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*J Immunol* 2011; 186:4546-4550; Prepublished online 14 March 2011;
doi: 10.4049/jimmunol.1004021
http://www.jimmunol.org/content/186/8/4546
Cutting Edge: Generation of Colitogenic Th17 CD4 T Cells Is Enhanced by IL-17+ γδ T Cells

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Th 17 cells have been implicated in the pathogenesis of colitis; however, a cellular mechanism by which colitogenic Th17 immunity arises in vivo remains unclear. In this study, we report that a subset of IL-17+ γδ T cells plays a crucial role in enhancing in vivo Th17 differentiation and T cell-mediated colitis. TCRβ−/− mice were highly susceptible to T cell-mediated colitis, whereas TCRβ−/− mice were resistant to the disease. Importantly, cotransfer of IL-17+ but not of IL-17− γδ T cells with CD4 T cells was sufficient to enhance Th17 differentiation and induce full-blown colitis in TCRβ−/− recipients. Collectively, our results provide a novel function of IL-17+ γδ T cell subsets in supporting in vivo Th17 differentiation and possibly in fostering the development of intestinal inflammation. The Journal of Immunology, 2011, 186: 4546–4550.

A mechanism underlying Th17 differentiation has been an area of extensive investigation. Factors involved in this process, including cytokines and transcription factors, have been identified (1); however, a cellular mechanism by which Th17 immunity arises in vivo still remains unclear. γδ T cells are rare T cell subsets primarily found in epithelial cell-rich tissues and lymphoid tissues (2). γδ T cells in naive animals express activated phenotypes and produce effector cytokines upon stimulation (2). They have been the targets of recent investigation because of their spontaneous IL-17A (referred to as IL-17 hereafter) expression without a differentiation process (3, 4). We have recently reported that IL-17 acquisition in γδ T cells occurs in the thymus via a TGF-β1–dependent mechanism (5). It was demonstrated that γδ T cells amplify Th17 immunity, thus exacerbating autoimmune encephalomyelitis (6). However, whether γδ T cell-mediated regulation also takes place in other Th17-associated inflammatory settings such as inflammatory bowel disease (IBD) remains unexplored.

Naïve CD4 T cells transferred into immunodeficient mice can differentiate into colitogenic effector cells in response to Ags derived from commensal microorganisms (7, 8). Effector CD4 T cells producing proinflammatory cytokines, especially IL-17, have been implicated to play a key role in the pathogenesis of colitis and IBD (9–11). A cellular mechanism involved in the generation of colitogenic Th17 T cells in vivo is not clear.

In this study, we report that γδ T cells support in vivo Th17 differentiation and the subsequent development of colitis induced by T cell transfer into immunodeficient animals. Interestingly, γδ T cell IL-17 production appears to be associated with Th17 differentiation and colitogenicity of CD4 T cells. Our results suggest that IL-17+ γδ T cells may represent potential targets to intervene inflammatory disorders in the intestine seen in patients with IBD.

Materials and Methods

Mice

C57BL/6, B6 Ly5.1, B6 Thy1.1, B6 TCRα−/−, and B6 Rag1−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). IL-17F–RFP Tg mice were previously described (12). IFN-γ–YFP Yeti mice (13) were kindly provided by Dr. Markus Mohrs (Trudeau Institute). TCRβ−/− mice were bred at the animal facility of the Lerner Research Institute. All animal procedures were conducted according to the guidelines of the Institutional Animal Care and Use Committee.

Cell sorting and adoptive transfer

Lymph node (LN) naïve CD4 T cells were obtained as previously reported (14). CD25neg CD44low naïve T cells were further sorted using an FACSaria cell sorter (BD Biosciences, San Jose, CA). A total of 2.5 × 10^5 naïve T cells was transferred alone or in combination with sorted γδ T cells to TCRβ−/− or TCRβδ−/− mice. After T cell transfer, mice were weighed weekly and monitored for signs of disease.

FACS analysis

Cells were stained with anti-CD4 (RM4-5), anti-γδ TCR (GL3), anti–IL-17A (XMG1.2), anti–IFN-γ (XMG1.2), anti-Thy1.1 (HIS51), anti-CD45.1 (A20), and anti-CCR6 (140706; BD Pharmingen, San Diego, CA). Cells were acquired using an FACScalibur or LSR II (BD Biosciences) and analyzed using FlowJo software (Tree Star, Ashland, OR).

