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REVIEW ARTICLE

Th17 and Allergy

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ABSTRACT
The identification of novel helper T (Th) cell subsets, i.e., IL-17-producing Th cells (Th17 cells) and regulatory T cells (Treg cells), provided new insight into our understanding of the molecular mechanisms involved in the development of infectious and autoimmune diseases as well as immune responses, and thus led to revision of the classic Th1/Th2 paradigm. Several current lines of evidence from gene-deficient mice indicate that IL-17 and Th17 cells, but not IFN-γ and Th1 cells, are responsible for the development of autoimmune diseases such as murine arthritis and encephalomyelitis, which have classically been considered to be Th1-mediated disorders. Th17 cells may also contribute to the pathogenesis of classically recognized Th2-mediated allergic disorders. In this review, we summarize the current knowledge regarding IL-17 and Th17 cells and discuss their potential roles in the pathogenesis of allergic disorders.

KEY WORDS
allergy, IL-17, Th17

INTRODUCTION

Allergic disorders, which affect approximately 30% of the population in developed countries, are genetically determined and/or environmentally affected multifactorial, refractory diseases. These disorders are associated with chronic inflammation characterized by influx of a large number of eosinophils and accumulation of mast cells in the lesions and increased IgE production.[1,2] Leukemic patients who did not have any allergic symptoms developed allergic diseases such as asthma or rhinitis after therapeutic transplantation of bone marrow stem cells from donors who had such allergic disorders,[3,4] suggesting that bone marrow-derived immune cells contribute to the development of allergic disorders. In particular, several lines of evidence generated in gene- and immune cell-deficient mice clearly demonstrated that helper T (Th) cells play central roles in the accumulation/activation of eosinophils and mast cells and in IgE production by B cells, contributing to the pathogenesis of allergic disorders.[1,2]

Since the discovery of two distinct types of Th cells (IFN-γ-producing Th1 cells and IL-4-producing Th2 cells) in mice by Coffman and colleagues in 1986,[5] mutual regulation between Th1 cells and Th2 cells (Th1/Th2 balance) has been considered to be important for homeostatic maintenance of the immune system in the whole body. For example, dysregulation of the Th1/Th2 balance leads to excessive Th1 cell or Th2 cell activation, resulting in the development of autoimmune diseases associated with accumulation of Th1 cells or induction of allergic diseases due to accumulation of Th2 cells, respectively.[6] Thus, the concept of a Th1/Th2 balance provided the basis for understanding the molecular mechanisms of immune responses and/or disease development and has been widely accepted as a "paradigm" of the immune system for the past two decades.[7]

On the other hand, changes in autoimmune diseases that could not be explained by the Th1/Th2 paradigm were also observed in several settings: unexpectedly, IFN-γ/IFN-γR-deficiency or-neutralization resulted in exacerbation, rather than attenuation, of development of autoimmune diseases such as murine encephalomyelitis,[8-10] arthritis,[11,12] uveitis[13,14] and nephritis,[15] which have classically been considered to be Th1-mediated diseases. Studies for non-Th1/Th2-cell-based mechanisms for the pathogenesis of autoimmune diseases identified two additional Th cell subsets, i.e., IL-17-producing Th cells (Th17 cells) and regulatory T cells (Treg cells). These cells provided new insight into the molecular mechanisms involved in immune responses and/or disease develop-

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ment, and led to revision of the classic Th1/Th2 paradigm in such settings. Likewise, these new T cell subsets, particularly Th17 cells, may also contribute to the pathogenesis of classically recognized Th2-mediated allergic disorders. In this review, we summarize current knowledge regarding IL-17 and Th17 cells and discuss their potential roles in the pathogenesis of allergic disorders.

TH17 CELLS ($TH_{IL\text{-}17}$ CELLS/THi CELLS)

In 2000, Infante-Duarte *et al.* first demonstrated that IL-17-producing T cells were a distinct Th population from Th1 cells and Th2 cells in both mice and humans.[16] Subsequently, the importance of IL-17-producing T cells rather than Th1 cells for the development of certain diseases, e.g., attenuated development of contact hypersensitivity[17] and arthritis,[18] was demonstrated in IL-17-deficient mice. These observations clearly indicated the involvement of IL-17 in the pathogenesis of such disorders, but the molecular mechanisms involving IL-17 and/or IL-17-producing T cells remained unclear. Based on the findings of Infante-Duarte *et al.*, Aggarwal *et al.* reported that IL-23 can promote *in vitro* IL-17 production by certain Th cells which are categorized as neither Th1 cells nor Th2 cells.[19] Then, Murphy *et al.* found that IL-23-deficient mice had fewer IL-17-producing T cells during autoimmune diseases such as collagen-induced arthritis and were able to completely suppress the disease development.[20] Almost simultaneously, Nakae *et al.* reported that IL-17-deficient mice exhibited marked suppression of the development of collagen-induced arthritis.[21] These observations indicate that IL-23-mediated IL-17 production, rather than IL-12-mediated IFN-γ induction, is crucial for the development of autoimmune diseases. In 2005, Langrish *et al.* provided direct *in vivo* evidence that IL-17-producing Th cells (called "$Th_{IL\text{-}17}$ cells") induced by IL-23, rather than IFN-γ-producing Th1 cells induced by IL-12, played a central role in the induction of murine encephalomyelitis.[22] In support of this notion, Harrington *et al.* and Park *et al.* established the basis of the molecular mechanism in the development of IL-17-producing T cells (called "Th17 cells" or "inflammatory T cells; Thi cells") that require distinct cytokines (IL-23 but not IL-12 and IL-4) and transcription factors from those of Th1 cells and Th2 cells.[23,24] In 2006, IL-6 in the presence of TGF-β rather than IL-23 was identified as the crucial combination for differentiation of IL-17-producing Th cells *in vitro*[25] and *in vivo*[26,27] in mice. Moreover, Ivanov *et al.* identified ROR-γt as a key transcription factor for Th17 cell differentiation.[28] In addition, in the absence of IL-6, IL-21 also induces IL-17-producing Th cell differentiation.[29-31] However, IL-6 plus TGF-β-mediated IL-17-producing T cells lack pathogenic activities since such IL-17-producing T cells also secrete an anti-inflammatory cytokine, IL-10.[32] In mice, IL-23 is required for the differentiation from IL-6 plus TGF-β-mediated IL-17$^+$, IL-10$^+$ nonpathogenic T cells to pathogenic Th17 cells which do not produce IL-10.[32] On the other hand, IL-27 suppresses Th17 cell-differentiation[33,34] by inducing IL-10- and IFN-γ-producing T-bet$^+$ Tr1 cells from IL-6 plus TGF-β-mediated IL-17$^+$, IL-10$^+$ T cells in mice.[32,35-37] Like IL-27, the newly identified IL-12-related cytokine IL-35, which is produced by Foxp$^+$ Treg cells, suppresses Th17 cell-mediated immune responses in mice, although the precise molecular mechanism remains unknown.[38,39] In contrast, human Th17 cell differentiation requires a cytokine milieu that is distinct from that needed for mouse Th17 cell differentiation: TGF-β is not essential for human Th17 cell differentiation. In humans, combination of IL-1β and IL-23, or IL-1β and IL-6, but not IL-1β, IL-6 or IL-23 alone, significantly induces Th17 cells as well as IL-17$^+$ and IFN-γ$^+$ Th cells.[40,41] The schemes of Th differentiation in humans and mice are summarized in Figure 1.

