functionally regulates Mϕs towards an anti-inflammatory phenotype (Kamada et al., 2005). These abnormal responses of intestinal Mϕ subsets to enteric bacteria in IL-$10^{-/-}$ mice may contribute to a Th1 cytokine bias and the development of intestinal inflammation. Interestingly, whole bacteria are a potent inducer of IL-12p70 production by Mϕs. Significant repression of IL-12p70 production was achieved by inhibition of phagocytosis, suggesting that intracellular pathogen recognition and signaling are involved in the induction of IL-12p70 in IL-$10^{-/-}$ Mϕs (Naruse et al., 2011). Also of note, histidine, which is one of the most common natural amino acids inhibited LPS-induced TNF-α and IL-6 production by IL-$10^{-/-}$ Mϕs, and dietary histidine ameliorated colitis in an IL-$10^{-/-}$ mouse transfer colitis model (Andou et al., 2009). Indeed, amino acid profiling has revealed decreased levels of plasma histidine concentration in patients with active Crohn's disease (Hisamatsu et al., 2012).

To date, many kinds of IBD animal models have been reported, but none of these completely reflect the characteristics of human IBD pathophysiology. Granuloma formation is the one of the most important pathological characteristics of human Crohn's disease. Mizoguchi et al. demonstrated that F4/80-positive immature CD11c^{+} DCs produce IL-23 and contribute to granuloma formation in a murine colitis model (Mizoguchi et al., 2007).

A Th1 immune response is predominant in Crohn's disease. Indeed, CD4^{+} T cells in the LP of Crohn's disease expressed T-bet and produced large amounts of interferon (IFN)-γ (Matsuoka et al., 2004). Sakuraba et al. demonstrated that DCs in the mesentric lymph nodes of patients with Crohn's disease strongly promoted a Th1 and Th17 immune response (Sakuraba et al., 2009). Not only mesentric lymph node DCs, but also LP-Mϕs, contribute to IBD pathogenesis, particularly that of Crohn's disease. The contribution of intestinal Mϕs (that express TREM-1 (triggering receptor expressed on myeloid cells 1)) to disease pathogenesis in a murine experimental colitis model, and patients with IBD, has been reported (Schenk et al., 2007). LP-Mϕs produce large amounts of IL-18 and promote Th1 immune responses in Crohn's disease (Kanai et al., 2001). Kamada et al. observed the infiltration of unique CD14^{+} intestinal Mϕs (CD14^{+}Mϕs) in the mucosa of Crohn's disease patients (Kamada et al., 2008). This subset expressed both Mϕ (CD14, CD33, CD68) and DC (CD205, CD209) markers and produced larger amounts of pro-inflammatory cytokines, such as IL-23, TNF-α, and IL-6, than typical intestinal resident Mϕs. Mϕ-derived IL-23 strongly enhances IFN-γ production by these cells in Crohn's disease (Kamada et al., 2008) suggesting that Mϕ-derived IL-23 is a key cytokine for the predominance of Th1 responses in Crohn's disease. CD14^{+} Mϕs also have antigen presenting functions and can stimulate the differentiation and proliferation of naïve CD4^{+} T cells obtained from peripheral blood (Kamada et al., 2009). Importantly, although in vitro differentiated DCs cannot induce the differentiation of peripheral blood naïve CD4^{+} T cells to Th17 cells, LP-CD14^{+}Mϕs from Crohn's disease patients can strongly promote T cell differentiation to both Th1 and Th17 cells in response to stimulation by whole bacteria (Kamada et al., 2009). TL1A/TNFSF15, a member of the TNF superfamily, was identified as a susceptibility gene for Crohn's disease, particularly in Japanese patients (Yamazaki et al., 2005; Cho, 2008). CD14^{+} Mϕs in Crohn's disease express membrane-boundTL1A/TNFSF15 that acts synergistically with IL-23 to promote the production of IFN-γ and IL-17 by LPMCs (Kamada et al., 2010).Thus, CD14^{+} Mϕ plays a central role in the promotion of an inflammatory cytokine network at mucosal sites of Crohn's disease (Fig. 5).

