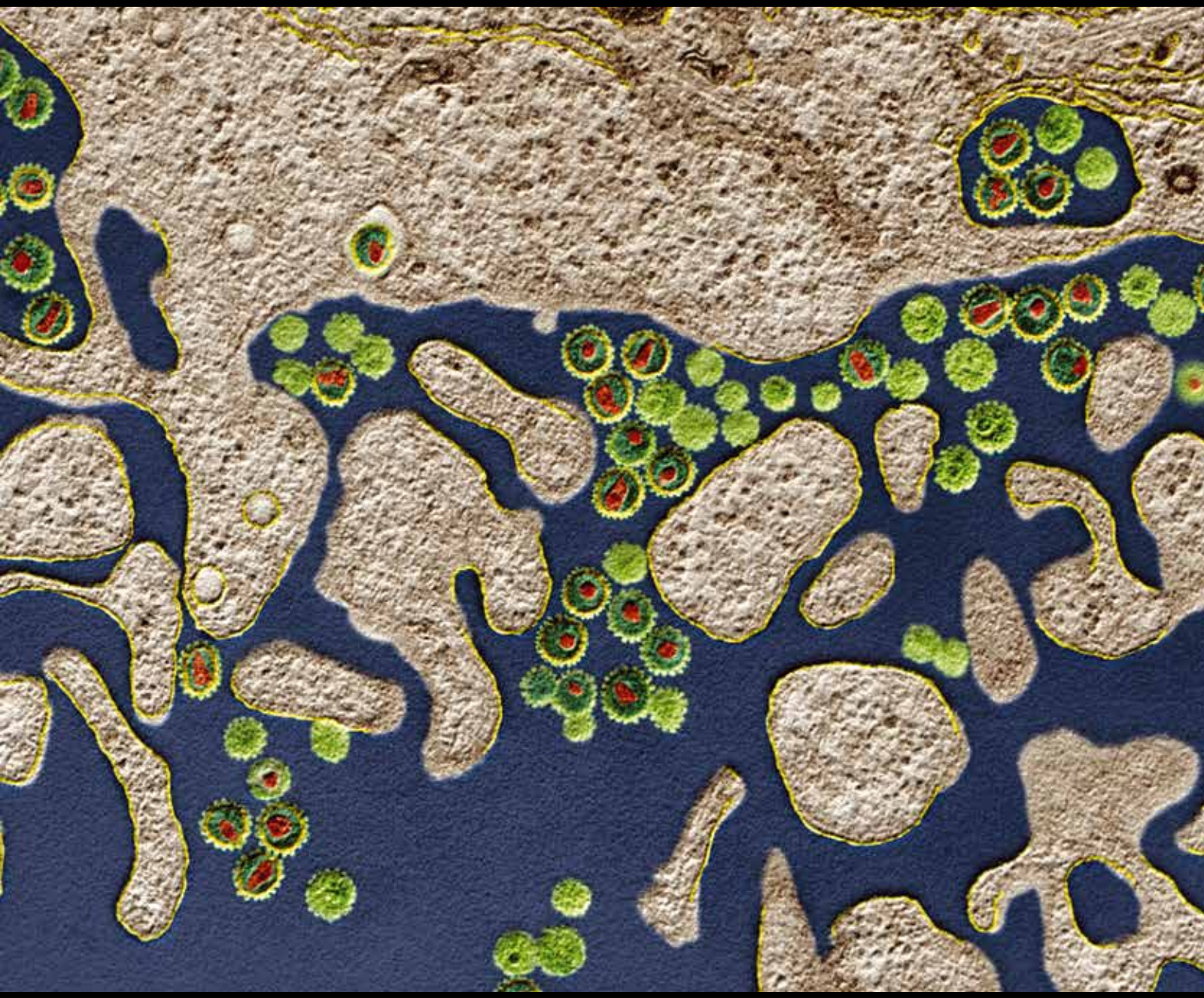
Journal of Immunology Research

The Role of T_H17-Associated Cytokines in Health and Disease

Guest Editors: William O'Connor Jr., Enric Esplugues, and Samuel Huber



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Editorial Board

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Editorial

The Role of T_H17-Associated Cytokines in Health and Disease

William O'Connor Jr.,[1] Enric Esplugues,[2] and Samuel Huber[3]

[1] *Center for Immunology and Microbial Disease, Albany Medical College, Albany, NY 12208, USA*
[2] *Immunology Institute, Mount Sinai School of Medicine, Icahn Medical Institute, 1425 Madison Avenue, New York, NY 10029, USA*
[3] *Medical Department, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany*

Correspondence should be addressed to William O'Connor Jr.; oconnow@mail.amc.edu

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The intriguing subset of effector CD4+ T cells termed T_H17 cells are now widely appreciated for their role in coordinating immune and inflammatory responses. The dynamic nature of the T_H17 cell subset allows for the adoption of inflammatory or regulatory functions as needed, in a microenvironment-dependent fashion. The ontogeny, tissue residence, migratory properties, and biological functions of these cells are areas of intense research focus given the broad spectrum of human disorders associated with aberrant T_H17-type responses.

While T_H17 cells are so named for their characteristic Interleukin 17 (IL-17) production, *bona fide* T_H17 cells of human or murine origin produce, at times, a cacophony of inflammatory mediators, which can include IL-17, IL-21, IL-22, and IL-26. As such, dissecting the biological consequences of robust T_H17 responses, properly or improperly controlled, has presented a number of challenges. Further confounding the study of T_H17 cells and individual cytokines are the many observations documenting non- T_H17 cell sources of these same cytokines. Given the complexity of T_H17 biology, we welcome the reports found within this issue, highlighting current findings and observations and illuminating several components of T_H17 cells and known associated cytokines.

N. Qu et al. provide an interesting overview of the roles T_H17 cells and their associated cytokines play in various inflammatory diseases. N. Y. A. Hemdan et al. build on this premise, addressing the T_H17 cell contributions to autoimmunity, in particular that which arises following exposure to xenobiotic substances.

In the absence of overt chronic inflammation, T_H17 cells predominantly reside at mucosal surfaces. H.-C. Tsai et al. provide an elegant update on the functions of T_H17 differentiation and on the functional consequences of IL-17 signaling in pulmonary inflammation. Y. Morishima et al. highlight the role of T_H17-associated cytokines in asthma, especially in steroid-resistant disease. Further interesting findings from the Hizawa laboratory suggest an important role for IL-17F in particular.

T_H17 cells, which are known to be induced in response to a variety of bacterial and fungal infections, may also be selectively depleted, as in the early stages of an HIV infection. S. L. Bixler and J. J. Mattapallil elegantly discuss potential mechanisms by which T_H17 cells are depleted or improperly regulated during HIV infection.

Further, T_H17 cells and T_H17-produced cytokines have also been associated with tumor immunity and conversely with promoting the initiation/progression of tumorigenesis. In this issue, D. Alizadeh et al. discuss how T_H17 cells and T_H17-associated cytokines may act directly or indirectly toward shifting local microenvironments to favor tumor promotion or tumor suppression. Focusing on AML, T. Tian et al. examined T_H17 cell frequencies in acute myeloid leukemia patients and discuss their observed stage-dependent variation.

It is our hope that you will find the articles within insightful; we have enjoyed reading all of these articles immensely.

William O'Connor Jr.
Enric Esplugues
Samuel Huber

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Review Article

Potential Involvement of IL-17F in Asthma

Kyoko Ota,[1] Mio Kawaguchi,[1] Satoshi Matsukura,[2] Masatsugu Kurokawa,[3] Fumio Kokubu,[2] Junichi Fujita,[1] Yuko Morishima,[1] Shau-Ku Huang,[4,5] Yukio Ishii,[1] Hiroaki Satoh,[1] and Nobuyuki Hizawa[1]

[1] *Division of Clinical Medicine, Department of Respiratory Medicine, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575, Japan*
[2] *Department of Respiratory Medicine, Showa University Fujigaoka Hospital, 1-30 Aoba-ku, Yokohama 227-8501, Japan*
[3] *Division of Allergy and Respiratory Medicine, Department of Internal Medicine, Showa University, School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan*
[4] *Johns Hopkins University, Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6801, USA*
[5] *National Health Research Institutes, 35 Keyan Road, Zhunan, Miaoli County 35053, Taiwan*

Correspondence should be addressed to Mio Kawaguchi; mkawaguchi@md.tsukuba.ac.jp

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The expression of IL-17F is seen in the airway of asthmatics and its level is correlated with disease severity. Several studies have demonstrated that IL-17F plays a pivotal role in allergic airway inflammation and induces several asthma-related molecules such as CCL20. IL-17F-induced CCL20 may attract Th17 cells into the airway resulting in the recruitment of additional Th17 cells to enhance allergic airway inflammation. We have recently identified, for the first time, that bronchial epithelial cells are its novel cell source in response to IL-33 via ST2-ERK1/2-MSK1 signaling pathway. The receptor for IL-17F is the heterodimeric complex of IL-17RA and IL-17RC, and IL-17F activates many signaling pathways. In a case-control study of 867 unrelated Japanese subjects, a His161 to Arg161 (H161R) substitution in the third exon of the IL-17F gene was associated with asthma. In atopic patients with asthma, prebronchodilator baseline FEV1/FVC values showed a significant association with the H161R variant. Moreover, this variant is a natural antagonist for the wild-type IL-17F. Moreover, IL-17F is involved in airway remodeling and steroid resistance. Hence, IL-17F may play an orchestrating role in the pathogenesis of asthma and may provide a valuable therapeutic target for development of novel strategies.

1. Introduction

Asthma is characterized by bronchoconstriction, airway hyperreactivity, inflammation, mucus hypersecretion, and remodeling. These processes are coordinated by a complex cytokine network. The clarification of the modulation of this cytokine network could contribute to the understanding of asthma pathogenesis and development of new therapeutic strategies. IL-17A, the original member of the IL-17 cytokine family, was first identified in 1993 and was initially recognized for its similarity to a sequence belonging to the open reading frame 13 of *Herpesvirus saimiri* (HVS13) [1, 2]. Moreover, five additional members, IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F, were discovered within a short period of time since 2000 to 2002 [3–9]. Structurally, the IL-17 cytokine family members have no sequence similarity to any other known cytokine or other mammalian proteins [2]. Similarly, the IL-17 receptor family (IL-17RA-RE) is not related to any of the other known cytokine receptors [2]. Thus, the IL-17 cytokine family appears to represent a distinct ligand-receptor signaling system. We and other groups discovered the human IL-17F gene from a human EST sequence, a genomic DNA clone, and T-cell cDNA sequences in 2001 [3, 8, 9]. The gene is localized on the same chromosome at the distance of about 50 kb from telomeric sequences of IL-17A gene, and both genes are in a tail-to-tail orientation [3]. Functional studies have suggested that IL-17F is involved in asthma pathology. Hence, increased understanding of the significance of IL-17F would help to

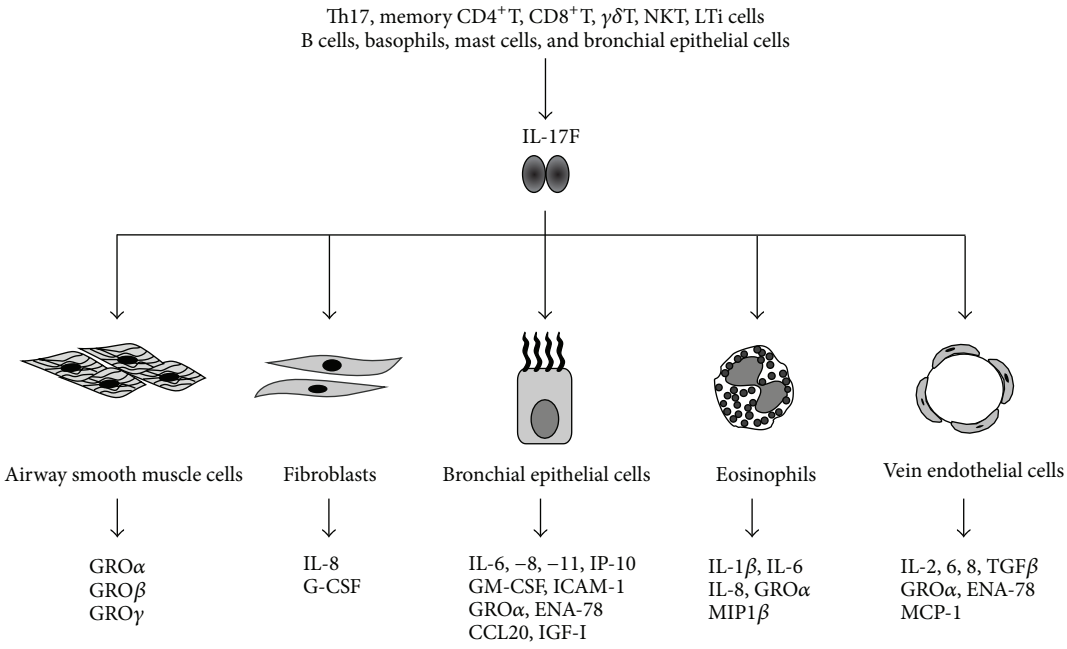

Figure 1: Biological activities of IL-17F. IL-17F has multiple biological activities. IL-17F is produced by several cell types including Th17 cells and bronchial epithelial cells. IL-17F can induce various asthma-related cytokines, chemokines, and adhesion molecules in bronchial epithelial cells, eosinophils, fibroblasts, airway smooth muscle cells, and vein endothelial cells, and thereby contributes to the pathogenesis of asthma.

uncover the molecular mechanisms of asthma. In this review, we discuss the finding that IL-17F has a key role in asthma pathology and is a novel drug target for asthma.

2. Structural Features

Among the IL-17 cytokine family members, IL-17F shows the highest amino acid sequence homology (50%) to IL-17A, while only 10–30% sequence identity is seen between IL-17A and the other family members [10]. These cytokines have their greatest similarity within the C-terminal 70 amino acids and have four well-conserved cysteines. The four conserved cysteines in the C-terminal half of the IL-17F sequence are shown to form a cystine knot structural motif in the crystal structure, and, interestingly, this cystine knot structure is similar to a common structural motif seen in several growth factors, such as bone morphogenic proteins (BMPs), TGF-β, nerve growth factor (NGF), and platelet-derived growth factor (PDGF) [9]. Of note, recent reports have demonstrated that IL-17A and IL-17F can be produced as heterodimers termed IL-17A/F [11]. These three cytokines are differentially expressed in activated $CD4^+$ T cells.

3. Cellular Source and Tissue Distribution

IL-17F is expressed in activated $CD4^+$ T cells, basophils, and mast cells, three important cell types involved in allergic airway inflammation [3]. Moreover, IL-17F is also derived from Th17 cells, a $CD4^+$ T-cell lineage distinct from Th1 cells and Th2 cells [12]. However, Th17 cells may not be the major cell source of IL-17F in lung diseases [13]. Recent studies have demonstrated that IL-17F is also produced by many cell types such as memory $CD4^+$ T cells, $CD8^+$ T cells, $\gamma\delta$T cells, NKT cells, B cells, and LTi cells [14–17]. These findings suggest that IL-17F is involved in the pathogenesis of a wide range of diseases beyond asthma. However, it remains to be determined which IL-17F-producing cell types contribute to asthma pathogenesis in response to various stimuli in human. So far, IL-17F has been thought to be derived from hematopoietic cells, but not nonhematopoietic cells such as lung structural cells. Recently, we have reported, for the first time, that bronchial epithelial cells are a novel cell source of IL-17F in response to IL-33 [18]. IL-33 is genetically and functionally associated with the pathogenesis of asthma [19, 20]. These findings suggest that bronchial epithelial cells play a central role in asthma, at least partially, as target and effector cells for IL-17F. In addition, IL-17F is detected in a wider range of tissues such as liver, lung, ovary, and fetal liver when compared with IL-17A [3]. This suggests that IL-17F has a more diverse biological function, despite the high degree of sequence homology with IL-17A.

4. Biological Activities

IL-17F has multiple biological activities (Figure 1). IL-17F is able to induce asthma-related cytokines, chemokines, and adhesion molecules in bronchial epithelial cells [3, 20–28]. In addition to bronchial epithelial cells, IL-17F is also able to stimulate lung structural cells such as vein endothelial cells, airway smooth muscle cells, and fibroblasts [7, 8, 21, 22, 29]. Interestingly, a recent report demonstrated that IL-17F acts upon eosinophils, one of the most important inflammatory

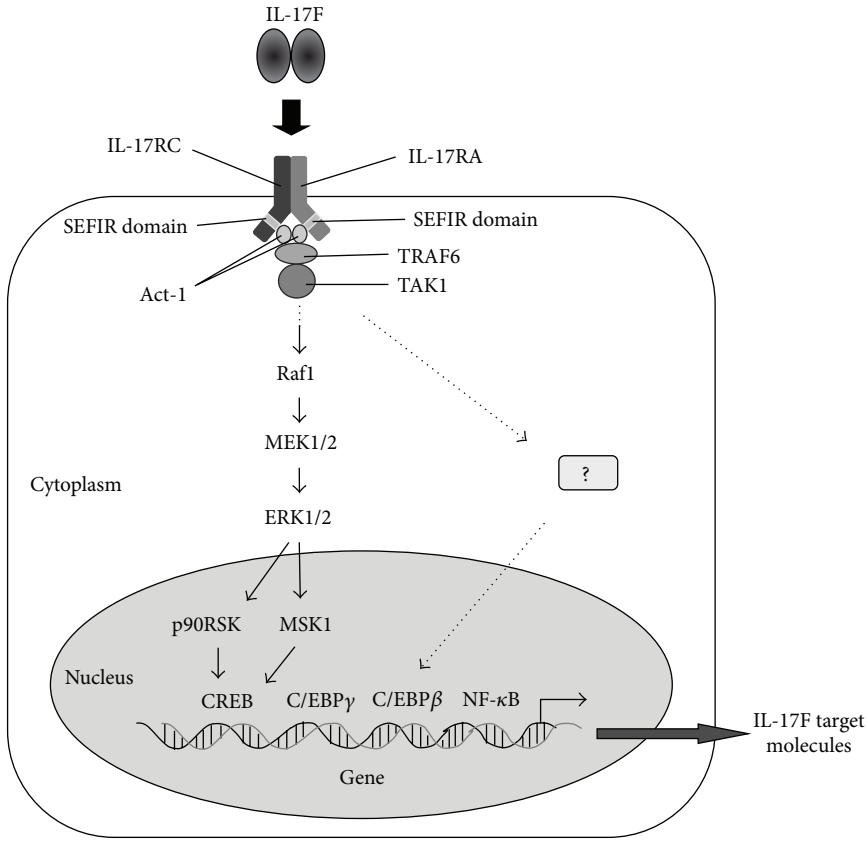

Figure 2: Signaling pathways induced by IL-17F. IL-17F utilizes a heterodimer of IL-17RA and IL-17RC as its receptor, and then IL-17RA engages the SEFIR domain-containing Act-1. Act-1 is required for recruitment of TRAF6, leading to the activation of TAK1. The Raf1-MEK1/2-ERK1/2-p90RSK/MSK1-CREB is the pivotal signaling pathway. On the other hand, IL-17F also activates transcriptional factors such as C/EBPβ, C/EBPγ, and NF-κB. Activation of these pathways leads to the expression of various inflammatory molecules.

cells in allergic airway inflammation and remodeling, to induce several cytokines and chemokines such as IL-1β, IL-6, IL-8, GROα, and MIP-1β [30]. These cell types may play crucial roles in asthma in response to IL-17F. IL-17F may develop and amplify allergic airway inflammation by facilitating the activation of inflammatory cells and lung structural cells through the induction of a wide range of molecules. Moreover, Th2 cytokines, IL-4 and IL-13, are able to enhance the biological activities of IL-17F [26–28, 31]. These findings suggest that the interaction of Th2 cytokines and IL-17F augments allergic airway inflammation.

5. Receptor and Signaling Pathway

Our understanding of the signaling pathway of IL-17F has gradually become clearer (Figure 2). Similar to IL-17A, the receptor for IL-17F is the heterodimeric complex of IL-17RA and IL-17RC [32]. Both IL-17RA and IL-17RC are necessary for the biological activity of IL-17F. Although human IL-17RA binds IL-17A effectively, it binds IL-17F with ~1000-fold lower affinity [33]. The relative binding affinity of IL-17F to IL-17RC is much stronger than to IL-17RA. Activation of the receptor by IL-17F leads to an interaction with Act-1 via the similar expression to fibroblast growth factor genes, IL-17 receptors, and TIR (SEFIR) domain [34]. This sequentially mediates activation of TNF receptor-associated factor- (TRAF-) 6, leading to the activation of TGFβ activated kinase (TAK) 1 [34, 35]. We have reported that the Raf1-MEK1/2-ERK1/2 pathway is a central upstream signaling pathway for IL-17F-induced cytokine and chemokine expression in bronchial epithelial cells and vein endothelial cells [21–28, 31]. In the downstream signaling pathway, we have also identified that mitogen- and stress-activated protein kinase1- (MSK1-) cyclic AMP response element binding protein (CREB) and p90 ribosomal S6 kinase- (p90RSK-) CREB are critical downstream signaling pathways [25–28, 31]. These pathways are located downstream of the Raf1-MEK1/2-ERK1/2 kinase cascade and are essential for cytokine expression by IL-17F. Further, IL-17F also activates transcriptional factors such as C/EBPβ, C/EBPγ, and NF-κB [34]. On the other hand, little is known about the signaling mechanisms of IL-17F expression. As shown in Figure 3, we have recently reported that bronchial epithelial cells are a novel cell source of IL-17F, and epithelial IL-17F expression is mediated via the activation of ST2-ERK1/2-MSK1 signaling pathway in response to IL-33 [18]. ST2 is a receptor for IL-33 [36]. However, other signaling molecules including transcriptional factors for IL-17F expression still remain undiscovered. These findings suggest

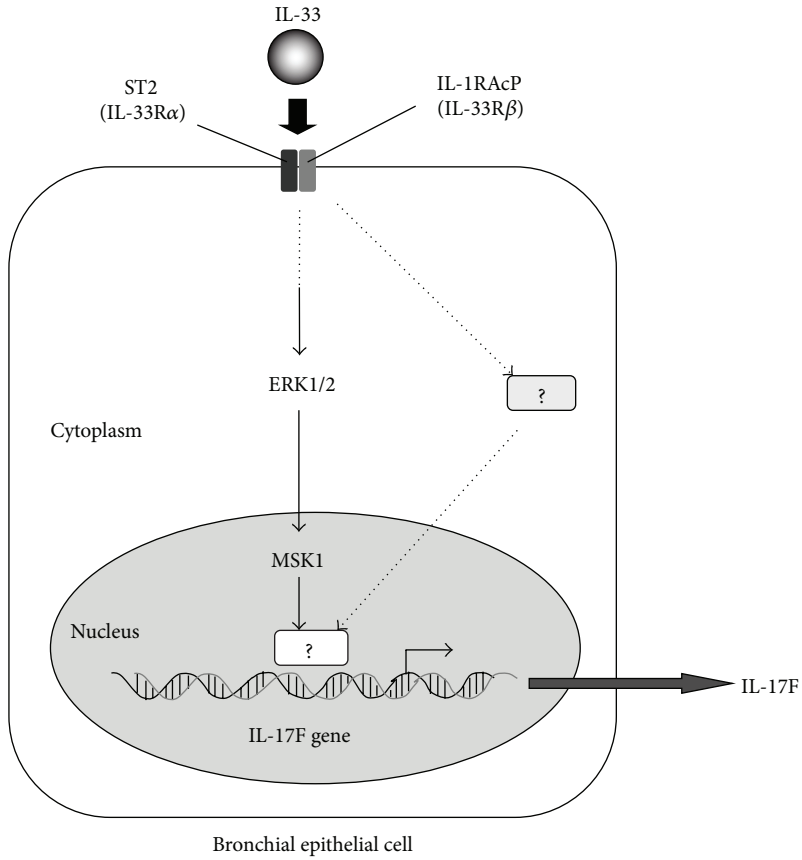

Figure 3: Signaling mechanism of IL-17F expression in bronchial epithelial cells. Bronchial epithelial cells are a novel cell source of IL-17F. The expression of IL-17F is induced by IL-33. IL-33 binds to its receptor, ST2, and then the ERK1/2-MSK1 signaling pathway is activated. However, other stimuli inducing IL-17F and their signaling pathways are largely unknown.

that these signaling pathways are potential pharmacological targets in the IL-17F-mediated airway inflammation.

6. Recruitment of Th17 Cells

Th17 cells play a pivotal role in a diverse group of immune-mediated diseases and host defense mechanisms [12]. Emerging evidence suggests that the Th17 cells provide a new insight into the molecular mechanisms of asthma [37]. Th17 cells have been isolated from bronchial tissues taken from patients during acute episodes of severe asthma [38]. Another study demonstrated that the percentages of Th17 cells in PBMCs are higher in allergic asthmatics than those in healthy subjects and show a tendency to increase with the disease severity [39]. However, it is unclear how Th17 cells migrate into the airway of asthmatics. We demonstrated that IL-17F induces CCL20 in bronchial epithelial cells [31]. Human Th17 cells predominantly express CCR6 [40]. This implies that its ligand, CCL20, is able to attract Th17 cells into the site of airway inflammation via CCR6. Taken together, it is possible that IL-17F-induced epithelial CCL20 attracts Th17 cells into the airway, and accumulated Th17 cells establish a positive feedback loop resulting in the recruitment of additional Th17 cells via the induction of IL-17F. Hence, IL-17F-producing cells may exert an effect on bronchial epithelial cells to induce CCL20 and attract Th17 cells via CCR6. Although *in vivo* study is needed to clarify this hypothesis, the IL-17F/CCL20 axis might be especially important in the pathophysiologic events of allergic airway inflammation.

7. Airway Neutrophilia

IL-17F is involved in neutrophilic inflammation in the airway [41, 42]. Although airway neutrophilia is one of the hallmarks of severe asthma, its mechanism is not well understood. In a mouse model of study, overexpression of IL-17F using an adenoviral gene transfer strategy in the mouse airways also leads to an increased number of neutrophils in bronchoalveolar lavage fluid (BALF) [41]. Another study using a different model has revealed that overexpression of IL-17F through intratracheal delivery of the IL-17F gene results in an increase in the number of neutrophils and macrophages in the airways [42]. Moreover, IL-17F-deficient mice have revealed that IL-17F is more critical than IL-17A in inducing airway neutrophilic inflammation to *Aspergillus oryzae* [34]. Specific inhibition of $CD4^+$ T cells, either with a CD4 Ab or

an IL-2R Ab, prevents allergen-induced recruitment of both eosinophils and neutrophils in animal models, suggesting $CD4^+$ T cells regulate airway neutrophilia [43, 44]. However, little is known about how $CD4^+$ T cells elicit neutrophil accumulation into the airway. IL-17F may be one of the key regulators for airway neutrophilia induced by $CD4^+$ T cells such as Th17 cells. Of interest is the finding that lung tissues from IL-17F gene transduced mice show substantial increases in the level of various inflammatory cytokines and chemokines, including IL-1β, IL-6, KC, and MIP-2 [41, 42]. These molecules are known to be involved in chemotaxis and activation for neutrophils. Additionally, *in vitro* studies have demonstrated that IL-17F is able to induce C-X-C chemokines, such as IL-8, ENA-78, and GROα, which are potent chemoattractants for neutrophils [3, 21, 22]. Neutrophil recruitment into the airway may be regulated through, at least partially, IL-17F-induced C-X-C chemokines. In contrast, C-C chemokines, such as eotaxin and RANTES, which are potent chemoattractants for eosinophils, are not produced by IL-17F, suggesting a selective role of IL-17F in neutrophil recruitment and activation in the airway [3].

8. Mucus Hypersecration and Airway Hyperreactivity

Asthma is characterized by mucus hypersecretion (goblet cell hyperplasia/metaplasia) and airway hyperreactivity that are consistently linked to asthma symptoms and morbidity. IL-17F may be involved in these pathological processes. Overexpression of IL-17F in the airway of mice resulted in the induction of goblet cell hyperplasia and the gene expression of MUC5AC, but only when the mice are challenged with antigen, and increased goblet cell hyperplasia is seen only in the small airways [42]. These results suggest that in addition to IL-13, IL-17F may also be an important contributor to mucus hypersecretion in asthma. Moreover, a significant increase in airway hyperreactivity was also noted in mice overexpressing IL-17F following Ag challenge, when compared to that of mice receiving mock control [42]. These findings suggest that IL-17F has an additive or enhancing effect on antigen-induced allergic inflammatory responses.

9. Airway Remodeling

We have reported that IL-17F induces profibrotic cytokines, IL-11 and IGF-I, in bronchial epithelial cells [27, 28]. IL-11 elicits subepithelial fibrosis, accumulation of fibroblasts, myofibroblasts and myocytes, and deposition of types I and III collagen [45]. IGF-I is able to induce collagen synthesis and smooth muscle hyperplasia and is also a potent mitogen for fibroblasts and smooth muscle cells [46–48]. The blockade of IGF-I inhibited the elevation of airway resistance, airway inflammation, increase in airway wall thickening, and the expression of ICAM-1. In humans, the expression of IGF-I is significantly increased within the airways of subjects with severe asthma when compared with those with mild asthma [49]. Its expression was inversely correlated to collagen thickening and the number of fibroblasts. Moreover, treatment with beclomethasone dipropionate significantly decreased the expression of IGF-I with reduction of the thickness of lamina reticularis. Blocking of IGF-I expression may contribute to prevent airway remodeling. In other studies, IL-17F has been shown to induce the expression of TGF-β in vein endothelial cells [8]. TGF-β is a profibrotic cytokine and has been implicated in the extracellular matrix changes observed in fibrosis. More recently, direct effect of IL-17F to airway smooth muscle (ASM) cells was demonstrated. IL-17F promotes migration of ASM cells via p38MAPK [50]. Th17 cells contribute to airway remodeling via excessive mucus expression and ASM proliferation [51]. These findings suggest the potential involvement of IL-17F in the process of airway remodeling.

10. Steroid Resistance

Recent studies have demonstrated that IL-17F is involved in steroid resistance in asthma. Th17 cells, but not Th2 cells, mediate steroid resistant airway inflammation and airway hyperreactivity in a mouse model of asthma [52]. In the setting of *in vivo* polarized Th17 cell transfer, chemokine secretion, cellular influx to the airways, and airway hyperreactivity are not sensitive to dexamethasone treatment. Other studies have reported that IL-17F induced expression of glucocorticoid receptor- (GR-) β mRNA in bronchial epithelial cells from healthy subjects as well as asthmatic patients [53]. GR-β acts as a dominant negative inhibitor of GR-α which is the active isoform of this receptor. Moreover, unlike healthy subjects, IL-6 induced by IL-17A and IL-17F was not inhibited by dexamethasone in bronchial epithelial cells from asthmatic patients. These findings suggest that steroid resistance in subjects with severe asthma may be due to, at least in part, IL-17F and Th17 cells.

11. Expression in the Airway of Asthmatic Patients

The expression of IL-17F is observed in the airway of asthmatic patients. Analyses of its expression in BAL cells from asthmatic subjects challenged with allergen or saline control show that while no detectable expression of IL-17F was seen in the BAL cells from saline-challenged sites, its expression was obviously seen in the BAL cells from allergen-challenged sites of all four study subjects [3]. IL-17F is expressed in both bronchial epithelium and inflammatory infiltrates [54, 55]. Immunocytochemistry showed that IL-17F positive cells in the subepithelial component and epithelium are significantly elevated in severe asthma compared with healthy and mild asthmatic subjects [55]. Additionally, an increased expression of epithelial IL-17F was correlated with disease severity. Moreover, another recent study demonstrated that asthmatic patients have a significantly higher level of serum IL-17F protein as compared to that of healthy subjects [56]. This implies that IL-17F can be used as a clinical biomarker of asthma diagnosis and management. Further validation is needed in the future.

12. Genetic Relevance

We investigated the genetic association of asthma with the common variants of IL-17F, using 867 unrelated Japanese subjects [57]. Five polymorphisms were studied, including the coding-region sequence variant SNP rs763780 (7488T>C), which causes a His-to-Arg substitution at amino acid 161 (H161R). A genotype-based χ^2 association analysis indicated a significant association between the H161R variant and asthma. Importantly, none of the asthmatic subjects were homozygous for H161R. The homozygosity of the H161R variant is associated with the protection against asthma; the odds ratio (OR) for asthma was 0.06 (95% confidence interval, 0.01–0.43, $P = 0.0039$) among H161R homozygotes compared with wild-type homozygotes. In atopic patients with asthma, prebronchodilator baseline FEV1/forced vital capacity (FVC) values also showed a significant association with the H161R variant [58]. This suggests that the H161R variant of IL-17f is associated with asthma severity. Moreover, *in vitro* functional studies demonstrated that, compared with wild-type IL-17F, the H161R variant is unable to activate ERK1/2 that is a critical signaling molecule of IL-17F [57] but, interestingly, is able to block the induction of IL-8 by wild-type IL-17F in a dose-dependent manner. These findings suggest that the H161R variant is a natural antagonist for the wild-type IL-17F and may be an attractive therapeutic target in IL-17F-mediated diseases. However, further study is needed to clarify the precise mechanisms by which H161R variant exerts its antagonistic effect. Interestingly, recent studies have demonstrated novel therapeutic options targeting IL-17A, IL-17F, and their signaling pathways. Inhibition of either IL-17RA or IL-17RC expression via siRNA revealed significant reduction of IL-17A/IL-17F-stimulated chemokine production [59]. Similarly the microRNA, miR-23b, suppresses IL-17A-associated autoimmune inflammation by targeting TGF-β-activated kinase 1/MAP3 K7 binding protein (TAB)2, TAB3, and IKK-α [60]. These molecules may provide therapeutic benefit for immune and inflammatory diseases.

13. Conclusions

IL-17F is one of the important cytokines involving in the pathophysiologic events of asthma. *In vivo* and *in vitro* studies have implicated that IL-17F shows multiple functions in the pathogenesis of airway allergic inflammation. In particular, CCL20 induced by IL-17F may enhance Th17-mediated airway inflammation via CCR6. Although biological function of IL-17F has become clear, its inducible factors still remain except for IL-33. It is suggested that further investigation of IL-17F is informative in pointing to novel approaches to the diagnosis and treatment of asthma.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Review Article

The Multifaceted Role of Th17 Lymphocytes and Their Associated Cytokines in Cancer

Darya Alizadeh,[1] Emmanuel Katsanis,[1,2] and Nicolas Larmonier[1,2]

[1] *Cancer Biology Graduate Interdisciplinary Program and Department of Pediatrics, Steele Children's Research Center, University of Arizona, 1501 N. Campbell Avenue, P.O. Box 245073, Tucson, AZ 85724-5073, USA*
[2] *Department of Immunobiology, BIO5 Institute and Arizona Cancer Center, University of Arizona, Tucson, AZ 85724, USA*

Correspondence should be addressed to Nicolas Larmonier; nrlarmon@email.arizona.edu

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While the role of T helper 17 lymphocytes (Th17) in the pathogenesis of autoimmune diseases and in infectious immunity has been relatively well defined, the impact of these cells and their associated cytokines on cancer development is still under debate. Although multiple reports have indicated that Th17 can promote anticancer immunity, others have argued that these cells may exhibit tumor-promoting properties. This dichotomy in the function of Th17 lymphocytes in cancer may be related to the versatile nature of these cells, being capable of differentiating into either proinflammatory Th1 or suppressive FoxP3-expressing Treg cells or hybrid T cell subsets depending on the underlying environmental conditions. In the current review, we examine the role of Th17 lymphocytes and Th17-associated cytokines in cancer and discuss how factors that control their final lineage commitment decision may influence the balance between their tumor-promoting versus tumor-suppressing properties.

1. Introduction

$CD4^+$ T helper (Th) lymphocytes are essential for the regulation of immune responses as they are endowed with the ability to modulate the function of $CD8^+$ cytotoxic T lymphocytes (CTLs) [1, 2], B cells [3], NK cells [4], macrophages, and dendritic cells [5, 6]. Following triggering of their T cell receptor (TCR) and in the presence of appropriate costimulatory signals and specific cytokines, naïve $CD4^+$ T lymphocytes differentiate into various effector or regulatory cells characterized by distinct functions and specific cytokine production profiles. For many years, it was believed that the expression of two mutually exclusive differentiation programs led to the polarization of naïve $CD4^+$ T cells towards either Th1 or Th2 lymphocytes [7, 8]. Terminally differentiated Th1 cells are characterized by the expression of the transcription factor Tbet and the production of IFNγ [9]. Th1 activate CTLs, macrophages and are required for the elimination of intracellular pathogens [7, 10]. Th1 cell lineage commitment is primarily triggered by IFNγ and IL-12 [11, 12]. Th2 lymphocytes, defined by transcription factor GATA3 expression and the secretion of IL-4, IL-5, IL-10, and IL-13, play an essential role in B cell-mediated humoral responses against extracellular pathogens and can inhibit Th1-dependent cellular immunity [13–15]. More recently, several subsets of $CD4^+$ T cells exhibiting immunosuppressive activity have been described (extensively reviewed elsewhere [16–21]). These so-called regulatory T lymphocytes (Tregs) may be generated during T cell development in the thymus (naturally occurring Treg) or may be induced in the periphery from naïve $CD4^+$ T cells (induced/adaptive iTreg) [22–26]. Treg generation essentially depends on transforming growth factor β (TGFβ), together with TCR, costimulatory signals, and IL-2 [27–29]. Extensive studies have demonstrated that the forkhead/winged helix transcription factor FoxP3 is fundamental for the development and function of Treg and remains one of the most specific molecular markers for these cells [21, 24]. Treg efficiently suppress effector T lymphocytes and may inhibit the function of B, NK, dendritic cells, or macrophages through different mechanisms [22]. They are, therefore, essential components of the regulatory networks controlling autoimmunity, infection, or cancer [30, 31].

In recent years, T helper 17 lymphocytes (Th17) have emerged as a new distinct effector $CD4^+$ T helper cell subset, prompting revision of the Th1/Th2 paradigm. Th17 produce large quantities of IL-17 and exhibit effector functions distinct from Th1 and Th2 lymphocytes. They play an important role in the clearance of pathogens that are not adequately handled by Th1 or Th2 lymphocytes. Th17 cells are potent inducers of tissue inflammation and have been identified as major contributors to the pathogenesis of multiple autoimmune conditions in animals and humans [32–34]. However, the role of Th17 in cancer is still being intensively discussed, with conflicting reports related to the pro-versus antitumoral effects of these cells. This discordance may be explained by different cytokine signature profiles inherent in the high degree of plasticity of these cells. We provide an overview on the requirements for Th17 development and the direct or indirect impact of Th17 lymphocytes and the cytokines they produce on antitumor responses.