IL-17 AND ALLERGIC ASTHMA

Allergic asthma is considered to be a Th2-dominant chronic inflammatory disease of the lungs.[42,43] Increased Th2-cytokine and IgE levels and accumulation/activation of Th2 cells, eosinophils and mast cells are observed in asthmatic lungs.[42,43]

IL-17 mRNA and/or proteins were reported to be increased in the lungs, sputum, bronchoalveolar lavage (BAL) fluids or sera from asthmatics,[44-50] and the levels of IL-17 correlated with the degree of severity of airway hypersensitivity in asthmatic patients,[47] implying a contribution of IL-17 to the pathogenesis of asthma. Supporting those observations, IL-17 can potentiate bronchial fibroblast, epithelial cell and smooth muscle cell activation. That is, IL-17 enhances IL-6, IL-8, IL-11 and CXCL1/Groα production by human bronchial fibroblasts,[45] β-defensin-2, ICAM-1, IL-8, CXCL1, CCL20, G-CSF, MUC5B and MUC5AC expression/production by human bronchial epithelial cells,[51-57] and IL-6 and IL-8 production by human airway smooth muscle cells (Fig. 2).[58,59] Nevertheless, these IL-17-mediated inflammatory mediators do not seem to contribute to the development of Th2-mediated eosinophil-dominant allergic asthma, since IL-8 and CXCL1/Groα are potent chemoattractant factors for neutrophils, and IL-6 and G-CSF are important for granulopoiesis, especially neutrophil development. In addition, IL-17 inhalation led to induction of neutrophilia rather than eosinophilia in the airways of rodents.[60,61] However, these observations may provide us with new insight into the pathogenesis of "non-Th2-type" asthma.

As "non-Th2-type" asthma, several investigators found asthmatic patients who had neutrophil-, rather than eosinophil-, rich pulmonary inflammation.[62-68] The heterogeneity in the symptoms of asthma has been classified as atopic and non-atopic[67] and eosino-