5.3. NKT and ILC (mucosal NK and LTi)

NKT cells are also a distinct subset of mucosal immune cells and have relationship to IBD. NKT cells respond rapidly to antigens presented on CD1d of APCs and secrete various cytokines, such as IL-13. Oxazolone-induced colitis mice are a Th2-dominant colitis model representing human ulcerative colitis (Boirivant et al., 1998). IL-13 is a key cytokine in the pathogenesis of oxazolone-induced colitis, as shown by the inflammation being prevented by the administration of an IL-13 inhibitor, IL-13Rα2-Fc (Heller et al., 2002). Mucosal NKT cells are the main IL-13-producing cells in oxazolone-induced colitis and drive Th2 inflammation. Importantly, increased numbers of non-classical NKT cells in the LP of ulcerative colitis produced large amounts of IL-13 (Fuss et al., 2004). IL-13 may play a role in the pathogenesis of ulcerative colitis by inducing epithelial cell damage (Kawashima et al., 2006, 2011).

ILC is an emerging category which includes NK cells and lymphoid tissue-inducer (LTi) cells and type 2 helper cells. These ILCs share the transcriptional repressor *Id2* and cytokine signals of IL-2. NK cells are now classified as IFN-γ producing ILCs and play an important role in systemic immune surveillance to protect hosts from neoplasms and infections. NK cells can rapidly detect and dispose of target cells such as tumor cells and infected cells. NK cells also contribute to immunity by producing several types of cytokines such as IFN-γ and TNF-α. Recently, mucosal NK cells were shown to be involved in mucosal homeostasis (Di Santo et al., 2010; Shi et al., 2011). Chinen et al. had previously identified that numbers of mucosal NK cells were increased in the LP of Crohn's disease (Chinen et al., 2007). Furthermore, increased numbers of c-kit^{+} lineage markers (lin)$^{-}$ cells were also observed in human adult intestine. These intestinal immune precursors expressed CD34, CD38, CD33, IL-2Rα, and IL-7Rα. The lin^{-} c-kit^{+} precursors mainly differentiated to CD56^{+} c-kit^{dim} cells in vitro, corresponding to intestinal NK cells, which are clearly distinguished from peripheral blood NK cells by expression patterns of surface molecules and cytokine production. These cells produced higher amounts of IFN-γ, while their cytotoxic activity was relatively low. Interestingly, both c-kit^{dim} cells and NK cells were increased in the inflamed mucosa of Crohn's disease (Chinen et al., 2007). Later, these findings were confirmed by the evidences that Lin^{-} CD45^{int}CD127^{hi}*RORC*$^{+}$LTi cells produce IL-17 and IL-22 and generate *RORC*$^{+}$ CD127^{hi} NK cells which retain the production of IL-17 and IL-22 (Cupedo et al., 2009).Further analysis showed that human intestinal mucosal CD3^{-}CD56^{+}NK cells could be classified into two subpopulations: NKp44^{+}NKp46^{-} (NKp44^{+}) and NKp44^{-} NKp46^{+} (NKp46^{+}) cells. In contrast to recent studies in mice, NKp46^{+} cells expressed RORγt and produced IL-22 (Satoh-Takayama et al., 2008), whereas human intestinal NKp46^{+} did not express RORC and produced higher amounts of IFN-γ. Importantly, the balance of NKp44^{+}/NKp46^{+} cells was disrupted in the intestinal mucosa of patients with Crohn's disease, where IFN-γ producingNKp46^{+} cells (conventional NK (cNK) cells) were dominant and IL-22 producing NKp44^{+} cells (ILC22 or NK22) are decreased (Takayama et al., 2010). These findings suggest that T cells and mucosal IFN-γ-producing cNK cells may contribute to excessive Th1 immune responses and the pathogenesis of Crohn's disease.

