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Th17 Cells Induce Colitis and Promote Th1 Cell Responses through IL-17 Induction of Innate IL-12 and IL-23 Production

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Both Th1 and Th17 cells have been implicated in the pathogenesis of inflammatory bowel disease and experimental colitis. However, the complex relationship between Th1 and Th17 cells and their relative contributions to the pathogenesis of inflammatory bowel disease have not been completely analyzed. Although it has been recently shown that Th17 cells can convert into Th1 cells, the underlying in vivo mechanisms and the role of Th1 cells converted from Th17 cells in the pathogenesis of colitis are still largely unknown. In this study, we report that Th17 cells from CBir1 TCR transgenic mice, which are specific for an immunodominant microbiota Ag, are more potent than Th1 cells in the induction of colitis, as Th17 cells induced severe colitis, whereas Th1 cells induced mild colitis when transferred into TCRβδ−/− mice. High levels of IL-12 and IL-23 and substantial numbers of IFN-γ+ Th1 cells emerged in the colons of Th17 cell recipients. Administration of anti–IL-17 mAb abrogated Th17 cell-induced colitis development, blocked colonic IL-12 and IL-23 production, and inhibited IFN-γ+ Th1 cell induction. IL-17 promoted dendritic cell production of IL-12 and IL-23. Furthermore, conditioned media from colonic tissues of colitic Th17 cell recipients induced IFN-γ production by Th17 cells, which was inhibited by blockade of IL-12 and IL-23. Collectively, these data indicate that Th17 cells convert to Th1 cells through IL-17 induction of mucosal innate IL-12 and IL-23 production. The Journal of Immunology, 2011, 186: 6313–6318.

Both Th1 cells, which produce IFN-γ, and Th17 cells, which produce IL-17 (IL-17A), IL-17F, IL-21, and IL-22, have been implicated as important mediators of inflammatory bowel disease (IBD) (1–5). It has been shown that IL-12 stimulates Th1 cell differentiation (6), whereas IL-6, TGF-β, and IL-23 promote Th17 cell development (7). There is increased production of IL-12 and IL-23 in the lesions of Crohn’s disease, and mesenteric lymph node (MLN) dendritic cells (DCs) from patients with Crohn’s disease induce both Th1 and Th17 immune responses (8–12). DCs from Crohn’s disease lesions express high levels of activated STAT4 and T-bet, the Th1-associated transcription factors indicative of IL-12 signaling (13, 14). The important role of Th17 cells, which express the IL-23 receptor on their surface, in the pathogenesis of IBD is supported by recent genome-wide association studies indicating that IL-23R and other genes involved in Th17 cell differentiation are associated with susceptibility to Crohn’s disease and ulcerative colitis (15–18). Anti–IL-12/IL-23p40 Ab therapy, which targets both Th1 and Th17 cells, is effective in Crohn’s disease (19, 20). Data from our own studies demonstrate that anti–IL-23p19 mAb prevents, as well as treats, colitis in an experimental model induced by adoptive transfer of microbiota Ag-specific T cells, further confirming a role for the IL-23/Th17 pathway in the pathogenesis of chronic intestinal inflammation (5). However, in patients with Crohn’s disease, a unique subset of CD14+ macrophages has been identified that contributes to the pathogenesis of Crohn’s disease by promoting IL-23–dependent IFN-γ production rather than IL-17 production by lamina propria (LP) mononuclear cells (21). Significant IL-17 mRNA upregulation is found in LP CD4+ T cells from patients with ulcerative colitis, while IFN-γ levels are increased in Crohn’s disease. These data argue somewhat against the concept that IL-23 contributes only to Th17 cytokine production (10), and they demonstrate that IL-23 can promote Th1 cell IFN-γ production as well. A number of reports have identified a subset of Th17 cells that coproduce the Th1 cytokine IFN-γ (22, 23). This is particularly prominent at sites of inflammation such as active Crohn’s disease (22). Those reports suggest that the complex relationship between Th1 and Th17 cells in IBD remains unclear. However, it is important to delineate the specific contributions of these cells to chronic intestinal inflammation, especially in regard to the persistence and progression of colitis.