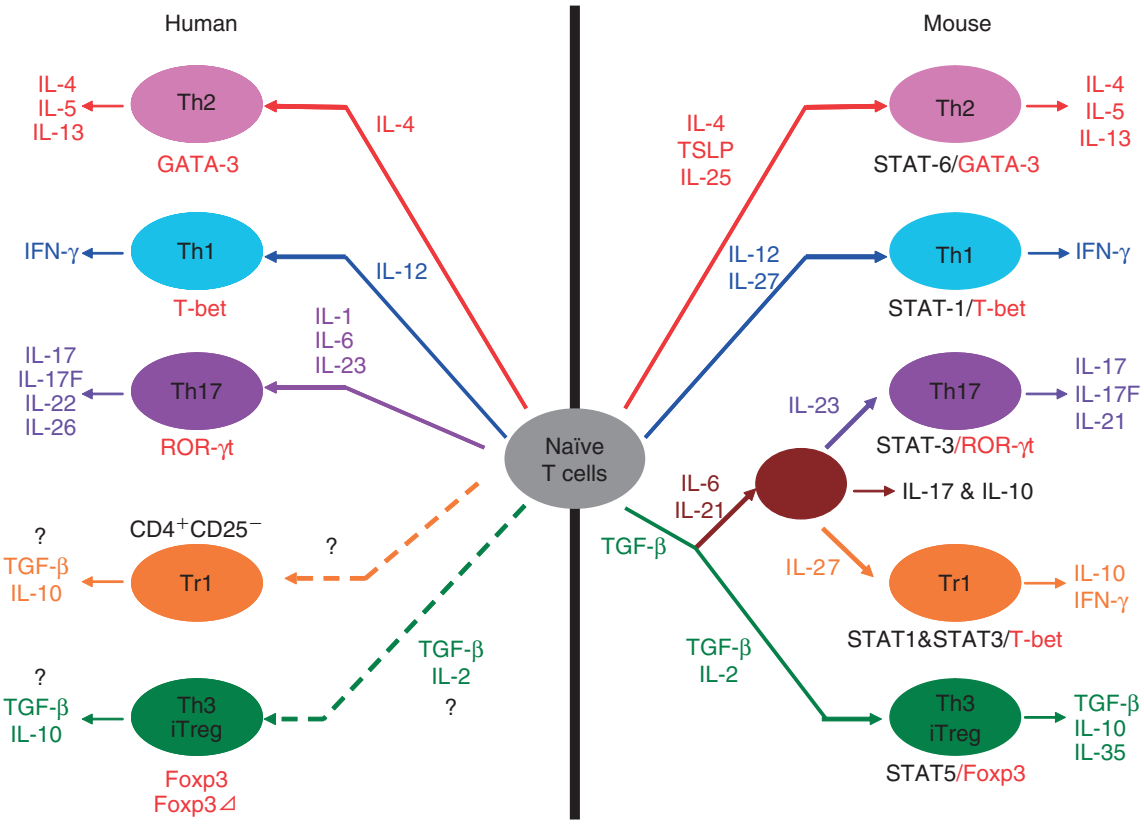

Fig. 1 The revised scheme of Th cell differentiation in humans and mice. In the mouse, naïve T cells differentiate into Th1 cells in the presence of IL-12 or IL-27, Th2 cells in the presence of IL-4, TSLP or IL-25, Th17 cells in the presence of IL-23 after TGF-β1 plus IL-6- or IL-21-mediated cell differentiation, Tr1 cells in the presence of IL-27 after TGF-β1 plus IL-6- or IL-21-mediated cell differentiation, and Th3/iTreg cells in the presence of TGF-β1 and IL-2. In man, naïve T cells differentiate into Th1 cells in the presence of IL-2, Th2 cells in the presence of IL-4 and Th17 cells in the presence of IL-1β plus IL-6 or IL-1β plus IL-23. The mechanisms of human Tr1 and Th3/iTreg cell differentiation remain unclear.

philic and non-eosinophilic.[64,68]

Amin *et al.* demonstrated that atopic asthmatics showed Th2-type airway inflammation characterized by increased IL-4$^+$ and IL-5$^+$ cells and eosinophils in bronchial biopsies and increased serum IgE levels, whereas non-atopic asthmatics exhibited non-Th2 type airway inflammation characterized by increased IL-8$^+$ cells and neutrophils in bronchial biopsies, and no elevation of serum IgE.[67] On the other hand, increased mast cell accumulation was observed in both atopic and non-atopic patients irrespective of the IgE level.[67] Clear structural differences were also observed between atopic and non-atopic patients: the degree of epithelial damage and the thickness of the tenascin and laminin layer were greater in atopic asthmatics than in non-atopic asthmatics (Fig. 3).[67]

Wenzel *et al.* examined the heterogeneity of infiltrated cells in lung biopsy from mild, moderate and severe asthmatic patients and healthy subjects.[64] Mild and moderate asthmatics showed predominantly eosinophilic inflammation, while severe asthmatics showed neutrophilic inflammation.[64] Moreover, these neutrophil-associated severe asthmatics also can be divided into two populations, eosinophil-negative and -positive.[64] The counts of other cell types (CD3$^+$ cells, CD4$^+$ cells, CD8$^+$ cells, CD68$^+$ cells and mast cells) in the biopsy were comparable in healthy subjects and mild, moderate and non-eosinophil but neutrophil-associated severe asthmatics.[64] However, these cells, including neutrophils and eosinophils, as well as pathological changes in the lungs were dramatically increased in eosinophil- and neutrophil-associated severe asthmatics compared with other populations.[64] Gibson *et al.* also presented further evidence of the heterogeneity of cell types and inflammatory mediator levels in the sputum from eosinophilic and non-eosinophilic asthmatics, having found increased neutrophils and elevated IL-8 in non-eosinophilic asthmatics (Fig. 3).[68] Importantly, the severity of asthma correlated well with the number of