LTi cells are now being classified as RORγt dependent ILCs and several ILC populations have been implicated in promoting innate immunity and intestinal inflammation via production of host protective and/or inflammatory cytokines (Eberl et al., 2004; Satoh-Takayama et al., 2008; Cella et al., 2009; Colonna, 2009; Buonocore et al., 2010; Cua & Tato, 2010; Sawa et al., 2010; Spits & Di Santo, 2011). LTi cells are the first hematopoietic cells recruited to the sites of secondary lymphoid tissue organogenesis during fetal development (Mebius et al., 1997).LTi cells in adult mice and humans constitutively express RORγt, which is a transcriptional factor that regulates the development of LTi cells (Eberl et al., 2004)and these cells also have the capacity to express IL-22 in vitro (Eberl & Littman, 2004; Takatori et al., 2009). Therefore, LTi cells in adults play a role not only in lymphoid tissue generation and maintenance, but also in host innate immunity. In mice infected with *Citrobacter rodentium*, CD4+ LTi cells were a dominant source of IL-22 and played a key role in promoting innate immunity in the intestine (Sonnenberg et al., 2011). Mouse ILCs produce IL-17, IL-22, and IFN-γ in response to IL-23 and this mediates innate colitis. Geremia et al. showed increased expression of IL17A and IL17F among intestinal

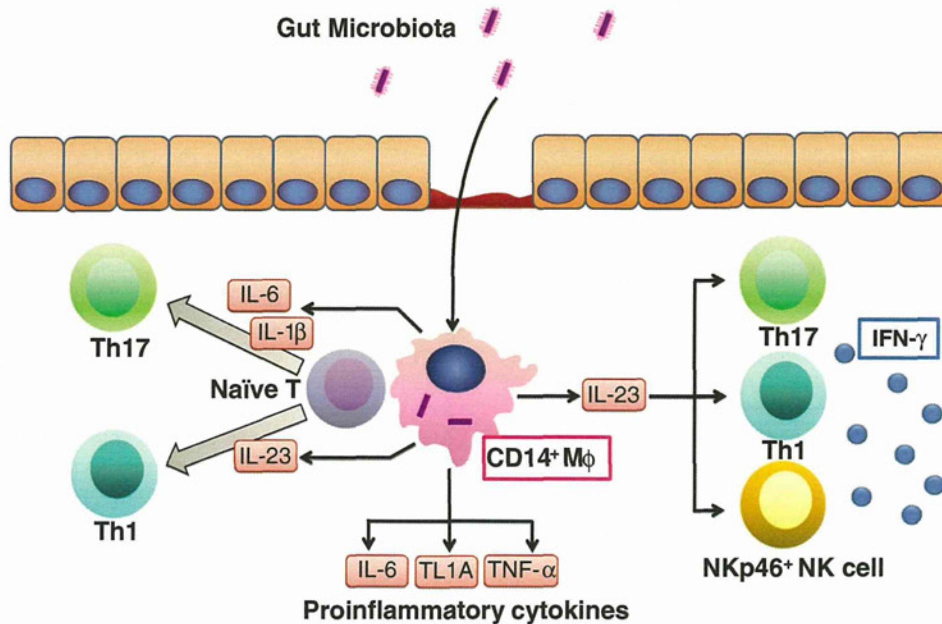

Fig. 5. Contribution of CD14+Mϕs in the pathogenesis of Crohn's disease. Lamina propria $CD14^+$ Mϕs produce large amount of TNFα, IL-23, and IL-6 resulting in induction of excess Th1 and Th17 responses. $CD14^+$ Mϕs as antigen presenting cells also promote Th1 and Th17 differentiations.

$CD3^-$ cells in IBD. IL17A and IL17F expression is restricted to $CD56^-$ ILCs, whereas IL-23 induces IL22 and IL26 in the $CD56^+$ ILC compartment. Furthermore, a significant and selective increase in $CD127^+$ $CD56^-$ ILCs in the inflamed intestine in Crohn's disease patients, but not in ulcerative colitis patients, has been observed. These results indicate that the selective accumulation of a phenotypically distinct ILC population may play a role in Crohn's disease pathogenesis (Geremia et al., 2011). ILCs also regulate selective containment of lymphoid-resident bacteria to prevent systemic inflammation associated with chronic diseases. Depletion of ILCs has been observed to result in peripheral dissemination of commensal bacteria and systemic inflammation. Disseminating bacteria were identified as *Alcaligenes* species originating from host lymphoid tissues. *Alcaligenes*-specific systemic immune responses were associated with Crohn's disease and progressive hepatitis C virus infection in patients (Sonnenberg et al., 2012).

