STAT3 signaling in type I collagen-expressing cells (Murakami et al., 2011). Based on these results, we propose that certain class II MHC-associated autoimmune diseases such as RA arise through a series of at least four steps (**Figure 2**): (1) T cell activation regardless of antigen specificity; (2) local events inducing a tissue-specific accumulation of activated T cells; (3) transient activation of the IL-6 amplifier, which is triggered by CD4+ T cell-derived cytokines such as IL-17A; and (4) enhanced sensitivity to T cell-derived cytokines and/or IL-6 in type 1 collagen+ cells in the target tissue. After these four steps, chronic activation of the IL-6 amplifier followed by the development of an autoimmune disease occurs. It is likely that each step interacts with the others, and the degree of the contribution of each to the pathogenesis varies with the disease. Our four-step model provides a plausible explanation for why tissue-specific antigens recognized by activated CD4+ T cells have not been identified in several autoimmune diseases, especially those associated with class II MHC molecules. It is likely that in diseases where tissue antigen-specific T cells play roles, tissue antigen-specific recognition by T cells would bypass the requirement of local events, even though these local events can still affect the accumulation of tissue antigen-specific T cells in the target tissue. Our four-step model, therefore, should be applicable to a wide range of autoimmune and other chronic inflammatory diseases (see last section).

CHRONIC ACTIVATION OF THE IL-6 AMPLIFIER CAN OVERRIDE HOMEOSTASIS IN TARGET TISSUES

The number of cytokine-secreting effector/memory CD4+ T cells increases with age due to an accumulation of pathogen-specific memory T cells and homeostatic proliferation of CD4+ T cells. These CD4+ T cells are increasingly localized in parenchymal organs like the alimentary tract, lung, and liver, rather than the lymphoid organs, meaning at steady state, memory/activated CD4+ T cells may migrate to and/or stay in non-lymphoid tissues that are at high risk for autoimmune diseases. Consistent with this, these diseases are more prevalent in older patients who have a larger population of memory/activated CD4+ T cells, some of which secrete cytokines because of homeostatic proliferation and/or chronic inflammations in other tissues (Hasler and Zouali, 2005; Larbi et al., 2008). Therefore, the first three steps will occur to some extent even in healthy subjects, although the degree will differ among individuals (see **Figure 2**). Autoimmune diseases like RA, however, do not develop in all individuals. Therefore, the relatively low rates of these disorders may reflect the fact that multiple genetic and environmental factors make a