2. T Helper 17 Lymphocytes: Cytokine Signature and Differentiation Profile(s)

2.1. Th17 Differentiation. Th17 cells are defined as $CD4^+$ T lymphocytes secreting substantial amounts of interleukin 17A (IL-17A) and expressing the transcription factor retinoic acid receptor-related orphan receptor gamma t (RORγt), which seems to act as a molecular determinant for their polarization [35, 36]. In addition, Th17 produce IL-21 and IL-22 [37] and, depending on the differentiation/environmental conditions secrete variable amounts of TNFα, IFNγ, and/or GM-CSF [32, 38–41]. Th17 foster B lymphocyte-mediated immunity [3], contribute to the migration and activation of macrophages [42], neutrophils [43], and regulate the activation and expansion of $CD8^+$ T cells [41, 44].

Th17 can be generated *in vitro* from naïve $CD4^+$ T lymphocytes using specific cytokines. In mice, the combination of TGFβ, IL-6, and IL-23, in presence of TCR and CD28 signals (antigen presenting cells, plate-bound anti-CD3 plus anti-CD28 or anti-CD3/anti-CD28 coated microbeads), is required and sufficient to drive the differentiation of naïve $CD4^+CD25^-$ T cells into Th17 (Figure 1) [45]. Studies have indicated that IL-6, by inhibiting TGFβ-driven induction of FoxP3, impairs Treg differentiation, leading to IL-17-producing RORγt^+ lymphocyte generation. However, other reports have also shown that, in IL-$6^{-/-}$ mice, Treg depletion increases the susceptibility of the animals to experimental autoimmune encephalomyelitis (EAE) as a result of enhanced pathogenic Th17 responses [39, 46]. These last observations suggest that Th17 lymphocytes can be generated in absence of IL-6. IL-21 was further identified as an alternative proinflammatory cytokine capable of suppressing TGFβ-mediated induction of FoxP3 expression in the absence of IL-6 [39, 47]. IL-21, produced in large amounts by Th17, promotes an autocrine amplification feedback loop enhancing Th17 generation especially in the absence of IL-6 [48]. The IL-23 receptor (IL-23R) is composed of IL-23R and IL-12Rβ1 [49]. Naïve $CD4^+$ T lymphocytes express very low levels of IL-23R. Conversely, Th17 are characterized by the expression of the IL-23R. It is therefore not surprising that, although IL-23 is not required for the initial Th17 lineage commitment, this cytokine fosters Th17 expansion and survival and contributes to their stabilization and proinflammatory properties [50]. Indeed, in IL-23p19-deficient mice, the number of Th17 is substantially decreased compared to their wild-type counterparts [51, 52]. In addition, IL-23 appears essential for the pathogenic properties of Th17 as demonstrated in collagen-induced arthritis (CIA) and EAE models [51, 53]. IL-23 is also essential for the generation of Th17 in prolonged *in vitro* cultures [50]. The proinflammatory cytokine IL-1β has also been reported as another important factor in the polarization of Th17 cells in proinflammatory environments. IL-1β induces interferon regulatory factor 4 (IRF4), which is a critical regulator of the IL-21 autocrine signaling loop [54, 55].

In humans, the conditions that would drive optimal Th17 differentiation remain unclear. Several reports have indicated that TGFβ may not be necessary for the generation of these cells [56–58] while other studies have argued for a critical role of this cytokine in Th17 differentiation [47, 59, 60]. A study by Yang et al. indicated that the combination of TGFβ with IL-21 but not IL-6 was effective in inducing Th17 differentiation [47]. Other reports have suggested that IL-1β alone or in combination with TGFβ is also required for human Th17 production [61]. Similar to the observations made in mice, the addition of IL-23 supports Th17 proliferation and stabilization [62].

2.2. Th17 Plasticity. Th1 and Th2 cells are relatively stable and terminally differentiated subsets: they essentially do not transdifferentiate into other specialized $CD4^+$ T helper cell lineages. On the other hand, one of the most striking characteristics of Th17 is their high degree of plasticity and their remarkable ability to give rise to other populations of either proinflammatory effector cells such as Th1 [63] or immunosuppressive $FoxP3^+$ Treg [64]. Interestingly, Th17 may themselves originate from $FoxP3^+$ Treg cells that have undergone "reprogramming" in specific environmental conditions [65]. Intermediary cell subpopulations expressing both FoxP3 and RORγt and demonstrating immunosuppressive activity have been identified [66].

TGFβ appears as a master regulator of the balance between Th17 and suppressive Treg differentiation. The role of TGFβ in Th17 polarization has, however, been questioned. Although some studies have indicated that TGFβ is required for the production of IL-17 by Th17 cells [60], others have reported that TGFβ may not be essential for the induction of Th17 [58]. Additional reports have demonstrated that the generation of Th17 and the development of Th17-mediated EAE are impaired in transgenic animals with T lymphocytes deficient in functional TGFβ receptor [67] or when TGFβ expression is ablated in T cells [68]. TGFβ alone induces the expression of FoxP3 and RORγt [69]. However, in the presence of IL-6, IL-21, or IL-23, FoxP3 expression is inhibited while RORγt expression is induced, resulting in Th17 generation instead of Treg. While it has been established that TGFβ is required for the initial production of IL-17 and for the induction of IL-23R expression [47, 57, 60],

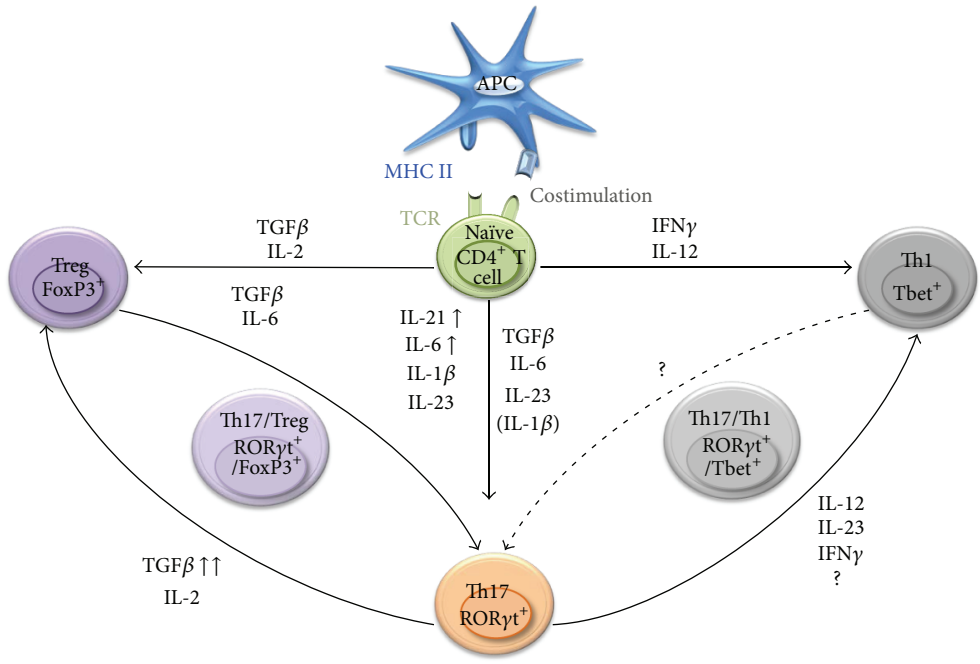

Figure 1: Specific cytokines drive the differentiation of specialized T helper lymphocytes. Naïve $CD4^+$ T lymphocytes, upon activation and in the presence of specific cytokines, differentiate into Th1, Th2, Th17, or Treg. The plasticity of Th17 and Treg enables them to transdifferentiate into Th17/Treg subsets. Th17 cells can also acquire a Th1-type phenotype leading to "hybrid" Th17/Th1 cells. The nature and concentration of the cytokines present in the differentiation milieu lead to the activation of distinct signaling cascades and transcription factors which control the developmental program of these specific lineages.

high concentration of TGFβ conversely impairs the expression of IL-23R [69]. Therefore, the outcome of the balance between Treg versus Th17 generation is likely dictated by the strength of the signals provided by TGFβ and thus depends, at least partially, on the concentration of this cytokine in the environment of the differentiating cells. Large amounts of TGFβ primarily promote the development of cells endowed with immunosuppressive activity (possibly even in the presence of low doses of IL-6 or IL-21), while intermediary or low concentration of TGFβ in combination with the proinflammatory cytokines IL-6 or IL-21 drives primarily the differentiation of naïve $CD4^+$ T cells into Th17 (Figure 1). This scenario may explain the observed Th17-Treg plasticity. Recent reports have also indicated that, in specific conditions, fully differentiated $FoxP3^+$ Treg may undergo "reprogramming" into effector helper T cells. These reprogrammed Treg are not immunosuppressive, produce proinflammatory cytokines (among which IL-2, TNFα, or IL-17), and may play an important role in anti-tumoral $CD8^+$ T cell activation [70]. Importantly, it has been shown that reprogrammed Treg may lose or maintain FoxP3 expression [70, 71]. Additional studies have reported that, in presence of IL-2 and IL-1β, human Th17 may be preferentially differentiated from naïve $FoxP3^+CD25^-CD4^+$ Treg rather than from naïve $FoxP3^-CD25^-CD4^+$ T cells [72, 73]. In these studies, Th17 differentiation was enhanced by IL-23 and TGFβ [73].

Th17 may also redifferentiate into Th1 lymphocytes. Indeed, IL-17-producing $CD4^+$ T lymphocytes expressing the Th1 lineage-specific transcription factor Tbet and producing IFNγ have been described (Figure 1) [74]. $Tbet^+$ Th17 cells have been identified in patients with multiple sclerosis [38] and IFNγ-producing human Th17 cells have been described [57]. These intermediary Th17/Th1 lymphocytes have been reported as pathogenic [32, 75] and as outlined in Section 3 can exhibit anti-tumoral activity [41, 74]. Initially, the recognition that IL-23 and IL-12 shared the common IL-12p40 (IL-12β) subunit and the observation that IL-23 induced not only IL-17 but low amounts of IFNγ led to the speculation that Th17 cells developed as a distal branch of the Th1 lineage. However, a recent study reported that Th17 precursors may produce IFNγ, independently of IL-23 and IL-12 signaling. These Th17 precursors are also capable of responding to IL-23 and IL-12 and, in the absence or in presence of low concentrations of TGFβ, can differentiate into cells characterized by enhanced production of IFNγ and minimal IL-17A and IL-17F secretion [76]. These studies confirmed that Th17 lymphocytes belong to a distinct cell lineage susceptible, however, to reprogramming into Th1 cells. This observed stability or plasticity of Th subsets seems controlled by epigenetic modifications regulating the expression of key transcription factors and cytokines specific for a dedicated Th lymphocyte lineage.

Thus, Th17 lymphocytes represent a highly heterogeneous cell population with a remarkable flexibility in their ability to differentiate into immunosuppressive Treg or effector proinflammatory Th1 depending on the environmental conditions. Since Treg are known to suppress anti-tumor immune responses and promote cancer development while Th1 enhance anti-tumoral immunity, it is therefore not surprising that Th17 have been reported to exhibit both pro- and anti-tumor activities.

3. IL-17-Producing Cells, Th17-Associated Cytokines, and Th17 Lymphocytes in Cancer

3.1. IL-17-Producing Cells, IL-17, and Major Th17-Associated Cytokines

3.1.1. IL-17-Producing Cells. IL-17A belongs to the IL-17 family, composed of 6 members (IL-17A-F) [77]. Although IL-17A and IL-17F are the signature cytokines defining $CD4^+$ Th17 cells, it should be noted that IL-17 is also produced by $\gamma\delta$T cells [78], natural killer (NK) T cells [79], CD8 T cells [80], macrophages [81], neutrophils, and eosinophils [82]. The role of IL-17 expressing non-$CD4^+$ T cells in cancer has been examined in several studies [81, 83, 84]. For instance, the adoptive transfer of *in vitro* generated $CD8^+$ T cells producing IL-17 (Tc17) in mice promoted anti-tumor immunity against B16 melanoma by fostering the recruitment of other inflammatory anti-tumoral cells such as CTL, Th1, neutrophils, or macrophages [85, 86]. In another report, mast cells accumulating in a murine hepatocellular carcinoma fostered the recruitment of myeloid-derived suppressor cells (MDSC) and induced IL-17 production by these MDSC. In turn, IL-17 secreted by MDSC attracted Treg to the tumor site and enhanced their suppressive function, therefore promoting tumor growth [84]. Additionally, tumor-associated macrophages expressing IL-17 were detected in human breast cancer tissues and their presence was directly associated with the degree of invasiveness of the tumor [81]. Whether these IL-17-producing non-Th17 cells may mediate pro- versus anti-tumoral effects does not solely depend on IL-17 as they produce variable amounts of a plethora of other cytokines with different activities. The abovementioned reports, underline the importance of distinguishing Th17 from IL-17-producing cells in general, as IL-17 targeting does not solely affect the role and function of Th17.

3.1.2. IL-17. IL-17A and IL-17F have been involved in proinflammatory cytokine and chemokine release by neutrophils, leading to tissue inflammation [82, 87]. The specific role of this cytokine in the development of malignancies remains elusive. Multiple reports have provided evidence that IL-17 promotes angiogenesis [88–90] and tumor development [89, 91, 92]. However, results from IL-17 deletion or ectopic expression remain conflicting. Several studies using IL-$17^{-/-}$ mice have demonstrated that the absence of IL-17 may promote tumor progression in mouse B16 melanoma [41] and MC38 colon carcinoma models [93]. The growth and propensity to give rise to lung metastases of MC38 tumors is augmented in IL-17-deficient mice, which is associated with decreased IFNγ^+ NK and IFNγ^+ tumor-specific T cells in the tumor draining lymph nodes and at the tumor sites [93]. IL-$17^{-/-}$ mice bearing B16 melanoma also exhibit increased lung metastases associated with reduced numbers of $CD4^+$, $CD8^+$ T cells, granulocytes, and $CD11c^+CD11b^+$ and $CD11c^+CD8a^+$ DCs at the tumor sites. Additionally, the activation status of $CD4^+$ T lymphocytes isolated from lung metastases was reduced [41]. Conversely, other studies performed with both B16 melanoma and MB49 bladder cancer models have argued that IL-17 deficiency resulted in reduced tumor burden [92]. A recent study has demonstrated that the growth of various tumors (EL4 lymphoma, Tramp-C2 prostate cancer, and B16-F10 melanoma) is significantly impaired in IL-$17R^{-/-}$ mice compared to their wild-type counterparts. In this study, IL-17R deficiency resulted in an increase in intratumoral $CD8^+$ T cells and reduced MDSC numbers in the tumor microenvironment. Interestingly, systemic pretreatment of animals with murine IL-17A exacerbated tumor growth [91].

Several human studies have highlighted the correlation between the level of IL-17 and poor prognosis in cancer patients [94, 95]. Increased numbers of IL-17-producing cells directly correlated to microvessel density in tumors and overall poor survival in hepatocellular carcinoma patients [94], as well as in non-small-cell lung cancer patients [95]. Consistent with these results, another study showed an increase in the level of IL-17 (most of which being secreted by $CD4^+$ T cells) in melanoma, breast, and colon cancer patients. Further characterization revealed that these tumor-derived IL-17 expressing cells were not immunosuppressive, but promoted tumor growth in an *in vitro* culture system [96]. Additionally, in colorectal carcinoma patients, a significantly higher frequency of IL-17-producing $CD4^+$ and $CD68^+$ cells were detected within the tumors when compared to the normal tissues. High expression of IL-17 was associated with increased microvessel density [88].

The angiogenic property of IL-17 has been an additional subject of debate. Indeed, several studies have linked IL-17 production to the induction of proangiogenic factors [88, 89, 92]. An early study conducted by Numasaki et al. demonstrated that the retroviral transduction of the IL-17 gene in cancer cells (MCA205 fibrosarcoma and MC38 colon adenocarcinoma) resulted in enhanced tumor growth *in vivo* while it had no effect on tumor cell proliferation *in vitro*. Tumors transduced with IL-17 exhibited significantly higher vascular density when compared to controls. IL-17 also enhanced the formation of vascular endothelial cells. Together these results indicate that IL-17 can participate in neoangiogenesis [89]. Nonetheless, it is important to underline that, while it can directly act as an angiogenic factor, IL-17 in combination with IFNγ increases the secretion of potent antiangiogenic factors such as CXCL9 and CXCL10 by cancer cells. The levels of CXCL9 and CXCL10 were associated with tumor-infiltrating effector T cells and improved outcomes in patients with ovarian cancer [37].

3.1.3. IL-21, IL-22, TNFα, and IFNγ. As outlined, the cytokine secretion profile of Th17 cells is variable in nature and

amount. We will therefore focus on the key factors produced by Th17 lymphocytes, which may influence anti-tumor immunity.

As mentioned in the previous section, IL-21 is involved in the generation of Th17 lymphocytes and is also produced by these cells. IL-6-induces IL-21 production in a STAT3-dependent and RORγ-independent manner. IL-17 and IL-21 production is impaired *in vivo* in IL-6-deficient mice [48]. IL-21 can synergize with IL-12 to enhance the cytotoxicity of peripheral blood mononuclear cells (PBMC) in patients with cervical intraepithelial neoplasia III and cervical cancer. In this study, the PBMC incubated with IL-21 and IL-12 effectively induced apoptosis of SiHa tumor cells [97]. Additionally a report by Søndergaard et al. demonstrated that the administration of IL-21 significantly hindered the growth of established subcutaneous B16 melanomas or Renca renal cell carcinomas. The anti-tumoral effect of IL-21 was mediated in this case by $CD8^+$ T lymphocytes [98].

IL-22 belongs to the IL-10 family and has often been reported as a cytokine produced by Th17 lymphocytes [99, 100]. In humans, IL-22 was initially characterized as a Th1 cytokine [101]. It was also reported that IL-22 could be secreted by $CD4^+$ T cells in the absence of IL-17 production [102]. The possibility of the existence of a dedicated IL-22 secreting $CD4^+$ T cell lineage (Th22) has been raised and whether "Th22" may belong to the Th17 family is currently being discussed [102–105]. Actually, it appears that naïve $CD4^+$ T lymphocytes in the presence of IL-6 but in the absence of exogenous TGFβ express high levels of IL-22 but minimal amount of IL-17 while IL-6 in combination with TGFβ triggers the polarization of "conventional" Th17 lymphocytes expressing large amounts of IL-17 but minimal levels of IL-22 [106]. These IL-22 secreting cells have been described for their protective function against infections [107]. However, the presence of IL-22-producing $CD4^+$ T cells has been correlated with poor survival in patients with gastric cancer [108]. IL-22 by itself has been described for both its pro- and anti-tumoral effects [103, 104, 109–111].

GM-CSF (granulocyte macrophage-colony stimulating factor) is endowed with anti-tumoral properties [112, 113]. It has been documented that GM-CSF is produced by highly pathogenic and proinflammatory Th17 cells in the setting of autoimmune diseases [114, 115]. GM-CSF production was dependent on the activity of the IL-12-IL-23 receptor complex and RORγt. Conversely, IFNγ, IL-12, and IL-27, known to inhibit RORγt expression, impeded GM-CSF secretion [114].

Th17 or hybrid Th17/Th1 lymphocytes can produce TNFα and IFNγ [38, 57]. Human tumor-infiltrating Th17 cells have been reported to produce high levels of TNFα and IFNγ [37]. These two cytokines are endowed with direct cytotoxic or cytostatic effects against tumor cells but are also involved in the activation of innate and adaptive immune cells, thus promoting anticancer immunity. Although TNFα is not essential for Th17 generation, it synergizes with IL-6 and IL-1β to amplify Th17 responses [67]. A significant positive correlation between the expression of genes involved in the TNFα signaling and those involved in Th17 pathways in patients with ovarian cancer was reported [42]. IFNγ is the hallmark of Th1 lymphocytes while Th17 cells generated *in vitro* typically produce minimal amounts of IFNγ. However, Th17 cells generated *in vivo*, especially during the development of autoimmune diseases, or adoptively transferred IL-17^+ Th17 cells can evolve towards IL-17^+ IFNγ^+ cells [36, 40, 116].

It should, however, be emphasized that conclusions drawn from the studies focusing on the effects of IL-17 should not be confused with those of Th17 cells since, as outlined above, several other non-$CD4^+$ T cell populations can produce this cytokine. Similar considerations hold true for other Th17-related cytokines such as IL-21, IL-22, GM-CSF, TNFα, or IFNγ.

3.2. Th17 Lymphocytes in Cancer: Foes or Allies? As previously outlined, the role of Th17 lymphocytes in cancer is still highly controversial (Figure 2). An important distinction should be made between "endogenous" Th17 cells present in cancer patients or mouse tumor models, which develop under the pressure of the complex tumor environment, and the adoptively transferred Th17 cells generated *in vitro* under well-defined cytokine conditions.

Th17 lymphocytes have been detected in patients with different types of malignancies, such as ovarian, pancreatic, or gastric cancers, but the role of these cells in disease progression and their prognosis value has been controversial [37, 117, 118]. Whether Th17 lymphocytes are induced *de novo* from naïve $CD4^+$ T cells or recruited at the tumor site or originate from "reprogrammed Treg" (see previous section and below) remains to be elucidated. In a report evaluating the nature of tumor-associated Th17 lymphocytes in ovarian cancer patients, it was demonstrated that the percentage of these cells correlated with the number of IFNγ^+ $CD4^+$ T cells, IL-17^+ IFNγ^+, IFNγ^+ $CD8^+$ T cells as well as NK cells and inversely correlated with the frequency of immunosuppressive Treg cells [37]. In another report focusing on prostate cancer patients, highly differentiated Th17 cells correlated with slower disease progression [119], which was contradicted by results from others obtained in hormone resistant prostate cancer patients [120]. In additional studies, an association between increased numbers of tumor-associated Th17 lymphocytes and survival was observed in ovarian and lung cancer patients [37, 121]. Similarly, a significant increase in Th17 cell numbers in the tumor environment has been reported in the mouse ID8 ovarian, Pan02 pancreatic, and B16 melanoma cancer models [42, 122, 123]. The physiological significance of this increase has been disputed. In an ovarian cancer model, TNFα-mediated induction of IL-17-producing $CD4^+$ cells led to the recruitment of myeloid cells into the tumor microenvironment and resulted in enhanced tumor growth [42]. In contrast, induced production of IL-6 in the tumor microenvironment, as a result of either indoleamine 2,3-dioxygenase (IDO) inhibition or the transduction of tumor cells with the IL-6 gene, led to the conversion of Treg to Th17 cells and regression of mouse B16 melanoma [123] or Pan02 pancreatic tumors [122], respectively.

Multiple studies have investigated the impact of *in vitro* generated Th17 cells on tumor growth following adoptive transfer, with variable outcomes. Initial studies by

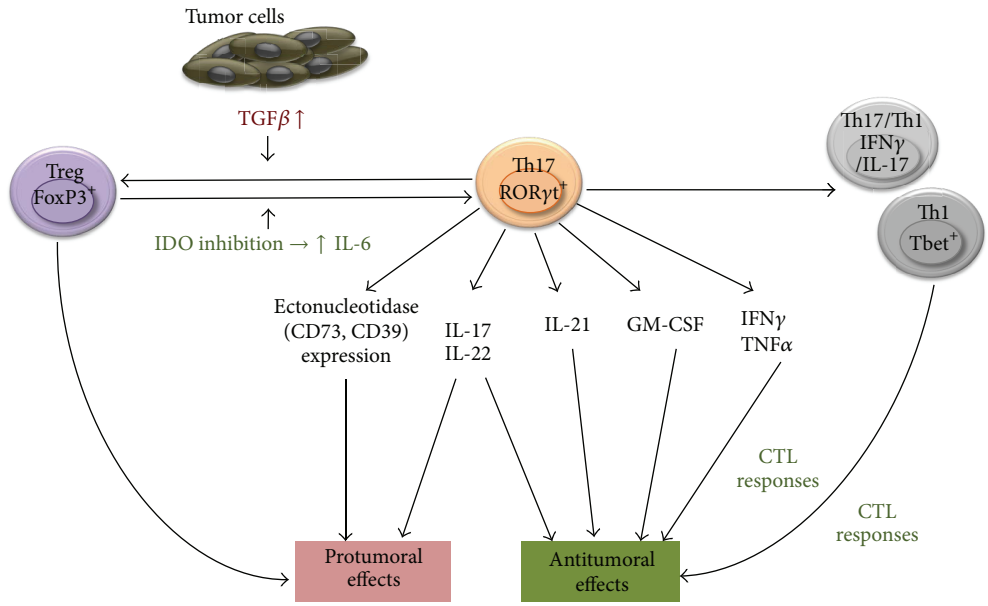

FIGURE 2: Pro-versus anti-tumoral effects of Th17 lymphocytes and the cytokines they produce on cancer development. Th17 lymphocytes produce cytokines which may promote or impair tumor development. Depending on the microenvironment Th17 may differentiate into Th1 or hybrid lymphocytes capable of controlling tumor growth or into protumoral Treg. IDO: indoleamine 2,3-dioxygenase.

Muranski et al. have evaluated the effects of Th17 generated from $CD4^+$ T cells isolated from TCR transgenic mice specific for the TRP-1 melanoma epitope. Administration of these Th17 lymphocytes led to the eradication of established B16 melanoma. The therapeutic effects of these cells were, however, substantially mediated by IFNγ [124]. In line with these results, subsequent studies indicated that adoptive transfer of *in vitro* generated Th17 lymphocytes impaired tumor development by eliciting robust tumor-specific $CD8^+$ T cell responses. Th17 cell therapy promoted the homing of dendritic cells to the tumor site and the draining lymph nodes [41]. Supporting the anti-tumoral role of *in vitro* polarized Th17, in a more recent study Muranski et al. demonstrated that adoptive Th17 cell therapy has the potential to eliminate established tumors. The anti-tumoral efficacy of these Th17 lymphocytes was dependent on their ability to produce both IFNγ and IL-17. Interestingly, the administered Th17 differentiated into cells, which exhibited a stem cell-like phenotype and Th1 properties (Tbet, IFNγ expression) but retained their ability to produce IL-17. Importantly, the therapeutic efficacy of Th17 lymphocytes generated from $Tbet^{-/-}$ or $IFN\gamma^{-/-}$ or $IL17^{-/-}$ mice was severely impaired [74]. In agreement with these reports, our own results have suggested that the adoptive transfer of Th17 efficiently combined with chemotherapy to treat established murine mammary carcinoma [125]. In one study, the possibility that *in vitro* generated Th17 cells may exhibit immunosuppressive function and promote tumor progression through the expression of ectonucleotidases has been proposed [126].

It should, however, be emphasized that in the majority of these studies, the generated populations of $CD4^+$ T cells were heterogeneous in nature and were not a pure subset of Th17 lymphocytes, advocating for a cautious interpretation of the above results. In addition, the concentration of cytokines (TGFβ, IL-6, and IL-23) represents a major source of variability between protocols used to generate Th17 cells *in vitro*. Therefore, standardized procedures are still needed to generate and purify homogeneous populations of $CD4^+$ T cells producing high levels of IL-17.

4. Conclusion: Manipulating the Differentiation Status of Th17 for Cancer Therapy

Although the role of Th17 in autoimmune diseases and infection has been relatively well documented, the impact of Th17 in cancer remains difficult to ascertain. The plasticity of the developmental program of these cells confers them with the ability to redifferentiate into suppressive Treg hindering anti-tumor immunity or alternatively into proinflammatory T helper cells such as Th1-like lymphocytes capable of activating tumor killer effector immune cells. This lack of clear lineage commitment explains the propensity of Th17 cells to be influenced in many different ways by the complex tumor microenvironment. The direction of Th17 eventual polarization is likely dictated by the concentration and ratio of cytokines and chemokines present in the tumor milieu, and by the presence and influence of other tumor-infiltrating immune cells. Since the tumor environment depends on the type, location, and stage of cancer, it is to be expected that Th17 function may vary according to these conditions. Controlling the level and type of the cytokines produced by

cancer cells in animal tumor models may help addressing the conditions required for the pro- or anti-tumoral activity of Th17 lymphocytes. In addition, it would be essential to further evaluate the contribution of Th17 cells in tumor immunity at different stages of cancer progression. The degree of plasticity of these cells and their unpredictable behavior *in vivo* makes the prospect of Th17-based cancer immunotherapy highly challenging. However, based on the promising results obtained in preclinical animal models, the prospect of treating patients with Th-17 cells polarized *in vitro* seems an attractive strategy which deserves to be evaluated in clinical trials. The recent discovery of the stem cell-like properties of Th17, which enables them to self-renew with the capacity to differentiate into Th1-like or Treg progeny, could have significant implications on the outcome of Th17-based therapy. However, although IFNγ-expressing Th17 lymphocytes mediate potent anti-tumor effects both in human and animals, it will conceivably be challenging to consistently and reproducibly redirect Th17 differentiation towards IFNγ-expressing Th1-like cells following adoptive transfer *in vivo*. Further studies are therefore required to more clearly understand the driving forces sustaining Th17 polarization into potent anti-tumor effector cells.

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Review Article

Th17-Associated Cytokines as a Therapeutic Target for Steroid-Insensitive Asthma

Yuko Morishima, Satoshi Ano, Yukio Ishii, Shigeo Ohtsuka, Masashi Matsuyama, Mio Kawaguchi, and Nobuyuki Hizawa

Division of Clinical Medicine, Department of Respiratory Medicine, Faculty of Medicine, University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575, Japan

Correspondence should be addressed to Yuko Morishima; mk01a231@md.tsukuba.ac.jp

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Steroid-insensitive asthma is an infrequent but problematic airway disease that presents with persistent symptoms, airflow limitation, or recurrent exacerbations even when treated with steroid-based therapies. Because of unsatisfactory results obtained from currently available therapies for steroid-insensitive asthma, a better understanding of its pathogenesis and the development of new targeted molecular therapies are warranted. Recent studies indicated that levels of interleukin (IL)-17 are increased and both eosinophils and neutrophils infiltrate the airways of severe asthmatics. IL-17 is a proinflammatory cytokine mainly secreted from helper T (Th) 17 cells and is important for the induction of neutrophil recruitment and migration at sites of inflammation. This review focuses on the pathogenetic role of Th17 cells and their associated cytokines in steroid-insensitive asthma and discusses the prospects of novel therapeutic options targeting the Th17 signaling pathway.

1. Introduction

Asthma is a very common disease that affects many people, men and women, young and old, worldwide. Although asthma is mostly well controlled by conventional therapies including inhaled corticosteroids, about 5–10% of asthma patients have a severe phenotype described as "fatal or near-fatal asthma," "severe asthma," "steroid-dependent asthma," "steroid-insensitive asthma," "difficult to control asthma," "poorly controlled asthma," "brittle asthma," or "irreversible asthma" [1]. The causes of these conditions are complex and most likely heterogeneous. Some can be explained by insufficient or inadequate treatment, while others explained by airway inflammation that is resistant to conventional treatment. Continuous exposure to aggravating factors and/or associated comorbid conditions may exert a deleterious influence on asthma control, but a certain type of airway inflammation may also contribute to standard therapy unresponsiveness. As such, the pathogenesis of uncontrollable asthma, especially steroid-insensitive asthma, has been a long-standing interest among researchers attempting to establish a novel strategy for the treatment of patients with persistent symptoms, irreversible airflow obstruction, or frequent exacerbations even under adequate treatment.

The current consensus on asthma is that the main underlying pathology is chronic airway inflammation in which inflammatory cells, such as eosinophils and helper T (Th) 2 lymphocytes, play a role. The Th1/Th2 paradigm has offered important insights into the pathogenesis of asthma, and there is no doubt that this classical Th1/Th2 theory mostly explains the immune responses in asthma. Based on the idea that asthma is associated with polarized Th2 responses, various clinical trials have been conducted to develop effective new therapeutic options by adjusting the Th1/Th2 cytokine balance. Several studies have demonstrated the benefits of an IL-4 variant, a soluble recombinant human interleukin (IL)-4 receptor, anti-IL-5 monoclonal antibodies, and anti-IL-13 monoclonal antibodies in controlling respiratory symptoms or in preventing either bronchospasm or eosinophilic airway inflammation in asthmatic patients [2–6]. However, some

studies failed to show complete improvements in therapeutic outcomes by blocking the biological actions of Th2 cytokines [7, 8], and some limitations have been recognized to exist in the Th1/Th2 paradigm.

Over the last two decades, our understanding of the pathogenic role of various Th cell subsets has greatly advanced. Several studies have recently described the characteristics of severe asthma to include the involvement of neutrophils as well as that of eosinophils [1, 9–12]. Neutrophilic airway inflammation appears to be insensitive to steroids [1, 11, 13] and may be related to Th17 rather than Th2 cytokines [14–17]. This review highlights the role of Th17 cells in the pathogenesis of steroid-insensitive asthma and discusses the possibilities of developing new therapeutic options targeting Th17 cells and their related cytokines.

2. General Features of Th17 Cells

2.1. Th17 Cell Differentiation. The network of differentiation factors and their interactions for generating Th17 cells are intricate and finely balanced, and they have gradually become understood. Th17 cells are derived from T cell precursors, naïve $CD4^+$ T cells common to Th1, Th2, and regulatory T (Treg) cells [18, 19]. The differentiation of naïve $CD4^+$ T cells into each cell type is triggered by a particular cytokine milieu during stimulation by cognate antigen. For Th17 cells, transforming growth factor (TGF)-β and IL-6, together with IL-21 and IL-23, may play a role in the induction of Th17-cell differentiation and activation [20, 21]. Since Th17 cells have a key role in the secretion of IL-21 and IL-6 produced by themselves or by stimulating other target cells, an amplification loop may exist to enhance Th17 cell differentiation through autocrine and paracrine regulation [22, 23]. Other proinflammatory cytokines such as IL-1β and tumor necrosis factor (TNF)-α also promote the development of Th17 cells [24, 25]. By contrast, several studies have demonstrated a different regulatory mechanism where TGF-β is not required for Th17 cell differentiation [26–28]. Nevertheless, the present consensus among researchers is that TGF-β is usually required for generating Th17 cells [29–31]. Upregulation and activation of the key transcription factors are also crucial for Th17 cell differentiation. Signaling transducer and activator of transcription 3 (STAT3) and retinoic acid-related orphan receptor-γt (RORγt), recently described as a Th17 master regulator, are both known to be important for Th17 cell differentiation and the production of related cytokines [20, 21, 32].

Th17 cell differentiation is also controlled by other T cell lineages and their associated cytokines. The Th1 cytokine interferon (IFN)-γ and Th2 cytokine IL-4 exert negative effects on the differentiation of Th17 cells [18, 19], while IL-9 exerts a promoting effect [33]. T-bet and Foxp3, master transcription factors for Th1 and Treg cell differentiation, respectively, may negatively regulate Th17 cell differentiation by interacting with RORγt to suppress its function [34, 35]. Interestingly, the multifunctional cytokine TGF-β, produced by every leukocyte lineage and also by nonimmune cells, has pleiotropic properties on the differentiation of Th17 and Treg cells. TGF-β displays distinct functions depending on the presence of IL-6. TGF-β alone leads to the differentiation of Treg cells that have suppressive effects on excessive Th1/Th2/Th17 immune responses, whereas TGF-β, together with IL-6, promotes the differentiation of Th17 cells. In addition, it was demonstrated that not only the combination but also the amount of cytokine stimuli is a critical determinant for T cell differentiation. Low concentration of TGF-β enhances the generation of Th17 cells, whereas high concentration promotes the development of Treg cells and inhibit that of Th17 cells [35].

2.2. Signature Cytokines of Th17 Cells, IL-17A, and IL-17F. Th17 cells selectively produce IL-17A, IL-17F, IL-21, and IL-22. Among these cytokines, IL-17A and IL-17F have critical roles in various immune responses such as host defense against pathogens and autoimmune and inflammatory conditions [36]. They are members of the IL-17 cytokine family that have high amino acid sequence homology and share a common receptor subunit, IL-17 receptor A (IL-17RA), and IL-17 receptor C (IL-17RC). Since IL-17A and IL-17F can form homo- and heterodimers because of their sequence homology [37], they may have similar functions. These include recruiting and activating neutrophils as well as stimulating other structural cells to release proinflammatory mediators, such as IL-6, IL-1β, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF), C-C motif (CC) chemokines, C-X-C motif (CXC) chemokines, antimicrobial peptides, and metalloproteinases [36, 38]. However, IL-17A plays important roles in the development of autoimmunity, inflammation and tumors and in host defense against bacterial and fungal infections, whereas IL-17F has a role mainly in mucosal host defense mechanisms [38]. It is therefore likely that the biological role of these cytokines in immune responses might not be identical.

3. Th17-Associated Cytokines and Steroid-Insensitive Asthma

3.1. Increased Expression of IL-17A in Patients with Asthma. Since some phenotypes of asthma cannot be explained using classical Th1/Th2 theory, numerous recent studies have suggested possible roles of IL-17A and IL-17F in asthma. Since the role of IL-17F in the pathogenesis of asthma is reviewed elsewhere in the same issue (see Ota et al. [39]), we describe the role of IL-17A in asthma. Increased levels of IL-17A (or IL-17) protein and messenger RNA were detected in the sputum [15, 40, 41], bronchoalveolar lavage fluids (BALF) [41], bronchial tissues [16, 42–44], peripheral mononuclear cells (PBMCs) [45, 46], and serum [17, 47, 48] from patients with asthma. It was demonstrated that expression of an IL-17A receptor unit, IL-17RA and IL-17RC [49], RORγt and RORC, which encodes RORγt, was also increased in the bronchial tissues and PBMCs of asthmatic patients [45, 46]. The expression levels of IL-17A correlated with airway hyperresponsiveness (AHR) [40] and clinical severity [16, 17,

43, 45, 47, 48], suggesting that IL-17A may contribute to the pathogenesis of a certain type of asthma.

3.2. Neutrophilic Airway Inflammation. Several studies have suggested the involvement of neutrophils in severe asthma [1, 9–12]. Since IL-17A is capable of inducing neutrophil influx directly into inflammatory sites or indirectly [36, 38], it is natural to consider a link between IL-17A and airway neutrophilia. In asthmatic patients, the expression levels of IL-17A correlated with the levels of C-X-C motif ligand (CXCL) 8 and neutrophilic airway inflammation [15]. By contrast, the results from another study demonstrated no association between IL-17A expression and neutrophilic inflammation despite an enhanced expression of IL-17A in bronchial submucosa of asthmatics [42]. However, a relationship between IL-17A expression and airflow limitation and sputum neutrophil counts were shown suggesting a potential role for IL-17A in a neutrophilic type of asthma [42].