Ex vivo stimulation

Tissue cells were ex vivo stimulated with PMA (10 ng/ml) and ionomycin (1 μM) for 4 h in the presence of 2 μM monensin (Calbiochem, San Diego, CA) during the last 2 h. Cells were immediately fixed with 4% paraformaldehyde, permeabilized, and stained with fluorescence-conjugated Abs. Isotype-matched control Ab was used for a staining control.

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Received for publication December 13, 2010. Accepted for publication February 23, 2011.

This work was supported by National Institutes of Health Grant AI074932 (to B.M.).

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The online version of this article contains supplemental material.

Abbreviations used in this article: DC, dendritic cell; IBD, inflammatory bowel disease; LN, lymph node; Treg, regulatory T cell.

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Results and Discussion

**TCRβ⁻/⁻** mice are highly susceptible but **TCRβδ⁻/⁻** mice are fully resistant to CD4-mediated colitis

To examine a role of γδ T cells in a CD4-mediated colitis model, naive CD4 cells were transferred into TCRβ⁻/⁻ and TCRβδ⁻/⁻ mice. TCRβ⁻/⁻ recipients developed severe weight loss with a massive cellular infiltration in the colon tissues, whereas TCRβδ⁻/⁻ recipients displayed no sign of weight loss only with mild inflammation in the colon (Fig. 1A–C). Consistent with the disease severity and histopathology, donor T cell accumulation in the lymphoid and gut tissues was markedly elevated in TCRβ⁻/⁻ compared with TCRβδ⁻/⁻ mice (data not shown). A dramatic increase in CD4 T cells that produce IL-17 was noticed in TCRβ⁻/⁻ recipients (Fig. 1D). Moreover, CD4 T cells producing IL-17F were also higher in TCRβ⁻/⁻ than in TCRβδ⁻/⁻ recipients (data not shown). Although the proportion of IFN-γ⁺ CD4 T cells was found to be very high in resistant TCRβδ⁻/⁻ mice (Supplemental Fig. 1), the absolute numbers of IFN-γ⁺-producing CD4 T cells was much higher in colitic TCRβ⁻/⁻ mice (data not shown). Therefore, severe colitis seen in TCRβ⁻/⁻ mice is associated with enhanced Th17 differentiation as reported in IBD patients (16), strongly suggesting that presence of γδ T cells in TCRβ⁻/⁻ mice may promote Th17 differentiation and colitogenicity of γδ T cells in TCRβ⁻/⁻ mice.

**Statistical analysis**

Results represent the mean ± SD. Statistical significance was determined by the Student t test (unpaired two-tailed) using the Prism 4 software (GraphPad, La Jolla, CA). A p value < 0.05 was considered statistically significant.

![Image](http://www.jimmunol.org/)

**FIGURE 1.** TCRβ⁻/⁻ recipients of naive CD4 T cells develop exacerbated colitis, whereas TCRβδ⁻/⁻ recipients are fully resistant to the colitis, and adoptive transfer of γδ T cells restores colitis in TCRβδ⁻/⁻ recipients. A, FACSPurified CD4⁺CD25⁻ CD44low Thy1.1 naive T cells (2.5 × 10⁵) were transferred into TCRβ⁻/⁻ or TCRβδ⁻/⁻ mice. In some experiments, FACSPurified γδ T cells (2.5 × 10⁵) were cotransferred with CD4 T cells into TCRβδ⁻/⁻ mice. Body weight was weekly monitored and presented as a percentage of the initial weight at day 0. Data shown are from five to eight individually tested mice. B, Colitis score of each group. C, Representative H&E staining of colon 5 wk posttransfer. Original magnification ×20. D, Total donor cell recovery of IL-17⁺ cells was calculated. Each symbol represents individually tested mouse. *p < 0.05, **p < 0.01, ***p < 0.001. LPL, lamina propria lymphocytes; mLN, mesenteric LN.