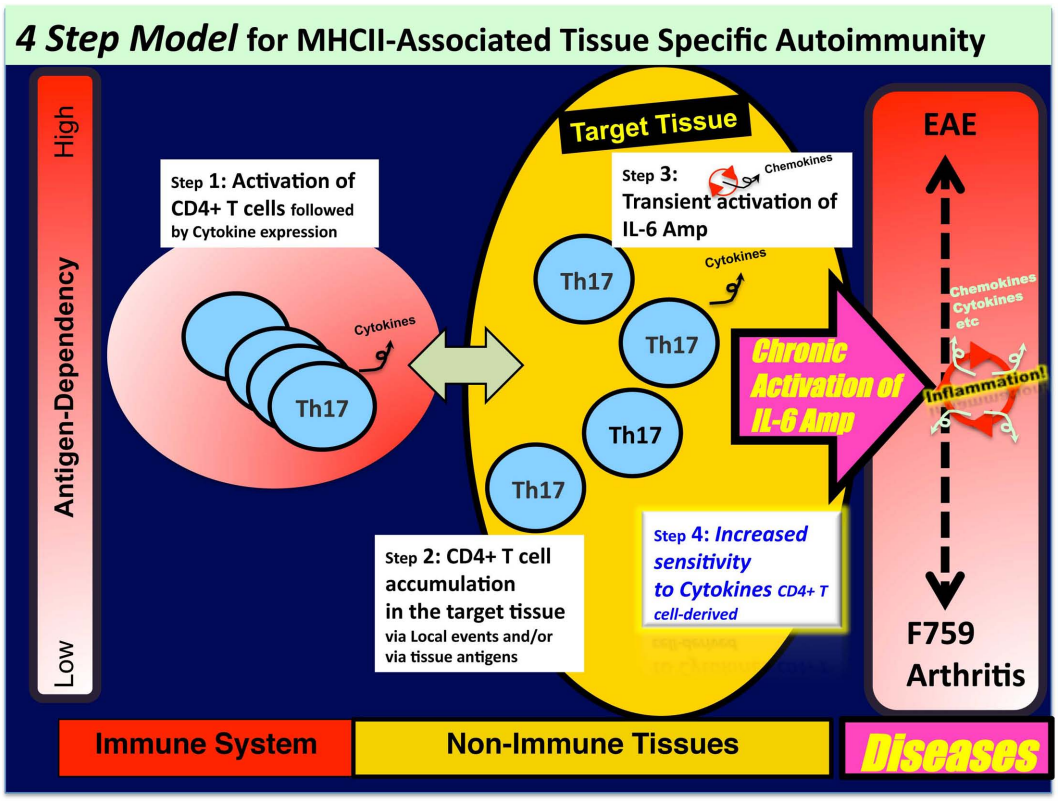


FIGURE 2 | A four-step model for MHC class II-associated autoimmune diseases. Certain class II MHC-associated autoimmune diseases arise through a series of four steps: (1) T cell activation regardless of antigen specificity; (2) local events inducing a tissue-specific accumulation of activated T cells; (3) transient activation of the IL-6 amplifier, which is triggered by CD4+ T cell-derived cytokines such as IL-17A; and (4) enhanced sensitivity to T cell-derived cytokines and/or IL-6 in type 1 collagen+ cells in the target tissue. Following these four steps, chronic activation of the IL-6 amplifier followed by the development of an autoimmune disease occurs. It is likely that each step interacts with the others, and the degree of the contribution of each to the pathogenesis varies with the disease.

significant contribution, particularly at the fourth step, for disease development.

POTENTIAL FACTORS THAT ACCELERATE TISSUE SENSITIVITY TO CYTOKINES INVOLVED IN AUTOIMMUNE DISEASES

Because the activation of the IL-6 amplifier is mediated by the synergistic activation of NF-κB and STAT3 molecules, we hypothesize factors that stimulate the signaling pathways regulating these two molecules in non-immune tissues/cells play a role in the development of MHC class II-associated autoimmune diseases and possibly other chronic inflammatory diseases (Hasler and Zouali, 2005; Larbi et al., 2008). These factors and the role of their cognate recognitions in CD4+ T cells are discussed below.

VIRUS/BACTERIA PRODUCTS AND EXOGENOUS TLR STIMULATORS

One example is products made by viruses or bacteria (Münz et al., 2009). For instance, HTLV1 infection is a significant risk factor for arthritis (Ishihara et al., 2004), while the transgenic expression of p40 Tax, a product of HTLV1 that activates NF-κB, causes a RA-like disease in mice (Iwakura et al., 1991). Indeed, forced expression of p40 Tax in F759 mice has been seen to enhance disease development (Ishihara et al., 2004). Moreover, many viral proteins, including the hepatitis C virus Core protein and EBNA2 from the Epstein–Barr virus, are strong STAT3 activators (Yoshida et al., 2002; Muromoto et al., 2009). Products from pathogens are also known to stimulate Toll-like receptors that lead to NF-κB activation. Since viruses and bacteria also have their own preferential target cells and/or tissues, their infections could determine the tissue specificity of a disease by enhancing cytokine sensitivity in the given tissue. Consistent with this, autoimmune diseases are sometimes induced after infections that also increase the number of activated, pathogen-specific, cytokine-secreting CD4+ T cells (Kivity et al., 2009).

MICROBLEEDING AND MECHANICAL STRESS IN THE TISSUES

Microbleeding in the joints may result in the accumulation of many different cell types including red blood cells, neutrophils, macrophages, and dendritic cells as well as memory/activated CD4+ T cells including Th17 cells. Here we focused on IL-17A expression from Th17 cells accumulating in the joints. However, one can argue that microbleeding can accelerate the inflammatory reaction by other means. For example, the environment of the joint synovium or the presence of dead cells may lead to the release of intracellular stimulants such as heme and/or danger-associated molecular patterns (DAMPs; Zhang et al., 2010). These stimulants secondarily enhance cell death by heme's toxic effect. They might also induce IL-6 and/or CCL20 expression based on the fact that DAMPs can activate the NF-κB pathway (Bianchi, 2007; Sims et al., 2010).