These clinical observations were extended and confirmed in experimental animal models. To examine whether IL-17 can induce neutrophil recruitment, recombinant IL-17 was directly administered into rodent airways, which resulted in increased numbers of neutrophils and CXC chemokine expression [50–52]. In allergic animal models, neutrophilic airway inflammation was induced by ovalbumin (OVA) exposure in two strains of OVA-specific T cell receptor (TCR) expressing transgenic mice, OTII and DO11.10 [53, 54], and was found to be attenuated in genetic mice lacking IL-17 [54]. Similar results were observed using Th17-polarized cells obtained from DO11.10 mice. Adoptive transfer of those cells into severe combined immunodeficiency (SCID) or naïve BALB/c mice induced the development of airway neutrophilia and AHR accompanied by increased expression of IL-17 and neutrophil chemoattractants such as CXCL 5, CXCL8, and G-CSF, after OVA challenge. These responses were abolished by depleting IL-17A using antibodies and in genetic mice lacking IL-17 receptor (IL-17R) [50, 55]. Lajoie et al. recently reported interesting data using two strains of mice, one with high susceptibility to develop allergen-induced AHR and the other with low susceptibility, each corresponding to severe and mild asthma, respectively. The susceptible strain manifested increased production of IL-17A and Th2 cytokines and severe AHR, which was attenuated by neutralizing IL-17A. In contrast, less susceptible strain exhibited a predominant Th2 cytokine profile and less severe AHR, which was aggravated by IL-17A administration [56]. These studies support the importance of IL-17A as a key regulator in generating neutrophilic inflammation and enhancing the severity of allergen-induced airway responses.

Understanding the underlying mechanisms that cause airway immune responses and promote Th17 polarization has been of great interest. We recently reported that the balance between two types of allergen-induced inflammation, neutrophilic and eosinophilic, is controlled by RORγt and GATA-3, Th17 and Th2 master transcription factors, respectively. RORγt-transgenic mice, which overexpress IL-17A, showed enhanced airway neutrophilia and AHR with increased expression of neutrophil chemoattractants in response to allergen exposure, whereas GATA-3-transgenic mice, which overexpress Th2 cytokines, developed enhanced airway eosinophilia and AHR [57]. Similarly, as described above, the transfer of *in vitro* polarized Th17 cells resulted in allergen-induced airway neutrophilia, while that of polarized Th2 cells resulted in allergen-induced AHR accompanied by airway eosinophilia [55]. Furthermore, the route of antigen sensitization, through the skin [58] or airway [59], but not peritoneum, and the duration of antigen exposure, longer rather than short [14, 60, 61], were important for the elicitation of Th17 responses in airway inflammation. These results suggest that the phenotypes of allergen-induced airway inflammation can be determined through the Th2/Th17 balance by both endogenous and exogenous factors.

3.3. Contribution of IL-17A to Steroid Insensitivity. There is no doubt that the presence of neutrophils in the airways is one explanation for steroid resistance in asthma. Neutrophilic inflammation tends to respond poorly to steroid therapy as steroids induce apoptosis in eosinophils but increase neutrophil release from the bone marrow, reduce egress of neutrophils from the circulating pool into the marginating pool, and prevent neutrophil apoptosis [62, 63]. Airway neutrophilia and IL-17A and CXCL8 expression were in fact not attenuated by steroid treatment in asthmatics [15]. Likewise, steroids had no effect on increased airway-infiltrating neutrophils and CXC chemokines in Th17-polarized mice following allergen exposure [55, 57] although some studies reported contrary results [14, 43, 51]. Interesting results have been obtained using primary epithelial cells from healthy subjects and asthmatics. Steroid treatment caused a significant reduction in IL-17A-induced IL-6 expression in epithelial cells obtained from normal controls but not in those from asthmatics [44]. Similarly, *in vitro* studies demonstrated that IL-17A-induced CXCL8 production in the bronchial epithelial cell line was normally sensitive to steroids. However, following pretreatment with IL-17A, TNF-α-induced CXCL8 production became insensitive [64]. An increase in glucocorticoid receptor (GR)-β expression [44] and reduction of histone deacetylase (HDAC) 2 activity [64] in target epithelial cells was suggested as a possible molecular mechanism for IL-17A-induced steroid insensitivity.

3.4. Airway Remodeling. Airway remodeling, such as subepithelial collagen deposition and increased airway muscle mass, together with excessive mucus secretion, is important components leading to irreversible air flow limitation that is insensitive to steroid treatment [1]. Several studies demonstrated that modifying the expression of IL-17A did not alter the degree of airway remodeling in an experimental mouse model of asthma [56, 57]. However, sensitized mice with prolonged allergen exposure developed airway remodeling, and its severity positively correlated with the number of $CD4^+IL\text{-}17^+$ cells and IL-17 concentration in the airways [61]. Similar results were also observed in Th17-transferred mice [61] and IL-17 transgenic mice [19]. These outcomes were supported by *in vitro* cultures of airway structural cells. IL-17 was shown to promote migration, proliferation, and

reduction of apoptosis in smooth muscle cells [65, 66], to stimulate the expression of mucin genes in epithelial cells [67], and to potentiate the production of profibrotic cytokines in fibroblasts and eosinophils [41, 68]. Although it is accepted that T cells, and particularly Th2 cells, play a critical role in the development of asthmatic airway remodeling, these results indicate a possibility that Th17 cells may also contribute to its pathogenesis.

3.5. Cross-Regulation between IL-17A and Th2 Responses. A reciprocal negative regulation between Th17 cells and Th2 cells has been considered to exist during immune responses [18, 19, 69]. Th17 cell differentiation is negatively controlled by Th2 cytokines [18, 19]. From another viewpoint, IL-17 administration reduced Th2-type responses, such as airway eosinophil recruitment, AHR, and expression of C-C motif chemokine ligand (CCL) 11 and CCL17, in allergen-exposed mice. Production of Th2 cytokines was also decreased by IL-17 in mediastinal lymph nodes from allergen-exposed mice [70]. In addition, treatment with anti-IL-17 neutralizing antibody enhanced bronchial eosinophil influx and IL-5 expression in an allergen-induced model of asthma [14, 70]. Conversely, CCL11 expression was increased in the lung tissues of IL-17 transgenic mice [19] and in IL-17A-treated airway smooth muscle cells in other experiments [71, 72]. Airway eosinophil recruitment was decreased in IL-17R deficient mice following allergen exposure [70]. These results indicate that, in contrast to exogenous IL-17, endogenous IL-17 is necessary for developing eosinophilic inflammation by inducing CCL11.

Consequently, it is difficult to describe a uniform contribution of IL-17 to the pathogenesis of asthma. It might not be appropriate to have a simple view that Th17 and Th2 cytokines reciprocally counteract each other. In addition, it would also be too straightforward to consider Th17-cell-mediated neutrophilic type of airway inflammation as severe asthma and Th2-cell-mediated eosinophilic type of inflammation as mild asthma. Indeed, Wenzel et al. reported that, when they classified asthmatics with inflammatory cell types in their sputum, patients with increased numbers of both neutrophils and eosinophils presented the most severe clinical features even when treated with steroids [73]. Sputum analysis also confirmed that patients with a simultaneous increase in IL-5 and IL-17A had significantly worse lung function parameters and that uncontrolled asthmatics tended to have higher IL-5 and IL-17A mRNA levels than controlled asthmatics [74]. Consistent with these clinical studies, sensitized mice, cochallenged with allergen and IL-17A, presented increased airway-infiltrating eosinophils and neutrophils and severe AHR, whereas those, challenged with allergen alone, presented only increased airway-infiltrating eosinophils and no AHR, and sensitized mice challenged with IL-17A alone developed moderate airway neutrophilia but not AHR [59]. In another animal study, asthma-susceptible mice were intratracheally exposed to IL-17A, IL-13, or a combination of both to elucidate the cross-regulation between IL-17A and Th2 responses during the development of airway inflammation. AHR was induced by treatment with IL-13, but not by IL-17A alone; however, a significant increase in AHR was demonstrated in mice treated with both IL-13 and IL-17A [56]. Taken together, the production of IL-17A may not simply suppress Th2 responses, but also enhance them to promote a severe phenotype of asthma in certain situations.

4. Therapeutic Considerations for Targeting the Th17 Signaling Pathway in Steroid-Insensitive Asthma

Steroids are potent immunosuppressive and anti-inflammatory agents, offering a pivotal role among currently available treatment options for asthma. However, they may function in a nonspecific manner. Since an effective approach to control steroid-insensitive asthma has not yet been developed, novel therapeutic agents that target specific molecular events are required. As some phenotypes of severe asthma are associated with excessive Th17 responses, adjusting Th17 signaling might offer effective therapeutic options for steroid-insensitive asthma. Considering the process of Th17 cell differentiation and function there are a number of theoretical candidate therapeutic targets. These include Th17-cell differentiation factors IL-6, IL-1β, and IL-23, Th17 cytokine IL-17A, Th17-cell specific transcription factor RORγt, IL-17A/F receptor IL-17RA, and Th17-cell downstream inflammatory mediators (Figure 1). However, since Th17 immune responses are also important for host defense and possibly antitumor immunity, their favorable roles, as well as pathogenic roles, should be considered when inhibiting Th17 signaling pathways in the clinic. Among these targets, several are currently being tested in clinical trials as described below.

4.1. Blocking IL-17A and IL-17RA. Based on results obtained from cellular, animal, and human studies, clinical studies in autoimmune and inflammatory diseases, such as psoriasis, rheumatoid arthritis, ankylosing spondylitis, uveitis, and Crohn's disease, have already been conducted to confirm anti-inflammatory effects by blocking IL-17A [75]. Compared with nonspecific immunosuppressive agents, targeting IL-17A is considered advantageous in only attenuating inflammation but not host defense, because IL-17F may have the potential to compensate for immunocompromised conditions [38].

Currently, numerous clinical studies examining the effectiveness of monoclonal antibodies against IL-17A are underway [75]. Phase II trials of secukinumab and ixekizumab demonstrated positive results for the substantial relief of symptoms, with satisfactory safety, in patients with Th17-related diseases, such as plaque psoriasis, psoriatic arthritis, rheumatoid arthritis, uveitis, and ankylosing spondylitis, but not those with Crohn's disease [76–82]. Brodalumab, a monoclonal antibody against IL-17RA, demonstrated favorable results as well in patients with plaque psoriasis [83, 84]. Equivocal findings were reported in the clinical trial of brodalumab in patients with moderate-to-severe asthma [85]. They showed benefits of brodalumab in improving clinical symptoms in the high bronchodilator reversibility subgroup, despite failing to achieve clinical or statistical

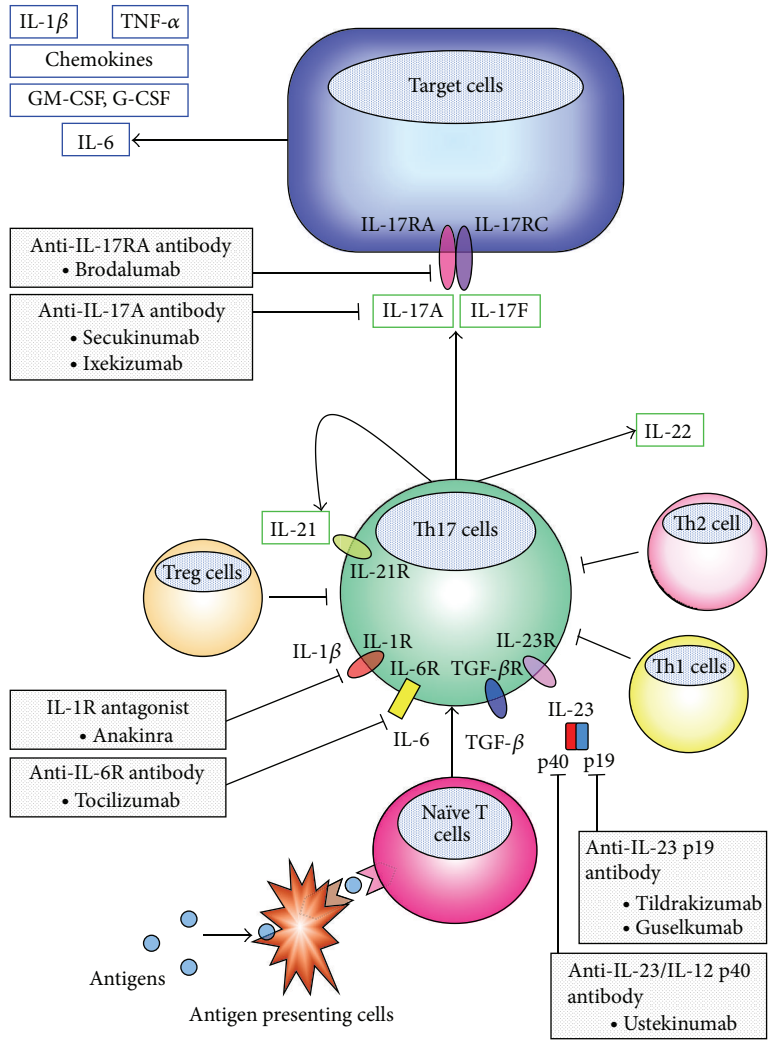

Figure 1: Targeting the Th17 pathway. Helper T (Th) 17 cells are derived from naïve $CD4^+$ T cells under the control of transforming growth factor (TGF)-β, interleukin (IL)-6, and IL-23 during stimulation by cognate antigen. These cytokines also stimulate Th 17 cells to produce IL-21, which affects Th17 cells themselves to activate a specific transcription factor, RORγt through autocrine regulation. Other proinflammatory cytokines, IL-1β and tumor necrosis factor (TNF)-α, may also promote Th17 development. RORγt regulates both Th17 cell differentiation and production of Th17-signature cytokines, IL-17A, IL-17F, IL-21, and IL-22. Among these cytokines, IL-17A and IL-17F play pivotal roles in the pathogenesis of asthma and share a common receptor subunit, IL-17 receptor A (IL-17RA), and IL-17 receptor C (IL-17RC). Several inhibitors of Th17 pathway are currently under clinical investigation. G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IL-1R, IL-1 receptor; IL-6R, IL-6 receptor, Treg, regulatory T.

improvements in the overall subjects with asthma. In addition, phase II trials of secukinumab for uncontrolled asthma have since been initiated, in which favorable results are expected (NCT01478360).

4.2. Blocking IL-23 and IL-1β. As IL-23 is crucial to the development of Th17 cells, a monoclonal antibody against the p40 subunit of IL-23/IL-12, ustekinumab, has also been investigated in clinical trials for the treatment of immune-mediated diseases such as psoriatic arthritis and Crohn's disease [86, 87]. Its efficacy, safety, and tolerability in the management of psoriasis are generally accepted; therefore, it is already approved to treat moderate-to-severe plaque psoriasis in several countries. Clinical trials using a monoclonal antibody against the p19 subunit of IL-23, expected to specifically inhibit IL-23, are underway to evaluate its efficacy in patients with plaque psoriasis and rheumatoid arthritis [75].

IL-1β may also be a therapeutic target because it has an important role in the development of Th17 responses. Anakinra, an IL-1 receptor (IL-1R) antagonist is already available for clinical use for rheumatoid arthritis and is now being investigated to determine whether to reduce endotoxin-induced airway inflammation (NCT01369017). Since endotoxin is believed to be associated with asthma exacerbations, anakinra may have a possibility to be one of the therapeutic options for asthma.

Although clinical trials of agents that block IL-23 or IL-1β have not been conducted in patients with asthma, future studies are required to elucidate the potential of treatments targeting IL-23 or IL-1β as novel therapeutic strategies for steroid-insensitive asthma.

4.3. Blocking IL-6. Recent studies showed that IL-6 is important for promoting Th17 cell differentiation and orchestrating downstream pathways of Th17 immune responses to cause inflammatory and autoimmune disorders such as rheumatoid arthritis and multiple sclerosis [23, 88, 89]. IL-17A also contributes to autoimmunity by triggering a positive feedback loop via IL-6 induction [23]. IL-17A-biased immune conditions may accelerate IL-6 production and in turn, an excessive amount of IL-6 may amplify upstream of Th17 immune responses to promote Th17-driven inflammation. Therefore, blocking this amplification loop might be important for the resolution of inflammation. In asthmatics, the expression of IL-6 and soluble IL-6 receptor (IL-6R) were increased in the serum and airways [90–92]. In addition, a novel variant of IL-6R was recently identified in a genome-wide study and was significantly associated with asthma risk [93]. In an animal study of allergic airway inflammation, we demonstrated that IL-6 production was increased in IL-17-induced steroid-insensitive airway inflammation and that both airway neutrophilia and AHR were effectively attenuated by treatment with an anti-IL-6R antibody [57]. These results indicated that IL-6, as well as IL-17, is a potential target for the treatment of Th17-driven steroid-insensitive airway inflammation.

Inhibition of the Th17 pathway by IL-6 blockade has recently been proposed as a treatment option in various autoimmune and inflammatory diseases [94]. A humanized anti-IL-6R antibody, tocilizumab, which was demonstrated to be therapeutically effective for rheumatoid arthritis, systemic juvenile idiopathic arthritis, and Castleman's disease, is already available for clinical use [88]. Consequently, it can be expected to become a novel therapeutic option for steroid-insensitive asthma, too.

5. Conclusions

Our understanding of the immunologic cascade in the pathogenesis of asthma has greatly advanced over the last 20 years, as various Th cell subsets have been identified. Numerous studies have been performed to determine the precise mechanism of treatment-insensitive severe asthma and to identify targets that may provide therapeutic benefits. From these studies, excessive Th17 responses have been shown to be key factors involved in steroid-insensitive asthma. In this review, we discussed that Th17-associated cytokines might be potential targets to alter excessive Th17 signaling and might offer advantages over classic therapies, such as steroids, for patients with severe asthma. Several clinical trials targeting Th17 cytokines have been initiated, and we should continue to focus on this issue to improve the outcomes of uncontrolled asthma that is resistant to conventional therapies.

Abbreviations

AHR:	Airway hyperresponsiveness
BALF:	Bronchoalveolar lavage fluids
CC:	C-C motif
CCL:	C-C motif chemokine ligand
CXC:	C-X-C motif
CXCL:	C-X-C motif ligand
G-CSF:	Granulocyte colony-stimulating factor
GM-CSF:	Granulocyte macrophage colony-stimulating factor
GR:	Glucocorticoid receptor
HDAC:	Histone deacetylase
IFN:	Interferon
IL:	Interleukin
IL-17RA:	IL-17 receptor A
IL-17RC:	IL-17 receptor C
IL-1R:	IL-1 receptor
IL-6R:	IL-6 receptor
LTi:	Lymphoid tissue inducer
NK:	Natural killer
NKT:	Natural killer T
OVA:	Ovalbumin
PBMCs:	Peripheral mononuclear cells (PBMCs)
RORγt:	Retinoic acid-related orphan receptor-γt
SCID:	Severe combined immunodeficiency
STAT3:	Signaling transducer and activator of transcription 3
TCR:	T cell receptor
TGF:	Transforming growth factor
Th:	Helper T
TNF:	Tumor necrosis factor
Treg:	Regulatory T.

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Research Article

Anti-IL-17 Antibody Improves Hepatic Steatosis by Suppressing Interleukin-17-Related Fatty Acid Synthesis and Metabolism

Weidong Shi,[1,2,3] Qiang Zhu,[4] Jian Gu,[1,2] Xiaoshan Liu,[1] Ling Lu,[1,2] Xiaofeng Qian,[1,2] Jian Shen,[5] Feng Zhang,[2] and Guoqiang Li[2]

[1] *Translational Medicine Research Center, Jiangning Hospital Affiliated to Nanjing Medical University, Nanjing 211100, China*
[2] *Key Laboratory of Living Donor Liver Transplantation of Ministry of Public Health, Nanjing 210029, China*
[3] *General Surgery, Second People's Hospital of Nantong, Nantong 226002, China*
[4] *Nanjing Children's Hospital Affiliated to Nanjing Medical University, Nanjing 210008, China*
[5] *Department of General Surgery, Second Affiliated Hospital of Nanjing Medical University, Nanjing 210000, China*

Correspondence should be addressed to Feng Zhang; zhangf@njmu.edu.cn and Guoqiang Li; liguoqiang@njmu.edu.cn

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To investigate the relationship between interleukin-17 and proteins involved in fatty acid metabolism with respect to alcoholic liver disease, male ICR mice were randomized into five groups: control, alcoholic liver disease (ALD) at 4 weeks, 8 weeks, and 12 weeks, and anti-IL-17 antibody treated ALD. A proteomic approach was adopted to investigate changes in liver proteins between control and ALD groups. The proteomic analysis was performed by two-dimensional difference gel electrophoresis. Spots of interest were subsequently subjected to nanospray ionization tandem mass spectrometry (MS/MS) for protein identification. Additionally, expression levels of selected proteins were confirmed by western blot. Transcriptional levels of some selected proteins were determined by RT-PCR. Expression levels of 95 protein spots changed significantly (ratio >1.5, $P < 0.05$) during the development of ALD. Sterol regulatory element-binding protein-lc (SREBP-1c), carbohydrate response element binding protein (ChREBP), enoyl-coenzyme A hydratase (ECHS1), and peroxisome proliferator-activated receptor alpha (PPAR-α) were identified by MS/MS among the proteins shown to vary the most; increased IL-17 elevated the transcription of SREBP-1c and ChREBP but suppressed ECHS1 and PPAR-α. The interleukin-17 signaling pathway is involved in ALD development; anti-IL-17 antibody improved hepatic steatosis by suppressing interleukin-17-related fatty acid metabolism.

1. Introduction

Alcoholic liver disease is becoming more and more widespread but the mechanisms that underlie the condition remain unknown. The typical characteristics of alcoholic liver disease are hepatic steatosis (otherwise known as fatty liver), hepatitis, fibrosis, and cirrhosis. Hospital mortality rates for alcoholic hepatitis can be as high as 60%, and mortality is often due to failure and cirrhosis of the liver [1].

Complex immune responses play an important role in the development of alcoholic liver disease [2]. Chronic long-term intake of alcohol impairs the protective mechanisms of the gut; this results in increased serum levels of lipopolysaccharide (LPS) in the area of the portal vein, which in turn activates Kupffer cells. The activated Kupffer cells secrete inflammatory cytokines, such as tumor necrosis factor α (TNF-α), which initiate a cascade of inflammatory events that can lead to inflammation.

T helper 17 (TH17) cells are a newly discovered subset of T helper cells that are independent of the traditional lineages of TH1 and TH2 cells. TH17 cells appear to be involved in many autoimmune diseases [3]. Cytokine IL-17 is secreted mainly by TH17 cells and has comprehensive biological functions. The liver tissue of patients with alcoholic liver disease contains a large number of cells that secrete IL-17, and the degree of injury shows a positive correlation with the number of IL-17-positive cells that have infiltrated into the liver [4].

Hepatic steatosis is characterized by the accumulation of triglycerides in the liver. Further studies are required to

determine whether IL-17 promotes hepatic steatosis by interfering with fatty acid synthesis and metabolism.

The purpose of this study was to explore the relationship between IL-17 and proteins involved in fatty acid synthesis and metabolism and to search for new targets for pharmacotherapy to treat hepatic steatosis.

2. Material and Methods

2.1. Sample Collection and Preparation. Male mice of the strain ICR (8 weeks of age; 18–25 g; obtained from the Animal Center of Nanjing Medical University) were housed under controlled environmental conditions with respect to temperature (20–22°C), light (12-h light/12-h dark cycle), and humidity (50–70%), and received food and water ad libitum in accordance with the National Research Council's guide (Permit number 17-0956) for the care and use of laboratory animals. In this study we used a flexible and stable mouse model of alcoholic liver disease that represented an improvement on the model of Tsukamoto-French [5]. The male mice were divided randomly into five groups: control ($n = 6$), ALD (4W) ($n = 8$), ALD (8W) ($n = 12$), ALD (12W) ($n = 14$), and Anti-IL-17 antibody treated ALD ($n = 6$; this group was equivalent to the ALD (12W) group but the animals were treated with Anti-IL-17 antibody for the 8th of the experimental period). All mice were sacrificed after 12 h of fasting. Blood samples were collected for biochemical assays. The liver was removed, rinsed with ice-cold saline, and weighed. It was then frozen immediately in liquid nitrogen and stored at −80°C until further analysis or fixed in formalin and embedded in paraffin for evaluation by hematoxylin and eosin (HE) staining.

To analyse the effect of IL-17 in vivo, 8-to 12-week-old male ICR mice were injected intravenously with 1 μg of IL-17 dissolved in saline. The mice were then sacrificed and the livers were collected 16 hours later, control mice were injected with saline only.

2.2. Analysis of Liver Enzymes. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured by using an assay kit (Transaminase C, WAKO, Osaka, Japan). Levels of Gamma-glutamyltransferase (GGT), alkaline phosphatase (ALK-P), and total bilirubin were assayed using an automatic biochemical analyzer.

2.3. Cytokine Determination by ELISA. Serum IL-17 levels were determined by using a mouse enzyme-linked immunosorbent assay (ELISA) kit (Biosource, San Jose, CA, USA). Analyses were performed in accordance with the manufacturer's instructions.

The blood samples were collected After 6 h of fasting because the cytokine levels increased more rapidly than the transaminase levels and returned to almost normal levels within 12 h.

2.4. Flow Cytometry Analysis. Thl7 cells accounted for the proportion of mononuclear cell detection in liver tissue. Take some fresh liver tissue and grind it after cuting it to pieces, make it into suspension cells. Cells were resuspened in 5% fetal bovine serum RPMI1640 and filted through a 200 mesh metal mesh filter. After that, lymphocyte isolation liquid (Ficoll) was used to obtain mononuclear cells. Cell survival rate was determined by trypan blue with the survival rate of (98 + 3.2)%. Mononuclear cells were stained with FITC rat anti-mouse—CD 4 and APC rat anti-mouse-IL-17 antibody (BD Pharmingen, San Diego,CA, USA). Result were detected through flow cytometry, negative tube was used as control.

2.5. Two-Dimensional Difference Gel Electrophoresis (2-DE) and Protein Identification by MALDI-TOF/TOF. Each sample containing 120 μg proteins was loaded in IPG strips (24 cm, pH 3–10, NL, GE Healthcare, San Francisco, CA, USA) for rehydration. Following isoelectric focusing, the IPG strips were equilibrated to maintain the fully reduced state of the proteins, and to prevent the reoxidation of thiol groups during electrophoresis. Second-dimensional electrophoresis was performed on 12.5% SDS gels in an Ettan-DALT-six electrophoresis unit (GE Healthcare, San Francisco, CA, USA) and gels were visualized by silver staining as described previously. In this experiment, data were generated from three independently run gels at each group (12 gels in total). The stained gels were scanned, and the ImageMasterTM 2-D Platinum Software (Version 5.0, Amersham Bioscience, Swiss Institute of Bioinformatics, Geneva, Switzerland) was used for spot detection, quantification, and comparative analyses. We averaged the values from the three independent samples, respectively, calculated the means and standard deviations, and assessed statistical significance with Student's t-tests using ImageMasterTM 2D platinum software. P values less than 0.05 were considered statistically significant.

The differentially expressed protein spots were then excised and identified. Briefly, the protein spots were dehydrated in acetonitrile (ACN), reduced using 10 mM DTT/25 mM NH4HCO3 at 56°C for 1 h, and subsequently alkylated with 55 mM iodoacetamide/25 mM NH4HCO3 in the dark at room temperature for 45 min. Gel fragments were thoroughly washed with 25 mM NH4HCO3, 50% ACN, and 100% ACN and dried in a SpeedVac. Dried gel fragments were reswollen with 2–3 μL trypsin solution (Promega, Madison, WI, USA) (10 ng/μL in 25 mM NH4HCO3) at 4°C for 30 min. Excess liquid was discarded and the gel plugs were incubated at 37°C for 12 h. Trifluoroacetic acid (TFA) at a final concentration of 0.1% was added to stop the digestive reaction. The digests were immediately spotted onto 600 μm AnchorChips (Bruker Daltonics, Bremen, Germany). Spotting was achieved by pipetting 1 μL of the analyte onto the MALDI target plate in duplicate and subsequently adding 0.05 μL of 2 mg/mL α-HCCA in 0.1% TFA/33% acetonitrile containing 2 mM (NH4)3PO4. All samples were analyzed on a time-of-flight Ultraflex II mass spectrometer (Bruker Daltonics) set to the positive-ion reflectron mode.

Each acquired mass spectrum (m/z range, 700–4000; resolution, 15000–20000) was processed using the FlexAnalysis v2.4 and Biotools 3.0 (Bruker Daltonics) software packages with the following specifications: peak detection algorithm: Sort Neaten Assign and Place (SNAP); S/N threshold: 3; and quality factor threshold: 50. Trypsic autodigestion ion picks (842.51, 1045.56, 2211.10, and 2225.12 Da) were used as

internal standards to validate the external calibration procedure. Matrix and/or autoproteolytic trypsin fragments were removed. The masses of the peptides obtained were cross-referenced with the NCBI mouse database with the use of Mascot (v2.1.03) in an automated mode that used the following search parameters: a significant protein score at $P < 0.05$; minimum mass accuracy: 100 ppm; trypsin as the enzyme; one missed cleavage sites allowed; cysteine carbamidomethylation, acrylamide modified cysteine, methionine oxidation and similarity of pI, and the relative molecular mass specified, with the minimum sequence coverage at 15%. Protein identification was confirmed by sequence information automatically obtained from the MS/MS analysis. Acquired MS/MS spectra were also processed using the software FlexAnalysisTM 2.4 using a SNAP method set at a signal-to-noise ratio threshold of 3.0. The MS/MS spectra were automatically searched in the NCBI mouse database by Mascot (v2.4). Search parameters for MS/MS data were set to 100 ppm for the precursor ion and 0.3 Da for the fragment ions. Cleavage specificity and covalent modifications were considered, as described above. The score was higher than the minimum significant individual ion score ($P < 0.05$). All significant MS/MS identifications by Mascot were manually verified for spectral quality and matching y and b ion series. When multiple entries corresponded to slightly different sequences, only the database entry that exhibited the highest number of matching peptides was included.

2.6. Western Blotting. Protein levels were determined by western blotting. The amount of protein was 20 μg. After the protein had been transferred to polyvinylidene fluoride membranes, the membranes were blocked in phosphate buffered saline (PBS) with 0.1% Tween, 5% (w/v) skim milk powder, and 10% horse serum for 2 hours and then incubated with the appropriate specific antibody (anti-sterol regulatory element binding protein-lc (SREBP-1c) antibody, 1 : 300; anti-carbohydrate response element binding protein (ChREBP) antibody, 1 : 150; anti-enoyl-coenzyme A hydratase (ECHS1) antibody, 1 : 300; anti-peroxisome proliferator-activated receptor alpha (PPAR-α) antibody, 1 : 100) overnight at 4°C. The membranes were then incubated in a 1 : 2500 dilution of horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at 37°C for 1 h. The results were visualized by using a chemiluminescence detection system (Pierce ECL Western Blotting Substrate, Thermo Scientific), followed by exposure to autoradiography film (Kodak Biomax XAR film). The expression in each sample was analyzed with Quantity One 4.4.0 software (Bio-Rad Laboratories, Hercules, CA, USA).

2.7. Cell Culture and Transient Transfection Assays. Human L02 hepatocytes and AML-12 cells (acquired from ATCC Rockefeller, MD, USA) were grown in Dulbecco's modified Eagle's medium (Cambrex, Verviers, Belgium) containing 10% fetal bovine serum (FBS) and antibiotics. Cells were treated with 5, 10, or 25 ng/mL IL-17 for 24 hours before reverse-transcription polymerase chain reaction (RT-PCR) analysis.

2.8. Fluorescence Quantitative RT-PCR Analysis. Primer Premier 5.0 software was used to design appropriate primers in accordance with the manufacturer's instructions. Total RNA was extracted from liver tissue with Trizol reagent (Gibco, Carlsbad, CA, USA) and used to prepare cDNA by reverse transcription. Aliquots of 0.4 μL of cDNA template were added to each 25 μL reaction mix. After labeling, each tube was placed in quantitative fluorescence detector (Rotor-Gene 3000) for PCR amplification. The concentration of each transcript of interest, together with that of β-actin, was calculated directly by the machine. The concentration of each transcript was normalized by dividing it by the concentration of β-actin to give the relative amount of transcript.

2.9. Statistical Analysis. Data are expressed as the mean ± standard error (SE). Analysis of variance was used to compare the means of three groups, followed by Newman-Keuls test to determine the statistical significance between two groups. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Hepatic Histology. Liver tissue from all groups was stained with HE (Figure 1). Typical steatosis was observed in the ALD (8, 12W) group as compared with the other groups. Some slightly form of Hepatic Hepatitis was observed in the ALD (12W) group. The form of steatosis was obviously ameliorated after treatment with Antibody-IL-17 from the 8th week.

3.2. Biochemical Indicators. Serum ALT, AST, and GGT levels were highest in the ALD (12W) group as compared with the other groups. All the ALD groups had higher serum ALT, AST, and GGT levels than the control group (Data not shown). In the process of establishing the mouse model, we discovered that the serum IL-17 level was highest in the 8th month followed by the 12th month (Figure 2).

3.3. The Level of IL-17 in Liver Tissue of Every ALD Group. To determine whether T lymphocytes from ALD groups might contribute to high circulating IL-17 level, we analyzed their PBMCs by flow cytometry (Figure 3). The proportions of IL-17+ cells in every group are (1.4)%, (2.3)%, (3.5)%, and (0.56)%, respectively.

3.4. Quantitative Proteomic Analysis. To identify protein molecules that were interesting with respect to alcoholic liver disease, we compared protein expression in the ALD and control groups by means of 2D-PAGE (Figure 4). On the basis of the statistical analysis, 95 protein spots changed significantly between the control and ALD groups. Among them, the expression of 35 proteins changed more than 1.5-fold, and six of these proteins showed more than a twofold change in abundance. These protein spots were characterized by tandem mass spectrometry (MS/MS). Among the 35 protein spots analyzed, 14 contained more than one protein; these spots were considered not to have been clearly identified and were excluded from further analysis. Six spots could not be

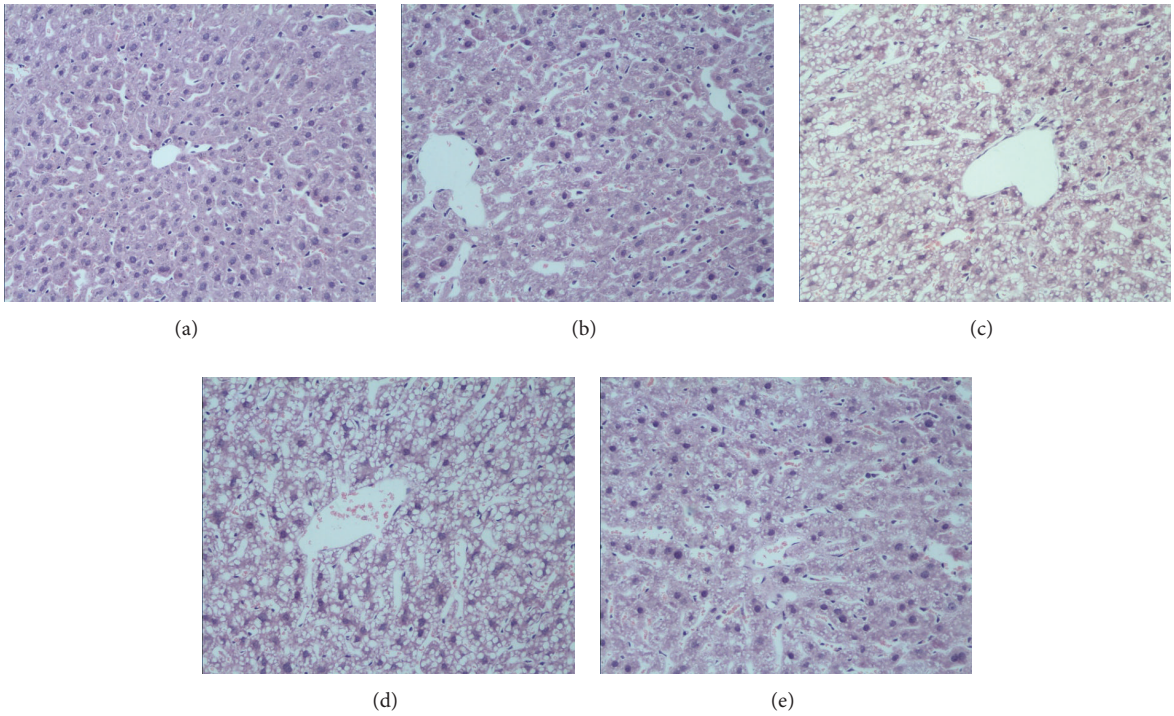

Figure 1: Hepatic pathology. Liver sections stained with hematoxylin and eosin from mice. (a) Control group, (b) ALD (4W) group, (c) ALD (8W) group, (d) ALD (12W) group, and (e) Anti-IL-17 antibody treated ALD group. Original magnification ×200. Data are representative of three separate experiments.

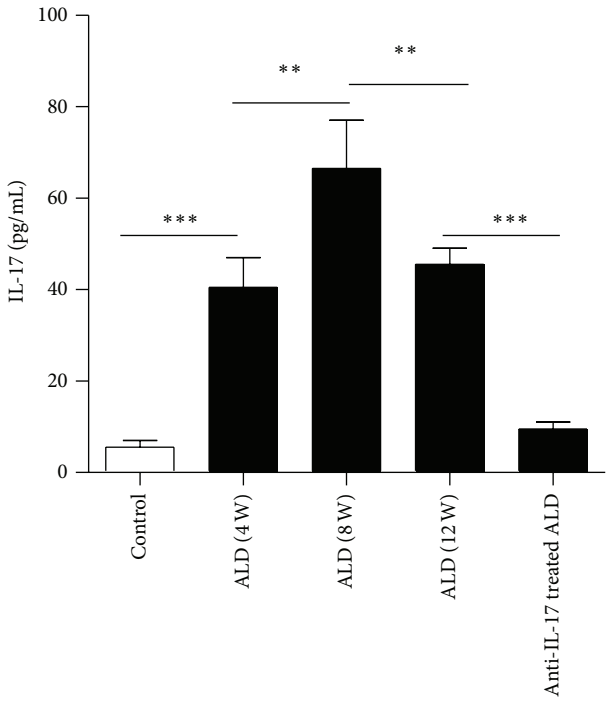

Figure 2: Serum IL-17 levels as determined by ELISA in the different stages of ALD. (a) Control group, (b) ALD (4W) group, (c) ALD (8W) group, (d) ALD (12W) group, and (e) Anti-IL-17 antibody treated ALD group. The data indicate Mean ± SEM of three separate experiments. $^{**}P < 0.01$; $^{***}P < 0.001$.

identified at all. The remaining 15 proteins were identified unambiguously. Among them, proteins 1438, 2447, 3211, and 3232 were identified as SREBP-1c, ChREBP, ECHS1, and PPAR-α, which are key proteins in fatty acid synthesis and metabolism. When the ALD groups were compared with the control group, the expression levels of SREBP-1c and ChREBP were elevated whereas the levels of ECHS1 and PPAR-α were reduced. The results of western blotting were in accordance with those of the quantitative proteomic analysis (Figure 5).