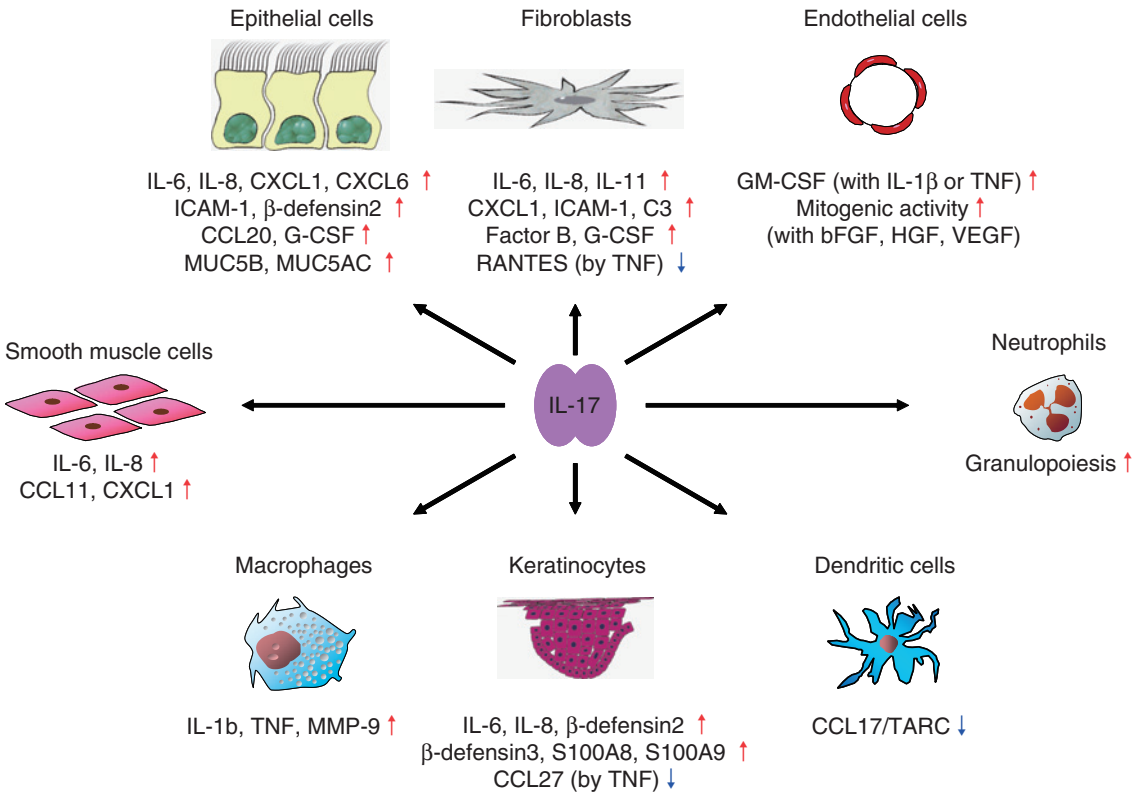

Fig. 2 IL-17 activities in various types of cells. IL-17 promotes (indicated as ↑) and suppresses (indicated as ↓) the expression and/or production of various molecules in epithelial, endothelial and smooth muscle cells, fibroblasts, keratinocytes, macrophages and dendritic cells.

neutrophils.[62-66,68] Otherwise, the dichotomous classifications by steroid sensitivity may be similar to the classification by eosinophilic and non-eosinophilic asthma. Eosinophilic mild/moderate asthmatics are sensitive to steroid treatment, while non-eosinophilic/neutrophilic or eosinophilic/neutrophilic severe asthmatics are resistant (Fig. 3).[69]

Douwes *et al.* reported that various studies from 1995 to 2000 using specimens from asthmatics who were defined as having eosinophil-dominant features actually contained data from non-eosinophil/neutrophil-dominant asthmatics.[70] Likewise, although IL-17 levels were significantly increased in the sputum of severe asthmatics,[49,50] the heterogeneity of asthmatics who showed increased IL-17 levels was not clearly characterized in some reports.[44-48] Thus, IL-17 may contribute to the pathogenesis of non-atopic and/or non-eosinophil/neutrophil-dominant asthma, and it may be a new marker for classification of such non-Th2 type asthma.

IL-17 IN A TH2 CYTOKINE-MEDIATED AND EOSINOPHIL-DOMINANT MOUSE ASTHMA MODEL

The well-established model of Th2-type asthma in mice is an allergic response against ovalbumin (OVA), fungal antigens and cockroach antigens. Sensitization with such antigens and subsequent intranasal or aerosol challenge with the same antigens in mice results in the development of airway inflammation and elevated airway hypersensitivity responses (AHR). However, the responses vary as a result of different sensitization protocols, immunization routes, antigens and mouse backgrounds.[71,72] For instance, in the case of OVA-induced AHR, the requirement for immune cells and mediators is affected by use of adjuvants during the sensitization. Although mast cells and B cell-derived IgE-mediated mast cell activation are considered to be responsible for the development of asthma, mast cells,[73,74] B cells[75] and IgE-FcεRI-,[76] IL-1-IL-1R-,[77,78] TNF-TNFR-[79] and CCR8[80,81]-mediated responses are not essential for induction of eosinophilic airway inflammation and AHR in mice sensitized with OVA emulsified in alum as an adjuvant. In contrast, such cells and mediators are essential for the induction of AHR and/or eosinophilic airway inflammation in mice sensitized by repeated injection of OVA without any adjuvant.[74,76-79,82] Moreover, even if the sensitization protocols are the same, the phenotypes differ among the genetic background.[71,72,83] For example, distinct responses are observed in TNF- and TNFR-deficient mice,[79,84-87] IL-1R1-deficient