Recently, substantial developmental plasticity of the Th17 lineage has been observed in human Th17 clones derived from intestinal isolates of patients with Crohn’s disease (22). There is also considerable plasticity late in the mouse Th17 program, which allows committed Th17 cells to transition from effectors that produce predominantly IL-17 to effectors that produce predominantly IFN-γ in a process driven by IL-12 and IL-23 via a STAT4- and T-bet–dependent manner (24–27). These elegant...
studies reveal a mechanism for the latent Th1-like responsiveness of Th17 cells, and they provide a basis for understanding the relationship between Th17- and Th1-mediated pathophysiology. However, many of the data defining Th17 cell conversion to Th1 cells are derived from in vitro studies. Whether IL-12 and IL-23 mediate Th17 cell conversion to Th1 cells in vivo, and, if so, where and how IL-12 and IL-23 are induced in vivo in the first place remain unknown. In this study, we demonstrate that Th17 cells from CBir1 TCR transgenic mice, which are specific for an immunodominant microbiota flagellin, induced colitis in TCRβδ−/− recipient mice. Furthermore, Th17 cells promoted Th1 cell development through IL-17 induction of mucosal IL-12 and IL-23 in inflamed colonic tissues.

Materials and Methods

Mice
C57BL/6 (B6) and B6.TCRβδ−/− (TCRβδ−/−) mice were obtained from The Jackson Laboratory. B6.CBir1 TCR transgenic (CBir1 Tg) mice (28) were generated and bred in the Animal Facility at the University of Alabama at Birmingham. All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Abs and reagents
Recombinant IL-6, TGF-B1, and IL-12 were from R&D Systems. Abs against IL-17 and IFN-γ, as well as anti-CD4-magnetic beads, were from BD Biosciences.

Generation of bone marrow-derived DCs
Bone marrow cells were isolated as described previously (29). Briefly, bone marrow cells were resuspended in complete RPMI 1640 media containing 10% heat-inactivated FBS (Atlanta Biologicals), 25 mM HEPES buffer, 2 mM sodium pyruvate, 50 mM 2-ME, 100 IU/ml penicillin, and 100 µg/ml streptomycin (Cellgro; Mediatech) and cultured in the presence of 20 ng/ml GM-CSF and 10% heat-inactivated FBS (Atlanta Biologicals) at 37°C. On day 8, bone marrow-derived DCs (BMDCs) were harvested and plated at 1 × 106/ml/well in 24-well plates in the presence or absence of 100 ng/ml recombinant IL-17 (R&D Systems).

Isolation of CD4+ T cells
CD4+ T cells were isolated using anti-mouse CD4 magnetic beads as previously described (30). Briefly, splenic cells were washed twice and incubated with anti-CD4 magnetic beads at 4°C for 30 min and then separated by magnetic field. When checked by flow cytometry, >95% of the cells were CD4+ T cells.

Preparation of LP lymphocytes
LP lymphocytes were isolated as previously described (31). Briefly, for removal of epithelial cells and intraepithelial lymphocytes, the intestines were washed, cut into small pieces, and then the pieces were incubated with calcium- and magnesium-free HBSS supplemented with 2% FBS and 5 mM EDTA (Sigma-Aldrich) on a magnetic stirrer at 37°C for 30 min. The tissues were then incubated with RPMI 1640 containing 5% FBS and 0.5 mg/ml collagenase type IV (Sigma-Aldrich) for 30 min at 37°C with stirring. The liberated cells were collected by passage through a stainless steel sieve. The isolated cells were pooled together and separated on a 40/75% discontinuous Percoll gradient (Amersham Pharmacia Biotech). The cell yield was typically ~2 × 106 lymphocytes per mouse with >90% cell viability.

