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RORγt-dependent IL-17A-producing cells in the pathogenesis of intestinal inflammation

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The hypothesis of helper T (T_h)1/T_h2 cytokine balance proposed by Mosmann and Coffman is often invoked to explain the development of inflammatory diseases, including inflammatory bowel diseases (IBD). Recently, however, a newly identified class of T_h cells—T_h17 cells, which produce T_h17 family cytokines—has been recognized as an essential subpopulation in the development of almost all kinds of human and animal inflammatory diseases, rather than T_h1 and T_h2 cells. A representative T_h17 family cytokine, interleukin (IL)-17A, is produced by not only T_h17 cells, but also by other types of cells, such as T-cell receptor γδ T cells, natural killer (NK) T cells, NK cells, myeloid cells, and innate lymphoid cells, which may also be critically involved in the initiation and persistence of IBD. Here we review recent advances in the study of such IL-17A-producing cells in the pathogenesis of IBD.

INTRODUCTION

Until recently, Crohn's disease (CD) and ulcerative colitis (UC) had been regarded as mediated by interferon (IFN)-γ-producing helper T (T_h)1 cells and interleukin (IL)-4/IL-13-producing T_h2 cells, respectively.[1] However, just after the discovery of T_h17 cells, which specifically produce T_h17 family cytokines, such as IL-17A, IL-17F, IL-21, and IL-22, many investigators reconsidered the T_h1/T_h2 cytokine balance hypothesis, concluding that T_h17 cells are the instrumental T_h cells involved in the pathogenesis of these diseases.[2,3] Importantly, it has also been considered that each T_h subpopulation is independently generated in the presence of specific cytokines via specific master transcription factors: T_h1 cells generate from naïve T cells in the presence of IL-12, and express T-bet (Tbx21); T_h2 cells generate in the presence of IL-4, and express GATA-3; and T_h17 cells generate in the presence of transforming growth factor (TGF)-β plus IL-6, and express RORγt.[3]

Development of experimental autoimmune encephalomyelitis had previously been thought to require IFN-γ-producing T_h1 cells; however, in 2003 Cua *et al.*[4] found that IL-23 (an IL-12p35/IL-23p19 heterodimer), rather than IL-12 (an IL-12p35/p40 heterodimer) is essential for experimental autoimmune encephalomyelitis development. Thereafter, T_h17 cells (which produce IL-17A) were proposed as newly identified T helper cells that act independently of T_h1 and T_h2 cells. Subsequent studies showed that T_h17 cells mediate not only other murine models of autoimmune diseases and inflammatory bowel diseases (IBD), but also human autoimmune diseases and IBD.[5–8]

T_H17 CELLS IN THE PATHOGENESIS OF HUMAN IBD

Before the discovery of T_h17 cells, Fujino *et al.*[9] reported that IL-17A expression is highly upregulated in $CD3^+$ T cells and $CD68^+$ macrophages in the inflamed mucosa of patients with IBD; similar reports followed.[10–14] In addition, other T_h17-associated molecules, such as IL-17F, IL-21, IL-22, IL-23, RORγt, and IL-23R, are upregulated in inflamed mucosa of IBD patients.[15–18] Rovedatti *et al.*[14] reported that not only mucosal IFN-γ^+IL-$17A^-$ T_h1 cells and IFN-γ^-IL-$17A^+$ T_h17 cells but also IFN-γ^+IL-$17A^+$ T_h1/T_h17 double-producing cells are highly expressed in the inflamed mucosa of UC and CD patients. Interestingly, they also showed that T_h1/T_h17 cells resided particularly in the $CD161^+$ subpopulation rather than in $CD161^-$ cells.[14] Our group showed that IL-17A increases in the inflamed mucosa of UC patients, while IFN-γ increases in the inflamed mucosa of CD patients.[12] Although our analysis covered only CD patients, we found that both IFN-γ and IL-17A are upregulated in $CD4^+$ T cells isolated from mesenteric lymph nodes.[19] In terms of T_h1 cells, however, most studies have shown normal IFN-γ levels in inflamed UC mucosa, although many studies offer evidence that IFN-γ expression is elevated in inflamed CD mucosa.[20] Therefore, it remains unknown whether T_h1 and T_h17 cells are dominant in the pathogenesis of IBD

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depending on the location, disease, and method used to assess cytokines.

We recently identified a unique $CD14^+CD33^+CD205^+CD209^+$ macrophage, which specifically increases in the inflamed IBD mucosa, especially in CD, and produces large amounts of IL-23, IL-6, and tumor necrosis factor-α. Of note, IL-23 promotes the production of IFN-γ and IL-17A by lamina propria (LP) mononuclear cells obtained from CD and UC tissues, respectively.[17] Subsequent analysis showed that increasing mucosal $CD14^+$ macrophages in CD patients could induce IFN-γ-producing T_h1 cells *in vitro*.[20]

Although IL-23 potentially maintains or induces T_h17 cells, it has a critical role in inducing T_h1 cells in some specific immunological conditions. Consistent with this, genome-wide association studies have recently revealed that the gene *IL-23R* has a significant association with not only UC but also CD.[21,22]