3.5. Fluorescence Quantitative RT-PCR Analysis. The effect of increased levels of IL-17 on the transcription of the genes for the above mentioned proteins was examined in vivo and in vitro. The results of the quantitative RT-PCR analysis showed that, both in vivo and in vitro, the transcription of SREBP-1c and ChREBP was elevated, whereas the transcription of ECHS1 and PPAR-α was suppressed as the level of IL-17 was increased (Figures 6 and 7).

4. Discussion

Imbalances of both the immune system and metabolism are involved in the development of alcohol-induced liver injury. However, so far, the underlying mechanisms have not been elucidated completely. The innate immune response and the adaptive immune response both participate in the etiology of alcoholic liver disease [2]. The innate immune system includes macrophages (Kupffer cells), dendritic cells (DC)

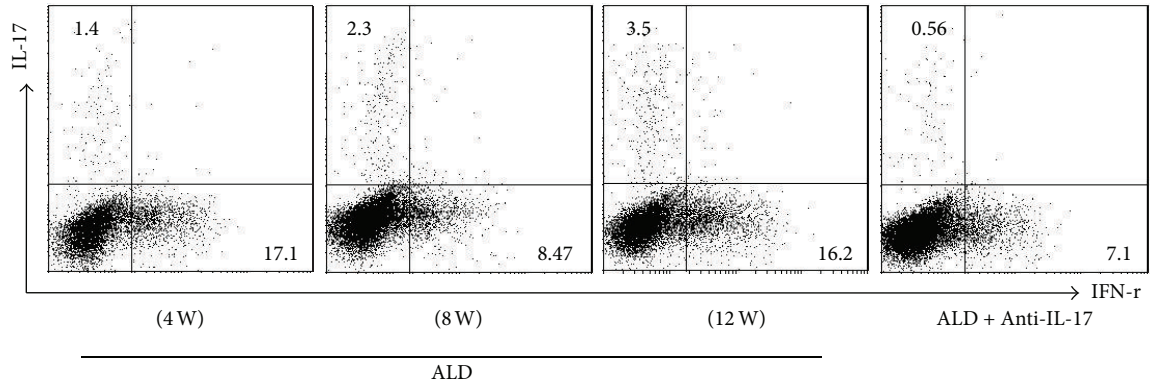

Figure 3: Activation of a peripheral IL-17-secreting phenotype in liver tissue of every ALD group. Data are representative of three separate experiments.

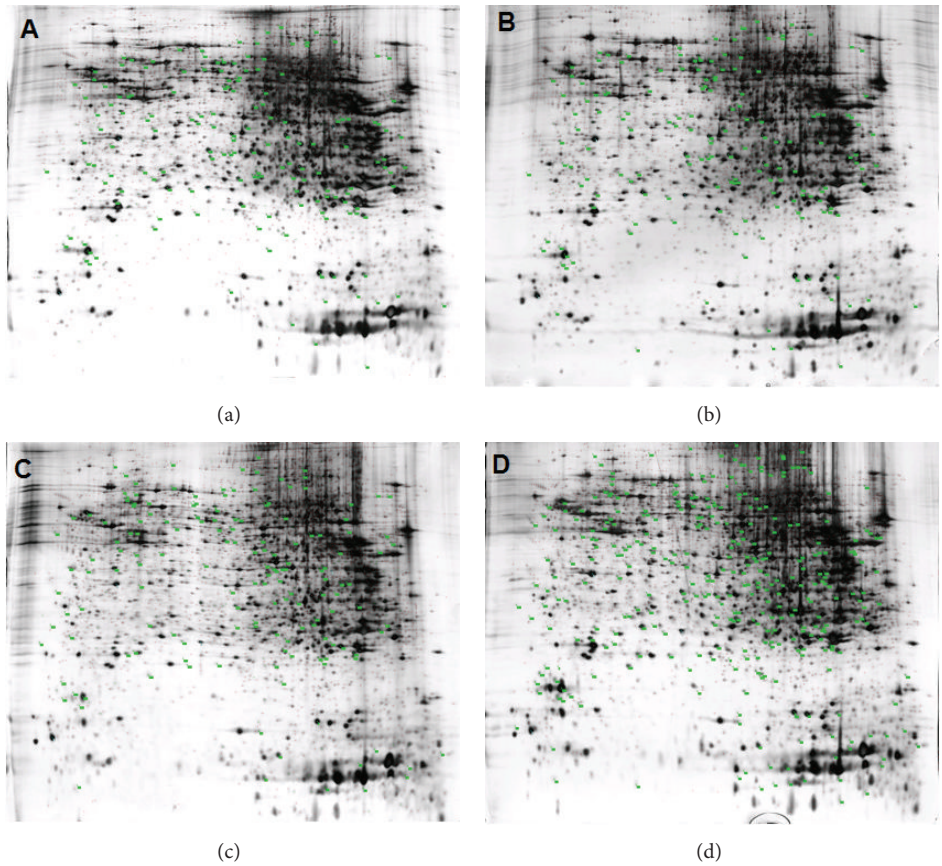

Figure 4: Differentially expressed protein spots displayed in 2DE images. Representative 2DE images from: (a) the ALD (4W) group, (b) the ALD (8W) group, (c) the ALD (12W) group, and (d) Anti-IL-17 antibody treated ALD group. Data are representative of three separate experiments.

cells, natural killer (NK) cells, natural killer T (NKT) cells, inflammatory cytokines, acute phase response proteins, and chemokines [6, 7]. The adaptive immune response, especially the autoimmune response, increases the severity of alcohol-induced liver injury.

IL-17, a more recently discovered cytokine, has a wide range of biological functions, such as the recruitment of neutrophils and the release of other inflammatory factors. These effects are mediated by IL-17 receptors, which are expressed in multiple tissues: vascular endothelial cells, peripheral

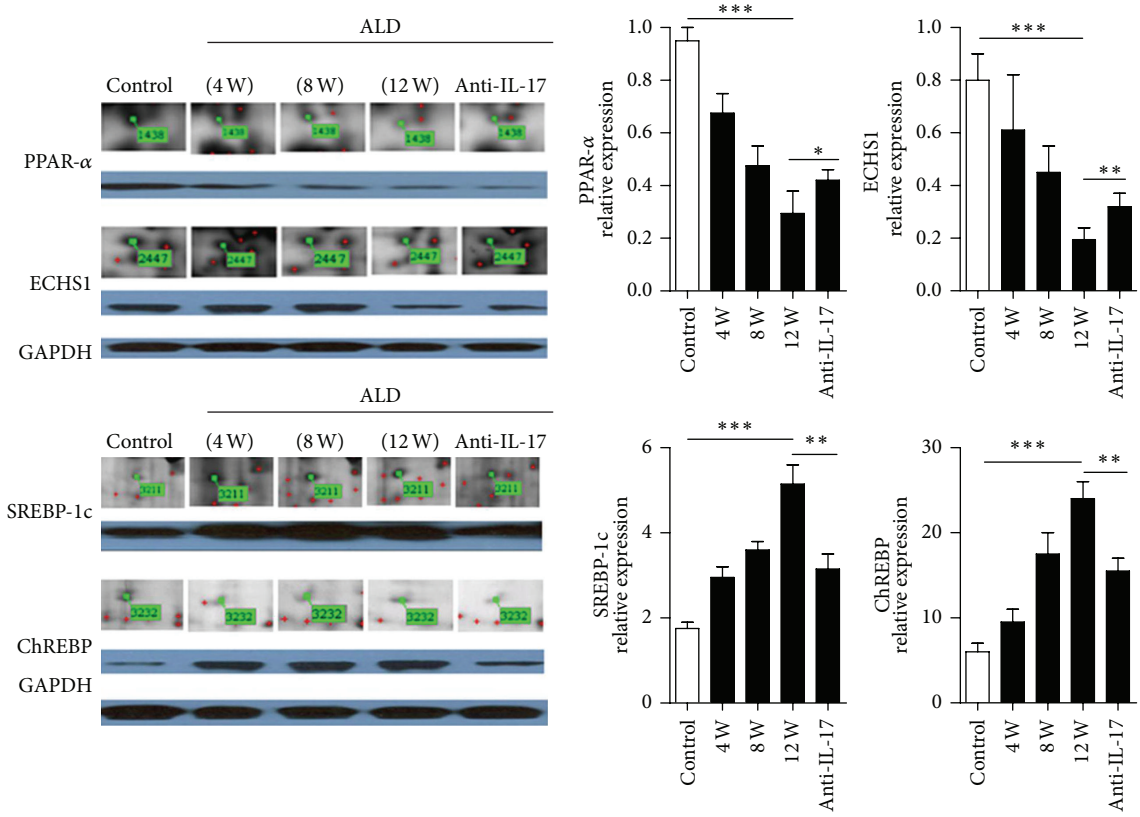

FIGURE 5: Western blot validation of four selected proteins. Hepatic expression levels of sterol regulatory element-binding protein-lc (SREBP-1c), carbohydrate response element binding protein (ChREBP), enoyl-coenzyme A hydratase (ECHS1), and peroxisome proliferator-activated receptor alpha (PPAR-α) in the ALD and control groups. The results correlated well with the quantification of the DIGE images, which is also shown. The data indicate Mean ± SEM of five separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

T cells, and B- cell lineages, together with fibroblast, lung, myelomonocytic, and marrow stromal cells [8–11]. It has been reported that large numbers of TH17 cells and high levels of serum IL-17 are found in many autoimmune diseases, including multiple sclerosis (MS), inflammatory bowel diseases, rheumatoid arthritis, Lyme disease, contact dermatitis, psoriasis, uveitis, and experimental autoimmune encephalomyelitis (EAE) [12–14]. IL-17 is involved in the development of alcoholic liver disease [8]. Our findings revealed that serum IL-17 levels increased progressively with increasing time in the mouse model of alcoholic liver disease and the serum levels of ALT, AST, and GGT also increased in parallelism.

In this study, we systematically analyzed the proteome during different stages of alcoholic liver disease in our mouse model, which might help to elucidate the mechanisms that are involved in the progression of the disease. We identified 95 protein spots that changed significantly between the control and ALD groups. By using MS/MS analysis, four of the proteins were identified as SREBP-1c, ChREBP, ECHS1, and PPAR-α.

SREBPs are transcription factors of the basic-helix-loop-helix leucine zipper (bHLH-Zip) family and bind to the DNA sequence TCACNCCAC, which constitutes the sterol regulatory element. SREBPs are encoded by the genes SREBP1 and SREBP2 [15]. Expression of the SREBP-1 gene produces two different protein isoforms, SREBP-1a and -1c. SREBP-1c is responsible for regulating the genes required for de novo lipogenesis [16].

ChREBP is a recently described transcription factor that also belongs to the bHLH-Zip family [17] and regulates carbohydrate metabolism in the liver [18]. Increased expression of ChREBP increases the activity of lipase, which leads to a high level of fatty acids in the liver [19].

ECHS1 functions in the second step of the mitochondrial fatty acid β-oxidation pathway of fatty acid metabolism. It catalyzes the hydration of 2-trans-enoyl-coenzyme A (CoA) intermediates to L-3-hydroxyacyl-CoAs. Oxidation of fatty acids occurs in the subcellular organelles, with β-oxidation confined to the mitochondria and peroxisomes and ω-oxidation occurring in the endoplasmic reticulum [20, 21]. Impairment of the function of ECHS1 inhibits the β-oxidation of fatty acids in the mitochondria and can account for the storage of excess lipid in the liver [22–24]. PPAR-α belongs to the peroxisome proliferator-activated receptor superfamily and regulates the transcription of many genes that encode enzymes involved in fatty acid β-oxidation.

It was of interest to determine whether the levels of these four proteins were correlated tightly with the serum IL-17 level. We found that as the level of IL-17 was increased, the

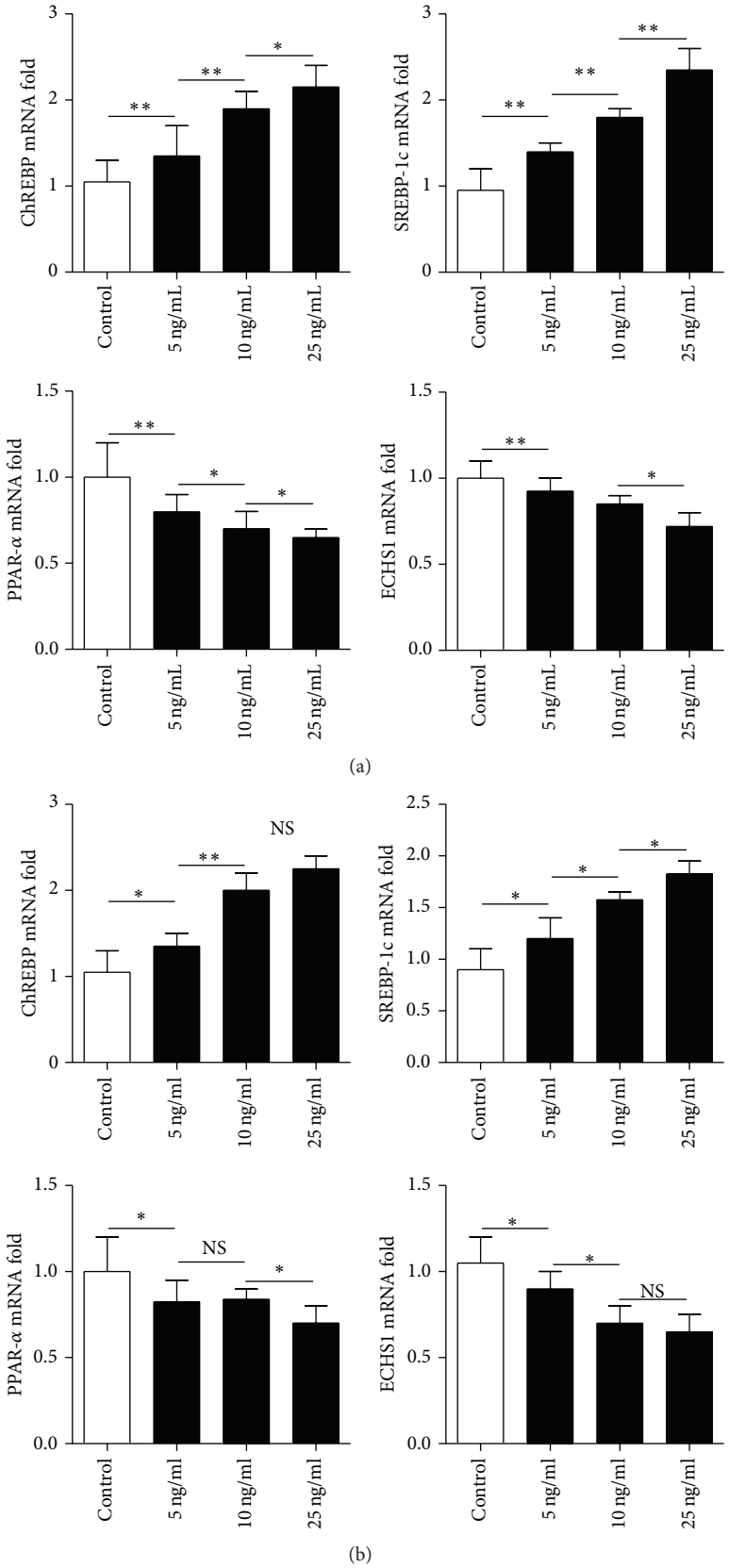

Figure 6: The effect of different serum levels of IL-17 in L02 cells (a) or in AML-12 cells (b) on the transcript levels for the four selected proteins. Gene expression levels in mice from the control group were set at 1. The data indicate Mean ± SEM of five separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; NS: no significance.

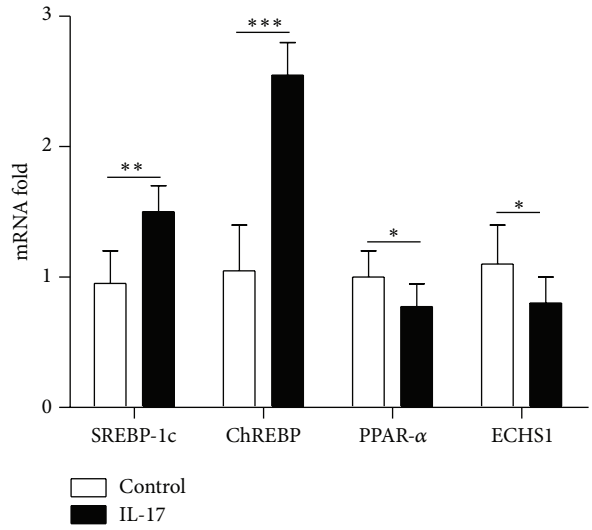

Figure 7: The effect of an intravenous dose of 1 μg of IL-17 on the transcript levels for the four selected proteins in vivo. The expression levels of SREBP-1c, ChREBP, ECHS1, and PPAR-α mRNA were determined in livers collected 16 hours after being intravenously injected with IL-17. Gene expression levels in mice from the control group were set at 1. The data indicate Mean ± SEM of three separate experiments. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

transcription of SREBP-1c and ChREBP was also increased, whereas the transcription of ECHS1 and PPAR-α was suppressed, both in vivo and in vitro. Allicin improved the degree of hepatic steatosis by reducing the level of IL-17 and thus affecting IL-17-related fatty acid synthesis and metabolism.

5. Conclusion

Our study indicated that the interleukin-17 signaling pathway is involved in the development of ALD. Increased IL-17 elevated the transcription of SREBP-1c and ChREBP but suppressed ECHS1 and PPAR-α; anti-IL-17 antibody improved hepatic steatosis by suppressing interleukin-17-related fatty acid metabolism.

Authors' Contribution

Weidong Shi, Qiang Zhu, and Jian Gu contributed equally to this work.

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Review Article

The Role of T Helper (T_H)17 Cells as a Double-Edged Sword in the Interplay of Infection and Autoimmunity with a Focus on Xenobiotic-Induced Immunomodulation

Nasr Y. A. Hemdan,[1,2] Ahmed M. Abu El-Saad,[2] and Ulrich Sack[3,4]

[1] *Department of Anesthesiology and Intensive Care Medicine, SG Sepsis Research, Center for Sepsis Control and Care, Jena University Hospital, 07740 Jena, Germany*
[2] *Department of Zoology, Faculty of Science, University of Alexandria, Moharram Bey, Alexandria 21511, Egypt*
[3] *Institute of Clinical Immunology, Medical Faculty, University of Leipzig, Johannisallee 30, 04103 Leipzig, Germany*
[4] *University of Leipzig, Translational Centre for Regenerative Medicine, Leipzig, Germany*

Correspondence should be addressed to Nasr Y. A. Hemdan; nhemdan@yahoo.de

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Extensive research in recent years suggests that exposure to xenobiotic stimuli plays a critical role in autoimmunity induction and severity and that the resulting response would be exacerbated in individuals with an infection-aroused immune system. In this context, heavy metals constitute a prominent category of xenobiotic substances, known to alter divergent immune cell responses in accidentally and occupationally exposed individuals, thereby increasing the susceptibility to autoimmunity and cancer, especially when accompanied by inflammation-triggered persistent sensitization. This perception is learned from experimental models of infection and epidemiologic studies and clearly underscores the interplay of exposure to such immunomodulatory elements with pre- or postexposure infectious events. Further, the T_H17 cell subset, known to be associated with a growing list of autoimmune manifestations, may be the "superstar" at the interface of xenobiotic exposure and autoimmunity. In this review, the most recently established links to this nomination are short-listed to create a framework to better understand new insights into T_H17's contributions to autoimmunity.

1. Introduction

Long-term exposure to xenobiotic substances induces hyperactivity of the immune system, thereby increasing the incidence of autoimmune diseases (AD), especially in infection-aroused systems. Circumstances dating back to earlier exposure, as in case of heavy-metal industry workers or current exposure as in individuals harboring amalgam teeth filling, favor incidence of inflammatory processes and most likely AD [1–4]. Exposure to infectious agents leads to the induction of various cellular pathways essential to the microbe's infectivity, survival, and virulence, thus making it difficult for the microbe to go undetected by the host's immune system [5]. Upon pathogen recognition, production of a proinflammatory response, primarily by macrophages, NK and NKT cells, is the subsequent event in the early phase of the infection [5, 6]. Further, the coordination between innate and adaptive immune defense systems ensures a successful eradication of pathogens, and such developed cytokine milieu determines the induction of a specific T-cell-mediated response that is critical for an effective and complete pathogen clearance. However, whether the induction of a strong host inflammation constitutes an adaptive advantage to the host or pathogen remains debated. Indeed, many disorders, including AD [7] and cancer [8], are associated with and maintained by chronic inflammation; for review, see [9, 10]. The association of cancer incidence with exposure to heavy metals, such as cadmium [11], or following attainment of chronic inflammation, as in case of colitis-associated cancer, has been widely anticipated.

Research on T_H17 cells has suggested a crucial role in autoimmunity. Despite developing autoimmune signs in the absence of detectable IL-17 levels, as in case of

choriomeningitis-virus-induced model of type 1 diabetes [12], a key role of T_H17 cells and their related molecules was underscored in many previously assigned "T_H1-mediated" AD including rheumatoid arthritis (RA), psoriasis, systemic lupus erythematosus (SLE), and multiple sclerosis (MS), as well as, the experimental autoimmune encephalomyelitis—EAE [7, 9, 13–15]. Variations in disease susceptibility or outcome may be a result of co-exposure to one or multiple xenobiotic substances or infectious pathogens, so that a xenobiotic-induced polarized immune response triggers the development of AD in genetically predisposed individuals [1, 2, 4, 16–19]. The IL-17 response, while constituting a protective arm defending the body against various infections, also functions as a double-edged sword constituting a risk factor that mediates the development and/or induction of AD, mostly manifested following pathogenic and xenobiotic-induced chronic inflammation; it then acts as a double-edged sword, constituting a risk factor that mediates the development and/or induction of AD, mostly manifested following pathogenic and xenobiotic-induced chronic inflammation. In the next sections, we revisit our view on the T_H17 cells' role in autoimmunity [9] and provide a brief description of the double-sided role of T_H17 cells and their related molecules IL-17, IL-21, and IL-22 and their participation at the initiation/induction of autoimmunity as a consequence of xenobiotic exposure.

2. T_H17 Cells and Their Associated Molecules Link Infection to Autoimmunity

T cells differentiate and expand into distinct lineages including T_H1, T_H2, iT_{Reg}, and T_H17 cells [9], whereas iT_{Reg} cells differentiate under subimmunogenic antigen presentation both during chronic inflammation and under normal homeostatic conditions of the gut and function to control severe chronic allergic inflammation and as a barrier to the eradication of tumors [20, 21]. T_H17 cells derive from $CD161^+$ precursors in umbilical cord blood and newborn thymus [22] and likely constitute the most prominent T cell subset at the crossroads of infection and autoimmunity. The contributions of T_H17 cells have prompted and were the results of intensive scientific research, which is reflected by a growing list of publications in this field (Figure 1), and have in turn led to identification of T_H17 cells' markers, as well as, their differentiation and commitment program [23]. Figure 2 demonstrates the major T cell subsets, their interaction with T_H17 cells and the main contributions of the latter.

Recently, several groups delivered compelling evidence of the effects of T_H17-associated cytokines, namely IL-17, IL-21, IL-22, and IL-23, on inflammatory responses elicited by extracellular, as well as, facultative and obligate intracellular pathogens including bacteria and fungi. Exemplified contributions of IL-17 response to some infectious diseases are summarized in Table 1.

2.1. T_H17 Cells-Associated Molecules and Their Contributions to Anti-Infectious Responses. In comparison to the frequent appearance of T_H1, the relative rarity of T_H17 in inflamed

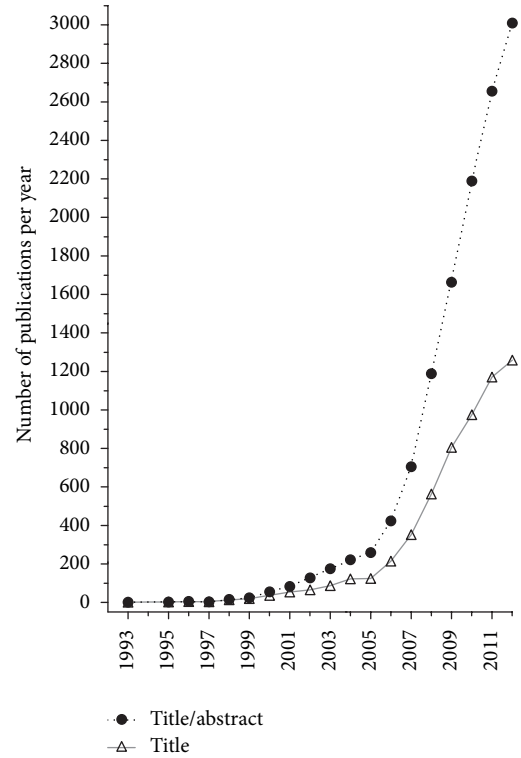

Figure 1: Frequencies of IL-17-producing T helper (T_H)17 cells and their related molecules as appeared in title or title/abstract of PubMed publications. The "keywords" {T_H17} OR {IL-17*} OR {IL-21} OR {IL-22} OR {IL-23*} OR {CTLA-8} OR {CCL20} OR {ROR*} and their synonyms combined with "publication date" {1993} through {2011} were given as search parameters.

tissues was attributed to their *RorC*-dependent expression of the oxidase IL4I1, which impairs CD3 signaling and hence constrains IL-2 production and cell proliferation [24]. As we recently reviewed, the recruitment of T_H17 cells to inflammatory tissues accompanies the expression of the chemokine receptor CCR6, in addition to CCR4, IL-23R (involved in the survival/maturation program of T_H17 cells) [9], and CD161 [22]. T_H17 cells are considered as potent inflammation inducers that, in addition to production of IL-17, differentially produce IL-6, IL-2, IL-8, IL-9, TNF-α, IL-17F, IL-21, IL-22, IL-26, IFN-γ, and the chemokine CCL20 and induce activation and recruitment of other cells including neutrophils that are pivotal in inflammation and AD [9, 25]. Through their cytokine/chemokine production, T_H17 cells act on a broad range of cell types initiating the expression of antibodies, metalloproteinases, prostaglandin E2 (PGE_2), and antimicrobial peptides and inducing cyclooxygenase 2 activity [9, 26], constituting, thereby, a link between innate and adaptive immune responses. In addition to its role as an arm of adaptive immunity, the current perception categorizes IL-17 also as an innate cytokine, produced mainly by NK cells [27] as well as by $\gamma\delta$ T cells [28–31], $CD8^+$ T cells, and mast cells [9, 32]. Indeed, detecting functional T_H17 cells and production of protective IL-17 during the early phase of the immune response [33–35] and the activation of T_H17 that

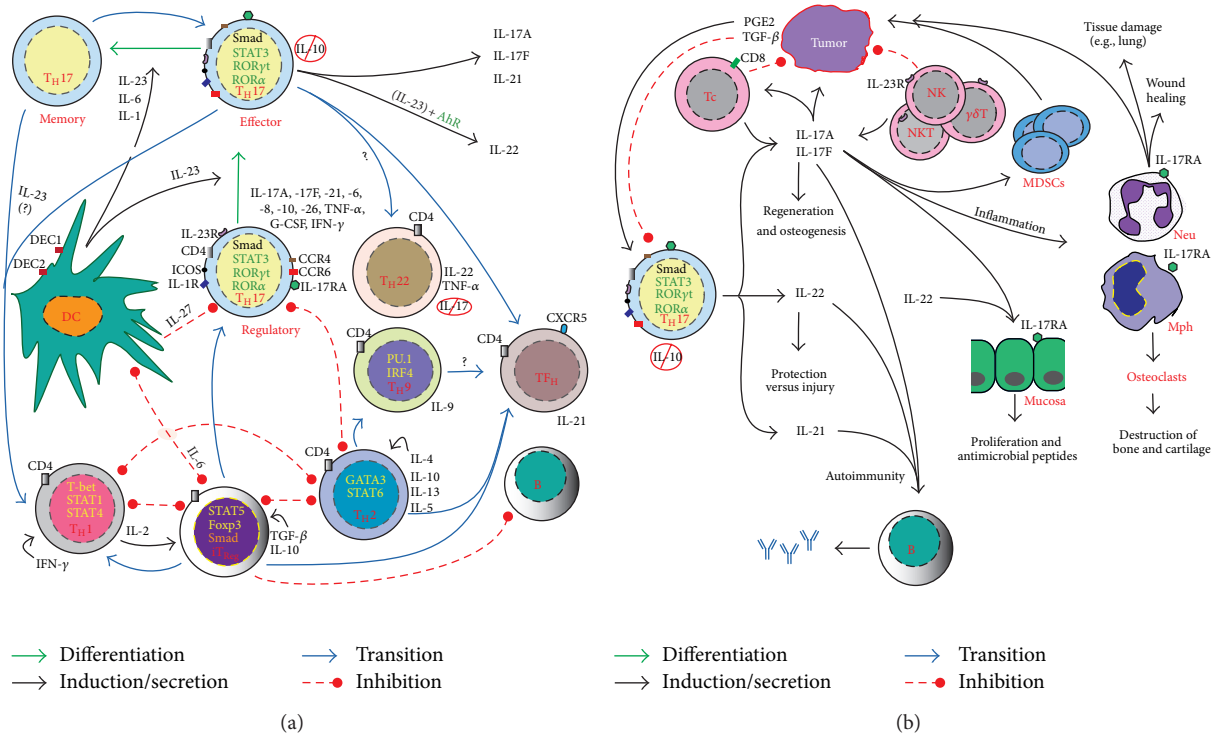

FIGURE 2: Differentiation and commitment of IL-17-producing T helper (T_H)17 cells in the midpoint of other coacting cells in favor of and against the harboring individual. Upon antigen recognition, presentation and cosignaling, naïve (T_H0) cells differentiate in the presence of distinct cytokine milieu into effecter (T_H1, T_H2, T_H9, T_H17, T_H22, and TFH) and T regulatory (T_{Reg}) cells. Signaling cytokine and other molecules activate lineage-unique transcription factors that ultimately mediate cell differentiation and maturation. Whereas activation of STAT1 induces T-bet expression, STAT6 signaling upregulates GATA3 expression; both cell lineages reciprocally regulate each other and regulate the generation of T_H17 cells through their hallmark effecter cytokines, IFN-γ and IL-4, respectively, though IFN-γ is produced by T_H17 cells in some disease settings and in response to certain infections. Differentiation of T_H17 necessitates costimulatory signals of CD28 and ICOS (the last is not mandatory) and the absence of T_H1 and T_H2 cytokines (IL-12/IFN- and IL-4) and their master transcription factors; a task taken over by TGF-β is to constrain T_H1 and T_H2 during T_H17 differentiation program. T_H17 and T_{Reg} are likely descendants of the same ancestor cell lineage, which differentiates in presence of low levels of TGF-β and IL-6/IL-21 or other proinflammatory cytokines (IL-1, TNF-α, and IL-18) into T_H17 cells; TGF-β signals through Smad2 protein pathway and is indispensable for induction of expression RORγt. High levels of TGF-β alone induce Foxp3 expression and hence T_{Reg} cell differentiation. Following their final commitment and upregulation of IL-23R, T_H17 cells require IL-23 signaling that is crucial for their survival and effecter functions including production of IL-22, as well as, cell plasticity including later production of IFN-γ. T_H22 may differentiate from T_H0 cells or through a local commitment of T_H17 cells homed in the epidermis. Contributions of T_H17 cells entail activations of aryl hydrocarbon receptor (AhR) signaling and production of IL-22 upon exposure to xenobiotic substances. IL-17 and IL-17F increase production of IL-6, IL-8, prostaglandin E2 (PGE2), monocyte chemotactic protein-(MCP-) 1, and the granulocyte colony-stimulating factor (G-CSF) by various cells including macrophages, fibroblasts, keratinocytes, and epithelial and endothelial cells and ultimately promote inflammatory diseases, AD, and/or cancer. These cytokines together with IL-21 and IL-22 are also implicated in mediating protective as well as pathogenic processes in various disease settings.

even precedes the differentiation of T_H1 cells [33], together with the later contribution of T_H17 [36], highlight the crucial importance of IL-17 and other T_H17-related cytokines in the early, as well as, the late phase of infection. The upregulation of TLR1 and TLR2 and dectin 1 by IL-17-producing $\gamma\delta$ T cells [37] supports this belief. Moreover, studies on nucleotide oligomerization domain knockout mice (Nod1$^{-/-}$ and Nod2$^{-/-}$) demonstrated that this "early" T_H17 response was Nod1- and Nod2-dependent, and hence they have been given the name innate (i)T_H17 cells [35]. Therefore, the new look of IL-17-producing cells comprises their contribution in building the first line of host defense, besides mediating and shaping adaptive responses required for ultimate clearance.

Based on a wealth of experimental data, the contribution of T_H17 cells to infection is manifold. As in the case of oral infection, the importance of IL-17 in protection against infection seems to be crucial to attain a mucosal barrier in the intestine as in case of *Salmonella* [30, 38], in mediating protection against oral brucellosis [39], promoting granulopoiesis through induction of granulocyte colony-stimulating factor G-CSF [40], and neutrophil influx through inducing neutrophil chemotactic CXCL8 (IL-8), macrophage chemotactic protein (MCP)-1, and macrophage inflammatory proteins- (MIP-) 1 and MIP-2 [9, 40, 41]. Additionally, we and others found that IL-17 activates phagocytosis and neutrophil cytotoxic activity [42]. Therefore, IL-17R$^{-/-}$ mice

Table 1: Mechanistic investigations on the role of T_H17 cells and their related molecules in various infectious diseases.

Diagnosis	Role	Observations on T_H17-associated molecules	Citations
		(1) Bacterial infections	
Bacillus subtilis	Pathogenic/protective	Increased lung inflammation and collagen deposition; delay in bacterial clearance in IL-17R$^{-/-}$ compared with WT counterparts	[118]
Francisella tularensis	Pathogenic	Intranasal inoculation induces T_H17 response and PGE_2 production in the lung; inhibition of PGE_2 production increased IFN-γ and decreased bacteremia	[119]
Saccharopolyspora rectivirgula	Pathogenic	Induction of IL-17-mediated hypersensitivity pneumonitis in mice; reduced lung inflammation and fibrosis in IL-17R$^{-/-}$ mice	[120, 121]
Klebsiella pneumoniae, Bordetella pertussis, & *S. pneumoniae*	Protective	Mounting of an IL-17 and IL-22 response; defects in T_H17 response increased susceptibility	[12, 122]
Staphylococcus aureus	Protective	High infection incidence correlated with defect in T_H17 response	[59]
Listeria monocytogenes	Protective	IL-17-mediated cross-protection following immunization with *M. pulmonis*; blockade of bacterial growth following transfer of IL-17-producing $\gamma\delta$ and double negative $\alpha\beta$ T cells into RAG2$^{-/-}$ mice	[30, 60]
Shigella flexneri	Protective	Restriction of bacterial growth mediated by T_H17 response	[28]
Citrobacter and Salmonella sp.	Protective	Innate T_H17 response-dependent protection; protective effect of IL-17 and IL-22; decrease in phagocytic activity and increase in bacterial burden upon IL-17 neutralization and its correlation with T_H17 response in Hg-exposed mice	[29, 35, 38] and Hemdan and Abul El-Saad, unpublished
Mycobacterium tuberculosis and M. bovis	Protective	IL-17$^{-/-}$ mice reveal a reduced IFN-γ production by CD4$^+$ T cells, impaired granuloma formation, and chemokine expression	[61]
Mycobacterium tuberculosis	Protective	Correlation of reduced T_H17 responses in patients with active tuberculosis with decreased expression of IL-6R on CD4$^+$ T cells	[123]
Chlamydia sp.	Protective	Enhanced bacterial growth and decreased mouse survival upon applying anti-IL-17 mAb	[34]
Inflammatory bowel disease—IBD (Crohn's disease and ulcerative colitis)	Pathogenic	Applying IL-17RA antagonist reversed the susceptible phenotype of C3H/HeN mice	[58]
	Pathogenic	Enhanced differentiation T_H17 and IL-17 expression levels and NK activities in IBD	[124, 125]
	Protective	IL-22 mediated protection against IBD	[54]
		(2) Protozoal infection	
Toxoplasma gondii	Pathogenic	IL-23-mediated IL-22 and MMP-2 upregulation in the ileum of infected mice; MMP-2 deficiency offered protection	[47]
	Protective	Increased mortality in IL-17$^{-/-}$ mice	[126]
		(3) Fungal infections	
Candida sp.	Protective	Involvement of IL-17, IL-17F, IL-22, and IL-23 in mediating natural defense against candidiasis	[45]
Aspergillus fumigatus		Induced IL-17 response mediates pathogen clearance	[127]
		(4) Viral infection	
Theiler's murine encephalomyelitis virus infection	Pathogenic	Induction of antiapoptotic molecules by IL-17 and thereby promoting persistent infection; boosting lytic function of CTLs and ameliorating disease upon neutralizing IL-17; association of lower T_H17 with higher virus-specific CD8$^+$ T cell responses in resistant mouse than in susceptible strain	[128]

Table 1: Continued.