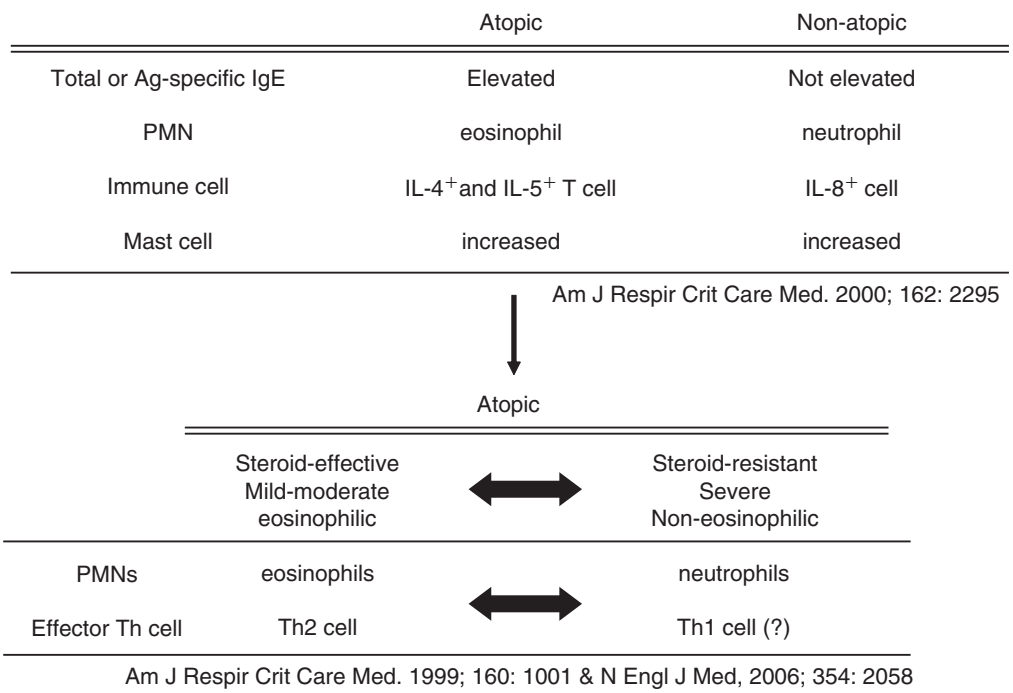

Fig. 3 Classification of "atopic and non-atopic" and "eosinophilic and non-eosinophilic" asthma. Atopic patients show elevated serum IgE levels and increased Th2 cells and eosinophils, while non-atopic patients show increased IL-8$^+$ cells and neutrophils without elevation of serum IgE. In addition, atopic patients can be roughly divided into two populations, eosinophilic and non-eosinophilic asthmatics. Eosinophilic asthmatics who are steroid-responsive and mild to moderate in severity show Th2 cytokine- and eosinophil-dominant inflammation, whereas non-eosinophilic asthmatics who are steroid-resistant and have refractory symptoms show non-Th2 cytokine- and neutrophil-dominant inflammation.

mice[78,88] and CCR8-deficient mice[80,81,89] between the 129 (Ola or Sv/J) ×B6 mixed, C57BL/6 and BALB/c backgrounds. However, irrespective of different sensitization protocols and mouse genetic backgrounds, similar features of pathogenesis characterized by Th2-dominant and eosinophil-rich pulmonary inflammation are observed: eosinophils make up approximately 80–90% of the total cells in BAL fluids, while neutrophils generally comprise less than 5–10%.

Using the intraperitoneal OVA with alum sensitization protocol, IL-17-deficient mice of a 129/Sv x B6 mixed background exhibited normal AHR as assessed by enhanced respiratory pause (Penh) responses,[17] although Penh evaluation is currently considered to be inappropriate for measurement of airway function.[90,91] A normal total BAL cell count, but increased IL-4 and IL-5 levels, in the BAL fluid were observed in IL-17-deficient mice of 129/Sv x B6 mixed background (Fig. 4A).[17]

Using a repeated intraperitoneal OVA sensitization protocol (without adjuvant), Hellings *et al.* demonstrated that BALB/c mice treated with anti-IL-17 neutralizing mAb (clone 50104 from R&D Systems; 50 μg/mouse intraperitoneally) before and during the phase of repeated OVA challenge exhibited comparable Penh responses and BAL eosinophil counts, but increased IL-5 levels in the BAL fluid, relative to mice treated with control Ab (Fig. 4B).[92] Notably, the BAL neutrophil count and also the blood and bone marrow neutrophils were reduced in anti-IL-17 mAb-treated BALB/c mice.[92]

Using a repeated epicutaneous OVA sensitization protocol (employing a gauze patch containing OVA solution, without adjuvant), He *et al.* reported that BALB/c mice treated with anti-IL-17 neutralizing mAb (clone 50104 from R&D systems; 100 μg/mouse intravenously) during the last epicutaneous sensitization showed reduced Penh responses, but a normal BAL eosinophil count in comparison with mice treated with control Ab (Fig. 4B).[93] Similar to the findings of Hellings *et al.*, the BAL neutrophil count was reduced by anti-IL-17 mAb treatment.[93] He *et al.* also demonstrated that the epicutaneous OVA sensitization potently induced Th17 cells in draining LNs and OVA-challenged lungs and neutrophil recruitment in BALs after OVA challenge (−20% neutrophils and −50% eosinophils in the total BAL cells), while the intraperitoneal OVA with alum sensitization induced

A

Th2 cytokine-mediated and eosinophil-dominant murine asthma

Reference	Mouse strain	Experimental protocol	Observations
Nakae et al. Immunity. 17, 375-387 (2002)	IL-17-deficient 129Sv x B6	1 11 22 23 24 Day 10 μg OVA in alum (i.p.) 100 μg OVA in saline (i.n)	Sensitization phase - Impaired OVA-specific T cell induction After challenge (24 h) - Reduced serum IgE - Normal Penh. - Normal number of BAL cells. - Increased IL-4 and IL-5 in BAL fluids.
Schnyder-Candrian et al. J Exp Med 203, 2715-2725 (2006)	IL-17R-deficient C57BL/6	1 7 14 16 18 Day 10 μg OVA in alum (s.c.) 10 μg OVA in saline (i.n)	Sensitization phase - Impaired OVA-specific T cell induction After challenge (48 h) - Reduced serum IgE - Reduced number of eosinophils in BAL. - Reduced EPO activity in BAL fluids.

B

Th2 cytokine-me-diated and eosinophil-dominant murine asthma

Reference	Mouse strain	Experimental protocol	Observations
Hellings et al. Am J Respir Cell Mol Biol. 28, 42-50 (2003)	BALB/c	50 μg anti-IL-17 mAb (i.p. 30 min before OVA challenge) 1 3 5 7 9 1113 32 33 34 35 36 37 38 39 40 Day 10 μg OVA in saline (i.p.) 10 mg/ml OVA in saline (aerosol, 5 min)	- Normal Penh. - Normal number of eosinophils in BAL. - Increased IL-5 in BAL fluids. - Reduced number of neutrophils in BAL. (3 h after last challenge)
He et al. Proc Natl Acad Sci USA 104, 15817-15822 (2007)	BALB/c	100 μg anti-IL-17 mAb (i.v.) 42 45 1–7 21–28 42–49 49 50 51 Day 100 μg OVA patch (e.c.) 1%OVA in saline (aerosol, 20 min)	- Reduced Penh. - Normal number of eosinophils in BAL. - Normal IL-4 and IL-13 mRNAs in lungs. - Reduced number of neutrophils in BAL. (24 h after last challenge)
Schnyder-Candrian et al. J Exp Med 203, 2715-2725 (2006)	BALB/c	1 7 14 16 18 Day 10 μg OVA in alum (s.c.) 10 μg OVA in saline +5 μg anti-IL-17 mAb (i.n)	- Increased Penh (24 h). - Increased number of eosinophils in BAL. - Augmented mucus secretion (48 h after last challenge)
Fujiwara et al. J Allergy Clin Immunol 119, 662-670 (2007)	T-bet/Rag-2-deficient BALB/c mouse + T-bet-deficient DO11.10 T cells	150 μg anti-IL-17 mAb (i.p. 12 h before OVA challenge) 1 14 29 30 Day 6 μg OVA in alum (i.p.) 50 mg/ml OVA in saline (aerosol, 20 min.)	- Normal number of eosinophils in BAL. - Reduced number of neutrophils in BAL. (36 h after last challenge)