T_H17 CELLS IN PATHOGENESIS OF ANIMAL MODELS OF IBD

Research stimulated by a study of experimental autoimmune encephalomyelitis and murine T_h17 cells[4] has produced evidence of a significant role for T_h17 cells in the pathogenesis of animal IBD models (**Table 1**). Transgenic mice that ubiquitously expressed IL-23p19 spontaneously developed chronic enterocolitis;[23] colitis in IL-10-deficient mice was exacerbated by IL-23 administration;[24] and SCID mice in which IL-17A-producing T_h17 cells from C3Bir mice were transferred, spontaneously developed severe colitis, which was blocked by administration of anti-IL-23p19 monoclonal antibody (mAb).[25] Similarly, development of colitis in IL-10-deficient mice[24] and *Helicobacter hepaticus* (*Hh*)-infected mice administered with anti-IL-10R mAb[26] was suppressed by anti-IL-23p19 mAb treatment. Irrespective of whether *Hh*-infected[26] or uninfected,[27] $IL\text{-}23^{-/-}\times RAG\text{-}1^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells did not develop colitis. Furthermore, $RAG\text{-}1^{-/-}$ mice transferred with $CD4^+CD25^-$ T cells from $ROR\gamma t^{-/-}$ mice did not develop colitis.[28] However, one paper reported that 2,4,6-trinitrobenzene sulfonic acid-induced colitis was exacerbated in IL-23-deficient mice compared with wild-type (WT) mice.[29]

In contrast, it is unsettled whether IL-17A has a pathological role in colitis. While $IL\text{-}17R^{-/-}$ mice developed less severe 2,4,6-trinitrobenzene sulfonic acid-induced colitis,[30] administering neutralizing anti-IL-17A mAb did not reduce severity of colitis in an adoptive transfer model of $CD4^+CD45RB^{high}$ T cells; however, coadministration of anti-IL-17A and anti-IL-6 significantly ameliorated the severity of intestinal inflammation.[24] Administration of anti-IL-17A mAb exacerbated dextran sulfate sodium-induced colitis,[31] while $IL\text{-}17A^{-/-}$ mice developed less severe dextran sulfate sodium-induced colitis.[32] There were no differences in colitis indices between $RAG\text{-}1^{-/-}$ mice transferred with naive T cells from WT, $IL\text{-}17A^{-/-}$, $IL\text{-}17F^{-/-}$, or $IL\text{-}22^{-/-}$ mice.[28] Interestingly, however, anti-IL-17A mAb–treated $RAG\text{-}1^{-/-}$ mice transferred with naive T cells from $IL\text{-}17F^{-/-}$ mice developed significantly less severe colitis than did control mAb–treated $RAG\text{-}1^{-/-}$ mice transferred with naive T cells from WT mice.[28] **Table 1** summarizes the previous papers regarding IL-23 and T_h17 family cytokines in animal models of IBD.

IL-17A SUPPRESSES GENERATION OF COLITOGENIC T_H1 CELLS

Against this complex background, O'Connor *et al.*[33] recently showed that IL-17A suppresses a murine model of colitis by blocking the development of T_h1 cells via IL-17R on naive $CD4^+$ T cells. First, they showed that $RAG\text{-}1^{-/-}$ mice transferred with $IL\text{-}17A^{-/-}$ $CD4^+CD45RB^{high}$ T cells developed more severe colitis and wasting diseases, with significantly increased colon IFN-γ production, than did mice transferred with WT $CD4^+CD45RB^{high}$ T cells. Furthermore, IL-17Rα expression in $CD4^+$ T cells was gradually upregulated during *in vitro* stimulation of T_h1 cell development; its generation was markedly suppressed by the addition of IL-17A. In addition, induction of T-bet was significantly promoted by *in vitro*–stimulated T_h1 cells using $IL\text{-}17A^{-/-}$ naive $CD4^+$ T cells as compared with WT naïve $CD4^+$ T cells. Collectively, O'Connor's group[33] concluded that IL-17A blocks differentiation of T_h1 cells via IL-17Rα on $CD4^+$ T cells. However, do other T_h17 family cytokines, such as IL-17F and IL-22, similarly affect development of T_h1 cells? Are there subpopulations that produce both IL-17A/IL-10 and IL-17A/IFN-γ? In this regard, Cua's group reported that IL-23 is a gateway cytokine to induce pathological or protective T_h17 cells in the presence or absence of this cytokine, respectively.[34] Furthermore, do T_h1 and T_h17 cells compete with each other in a cell–cell-dependent or an independent manner?

INTERFERENCE BETWEEN COLITOGENIC T_H1 AND T_H17 CELLS

To investigate how colitogenic T_h1 and T_h17 cells collaborate or interfere with each other in the process of development of colitis *in vivo*, our group used two different colitis models: a T_h1-dominant adoptive transfer model, and a T_h1- and T_h17-mixed IL-10-deficient mouse model. We co-transferred the same number of $CD4^+$ T cells isolated from colitic $RAG\text{-}2^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells and colitic $IL\text{-}10^{-/-}$ mice, or mice transferred with one type alone, into new $RAG\text{-}2^{-/-}$ mice. Interestingly, co-transferred mice developed colitis to an extent similar to mice transferred with one type of cells, and $CD4^+$ T cells were well mixed in a ratio of approximately 1:1 in the inflamed colons of co-transferred mice.[35] However, the proportions of IFN-γ- and IL-17A-expressing $CD4^+$ T cells in co-transferred mice were significantly decreased compared with single-transferred mice, suggesting interference between colitogenic T_h1 and T_h17 cells *in vivo*.[35] Interestingly colitic $RAG\text{-}2^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells gradually became healthy, even after parabiosis surgery with colitic $IL\text{-}10^{-/-}$ mice. The two types of $CD4^+$ T cells were also well mixed in colonic LP, and the percentages of IFN-γ- and IL-17A-expressing LP $CD4^+$ T cells tended to decrease after parabiosis surgery, which is one reason for the amelioration of colitis.[33] All these experiments indicate that disease phenotypes of colitis grossly depend on