Diagnosis	Role	Observations on T_H17-associated molecules	Citations
Respiratory syncytial virus (RSV)	Pathogenic	Elevated IL-6 and IL-17 levels in tracheal aspirate samples from severely ill infants and in infected mice; IL-17 blockade decreased the exacerbated disease via increasing RSV-specific $CD8^+$ T cells, T-bet, IFN-γ, eomesodermin, and granzyme B	[129]
HBV	Pathogenic/protective	Distinct effects associated with heterogeneous T_H17 populations: IL-17 with inflammation and ALT levels, IL-22 with protection of hepatocytes, and IL-21 with virus clearance	[130]
HCV	Pathogenic	Hepatitis-C-virus-infected patient revealed upregulated T_H17 cell cytokines that became downregulated by combined treatment with pegylated IFN and ribavirin	[131]
Simian immunodeficiency virus (SIV)/HIV	Pathogenic	Induction of TGF-β and IL-18 during the acute phase in SIV-infected rhesus macaques proposed to be associated with induction of IL-17-producing NKT cells	[132]
	Protective	Association of disease progression with loss of T_H17 and induction of T_{Reg} cells; T_H17 cell frequency correlated negatively with viral load	[63, 133, 134]
Herpes simplex virus (HSV-1)		Infiltration of T_H1 preceded T_H17 cells, the latter showed lower responsiveness ability to HSV-1; diminished stromal keratitis severity in IL-$17R^{-/-}$-infected mice and upon IL-17 neutralization in WT mice	[135]
		(5) Nematode infection	
Trichinella spiralis	Pathogenic	Correlation of T_H17 response with increase of smooth muscle contraction probably causing gut dysfunction; association of IL-17/IL-23 axis induction with increased mortality in mice coinfected with malaria and nematode	[136]

revealed increased systemic dissemination of *S. typhimurium* from the gut [29]. The same strategy seems to be attained to combat extracellular pathogens such as *Klebsiella pneumoniae* [12] and fungal infections (e.g., *Candida* sp.), mainly due to defective IL-17 immunity [43, 44], mediated by eliciting production of autoantibodies (AAs) against IL-17, IL-17F, and IL-22 that contribute to chronic mucocutaneous candidiasis [45, 46]. A third arm of T_H17 cells is built through IL-22, which is also produced by other cell types including NK22 and lymphoid tissue inducer cells [47], as well as by skin homing T_H22 cells [48, 49]. Besides its accepted role against infection, it induces tissue repair offering protection against injury [49]. Although both IL-22 and IL-17 or IL-17F synergize to stimulate expression of human beta-defensin-(HBD-) 2, S100 calcium binding protein A9 (S100A9) and enhanced the expression of S100A7 and S100A8 [9], IL-22, rather than IL-17, seems to contribute more to the epidermal and mucosal immunity [47, 49]. It synergizes with TNF-α to induce secretion of initial complement factors C1r and C1s, antimicrobial peptides S100A7 and HBD-2, and antimicrobial chemokines CXCL-9/-10/-11 in primary human keratinocytes [50]. In a three-dimensional skin infection model, stimulation of keratinocytes with T_H22 supernatants or by adding IL-22 plus TNF-α effectively inhibited *C. albicans* growth and maintained epithelial survival, and the combinatorial stimulation of keratinocytes with IL-22 plus TNF-α most effectively conserved the integrity of the epidermal barrier as compared with IFN-γ, IL-17, IL-22, or TNF-α alone [50]. IL-22 also functions to induce an acute phase systemic response that extends beyond IL-22R-expressing cells and revealed diverse significant impact on coagulation and cellular constituents of blood, in addition to induction of thymic atrophy, body weight loss, and renal proximal tubule metabolic activity and biochemical changes in the liver, including induction of fibrinogen, CXCL1, and serum amyloid A [51]. Besides its contribution to protection against bacterial infection [50, 52], IL-22 plays an important role in protection against viral infection, for example, hepatitis B virus [53]. On the other side, IL-22 is implicated in the induction of IBD [54] and AD such as experimental autoimmune myocarditis [9] and psoriatic disease through the induction of keratinocyte proliferation and cytokine and chemokine release [7]. This reflects the dark side of the T_H17 story; that is, a promoted T_H17 response may reflect a current or predict incidence of AD.

2.2. T_H1-T_H17 Cells Interaction during Infection. Although a protective role against intracellular bacteria such as *Listeria monocytogenes* [55] or *S. typhimurium* (N. Y. A. Hemdan and A. M. Abu El-Saad, unpublished data) may be attributed to T_H17 response, this may be rather compensatory to a defective IL-12/IFN-γ axis as previously demonstrated by N. N. Orgun et al. [56], or a complementary function to indirectly induce type 1 response mediated by APCs endowing thereby a protection against infection, as in case of the obligate intracellular bacteria *Chlamydia muridarum* [57]. In case of infection with *S. typhimurium* and *C. muridarum*, neutralizing IL-17 significantly reduced pathogen-specific T_H1 but promoted higher T_H2 responses. DCs isolated from IL-17-neutralized

mice demonstrated lower expression of CD40, MHC II, and IL-12 production, but higher level of IL-10 compared with control mice [57]. Furthermore, neutralizing IL-17 in case of *S. typhimurium* significantly reduced phagocytosis as well as T_H1 cytokine production (N. Y. A. Hemdan and A. M. Abu El-Saad, unpublished data). Moreover, delivery of an IL-17R antagonist that resulted in a 50% reduction in the neutrophilic infiltration in lungs following *Chlamydia* infection reversed the susceptible phenotype of C3H/HeN mice [58], indicating a key role of IL-17 in induction of neutrophil infiltration. The compromised IL-17 response in HIS (Job's syndrome) patients that contributed to higher susceptibility to *Staphylococcus aureus* infection [59] is evidenced by a recent finding that coinfection with influenza A abrogated host defense, which was rescued by overexpression of IL-23 and markedly improved bacterial clearance [52]. Influenza A was found to inhibit T_H17 differentiation and substantially decreased IL-17, IL-22, and IL-23 production after *S. aureus* infection. Interestingly, IL-17-mediated cross-protection against secondary *L. monocytogenes* infection has been demonstrated following immunization with *Mycoplasma pulmonis* [60].

In addition to the function of T_H17 cells as a substitute for a defective T_H1 response, synergism between T_H17 and T_H1 cells is proposed following infection or postvaccination challenge with *Mycobacterium* sp., based on the observation that IL-$17^{-/-}$ mice revealed a reduced IFN-γ production by $CD4^+$ T cells and impaired granuloma formation and expression of chemokines CXCL9, CXCL10, and CXCL11 [61]. Also, an enhanced T_H1 memory response in the lungs of vaccinated mice infected with *M. tuberculosis* was dependent upon IL-23/IL-17 axis [61]. In a model of TCR $\alpha\beta^{-/-}$ mouse [62], where adoptive transfer of either T_H1 or T_H17 cells restored bacterial burdens and innate immune cell infiltrates to wild-type animals level, T_H17 transferred cells revealed plasticity within the CNS compartment with an ultimate T_H1-like cytokine profile, and this might be the reason for restoration of a strong innate immune response against infection with pyogenic bacteria; for review on various forms of T_H17 cell plasticity, refer to Hemdan [10]. Furthermore, the importance of T_H17 cells in combating HIV-associated bacterial infections has been recently elucidated [63].

2.3. Interaction of T_H17 Cells with Commensal Bacteria. Of a crucial importance is the recent clue linking the induction of T_H17 response with gut commensal bacteria. Colonization of the small intestine of mice with a single commensal microbe, segmented filamentous bacterium (SFB), was sufficient to induce IL-17- and IL-22-producing T_H17 cell responses in the lamina propria, and this was correlated with enhanced expression of inflammation- and antimicrobial-associated genes and increased resistance to the intestinal pathogens *Citrobacter* and *Salmonella* [35, 64]. Induction of T_H17 cells mediated autoimmune arthritis in K/BxN mice [65], whereas when the same mice were held under germ-free conditions, autoimmune arthritis was strongly attenuated and mice revealed reductions in serum AAs titers, splenic AAs-secreting cells, germinal centers, and splenic T_H17 cells as well as the lack of T_H17 cells in the small intestinal lamina propria [65]. These findings suggest the role of T_H17 cells not only in defending the gastrointestinal tract against pathogens, but also in mediating AD (Table 1). How does the immune system monitor the resident intestinal microbes and coordinate between host defense and tolerance and how do dysregulated host-microbe interactions lead to intestinal inflammation were recently discussed [66]. The increased production of IL-17 and IL-23 by PBMCs derived from patients of primary Sjogren's syndrome upon TLR2, TLR4, and TLR6 stimulation [67] highlights the link between TLR ligation and autoimmune induction in such disease settings, where the participation of T_H17 cytokines in their pathogenesis is evident [68, 69].

2.4. T_H17 Cells in the Bone Disease. One of the most important contributions of T_H17 cells involves bone metabolism and bone disease. The coincidence of chronic inflammation and osteoporosis (OP) or osteoarthritis (OA) is quite anticipated and raised a debate about IL-17's contribution. Several hallmark inflammatory mediators including TNF-α, IL-1, IL-6, IFN-γ, receptor activator of NF-κB (RANK), and RANK ligand (RANKL) are of crucial importance not only at the primary inflammation site, but also in bone metabolism [70, 71]. Although a protective role of IL-17 against bone loss has been described [72], induction of osteoclastogenesis by T_H17 cells has been suggested in various inflammatory models [73]. Proinflammatory cytokines correlated with osteoclastogenic or antiosteoclastogenic manifestations in human OP and OA, for example, negative correlations of hip bone mineral density (BMD) with TNF-α in OA and with RANKL/RANK in OP [70]. In a mouse model of type II diabetes, whereas osteocalcin and osteoprotegerin (osteoblast-specific bone forming markers) were decreased, osteoclast-driven bone resorption markers such as IL-6 and RANK were elevated and coincided with enhanced RANKL and IL-17 expression by $CD4^+$ cells; IL-17 induction was directly promoted upon leptin treatment [74]. The authors proposed that leptin and IL-6 stimulate IL-17 production and, thereby, induce RANKL-mediated osteoclastogenesis. A direct link of IL-17 to osteoclast induction was proved in cultures of PBMCs drained from patients with Crohn's disease [71]. Altogether, IL-17 may be a valuable target for controlling bone diseases, at least those accompanying chronic inflammations as in Crohn's disease or inflammatory arthritis.

Overall, in addition to expecting counterprotective impacts of T_H17-associated cytokines, the induction of T_H17 response seems to be an intrinsic feature originally evolved to fight bacterial, viral, and fungal infections. However, what drives such an immune arm to react against the body's own elements, that is, the loss of tolerance, remains elusive. Upon infection, it seems to be a failure to eliminate the invader, whereby an inflammation-potent cell response is amplified, whose army calls for other inflammation competent cells that might have lost the ability to recognize the body's own MHC molecules and therefore attack the self and/or induce production of AAs. Such a modified response attained through a persistent infection constitutes an additional load against the

system's strategy of pathogen clearance and the culmination of the immune response to its steady state thereafter. In other words, boosting such a potent inflammatory cell type as T_H17 through an initial inflammation, for example, through inflammatory cytokine-mediated induction of NF-κB, see next, should normally be accompanied by induction of a regulation program; otherwise autoimmunity occurs. On the basis of current understanding, we propose that the T_H17-driven autoimmune response is manifold, attaining its incidence through (i) activating T_H1 responses and the later conversion of T_H17 themselves into T_H1-like cells or double T_H1/T_H17 cytokine producers having the inflammatory potency of both subsets; (ii) activating B cells and their production of AAs, especially through IL-21-dominated responses; (iii) inducing inflammatory cells like macrophages and neutrophils and their recruitment through induction of chemokines, facilitating thereby tissue destruction and release of intrinsic cellular factors, which, in turn, leads to local or systemic induction of the autoimmune traits; and (iv) promoting cytotoxicity of NK and $CD8^+$ cells and the conversion of the latter cells into IL-17 producers that further magnify the whole response. On the basis of current knowledge, introducing the T_H17 efficacy as a potent inflammatory lineage, targeting IL-23/T_H17 axis, may be a promising approach that paves the way for additive and alternative treatment of chronic inflammation and AD [75].

3. T_H17 Cells Are Key Players in Heavy-Metal-Elicited Autoimmunity

Whereas some heavy metals (e.g., copper, selenium, iron, and zinc) are essential to maintain our metabolism, the majority of heavy metals are non-essential, for example, arsenic (As^{3+}, As^{4+}), cadmium (Cd^{2+}), chromium (Cr^{3+}, Cr^{4+}), mercury (Hg^{2+}), and lead (Pb^{2+}), and are ranked among the most highly toxic substances. Great evidence exists that various heavy metals elicit immunomodulation increasing thereby the incidence of human AD and cancer [3, 18, 76]. It has been recently found that patients with autoimmune thyroiditis (AT) and other AD, including MS, psoriasis, SLE, and atopic eczema, showed increased lymphocyte reactivity to inorganic Hg^{2+}, Ni^{3+}, and other metals and that replacement of amalgam in Hg^{2+}-allergic subjects resulted in improvement of health in about 70% of AT patients [3]. Furthermore, recent data implied that exposure of mice to low micromolar concentrations of Cd^{2+} and Hg^{2+} induces a robust T_H17 response, that was also inferred by mild but significant increase of IL-17 profile in serum of individuals occupationally exposed to the same metals, as well as a robust *ex vivo* T_H17 response (N. Y. A. Hemdan et al., unpublished data).

3.1. Ligation of Metal Ion with the Aryl Hydrocarbon Receptor. Like other xenobiotic stimuli [77], one mechanism so far delineated is the ligation of metal ion with the aryl hydrocarbon receptor (AhR) as in the case of Cd^{2+}, As^{3+}, Cr^{6+} [78, 79], and Pb^{2+} [80]. Such ligand-specific activation of the AhR was found to regulate the balance between T_H17 and T_{Reg} cell responses [81]. Whereas AhR activation by TCDD (dioxin) induces functional T_{Reg} cells that suppress EAE, activation by 6-formylindolo[3,2-b]carbazole induces T_H17 cells and ultimately the disease severity. AhR is expressed by T_H17 cells, $\gamma\delta$ T cells, and DCs [82, 83] and is indispensible for IL-22 production as evidenced by $AhR^{-/-}$ mouse studies [82] and by downregulation of the AhR on RNA-mediated interference, as well as, by applying AhR agonists [48], where it substantially altered the balance of IL-22- versus IL-17-producing cells. In DCs, activation of AhR induces expression of IDO1 and IDO2 that mediate induction of T_{Reg} cells [83]. Therefore, we hypothesized that exposure to heavy metals may mediate autoimmune initiation/induction through metal ligation of AhR. Recent works of other groups and our unpublished data indicate the association of IL-22 with the appearance of autoimmune signs, as inferred in the pathogenesis of psoriasis [7]. These data raise the AhR as a sequential segment linking such potent inflammatory T_H17 cells with the heavy-metal-induced autoimmune induction and reveal a mechanism for further differentiation of T_H17 into T_H22 under organ- or pathogen-specific conditions, including AhR ligation pathways. Therefore, targeting AhR may offer a possibility for differential regulation of T_H17 cytokines and thereby reduction of autoimmune susceptibility in heavy-metal occupationally exposed individuals; however, the paradoxical effect of various AhR ligands should be considered.

AhR-mediated immunomodulation by heavy metals may involve several mechanisms; one of which is the regulation of CYP1A1 expression, for example, in the case of Pb^{2+} [80] that coincided with increase of heme-oxygenase- (HO-) 1 mRNA level and production of reactive oxygen species (ROS). Induction of CYP1A1 expression by AhR ligation is well documented; its downstream signal mediates cellular responses probably through modifying cytokine secretion, including IL-6 [84], IL-17, and IL-22 [37, 82, 85]. Induction of oxidative stress has been reported in case of Pb^{2+} [86], Cd^{2+} [87–89], and Hg^{2+} exposure [90], inferred by reduced activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) and reduction of glutathione (GSH). Smoking, a major source of Cd [91], also initiates ROS production accompanied by augmented cell signaling pathways implicated in the pathogenesis of AD such as psoriasis that implicates mitogen-activated protein kinase (MAPK), NF-κB and Janus kinase JAK/STAT [92], and reduced antioxidants malondialdehyde and SOD [93]. Oxidative stress comprising production of free radicals such as reactive oxygen and nitrogen species is closely related to inflammation and discussed as an underlying mechanism of inflammatory diseases, accompanying activation of the leucocytes and generation of peroxynitrite, at the early stages of induction, or appearing before the incidence of various AD such as IBD [94, 95], systemic sclerosis [96], SLE [97], psoriasis [93, 98], and in cardiovascular inflammation [99]. Therefore, depletion of endogenous antioxidants such as GSH, Cu, and ZnSOD was manifested in experimental models of IBD [100], accompanied, however, by induction of HO-1. The latter catalyzes CO production, and, although it

is considered as a prooxidant due to iron released from HO activity [101], it may be a compensatory response to oxidative stress and chronic inflammation [102], whose protective function has been elucidated in various AD including MS [103].

The protective role of antioxidants in combating inflammation has been clarified in various models by applying exogenous antioxidants or by manipulating expression of endogenous antioxidants. Depletion of NF-E2-related factor 2 (Nrf2), for instance, markedly enhanced susceptibility of experimental IBD [104]. Moreover, by disrupting GSH metabolism through targeting GSH peroxidase (GPx) 1 and GPx2 derived development of colitis [105], or by depleting GSH though curcumin-elicited glyoxalase 1 activity inhibition that enhanced the anti-inflammatory response as well as anticarcinogenetic potency [106], it became clear how close the metabolic stress is related to cell response and cell survival, an assumption that is confirmed by many studies [107]. Unfortunately, although the role of T_H17 cells-related products such as IL-17 in the previous experimental settings has not been addressed, T_H17 together with T_H1 cells are drawn as being major players in the IBD as well as in other inflammatory concerts [108]. Therefore, the elevation of ROS levels links heavy metal exposure to induction of inflammation and cancer. Indeed, induction of T_H17 cell response by heavy metals is likely a downstream event of hydrogen-peroxide- (H_2O_2-) mediated IL-6 induction, which is found to protect resident lung cells from ROS-induced injury [109]. Ye et al. [110] found that fumarates induced type II DCs as a result of initial GSH depletion followed by induction of HO-1, which interacts with AP-1 and NF-κB sites of *Il23p19* promoter and inactivates STAT1 and thereby improves T_H1- and T_H17-mediated AD including MS and psoriasis.

3.2. Modification of NF-κB Signaling. A key pathway through which heavy metals, amongst other xenobiotic substances, exert impacts on the immune response occurs via modifying NF-κB signaling. Several distinct NF-κB activation pathways are identified, including responses to various cell stresses and stimuli such as proinflammatory cytokines TNF-α and IL-1, bacterial products, genotoxic stimuli such as ionizing radiation and some chemotherapeutic drugs [88], in addition to exposure of various cell types to heavy metals such as Cd^{2+} [88, 111]. This may highlight NF-κB activation as a trait of carcinogenicity assigned to heavy metals including Cd^{2+} [112]. The increased production of cytokines, for example, IL-6 and IL-17, in murine models and in heavy-metal-exposed individuals (Hemdan & Abul El-Saad, unpublished data), might ultimately lead to excessive induction of NF-κB-mediated chronic inflammation [113]. This is consistent with the involvement of IL-17 in the differentiation of plasma cells mediated by NF-κB-regulated TF Twist-1 [114]; we recall the correlation of higher IL-17 levels with the severity of various AD and the appearance of autoimmune signs accompanying exposure to heavy metals. Therefore, delicate intervention to regulate NF-κB activation may help prevent chronic inflammation, simultaneous tissue cell damage, and reduce incidence of AD in individuals occupationally exposed to heavy metals or those with an accidental exposure history.

3.3. Disruption of Ca^{2+} Homeostasis. A third event by which heavy metals like Cd^{2+} modify cell survival and function is modifying Ca^{2+} displacement and ultimately adherence/tight junctions, mediated by disrupting expression and translocation of E-cadherin/β-catenin, in a way that mimics Wnt-signaling [88, 115], providing a clue for the carcinogenicity of heavy metals. Previous studies [116] as well as our unpublished data identified some protein kinases including ROCK-II as a target of Cd^{2+}- and Hg^{2+}-mediated induction of IL-17, which activates NF-κB through CIKS/Act1 adaptor proteins, inducing thereby chronic inflammation and cancer.

3.4. Modification of Zinc Metabolism. A further important element in the interplay of heavy metals such as Cd^{2+} with immunomodulation is represented by its interaction with zinc. Zinc was found to inhibit cancer through regulating various oncogenic pathways including NF-κB, AP-1, Notch-1, and PI3K/Akt, apoptosis, cytotoxicity, regulating tumor suppressors such as p53 and macrophage phagocytic activities and increased production of ROS and inflammatory cytokines TNF-α, IL-1β, IL-8, VCAM, and MCP-1 [112]. Similarly, Zn probably inhibits STAT3 activation and thereby T_H17-mediated collagen-induced arthritis [117]. Via targeting such oncogenic pathways, Zn supplements may participate in attaining promising antitumor approaches, at least in cases where Cd^{2+} is considered to have a carcinogenic potential.

4. Concluding Remarks

Ongoing research provides a preponderance of evidence that T_H17 cells and related molecules act as double agents both in favor of but also against the harboring individual. They elicit various antimicrobial mechanisms on one hand, but, on the other hand, when dysregulated, likely triggered by xenobiotic agents including pollutants and infectious agents, initiate/promote chronic inflammatory/autoimmune manifestations. A delineation of the underlying mechanisms that culminate into hyperactivation of T_H17 cells and the resultant production of related mediators would facilitate the development of potential therapeutic approaches to combat their deteriorating effects but simultaneously allow their benefits to act. Therefore, various research directions gave more attention in the last decade to T_H17-cells-related molecules to help attain and evaluate valuable therapeutic strategies. Manipulating T_H17 differentiation and function by targeting differentiation/promoting cytokines, transcription factors, or commensal-bacteria-elicited immune induction may be valuable for treating AD; however, the risk of increasing the vulnerability of attacking infections should remain in focus. Therefore, it may be of worth to apply prophylactic antibacterial and antifungal therapy in case of treating patients of AD with IL-17/-22/-23 inhibitors.

Take-Home Messages

(i) T_H17 cells and their related products act as double agents both to mediate various antimicrobial mechanisms and to initiate/promote chronic inflammatory/autoimmune manifestations.

(ii) Simultaneous or successive exposure to xenobiotic substances and infectious agents renders the genetically susceptible individual vulnerable to autoimmune incidence via induction of inflammatory mediators.

(iii) A compromise should be met to facilitate development of potential therapeutics aiming at targeting AD via inhibiting differentiation of T_H17 cells and/or commitment factors for the benefit of attaining the antimicrobial response intact, for example, by applying prophylactic therapy combined with IL-17/-22/-23 inhibitors.

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Clinical Study

Aberrant T Helper 17 Cells and Related Cytokines in Bone Marrow Microenvironment of Patients with Acute Myeloid Leukemia

Tian Tian,[1] Shuang Yu,[1] Min Wang,[1] Cunzhong Yuan,[2] Hua Zhang,[3] Chunyan Ji,[1] and Daoxin Ma[1]

[1] *Department of Hematology, Qilu Hospital, Shandong University, 107 West Wenhua Road, Jinan 250012, China*
[2] *Department of Obstetrics and Gynecology, Qilu Hospital, Shandong University, Jinan 230001, China*
[3] *Institute of Basic Medicine, Shandong Academy of Medical Sciences, Jinan 230001, China*

Correspondence should be addressed to Daoxin Ma; daoxinma@sdu.edu.cn

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In this study, we mainly investigate the role of Th17 cells, Th1 cells, and their related cytokines in the pathophysiology of AML. BM and PB were extracted from ND, CR, and relapsed-refractory AML patients and controls. Th subsets frequencies were examined by flow cytometry. BM plasma Th-associated cytokines levels were determined by ELISA. The frequencies of Th17 and Th1, and IFN-γ or TGF-β concentrations were significantly decreased in ND compared with CR patients or controls. Th17 percentage was significantly lower in BM than in PB for ND patients but was higher in BM for CR patients. However, in CR or relapsed-refractory patients, Th1 percentage in BM was higher than that in PB. Moreover, BM IL-17A level showed a decreased trend in ND patients. A significant elevation of plasma IL-6 level was found in ND compared with CR patients or controls. IL-17A showed the positive correlation with IL-6 concentration. And Th17 cells frequencies and TGF-β1 concentration were increased in BM from AML patients achieving CR after chemotherapy. Moreover, a significant decrease of BM plasma TGF-β1 level was found in M3 patients compared with the other subtypes. Our findings suggest that Th17 and related cytokines may be implicated in AML pathogenesis.

1. Introduction

Acute myeloid leukemia (AML) is a life-threatening hematopoietic stem cell neoplasm characterized by increased number of myeloid cells in the bone marrow and an arrest in their maturation, frequently resulting in fatal infection, bleeding, or organ infiltration, with or without leukocytosis [1–3]. The etiology of AML is heterogeneous and complex, but it is widely accepted that both environmental and genetic factors play significant roles in the development of AML. Immune system disorders have increased our understanding of leukemogenesis [4]. However, little is known about the immunopathological events, especially the abnormal T helper (Th) subsets, leading to the initiation and progression of this disease.

A novel IL-17-producing Th subset, termed Th17 cells, has been described in recent years [5–7]. In mice, the differentiation of Th17 cells is driven primarily by the cytokines transforming growth factor- (TGF-) β and IL-6, and it is known that IL-23 is necessary for the pathogenicity of Th17 cells [5, 8–10]. Retinoic acid-related orphan nuclear receptor gamma t (ROR-γt) is a transcription factor that is considered to be important for the initiation and maintenance of Th17 cell lineage [11, 12] and regulating the differentiation of Th17 subset. However, less is known in human than in mice. Volpe et al. [13] have reported that TGF-β, IL-23, and proinflammatory cytokines (IL-1β and IL-6) were all essential for human Th17 differentiation. Nevertheless, Acosta-Rodriguez et al. [14] found that for human naive $CD4^+$ T cells, ROR-γt expression and Th17 polarization were induced by IL-1β and enhanced by IL-6 but were suppressed by TGF-β and IL-12.

Accumulating evidence demonstrated that Th17 cells play critical roles in several animal models of autoimmunity,

such as experimental allergic encephalomyelitis (EAE) [15] and murine arthritis models [16, 17]. Besides, Th17 cells are considered to be involved in many human inflammatory diseases, including multiple sclerosis, psoriasis, and inflammatory arthritis [18–21]. What is more, Th17 cells and IL-17 have a regulatory role in normal hematopoiesis [22]. It has been established that Th17 cells participate in solid tumors [23]; however, the specific role of Th17 in cancer is debatable. Results from two studies in prostate and ovarian cancer patients suggested both beneficial and harmful implications of Th17 cells in tumor development [24, 25], while another ovarian cancer research [26] showed that Th17 may provide protection to human tumor immunity through inducing Th1-type chemokines and recruiting effector cells to the tumor microenvironment. Recently, Zhang et al. [27] showed a prominently increased frequencies of Th17 and IL-17 level in patients with uterine cervical cancer and cervical intraepithelial neoplasia. However, studies about circulating Th17 cells in AML are divergent. Wu et al. showed that Th17 frequency was significantly increased in the peripheral blood of patients with AML compared with controls [28], while Fan et al. demonstrated that Th17 frequencies and IL-17A levels in ND and CR AML patients were lower than healthy controls [29]. Up to now, there was no study about Th17 cells in bone marrow (BM) microenvironment of AML. Considering the important role of BM microenvironment in the hematopoiesis and the formation of the primitive cells, it is necessary to do further researches to interpret the specific role of Th17 cells in BM microenvironment of AML.

In this study, we examined the frequencies of Th17 and Th1 cells and the concentrations of related cytokines (IL-17A, IL-6, TGF-β1, and IFN-γ) in BM or PB of patients with different AML stages and controls and evaluated their involvement in the pathogenesis and progression of AML.

2. Materials and Methods

2.1. Patients and Controls. Forty-nine newly diagnosed (ND) (23 females and 26 males; age range, 21–83 years; median age, 43 years), 18 relapsed-refractory (8 females and 10 males; age range, 18–63 years; median age, 41 years), and 38 complete remission (CR) AML patients (19 females and 19 males; age range, 19–71 years; median age, 45 years) were enrolled in this study. AML patients were diagnosed according to the French-American-British (FAB) classification system [30]. CR was defined based on International Working Group Criteria [31]. Relapsed-refractory patients failed to achieve CR after two courses of standard induction chemotherapy or relapsed in 6 months after the first CR. Because bone marrow aspiration is a quite invasive procedure, individuals with slight iron deficiency anemia, having no immunological changes, were used as controls. The control group consisted of 19 individuals (14 females and 5 males; age range, 18–67 years; median age, 39 years). Participants' characteristics were provided in Table 1. This study was approved by the Medical Ethical Committee of Qilu Hospital, Shandong University, China. Informed consent was obtained from all patients before enrollment in the study in accordance with the Declaration of Helsinki.

2.2. Treatment Regimen. Newly diagnosed patients with acute promyelocytic leukemia (APL, subtype M3) received all-trans retinoic acid with or without concurrent induction chemotherapy. Newly diagnosed patients with non-M3 AML subtypes underwent standard induction chemotherapy with one of the anthracyclines (doxorubicin or idarubicin) for 3 days and cytarabine for 7 days and received consolidation therapy with high-dose cytarabine with or without the anthracycline after achieving CR.

2.3. Bone Marrow and Peripheral Samples. BM and PB samples from different stages of AML patients and controls were collected in heparin-anticoagulant vacutainer tubes. Plasma of BM was obtained after centrifugation and stored at −80°C for measurement of cytokine levels.

2.4. Flow Cytometric Analysis of Th17 and Th1 Cells. Intracellular cytokines were studied by flow cytometry to reflex the cytokine-producing cells. Briefly, heparinized whole BM (400 uL) with an equal volume of Roswell Park Memorial Institute (RPMI)-1640 medium was incubated for 4 h at 37°C in 5% CO_2 in the presence of 2.5 ng/mL of phorbol myristate acetate (PMA), 1 mg/mL of ionomycin, and 1.7 mg/mL of monensin (all from Alexis Biochemicals, San Diego, CA, USA). PMA and ionomycin are pharmacologic T-cell-activating agents that mimic signals generated by the T-cell receptor (TCR) complex and have the advantage of stimulating T cells of any antigen specificity. Monensin is used to block the intracellular transport mechanisms, thereby leading to an accumulation of cytokines in the cells. After incubation, the cells were stained with Alexa Fluor 647 anti-human CD4 monoclonal antibody at room temperature in the dark for 20 min. The cells were next stained with PerCP/Cy5.5 anti-human IL-17A or anti-human IFN-γ monoclonal antibody after fixation and permeabilization. All antibodies were obtained from BioLegend (San Diego, CA, USA). Isotype controls were utilized to enable correct compensation and to confirm antibody specificity. Stained cells were analyzed by flow cytometric analysis using a FACS Calibur cytometer equipped with CellQuest software (BD Bioscience Pharmingen, San Jose, CA, USA). Considering the relatively less number of lymphocytes in BM microenvironment of AML patients, we circled and collected 5000 $CD4^+$ cells during the step of FACS cell collection. For analysis, we first gated $CD4^+$ lymphocytes, then analyzed the proportion of Th17 ($CD4^+$IL-17^+) and Th1 ($CD4^+$IFN-γ^+) cells in $CD4^+$ lymphocytes.

2.5. IL-17, TGF-β1, IL-6, and IFN-γ Enzyme-Linked Immunosorbent Assay (ELISA). BM plasma Th17-related cytokines (IL-17A, total TGF-β1, and IL-6) and IFN-γ levels were determined using ELISA method according to the manufacturer's recommendations (lower detection limit 0.5 pg/mL, 9 pg/mL, 0.92 pg/mL, 0.99 pg/mL, resp.; all ELISA kits are from eBioscience).

2.6. Statistical Analysis. Results were expressed as mean ± SD or median (range). Statistical significance among patients in the four groups was determined by ANOVA, and difference

Table 1: The characteristics of subjects.

	ND AML patients ($n = 49$)	CR AML patients ($n = 38$)	Relapsed-refractory AML patients ($n = 18$)	Controls ($n = 19$)
Age (years)	21–83	19–71	18–63	18–67
Gender (male/female)	26/23	19/19	10/8	5/14
WBC ($*10^9$/L)	31.06 ± 53.64	5.30 ± 2.13	40.12 ± 61.02	6.5274 ± 2.42
BM leukemic blast (%)	72.61 ± 40.91	1.17 ± 1.15	58.27 ± 29.13	
FAB subtype				
M1	2	0	0	
M2	7	2	1	
M3	9	14	1	
M4	9	9	5	
M5	22	13	11	

ND: newly diagnosed; CR: complete remission; WBC: white blood cell; FAB: French-American-British; BM: bone marrow.

between two groups was determined by Newman-Keuls multiple comparison test (q test) unless the data were not normally distributed, in which case Kruskal-Wallis test (H test) and Nemenyi test were used. The Pearson or Spearman correlation test was used for correlation analysis depending on data distribution. P value < 0.05 was considered statistically significant.

3. Results

3.1. Abnormal Th17 Cells in AML Patients. We analyzed the frequency of Th17 cells based on cytokine patterns after in vitro activation by PMA plus ionomycin in short-term culture. The expression of a typical dot-plot of Th17 cells in representative ND, relapsed-refractory, CR AML patients, and controls was shown in Figure 1. In different stages of AML, Th17 cells frequencies were statistically decreased in ND patients (1.76 ± 0.96%) compared to CR (5.082 ± 2.4%; $^{***}P < 0.0001$) or relapsed-refractory AML patients (3.97 ± 2.17%; $^{*}P = 0.0011$) or controls (3.63 ± 1.37%; $^{***}P < 0.0001$) (Figure 2(a)). Compared with BM plasma IL-17A level in controls (median, 0.95 pg/mL; range, 0.19–3.14 pg/mL), though there was a decreased trend in ND (median, 0.72 pg/mL; range, 0.19–3.83 pg/mL), relapsed-refractory (median, 0.77 pg/mL; range, 0.14–2.03 pg/mL), or CR (median, 0.45 pg/mL; range, 0.14–3.03 pg/mL) AML patients, no statistical significance was observed (Figure 2(b)). No significant correlation was found between Th17 and plasma IL-17A levels in all the groups.

Meanwhile, we compared the Th17 cells between BM and PB in AML patients. The results showed that in ND AML patients, Th17 percentage was lower in BM (1.76 ± 0.96%) than in PB (3.18 ± 2.53%), but did not reach the statistical difference ($P = 0.08$). In CR patients, Th17 percentage was markedly higher in BM (5.08 ± 2.4%) than in PB (3.07 ± 1.38%; $^{*}P = 0.0005$) (Figure 3(a)).

3.2. Abnormal Th1 Cells in AML Patients. We also analyzed Th1 frequencies in different stages of AML patients. The expression of a typical dot-plot of Th1 cells was shown in Figure 1. In different stages of AML, Th1 frequencies were statistically decreased in ND patients (11.22 ± 7.99%) compared to CR (24.84 ± 12.72%; $^{***}P < 0.0001$) or relapsed-refractory AML patients (17.23 ± 7.52%; $^{*}P = 0.02$) or controls (21.95 ± 11.86%; $^{*}P = 0.001$) (Figure 2(c)). Moreover, Th1 percentage was markedly decreased in relapsed-refractory stage than in CR stage ($^{*}P = 0.01$). Compared with CR (median, 2.34 pg/mL; range, 1.48–18.37 pg/mL) patients, a significant decreased BM plasma IFN-γ level was found in ND AML patients (median, 1.55 pg/mL; range, 0.1–9.77 pg/mL; $^{*}P = 0.03$) (Figure 2(d)).

Meanwhile, we compared the Th1 cells between BM and PB. The results showed that Th1 percentage was significantly higher in BM (24.84 ± 9.51%; 17.23 ± 7.52%, resp.) than in PB (14.51 ± 9.08%; 12.52 ± 4.56%, resp.) in CR ($^{*}P = 0.0011$) or relapsed-refractory patients ($^{*}P = 0.049$) (Figure 3(b)).

3.3. Increased IL-6 and Decreased TGF-β1 Concentration in Plasma from ND AML Patients. Concentrations of bone marrow plasma IL-6 and TGF-β1 were measured by ELISA. For IL-6 level, there was a significant increase in ND patients (median, 5.34 pg/mL; range, 1.92–175.66 pg/mL) compared to controls (median, 2.78 pg/mL; range, 2.36–31.81 pg/mL; $^{*}P = 0.0071$) or CR patients (median, 3.25 pg/mL; range, 2.63–25.62 pg/mL; $^{*}P = 0.01$). Moreover, we observed an increased trend in relapsed-refractory patients (median, 4.25 pg/mL; range, 2.60–15.83 pg/mL) compared with controls or CR patients (Figure 4(a)).

For TGF-β1, the level was markedly decreased in ND (median, 2222.95 pg/mL; range 289.8–13883.7 pg/mL) and relapsed-refractory AML patients (median, 2462.35 pg/mL; range, 677.2–4799.8 pg/mL) compared with CR patients (median, 9273.68 pg/mL; range, 1092.8–40438.5 pg/mL; $^{***}P < 0.0001$; $^{*}P = 0.0003$; resp.) or controls (median, 7510.33 pg/mL; range, 3510–26205.9 pg/mL; $^{***}P < 0.0001$; $^{*}P = 0.0002$, resp.) (Figure 4(b)).

3.4. Correlation between Th17 Cells and Related Cytokines in BM Environment of ND AML Patients. Our research failed

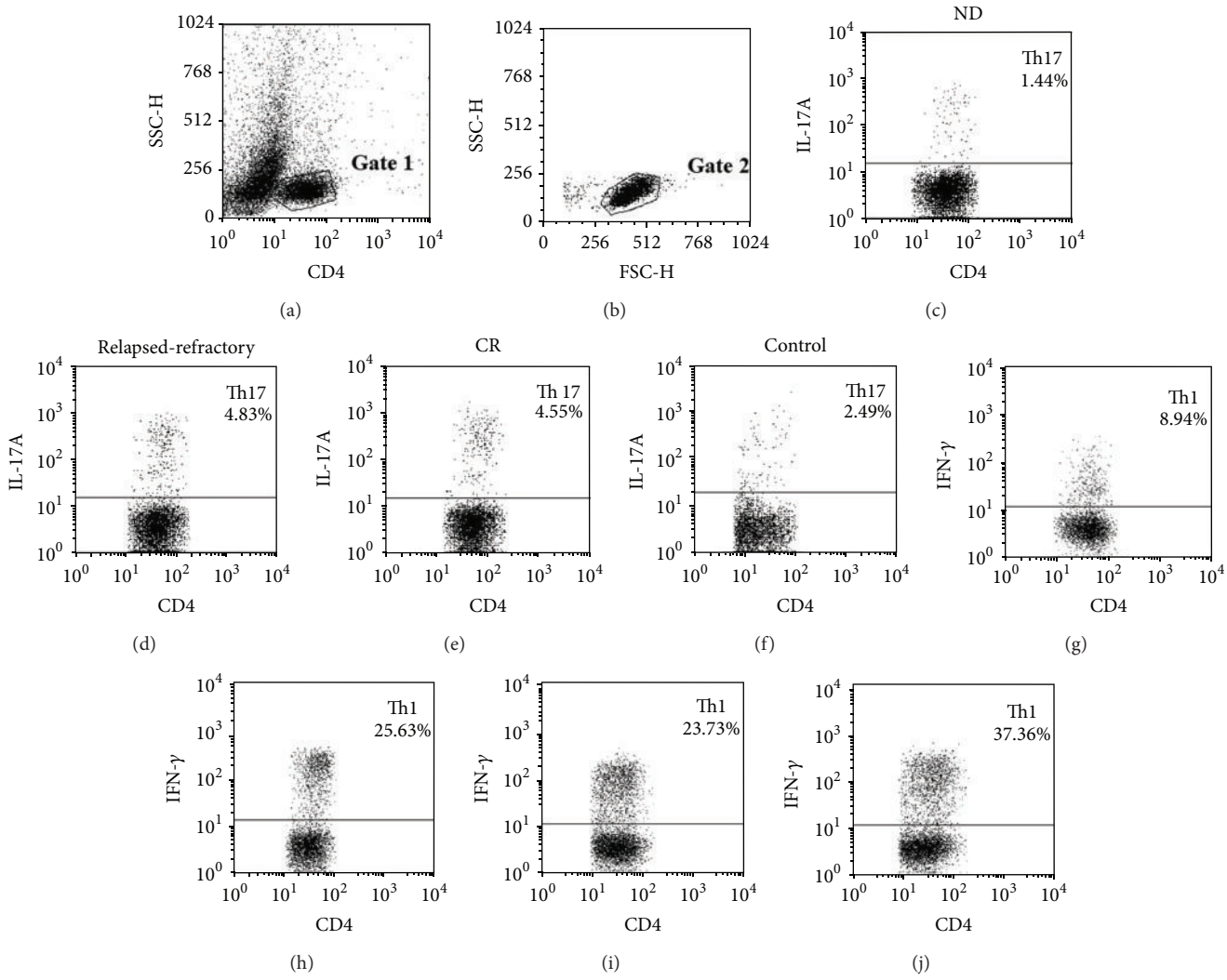

Figure 1: BM Th17 and Th1 cells in representative patients with ND, CR, and relapsed-refractory AML and controls. (a) 5000 $CD4^+$ cells were collected by flow cytometry. (b) $CD4^+$ lymphocytes were gated. (c), (d), (e), and (f) The percentages of BM Th17 ($CD4^+$ IL-17^+) cells in ND, CR, and relapsed-refractory AML patients and controls. (g), (h), (i), and (j) The percentages of BM Th1 ($CD4^+$ IFN-γ^+) cells in ND, CR, and relapsed-refractory AML patients and controls.

to show any statistical correlation between Th17 and IL-17A ($r = 0.04$, $P = 0.86$) or IL-6 ($r = 0.125$, $P = 0.51$) or TGF-β1 ($r = 0.077$, $P = 0.76$) concentration (Figures 5(d) and 5(e)). However, it demonstrated that IL-17 concentration showed a positive correlation with the level of IL-6 ($r = 0.5415$, $P = 0.0009$) (Figure 5(a)) in ND AML patients, but did not reach statistical correlation in CR, relapsed-refractory, or control groups. There was marginally statistical correlation between TGF-β1 and IL-6 concentrations ($r = 0.277$, $P = 0.0836$) (Figure 5(b)). No significant correlation was found between TGF-β and IL-17A ($r = 0.194$, $P = 0.27$) (Figure 5(c)).