Tissue sensitivity to mechanical stress that arises with age is another potential trigger or enhancer for autoimmune diseases. We have shown that an experimental compression enhances arthritis development in F759 mice in the presence of Th17 cell transfer (unpublished data), suggesting that such stress can induce local events like microbleeding and/or IL-6 expression via activation of NF-κB.

GENETIC FACTORS/MUTATIONS AFFECTING SIGNALING MOLECULES IN NF-κB AND STAT3 PATHWAYS

Moreover, MHC class II-associated autoimmune diseases might be associated with various genetic aberrations including a somatic mutation in gp130 molecules that induces STAT3 hyperactivation, and mutations in NF-κB and its regulators that lead to dysregualted NF-κB signaling (Lenz et al., 2008; Compagno et al., 2009; Rebouissou et al., 2009). This may not apply in humans, however, as to date we have not identified mutations in the cytoplasmic region of gp130 in patients. Nevertheless, there still remains evidence that STAT3 abnormalities are involved, as demonstrated in the B cells of patients with hyper-immunoglobulin E syndrome (Minegishi et al., 2007). Because F759 mice lack SOCS3-mediated negative feedback only in the gp130 signaling pathway, it is reasonable to speculate that specifically deleting SOCS3 in non-hematopoietic cells could also increase the risk of autoimmune and/or other chronic inflammatory diseases. Consistent with this notion, SOCS3 deficiency in liver cells increased the degree of liver fibrosis (Ogata et al., 2006). Although this phenotype is should not be classified specifically as an autoimmune syndrome, the fibrosis likely mirrors the effects of NF-κB/STAT3 mutations. Finally, dysregulated NF-κB/STAT3 activation in non-immune cells such as type I collagen^{+} fibroblasts may trigger a feedback loop that increases IL-6 expression to induce inflammation like that seen in F759 mice.

ROLE OF OTHER CYTOKINES DERIVED FROM CD4+ T CELLS

Cytokines other than IL-17A may also contribute to class II MHC-associated diseases by enhancing IL-6 signaling in affected tissues and cells like type 1 collagen^{+} cells (the fourth step), as non-polarized activated CD4+ T cells, Th1, and IL-17A$^{-/-}$ Th17 cells too induce a mild form of arthritis in F759 mice (Murakami et al., 2011). TNFα, for example, may contribute to localized class II MHC-associated autoimmune diseases via NF-κB activation followed by IL-6 amplifier activation. In support of this idea, numerous studies have demonstrated the efficacy of targeting TNFα when treating RA and other chronic autoimmune diseases, the majority of which involve class II MHC molecules (Feldmann and Maini, 2001). Furthermore, activated CD4+ T cells are known to express TNFα (Cherwinski et al., 1987; Constant and Bottomly, 1997; Brennan et al., 2002; Williams et al., 2008), while we have found a lack of TNFα attenuates arthritis in F759 mice (unpublished data). Moreover, it is interesting that LPS administration around the joints induces arthritis in mice that have an excess number of Th1 cells (Nickdel et al., 2009). This may suggest that LPS-mediated IL-6 production induces a local accumulation of Th1 cells followed by activation of the IL-6 amplifier.

ROLE OF COGNATE RECOGNITIONS BY CD4+ T CELLS

Activation of the IL-6 amplifier is also involved in the development of EAE (Ogura et al., 2008). Because the model for EAE is dependent on tissue specific, MOG-derived peptides, these results suggest that antigen specificity by effector CD4+ T cells and IL-6 amplifier activation in the affected tissue do not always function independently of each other in tissue-specific autoimmune diseases. If this is the case, cognate antigen-recognition by effector CD4+ T cells could occur upstream of the enhanced IL-6

signaling in the affected tissue such that the antigen specificity of the effector CD4+ T cells functions initially to target CD4+ T cells around said tissues. In other words, the antigen specificity might bypass initial local events (like microbleeding) to induce tissue-specific accumulation of activated T cells, although some local events might increase the efficacy of the tissue accumulation even in diseases like EAE (step 2 in **Figure 2**). The resulting pool of activated CD4+ T cells around the affected tissues could enhance local IL-6 signaling, which would then act as a source for cytokines via the IL-6 amplifier. Thus, regardless of the stimulus for the local accumulation of effector CD4+ T cells, the resulting inflammatory disease is associated with class II MHC molecules if cytokines from the activated CD4+ T cells are involved in the disease development.