Table 1 Role of T_h17 cells in murine models of IBD

Pathogenic role of IL-23	
Wiekowski *et al.*[23]	IL-23p19 transgenic mice spontaneously develop enterocolitis
Kullberg *et al.*[26]	No colitis in *Hh*-infected RAG-$1^{-/-}$×IL-$23^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells
Anti-IL-23 mAb suppresses colitis in anti-IL-10R-treated Hh*-infected mice*	
Yen *et al.*[24]	IL-23 administration exacerbates colitis in IL-$10^{-/-}$ mice, while anti-IL-23 mAb administration suppresses colitis in those mice
Elson *et al.*[25]	Anti-IL-23 mAb treatment suppresses the development of colitis in RAG-$1^{-/-}$ mice transferred with T_h17 cells from colitic C3Bir mice
Izcue *et al.*[27]	No colitis in RAG-$1^{-/-}$ x IL-$23^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells
Protective role of IL-23	
Becker *et al.*[29]	Severer TNBS-induced colitis in IL-$23^{-/-}$ mice
Pathogenic role of RORγt	
Leppkes *et al.*[28]	No colitis in RAG-$1^{-/-}$ mice transferred with RORγt$^{-/-}CD4^+CD25^-$ T cells
Pathogenic role of IL-17A	
Zhang *et al.*[30]	Milder TNBS-induced colitis in IL-$17R^{-/-}$ mice
Ito *et al.*[32]	Milder DSS-induced colitis in IL-$17A^{-/-}$ mice
Non-effective role of IL-17A	
Izcue *et al.*[27]	No change of colitis in RAG-$1^{-/-}$ mice transferred with IL-$17A^{-/-}$ $CD4^+CD45RB^{high}$ T cells
Leppkes *et al.*[28]	Anti-IL-17A treatment does not affect colitis in RAG-$1^{-/-}$ mice transferred with $CD4^+CD25^-$ T cells
Yen *et al.*[24]	Anti-IL-17A treatment does not affect colitis in RAG-$1^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells (coadministration of anti-IL-17A and anti-IL-6 mAbs ameliorates colitis)
Protective role of IL-17A	
Ogawa *et al.*[31]	Anti-IL-17A administration exacerbates DSS-induced colitis
O'Connor *et al.*[33]	Exacerbation of colitis in RAG-$1^{-/-}$ mice transferred with IL-$17A^{-/-}$ or IL$17R^{-/-}$ $CD4^+CD45RB^{high}$ T cells

DSS, dextran sulfate sodium; *Hh*, *Helicobacter hepaticus*; IBD, inflammatory bowel disease; IL, interleukin; mAb, monoclonal antibody; T_h, helper T cell; TNBS, 2,4,6-trinitrobenzene sulfonic acid.

a balance of cytokine interference, including the competition between T_h1 and T_h17 cells.

LINEAR DEVELOPMENTAL PATHWAY FROM T_H17 TO T_H1 CELLS IN THE DEVELOPMENT OF COLITIS

In the current immunology, the new T_h cell subsets, such as IL-10-producing Tr1 cells,[36] follicular helper T cells, which support antibody-producing B cells,[37] IL-9-producing Th9 cells,[38] and IL-22-producing T_h22 cells,[39] are emerging besides T_h1, T_h2, T_h17, and $CD4^+CD25^+Foxp3^+$ T regulatory (T_{reg}) cells,[40] and the plasticity between those cells is also now extensively under investigation.[7] Initially, it was thought that T_h1 and T_h17 cells are generated from naive $CD4^+$ T cells independently, and that terminally differentiated T_h cells seldom re-differentiate to other T_h subsets. Following the discovery of induced pluripotent stem cells,[41] which are reprogrammed from terminally differentiated cells, it became evident that many types of cells can re-differentiate to others; e.g., even protective T_{reg} cells can re-differentiate to pathogenic T_h1, T_h17, and follicular helper T cells.[42]

Against this background, Weaver's group recently reported that T_h1 cells are generated from IL-17F-expressing T_h17 cells in the late stages of colitis development.[43] They first showed that IL-17F reporter mice-derived IL-$17F^+$ (green fluorescent protein, GFP^+) IFN-γ^- T_h17 cells generated in the presence of TGF-β and IL-6 converted to IL-$17A^-$/IFN-γ^+ "T_h1-like" cells in the absence of TGF-β and in the presence of IL-23. Then, the *in vitro*–manipulated IL-$17F^+CD4^+$ T_h17 cells were transferred into RAG-$1^{-/-}$ mice. In this setting, mice not only developed colitis but also retained IL-$17A^-$IFN-γ^+ T_h1-like cells in the inflamed mucosa, indicating that committed T_h17 cells give rise to progeny that lost IL-17A expression and upregulated IFN-γ expression in the late stage of colitis development. Although these findings explain why both T_h1 and T_h17 cells are

mandatory for the development of colitis by this linear pathway from T_h17 to T_h1 cells, they did not characterize the expression of RORγt and T-bet in the "T_h1-like" IL-17A$^-$IFN-γ$^+$ CD4$^+$ T cells in the inflamed mucosa of colitic RAG-1$^{-/-}$ mice. In addition, it remains unclear if *in vitro*–manipulated IL-17F$^+$CD4$^+$ T cells mirror the bona fide T_h17 cells that emerged in the early stage of colitis *in vivo*.