Flow cytometry
As described previously (28), cells were stimulated for 5 h with PMA (50 ng/ml) and ionomycin (750 ng/ml), with monensin added for the last 3 h culture. The cells were fixed and permeabilized using Cytofix/Cytoperm solution (BD Pharmingen). Staining was performed for IL-17 and IFN-γ using fluorescence-conjugated Abs, and the cells were analyzed on a FACSCalibur flow cytometer (BD Biosciences) using FlowJo software (Tree Star).

Ex vivo organ culture
Ex vivo organ fragments of colon and small intestine were performed by removing and longitudinally opening the small and large intestines. After washing with cold RPMI 1640 three times, three 3-mm circular full-thickness pieces of small intestine and colon were obtained using a 3-mm dermal punch (Baker-Cummins). Each fragment was then placed in 0.5 ml complete RPMI 1640 in separate wells of a 48-well plate and incubated for 24 h at 37°C in humid air with 5% CO2. Culture supernatants from each biopsy were collected and stored at −80°C before analysis for cytokine content.

Statistical analysis
For comparisons between samples, levels of significance were determined by a Student t test in Prism 5.0 (GraphPad Software). Where appropriate, means ± SEM are represented on graphs. The p values are indicated as follows: *p < 0.05, **p < 0.01.

Results

Naive CBir1 Tg T cells differentiate into both Th1 and Th17 cells in the intestine during the induction of colitis in TCRβδ−/− mice
We have previously shown that microbiota Ag-specific CD4+ T cells from CBir1 Tg mice (28) that are specific for CBir1 flagellin, an immunodominant commensal Ag (32), induced colitis in immunodeficient mice (33, 34). With the progression of colitis, IFN-γ-γ T cells, IL-17+ T cells, IFN-γ-IL-17+ T cells, as well as Foxp3+ T cells, Foxp3+IFN-γ+ T cells, Foxp3+IL-17+ T cells, and Foxp3+IFN-γ-IL-17+ T cells, emerged in the intestinal lamina propria of recipient mice (33). To investigate Th1 and Th17 cell differentiation of microbiota Ag-specific CD4+ T cells in the intestines.

FIGURE 1. Naive CBir1 Tg mice were injected with PBS as control. The recipients were sacrificed at different time points. A. LP CD4+ T cell intracellular IFN-γ and IL-17 production was determined by flow cytometry. Plot numbers represent the percentage of CD4+ T cells in the respective quadrants. B. Absolute numbers of IFN-γ- and IL-17+ CD4+ T cells in MLN and LP. C. Aggregate data of percentages of LP IFN-γ- and IL-17+ T cells from three independent experiments. D. Colonic histopathology was assessed at different time points after cell transfer. Pathology scores are shown. One representative of three independent experiments is shown. **p < 0.01.
intestine and their role in the pathogenesis of colitis, $1 \times 10^6$ naive CD4$^+$ T cells from CBir1 Tg mice were transferred i.v. into TCR$\beta^+\delta^-/-$ mice. Unlike SCID mice or RAG-deficient mice, which lack both T cells and B cells, TCR$\beta^+\delta^-/-$ mice only lack T cells but have a fully functional innate immune system, B cell repertoire, and NK cells. Using TCR$\beta^+\delta^-/-$ mice as recipients allows us to dissect the role of transferred T cells in colitis development in mice with a relatively intact immune system. A group of TCR$\beta^+\delta^-/-$ mice injected with PBS served as control. Five recipient mice per group were sacrificed 1, 2, 4, or 8 wk after cell transfer, and IFN-\(\gamma\) and IL-17 production by LP T cells were assessed. One week after adoptive transfer, 4.7% of transferred CBir1 Tg CD4$^+$ T cells in the LP were IFN-\(\gamma\) single-positive (Th1) and 10.7% were IL-17 single-positive (Th17). Interestingly, a significant number of transferred CBir1 Tg CD4$^+$ T cells (3.6%) expressed both IFN-\(\gamma\) and IL-17 (Th1+17 cells) (Fig. 1A). The percentages and absolute numbers of these populations increased over time (Fig. 1B, 1C). Eight weeks after cell transfer, 17.9% were IFN-\(\gamma\) single-positive, 28.3% were IL-17 single-positive, and 16.7% were IFN-\(\gamma\) and IL-17 double-positive cells (Fig. 1A, 1C).