Collectively, a new category of immune cells, ILCs, may contribute to gut homeostasis and ecology. IL-23 produced by activated DCs and Mϕs stimulates IL-22 production by ILCs. IL-22 may induce an anti-microbial peptide, RegIII-γ, production in IECs. IEC derived factors including IgA, defensins, and RegIII-γ play a protective and homeostatic role in commensal bacteria ecology of gut (Fig. 6). Therefore, abnormal immune responses of ILCs may change intestinal immune environment and cause intestinal inflammation.

5.4. Lamina propria colitogenic T cells

Lamina propria effector memory $CD4^+$ T cells are directory involved in IBD pathogenesis. One of the most important clinical characteristics of human IBD is a pattern of repeated remission and relapse. To date, several factors including a high fat diet, infection, non-steroidal anti-inflammatory drugs, antibiotics, and mental stress have been reported as triggers contributing to the mechanisms of relapse and chronicity. However, these mechanisms remain unclear, in spite of recent research progress in the investigation of IBD pathogenesis. It has been suggested that some triggers (e.g. bacterial antigens) are re-recognized by APCs and the population of antigen-specific effector memory T cells expands, leading to relapse. This hypothesis is quite reasonable, but it raises the question of where the effector memory T cells are "hidden" during remission. Nemoto et al. demonstrated that bone marrow acts as a reservoir for colitogenic $CD4^+$ T cells (Nemoto et al., 2007). In addition, colitogenic $CD4^+$ T cells continuously recirculate in peripheral blood, supporting the efficacy of molecular targeting therapy for adhesion molecules (Tomita et al., 2008a). Interestingly, peripheral lymph nodes are not necessary for recirculation and storage of colitogenic $CD4^+$ T cells. FTY720, a chemical agent that suppresses T cell trafficking at the lymph nodes, also can inhibit recirculation of colitogenic $CD4^+$ T cells at the bone marrow (Fujii et al., 2008). IL-7 plays an important role in the survival of colitogenic $CD4^+$ T cells and the persistence of T cells associated with chronic colitis (Totsuka et al., 2007; Tomita et al., 2008b). Consistent with this observation, IL-7Rα expression in colitogenic $CD4^+$ T cells is significantly up-regulated (Shinohara et al., 2011).

The hypothesis of a Th1/Th2 cytokine balance has been used to explain the pathogenesis of chronic inflammatory disorders such as IBD. However, the recent discovery of a new class of Th cells, Th17 cells, which produce IL-17 family cytokines, has raised a new paradigm: namely, thatTh17 cells are an essential T cell subpopulation in the development of chronic inflammatory disorders in humans and mice. In mice, Th17 cells are generated from naïve T cells in the presence of TGF-β and IL-6 and express a specific transcription factor, retinoic acid-related orphan receptor (ROR)γt (Bettelli et al., 2006; Mangan et al., 2006; Veldhoen et al., 2006; Bettelli et al., 2008). Recent reports suggest that the combination of IL-6, IL-23, and IL-1β also effectively induces IL-17 production in naive T cells, independent of TGF-β (Ghoreschi et al., 2010). In humans, the mechanism of Th17 development is more complicated. It is difficult to induce Th17 cells from naïve peripheral blood T cells using in vitro differentiated conventional DCs, even in the presence of TGF-β and IL-6. Acosta-Rodriguez et al. reported that IL-1β and IL-6 are essential for the development of Th17 cells inhumans (Acosta-Rodriguez et al., 2007). However, Manelet al. succeeded in inducing Th17 cells from naïve T cells obtained from cord blood in the presence of TGF-β, IL-1β,and IL-21 or IL-23 (Manel et al., 2008).