**FIGURE 2.** Increased IL-17⁺ γδ T cells after CD4 T cell transfer. TCRβ⁻/⁻ mice were transferred with naive CD4 T cells as described above. Spleen and LN cells were harvested every week posttransfer and ex vivo stimulated, and γδ T cell cytokine expression was examined. The results shown represent the mean ± SD of four to five individually tested mice. *p < 0.05, **p < 0.01.
CD4 T cells. Of note, T cell-deficient mice, particularly TCRβ−/−, are known to spontaneously develop colitis starting at ~4 mo of age (17). However, both strains of mice used in these experiments were ~2 mo of age and continuously gained weight without T cell transfer (Fig. 1A), demonstrating that the spontaneous colitis is not involved.

γδ T cell cotransfer renders TCRβδ−/− recipients susceptible to colitis

To directly test if γδ T cells are responsible for the enhanced pathology seen in TCRβδ−/− mice, purified γδ T cells were cotransferred with CD4 T cells into TCRβδ−/− recipients. γδ T cell cotransfer rendered resistant TCRβδ−/− mice highly susceptible to colitis, thus the recipients developed weight loss (Fig. 1A) and severe colitis (Fig. 1B, 1C). Donor CD4 T cell accumulation in the lymphoid and gut tissues was dramatically elevated to a level seen in TCRβδ−/− recipients (data not shown). Furthermore, CD4 T cells producing IL-17 significantly increased in all tested tissues after γδ T cell cotransfer (Fig. 1D, Supplemental Fig. 1), strongly supporting the hypothesis that Th17 differentiation and colitogenicity of CD4 T cells is greatly enhanced by γδ T cells in vivo. γδ T cell transfer alone without CD4 T cells did not result in any noticeable weight loss (data not shown), further supporting the notion that γδ T cells amplify Th17 differentiation, aggravating colitis. Notably, the onset of weight loss in TCRβδ−/− recipients was substantially faster compared with that in TCRβδ−/− mice receiving γδ T cells (Fig. 1A), which is probably attributed to high levels of γδ T cells generated in TCRβδ−/− mice (17).

IL-17+ but not IL-17− γδ T cells promote T cell-mediated colitis

To examine a mechanism underlying γδ T cell-mediated Th17 differentiation, we next measured γδ T cell cytokine production in TCRβδ−/− mice before and after CD4 T cell transfer. Interestingly, γδ T cells that produce IL-17 significantly increased following T cell transfer (Fig. 2). The increase was specific for IL-17+ γδ T cell subsets because IFN-γ− γδ T cells remained unchanged. Moreover, a noticeable increase was observed even 2 wk after transfer, suggesting that IL-17+ γδ T cells may play a role in altering the inflammatory responses elicited by T cells.

Phenotypic dichotomy associated with IFN-γ− or IL-17− producing γδ T cells has been recently reported: IFN-γ−-producing γδ T cells are CD27+CCR6−, and IL-17−-producing γδ T cells are CD27−CCR6+ (18, 19). When CCR6+ and CCR6− γδ T cells were sorted and examined for gene expression, the expression of il17a and il17f genes was primarily associated with CCR6+ γδ T cells, whereas ifng expression was primarily found in CCR6− γδ T cells (Supplemental Fig. 2). To test if IL-17−-producing and IFN-γ−-producing γδ T cells play different roles in T cell differentiation and colitis induction, FACS-sorted CCR6+ or CCR6− γδ T cells were transferred into TCRβδ−/− recipients with CD4 T cells. Cotransfer of CCR6+ (i.e., IL-17+) γδ T cells resulted in a significant weight loss (Fig. 3A). By stark contrast, weight loss resulting from CCR6− γδ T cell cotransfer was relatively mild (Fig. 3A). Colitis and CD4+ cell infiltration was dramatically pronounced in mice that received CD4+ plus CCR6+ γδ T cells compared with recipients of CD4+ plus CCR6− γδ T cells or CD4 T cells alone (Fig. 3B, 3C). Notably, a difference in relative weight loss in CCR6+ γδ T cell recipients...
compared with the recipients of CD4 T cells alone was already noticeable 21 d posttransfer (Fig. 3A). Moreover, the cellular infiltration in the colon of CCR6+ γδ T cell recipients was often extended into the outer muscular layer of the colon (Fig. 3B), suggesting more severe colitis after transfer of γδ T cells enriched for IL-17-producing cells. Importantly, both γδ T cell subsets were equally accumulated in the lamina propria of immunodeficient recipients when measured 5 wk posttransfer (data not shown), suggesting that enhanced susceptibility of CCR6+ γδ T cell recipients is not attributed to differential migration of γδ T cells in vivo. Consistent with colitis, IL-17 expression in CD4 T cells was significantly elevated in mice that received CCR6+ γδ compared with those that received CCR6− γδ T cells (Fig. 3D, 3E), whereas IFN-γ expression was comparable in all tested groups (data not shown). Moreover, the absolute numbers of IL-17+ CD4 T cells were significantly greater in CD4/CCR6+ γδ T cell recipients than those in CD4/CCR6− γδ T cell or CD4 T cell recipients (data not shown). Therefore, the presence of IL-17+ γδ T cells greatly enhanced Th17 differentiation and subsequent development of T cell-mediated colitis.