THE IL-6 AMPLIFIER BEYOND MHC CLASS II-ASSOCIATED DISEASES AND DISORDERS

Indeed, MHC class II genes are associated with a number of human diseases and disorders that extend beyond typical autoimmune diseases including metabolic syndrome, psychotic illnesses, and other inflammatory diseases. Diseases and disorders in the Genetic Association Database at the National Institute of Aging (http://geneticassociationdb.nih.gov/cgi-bin/index.cgi) found to be associated with the MHC class II genes described above are wide and varied (**Table 1**). These associations and our four-step model suggest that at least some subfamilies of these diseases and disorders might be affected by the activation status of the IL-6 amplifier, which is triggered by cytokines from CD4+ T cells. However, we also hypothesize that the importance of the IL-6 amplifier activation extends beyond MHC class II-associated diseases and disorders to include those that arise from inflammation induction. One reason is the amplifier's synergistic activation of NF-κB and STAT3 in type 1 collagen+ cells. In other words, it is possible that not all NF-κB and/or STAT3 stimulators are supplied by activated CD4+ T cells. Therefore, chronic activation of NF-κB and STAT3 induced by genetic and/or environmental factors may give rise to similar effects exerted by chronic activation of the IL-6 amplifier. It is likely that activation of NF-κB and STAT3 by genetic and environmental factors other than T cell products triggers certain chronic inflammatory diseases that are not readily apparent to be associated with MHC class II genes. Examples include adult Still's disease and Castleman's disease. Indeed, these diseases do associate with the IL-18 gene (Sugiura et al., 2002), an NF-κB stimulator, and with the IL-6 gene, suggesting perhaps that IL-18- and IL-6-mediated IL-6 amplifier activation plays a role. In these cases, local events can determine the tissue specificity for the development of diseases like F759 arthritis even in the absence of tissue antigen-recognition by activated T cells (Hirano, 2010; Murakami et al., 2011).

To summarize, we have investigated how MHC class II-associated tissue-specific autoimmune arthritis develops and propose a four-step model to explain the process. This model provides a possible explanation for why tissue-specific antigens recognized by activated CD4+ T cells have not been identified in many tissue-specific autoimmune diseases associated with class II MHC molecules including RA. This may be explained by our four-step model, which highlights the idea that the tissue itself can determine the tissue specificity of the autoimmune disease. Additionally, genetic and environmental factors affecting the target tissue are involved. These results have led us to propose that direct activation of the IL-6 amplifier by STAT3 and NF-κB can result in chronic inflammatory diseases that are not apparently associated with MHC class II. Thus, we expect our four-step model will provide new and important insights on the immunological mechanisms that underlie autoimmune disease as well as other chronic inflammatory diseases.

ACKNOWLEDGMENTS

We thank Drs D. Kamimura, H. Ogura, and especially P. Karagiannis (Osaka University, Osaka, Japan) for carefully reading the manuscript. This work was supported by KAKENHI (Masaaki Murakami and Toshio Hirano), the JST-CREST program (Masaaki Murakami and Toshio Hirano), and the Osaka Foundation for the Promotion of Clinical Immunology (Masaaki Murakami).

Table 1 | The MHC class II genes are associated with various diseases and disorders.

Autoimmune diseases	Celiac disease Multiple sclerosis	Crohn's disease Rheumatoid arthritis	Grave's disease Sjogren's syndrome	Juvenile idiopathic arthritis Thyroid autoimmunity	Lupus/glomerulonephritis Type 1 diabetes/IDDM
Metabolic syndrome-related diseases	Cholangitis	Hypertension	Lacunar stroke	Sclerosing	Type 2 diabetes/NIDDM
Psychotic illnesses	Narcolepsy	Schizophrenia			
Inflammatory diseases	Addison's disease Dermatomyositis Liver cirrhosis Recurrent pregnancy loss	Allergies Endometriosis Nasal polyposis Sarcoidosis	Asthma GVHD Osteoarthritis Ulcerative colitis	Atopy Hepatitis Periodontitis Vitiligo	Cardiomyopathy Inflammatory bowel disease Psoriasis

We investigated whether the MHC class II genes are associated with various diseases and disorders using the Genetic Association Database at the National Institute of Aging.