Previously, it was believed, although not experimentally proved, that both T_h1 and T_h17 cells are critically involved in the model of adoptive transfer of naive CD4$^+$ T cells into immunodeficient mice, and CD4$^+$CD25$^+$Foxp3$^+$ T_{reg} cells suppress development of this model, possibly by suppressing generation and maintenance of both colitogenic T_h1 and T_h17 cells.[44] We noticed that IL-17A$^+$IFN-γ$^+$ T_h17/T_h1 cells reside in only the inflamed mucosa of this model, but not in normal mucosa of WT mice. When RAG-2$^{-/-}$ mice were transferred with CD4$^+$CD45RB^{high} T cells derived from RORγt–GFP reporter mice, we first confirmed that they developed colitis, but only ~10% of CD3$^+$CD4$^+$ T cells were GFP$^+$ (RORγt$^+$), suggesting that this model would be T_h1 dominant as whole CD3$^+$CD4$^+$ T cells were CD44^{high}CD62L$^-$IL-7Rα^{high} effector-memory T cells. Thereafter, GFP$^+$ (RORγt$^+$) or GFP$^-$ (RORγt$^-$) CD4$^+$ T cells were isolated from inflamed mucosa of those mice using a fluorescence-activated cell sorter. The GFP$^-$ cells consisted of only IL-17A$^-$IFN-γ$^+$ T_h1 cells and did not include IL-17A$^+$IFN-γ$^-$ T_h17 and IL-17A$^+$IFN-γ$^+$ T_h17/T_h1 cells, while—surprisingly—GFP$^+$ cells not only consisted of T_h17 and T_h17/T_h1 subsets, but also had T_h1 cells in the highest ratio among these three subpopulations. Furthermore, both GFP$^-$ and GFP$^+$ cells expressed Tbx21, which is the gene product of *T-bet*, suggesting that GFP$^-$ cells were classical T_h1 cells, while GFP$^+$ cells included RORγt$^+$T-bet$^-$ T_h17, RORγt$^+$T-bet$^+$ T_h17/T_h1, and RORγt$^+$T-bet$^+$ T_h1-like cells, as the major subset of GFP$^+$ cells was IL-17A$^-$IFN-γ$^+$.

To further assess the roles of GFP$^-$ and GFP$^+$ cells in terms of colitogenecity, these cells were again transferred into new RAG-2$^{-/-}$ mice. In both cases, the re-transferred mice developed colitis to a similar extent. When the expression of GFP was examined after re-transfer, we found that LP CD4$^+$ T cells isolated from mice transferred with GFP$^-$ cells remained GFP-negative and preferentially produced IFN-γ, but not IL-17A, suggesting that re-differentiation from $T_h1 \rightarrow T_h17$ cells did not occur in this setting. In contrast, in mice transferred with GFP$^+$ cells, approximately half of cells lost GFP expression. Moreover, among GFP-retaining cells, most had IL-17$^-$IFN-γ$^+$ as the major subset, with IL-17A$^+$IFN-γ$^-$ T_h17 and IL-17A$^+$IFN-γ$^+$ T_h17/T_h1 cells as minor subsets, whereas almost all cells that lost GFP expression were IL-17A$^-$IFN-γ$^+$ T_h1 cells. Collectively, these results clearly showed that there is a distinct developmental pathway from $T_h17 \rightarrow T_h1$ cells, via possibly T_h17/T_h1 and T_h1-like cells during the development of colitis *in vivo* (**Figure 1**).[45] However, it is also possible that the lymphopenic host microenvironment favors transformation from T_h17 to alternative T_h1 cells, and this linear pathway may not occur under physiologically lymphosufficient conditions.

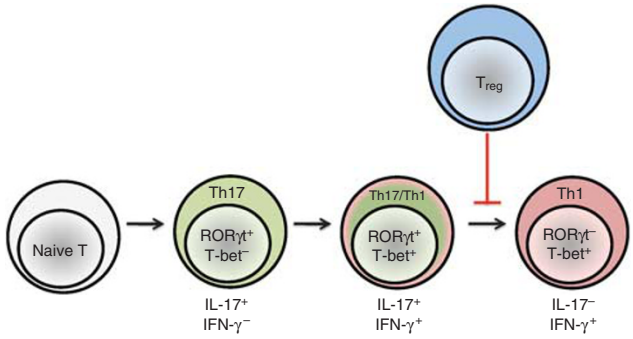

Figure 1 Alternative T_h1 cell differentiation from T_h17 cells during colitis development. T_{reg} cells suppress the path of $T_h17 \rightarrow$alternative T_h1 cells, resulting in a high proportion of T_h17 and T_h17/T_h1 cells in the presence of T_{reg} cells. IFN-γ, interferon-γ; IL-17, interleukin-17; T_h, helper T cell; T_{reg}, T regulatory cell.

Furthermore, recent work by Powrie's group nicely demonstrated that IL-23 promotes both accumulation of intestinal T_h17 cells, and emergence of a IL-17A$^+$IFN-γ$^+$ T_h17/T_h1 population; while blockage of IL-23/IL-23R axis suppresses the development of colitis induced by transfer of CD4$^+$CD45RB^{high} T cells into RAG-1$^{-/-}$ mice,[46] and particularly reduces the ratio of T_h17/T_h1 population. These results suggest that T_h17/T_h1 cells are the true pathological cells in this model. Our results, however, imply that IL-23 is critical to the linear developmental pathway of alternative T_h1 cells, and particularly promotes the path from T_h17 to T_h17/T_h1 cells. We also found that IL-17A-producing T_h17 cells did not decline more rapidly than IFN-γ-producing T_h1 cells during the *in vivo* competition between T_h1 and T_h17 cells after parabiosis between the two models of colitis and co-transfer experiment—not what one might expect from results showing that T_h17 cells transform into T_h1 cells. Further studies are warranted to resolve these interesting issues.