3.5. Elevated Frequencies of Th17 Cells in BM from AML Patients Achieving CR after Chemotherapy. To further understand the influence of chemotherapy on AML BM microenvironment, we observed the whole treatment process in 9 AML patients. CR was obtained after the standard induction chemotherapy. After chemotherapy, Th17 frequencies were markedly increased (median, 3.36%; range, 1.41–8.03% versus median, 1.96%; range, 0.95–2.79%; $^*P = 0.0148$) (Figure 6(a)). Moreover, plasma TGF-β1 level was also significantly elevated in CR stage (median, 6753.6 pg/mL; range, 2249.5–17092.3 pg/mL versus median, 832.7 pg/mL; range, 315.5–9979.6 pg/mL; $^*P = 0.0298$) (Figure 6(b)).

3.6. BM Plasma TGF-β1 Level among Different FAB Subtypes of AML. Because of different characteristics for various AML subtypes, we compared the Th17 subset and related cytokines in M3 with the other subtypes. We found a significant decrease of BM plasma TGF-β1 level in M3 patients compared with the other subtypes (1137.4 ± 1016.3 pg/mL versus 3809 ± 3388.6 pg/mL, $^*P = 0.0005$) (Figure 7). We did not obtain the statistical difference of the other Th cells and related cytokines between these two groups.

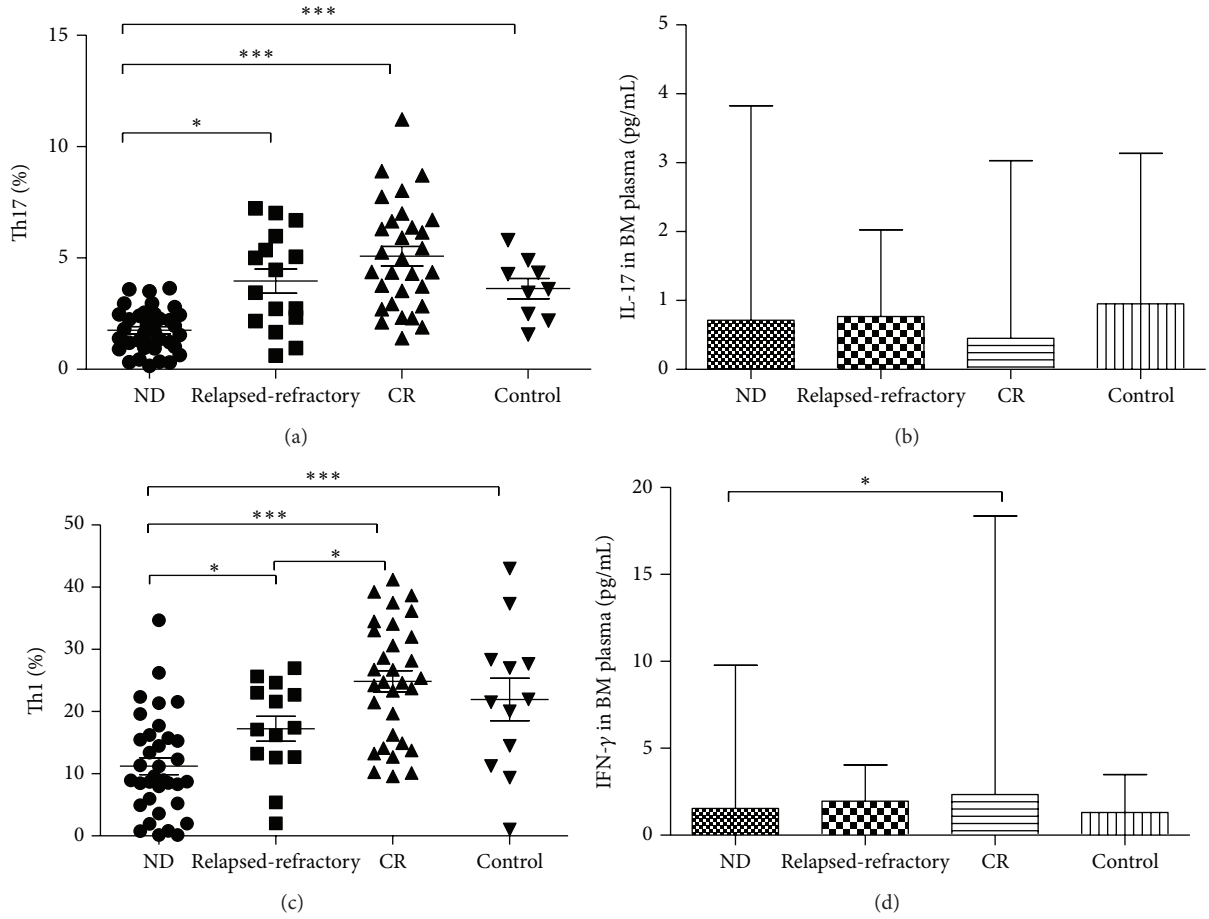

Figure 2: Th subsets and their related cytokines in ND, relapsed-refractory, and CR AML patients and controls. (a) The percentage of BM Th17 cells was significantly decreased in ND AML patients compared with CR patients or controls after stimulation with phorbol myristate acetate, ionomycin, and monensin for 4 h. (b) The level of BM plasma IL-17A showed the decreased trend in the ND, relapsed-refractory, or CR AML patients compared with controls, though no statistical significance exists. (c) The percentage of BM Th1 cells was significantly decreased in ND AML patients compared with relapsed-refractory or CR patients or controls. (d) The level of BM plasma IFN-γ was decreased in the ND AML patients compared with CR patients.

4. Discussion

In this study, we firstly observed that aberrant Th17 or Th1 subset and associated cytokines in BM microenvironment are involved in AML pathogenesis, and chemotherapy partly ameliorates this turmoil.

Th17 and their effector cytokines are being recognized as important mediators in autoimmune and inflammatory diseases, and our studies had demonstrated that Th17 cells were elevated in idiopathic thrombocytopenia (ITP) patients [32]. Previous studies had investigated Th17 cells in both murine and human solid tumors. However, the nature and the role of Th17 cells in cancer immunity remained elusive. In peripheral blood, Kryczek et al. [25] reported that the levels of Th17 were significantly increased both in prostate-tumor-bearing mice and epithelial ovarian carcinomas patients. Another researches in gastric cancer, uterine cervical cancer patients [27, 33] also found significantly elevated frequencies of Th17 cells in peripheral blood as well as in tumor draining lymph nodes. In blood malignant disease, our research showed that Th17 frequencies were increased in early-stage myelodysplastic syndrome (MDS) compared with the late-stage MDS or controls [34]. About the specific role of Th17 cells in peripheral blood of AML patients, the results were controversial, and so far no research was investigated in BM microenvironment of AML patients. Consistent with Fan et al.'s research that Th17 frequencies and IL-17A levels were significantly decreased in peripheral blood of ND and CR AML patients [29], our results demonstrated that Th17 cells were markedly decreased in BM microenvironment of ND patients compared with CR, relapsed-refractory patients or controls. What is more, the IL-17A in BM plasma showed a decreased trend in ND and relapsed-refractory AML patients. The possible explanation is that in addition to Th17 cells, other subset of T cells, including $CD8^+$ T, NK T, and TCR$\gamma\delta$ cells, have been demonstrated to produce IL-17A. Even non-T cells, such as neutrophils and lymphoid tissue inducer-like cells, can also be an innate source of IL-17A [35]. Our result in the same cohort of AML patients showed that decreased Th17 frequencies were partly corrected after

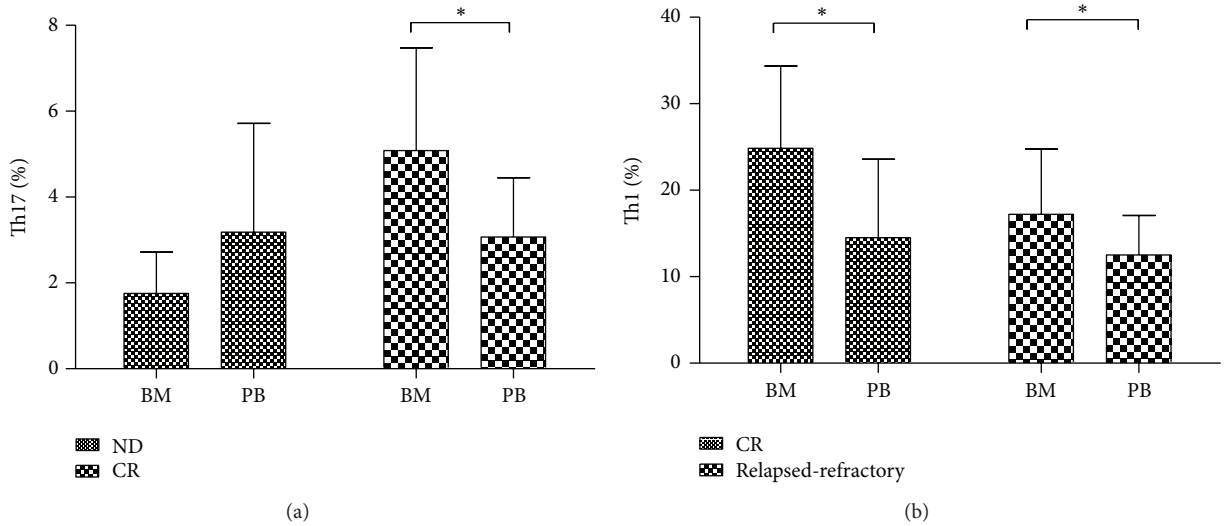

Figure 3: The comparison of Th17 or Th1 cells in BM and PB in different stages of AML patients. (a) Th17 cells percentage was markedly higher in BM than in PB in CR AML patients. (B) Th1 cells percentage was significantly increased in BM compared with in PB of CR or relapsed-refractory patients.

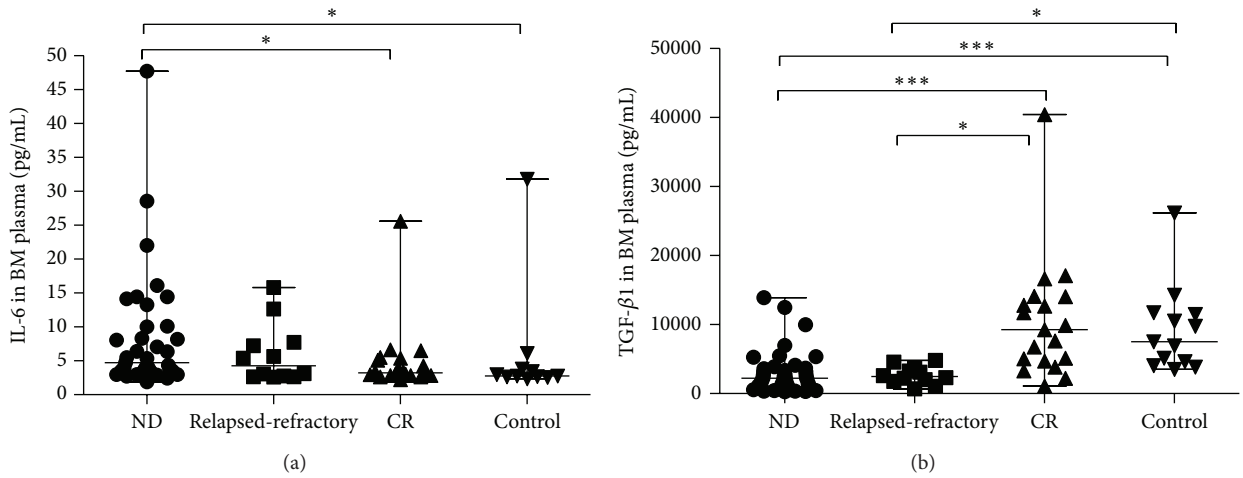

Figure 4: Th17-related cytokines in AML patients and controls. (a) The level of IL-6 was significantly higher in ND AML patients than in CR patients or controls ($^{*}P < 0.05$). (b) A statistical decrease of plasma TGF-β1 level in ND or relapsed-refractory AML patients was found compared with CR patients or controls ($^{*}P < 0.05$, $^{***}P < 0.0001$).

standard chemotherapy, indicating the importance of Th17 in AML pathogenesis and measurement of Th17 frequencies may be valuable for evaluating therapeutic effect. In light of these results, the decrease of Th17 cells in ND AML patients may be explained as a depressed immune response, and the elevation of Th17 in the CR patients indicated a protective reaction of the immune system accompanied by the chemotherapy. In any case, Th17 cells may participate in the progression of AML. These results also suggest that the number of Th17 cells may relate to tumor burden, which may be as a prognostic target. Obviously, further researches are needed to explain the specific role of Th17 in AML patients.

Moreover, considering the decreased Th17 in BM compared with in PB of the ND AML patients; the possible interpretation is that leukemic cells inhibit the production and differentiation of normal immune cells, such as Th17 cells, which causes the lower percentage of Th17 cells in BM of ND AML patients. In CR AML patients, the leukemia cells were decreased greatly which results in the decreased inhibition, and Th17 cells percentage was elevated in BM than in PB.

Our result showed that Th1 cells were significantly decreased in ND or relapsed-refractory patients compared with CR or controls, which was consistent with the reduced immune function in AML patients. We also found that Th1 percentage was higher in BM than in PB from CR or relapsed-refractory patients. And Th1 cells showed the high trend in BM than in PB in ND patients, even not reaching the statistical significance. All these results indicated that

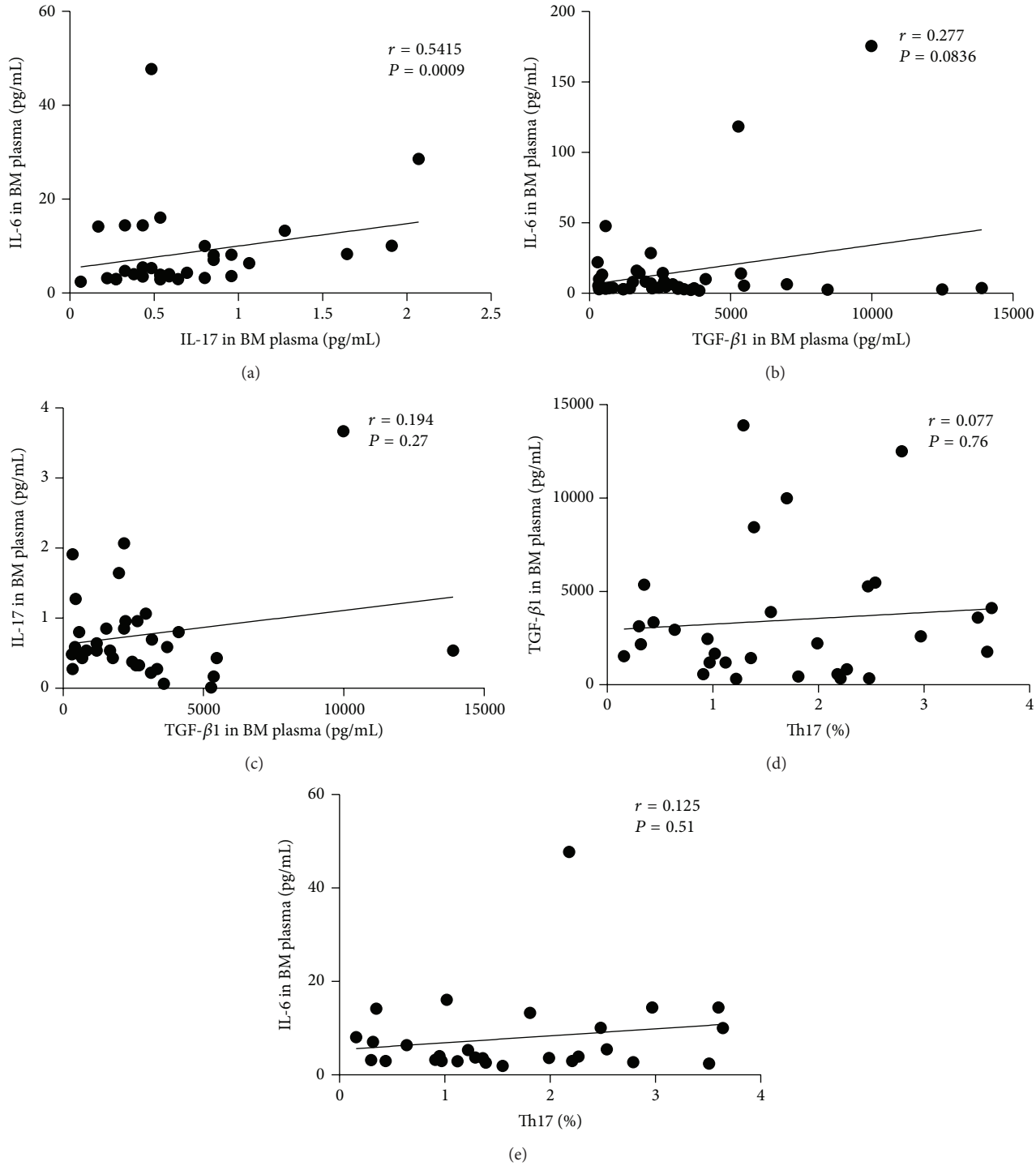

Figure 5: Correlations between the related cytokines in ND AML patients. (a) A positive correlation was found between IL-6 and IL-17A concentration ($r = 0.5415$, $P = 0.0009$) in ND AML patients. (b) Marginal correlation existed between TGF-β1 and IL-6 concentration ($r = 0.277$, $P = 0.0836$). (c, d, e) No significant correlation was found between TGF-β1 and IL-17A, Th17 and TGF-β1, or Th17 and IL-6.

Th1 may be a therapeutic target for AML patients. Further researches are needed to specify the role of Th1 subset in AML.

To date, the knowledge of Th17 differentiation originates from experimental animals, whereas very little information exists about human Th17 cells. In mice, TGF-β is the cytokine critical for Th17 initiation, and IL-6 acts as a critical cofactor for Th17 cell differentiation [5, 8, 9]. In human being, the opinions about the role of TGF-β or IL-6 in Th17 cells differentiation were divergent. Our results

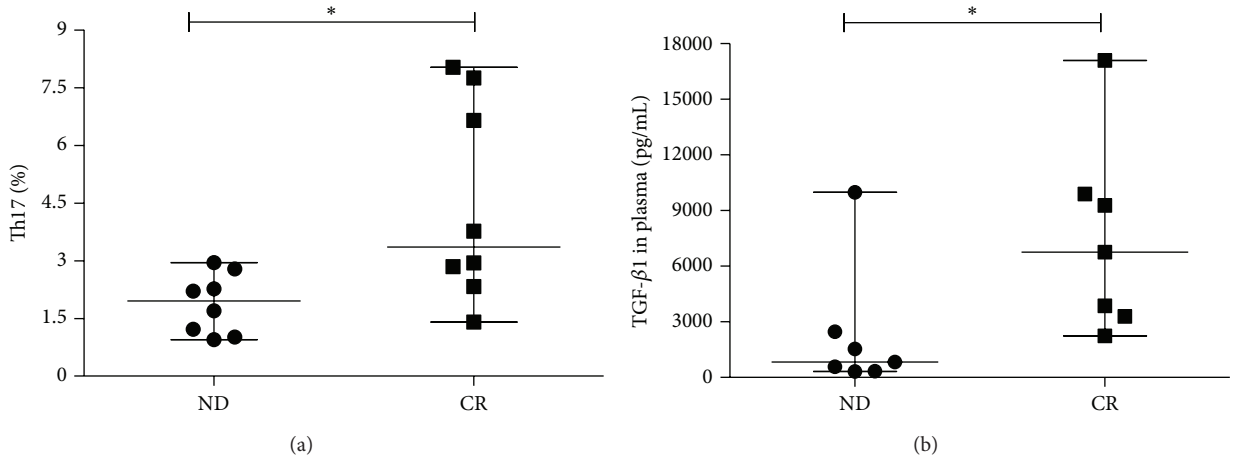

Figure 6: Th17 and associated cytokines in the same AML cohort. (a) Th17 percentage was observed significantly lower in ND stage than in CR stage of the same AML patients ($^*P < 0.05$). (b) A markedly lower TGF-β1 concentration was observed in ND stage than in CR stage of the same AML patients ($^*P < 0.05$).

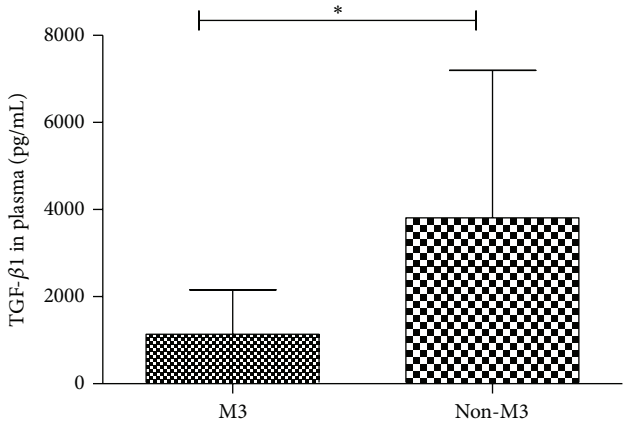

Figure 7: BM plasma TGF-β1 level among different FAB subtypes of AML. A significant decrease of BM plasma TGF-β1 level in M3 patients compared with the other subtypes ($^*P = 0.0005$).

showed that concentration of IL-6 was markedly elevated whereas TGF-β1 was significantly decreased in ND AML patients. Considering the decreased frequencies of Th17 in ND patients, we speculated that TGF-β1 may play a central role in the Th17 differentiation, which was consistent with three independent reports that TGF-β was critical for human Th17 cell differentiation [13, 36, 37]. Simultaneously, in ND AML patients, IL-6 concentration showed a positive correlation to the IL-17A levels, and a marginal correlation with TGF-β1, which may show that IL-6 and TGF-β1 cytokines coordinately promote the differentiation of Th17 cells and generation of IL-17A. Moreover, the variation of the Th17-related cytokines implies that they may play a pathological or protective role in AML process, or work as an effector cytokine. However, we must mention a caveat that TGF-β1 is produced in a latent form, and needs to be activated in order to exert its action. The active form of TGF-β1 is unstable. Therefore, it is always difficult to correlate TGF-β1 serum levels with biological outcomes in vivo.

Recently, more and more evidence supports deregulated TGF-β1 signaling in leukemogenesis, especially in M3. Our study showed that TGF-β1 was lower in M3 patients than in other subtypes. The results were consistent with a study that combined treatment with TGF-β1, and 1,25-dihydroxyvitamin D3 can cause terminal monocytic maturation in monocytic leukemic cell lines [38]. These all indicate the therapeutic potential of TGF-β1 in M3, even in all AML patients.

In summary, the downregulation of Th17 cells, Th1 cells, TGF-β1, and the secreted cytokine IL-17A and the upregulation of IL-6 concentration showed a strong relationship with AML activity. And standard induction chemotherapy may partly ameliorate the abnormal changes. All these results open a new avenue in the study of tumor immunotherapy. Further studies are awaited to clarify their specific roles in the pathophysiology of AML occurrence and development, finally providing the perspective for clinical treatment.

Acknowledgments

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Review Article

IL-17A and Th17 Cells in Lung Inflammation: An Update on the Role of Th17 Cell Differentiation and IL-17R Signaling in Host Defense against Infection

Hsing-Chuan Tsai, Sharlene Velichko, Li-Yin Hung, and Reen Wu

Center for Comparative Respiratory Biology and Medicine, University of California, Davis, CA 95616, USA

Correspondence should be addressed to Reen Wu; rwu@ucdavis.edu

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The significance of Th17 cells and interleukin- (IL-)17A signaling in host defense and disease development has been demonstrated in various infection and autoimmune models. Numerous studies have indicated that Th17 cells and its signature cytokine IL-17A are critical to the airway's immune response against various bacteria and fungal infection. Cytokines such as IL-23, which are involved in Th17 differentiation, play a critical role in controlling *Klebsiella pneumonia* (*K. pneumonia*) infection. IL-17A acts on nonimmune cells in infected tissues to strengthen innate immunity by inducing the expression of antimicrobial proteins, cytokines, and chemokines. Mice deficient in IL-17 receptor (IL-17R) expression are susceptible to infection by various pathogens. In this review, we summarize the recent advances in unraveling the mechanism behind Th17 cell differentiation, IL-17A/IL-17R signaling, and also the importance of IL-17A in pulmonary infection.

1. Background and Overview of Th17 Cells and IL-17A

$CD4^+$ T cells are central mediators of cellular immunity. For many years, $CD4^+$ T cells were classified as either T helper (Th)1 or Th2 cells by their effector cytokines and functions [1, 2]. Th1 cells, which express Interferon-γ (IFN-γ), are responsible for the control of cellular immune responses and tissue inflammation, whereas Th2 cells, which express IL-4, IL-5, and IL-13, are responsible for the regulation of humoral immunity and allergic disease. The discovery of Th17 cells revolutionized our concept of immunopathology and immune regulation. Th17 cells produce proinflammatory cytokines [3, 4] such as IL-17A, IL-17F, IL-22, IL-26, tumor necrosis factor-α (TNF-α), chemokine (C–C motif) ligand 20 (CCL20) [5], and granulocyte macrophage colony-stimulating factor (GM-CSF) [6]. Although these cytokines all have proinflammatory features, they act on different target cells and therefore contribute to different diseases [7–9]. Th17 cells have been implicated in a wide variety of inflammatory conditions, such as autoimmune diseases, chronic inflammation, and pathogen infection [10].

The differentiation of naive T cells to Th17 cells is regulated by multiple signals. The engagement of TCR receptors (Signal 1) and costimulatory molecules (Signal 2) initiates naive T cell differentiation, and then cytokines produced by the innate immune system (Signal 3) direct the differentiation to particular Th subsets. The proinflammatory cytokines IL-1β, IL-6, IL-21, and IL-23, and the anti-inflammatory cytokine, transforming growth factor-β (TGF-β), coordinate to trigger Th17 cell differentiation in a RAR-related orphan receptor-γt (RORγt) dependent manner [11, 12].

IL-17A is the signature effector cytokine of Th17 cells and contributes to Th17-mediated diseases. Although first identified in $CD4^+$ T cells, IL-17A can also be produced by $CD8^+$ T cells [13] and innate cells, such as $\gamma\delta$T cells [14, 15], NK1.1-iNKT cells [16], neutrophils [17], and also innate lymphoid cells (ILCs) [18–20]. IL-17A appears to act primarily on nonhematopoietic cells such as endothelial cells [21, 22], epithelial cells [23–25], and fibroblasts [26, 27], due to the restricted expression of one of its receptor subunits, IL-17RC [28, 29]. Systemically, IL-17A and IL-17F have been reported to play a pathogenic role in certain autoimmune diseases, including multiple sclerosis and rheumatoid arthritis [30–32].

However, its role at mucosal surfaces appears to be dualistic. While high expression of IL-17A has been linked to inflammatory diseases of the mucosal surface, such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease (COPD) in the airway, as well as inflammatory bowel disease, it appears to play an important protective role against infection, particularly by extracellular bacterial pathogens [26, 33–40]. Here, we will summarize recent studies on Th17 cell differentiation as well as IL-17R signaling and highlight the role of Th17/IL-17A in pulmonary infection. The roles of innate IL-17A-producing cells in the pulmonary infection will also be discussed.

2. Factors Involved in Th17 Cell Development

Although IL17A production by $CD4^+$ T cells was first described 20 years ago, Th17 cells were not recognized as a distinct $CD4^+$ cell lineage until 2005 [41, 42]. Th17 cell differentiation has primarily been characterized in the murine system [4]. In mice, IL-6 and TGF-β initiate Th17 cell differentiation by activating STAT3 and inducing IL-23R expression. IL-23 is then responsible for Th17 cell maintenance and expansion [11, 43–45]. In addition, Th17 cells also secrete IL-21, an autocrine mechanism to sustain and promote their own differentiation via a STAT3-mediated manner [46, 47]. IL-1β was initially thought to play an accessory role in mouse Th17 cell differentiation but recently it has been demonstrated to play a critical role in the early differentiation stages of mouse Th17 cells [48].

However, human Th17 cell differentiation is intrinsically different from murine Th17 because IL-23R is already expressed on human naive T cells, prior to differentiation [49]. IL-1β and IL-23 are sufficient to induce human Th17 cells from $CD4^+CD161^+$ cells derived from umbilical cord blood [50], whereas the role of TGF-β has been controversial [51, 52]. Recently, it has become clear that TGF-β plays an auxiliary role in the suppression of Th1 and Th2 cells [53]. TGF-β orchestrates with proinflammatory cytokines to promote Th17 cell differentiation in a dosage-dependent manner [54]. At low concentrations, TGF-β induces RORγt expression and promotes the expression of RORγt-inducing genes. However, at high concentrations, robust forkhead box P3 (FOXP3) expression induced by TGF-β suppresses Th17 cell differentiation by antagonizing RORγt function [55]. In our laboratory, we also found that human naive T cells responded differentially to the concentration of TGF-β, depending on the individual donor (Tsai, HC, unpublished data).

Through cytokine signaling or environmental factors, multiple transcription factors (TFs) are induced to drive Th17 differentiation [56], such as STAT3, Runt-related transcription factor 1 (Runx1) [57], RORα, RORγt, aryl hydrocarbon receptor (AHR), interferon regulatory factor 4 (IRF4), and basic leucine zipper transcription factor (BATF) [58]. These TFs not only regulate IL-17A expression but the expression of other Th17-associated genes as well. The expression of Th17-signature cytokines, such as IL-17A, IL-17F, and IL-22, is differentially regulated by Th17-lineage transcription factors. RORγt is the "master regulator" for Th17 differentiation and also directly binds to cis-regulatory elements of the IL17A/F gene [59, 60]. AHR responds to a physiological ligand, tryptophan photoproduct 6-formylindolo[3,2-b]carbazole (FICZ), to promote both IL17A and IL22 expression [61]. The expression of IL17A and IL22 is differentially regulated by TGF-β. IRF4 regulates not only IL17A expression [62, 63] but also Th2 cytokine expression [64]. Thus, IRF4 may regulate Th2 and Th17 differentiation by interacting with different transcription factors. The STAT family also plays a vital role in Th17 differentiation. STAT3 is activated by Th17 promoting cytokines (IL-6, IL-21, and IL-23) and directly binds to the promoter of the *Il17a-Il17f* locus, as well as the *Il21* gene [45]. On the other hand, other Stat molecules, including Stat1, activated by IL-27 [65, 66], and Stat5a/b, activated by IL-2 [67], play inhibitory roles in Th17 differentiation. Recently, the reciprocal action of STAT3 and STAT5 on the *Il17a* loci has been reported [68]. STAT3 and STAT5 have been demonstrated to compete for the same binding sites of the *Il17a-Il17f* locus [68]. The relative ratio of STAT3/STAT5 affects the intensity of IL-17A and IL-17F expression in Th17 cell differentiation [68].

The differential regulation of Th17 cytokines also reflects their different roles in physiological conditions and disease pathogenesis [7, 69]. For instance, Yang and colleagues [8] suggested that IL-17A was required to induce EAE, whereas IL-17F was required to induce airway neutrophilia in allergic airway animal models. Additionally, it was demonstrated that IL-22 but not IL-17A was required to protect mice from *Citrobacter rodentium* infection [70].

The understanding of Th17 cell differentiation has been applied to the development of therapies targeted to Th17-mediated autoimmune diseases [71]. Synthetic or natural forms of RORγt inverse agonists have been studied to suppress IL-17A expression. SR1001, one of the inverse agonists, was shown to be efficacious in the experimental autoimmune encephalomyelitis (EAE) model in rodents [72]. The comprehensive regulation of different Th17-related gene expression urgently needs to be studied for the development of more specific therapy in diseases.

3. IL17 Family and Their Receptors

Interleukin-17A was first identified in activated rodent T lymphomas, termed CTLA-8, and subsequently identified in humans in 1995 [73, 74]. At the time, it was noted that IL-17A had a unique structure among the interleukin cytokines. Five related cytokines were subsequently discovered through genome database searches and degenerative RT-PCR techniques [75]. The IL-17A cytokine family members (IL-17A, IL-17B, IL-17C, IL-17D, IL17E/IL-25, and IL-17F) share 20–50% homology at the amino acid level [76]. IL-17F is the most closely related member to IL-17A, and the IL-17F gene is located in the same chromosomal region as *Il17a* in humans (6p12). The resultant protein is approximately 44% homologous to the IL-17A protein and forms as homodimers and heterodimers with IL-17A, and binds a shared receptor heterodimer, IL-17RA/IL-17RC [28, 77–81]. IL-25 is the most

distantly related member of the IL-17 family, with only 20% homology to the IL-17A protein. IL-25 also binds to a different receptor heterodimer, IL-17RA/IL-17RB [82]. IL-17B, IL-17C, and IL-17D are less well characterized. IL-17B and IL-17C were reported to be associated with TNF-α production and inflammatory arthritis [83]. In recent studies, IL-17C has been demonstrated to bind to IL-17RA/RE and has similar biological functions to IL-17A [84–86]. Similar to IL-17A, Act1 activation is required for these IL-17C-induced responses [84]. In an EAE model, IL-17C deficient mice exhibited less severe disease; this phenomenon demonstrates the pathogenic role of IL-17C in EAE. IL-17C also promoted Th17 responses via IL-17RE signaling in an EAE model [84]. IL-17C was reported to induce the expression of cytokines, chemokines, and antimicrobial peptides by epithelial cells. Overall, IL-17C is important in host defenses against pathogens [85, 86]. IL-17D is preferentially expressed by the nonimmune cells that compose skeletal muscle, adipose and lung tissue. It induces IL-6, IL-8, and GM-CSF expression in endothelial cells and suppresses hematopoiesis [87]. Since Th17 cells express only IL-17A and IL-17F, we will highlight their roles and what is known regarding IL-17R signaling in the following discussion.

3.1. IL-17R Signaling. The IL-17R family is composed of five receptors (IL-17RA-IL-17RE) and the ligand-receptor pairing is not completely understood for all members. Extensive biochemical studies have been executed to characterize IL-17 binding to its receptors [88]. Briefly, IL-17A and IL-17F can form homodimers or heterodimers (IL-17A/A, IL-17A/F, IL-17F/F) to bind to a heteromeric receptor complex composed of IL-17RA and IL-17RC. Surface plasmon resonance (SPR) studies revealed that the different dimers have different affinities for the receptor subunits [28, 77, 89]. X-ray crystallographic, fluorescence resonance energy transfer (FRET) and SPR analyses suggest that IL-17RA homodimers are preassembled as "inactive" receptors on the cell membrane in the absence of ligand binding and that ligand binding shifts the favorability towards the formation of an IL-17RA and IL-17RC heterodimer [78, 79, 89].

In 2003, a bioinformatics approach was used to identify a conserved domain present in IL-17RA and the other IL-17 receptor family members, that was distantly related to the TIR domain in Toll-like receptor (TLR) and IL-1 receptor (IL-1R) signaling [90]. Because of the similarity to the TIR domain, this domain, termed the SEFIR (SEF/IL-17R) domain, was proposed to belong to a superfamily with the TIR domain, termed the STIR superfamily. In TLR signaling, the TIR domain mediates the binding of adaptor proteins such as MyD88 and Mal/TRAP to the receptor via homotypic interactions between their respective TIR domains [91, 92]. However, the SEFIR domain lacks the TIR box 3 subdomain and the BB-loop [90], which are critical for the protein-protein interaction of TLR signaling [93]. MyD88 and Mal are not thought to be involved in IL-17 signaling. Although lacking BB loop, a TIR-like loop (TILL) at the C′-terminal side of SEFIR domain in IL17RA, which sequence is homologous to BB loop, may provide the surface for protein-protein interaction [94, 95]. Another SEFIR-domain containing protein, Act1 (also known as CIKS) was later identified as an essential mediator of IL-17 signaling via its interaction with IL-17R [28, 96]. The shRNA knockdown of Act1 expression was shown to attenuate IL-17A signaling in mouse embryonic fibroblasts (MEFs) [97]; likewise, Act1-null mouse embryonic fibroblasts were shown to be unresponsive to IL-17A stimulation [98]. The direct interaction of Act1 and IL-17RA was demonstrated through coimmunoprecipitation experiments and shown to be dependent on the SEFIR domain [97, 98]. Act1 KO mice were shown to have reduced EAE and DSS-colitis induced disease severity, similar to IL-17A KO mice [98]. It was subsequently shown that Act1 can also interact with IL-17RC, as well as IL-17RB, in a SEFIR domain dependent manner [28, 96]. Act1 was also shown to be an essential mediator of IL-25 signaling [96]. Now, it is clear that the CC′ loop of the SEFIR domain is critical for the SEFIR-SEFIR binding [99]. In addition to the SEFIR domain, it has recently been shown that the C-terminal region beyond the SEFIR domain, for both IL-17RA and IL-17RC, is also necessary for the full activity of IL-17A [100].