As shown previously, the recipients developed mild colitis as early as 4 wk and severe colitis 8 wk after cell transfer (Fig. 1D). Collectively, these data demonstrate that CD4$^+$ T cells reactive to a single dominant microbiota Ag can differentiate into both Th1 and Th17 cells in the intestine and induce colitis. Large numbers of IFN-\(\gamma\) and IL-17 double-positive CD4$^+$ T cells present in the LP of colitic mice may play a role in the colitis development, as they are not found in the LP of normal control mice (data not shown).

CBir1-specific Th17 cells are more potent than Th1 cells in induction of colitis

Both Th1 and Th17 cells have been implicated in the pathogenesis of colitis. To determine the role of CBir1 flagellin-specific Th1 and Th17 cells in the induction of colitis in this model, CBir1-specific Th1 and Th17 cells were polarized in vitro by culture of CD4$^+$ T cells from CBir1 Tg mice with CBir1-pulsed APCs under standard Th1 conditions (IL-12 and anti–IL-4 mAbs) or Th17 conditions (IL-6, TGF-\(\beta\), anti–IL-4 mAbs, and anti–IFN-\(\gamma\) mAbs), respectively. Seven days later, T cell cytokine production was analyzed by intracellular staining. As expected, activation of naive CBir1 Tg CD4$^+$ T cells under Th1 conditions induced the development of a polarized Th1 phenotype with high levels of IFN-\(\gamma\) and little IL-17. Activation of naive CBir1 Tg CD4$^+$ T cells under Th17 conditions resulted in the development of a severe colitis with high levels of IL-17 and low levels of IFN-\(\gamma\) (Fig. 2A). These polarized CBir1-specific Th1 and Th17 cells were then transferred into TCR$\beta^+\delta^-/-$ mice separately. Eight weeks after cell transfer, recipient mice were sacrificed and intestinal histopathology was assessed. The recipient mice of CBir1-specific Th1 cells developed mild colitis, whereas the recipients of CBir1-specific Th17 cells developed severe colitis (Fig. 2B, 2C). There were more CD4$^+$ T cells in MLN and LP of Th17 recipient mice compared with Th1 recipients (Fig. 2D). Collectively, these data indicate that CBir1-specific Th1 and Th17 cells may function differently in the pathogenesis of colitis, and specifically, Th17 cells are more potent than Th1 cells in the induction of colitis. At present, we still do not know whether the difference in pathology is related to the differences in proliferative potential of these two different types of T cells or to the pathogenicity of their signature cytokines. Of note, despite the presence of CBir1 Tg T cells in

**FIGURE 2.** CBir1 Tg Th17 cells are more potent than Th1 cells in the induction of colitis. To determine the ability of Th17 and Th1 cells to induce colitis, CBir1 Tg Th17 and Th1 cells were polarized in vitro and transferred into TCR$\beta^+\delta^-/-$ mice under standard Th1 conditions (A) and Th17 conditions (B), and $1 \times 10^6$ Th17 and Th1 cells were transferred into groups of five TCR$\beta^+\delta^-/-$ mice separately. Eight weeks after transfer, the recipient mice were sacrificed and assessed for histopathology. Colonic histopathology (B) and pathology scores (C) are shown. H&E, original magnification \(\times 20\). **\(p < 0.01\). D, Absolute numbers of CD4$^+$ T cells in MLN and LP are shown. Data are representative of one of three independent experiments. **\(p < 0.01\).
both small and large intestinal LP, no inflammation was observed in the small intestines of recipient mice that received either Th1 or Th17 cells (data not shown). Interestingly, A4 bacteria, which produce CBir1-like flagellins (35) and activate CBir1 Tg T cells, were only detected in the cecum and colon but not in the small intestine (data not shown), indicating that the lack of inflammation in the small intestine is due to the absence of Ag-induced TCR activation, which is required for the induction of colitis.