Is this linear developmental pathway from T_h17 to T_h1 cells the only one to mediate colitis? On the basis of the independent developmental pathway of T_h1 and T_h17 cells, it is likely that RAG-1$^{-/-}$ mice transferred with naive T cells from T-bet$^{-/-}$ or RORγt$^{-/-}$ mice can develop T_h17- or T_h1-mediated colitis, respectively. In this regard, however, RAG-1$^{-/-}$ mice transferred with naive T cells from T-bet$^{-/-}$ or RORγt$^{-/-}$ mice reportedly did not develop colitis at all.[28,47] This discrepancy suggests that the linear developmental pathway is essential for colitis development. We called colitogenic T_h1 cells that arise from T_h17 cells via such a linear pathway "alternative" T_h1 cells; they may be genuinely pathological T_h1 cells in colitis development. Interestingly, this hypothesis allows that the adoptive transfer model of colitis depends more on IL-23 than IL-12.[26] Further study is needed to determine whether classical T_h1 cells generated in RORγt-independent manner are truly involved in colitis development.

T_{REG} CELLS BLOCK THE DEVELOPMENTAL PATHWAY FROM T_H17 TO T_H1 CELLS

The next question arising is how CD4$^+$CD25$^+$Foxp3$^+$ T_{reg} cells affect the linear developmental pathway from T_h17 to

alternative T_h1 cells in the development of colitis. To examine this, we transferred Ly5.2$^+$CD4$^+$CD45RB^{high} T cells, with or without Ly5.1$^+$CD4$^+$CD25$^+$ T_{reg} cells, from RORγt–GFP reporter mice into RAG-2$^{-/-}$ mice. As expected, mice co-transferred with CD4$^+$CD45RB^{high} T cells and T_{reg} cells did not develop colitis, possibly due to the suppressive activity of T_{reg} cells, whereas mice transferred with CD4$^+$CD45RB^{high} T cells alone did develop colitis. Surprisingly, however, the proportions of RORγt-expressing IL-17A$^+$IFN-γ$^-$ T_h17 and IL-17A$^+$IFN-γ$^+$ T_h17/T_h1 cells in LP of the co-transferred mice were significantly higher than those of single-transferred mice, while, as expected, the proportion of RORγt-negative IL-17A$^-$IFN-γ$^+$ T_h1 cells in the co-transferred mice was significantly decreased compared with the single-transferred mice; this suggests that T_h17 and T_h17/T_h1 cells are non-colitogenic or pre-colitogenic, whereas T_h1 cells are colitogenic effector-memory T cells. Also, irrespective of GFP expression, LP CD3$^+$CD4$^+$ T cells in colitic mice transferred with CD4$^+$CD45RB^{high} T cells alone expressed Tbx21, whereas LP CD3$^+$CD4$^+$ T cells in non-colitic mice transferred with CD4$^+$CD45RB^{high} T cells and T_{reg} cells did not express Tbx21,[45] indicating that T_{reg} cells suppress not only the pathway between T_h17/T_h1 and T_h1 cells, but also the induction of Tbx21 in colitogenic effector-memory T cells (**Figure 1**). Furthermore, consistent with the results of Weaver's study showing that this transformation from T_h17 to T_h1 cells occurs in the absence of TGF-β and increases in mice treated with anti-IL-23R,[43] it is possible that TGF-β derived from T_{reg} cells suppresses the development from T_h1, T_h17/T_h1 to alternative T_h1 cells. Another possible mechanism of action of T_{reg} cells is shown in Chen and colleagues' recent demonstration that IL-2 consumption by IL-2Rα (CD25)-expressing T_{reg} cells increases T_h17 cell development in Foxp3.luciDTR mice, in which diphtheria toxin selectively depletes Foxp3$^+$ T_{reg} cells.[48] Pandiyan and colleagues reported a similar result.[49] In this regard, these two papers may explain the previous finding that IL-2 suppresses the development of T_h17 cells.[50]