Fig. 4 Effects of IL-17 inhibition on Th2 cytokine-mediated and eosinophil-dominant murine asthma. **A**) Phenotypes in IL-17-deficient or IL-17R-deficient mice. **B**) Phenotypes in mice treated with anti-IL-17 neutralizing mAb.

weak Th17 responses and neutrophil recruitment (less than 5% neutrophils and −70% eosinophils in the total BAL cells).[93]

Thus, these observations indicate no or little contribution of IL-17 to the induction of Th2-mediated eosinophil recruitment and AHR in murine asthma.

On the contrary, using the subcutaneous OVA with alum sensitization protocol, Schnyder-Candrian *et al.* demonstrated that IL-17R (IL-17RA)-deficient mice of a C57BL/6 background revealed profoundly impaired eosinophil recruitment and a reduced IL-5 level in the BAL fluid (Fig. 4A).[94] The authors concluded that the reduced responses were caused by impaired antigen-specific Th cell induction during the sensitization phase in IL-17R-deficient mice.[94] IL-17-deficient mice (129/SvxB6 mixed background) also showed impaired antigen-specific Th cell induction during intraperitoneal sensitization with OVA emulsified in alum, but normal AHR and airway inflammation.[17] The distinct phenotypes between IL-17-deficient mice and IL-17R-deficient mice may be caused by the differences in their genetic backgrounds (129/SvxB6 mixed background vs. C57BL/6 background) and immunization routes (intraperitoneally vs. subcutaneously). He *et al.* showed that the intraperitoneal OVA with alum immunization could induce Th17 cells in the spleen, although the levels were less than those by repeated epicutaneous OVA immunization without any adjuvant.[93] On the other hand, Schnyder-Candrian *et al.* showed that subcutaneous OVA with alum immunization could not induce IL-23-responding Th17 cells in the spleen, although it did induce Th17 cells in the draining LNs.[94] Thus, the degree of induction of splenic Th17 cells after OVA sensitization is influenced by the immunization route (epicutaneouslly >> intraperitoneally > subcutaneously) and may affect the different contributions of pathology to Th2-mediated and eosinophil-dominant murine asthma.

IL-17F, which, like IL-17 is a member of the IL-17 cytokine family of molecules, binds to IL-17R (consisting of IL-17RA and IL-17RC) and has similar pathological activities to IL-17. IL-17F overexpression in lungs induces airway neutrophilia.[95] Therefore, it can be speculated that the distinct phenotypes between IL-17-deficient mice (characterized by Nakae *et al.*[17]) and IL-17R-deficient mice (characterized by Schnyder-Candrian *et al.*[94]) may be explained by a compensatory effect of IL-17F on IL-17-deficiency.

Schnyder-Candrian *et al.* also investigated the role of IL-17 in the challenge phase of OVA-induced murine asthma. The OVA challenge in the presence of anti-IL-17 mAb (clone 50104 from R&D Systems; 5 μg/mouse, intranasally) in the challenge phase resulted in exacerbated eosinophil recruitment in BAL fluids (Fig. 4B).[94] In contrast, OVA challenge in the presence of rIL-17 led to attenuated responses.[94] This inhibitory activity of IL-17 is attributed to down-regulation of chemokine CCL17 (TARC) production by bone marrow-derived cultured DCs and lung cells.[94] These observations suggest that IL-17 plays a protective role in the induction of Th2-mediated and eosinophil-dominant inflammation in the challenge phase of murine asthma. However, except for the different routes of OVA sensitization as described above, the apparent differences in the effects of anti-IL-17mAb treatment reported by Hellings *et al.*[92] and He *et al.*[93] versus by Schnyder-Candrian *et al.*[94] may have reflected, at least in part, the different timing and routes of Ab injection, i.e., systemic Ab administration (intravenously and intraperitoneally) by Hellings *et al.*[92] and He *et al.*,[93] and local Ab administration (intranasally) by Schnyder-Candrian *et al.* (Fig. 4 B).[94]

Taken together, IL-17 may play a role in Th2 cytokine-mediated and eosinophil-dominant mouse asthma by promoting allergen-specific Th cell induction in the sensitization phase and by inhibiting the local allergic response in the challenge phase, dependent at least in part on the experimental protocol used.

IL-17 IN A TH17 CELL-MEDIATED AND NEUTROPHIL-DOMINANT MOUSE ASTHMA MODEL USING DO11.10 AND OTII MICE

As described above, IL-17 may contribute to the pathogenesis of neutrophil-rich non-atopic or severe asthma rather than that of eosinophil-rich atopic or mild/moderate asthma. However, for investigating the potential role of IL-17 in neutrophil-associated asthma, the Th2-mediated and eosinophil-dominant murine asthma model may not be appropriate.