CBir1-specific Th17 cells convert into Th1 cells in TCRβδ−/− recipient mice

Th17 cells have demonstrated substantial plasticity. However, whether their signature cytokine IL-17 regulates Th17 cell stability has not been defined, and its role in the pathogenesis of colitis is also controversial as both protective and pathogenic functions of IL-17 have been reported in different experimental models of colitis (36–38). To determine whether microbiota Ag-specific Th17 cells convert to Th1 cells in the intestine and the role of IL-17 in Th17 cell conversion and induction of colitis, Th17 cells were generated from CBir1 Tg mice in vitro (Fig. 3A) and transferred into TCRβδ−/− mice. Groups of five recipient mice were treated with anti–IL-17 or control mAbs. Eight weeks later, total CD4+ T cells in the MLN and LP and T cell cytokine production in the LP and colonic histopathology were examined. As shown in Fig. 3B, substantial CD4+ T cells accumulated in the LP of Th17 recipient mice treated with control mAb, and treatment with anti–IL-17 mAb decreased CD4+ T cell accumulation in the LP. Adoptively transferred Th17 cells in the recipient mice treated with control mAb expressed IFN-γ, and a large proportion of T cells coexpressed both IL-17 and IFN-γ in the LP. Treatment with anti–IL-17 mAb did not affect IL-17 expression, but it reduced the expression of IFN-γ in T cells (Fig. 3C). Consistently, colonic tissue of colitic Th17 recipient mice that were treated with control mAb produced high levels of IL-17 and IFN-γ (Fig. 3D).

Although production of IFN-γ was significantly inhibited by treatment with anti–IL-17 mAb, it is interesting that while there were fewer CD4+ T cells in the LP of anti–IL-17 mAb-treated recipient mice, the colonic tissues produced similar levels of IL-17 ex vivo in both groups of recipient mice (Fig. 3D). The colonic tissues contain not only Th17 cells but also many different types of cells that could produce IL-17, including recently identified IL-17-producing innate cells (39–41). This raises the possibility that transferred Th17 cells could promote IL-17 production by such innate cells. Collectively, these data indicate that IL-17 induces T cell IFN-γ production, probably through promoting Th17 cell conversion to Th1 cells. Although high levels of IL-17 have been detected in patients with Crohn’s disease, its role in the pathogenesis of IBD is still controversial. As shown in Fig. 3E and 3F, Th17 cell recipient mice treated with control mAb developed severe colitis. In contrast, anti–IL-17 mAb treatment abrogated colitis development, demonstrating a protective role of IL-17 in the development of colitis, at least in this model.

**IL-17 stimulates DC IL-12 and IL-23 production, and anti–IL-17 mAb inhibits colonic IL-12 and IL-23 production in colitic mice**

It has been shown that IL-12 and IL-23 mediate Th17 cell conversion to Th1 cells in vitro (24). To determine the role of IL-12 and IL-23 in Th17 cell conversion to Th1 cells in the intestines of CBir1-specific Th17 recipient mice, colonic IL-12 and IL-23 production was determined by ex vivo organ culture. High levels of IL-12 and IL-23 were detected in colonic tissue of control mAb-treated Th17 recipients but not of control TCRβδ−/− mice that received PBS, and treatment with anti–IL-17 mAb decreased colonic IL-12 and IL-23 production (Fig. 4A and data not shown).

**FIGURE 4.** IL-17 mediates Th17 cell induction of colonic IL-12 and IL-23 in vivo and stimulates DC IL-12 and IL-23 production in vitro. A. In vitro-polarized CBir1 Tg Th17 cells (1 × 10^6) were transferred into TCRβδ−/− mice that were administered with anti–IL-17 mAb or control mAb at the time of transfer and weekly thereafter for 8 wk. Colonic tissues of Th17 recipients were cultured for 24 h, and IL-12 and IL-23 production in the supernatants was measured by ELISA. Supernatants were collected 24 h later, and IL-12 and IL-23 levels were determined by ELISA. *p < 0.05, **p < 0.01 compared with IL-17–treated BMDCs. B. BMDCs were also harvested at different time points after treatment, and IL-12 and IL-23 mRNA expression was determined by RT-PCR. Data are representative of one of two independent experiments with similar results. D. Aggregate data of density from two independent experiments.