RORγT-DEPENDENT INNATE LYMPHOID CELLS IN ANIMAL MODELS OF IBD PATHOGENESIS

Besides T_h17 lymphocytes, immune cells, such as natural killer (NK) cells, NKT cells, TCR γδ-expressing T cells, macrophages, and Paneth cells, can produce IL-17A.[51] Of particular importance, these more innate immune cells, unlike T_h17 cells, immediately secrete IL-17A in response to specific alert molecules such as pathogen-associated molecular patterns and cytokines such as IL-23. However, the involvement of these "non-Th17 cell IL-17A-producing cells" to colitis pathogenesis is not fully understood and are now an area of intense research. Furthermore, evidence is emerging of a new IL-17A-producing cell population, innate lymphoid cells (ILCs), that have crucial roles in not only the formation and maintenance of gut-associated lymphoid tissue (GALT), but also colitis pathogenesis in animals and humans.[52] There are various kinds of ILCs categorized by location, cytokine production, and expression of specific surface markers and intracellular transcription factors, including lymphoid tissue inducer (LTi) cells, which are involved in GALT formation during the fetal period. Similar LTi cells are also present in the gut and tonsils after birth, and are called adult LTi-like (hereafter described as LTi-like) cells.[53] These cells may have important roles in the generation of cryptopatches and isolated lymphoid follicles, and in the maintenance of all GALT after birth, but experimental proof for the role of LTi-like cells after birth is lacking. The LTi/LTi-like cells constitutively express RORγt; consistently RORγt$^{-/-}$ mice completely lack GALT. Therefore, RORγt is a master transcription factor for not only T_h17 cells, but also LTi/LTi-like cells. These cells also promptly produce IL-17A in response to IL-23, which may be induced in activated antigen-presenting cells such as macrophages and dendritic cells. Although it is known that LTi cells produce both IL-17A and IL-22, several groups have identified NK receptor (NKR)-expressing "LTi-like"-like cells that express RORγt and produce IL-22 but not IL-17A in the tonsils and intestines. These cells are now designated (not uniformly) as NK22, NKR-LTi, NCR-22, NKR$^+$RORγt$^+$ ILC, and ILC22 cells. These cells specifically express NKp46 but not NKp44 in mice, and in contrast, NKp44 but not NKp46 in humans.[54,55] Importantly, IL-22 is a IL-10 family cytokine, but its specific receptor seems to be expressed only on non-hematopoietic cells, such as epithelial cells, whereas IL-10R is specifically expressed on hematopoietic cells. Consistent with this, IL-22$^{-/-}$ mice and RORγt$^{-/-}$ mice develop more severe colitis with marked epithelial damage in the dextran sulfate sodium-induced colitis model than do control WT mice,[55,56] and IL-22$^{-/-}$ mice develop more severe intestinal epithelial damage in the *Citrobacter* infection colitis model,[57] suggesting that ILC22 (NK22) cells are critical to epithelial repair (**Figure 2**). However, it remains unclear whether ILC22 cells develop from LTi/LTi-like cells[55] or directly from RORγt$^+$ ILC precursor cells[58] that have not yet been identified. Nevertheless, it seems that LTi/LTi-like cells preferentially reside in GALT and have important roles in generating and maintaining GALT, while ILC22 cells diversely reside in intestinal LP to promote epithelial repair.

The next critical issue is whether ILC cells are pathologically involved in IBD development. In this regard, Powrie's group first described pathological ILC cells in two models of innate colitis (**Figure 2**).[59] In the first model of *Hh*-infected RAG-1$^{-/-}$ mice, the proportion and absolute number of unique Thy1^{high}Sca-1$^+$ ILC cells that produce not only IL-17A and IL-22 but also IFN-γ (ILC17 or ILC17/1 cells) were markedly increased in the colonic LP. Like physiological-condition LTi/LTi-like cells, ILC cells in colitic *Hh*-infected RAG-1$^{-/-}$ mice are Lin (CD11b, Gr1, B220)$^-$ RORγt$^+$NKp46$^-$IL-7Rα$^+$CCR6$^+$, but unlike LTi/LTi-like cells, they are Sca-1$^+$c-kit$^-$CD4$^-$. It is not known whether those pathological ILC cells are derived from physiological-condition LTi/LTi-like cells or are generated from RORγt$^+$ ILC precursor cells (**Figure 2**). Administration of anti-Thy1 mAb to target those Thy1^{high}Sca-1$^+$ ILC cells suppressed the development of *Hh*-induced innate colitis in RAG-1$^{-/-}$ mice, although it is unclear if anti-Thy1 treatment targets only the pathological ILC cells in this model, as Thy1 is broadly expressed on various cells (such as NK cells). Of note, pathological ILC cells expressed T-bet and RORγt, suggesting that they are counterparts of

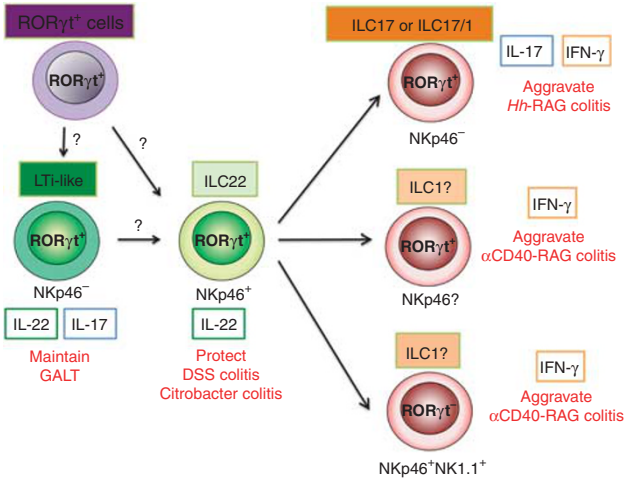

Figure 2 Role of newly identified RORγt-dependent ILC in the development of animal models of colitis. The various kinds of ILCs are categorized by location, cytokine production, and expression of specific surface markers and intracellular transcription factors; LTi cells and possibly adult LTi-like cells are involved in the formation and maintenance of GALT. ILC22 (NK22, NKR-LTi, NCR-22, or NKR$^+$ RORγt$^+$ ILC) cells may be involved in IL-22-mediated epithelial repair. However, it is unclear whether ILC22 cells develop from LTi/LTi-like cells[55] or directly from RORγt$^+$ ILC precursor cells.[58] Several studies described colitogenic ILC cells (ILC1, ILC17/1, or ILC17) in two models of innate colitis. GALT, gut-associated lymphoid tissue; IFN-γ, interferon-γ; IL, interleukin; ILC, innate lymphoid cell; LTi, lymphoid tissue inducer; NK22, natural killer-22; NKR-22, natural killer receptor-22; T_h, helper T cell; T_{reg}, T regulatory cell.