REFERENCES

Andersson, I. E., Batsalova, T., Dzhambazov, B., Edvinsson, L., Holmdahl, R., Kihlberg, J., and Linusson, A. (2010). Oxazole-modified glycopeptides that target arthritis-associated class II MHC A(q) and DR4 proteins. *Org. Biomol. Chem.* 8, 2931–2940.

Atsumi, T., Ishihara, K., Kamimura, D., Ikushima, H., Ohtani, T., Hirota, S., Kobayashi, H., Park, S., Saeki, Y., Kitamura, Y., and Hirano, T. (2002). A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. *J. Exp. Med.* 196, 979–990.

Awasthi, A., and Kuchroo, V. K. (2009). Th17 cells: from precursors to players in inflammation and infection. *Int. Immunol.* 21, 489–498.

Bettelli, E., Oukka, M., and Kuchroo, V. K. (2007). T(H)-17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* 345–350.

Bianchi, M. E. (2007). DAMPs, PAMPs and alarmins: all we need to know about danger. *J. Leukoc. Biol.* 81, 1–5.

Brennan, F. M., Hayes, A. L., Ciesielski, C. J., Green, P., Foxwell, B. M., and Feldmann, M. (2002). Evidence that rheumatoid arthritis synovial T cells are similar to cytokine-activated T cells: involvement of phosphatidylinositol 3-kinase and nuclear factor kappaB pathways in tumor necrosis factor alpha production in rheumatoid arthritis. *Arthritis Rheum.* 46, 31–41.

Chapuy-Regaud, S., Sebbag, M., Baeten, D., Clavel, C., Foulquier, C., De Keyser, F., and Serre, G. (2005). Fibrin deimination in synovial tissue is not specific for rheumatoid arthritis but commonly occurs during synovitides. *J. Immunol.* 174, 5057–5064.

Cherwinski, H. M., Schumacher, J. H., Brown, K. D., and Mosmann, T. R. (1987). Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med.* 166, 1229–1244.

Compagno, M., Lim, W. K., Grunn, A., Nandula, S. V., Brahmachary, M., Shen, Q., Bertoni, F., Ponzoni, M., Scandurra, M., Califano, A., Bhagat, G., Chadburn, A., Dalla-Favera, R., and Pasqualucci, L. (2009). Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature* 459, 717–721.

Constant, S. L., and Bottomly, K. (1997). Induction of Th1 and Th2 CD4+ T cell responses: the alternative approaches. *Annu. Rev. Immunol.* 166, 297–322.

Cua, D. J., Sherlock, J., Chen, Y., Murphy, C. A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T., Zurawski, S., Wiekowski, M., Lira, S. A., Gorman, D., Kastelein, R. A., and Sedgwick, J. D. (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421, 744–748.

Falgarone, G., Semerano, L., Rullé, S., and Boissier, M. C. (2009). Targeting lymphocyte activation to treat rheumatoid arthritis. *Joint Bone Spine.* 76, 327–332.

Feldmann, M., and Maini, R. N. (2001). Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu. Rev. Immunol.* 19, 163–196.

Fukada, T., Hibi, M., Yamanaka, Y., Takahashi-Tezuka, M., Fujitani, Y., Yamaguchi, T., Nakajima, K., and Hirano, T. (1996). Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. *Immunity* 5, 449–460.

Glimcher, L. H., and Murphy, K. M. (2000). Lineage commitment in the immune system: the T helper lymphocyte grows up. *Genes Dev.* 14, 1693–1711.

Gudmundsdottir, H., and Turka, L. A. (2001). A closer look at homeostatic proliferation of CD4+ T cells: costimulatory requirements and role in memory formation. *J. Immunol.* 167, 3699–3707.

Harrington, L. E., Hatton, R. D., Mangan, P. R., Turner, H., Murphy, T. L., Murphy, K. M., and Weaver, C. T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6, 1123–1132.

Hasler, P., and Zouali, M. (2005). Immune receptor signaling, aging, and autoimmunity. *Cell. Immunol.* 233, 102–108.

Hirano, T. (1998). Interleukin 6 and its receptor: ten years later. *Int. Rev. Immunol.* 16, 249–284.

Hirano, T. (2002). Revival of the autoantibody model in rheumatoid arthritis. *Nat. Immunol.* 3, 342–344.

Hirano, T. (2010). Interleukin 6 in autoimmune and inflammatory diseases: a personal memoir. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 86, 717–730.