Several reports have suggested that Th17 cells play a role in the pathogenesis of murine models of colitis. IL-23p19 transgenic mice spontaneously developed chronic colitis (Wiekowski et al., 2001). In $IL\text{-}10^{-/-}$ mice, which develop chronic Th1/Th17 dominant colitis, IL-23 administration exacerbated intestinal inflammation (Yen et al.,

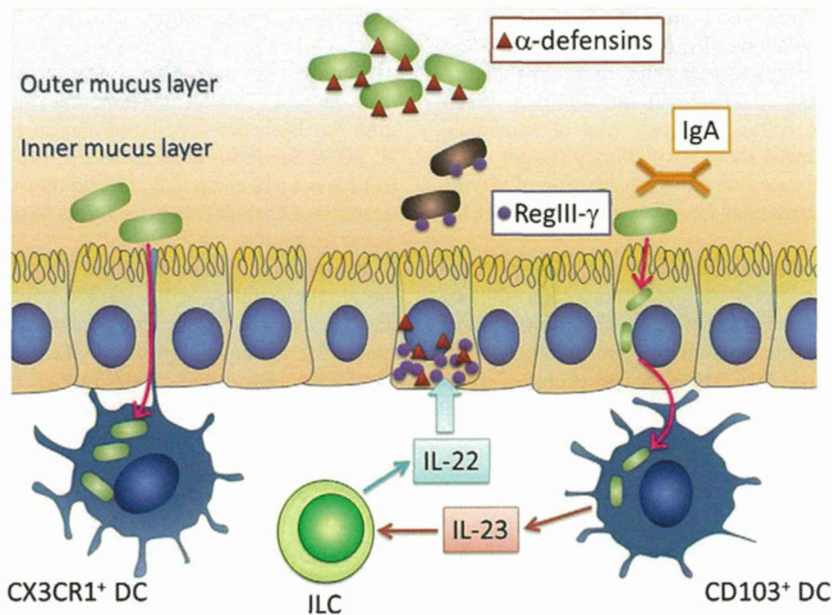

Fig. 6. ILCs protect the host by induction of IEC-derived RegIII-γ. ILCs produced IL-22 which is dependent on IL-23. IL-22 promotes production of IEC-derived RegIII-γ which contributes to intestinal mucosal ecology.

2006). The transfer of IL-17A-producing Th17 cells from C3Bir mice caused severe chronic colitis in SCID mice, which was inhibited by anti-IL-23p19 monoclonal antibody (mAb) treatment (Elson et al., 2007). The transferof $CD4^+$ $CD25^-$ T cells from $ROR\gamma t^{-/-}$ mice into RAG-$1^{-/-}$ mice did not result in colitis, while IL-17A induced colitis in this model (Leppkes et al., 2009). IL-17 receptor-deficient mice were protected from acute trinitrobenzene sulfonic acid-induced colitis (Zhang et al., 2006). These findings suggest that Th17 cells may contribute to the pathogenesis of chronic intestinal inflammation. However, it is still controversial whether IL-17A plays a pathological role in murine colitis. Administration of neutralizing anti-IL-17A mAb did not reduce the severity of colitis in an adaptive transfer model of $CD4^+CD45RB^{high}$ T cells (Yen et al., 2006) and exacerbated DSS-induced colitis (Ogawa et al., 2004). Furthermore, RAG-$1^{-/-}$ mice reconstituted with $CD45RB^{hi}$ T cells from IL-17 receptor-deficient mice exhibited an accelerated wasting disease (O'Connor et al., 2009). Thus, the contribution of IL-17 itself, and of Th17 cells, in the pathogenesis of colitis remains unresolved. It has become evident that Th17 cells can be classified into several subpopulations according to their cytokine profile. McGeachy et al. reported that Th17 cells could be divided into two types: Th17 cells producing IL-17 and IL-10, and Th17 cells developed under IL-23 stimulation producing IL-17 and pro-inflammatory cytokines (McGeachy et al., 2007). Furthermore, recent studies have suggested interactions between Th1 and Th17 cells. IL-17A suppressed inflammation in a murine model of colitis by blocking the development of Th1 cells (O'Connor et al., 2009). Mikami et al. demonstrated the existence of interference between colitogenic Th1 and Th17 cells in vivo. In experiments of a co-transferred $CD4^+$ T cells mice model (colitogenic$CD4^+$ T cells from colitic RAG-$2^{-/-}$ mice transferred with $CD4^+$ $CD45RB^{high}$ cells and colitogenic $CD4^+$ T cells from colitic IL-$10^{-/-}$ mice). The proportions of IFN-γ and IL-17A producing $CD4^+$ T cells in co-transferred mice were significantly decreased compared with single-cell transferred mice (Mikami et al., 2010). Although to date it has been thought that each Th subpopulation is generated independently, current progress in immunology research has highlighted the plasticity between these Th cell lineages. Lee et al. demonstrated that Th1 cells could be generated from IL-17 F-expressing Th17 cells in the presence of IL-23 in vivo (Lee et al., 2009). Sujino et al. proved the existence of plasticity between Th1 and Th17 cells in vivo using RORγt-GFP reporter mice. They identified a distinct developmental pathway from Th17 to alternative Th1 cells via Th17/Th1 and Th1-like cells during colitis (Sujino et al., 2011). Ono et al. also demonstrated that naturally occurring Th17 cells have regulatory activities in normal mouse intestine, in contrast with colitogenic Th17 and Th17/Th1 cells during inflammation (Ono et al., 2012).