IL-17A/IFN-γ-double–producing T-bet$^+$RORγt$^+$ T_h1/T_h17 cells. This group attempted using another innate colitis model,[58] in which RAG-1$^{-/-}$ mice are administered anti-CD40 mAb to activate antigen-presenting cells. In this second model, increased Thy1^{high}Sca-1$^+$ ILC cells also emerged in inflamed colon mucosa, and anti-Thy1 mAb treatment blocked the development of colitis. Interestingly, unlike the model of *Hh*-infected RAG-1$^{-/-}$ mice, these ILC cells produced IFN-γ and IL-22, but not IL-17A, although they expressed RORγt. Therefore, this second type of ILC cells (possibly ILC1) that emerged in anti-CD40-administerred mice is not identical to the ILC17 or ILC17/1 in *Hh*-infected RAG-1$^{-/-}$ mice. Furthermore, RORγt$^{-/-}$× RAG-1$^{-/-}$ mice were totally resistant to this anti-CD40 mAb administration model, suggesting that RORγt is essential for the generation or maintenance of the second type of pathological ILC1 cells; i.e., ILC cells are essential for colitis development, following the activation of antigen-presenting cells via CD40 molecules to produce IL-23. Further studies should address the developmental pathway and plasticity of colitogenic ILC cells, their relationship to T-bet, the reason those two colitogenic ILC cells produce IL-22, and the distinct location of those cells.

Subsequently, Diefenbach's group elegantly showed a sequential developmental pathway for LTi-like cells→RORγt$^+$ NKR–ILC (NKp46$^+$)→IFN-γ-producing RORγt$^-$ NKR–ILC (NKp46$^+$) in the process of anti-CD40 mAb-induced innate colitis, using RORγt fate-map mice.[55] These colitogenic IFN-γ-producing RORγt$^-$ NKR–ILC cells differ from conventional NK (cNK) cells, as they could be depleted by anti-NK1.1 mAb, but not by anti-asialo GM1 mAb, while cNK cells were depleted by either anti-NK1.1 mAb or anti-asialo GM1 mAb. Consistent with this, administration of anti-NK1.1 mAb, but not anti-asialo GM1, ameliorated this colitis model.[54] It is not known whether RORγt$^-$ NKR–ILC cells in colitic mice after anti-CD40 mAb administration express NK1.1. Although the Diefenbach team showed that, before anti-CD40 mAb administration, half of the RORγt$^+$ NKR–ILC cells in fate-map mice expressed NK1.1, it would be interesting to know if the depletion of NKp46$^+$ cells affects development of this model. Other groups have shown that murine RORγt$^+$ ILC22 cells lose NK1.1 expression compared with cNK.[55] Otherwise NK1.1-expressing RORγt$^+$ NKR–ILC cells may be able to differentiate to colitogenic RORγt$^-$ NKR–ILC cells, or colitogenic RORγt$^-$ NKR–ILC cells may re-express NK1.1 after anti-CD40 mAb administration.

Apart from RORγt-expressing ILC cells, RORγt-independent ILC cells were recently identified by several groups. These ILC cells, such as natural helper cells, nuocytes, and innate helper type 2 (Ih2) cells, are called ILC2, as they produce T_h2 family cytokines, such as IL-5 and IL-13, in response to IL-25 or IL-33.[60–62] The natural helper cells reside in adipose tissue in the peritoneal cavity as "fat-associated lymphoid clusters", and are as dependent on common gamma (γc) receptors as LTi/LTi-like cells are, but—unlike LTi/LTi-like cells—are independent of RORγt, indicating that ILC2 subsets are generated from hematopoietic precursor cells in an RORγt-independent manner. However, it is unclear whether this new ILC2 subset is involved in the pathogenesis of human IBD and their animal models. The natural helper cells in fat-associated lymphoid cluster in the mesentery may have a pathological role in forming mesenteric fat lapping—a particular finding in CD. Interestingly, nuocytes[61] and Ih2 cells[62] are found in the small intestine and mesenteric lymph nodes, in addition to the spleen, suggesting the intestinal pathology involvement.

Accumulating evidence suggests that ILC cells are closer to innate immune cells than to acquired immune cells. Notably, various ILC subsets have corresponding partner T_h cells: IFN-γ-producing and RORγt-expressing (or previously expressing) ILC1 cells, IL-4/IL-5/IL-13-producing and GATA3-expressing ILC2 cells, IL-17A-expressing and RORγt-expressing ILC17 cells, and IL-22-expressing and RORγt-expressing ILC22 cells may correspond to NK cells, T_h1 cells, T_h2 cells, T_h17 cells, and T_h22 cells, respectively. Also, T_h17/T_h1 cells, which often emerge in pathological conditions, may be the counterparts of IL-17A/IFN-γ-double–producing and RORγt/T-bet-double–expressing ILC17/1 cells that increase in inflamed mucosa of *Hh*-infected RAG-1$^{-/-}$ mice. The Foxp3-expressing ILC cells may emerge as a type of regulatory ILC cell in the future.