Hirano, T., Matsuda, T., Turne, M., Miyasaka, N., Buchan, G., Tang, B., Sato, K., Shimizu, M., Maini, R., Feldmann, M., and Kishimoto, T. (1988). Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. *Eur. J. Immunol.* 18, 1797–1801.

Imboden, J. B. (2009). The immunopathogenesis of rheumatoid arthritis. *Annu. Rev. Pathol.* 4, 417–434.

Ishihara, K., Sawa, S., Ikushima, H., Hirota, S., Atsumi, T., Kamimura, D., Park, S., Murakami, M., Kitamura, Y., Iwakura, Y., and Hirano, T. (2004). The point mutation of tyrosine 759 of the IL-6 family cytokine receptor gp130 synergizes with HTLV-1 pX in promoting rheumatoid arthritis-like arthritis. *Int. Immunol.* 16, 455–465.

Iwakura, Y., Tosu, M., Yoshida, E., Takiguchi, M., Sato, K., Kitajima, I., Nishioka, K., Yamamoto, K., Takeda, T., Hatanaka, M., Yamamoto, H., and Sekiguchi, T. (1991). Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. *Science* 253, 1026–1028.

Jang, E., Kim, H. R., Cho, S. H., Paik, D. J., Kim, J. M., Lee, S. G., and Youn, J. (2006). Prevention of spontaneous arthritis by inhibiting homeostatic expansion of autoreactive CD4+ T cells in K/BxN mouse model. *Arthritis Rheum.* 54, 492–498.

Kamimura, D., Ishihara, K., and Hirano, T. (2003). IL-6 signal transduction and its physiological roles: the signal orchestration model. *Rev. Physiol. Biochem. Pharmacol.* 149, 1–38.

Khiong, K., Murakami, M., Kitabayashi, C., Ueda, N., Sawa, S., Sakamoto, A., Kotzin, B. L., Rozzo, S. J., Ishihara, K., Verella-Garcia, M., Kappler, J., Marrack, P., and Hirano, T. (2007). Homeostatically proliferating CD4 T cells are involved in the pathogenesis of an Omenn syndrome murine model. *J. Clin. Invest.* 117, 1270–1281.

King, C., Ilic, A., Koelsch, K., and Sarvetnick, N. (2004). Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell* 117, 265–277.

Kivity, S., Agmon-Levin, N., Blank, M., and Shoenfeld, Y. (2009). Infections and autoimmunity–friends or foes? *Trends Immunol.* 30, 409–414.

Larbi, A., Fülöp, T., and Pawelec, G. (2008). Immune receptor signaling, aging and autoimmunity. *Adv. Exp. Med. Biol.* 640, 312–324.

Lenz, G., Davis, R. E., Ngo, V. N., Lam, L., George, T. C., Wright, G. W., Dave, S. S., Zhao, H., Xu, W., Rosenwald, A., Ott, G., Muller-Hermelink, H. K., Gascoyne, R. D., Connors, J. M., Rimsza, L. M., Campo, E., Jaffe, E. S., Delabie, J., Smeland, E. B., Fisher, R. I., Chan, W. C., and Staudt, L. M. (2008). Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science* 319, 1676–1679.

Marrack, P., Kappler, J., and Kotzin, B. (2001). Autoimmune disease: why and where it occurs. *Nat. Med.* 7, 899–905.

Mathis, D., and Benoist, C. (2004). Back to central tolerance. *Immunity* 20, 509–516.

Matsumoto, I., Staub, A., Benoist, C., and Mathis, D. (1999). Arthritis provoked by linked T and B cell recognition of a glycolytic enzyme. *Science* 286, 1732–1735.

Minegishi, Y., Saito, M., Tsuchiya, S., Tsuge, I., Takada, H., Hara, T., Kawamura, N., Ariga, T., Pasic, S., Stojkovic, O., Metin, A., and Karasuyama, H. (2007). Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 448, 1058–1062.

Mocci, S., Lafferty, K., and Howard, M. (2000). The role of autoantigens in autoimmune disease. *Curr. Opin. Immunol.* 12, 725–730.

Mosmann, T. R., and Coffman, R. L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7, 145–173.

Münz, C., Lünemann, J. D., Getts, M. T., and Miller, S. D. (2009). Antiviral immune responses: triggers of or triggered by autoimmunity? *Nat. Rev. Immunol.* 9, 246–258.