The pathological role of Th17 cells in human disease remains unclear, and there is recent data which show differences in the functional roles of Th17 cells between mice models and human diseases (Steinman, 2008) and the existence of several subpopulations of IL-17 producing T cells in humans. Fujino et al. reported that IL-17A expression is up-regulated in $CD3^+$ T cells and $CD68^+$ Mϕs in the inflamed mucosa of patients with IBD (Fujino et al., 2003). $CD14^+CD68^+$ Mϕs inthe inflamed mucosa of Crohn's disease patients were observed to produce IL-23, IL-6, TNF-α, and TL1A/TNFSF15 in response to stimulation by whole bacteria and to promote the differentiation of naïve T cells to both Th1 and Th17 cells as antigen presenting cells (Kamada et al., 2008, 2009, 2010). Kobayashi et al. also demonstrated that IL-23 from $CD14^+CD68^+$Mϕ enhanced Th17 immunological responses in ulcerative colitis (Kobayashi et al., 2008). Thus, although several observations support a role for Th17 in IBD pathogenesis, to date the contribution of Th17 cells is still controversial. Indeed, the efficacy of anti-IL17A mAb has not yet been proven in patients with IBD.

5.5. Intestinal mucosal B cells

T cell receptor (TCR) α mutants represent a Th2-dominant chronic colitis, often used as a model of human disease ulcerative colitis (Mombaerts et al., 1993). Surprisingly, the spontaneous chronic colitis in $TCR\alpha^{-/-}$ mice (mediated by$CD4^+TCR\alpha^-\beta^+$ T cells) showed more severe inflammation in the absence of mature B cells, suggesting a suppressive role of B cells. Mature B cells play an important role in the development of chronic colitis in $TCR\alpha^{-/-}$ mice by directly regulating the pathogenic $CD4^+TCR\alpha^-\beta^+$ T cells (Mizoguchi et al., 2000). Mizoguchi et al. identified a B cell subset induced in gut-associated lymphoid tissues which is characterized by CD1d up-regulation. This B cell subset produced IL-10, and suppressed intestinal inflammation (Mizoguchi et al., 2002).

The association of mucosal B cells with human IBD pathogenesis remains unclear. Several sub-populations of mucosal B cells, including pro-inflammatory or immuno-regulatory B cells, may exist in the human intestine. This complexity is reflectedin the results of B cell targeting therapy in ulcerative colitis. A clinical trial of rituximab, which is an anti-CD20 mAb, showed short-term efficacy (Leiper et al., 2011), while in contrast several cases showingexacerbation of ulcerative colitis after rituximab therapy have been reported (Goetz et al., 2007; Ardelean et al., 2010).

Ulcerative colitis is characterized by infiltration of IgG-producing plasma cells. Recently, Uo et al. reported that IgG plasma cells infiltrate the inflamed mucosa via CXCR4, and critically influence pathogenesis of ulcerative colitis by exacerbating mucosal inflammation through the activation of "pathogenic" intestinal $CD14^{+}$macrophages via IgG-IC-FcγR signaling (Uo et al., 2012).