Even though CCR6 expression marks IL-17+ γδ T cells, γδ T cells may acquire or lose IL-17−producing potentials after transfer into TCRβ/−/− recipients, where they undergo homeostatic proliferation (20). To verify this possibility, purified CCR6+ and CCR6− γδ T cells were separately transferred into Rag−/− mice, allowed to proliferate, and examined for cytokine expression 2 wk after transfer. Surprisingly, CCR6+ γδ T cells remained IL-17+ cells, whereas CCR6− γδ T cells failed to convert into IL-17+ cells and remain IFN-γ-producing cells (Fig. 4). Furthermore, gene expression profiles measured by quantitative PCR remained unchanged (data not shown). Therefore, IL-17−/IFN-γ−producing γδ T cell subsets are not reversible in vivo even during homeostatic proliferation; thus, enhanced Th17 differentiation and colitis seen in CCR6+ γδ T cell recipients appears to be strongly associated with IL-17 produced by γδ T cells.

How IL-17−producing γδ T cells play such proinflammatory roles? Inflammatory IL-6/IL-23−producing dendritic cells (DCs) support Th17 immunity and T cell-mediated colitis (21). Because IL-17 can induce those cytokines from DCs (22), it is possible that IL-17 produced by γδ T cells may induce factors that favor Th17 differentiation from DCs. Although IL-17−/− (or IL-17F−/−) CD4 T cells were shown to equally induce colitis (23, 24), the fact that retinoic acid–related orphan receptor γt/−− CD4 T cells fail to induce Th17 immunity and colitis strongly suggests both the importance of IL-17 and the redundancy between IL-17 and IL-17F in inducing colitis. Because IL-17−producing γδ T cells also express IL-17F (data not shown), it will be important to examine if IL-17 and IL-17F derived from γδ T cells play redundant (or synergistic) roles in supporting Th17 differentiation and colitis. We are currently investigating this issue.

γδ T cell functions are highly divergent in vivo. γδ T cells mediate a regulatory effect in preventing generation of autoantigen–specific Th17 CD4 T cells in an autoimmune uveitis model (25). In contrast, γδ T cells promote the generation of myelin–specific Th17 differentiation, exacerbating the development of experimental autoimmune encephalomyelitis (26). In the context of intestinal inflammation, intestinal resident γδ T cells have been proposed to play exacerbating roles in colitis developed in TCRβ−/− mice (27). It was recently reported that γδ T cells may augment autoimmune responses by suppressing regulatory T cell (Treg) functions as well as preventing the generation of inducible Treg cells (26). Whether γδ T cell–mediated suppression of Treg functions plays a role in the current model system remains to be determined.

Collectively, our results suggest that IL-17+ γδ T cells promote in vivo Th17 differentiation and may contribute to subsequent development of intestinal inflammation. IL-17+ γδ T cell subsets may represent a potential target for treating chronic inflammatory disorders associated with Th17 effector cells such as IBD.

Acknowledgments
We thank Dr. Nina Volokh and Jennifer Powers for technical assistance in carrying out immunohistochemistry and for cell sorting, respectively.

Disclosures
The authors have no financial conflicts of interest.

References