Murakami, M., Kamimura, D., and Hirano, T. (2004). New IL-6 (gp130) family cytokine members, CLC/NNT1/BSF3 and IL-27. *Growth Factors* 22, 75–77.

Murakami, M., Okuyama, Y., Ogura, H., Asao, S., Arima, Y., Tsuruoka, M., Harada, M., Kanamoto, M., Iwakura, Y., Takatsu, K., Kamimura, D., and Hirano, T. (2011). Local microbleeding facilitates IL-6– and IL-17–dependent arthritis in the absence of tissue antigen recognition by activated T cells. *J. Exp. Med.* 208, 103–114.

Muromoto, R., Ikeda, O., Okabe, K., Togi, S., Kamitani, S., Fujimuro, M., Harada, S., Oritani, K., and Matsuda, T. (2009). Epstein-Barr virus-derived EBNA2 regulates STAT3 activation. *Biochem. Biophys. Res. Commun.* 378, 439–443.

Nakagawa, T., Tsuruoka, M., Ogura, H., Okuyama, Y., Arima, Y., Hirano, T., and Murakami, M. (2010). IL-6 positively regulates Foxp3+CD8+ T cells in vivo. *Int. Immunol.* 22, 129–139.

Nickdel, M. B., Conigliaro, P., Valesini, G., Hutchison, S., Benson, R., Bundick, R. V., Leishman, A. J., McInnes, I. B., Brewer, J. M., and Garside, P. (2009). Dissecting the contribution of innate and antigen-specific pathways to the breach of self-tolerance observed in a murine model of arthritis. *Ann. Rheum. Dis.* 68, 1059–1066.

Nishihara, M., Ogura, H., Ueda, N., Tsuruoka, M., Kitabayashi, C., Tsuji, F., Aono, H., Ishihara, K., Huseby, E., Betz, U. A., Murakami, M., and Hirano, T. (2007). IL-6-gp130-STAT3 in T cells directs the development of IL-17+ Th with a minimum effect on that of Treg in the steady state. *Int. Immunol.* 19, 695–702.

Ogata, H., Chinen, T., Yoshida, T., Kinjyo, I., Takaesu, G., Shiraishi, H., Iida, M., Kobayashi, T., and Yoshimura, A. (2006). Loss of SOCS3 in the liver promotes fibrosis by enhancing STAT3-mediated TGF-beta1 production. *Oncogene* 25, 2520–2530.

Ogura, H., Murakami, M., Okuyama, Y., Tsuruoka, M., Kitabayashi, C., Kanamoto, M., Nishihara, M., Iwakura, Y., and Hirano, T. (2008). Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity* 29, 628–636.

Ohtani, T., Ishihara, K., Atsumi, T., Nishida, K., Kaneko, Y., Miyata, T., Itoh, S., Narimatsu, M., Maeda, H., Fukada, T., Itoh, M., Okano, H., Hibi, M., and Hirano, T. (2000). Dissection of signaling cascades through gp130 in vivo: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. *Immunity* 12, 95–105.

O'Shea, J. J., Ma, A., and Lipsky, P. (2002). Cytokines and autoimmunity. *Nat. Rev. Immunol.* 2, 37–45.

Park, H., Li, Z., Yang, X. O., Chang, S. H., Nurieva, R., Wang, Y. H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6, 1133–1141.

Polgár, A., Falus, A., Koó, E., Ujfalussy, I., Szeszták, M., Szuts, I., Konrád, K., Hodinka, L., Bene, E., Mészáros, G., Ortutay, Z., Farkas, E., Paksy, A., and Buzás, E. I. (2003). Elevated levels of synovial fluid antibodies reactive with the small proteoglycans biglycan and decorin in patients with rheumatoid arthritis or other joint diseases. *Rheumatology (Oxford)* 42, 522–527.

Rebouissou, S., Amessou, M., Couchy, G., Poussin, K., Imbeaud, S., Pilati, C., Izard, T., Balabaud, C., Bioulac-Sage, P., and Zucman-Rossi, J. (2009). Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. *Nature* 457, 200–204.

Sakaguchi, S., and Sakaguchi, N. (2005). Animal model of arthritis caused by systemic alteration of the immune system. *Curr. Opin. Immunol.* 17, 589–594.