**FIGURE 5.** CM from colonic tissues of colitic Th17 recipients induce Th17 cell conversion to Th1 cells through IL-12 and IL-23 production. A. Colonic tissues of colitic Th17 cell recipient mice were cultured in complete RPMI 1640, and the culture supernatants were collected after 24 h to serve as CM. In vitro-polarized CBir1 Tg Th17 cells were restimulated with CBir1 flagellin-pulsed APCs in the absence or presence of 20% colonic CM with anti–IL-12, anti–IL-23, or both. Five days later, IL-17 and IFN-γ production was assessed by intracellular staining. One representative of three independent experiments is shown. B. Aggregate data of percentages of cytokine-expressing T cells from three independent experiments. *p < 0.05, **p < 0.01 compared with CM-treated group.
indicating that Th17 cells induce mucosal IL-12 and IL-23 production, likely through IL-17.

IL-12 and IL-23 are produced mainly by activated APCs, including DCs. To determine the effect of IL-17 on DC IL-12 and IL-23 production, BMDCs were treated with IL-17. Supernatant was collected after 24 h culture, and the cells were harvested at different time points. IL-12 and IL-23 protein and mRNA expression were determined by ELISA and RT-PCR, respectively. As shown in Fig. 4B, IL-17 induced BMDC IL-12 and IL-23 protein production. Interestingly, IL-17 differentially stimulated IL-12 and IL-23 mRNA expression: IL-23 mRNA was induced at early time points, peaked at 8 h, and diminished at later time points, whereas expression of IL-12 mRNA occurred later, having peaked at 16–24 h (Fig. 4C, 4D). These data are consistent with a recent report showing that IL-17 induced DC IL-12 production and promoted T cell IFN-γ production (42).

**Conditioned media from colons of colitic Th17 recipients induce Th17 cell conversion to Th1 cells**

To determine the role of colonic IL-12 and IL-23 from colitic Th17 recipients in promoting Th17 cell conversion to Th1 cells in the intestine, colonic tissues of Th17 cell recipient mice were cultured with complete RPMI 1640, and the culture supernatants were collected after 24 h to serve as conditioned media (CM). Culture supernatants from colonic tissues of TCRβδ−/− mice that were given PBS were also collected and served as control. In vitro-polarized CBIR1-specific Th17 cells were restimulated with CBIR1 flagellin-pulsed APCs in the absence or presence of 20% colonic CM. Five days later, CD4+ T cell IL-17 and IFN-γ production was assessed by intracellular staining. As shown in Fig. 5, addition of colonic CM from colitic Th17 recipients promoted in vitro-polarized Th17 cell IFN-γ production, whereas control CM did not affect Th17 cell IFN-γ production (data not shown). Blockade of IL-12 or IL-23 greatly reduced IFN-γ production by Th17 cells, and IFN-γ production was further inhibited by blocking both IL-12 and IL-23 (Fig. 5). Taken together, these data demonstrate that IL-12 and IL-23 are required in order for colonic CM from colitic Th17 recipients to promote IFN-γ production by Th17 cells.

**Discussion**

Although accumulating evidence indicates important roles of Th17 cells in chronic intestinal inflammatory conditions, there is considerable controversy as to whether their signature cytokine IL-17 is essential in the pathogenesis of colitis. Our data show that Th17 cells, specific for a single microbiota Ag, induce colitis. IL-17 plays a crucial role in the pathogenesis of chronic intestinal inflammation. Importantly, IL-17 promoted IFN-γ production by T cells in the LP of colitic Th17 cell recipient mice through induction of colonic DC IL-12 and IL-23 production.