RORγT-DEPENDENT ILCs IN HUMAN IBD PATHOGENESIS

A recent study shows that IL-17A-single, IFN-γ-single, or IL-17A/IFN-γ – double cells, which produce Lin$^-$CD45$^+$CD127$^+$ CD56$^-$ and are similar to LTi-like cells in terms of cell surface markers, are markedly increased in inflamed mucosa of CD, but not UC or non-IBD control[63] (**Figure 3**). These cells may be the counterparts of Thy1^{high}Sca-1$^+$ ILC1, ILC17 or ILC17/1 cells that emerge in inflamed mucosa of colitic *Hh*-infected

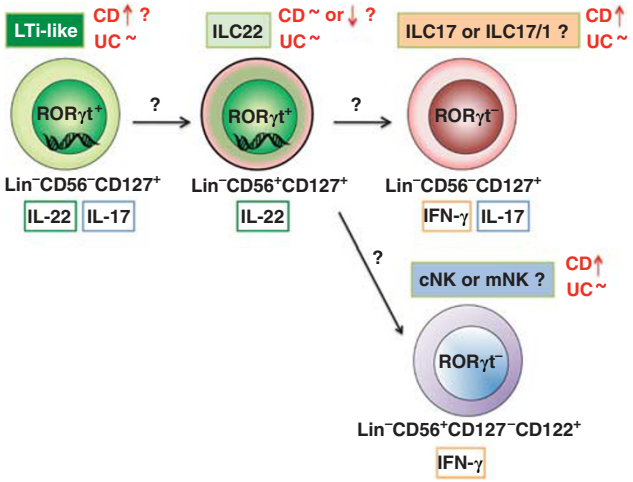

Figure 3 Role of LTi-like cells and NK cells in human IBD. IL-17A/IFN-γ-double–producing $Lin^-CD45^+CD127^+CD56^-$ cells are markedly increased in inflamed CD mucosa.[63] However, whether LTi-like cells and ILC22 cells are increased or decreased in inflamed IBD mucosa is still debatable;[63,64] and the origin of mNK cells is still undetermined. CD, Crohn's disease; ILC, innate lymphoid cells; LTi, lymphoid tissue inducer; UC, ulcerative colitis.

RAG-$1^{-/-}$ mice and/or anti-CD40 mAb-injected mice, rather than IL-22/IL-17A-producing LTi-like cells that reside in the intestine in physiological condition. However, as the absolute numbers of these inflammatory ILC subpopulations are very small in inflamed IBD lesions, it is largely unclear if those cells are critically involved in human IBD pathogenesis, and further studies are needed to determine their exact role. According to this same group, the proportion of $Lin^-CD45^+CD127^+CD56^+$ cells (analogous to human ILC22 cells) was comparable among CD, UC, and controls.[63] However, we previously found two types of NK cells with different surface markers residing in the human intestinal mucosa: $NKp44^+NKp46^-CD56^+CD122^-CD127^+$ and $NKp44^-NKp46^+CD56^+CD122^+CD127^-$. The former $NKp44^+$ cells express RORC and IL-22, whereas the latter $NKp46^+$ cells express IFN-γ, suggesting that $NKp44^+$ and $NKp46^+$ cells are IL-22-producing ILC22 cells and IFN-γ-producing cNK cells, respectively.[64] Interestingly, the proportion of $NKp44^+$ cNK cells in inflamed CD mucosa was significantly decreased compared with controls, whereas the proportion of $NKp46^+$ ILC22 cells was conversely increased compared with controls, indicating that the imbalance between protective $NKp44^+$ ILC22 cells and colitogenic $NKp46^+$ cNK cells in intestinal mucosa is involved in CD pathogenesis in at least one critical pathological pathway. Surprisingly, however, the expression of IL-23Rα on $NKp46^+$ cells was significantly higher than that on $NKp44^+$ cells, and $NKp46^+$ cells strongly responded to IL-23 to produce IFN-γ, suggesting that $NKp46^+$ cNK cells in the intestinal sites are differentiated from $NKp44^+$ ILC22 cells. In this regard, we previously identified $Lin^-c\text{-}kit^+CD127^+CD25^+Id2^+LT\alpha^+$ cells, which are similar to LTi-like cells, in human intestinal mucosa, and these cells differentiate into IFN-γ-producing NK cells.[65] Therefore, unlike cNK cells in extraintestinal locations, mucosal NK cells in humans may be independently generated from self-renewal NK precursor cells in intestinal mucosa to LTi-like cells, ILC22 cells, and IFN-γ-producing NK cells[52] (**Figure 3**), although it remains largely unknown why those mucosal IL-23R-expressing ILC-like $NKp44^+$ cNK cells expressed CD122 but not CD127.

CONCLUSIONS

- Although IL-17A is thought to be preferentially produced by T_h17 cells and to have a critical role in autoimmune disease development, it seems to inhibit the development of T_h1 cells in some circumstances.
- Colitogenic T_h1 and T_h17 cells seem to interfere with each other *in vivo* under inflammatory conditions. At least in inflammatory conditions, colitogenic T_h1 and T_h17 cells are not independently generated; rather the linear sequential developmental pathway from T_h17 to T_h1 cells seems to dominate. Further study is needed to determine whether the classical T_h1 cells directly generated from naive T cells in an RORγt-independent manner are also involved in IBD pathogenesis.
- The pathway from T_h17/T_h1 cells to alternative T_h1 cells is suppressed by T_{reg} cells, resulting in the accumulation of T_h17 cells by T_{reg} cells.
- A newly identified immune cell subset, ILC, may be involved in both the generation and maintenance of GALT, and pathogenesis of chronic intestinal inflammation.

Much new evidence about IL-17A-producing immune cells will likely soon emerge. We can be cautiously optimistic that these new findings will solve many puzzles of IBD pathogenesis, and generate novel therapeutic strategies for these diseases.

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DISCLOSURE

The authors declared no conflict of interest.

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