BALB/c-DO11.10 transgenic mice, which overexpress OVA-specific TCR genes in $CD4^+$ T cells, exhibited airway inflammation after OVA inhalation without prior sensitization with OVA.[17,96,97] Like non-atopic asthmatics, the inflammation in OVA-inhaled DO11.10 mice is characterized by a predominant influx of neutrophils rather than eosinophils in the airways, without an increase in serum IgE.[96,97] Similarly, OVA-specific TCR-expressing C57BL/6-OTII transgenic mice[98] also exhibited neutrophil-dominant airway inflammation after OVA inhalation without prior OVA sensitization.[99] Moreover, Th1 cells and Th17 cells, but not Th2 cells, are increased in BAL cells from OVA-inhaled DO11.10 or OTII mice.[99] In association with this notion, IFN-γ and IL-17, but not IL-4, IL-5 or IL-13, are increased in the BAL fluids of these mice.[96,99] After OVA inhalation, prior treatment with anti-IFN-γ neutralizing Ab did not exert any influence upon neutrophil recruitment, but significantly augmented eosinophil recruitment, in the BAL fluids of DO11.10 mice.[96] IFN-γ-deficient OTII mice exhibited increased neutrophils as well as eosinophils in BAL fluids after OVA inhalation.[99] In contrast, airway neutrophilia and AHR were profoundly impaired in IL-17-

deficient DO11.10 mice[17] and IL-17-deficient OTII mice.[99] These observations indicate that IL-17/Th17 cells rather than IFN-γ/Th1 cells are responsible for airway neutrophilia in the non-atopic asthma-like model using DO11.10 and OTII mice (DO11.10/OTII model). In particular, the IL-17-induced airway neutrophil influx was highly dependent on TNF.[99]

In accordance with there being no elevation of OVA-specific IgG_1 and IgE in the DO11.10/OTII model, Ig/Ag-FcR signals were not necessary for the airway neutrophilia.[99] However, notably, Ig/Ag-FcR-independent mast cell activation was crucial for the event: Th17 cell-derived IL-17 promoted Ig/Ag-FcR-independent mast cell-TNF production, resulting in enhanced airway neutrophilia in the OTII model that resembled non-atopic asthma.[99]

The importance of the Th17/IL-17-mast cell/TNF axis is also shown by the neutrophil recruitment in Th2 cytokine-mediated, IgE/Mast cell-dependent and eosinophil-dominant murine asthma induced by repeated OVA sensitization without any adjuvant.[79,100]

Both IL-17 and TNF can induce IL-8 and IL-6 production by human bronchial epithelial cells, but the extent of that induction is much less than that by TNF.[101] However, IL-17 can amplify TNF-mediated IL-8 and IL-6 production.[101] Additive or synergistic effects of IL-17 on TNF-mediated immune responses have also been observed with several cell types.[102,103]

Like IL-17, TNF can induce airway neutrophilia and AHR in healthy subjects.[104] TNF levels in sputum from non-eosinophilic (neutrophilic) asthmatic patients are higher than those in eosinophilic patients.[105] TNF-positive mast cells were increased in the airway submucosa in biopsies from asthma patients.[106,107] Anti-TNF therapy reduced the severity of the disease in severe asthmatics (including small reductions in both eosinophils and neutrophils in the sputum),[107-109] but not the pulmonary eosinophilia in patients with mild or moderate asthma.[110] Thus, the Th17/IL-17-mast cell/TNF axis may be involved in the development of not only non-atopic asthma but also severe asthma.

IL-17 IN A TH17 CELL-MEDIATED AND NEUTROPHIL-DOMINANT MOUSE ASTHMA MODEL CREATED BY T CELL TRANSFER

After OVA inhalation, airway inflammation can be induced by passive transfer of T cells from DO11.10 mice into naïve mice that had not been sensitized with OVA. Indeed, Cohn *et al.* demonstrated that naïve BALB/c mice engrafted *in vitro* with DO11.10 Th2 cells induced by IL-4 (DO11.10 Th2 cells → BALB mice), or with DO11.10 Th1 cells induced by IL-12 (DO11.10 Th1 cells → BALB mice), exhibited eosinophil-dominant or neutrophil-dominant airway inflammation.[111] Based on the observations in the intact DO11.10/OTII mouse model noted above, however, the Th1-mediated neutrophilia may be influenced by other cell contaminations such as Th17 cells. In support of this, it was reported that BALB/c mice engrafted with DO11.10 Th17 cells, which were generated with TGF-β, IL-6, IL-1β, TNF and IL-23 *in vitro* (DO11.10 Th17 cells → BALB mice), showed airway neutrophilia after OVA inhalation.[112] Interestingly, the authors also demonstrated the involvement of IL-17 and IL-17F in airway neutrophilia. Both recombinant IL-17 and IL-17F inhalation induced airway neutrophilia in mice, but the effects of IL-17 were much more potent than those of IL-17F. Furthermore, the airway neutrophilia in OVA-inhaled DO 11.10 Th17 cells → BALB mice was suppressed by treatment with anti-IL-17 mAb but not anti-IL-17F mAb, indicating that Th17 cell-derived IL-17 rather than IL-17F plays the central role in such settings.[112]

T-bet, which is a transcription factor, plays a central role in Th1 cell development (Fig. 1).[113] T cell/B cell-deficient Rag-2-deficient mice engrafted with T-bet-deficient DO11.10 $CD4^+$ T cells, which include many Th17 cells and Th2 cells but few Th1 cells in comparison with wild-type DO11.10 $CD4^+$ T cells, exhibited increased neutrophils as well as eosinophils in the BAL fluid after OVA challenge.[114] The increase in neutrophils, but not the increase in eosinophils, in the BAL fluid from Rag-2-deficient mice engrafted with T-bet-deficient DO11.10 $CD4^+$ T cells was attenuated by treatment with anti-IL-17 mAb (clone 50104 from R&D Systems; 150 μg/mouse, intraperitoneally) (Fig. 4B).[114] Thus, these observations indicate that Th17 cells (IL-17 but not IL-17F), rather than Th1 cells and Th2 cells, are responsible for neutrophil recruitment in the passive Th cell transfer murine asthma model.