Sawa, S., Kamimura, D., Jin, G. H., Morikawa, H., Kamon, H., Nishihara, M., Ishihara, K., Murakami, M., and Hirano, T. (2006). Autoimmune arthritis associated with mutated interleukin (IL)-6 receptor gp130 is driven by STAT3/IL-7-dependent homeostatic proliferation of CD4+ T cells. *J. Exp. Med.* 12, 1459–1470.

Sawa, Y., Arima, Y., Ogura, H., Kitabayashi, C., Jiang, J. J., Fukushima, T., Kamimura, D., Hirano, T., and Murakami, M. (2009). Hepatic interleukin-7 expression regulates T cell responses. *Immunity* 30, 447–457.

Sims, G. P., Rowe, D. C., Rietdijk, S. T., Herbst, R., and Coyle, A. J. (2010). HMGB1 and RAGE in inflammation and cancer. *Annu. Rev. Immunol.* 24, 267–388.

Skapenko, A., Leipe, J., Lipsky, P. E., and Schulze-Koops, H. (2005). The role of the T cell in autoimmune inflammation. *Arthritis Res. Ther.* 7, S4–S14.

Steinman, L. (2001). Multiple sclerosis: a two-stage disease. *Nat. Immunol.* 2, 762–765.

Sugiura, T., Kawaguchi, Y., Harigai, M., Terajima-Ichida, H., Kitamura, Y., Furuya, T., Ichikawa, N., Kotake, S., Tanaka, M., Hara, M., and Kamatani, N. (2002). Association between adult-onset Still's disease and interleukin-18 gene polymorphisms. *Genes Immun.* 3, 394–399.

Surh, C. D., and Sprent, J. (2000). Homeostatic T cell proliferation: how far can T cells be activated to self-ligands? *J. Exp. Med.* 192, F9–F14.

Suthaus, J., Tillmann, A., Lorenzen, I., Bulanova, E., Rose-John, S., and Scheller, J. (2010). Forced homo- and heterodimerization of all gp130-type receptor complexes leads to constitutive ligand-independent signaling and cytokine-independent growth. *Mol. Biol. Cell* 21, 2797–2807.

Takizawa, Y., Suzuki, A., Sawada, T., Ohsaka, M., Inoue, T., Yamada, R., and Yamamoto, K. (2006). Citrullinated fibrinogen detected as a soluble citrullinated autoantigen in rheumatoid arthritis synovial fluids. *Ann. Rheum. Dis.* 65, 1013–1020.

Van Steendam, K., Tilleman, K., De Ceuleneer, M., De Keyser, F., Elewaut, D., and Deforce, D. (2010). Citrullinated vimentin as an important antigen in immune complexes from synovial fluid of rheumatoid arthritis patients with antibodies against citrullinated proteins. *Arthritis Res. Ther.* 12, R132.

Veldhoen, M., Hocking, R., Atkins, C., Locksley, R., and Stockinger, B. (2006). TGF in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24, 179–189.

Williams, M. A., Ravkov, E. V., and Bevan, M. J. (2008). Rapid culling of the CD4+ T cell repertoire in the transition from effector to memory. *Immunity* 28, 533–545.

Yoshida, T., Hanada, T., Tokuhisa, T., Kosai, K., Sata, M., Kohara, M., and Yoshimura, A. (2002). Activation of STAT3 by the hepatitis C virus core protein leads to cellular transformation. *J. Exp. Med.* 196, 641–653.

Zhang, L., Nakayama, M., and Eisenbarth, G. S. (2008). Insulin as an autoantigen in NOD/human diabetes. *Curr. Opin. Immunol.* 20, 111–118.

Zhang, Q., Raoof, M., Chen, Y., Sumi, Y., Sursal, T., Junger, W., Brohi, K., Itagaki, K., and Hauser, C. J. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464, 104–107.

Zhu, J., Yamane, H., Cote-Sierra, J., Guo, L., and Paul, W. E. (2006). GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. *Cell Res.* 16, 3–10.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 April 2011; paper pending published: 24 May 2011; accepted: 06 June 2011; published online: 16 June 2011.

Citation: Murakami M and Hirano T (2011) A four-step model for the IL-6 amplifier, a regulator of chronic inflammations in tissue-specific MHC class II-associated autoimmune diseases. Front. Immun. ***2****:22. doi: 10.3389/fimmu.2011.00022*

This article was submitted to Frontiers in Inflammation, a specialty of Frontiers in Immunology.