Act 1 has a TNFR-associated-factor- (TRAF-) binding domain at the amino terminus and a coiled-coil domain containing the SEFIR domain at the carboxyl terminus. TRAF3 and TRAF6 have both been shown to associate with IL-17RA. TRAF6 associates indirectly with IL-17RA through Act1 and, in most cases, positively mediates IL-17A signaling [101]. Recently, TRAF3 has been shown to interact directly with IL-17RA, via a TRAF-binding domain at the distal C-terminus of the receptor's intracellular domain, as well as with the intracellular domain of IL-17RC [102]. Most intriguingly, TRAF3 has been shown to inhibit IL-17A signaling and IL-17 mediated EAE, the first demonstration of a negative regulatory feedback mechanism for IL-17A. Although the mechanism of this negative regulation is not completely clear, it may in part be due to the fact that TRAF3 binding to the distal domain of IL-17RA appears to interfere with Act1-TRAF6 binding to the SEFIR domain of IL-17RA.

In addition, Act1 is also a U-box type E3 ubiquitin ligase and it ubiquitinates TRAF6. The TRAF6 ubiquitination is required for the IL-17A-induced activation of nuclear factor-κB (NF-κB) [103]. The canonical NF-κB pathway is the most well-described downstream signaling pathway of IL-17A. Indeed, IL-17A induces phosphorylation of p65 at Ser^{536}; our lab and others have demonstrated p65 and p50 translocation into the nucleus following IL-17A stimulation [23, 104]. Mutation of NF-κB binding sites in the promoter region of the IL-17A target gene, human beta defensin 4 (*DEFB4*), severely attenuates promoter activation in response to IL-17A stimulation in airway epithelial cells [105]. NF-κB is also the major pathway responsible for IL-17 induced early response genes (<4 hours) [104]. However, NF-κB cannot be the sole pathway responsible for IL-17A's effects. For example, in comparison to "classical" NF-κB activating cytokines such as TNF-α and IL-1β, activation of p65-p50 NF-κB by IL-17A is relatively weak; yet, induction of DEFB4 in airway epithelial cells by IL-17A is much greater than either TNF-α or IL-1β [23]. It is plausible that it is the synergistic induction of multiple transcription

factors including NF-κB, which is responsible for IL-17A's effects. Indeed, other transcription factors, such as AP-1 and C/EBPδ (CCAAT/enhancer binding protein δ), have been shown to be activated by IL-17A [94, 106]. In addition, all three mitogen activated protein (MAP) kinase pathways, JNK (JUN Ntermainal kinase), ERK (extracellular signal-related kinase) and p38, have been described in the literature as being activated by IL-17A [107]. The relative contribution of the individual pathways appears to depend both on the cell type being studied, as well as on the target gene being studied. In airway epithelial cells specifically, our lab has demonstrated that JAK1/2 and PI3-kinase, Act1/TRAF6/TAK1/NF-κB, and MEK1/2 (MAP kinase kinase1/2)-ERK are all involved in IL-17 mediated gene expression, and that the pathway involved varied depending on the target gene in question [23, 108–110]. Other labs have shown the involvement in p38 in IL-17 mediated IL-6 and IL-8 gene expression by airway epithelial cells as well [111, 112]. Act1 has been shown to be necessary for IL-17A induced NF-κB and C/EBPδ activation, as well as JNK and p38 activation [98]. Interestingly, IL-17 induced ERK activation appears to be Act1 independent [98, 104].

IL-17A utilizes two different methods to increase target gene expression. The first is by transcriptional activation; we have previously demonstrated that this is the case for *DEFB4* and *CCL20* induction by IL-17A in airway epithelial cells using promoter-luciferase reporter assays [105, 109]. The second method of increasing gene expression is by stabilization of the target mRNA via a tristetrapolin/AUUUA-independent mechanism. This has been demonstrated in HeLa cells for both IL-17A induced *CXCL1* and *NFIKBZ* expression [113–115]. The mRNA stabilization pathway appears to be dependent on Act1, but independent of TRAF6, the first demonstration of Act1-dependent, TRAF6-independent IL-17 signaling.

More fine-tuned control of Act1 and IL-17R signaling has been recently described. Act1 exists in multiple phosphorylated forms, which display different functions. In 2010, Act1 was found to be phosphorylated upon IL-17A stimulation [116]. The inducible kinase IKKi (inducible inhibitor of NF-κB (IκB) kinase; also known as IKKε) forms a complex with Act1 and IL-17R and catalyzes the phosphorylation of Act1 at Ser^{311}, adjacent to its putative TRAF-binding motif [117]. The phosphorylated form of Act1 appears to have different affinities to various TRAF proteins. Mutation of IKKi or substitution of S311A of Act1 abolished Act1's interaction with TRAF2 and TRAF5, but not TRAF6. This phosphorylated form of Act1 has also been shown to be important for IL17R-Act1-TRAF2/5-mediated mRNA stability. Neither IKKi nor phosphorylation of Ser^{311} on Act1 is required for IL-17A-induced activation of NF-κB. However, IKKi is still responsible for the IL-17A-induced expression of pro-inflammatory genes (*Cxcl1, Cxcl2, Tnf, Il6,* and *Csf3*), resulting in neutrophilia and pulmonary inflammation. A different story is found for other phosphorylated forms of Act1. Three additional serines on human Act1 (Ser^{162} (not phosphorylated by IKKi), Ser^{220}, and Ser^{233}) and mouse Act1 (Ser^{147}, Ser^{209}, and Ser^{222}) are phosphorylated by IKKi and TBK1 (TANK binding kinase 1, another IKK-related kinases) [118]. TBK1 and IKKi play redundant roles in the phosphorylation of these three sites sites and act to suppress IL-17A-induced activation of NF-κB. In this study, the authors reported that IL-17A-induced Act1 phosphorylation is TRAF6-dependent and serves to suppress IL-17A-induced gene production, such as *ccl20, ccl3, cxcl2*, and *KC*. Interstingly, IKKi appears to regulate IL-17-induced Act1 phosphorylation at different sites via both TRAF6-dependent and TRAF6-independent pathways. More research regarding kinase-mediated Act1 phosphorylation and their specific roles in IL-17A-induced inflammatory response is needed.

3.2. Beyond IL-17RA: IL17-RC and IL-17RD. IL-17RA serves as the common receptor for IL-17 family members, in a manner similar to that of gp130 in IL-6 family signaling. IL-17RA is the most well-characterized IL-17R subunit because of its critical role in IL-17 and IL-25 induced signaling. However, in addition to IL-17RA, IL-17RC and IL-17RD have also been shown to have distinct functions in IL-17-mediated signaling.

IL-17RC was identified by a homology search of a mammalian expressed sequence tag database and found to share 22% sequence homology with IL-17RA [29, 119]. Unlike IL-17RA, IL-17RC has no obvious TILL structure in its cytoplasmic domain; whether Act1, TRAF6, or other signaling intermediates are recruited to IL-17RC are unclear [88]. Although the CC′ loop, which is responsible for the interaction of Act1/IL-17RA, is also conserved in IL-17RC [99], no direct evidence supports the interaction of Act1 and IL-17RC. Intriguingly, IL-17RA and IL-17RC have strikingly distinct tissue expression patterns. In contrast to IL-17RA, IL-17RC is preferentially expressed in nonimmune cells of the prostate, liver, kidney, thyroid, joints, and lung [77, 119–121]. In term of biological functions, IL-17RA and IL-17RC have differential affinity to IL-17A and IL-17F [28, 77, 89]. In humans, IL-17RA binds to IL-17F with extremely low affinity but IL-17RC has higher affinity binding to IL-17F than IL-17A. In mice, IL-17RA binds both IL-17A and IL-17F but IL-17RC only binds to IL-17F. Therefore, both IL-17RA and IL-17RC are required for IL-17F signaling. With the exception of IL-17RA, IL-17RC has various spliced isoforms and the affinity of IL-17RC splice variants to IL-17A and IL-17F are variable [29, 77]. Since some IL-17RC variants have no affinity to both IL-17A and IL-17F, it is possible that IL-17RC may have other ligands as well. The existence of soluble forms of IL-17RC have been demonstrated in humans but their physiological roles as well as that of the other variants are still unclear. Soluble IL-17RC has been proposed as a decoy receptor to inhibit IL-17R signaling but no evidence supporting this hypothesis yet exists [119]. Although little is known about exactly how IL-17RC participates in signaling, the cytoplasmic tail of the extended SEFIR domain of IL-17RC is essential for functional IL-17A-dependent signaling and IL-17RC knockout mice are susceptible to *Candida albicans* [100]. IL-17RC has also been reported to play a role in a number of human diseases. The levels of IL-17A, IL-17F, IL-17RA, and IL-17RC are also high in the sera and inflamed synovium of patients with rheumatoid arthritis [122–124]. However, the specific role of IL-17RC in these diseases has not been clarified.

IL-17RD was also known as SEF (similar expression to the FGFR) due to its similar expression pattern to the fibroblast growth factor receptor during zebrafish development [90]. The role of IL-17RD in FGF signaling and development is beyond the scope of this review. Previous findings showed that basal IL-17RD expression is higher than IL-17RA; in addition, IL-17A stimulation enhances the expression of other IL-17R family members, but not IL-17RD expression [125]. It has been indicated that IL-17RD may interact with IL-17RA to mediate IL-17A signaling, but the mechanism by which the interaction occurs has not yet been elucidated [126]. Recently, it has been reported that the orphan receptor IL-17RD differentially regulates IL-17A-induced NF-κB and p38 MAPK signaling. IL-17RD utilizes its SEFIR domain to sequester Act1 from interacting with IL-17RA and TRAF6, thereby negatively regulating NF-κB signaling. IL-17RD may therefore act as a basal braking system to prevent IL-17A mediated NF-κB activation. Conversely, IL-17RD promotes IL-17A-induced activation of p38 MAPK to induce the expression of the neutrophil-attractive chemokine, CXCL2, so the net effect of IL-17RD in IL-17A-mediated neutrophilia is unclear [127].

4. IL-17A-Induced Gene Expression in the Airway

IL-17A acts on a variety of cell types [128]. The best characterized IL-17A-targeted cells are nonimmune cells, such as epithelial cells and mesenchymal cells. In addition, IL-17A also acts on some immune cells. Immune cells express IL-17RA but not IL-17RC, and some studies have described that IL-17 synergized with B cell activation factor to promote B cell survival and proliferation [129] and that IL-17A induced matrix metalloproteinase 9 (MMP-9) expression in monocytes/macrophages [130, 131]. Here, we focus our discussion on the recent discoveries regarding IL-17A-mediated gene expression in airway epithelial cells and other cells in the airway.

In the context of airway epithelial cells, IL-17A-targeted genes can be roughly divided in three different categories: antimicrobial molecules, chemokines/cytokines, and adhesion molecules. A number of proteins with antimicrobial properties have been described as being upregulated by IL-17A, including CCL20, DEFB4, MUC5B/AC, S100A7, S100A8, and LCN2/24p3 (lipocalin 2) [25]. These proteins are important for the protective effect of IL-17 against extracellular pathogens. In addition, IL-17A stimulates the production of a number of chemokines and cytokines by airway epithelial cells. Some, such as CXCL1, CXCL2, IL-6, IL-8, KC, and GM-CSF, play a critical role in IL-17A's ability to recruit neutrophils to the airway [118, 125, 132–134]. Others, such as CCL20 and IL-19, have the ability to recruit or influence the differentiation of cells of the adaptive immune system, such as Th17 and Th2 cells, respectively [135, 136]. Additionally, in vitro studies showed that IL-17A induces CCL28 expression in the human airway epithelium, which causes the migration of IgE-secreting B cells [137]. Finally, it has been demonstrated that IL-17A can enhance the proliferation of airway epithelial cells, although the target genes responsible for this effect have yet to be identified [138]. IL-17A can also increase the expression of ICAM-1 in airway epithelial cells and claudin-1 and claudin-2 in intestinal epithelial cells, important adhesion, and cell junction molecules [139, 140].

Besides airway epithelial cells, IL-17A also directly acts on airway smooth muscle (ASM) [141] and lung microvascular endothelial cells (LMVECs) [142–144]. IL-17A directly enhances ASM contraction through the IL-17RA/RC complex on the basis of a NF-κB/RhoA/ROCK2 signal cascade in murine models of house dust mite-induced and ovalbumin-induced asthma. IL-17A mediated ASM contraction has also been confirmed in human lung tissue [141]. IL-17RA and IL-17RC are also expressed on the surface of LMVECs and IL-17A significantly induces CXCL1 production in LMVECs. In synergy with IL-1β and TNF-α, IL-17 also enhances CXCL5 and CXCL8 expression in these cells [144].

One of the most striking features of IL-17A signaling is its ability to synergize with other proinflammatory cytokines, as well as with TLR signaling pathways. In the literature, it has been reported that TNF-α, IL-1β, IL-22, Oncostatin M, IFNγ, BAFF, and CD40L can all synergize with IL-17A to upregulate IL-17A target genes or their respective target genes [129, 139, 145–147]. In addition, IL-17A also synergizes with TLR2 and TLR4 ligands to increase IL-8 production in a human cystic fibrosis bronchial epithelial cell lines [148]. In an *in vivo* context, this may be where IL-17A induces its most potent effects, within the cytokine milieu of an inflammatory setting to further potentiate the inflammatory response. The mechanism by which this synergism occurs is not yet known, but deserves further study.

5. IL-17A in Pulmonary Infection

Numerous studies have identified a protective role of IL-17A in immunity against various infections, including the infection of intracellular [133, 149–151] and extracellular bacteria [152, 153], fungi [154, 155], and even parasites [156]. In murine models of airway infection, IL-17A has been shown to play a critical role in the defense against extracellular bacterial pathogens, such as *K. pneumonia*, and *Pseudomonas aeruginosa* [152, 153, 157]. It has also been reported to play a protective role against intracellular bacterial pathogens, such as *Chlamydia muridarum*, and *Mycoplasma pneumonia*. Although the exact mechanism of this protection is unclear, the deficiency of IL-17 signal or other Th17-associated cytokines in various infection models has shown that neutrophil recruitment is impaired in infected tissue, which is also linked to the reduction of CXC chemokines expression [133, 149–151]. Additionally, Th17 cell response has also been reported to contribute to the mucosal vaccine response against pathogens [158, 159]. Mice vaccinated with antigen from *Mycobacteria tuberculosis* (*Mtb*) provoke a Th17 response, and the CXCL-13 induction by IL-17A is critical in the protection against Mtb infection [159]. IL-17A has also been reported to play a protective role at other mucosal surfaces, with other types of pathogens as well, *Candida albicans* infection in the oral cavity and *Salmonella*

dissemination in the intestines [155, 160]. Interestingly, one intracellular respiratory pathogen, *Chlamydia pneumoniae*, has developed a defense against IL-17A signaling. It encodes a protein, CP0236, which binds to the essential IL-17 signaling mediator protein Act1 (also known as CIKS and TRAF3IP2) and sequesters it in bacterial inclusion bodies, leaving it unavailable to mediate IL-17 signaling [161]. The adaption of anti-IL-17 strategies by bacterial pathogens underlines the importance of IL-17A signaling in host defense.

6. Innate IL-17A-Producing Cells in Host Defense and Pulmonary Infection

The significance of IL-17A production by innate immune cells in host defense against infection as well as development of autoimmune diseases has been demonstrated and reviewed [162–164]. Cells of the innate immune system are abundant in the skin and at mucosal surfaces and respond rapidly to pathogenic infection, providing the first line of defense. Interestingly, innate IL-17A-producing cells share some characteristics with Th17 cells. For instance,s $\gamma\delta$T cells respond to IL-1β, IL-18, and IL-23 [165–167] and share some common transcription factors with Th17 cells, such as AHR, RORγt, and STAT3 [59, 168, 169]. ILCs can be divided into three functionally distinct types; RORγt is required for the differentiation and maintenance of type 3 ILCs [170, 171]. These RORγt + ILCs express either IL-17A or IL-22, and some of them express both IL-17A and IL-22 [18–20, 172, 173]. iNKT cells constitutively express IL-23R and RORγt [174, 175] and produce IL-17A upon stimulation by IL-1β, IL-18, IL-23, and TGF-β [174, 176, 177]; however, unlike Th17 cells, iNKT cells do not respond to IL-6 stimulation [174].

Early IL-17A production by these innate cells provides an initial response to pathogens to recruit neutrophils within 4–8 hours after infection. In the lung, $\gamma\delta$T cells have been demonstrated to be the major source of early IL-17A production in response to some infections, such as *K. pneumonia* [178], *M. tuberculosis* [15, 179], and *Mycobacterium bovis* [180]. In the *M. bovis*-infected mouse model, the IL-17A secretion by $\gamma\delta$T cells is essential for mature granuloma formation and resolution of infection [180]. IL-17A-producing iNKT cells comprise up to 40% of pulmonary iNKT cells [16] and may also be responsible for the infection with *Streptococcus pneumonia* [181]. In addition, early IL-17A responses may also promote subsequent adaptive immune responses. IL-17A has been reported to induce chemokines to attract Th1 cells into the lung [182, 183], and this secondary response may provide more efficient pathogen clearance. In an EAE model, the depletion of $\gamma\delta$T cells is responsible for the development of fewer antigen-specific Th17 cells [165]. Therefore, innate IL-17-producing cells may also enhance or direct the development of later Th17 responses.

7. Summary and Perspectives

To summarize, Th17 effector cytokines such as IL-17 are differentially regulated via multiple transcription factors and play different roles in diseases. Through multiple IL-17R subunits and the posttranslational modification of Act1, IL-17A mediates tissue inflammation and host defense in many facets of signaling regulation. IL-17A induced production of pro-inflammatory cytokines, chemokines, and antimicrobial peptides by multiple cell types in the airway is critical for mounting successful host defense against pathogens. Due to advances in Th17/IL17A research, efforts are now underway to apply some of these findings to the clinical setting, particularly in the setting of autoimmune diseases. A delicate balance is needed to dampen the pathogenic effects of Th17/IL-17A in inflammatory and autoimmune diseases while maintaining the important role it plays in airway host defense. A more comprehensive understanding of Th17 cell differentiation and their functions is urgently needed to provide specific molecular targets to constrain disease-specific cytokine production from Th17 cells but retain other functions of Th17 cells. In addition, the role of innate IL-17A-producing cells in contributing to the resolution of infection and the progression of inflammation cannot be overlooked. Studies on the regulation mechanism of innate-IL-17-producing cells and the clinical relevance of these cells are still limited. A comprehensive understanding of these innate IL-17 cells may be useful in the development of disease therapy. Additionally, a thorough knowledge of cell-type specific IL-17 signaling mechanism also provides alternative therapeutic potentials in IL-17A-mediated diseases.

Conflict of Interests

The authors have no direct financial interest or relationship to the subject matter of this report.

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Review Article

Pivotal Roles of T-Helper 17-Related Cytokines, IL-17, IL-22, and IL-23, in Inflammatory Diseases

Ning Qu,[1] Mingli Xu,[2] Izuru Mizoguchi,[3] Jun-ichi Furusawa,[3] Kotaro Kaneko,[3] Kazunori Watanabe,[3] Junichiro Mizuguchi,[4] Masahiro Itoh,[1] Yutaka Kawakami,[2] and Takayuki Yoshimoto[3]

[1] *Department of Anatomy, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan*
[2] *Division of Cellular Signaling, Institute for Advanced Medical Research School of Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*
[3] *Department of Immunoregulation, Institute of Medical Science, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo160-8402, Japan*
[4] *Department of Immunology, Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan*

Correspondence should be addressed to Takayuki Yoshimoto; yoshimot@tokyo-med.ac.jp

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T-helper 17 (Th17) cells are characterized by producing interleukin-17 (IL-17, also called IL-17A), IL-17F, IL-21, and IL-22 and potentially TNF-α and IL-6 upon certain stimulation. IL-23, which promotes Th17 cell development, as well as IL-17 and IL-22 produced by the Th17 cells plays essential roles in various inflammatory diseases, such as experimental autoimmune encephalomyelitis, rheumatoid arthritis, colitis, and Concanavalin A-induced hepatitis. In this review, we summarize the characteristics of the functional role of Th17 cells, with particular focus on the Th17 cell-related cytokines such as IL-17, IL-22, and IL-23, in mouse models and human inflammatory diseases.

1. Introduction

$CD4^+$ T-helper (Th) cells play a central role in initiating and maintaining diverse immune responses. Functionally distinct Th cells are induced when naïve T cells are stimulated via T cell receptor engagement in conjunction with costimulatory molecules and cytokines produced by innate immune cells. Classically, Th1 cells regulate cellular immunity via production of interferon (IFN)-γ, whereas Th2 cells regulate humoral immunity via production of interleukin (IL)-4, IL-5, and IL-13 [1, 2]. Regulatory T cells (Tregs), a third subset of $CD4^+$ T cells, regulate the activation and expansion of these lineages via expression of forkhead box P3 and/or their capacity to produce cytokines such as transforming growth factor (TGF)-β, IL-10, and IL-35 [3, 4]. Recently, the identification of a novel lineage of helper T cells, Th17, has broken the long-held paradigm regarding the roles of the other three lineages (Th1, Th2, and Treg) (Figure 1). Distinguished by the production of IL-17 (also called IL-17A), these Th17 cells are developed from naïve $CD4^+$ T cells under the influence of a network of inflammatory cytokines, including IL-1, IL-6, and TGF-β, which support the commitment to this lineage. Although IL-23 was previously reported to be necessary for Th17 differentiation, it is currently thought that IL-23 plays an important role in the survival and expansion of pathological Th17 cells [5–9].

Th17 cells were first defined by their expression of IL-17A, but they have since been shown also to preferentially express IL-22, as well as IL-17F, IL-21, GM-CSF, and potentially TNF-α and IL-6 [10, 11]. However, it is becoming apparent that the IL-22 expression profile differs from that of IL-17A. Whereas TGF-β and IL-6 are both necessary for induction of IL-17A, IL-22 can be induced via IL-6 alone, and increasing amounts of TGF-β are actually inhibitory to the expression of IL-22 [12]. Accumulating data suggest that Th17 cells play a significant role in infectious diseases, autoimmune conditions,

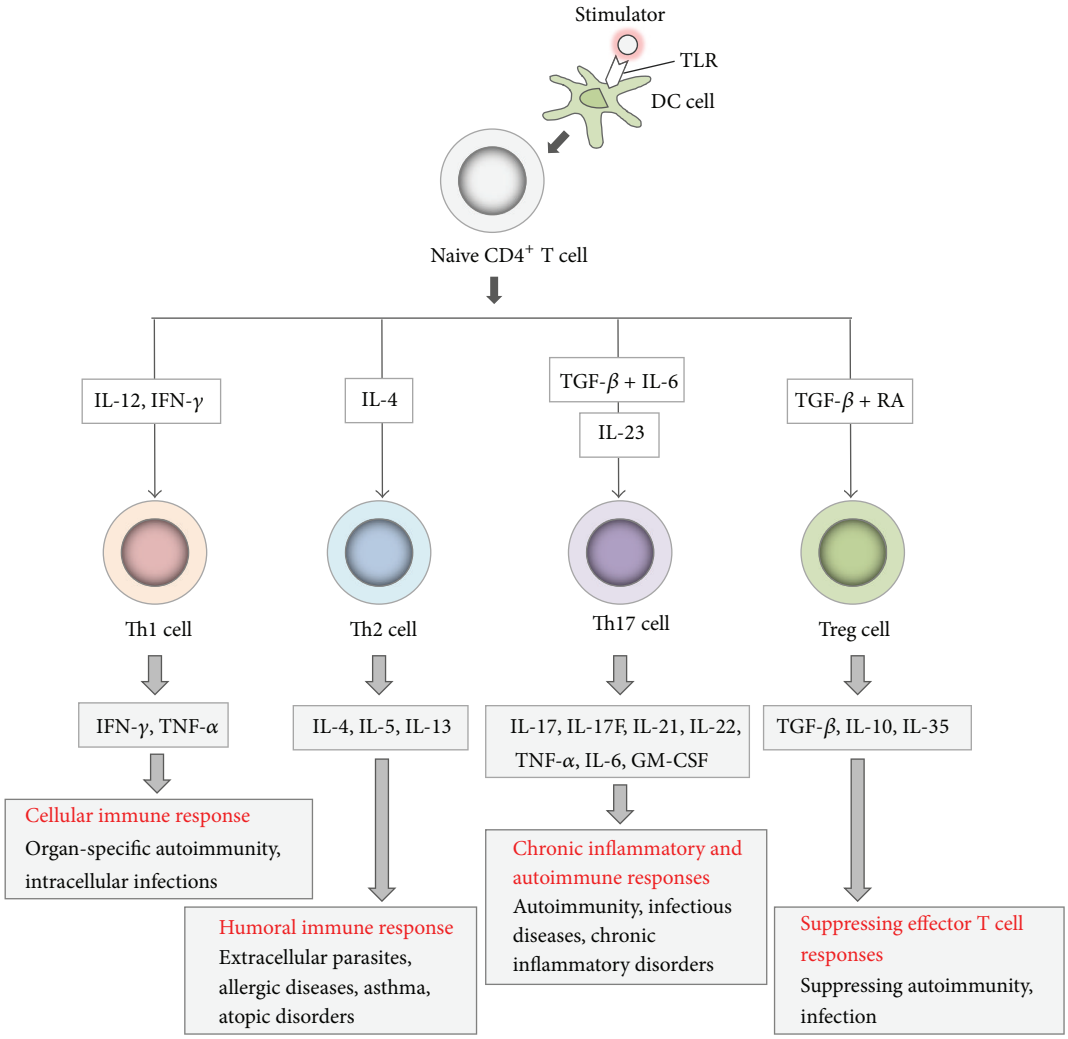

Figure 1: Differentiation of naïve $CD4^+$ T cells. Upon certain stimulating conditions, naïve $CD4^+$ T cells differentiate into different subpopulations, such as Th1, Th2, Th17, and regulatory T cells (Tregs). Th1: T-helper 1 cell; Th2: T-helper 2 cell; Th17: T-helper 17 cell; IL: interleukin; TGF-β: transforming growth factor-β; IFN-γ: interferon-γ; TNF-α: tumor necrosis factor-α; GM-CSF: granulocyte macrophage colony-stimulating factor; DC: dendritic cell; RA: retinoic acid.

adoptive immune response, and mucosal immunity [13–16]. The polarization of Th17 cells relies critically upon the actions of cytokines (e.g., IL-23) secreted by antigen-presenting cells (APCs) [14, 17, 18]. In addition to the inflammatory diseases, IL-23 also plays essential roles during tumorigenesis [19].

Based on evidence that Th17 cells can mediate inflammation and tissue destruction [20, 21], there has been intense interest in defining their origins and functions and developing strategies to block their pathological effects. In this review, we highlight studies that provide significant evidence for a role of Th17 cells in human diseases and animal models, and we briefly review the role of Th17 cells by focusing on the production of cytokines in inflammatory diseases (Figure 2).

2. Th17 Cells in Inflammatory Skin Diseases

Inflammatory skin diseases include psoriasis, allergic contact dermatitis, and atopic dermatitis. Psoriasis is a complex autoimmune skin disease characterized by interactions between dendritic cells (DCs), T cells, and keratinocytes [22, 23]. Although mice with epidermal acanthosis and dermal inflammation induced by IL-23 injection into the ear are not an exact model for psoriasis, many of the features in this model, such as IL-22 upregulation and STAT3 activation, are similar to the features evident in psoriasis.

In psoriasis, IL-23 is produced at high levels by DCs and keratinocytes, and this cytokine stimulates Th17 cells to produce IL-17A and IL-22. Several groups reported that psoriatic lesions showed increased mRNA levels of the IL-23/Th17 axis, including IL-23p19, IL-12/23p40, IL-22, IL-17A, and IL-17F, whereas mRNA levels of IL-12p35 and IL-4 were not elevated [24–26]. Furthermore, evidence for the role of IL-23 in the pathogenesis of psoriasis was substantiated by the initiation of the psoriasis-like disease acanthosis following repeated injections of IL-23 in mice [12]. More recent studies have also revealed that polymorphisms in the IL-12/23p40 and IL-23 receptor (IL-23R) are associated with psoriasis [27]. Ustekinumab, an anti-IL-12/23p40 antibody, has been used to

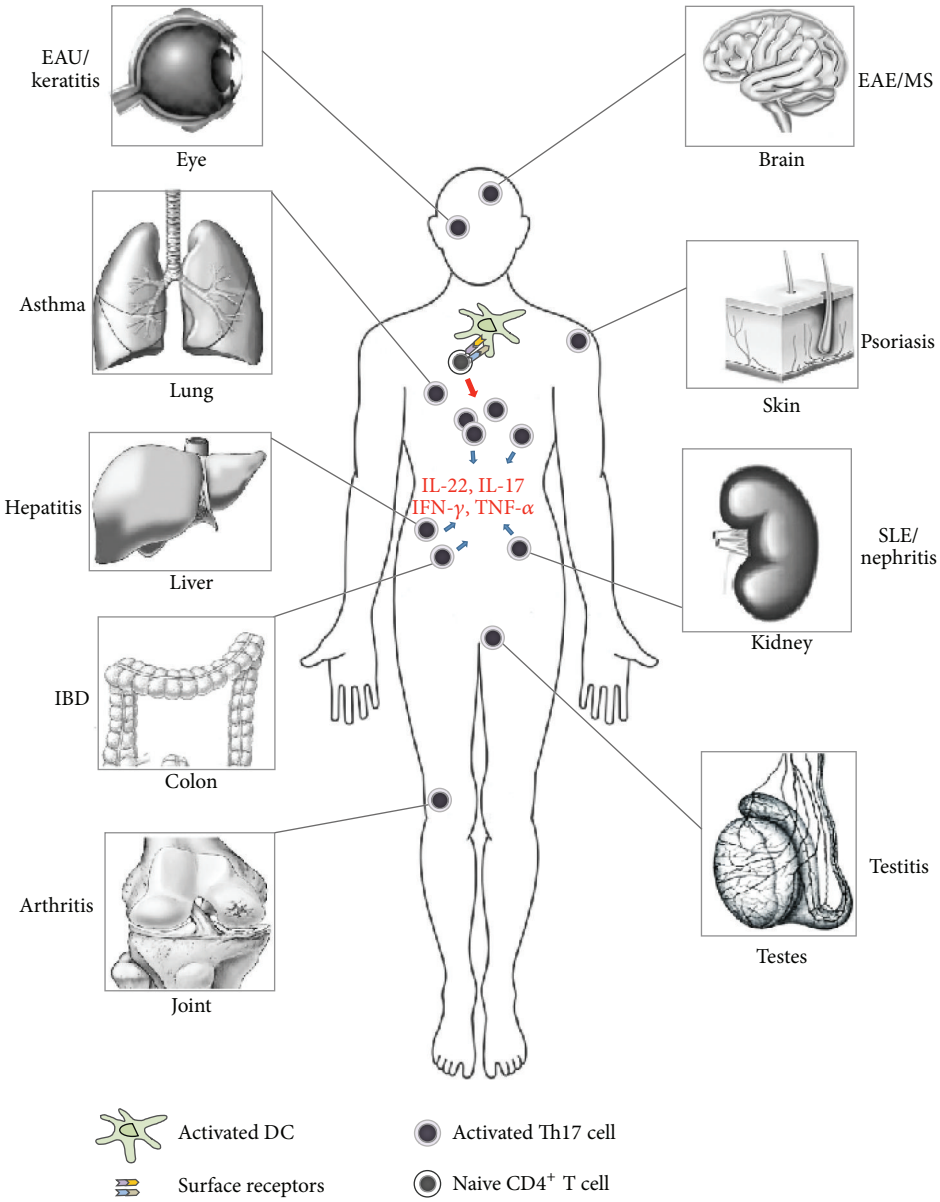

Figure 2: Schematic representation of Th17 cell-induced inflammatory diseases in humans. Inflammation mediated by Th17 cells has been identified in several human organs or tissues, including the eye, brain, skin, liver, colon, kidney, testes, joint, and lung. Numerous cytokines induced by activated Th17 cells, such as IL-22, IL-17, IFN-γ, TNF-α, and IL-6, play essential roles during the inflammatory diseases. These cytokines lead to the onset of the uveitis, autoimmune encephalomyelitis, psoriasis, hepatitis, inflammatory bowel disease, nephritis, testitis, rheumatic arthritis, and asthma. The counteraction between protective cytokines and pro-inflammatory cytokines decides the final outcome in the organ or tissue.

treat plaque psoriasis [28]. In transgenic mice, overexpression of individual subunits of IL-23 led to inflammation [29]. In another mouse study, recombinant IL-23 injected into normal skin produced erythematous skin with histologic characteristics of psoriasis [30].

IL-22 is a key cytokine produced by Th17 cells, and it plays an important role in maintaining homeostasis and remodeling epithelial tissues. The importance of IL-22 has been highlighted in the pathogenesis of psoriasis [12]. IL-22 mRNA expression is upregulated in psoriatic skin as compared to normal skin, whereas the levels of IL-22 mRNA in peripheral blood mononuclear cells from psoriatic patients and normal controls were similar [31]. Using IL-22-deficient mice, Zheng et al. showed that in the absence of IL-22, IL-23-mediated dermal inflammation was reduced [12]. Another study also showed that IL-22 is required for psoriasis-like lesions in the mouse Imiquimod model. Imiquimod-induced scaly skin lesions were almost totally absent in IL-22-deficient mice or in mice treated with anti-IL-22 antibody. Importantly, IL-22 mediates keratinocyte activation via phosphorylation of STAT3, leading to acanthosis that is associated with a psoriatic phenotype [12, 32].

In addition, injection of IL-23 enhances IL-17A expression in mouse skin, but pretreatment of anti-IL-17A antibody

does not ameliorate the formation of psoriatic lesions [30]. This observation suggests that IL-17A is dispensable during IL-23-dependent psoriasis. Skin biopsy samples from patients with psoriasis showed elevated expression levels of IL-17 together with high expression of IL-23 and IL-22 [12, 33]. Although there was no difference between the levels of IL-17 in the sera of patients with psoriasis and in controls, there was a correlation between serum levels of IL-17 and the severity of psoriasis [34].

3. Th17 Cells in Inflammatory Bowel Diseases

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a chronic inflammatory disease of the gastrointestinal tract. IBD is caused by aberrant innate and/or adaptive immune responses [35]. IBD had long been described as a Th1-mediated disease because IFN-γ is essential for disease progression [36]. However, the recent discovery of Th17 cells has revealed a key role of this subset of T cells in IBD.

IL-23 is essential for the development of IBD in mouse models [37, 38], and protective IL-23R polymorphisms in the human population were identified through a genome association study [39]. However, IL-22 stimulates epithelial cell growth, goblet cell hyperplasia, and antimicrobial production. IL-22-mediated protective effects were seen in the T cell transfer colitis model [40]. IL-22 is highly upregulated in the sera and lesions of patients with either Crohn's disease or ulcerative colitis [41]. Moreover, activation of aryl hydrocarbon receptor (AHR) results in the elevated production of IL-22 in particular and reduction of Th1 and Th2 cytokines [42, 43]. Blockade of IL-22 by using its neutralizing antibody reversed the therapeutic effect of 6-formylindolo (3, 2-b) carbazole on the trinitrobenzenesulfonic acid-induced colitis in mice. Thus, induction of IL-22 is one of the major mechanisms controlling pathogenesis in the gut through the AHR signaling pathway [44].

On the other hand, IL-17 is produced in healthy gut. A recent study suggested that IL-17F, but not IL-17A, was required to induce severe immunopathology in the dextran sulfate sodium-induced colitis model [45]. In contrast, anti-IL-17A monoclonal antibody treatment was demonstrated to aggravate dextran sulfate sodium-induced colitis, and blockade of IL-17A in colitis of IL-10 knockout mice was inefficient in reducing disease unless IL-6 was also neutralized [46, 47]. Another study demonstrated that adoptive transfer of IL-17A-deficient naïve $CD4^+$ T cells or transfer of IL-17 receptor-deficient T cells to recipient immunedeficient mice induces severe colitis [48], suggesting that IL-17 exerts a protective effect on T cells. Collectively, these results indicate that Th17 cytokines have both anti- and pro-inflammatory effects in the gut, depending on the microenvironments.

4. Th17 Cells in Experimental Autoimmune Encephalomyelitis/Multiple Sclerosis

Experimental autoimmune encephalomyelitis (EAE), which resembles an autoimmune inflammatory disease of human multiple sclerosis (MS), was classically believed to be mediated by Th1 cells and inflammatory macrophages. However, the concept that Th1 response is centrally important for autoimmunity was challenged by evidence that animals lacking a functional Th1 response still develop aggravated autoimmune encephalomyelitis. Recent studies demonstrated an association between the development of demyelinating plaques and the accumulation of Th17 cells in EAE and MS.

IL-23 plays a pivotal role in the development of EAE. Mice deficient in IL-23p19 or IL-23R knockout mice were resistant to EAE [5, 49, 50]. Moreover, IL-23R is expressed in macrophages infiltrating the central nervous system, and macrophages expressing IL-23R in response to IL-23 produce IL-22 and IL-17 [5, 50].

In addition, IL-17 (IL-17A) also plays a pro-inflammatory role during the development of EAE, as shown by several lines of evidence. First, IL-17F knockout mice with normal levels of IL-17A showed only marginally reduced EAE [45]. Second, IL-17A knockout mice with normal levels of IL-17F showed milder disease [51]. Finally, IL-17A knockout mice with reduced levels of IL-17F exhibited clearly reduced EAE [15]. Moreover, administration of anti-IL-17A antibody could attenuate EAE but not completely prevent this disease [52].

Although IL-22 can be induced from Th17 cells by IL-23 during inflammation, IL-22 seems to have no effect on the development of EAE. Kreymborg et al. showed that IL-22 knockout mice are not protected from EAE [53].

In MS patients, IL-17 mRNA and protein levels were increased in both brain lesions and mononuclear cells isolated from blood and cerebrospinal fluids [54, 55]. Although these observations suggest that IL-17 may contribute to the development of MS in humans, further research is needed to elucidate the precise role of this cytokine in the pathogenesis of MS. In addition, because IL-23 plays a pivotal role in EAE, administration of monoclonal antibody specific for IL-23p19 instead of IL-17A or IL-17F might prevent this disease [56]. Based on these results, neutralization of IL-23 may be an effective therapeutic approach to treat EAE/MS.