Both protective and pathogenic functions of IL-17 have been reported in different experimental models of colitis (36–38). IL-17 and/or IL-17F deficiency did not prevent colitis mediated by transfer of regulatory T cell-depleted CD4+ T cells (43, 44). Adoptive transfer of IL-17−/− CD45RBβ− T cells, compared with wild-type counterparts, induced a more severe wasting disease when transferred into RAG−/− mice (37). In contrast, IL-17 deficiency resulted in resistance to dextran sulfate sodium-induced colitis, indicating a pathogenic role of IL-17 in intestinal inflammation (38). Moreover, recent genetic studies have shown that IL-23 and its receptor (IL-23R), as well as RORγt, which are essential for the maintenance and differentiation of Th17 cells, are required for IBD (45, 46). Interestingly, it has also been shown that polymorphisms in the IL-23R gene are strongly associated with either protection from or susceptibility to Crohn’s disease (18, 47). In our present study, transfer of CBIR1-specific Th17 cells induced colitis in TCRβδ−/− mice, and neutralizing IL-17 inhibited colitis development (Figs. 2, 3), indicating a proinflammatory role of Th17 cells and their signature cytokine IL-17 at least in this model. These data, although conflicting, may actually indicate a dual role of Th17/IL-17 in homeostatic and inflammatory settings: Th17/IL-17 is normally involved in the maintenance of intestinal homeostasis by protecting the host from inflammation through induction of antimicrobial peptides (48, 49); however, under certain circumstances, Th17/IL-17 is involved in the pathogenesis of intestinal inflammation by recruiting neutrophils and stimulating production of various proinflammatory cytokines (50, 51).

IL-12 and IL-23 have been shown to promote Th17 cell conversion to Th1 cells via a STAT4- and T-bet–dependent manner in vitro (24–27). However, whereas how IL-12 and IL-23 are induced in vivo in the first place are still undefined. Our data demonstrate that colonic tissues of colitic Th17 recipient mice produced high levels of IL-12 and IL-23 (Fig. 4). CM of such colonic tissues promoted Th17 cell conversion to Th1 cells through IL-12 and IL-23, as blockade of CM IL-12 and IL-23 inhibited IFN-γ production by Th17 cells (Fig. 5). Intriguingly, IL-17 produced by Th17 cells stimulated DC IL-12 and IL-23 production, and neutralizing IL-17 by administration of anti–IL-17 mAb inhibited colonic IL-12 and IL-23 production as well as IFN-γ production by T cells in Th17 recipient mice (Fig. 4). These data provide direct in vivo evidence that Th17 cells can promote their own conversion to IFN-γ-producing Th1 cells by induction of local DC IL-12 and IL-23 production. This is consistent with a recent report that IL-17 is required for Th1 immunity against the intracellular pathogen Francisella tularensis (42). Notably, anti-IL-17 reduced the absolute numbers of single IFN-γ-producing cells (Fig. 3C); however, the reduction of IFN-γ was not complete. This suggests that other mechanisms by which Th17 cells convert to Th1 cells may exist. Among many possibilities, IL-17F, IL-21, or IL-22 could be most likely involved in such process. Although it is still unclear whether Th17 cells act similarly in patients with IBD, recent reports support the notion that Th17 cells in IBD patients may convert to IFN-γ-producing T cells via induction of mucosal DC IL-12 and IL-23 production, as there is substantial developmental plasticity in human Th17 clones derived from intestinal isolates of patients with Crohn’s disease (22), and MLN DCs from Crohn’s disease patients induce both Th1 and Th17 immune responses, probably through production of IL-12 and IL-23 (8–12). Th17 cell conversion to Th1 cells could provide the pathogenic effect of both T cell subsets for the induction of intestinal inflammation. This can be at least one mechanism for Th17 cells to be more potent than Th1 cells in the induction of colitis.

**Disclosures**

The authors have no financial conflicts of interest.

**References**

Th17/IL-17 INDUCTION OF COLITIS AND Th1 RESPONSES

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