IL-17 IN CHEMICALLY INDUCED MOUSE ASTHMA MODEL

Airway inflammation induced by chemicals such as toluene diisocyanate (TDI),[115,116] hezamethylene diisocyanate (HDI)[117,118] and dinitrofluorobenzene (DNFB)[119,120] is used as a murine model resembling occupational asthma or sick building/house syndrome and characterized by an influx of neutrophils. Although TNF and mast cells are responsible for the DNFB-induced neutrophilic airway inflammation,[119,120] the possible contribution of IL-17 to the diseases remains unknown. However, it was shown that IL-17 is important for the neutrophil recruitment in TDI-induced airway inflammation.[121] These limited observations suggest that IL-17 may be involved in the pathogenesis of occupational asthma or sick building/house syndrome.

IL-17 IN ALLERGIC RHINITIS, CONJUNCTIVITIS AND FOOD ALLERGY

Allergic rhinitis, conjunctivitis and food protein-induced allergic enterocolitis are considered to be typical Th2 cytokine-dominant diseases. It was re-

ported that IL-17 was detectable in the nasal fluids from patients with allergic and viral rhinitis, although the IL-17 levels were much higher in viral rhinitis than in allergic rhinitis.[122] Both eosinophils and neutrophils had infiltrated the inflammatory lesions of patients with conjunctivitis, and increased IL-8 levels and mast cell activation were also observed.[123,124] In certain cases, patients with food-protein enterocolitis syndrome exhibited a significant increase in neutrophils.[125] In humans and rodents, the roles of IL-17 and Th17 cells in such allergic diseases remain to be elucidated. However, the limited observations to date imply that IL-17 may contribute to neutrophil recruitment by inducing IL-8 or mast cell activation.

IL-17 IN ALLERGIC DERMATITIS

It was recently shown that IL-17 and Th17 cells contribute to the pathogenesis of psoriasis, which is an autoimmune skin disorder.[126] IL-17 can promote IL-6, IL-8, Groα, GM-CSF, SCF and ICAM-1 expression/production[127-129] by keratinocytes and CCL20 (MIP-3α) expression by dermal fibroblasts, dermal microvascular endothelial and dendritic cells, and keratinocytes.[130] Although IL-17 was increased more in skin biopsy specimens from patients with acute-phase atopic dermatitis compared with the chronic phase,[131] the precise roles of IL-17 and Th17 in the disease development remain to be elucidated.

Classically, contact dermatitis (contact hypersensitivity; CHS) was regarded as one form of delayed-type hypersensitivity (DTH) reaction. However, based on the findings in gene-deficient mice, it is known that CHS develops due to different molecular mechanisms from DTH.[132] MHC class I-deficient mice, which lack $CD8^+$ T cells, showed attenuated CHS responses, while MHC class II-deficient mice, which lack $CD4^+$ T cells, showed exacerbated disease.[133] These observations indicate that $CD8^+$ T cells are an effector, while $CD4^+$ T cells are a regulator, in such settings. On the other hand, studies using CD4-deficient and CD8-deficient mice indicated that both $CD4^+$ T cells and $CD8^+$ T cells are required as effector cells for CHS responses.[134] Supporting this, it was further reported that MHC class II-deficiency also led to attenuation of CHS.[135]

As in the development of autoimmune diseases, CHS responses are also considered to induce excessive IFN-γ-producing Th1 cells and Tc1 cells.[132,134] However, CHS responses induced by oxazolone,[136] TNCB[136,137] and DNFB[138,139] developed normally in IFN-γ- and IFN-γR1-deficient mice. IFN-γR2-deficient mice (129xB6 mixed background) showed reduced CHS responses induced by FITC,[140] while IFN-γ-deficient mice (C57BL/6 background) exhibited normal CHS responses induced by FITC and oxazolone (Nakae S; unpublished observation). Thus, these observations indicate that IFN-γ is not essential for the development of CHS, and at least, CHS does not seem to be a Th1/Tc1-mediated response. Oxazolone-induced CHS developed normally in IL-4-deficient mice having a 129xB6,[141] BALB/c[142] or C57BL/6[143] background, while TNCB- or DNFB-induced CHS was decreased in IL-4-deficient mice having a BALB/c or C57BL/6 background,[142-144] but not in IL-13-deficient mice having a 129xB6 mixed background.[145] Mice deficient in STAT-6, which is essential for IL-4 and IL-13 signal transduction, exhibited attenuated CHS responses induced by oxazolone, TNCB, DNFB or FITC.[135,146] Thus, these observations indicate that Th2-cytokines, rather than Th1-cytokines, are important for the chemical induction of CHS responses, although the responses are affected by the mouse background and chemicals used.

Nickel-specific Th clones which were established from patients with allergic contact dermatitis expressed IL-17,[128,129] implying a contribution of IL-17 and Th17 cells to the disease. IL-17-deficient mice of 129xB6 mixed background exhibited reduced CHS induced by DNFB and TNCB.[17] FITC- and oxazolone-induced CHS responses were also attenuated in IL-17-deficient mice having a C57BL/6 background (Nakae S; unpublished observations). The attenuated CHS responses were caused by a defect in hapten-specific Th cell induction.[17] Moreover, it was shown that IL-17-producing $CD8^+$ T cells (Tc17 cells), but not Tc1 cells, are a central player in the induction of CHS responses.[147]

Taken all together, both Th2-cytokines and Th17-cytokines, but not Th1-cytokines, are responsible for the development of CHS in mice.

CONCLUSIONS

In addition to its role in the development of autoimmune diseases, IL-17 may play roles in the development of various allergic diseases that have classically been considered to be Th1- or Th2-mediated disorders. Moreover, IL-17 may be a new marker of disease progression and a new target for therapy.

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