5. Th17 Cells in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with the destruction of affected joints, and it represents one of the most common autoimmune-related diseases. Although RA had long been classified as a Th1-mediated disease, it is now thought to be a primarily Th17-driven disease [57].

Initial evidence for a pathogenic role of IL-17 in RA came from reports that IL-17 was increased in the sera and synovial fluids of RA patients [58–60]. Long-term intra-articular administration of IL-17 via gene transfer reproduced the key features of RA, including massive inflammation, bone erosions, and cartilage damage [61]. As with psoriasis, there is also increased IL-22 and IL-23 in the synovium of RA patients [62, 63]. Notably, the increase in IL-17 and IL-23 appears to be specific for RA, but not for osteoarthritis [64, 65]. Conversely, inhibition of IL17 by antibodies against IL-17A or its receptor IL17RA protected against the development of arthritis [66].

Because cyclosporine A can inhibit the production of IL-17 by memory Th17 cells in healthy donors and RA patients [67], this could be an effective strategy to limit the disease. Furthermore, mice lacking IL-17RA develop a very mild form of experimental arthritis [68]. TNF has been shown to be a key cytokine in the collagen-induced arthritis model. Although TNF contributes to the pathogenesis of the early stages of the disease, it is not involved in the later stages. In contrast, IL-17 has a role throughout all stages of chronic disease [69]. This finding is another indication that IL-17 contributes to the chronicity of RA. Therapeutic strategies that specifically block Th17 cell development are expected to be highly effective in treating RA patients.

6. Th17 Cells in Renal Inflammation

Several recent studies have emphasized the functional importance of Th17-induced immune response in renal inflammatory diseases. We discuss the potential roles of the Th17 immune response in experimental murine models and humans.

6.1. Th17 Cells in Experimental Animal Models with Nephritis. The first evidence for the importance of TH17 cells in renal inflammation was provided by a murine model of crescentic glomerulonephritis [70, 71]. Recently, Th17 cells were identified in murine kidneys after ureteral obstruction [72]. In addition, the IL-23/IL-17 pathway was demonstrated to contribute significantly to renal tissue injury in experimental glomerulonephritis by analysis of nephrotoxic nephritis in both IL-23p19 and IL-17 knockout mice [70]. Moreover, IFN-γ plays a protective role in experimental autoimmune anti-glomerular basement membrane (anti-GBM) glomerulonephritis, as revealed by the fact that IFN-γ-deficient mice develop more severe anti-GBM disease [73]. In contrast, IL-23p19 and IL-17A knockout mice are protected from anti-GBM disease after treatment with anti-mouse GBM antibodies [70]. In addition, by using IL-12p35, IL-12p40, and IL-23p19 knockout mice, Ooi et al. demonstrated that mice deficient in IL-23, but not IL-12, were protected from glomerulonephritis [74]. Neutrophils were recently identified to be an early source of IL-17 in renal inflammation in a mouse kidney ischemia reperfusion injury model [75].

6.2. Th17 Cells in Human Renal Inflammation. There is only limited evidence of the involvement of Th17 cells/IL-17 in the pathogenesis of renal autoimmunity in humans. The contribution of IL-17 to inflammatory reactions in the kidney was initially reported in an *in vitro* study of patients suffering from renal transplantation graft rejection [76]. Recently, upregulation of IL-17 mRNA expression in the urinary sediment of patients with systemic lupus erythematosus (SLE) and increased percentage of Th17 cells in patients with active SLE were reported [77, 78]. Although serum IL-17 levels were significantly increased in SLE patients compared with normal controls, associations between serum IL-17 levels and clinical parameters were demonstrated [79]. Another study reported that a lower percentage of Th22 cells and higher percentage of Th17 cells are present in patients with lupus nephritis compared with healthy controls [80]. Th22 cells are a new subset of $CD4^+$ T helper differentiated from naïve T cells and characterized by secretion of IL-22 but not IL-17 or IFN-γ [43, 81]. IL-22 may play a protective role in preventing the development of lupus nephritis, although future research is necessary to identify the real role of IL-22 in SLE.

7. Th17 Cells in Hepatitis

7.1. Th17 Cells in Experimental Hepatitis Models. Intravenous administration of Concanavalin A (Con A) results in rapid liver inflammation and necrosis [82]. Many features of Con A-induced liver injury are believed to mimic human autoimmune and viral liver disorders. Numerous experiments have also shown that IL-22 plays a protective role in mice with hepatitis [83, 84]. However, there are conflicting reports regarding the susceptibility of *IL-17*-deficient mice to Con A-induced acute hepatitis [84, 85]. IL-17 is critical in the induction of liver injury and is induced during Con A hepatitis [86, 87]. Moreover, both IL-17A and IL-17F function via the IL-17 receptor (IL-17R), and both *IL-17A* and *IL-17F* are overexpressed in IL-17R-deficient mice, suggesting that a feedback loop acts on Th17 cells [88, 89]. In addition, IL-17 activates other cell types in the liver to produce pro-inflammatory cytokines beneficial to hepatocyte apoptosis [90].

Notch is an evolutionarily conserved molecule that controls the cell fate decision in a variety of cells [91, 92]. We previously demonstrated that Notch signaling drives IL-22 secretion by stimulating the AHR [93]. Mice that are deficient in RBP-J, a key mediator of Notch signaling, are highly susceptible to the detrimental immunopathology associated with Con A-induced hepatitis with little IL-22 production [93] (Figure 3). Although IL-6 has the ability to induce IL-22 production [12], and IL-6-deficient mice were shown to be highly susceptible to liver damage [94], these mice were reported to have no impairment in IL-22 expression during Con A-induced hepatitis [84]. IL-23 also has the ability to induce the production of IL-22 [12] and IL-17 [95]. However, there are conflicting reports regarding the role of IL-17 in Con A-induced hepatitis and the susceptibility of IL-17-deficient mice to hepatitis [84, 85]. Therefore, the role of IL-23 in the induction of IL-22 and IL-17 production and liver damage during Con A-induced hepatitis using IL-23p19- and IL-17-deficient mice was investigated [86]. These results revealed that the endogenous IL-23 plays a protective role in hepatitis in an IL-22-dependent manner, whereas exogenous IL-23 plays a pathological role in IL-17-dependent and -independent manners. Further studies are necessary to elucidate the precise role of exogenous IL-23 in Con A-induced hepatitis.

7.2. Th17 Cells in Human Liver Diseases. Chronic hepatitis B virus (HBV) or hepatitis C virus infection leads to liver disease. Such infective disease is associated with T cell activation and the secretion of numerous pro-inflammatory cytokines, such as IFN-γ. Nonetheless, although IL-22 shows

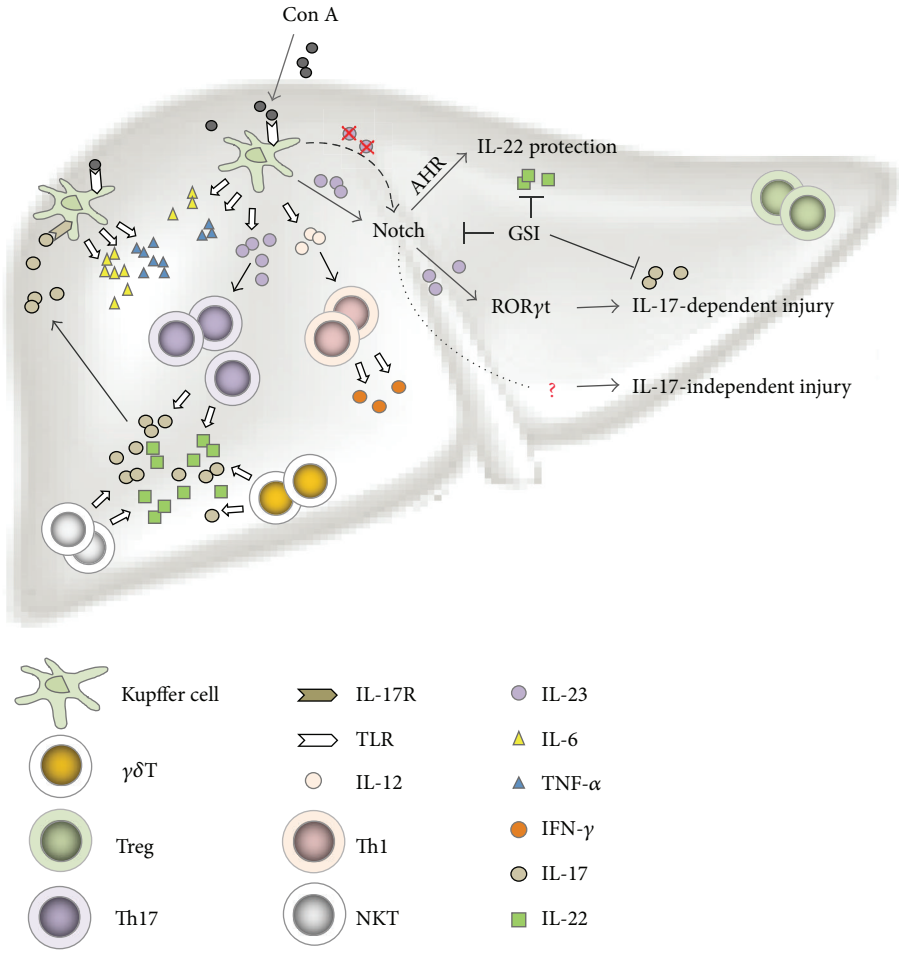

Figure 3: Schematic diagram of the role of activated Th17 cells during Con A-induced hepatitis. Con A injection induces IL-23 expression from Kupffer cells (also inducing IL-12, IL-6, TNF-α, and other cytokines) in the liver, then activates Notch signaling in activated Th17 cells (and other types of T cells). AHR-dependent production of IL-22 is pivotal for protection, and RORγt-dependent production of IL-17 is critical for pathogenesis. The IL-17/IL-17R signaling pathway also exacerbates hepatitis by inducing TNF-α and IL-6. Con A: Concanavalin A; AHR: aryl hydrocarbon receptor; RORγt: retinoic acid-related orphan receptor γt; TLR: toll-like receptor; GSI: γ-secretase inhibitor; IL-17R: interleukin-17 receptor.

a marked protective role in Con A-induced hepatitis, IL-22 also enhances the pro-inflammatory activity of TNF-α expressed in the liver after transfer of HBV-specific T cells [96]. Another study reported that IL-22 neutralization ameliorates liver damage after transferring HBV-specific T cells by using a transgenic mouse model of HBV replication [97]. During acute liver inflammation, IL-22 protects hepatocytes from injury, possibly through STAT3-mediated upregulation of prosurvival and proliferative responses. During chronic inflammation, IL-22 may also help to limit damage and allow survival of damaged hepatocytes that are precursors for hepatocellular carcinomas [98]. Future research is necessary to examine the role of IL-22 in chronic inflammation and the development of liver cancer. In patients with chronic HBV infection, Th17 cells are highly increased in both peripheral blood and liver, and they exhibit a potential to aggravate liver damage during chronic HBV infection [99]. Thus, Th17 cells may be involved in both the pathogenesis and anti-inflammatory responses in human liver diseases.

8. Th17 Cells in Ophthalmic Inflammation

8.1. Th17 Cells in Experimental Autoimmune Uveitis. The eye is an immune-privileged organ, and immune privilege is a complex phenomenon that involves multiple components. Uveitis is a sight-threatening intra-ocular inflammatory disease that is predominantly mediated by Th1 and Th17 [100]. Experimental autoimmune uveitis (EAU) is an animal model of human autoimmune uveitis, and activated Th1 and Th17 cells are considered to play a major role in initiating the intraocular inflammation [101].

The initial evidence indicated that Th1 cells predominantly produce IFN-γ in experimental and clinical uveitis [102–104]. However, it is now clear that IL-17-producing Th17 cells, but not IFN-γ-producing Th1 cells, are the true mediators of tissue-specific ocular pathogenesis [81, 105]. Neutralization of IL-17, but not IFN-γ, in mice prevents and ameliorates EAU [104, 106]. Several recent studies suggested that IL-17 has both pro- and anti-inflammatory effects on the

development of EAU [107, 108]. Furthermore, a protective role of IL-22 by inducing regulatory CD11b$^+$ APCs has been described in EAU [109]. In addition, CD4$^+$ T cells are necessary for initiating EAU, and depletion of CD4$^+$ T cells prevents EAU development. Furthermore, antigen-specific CD8$^+$ T cells also act as regulatory cells to suppress EAU [110, 111]. Similar to other autoimmune animal models, costimulatory signals such as CD40, CD80, and CD86 are also involved in the course of EAU, and blockade of these signals ameliorates intra-ocular inflammation [112–116].

8.2. Th17 Cells in Keratitis. IL-17A-producing cells are present in the midperipheral cornea in a mouse model of dry eye disease, as well as in corneas from patients with herpetic stromal keratitis (SK) [117–119]. The cornea infection with herpes simplex virus (HSV) 1 leads to SK, a blinding immune-inflammatory lesion of the eye. IL-17 is upregulated after HSV infection of the cornea [120]. HSV infection of IL-17R knockout mouse as well as IL-17 neutralization in wild-type mouse showed reduced SK damage [120]. In addition, administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin, which is a ligand for AHR, caused a significant induction of Tregs and inhibited the differentiation of Th1 and Th17 cells, resulting in suppression of the severity of SK damage [121].

Staphylococcus aureus and *Pseudomonas aeruginosa* often cause bacterial keratitis, and these bacteria predominantly invade corneal epithelial cells [122]. IL-6, one of the major cytokines responsible for differentiating into Th17 cells, is expressed in the corneal epithelial and conjunctival cell lines [123]. Desiccating stress in the murine dry eye model, similar to human dry eye, also causes ocular surface inflammation characterized by increasing IL-6 and IL-17A expression [118]. In general, IL-17RA is constitutively expressed in cornea and conjunctiva. When infected with *S. aureus*, human corneal epithelial cells were demonstrated to increase the production of IL-6 but show no change of IL-17A and IL-17RA *in vitro* [124].

9. Th17 Cells in Testes

The testis is an immunologically privileged site where germ cell antigens are protected from autoimmune attack [125–128]. Multiple mechanisms prevent autoimmune disease in the testes, including the structure of the blood-testis barrier and secretion of immunosuppressive factors mainly by macrophages, Sertoli, peritubular, and Leydig cells. Studies established the presence of several T cell subsets (CD4$^+$ and CD8$^+$ $\alpha\beta$ T cells, $\gamma\delta$ T cells, and NK cells) and Tregs, as modulators of immune response acting through local and systemic mechanisms, in normal testicular interstitium of human and rodents [129, 130]. However, the testicular environment does not preclude inflammatory reactions and recruitment of tissue-specific T lymphocytes, which appear to be crucial components of the inflammation cascade [131, 132]. In fact, testicular inflammatory disorders leading to impairment of spermatogenesis are thought to be a primary reason for male infertility [133–135]. The recruitment of immune cells in testicular interstitium (mainly DCs, macrophages, and T cells) and secretion of pro-inflammatory cytokines (IL-6, IFN-γ, TNF-α, IL-12, IL-17, and IL-23), which disrupt the normal testicular immunosuppressive microenvironment, occur during inflammation induced by infectious agents or develop in different pathologies, such as experimental autoimmunity orchitis (EAO) [136]. In the rat testis of EAO, it was demonstrated that not only CD4$^+$ cells (Th17) but also CD8$^+$ T cells produce IL-17 (Tc17). Both CD4$^+$ and CD8$^+$ T cells are the major contributors during the onset and chronic phases of EAO [136]. In human azoospermic testis with chronic inflammation, Th17 cells, which are orchestrated by IL-23 produced from APCs, are critically involved in chronic inflammation [137]. Such patients have increased levels of Th17 cells, their cytokines such as IL-17A, IL-21, and IL-22, and IL-23-producing CD11c$^+$ DCs and CD68$^+$ macrophages [137]. Moreover, because IL-17 was expressed not only in normal testis but also in higher levels in azoospermic testis, IL-17 might be involved in the maintenance of testicular immune privilege and spermatogenesis [137, 138]. In addition, pro-inflammatory cytokines including IL-1 and IL-6 have direct effects on spermatogenic cell differentiation and testicular steroidogenensis within the normal testis [139]. However, increased numbers and expression level of IL-17A-immunoreactive cells in azoospermic testis with chronic inflammation indicate that overexpression of IL-17A can substantially damage the blood-testis barrier and probably destroy normal spermatogenesis and germ cells, which in turn could ultimately lead to azoospermia.

IL-17-deficient mice showed decreased antigen-specific T cell activation and antibody production in models of autoimmune and allergic diseases [140]. In addition to the signature cytokine IL-17A (IL-17), Th17 cells also produce IL-17F, IL-21, and IL-22, which would also allow Th17 cells to communicate with a wide variety of immune and non-immune cells [14]. A recent study demonstrated that the small molecule halofuginone can selectively inhibit mouse and human Th17 cell differentiation and autoimmune inflammation *in vivo* through a cytoprotective signaling pathway [141]. An understanding of the development, function, and regulation of Th17 cells in testicular immunopathology is critical for designing better strategies for the treatment of immunological male infertility.

10. Th17 Cells in Allergic Airway Disease

Asthma is characterized by an inflammatory reaction associated with increased production of Th2 type cytokines, such as IL-4 and IL-13.

10.1. Th17 Cells in Mouse Models. Numerous studies have shown that Th17 cytokines play an essential role in allergic airway disease, and the role of Th17 cells was investigated in several mouse models. A study of IL-17RA knockout mice demonstrated decreased ovalbumin-induced airway eosinophilia and Th2-related cytokines [142]. IL-17A knockout mice showed attenuated airway eosinophilia and neutrophilia, whereas IL-17F knockout mice demonstrated elevated eosinophil recruitment. These findings suggest that

IL-17 drives the allergic Th2 response. Other studies also confirmed that IL-17 promotes ovalbumin-induced Th2 responses by synergizing with IL-4 and IL-13 [143]. Unlike IL-17, which has a pro-inflammatory role during allergic airway disease, IL-22 seems to suppress Th2-mediated inflammation. Treatment with anti-IL-22 antibody exacerbated airway eosinophilia, suggesting that IL-22 may have anti-inflammatory properties in airway disease [144]. In contrast, IL-23 knockout mice showed ameliorated eosinophilia compared to IL-23 overexpression mice [145, 146].

10.2. Th17 Cells in Human Asthma. The role of Th17 cells in human asthma remains largely elusive. In humans, increased expression of IL-17A and IL-17F was detected in bronchial submucosa, and examination of sputum in patients with asthma demonstrated that neutrophils were present, particularly in severe forms of this disease [147]. Furthermore, Lajoie et al. demonstrated a direct link among C5aR signaling, IL-17A production, and severe airway hyperresponsiveness; the sensitivity of airway hyperresponsiveness noted in mice after C5aR blockade is completely reversed by concurrent IL-17A blockade [148]. In addition, polymorphisms in the *IL-17A* gene related to asthma risk have been reported [149]. Further studies are necessary to clarify whether IL-17 is a safe therapeutic target for asthma therapy.

11. Conclusion

Th17 cells, which are directly involved in and mediate chronic inflammation, are characterized by the production of cytokines such as IL-17 and IL-22 as well as the recruitment of neutrophils and other inflammatory cells. Under certain circumstances, the same cytokine plays opposite roles in different tissues. For instance, IL-22 plays a protective role in Con A-induced acute hepatitis but a pro-inflammatory role in psoriasis. In different tissues, the counteraction between protective cytokines and pro-inflammatory cytokines should determine the final outcome of the immune responses. Although some conflicting findings still need to be resolved, targeting Th17 cells and their related cytokines such as IL-17, IL-22, and IL-23 may be an effective therapeutic approach for chronic inflammation in the future.

Authors' Contribution

N. Qu and M. Xu contributed equally to this work.

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Review Article

Loss and Dysregulation of Th17 Cells during HIV Infection

Sandra L. Bixler and Joseph J. Mattapallil

Department of Microbiology & Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA

Correspondence should be addressed to Sandra L. Bixler; sandra.bixler@usuhs.edu and
Joseph J. Mattapallil; joseph.mattapallil@usuhs.edu

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Bacterial translocation across the damaged mucosal epithelium has emerged as a major paradigm for chronic immune activation observed during HIV infection. T helper 17 (Th17) cells are a unique lineage of T helper cells that are enriched in mucosal tissues and are thought to play a central role in protecting the integrity of the mucosal barrier and maintaining immune homeostasis at mucosal sites. Th17 cells are lost very early during the course of HIV infection, and their loss has been shown to correlate with bacterial translocation. Interestingly, Th17 cells are unable to completely recover from the early destruction even after successful antiretroviral therapy (ART). Here, we review some of the potential mechanisms for the loss and dysregulation of Th17 cells during HIV infection.

1. Introduction

T helper 17 (Th17) cells have emerged as a key player in host-pathogen interplay at the mucosal surface. The lack of Th17 cells has been associated with recurring bacterial and fungal infections that are a hallmark of hyper-IgE syndrome [1, 2]. Th17 cells are enriched at mucosal sites [3–5] where they are thought to play a role in maintenance of immune homeostasis in response to commensal organisms and protect against pathogens that may gain entry via these surfaces [6]. Studies have shown that a paucity of Th17 cells in mucosal tissues is associated with systemic translocation of bacteria across the intestinal epithelial barrier [7].

Th17 cells are a unique lineage of T helper cells that are induced under anti-Th1/Th2 polarizing conditions and preferentially produce interleukin-17 (IL-17) [8–12] and express markers such as CD26, CD161, and interleukin-4-inducible gene [11, 13–15]. This newly identified subset of Th17 cells was later found to be the key effector T-cell subset mediating experimental autoimmune encephalitis (EAE) in mice [16, 17]. Deletion of Th1 cells was found to exacerbate the symptoms of EAE, and this finally led to identification of Th17 cells as the primary cells mediating the development of EAE [18–20].

IL-17 produced by Th17 cells serves as a chemoattractant for neutrophils to sites of infection and inflammation [21, 22]. IL-17 also promotes tight junction formation at mucosal surfaces through the upregulation of claudin-1, claudin-2, and zona occludens-1 expression, which are all key proteins essential for maintenance of epithelial barrier integrity [23, 24]. Studies have demonstrated that IL-17 increases the production of antimicrobial peptides such as β-defensins that play critical roles in defense against microbial pathogens [25–28]. Th17 cells also produce a number of other cytokines such as IL-22 and IL-21 that have been shown to synergize with IL-17 and enhance the expression of antimicrobial peptides in mucosal tissues [26]. Additionally, IL-22 has been demonstrated to be critical for enterocyte homeostasis [29]. Numerous studies have shown that Th17 cells express CCR4, CCR6, CCR9, and $\alpha 4\beta 7$ [30–33] suggesting that these cells preferentially migrate to mucosal tissues.

Th17 cells play a critical role in protection against pathogens though they have been implicated in several autoimmune and inflammatory disorders, including asthma and allergy [34], psoriasis [35, 36], and inflammatory bowel disease [37, 38]. Interestingly, recent studies have shown that other cells such as CD8 T cells called T-cytotoxic-17 (Tc17) cells were capable of producing IL-17. Huber et al. [39]

showed that IL-17 secretion by CD8 T cells supported Th17-mediated autoimmune encephalomyelitis, whereas Saxena et al. [40] demonstrated that Tc17 cells potentiated Th1-mediated diabetes in the mouse model. Other studies have implicated Tc17 cells in vaccine-mediated immunity against fungal pathogens [41].

2. Th17 Cells during HIV Infection

HIV and SIV infections are characterized by massive loss of T helper cells, particularly at mucosal sites that persists during the course of infection, with little or no repopulation even after long periods of antiretroviral therapy [32, 42–53]. Destruction of mucosal CD4+ T cells is accompanied by dramatic alterations of the mucosal microenvironment, and is characterized by a preferential loss of Th17 cells, intestinal dysfunction and malabsorption, loss of mucosal epithelial barrier integrity, and severe enteropathy [54].

The exact mechanisms for the loss of Th17 cells are still under investigation. Brenchley et al. [4] reported that Th17 cells in the mucosa express high levels of CCR5, the coreceptor for HIV, and appear to be preferentially depleted despite the fact that they were not preferentially infected. On the other hand, Hed et al. using phenotypic markers such as CCR6 expression to delineate Th17 cells reported that direct infection by HIV likely played a central role in their depletion [55]. Ndhlovu et al. [56] demonstrated that IL-17 expression was dependent on the extent of infection in HIV-1+ children whereas HIV-infected patients with a plasma viral load below 50 copies/mL had detectable IL-17 expression. Other studies [57] have shown that HIV-1 specific Th17 cells were present in the acute stage of HIV infection yet were undetectable during chronic infection. The exact role that virus-specific Th17 cells play in HIV infection is still under investigation. Interestingly, however, HIV long-term nonprogressors appear to preserve their Th17 subsets [58]. In spite of ongoing debate about the exact mechanisms for the loss of Th17 cells, it is clear from a large number of studies in HIV-infected subjects and nonhuman primates with pathogenic SIV infections that Th17 cells are depleted to some degree during infection and their depletion contributes to the pathogenesis of HIV infection. Recent studies have shown that the Tc17 cells like their counterparts are also depleted during chronic HIV and SIV infections [59, 60].

In a landmark study, Brenchley et al. [61] showed that HIV infection was accompanied by translocation of microbial products across the lumen of the intestinal mucosa into systemic circulation. These translocated microbial products are believed to be a major cause for chronic immune activation and disease progression characterized by increased cell turnover [61–63]. A number of studies in HIV-infected patients and nonhuman primate models have demonstrated that the loss of Th17 cells from the mucosa most likely plays a major role in microbial translocation. Raffatellu et al. [7] showed that Th17 cell deficiency during SIV infection was associated with systemic translocation of *Salmonella*. Likewise, pathogenic SIV infections are accompanied by a severe loss of Th17 cells at mucosal sites within the first few weeks of infection that persists in chronic infection [64]. In contrast to pathogenic infections, natural hosts of SIV infection such as sooty mangabeys and African green monkeys were found to preserve their Th17 cells following infection and display little or no immune activation even when viral replication is high [4].

The effect of HIV and SIV infections on the loss of Th17 cells has been well documented. Not much is, however, known about the ability of Th17 cells to effectively repopulate either during the course of infection or after therapy. Ciccone et al. demonstrated that long-term highly active antiretroviral therapy (HAART) was somewhat successful in achieving Th17 repopulation in both peripheral blood and the mucosa [58]. On the other hand, Macal et al. [65] suggested that Th17 repopulation was dependent on overall levels of CD4+ T cell restoration in the gastrointestinal-associated lymphoid tissue (GALT). Gaardbo et al. [66] reported that ~20% of the HIV patients on antiretroviral therapy failed to completely reconstitute their CD4+ T cells which was accompanied by an incomplete repopulation of Th17 cells. Mavigner et al. [33] showed that incomplete mucosal immune reconstitution was associated with defective gut homing of CCR9+β7+ CD4+ T cells, a population that harbored Th17 cells. This was likely due to the altered expression of the CCR9 ligand CCL25 in the small intestinal mucosa of HIV-infected individuals. He et al. [67] reported that HIV-infected patients had significantly low levels of Th17 cells that were partially restored after 6 months of HAART though higher levels were observed after 1 year of therapy. Likewise, elite control of HIV infection has been associated with higher levels of Th17 cells [68]. However, others have demonstrated that HAART failed to restore Th17 cells in HIV-infected patients undergoing therapy [55, 69]. The inability to effectively repopulate Th17 cells unlike other subsets such as Th1 or Tregs suggests that mechanisms that likely affect either the induction or differentiation of Th17 cells may be involved in the poor repopulation of Th17 cells.

Even though HAART has had limited effect on Th17 repopulation, recent studies suggest that using probiotics can potentially enhance gastrointestinal immunity, enhance CD4+ T cell numbers, and lead to the restoration of Th17 cells in the mucosa [70]. Klatt et al. [71] showed that treatment of SIV infected pigtail macaques with probiotics/prebiotics for 60 days along with antiretroviral therapy was accompanied by an increase in IL-23 producing cells and higher levels of multifunctional Th17 cells in the mucosa as compared to animals that only received antiretroviral therapy. Likewise, Gonzalez-Hernandez et al. [72] showed that symbiotic treatment of HIV-infected subjects with a combination of pre- and probiotics significantly decreased microbial translocation and inflammation and improved the immunological status of patients leading to a better long-term outcome. However, another randomized clinical trial [73] reported no major changes in either microbial translocation or markers of immune activation. It is not clear if a better outcome would have been observed with either longer periods of symbiotic treatment or if patients were on antiretroviral therapy at the time of symbiotic therapy. Additional studies are needed to assess the beneficial role of symbiotic therapy on Th17 reconstitution.

3. Regulation of Th17 Cells and HIV Infection

Like the other T helper subsets, Th17 cells are memory CD4+ T cells [30, 69] that differentiate from naïve CD4+ T cells after TCR stimulation and costimulation by antigen presenting cells (APC) in the presence of Th17 promoting cytokines [74–76].

Development of Th17 cells requires key cytokine signals, several of which are produced by APCs following activation of toll-like receptors (TLRs) by pathogen-associated motifs. Activation of TLR 1/2, TLR 3, TLR 4, TLR7/8, and TLR9 have been shown to promote development of Th17 cells [74–78]. Fukata et al. [79] also demonstrated a role for MyD88 induction in Th17 differentiation. Reynolds et al. [80] showed that Th17 cells express high levels of TLR2 and stimulation with TLR2 agonists in the presence of Th17-promoting cytokines led to increased IL-17 production and expression of Th17-associated gene products. Signaling through other molecules such as dectin-1 and DC-SIGN has also been shown to promote Th17 development [81–85].

Initial studies identified IL-6, IL-21, IL-23, and TGFβ as critical cytokines essential for the induction of Th17 cells. A number of studies using mouse models suggested that IL-6 and TGFβ were essential for the initial differentiation of Th17 cells. Unlike mouse, however, the studies in humans have suggested that any of the four cytokines along with IL-1β in different combinations were sufficient to induce Th17 cells [85–87]. Of the four Th17 promoting cytokines, IL-6 and TGFβ appear to be critical for the polarization of Th17 cells as Th17 cells produce IL-17 and IFNγ in the absence of TGFβ.

IL-6 binding to the IL-6 receptor initiates signaling through STAT3 and RORγt transcription factors leading to the STAT3-mediated activation of the IL-17 promoter and the induction of IL-21 and IL-23 receptor expression, two factors that are important for subsequent stages of Th17 development [88]. The essential requirement of IL-6 for the generation of Th17 cells came from studies showing that expression of mutant gp130 IL-6R [89] or treatment with an anti-IL-6 antibody prevented Th17 polarization [76, 90].

Unlike IL-6, the ability of TGFβ to polarize Th17 cells appears to be dependent on the concentration of TGFβ in the environment; low concentrations of TGFβ in the presence of other Th17-promoting cytokines drives RORγt expression and induces Th17 cells. On the other hand, high concentrations of TGFβ in the absence of other Th17 inducing cytokines promote the development of T regulatory (Treg) cells and inhibit Th17 development through an effect on the Treg transcription factor FoxP3. TGFβ1 deficient mice have low levels of Th17 cells and circulating IL-17 [91] whereas treatment with anti-TGFβ1 antibodies were found to inhibit the generation of Th17 cells [92].

The second stage of Th17 differentiation is mediated by IL-21, a member of the common gamma chain family of cytokines. IL-21 is an autocrine cytokine that provides a positive feedback mechanism for the induction of Th17 cells [93, 94] and has been shown to inhibit FoxP3, thereby skewing the development away from Tregs. IL-21 has been shown to promote the induction of IL-17 and block IFNγ production [93–96] whereas other studies have shown that IL-21 knockout mice or IL-21R-deficient mice fail to develop Th17 cells when stimulated with IL-6 [93–97]. Interestingly, one study reported that IL-21 can subvert the requirement for IL-6-mediated stimulation for inducing Th17 cells by promoting an alternative pathway; a combination of IL-21 and TGFβ was found to induce Th17 cells in IL-6 deficient mice [98].

Like IL-21, IL-23 appears to be critical for the differentiation of Th17 cells during later stages of development. IL-23 is a heterodimeric cytokine comprised of the IL-12p40 and p19 subunits that is induced by stimulation of dendritic cells and macrophages with different TLR2 and dectin-1 ligands [84, 85, 99]. IL-23 binds to the IL-23 receptor which is primarily expressed by activated memory T cells [100]. Initial studies suggested that IL-23 was essential for the Th17 polarization. Later studies, however, demonstrated that it was not required for initial differentiation of Th17 cells but was essential for the survival and expansion of Th17 cells [8, 9, 101]. Importantly, naïve CD4+ T cells were found to lack the IL-23 receptor. This further supports a role for IL-23 in the later stages of Th17 differentiation.

Interestingly, both HIV and SIV infections are characterized by high levels of IL-6 and TGFβ [102–104]. Conversely, IL-21 producing CD4+ T cells are lost very early in infection [105–107] though other cellular subsets such as CD8 T cells have been shown to upregulate IL-21 production [107–111]. The presence of high levels of Th17 promoting cytokines during HIV and SIV infections suggests that the failure to induce Th17 cells during infection is likely mediated by mechanisms unrelated to availability of these cytokines.

Recent studies have shown that the loss of Th17 cells was accompanied by an expansion of Treg cells. These studies have suggested that the accumulation of byproducts of tryptophan metabolism promotes the development of Treg's and inhibits Th17 cells. Indoleamine deoxygenase (IDO), a rate-limiting enzyme that mediates the catabolism of tryptophan, has been shown to be significantly upregulated during HIV and SIV infections [68, 112–115]. Likewise the frequency of Tregs was reported to be altered during HIV infection and during HAART [116–120] whereas effector IL-17 absolute cell numbers were significantly lower in all HIV(+) subjects tested and were not restored after therapy. On the other hand, Brandt et al. [68] showed that the ratio of Th17/Treg in elite controllers did not differ from that of uninfected controls, whereas the ratio was lower in viremic patients and patients on HAART.

It is not clear if HIV infection alters the signaling pathways that promote the induction of Th17 cells. RORγt is a lineage specific transcription factor associated with Th17 differentiation [88, 121, 122] whose expression is regulated by signal transducers and activators of transcription-3 (STAT3) [123, 124]. They bind to ROR-dependent enhancer elements in conserved noncoding sequence (CNS)-2, which is located upstream of the IL17A promoter [124]. Rueda et al. [125] examined expression of T helper lineage-specific transcription factors in the GALT from healthy uninfected volunteers, HIV-infected untreated, and patients undergoing HAART and found that the ratio of RORγt to FoxP3 expression

shifted in favor of FoxP3 in untreated patients, though RORγt expression itself was not changed among the groups.

Numerous studies have demonstrated the importance of the Janus-associated kinases (JAK)/STAT3 signaling pathway in the development of Th17 cells [126, 127]. Binding of Th17 promoting cytokines to their cognate receptors initiates the signaling cascade that leads to receptor dimerization and recruitment of JAK culminating in the activation and phosphorylation of STAT3. Activated pSTAT3 dimerizes and translocates to the nucleus where it binds to the IL-17 promoter and drives the induction of IL-17. Studies have shown that STAT3 knockout mice failed to develop Th17 cells [123, 128], whereas patients with Jobs' syndrome lack functional STAT3 and display impaired Th17 development [2].

STAT3 is negatively regulated by a number of factors such as the suppressor of cytokine signaling-3 (SOCS3), protein inhibitor of activated STAT3 (PIAS3), and protein tyrosine phosphatase (SHP-2). Overexpression of SOCS3 has been shown to inhibit Th17 development while SOCS3 conditional knockouts were shown to have higher levels of Th17 cells [129]. Interestingly while SOCS3 is activated by Th17 promoting cytokines IL-6, IL-21, and IL-23 [92, 129], TGFβ has been shown to inhibit SOCS3 induction by IL-6 and IL-23, thereby promoting the activation of STAT3 and subsequent induction of Th17 cells [92].

CD4+ T cells from HIV-infected patients have been shown to express high levels of SOCS3 mRNA [130] though SOCS3 protein levels were lower. Higher levels of SOCS3 mRNA have been reported in the gastrointestinal tissues of SIV-infected rhesus macaques [131]. Interestingly, increased levels of SOC3 have been shown to aid in HIV replication [132] whereas high levels of SOCS3 in hepatic cells have been associated with nonresponsiveness to therapy in HIV/HCV infected individuals [133]. Moutsopoulos et al. [134] reported that high levels of SOCS3 protein in mucosa-associated lymphoid organ such as the tonsils are associated with increased susceptibility to HIV infection.

Unlike SOCS3, PIAS3 has been shown to directly interact with pSTAT3 and inhibit its binding to target DNA thereby interfering with the STAT3-mediated activation of target genes [135, 136]. Others have shown that PIAS3 directly inhibits the transactivation of STAT3 [137]. PIAS3 transcript levels were found to be absent in Th17 cells as compared to Th1 or Th2 cells in mice, and knockdown of PIAS3 with siRNA resulted in severe EAE suggesting an important role for PIAS3 in Th17 regulation [138]. Recent studies have shown that PIAS3 mRNA levels were significantly upregulated in CD4 T cells from SIV-infected rhesus macaques and high levels of PIAS3 was found to significantly correlate with immune activation and markers of microbial translocation [139]. Not much is known about the effect of HIV infection on PIAS3 and if PIAS3 plays a role in dysregulating the induction of Th17 cells.

Like SOCS3 and PIAS3, SHP2 negatively regulates IL-17 production. However, unlike the other two, SHP2 interferes with IL-6 signaling-mediated activation of STAT3. SHP2 is recruited to receptors following cytokine signaling and JAK activation. Studies have shown that SHP2 is recruited to gp130 domain of the IL6 receptor following IL-6 signaling and dephosphorylates pSTAT3, preventing its dimerization and translocation to the nucleus [140, 141]. The exact role of SHP2 in preventing the induction of Th17 cells during HIV infection is not clear. However, studies have shown that HIV-mediated signaling through CCR5 and C-type lectin domain-4 (DCIR) results in recruitment of SHP-2 whereas HIV gp120 binding has been shown to increase SHP2-mediated signaling [142].

4. Summary

Th17 cells play an essential role in host immunity and are key players in protecting the mucosal integrity. Their loss during HIV infection is associated with translocation of microbial products across the damaged mucosal epithelium leading to immune activation and poor long-term outcome in HIV-infected patients. While progress has been made in understanding the role of Th17 cells in HIV infection, there are significant gaps in the field regarding the exact mechanisms that prevent full Th17 reconstitution during therapy. A better understanding of how these key molecular mechanisms are altered during HIV infection and the role these altered mechanisms play is essential to develop better therapeutic approaches to repopulate Th17 cells and overcome the deleterious effects associated with the loss of Th17 cells during HIV infection.

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