6. Development of immune regulatory therapy for IBD

As a result of progress in the research of IBD pathogenesis, immune regulatory therapies to effectively manage the symptoms of patients with IBD have been developed. In recent years, molecular targeting therapy has been developed in IBD therapeutics, including monoclonal antibodies (mAbs) and small compounds as specific inhibitors of target molecules.

6.1. Immune regulatory therapy for IBD

Corticosteroids have been used for induction therapy in both ulcerative colitis and Crohn's disease. In steroid dependent and steroid resistant patients, 6-mercaptopurine (6-MP) and azathioprine (AZA) have been used for maintenance therapy (Hanauer & Present, 2003). Several clinical studies of cyclosporine A, a calcineurin inhibitor, for induction or rescue therapy in patients with severe ulcerative colitis refractory to steroid therapy have been reported (Lichtiger et al., 1994; Kobayashi et al., 2010; Mocciaro et al., 2012; Sjoberg et al., 2012). Recently, the efficacy of oral tacrolimus, a newer calcineurin inhibitor, in patients with refractory ulcerative patients has been reported (Ogata et al., 2006, 2012). Thus, therapeutic strategies that regulate immune response have been a central component of IBD therapeutics.

6.2. Development of anti-TNFα mAbs in IBD

One of the most successful examples of immuno-molecular targeting therapies in IBD is an anti-TNFα mAb, which has "opened the door" on the era of biologics. The turning point was a report in 1993 of a 12-year-old female Crohn's disease patient from the Netherlands (Derkx et al., 1993). Following this single case report, the somewhat surprisingly successful results of a clinical trial of anti-TNFα mAb were reported in 1995 (van Dullemen et al., 1995).After several clinical trials that supported the efficacy of a chimeric (75% human, 25% mouse) anti-TNFα mAb, infliximab (Targan et al., 1997; Present et al., 1999; Rutgeerts et al., 1999), a large-scale randomized trial (ACCENT I) was performed and indicated the efficacy of infliximab in induction and maintenance therapy for active Crohn's disease (Hanauer et al., 2002). Following the success of infliximab, other anti-TNFα mAbs were developed. Adalimumab (Hanauer et al., 2006; Colombel et al., 2007), a human anti-TNFα mAb, and certolizumabpegol (Schreiber et al., 2005; Sandborn et al., 2007), a polyethylene-glycolated Fab' fragment of anti-TNFα mAb, also demonstrated efficacy. Anti-TNFα mAbs also showed efficacy in patients with refractory ulcerative colitis (Rutgeerts et al., 2005; Reinisch et al., 2011; Sandborn et al., 2012) suggesting an overlap of pathophysiology between Crohn's disease and ulcerative colitis.

6.3. Novel molecular targeting therapy in IBD

Success of anti-TNFα mAb therapies has encouraged the development of molecular targeting therapy in IBD. An anti-IL-12p40 mAb has been developed and clinical trials are ongoing (Mannon et al., 2004; Sandborn et al., 2008). IL-12p40 is bound to a p35 chain in IL-12 and a p19 chain in IL-23, and these are key cytokines in the pathogenesis of Crohn's disease in terms of promotion of Th1 and Th17 immune responses.

Adhesion molecules have been highlighted as a novel therapeutic target. Natalizumab, a humanized monoclonal antibody against α4 integrin that inhibits leukocyte adhesion and migration into inflamed tissue, has demonstrated some efficacy in Crohn's disease (Sandborn et al., 2005; Feagan et al., 2007; Targan et al., 2007), although the potential for progressive multifocal leukoencephalopathy caused by the reactivation of the JC virus means that caution is required (Van Assche et al., 2005). A clinical trial of GSK-1605786 (CCX-282; Traficet-EN), a selective antagonist of the CC chemokine receptor (CCR9) which also inhibits lymphocyte homing, is ongoing.

Not only cytokines and surface molecules, but signal transduction molecules could also be therapeutic targets for chronic inflammatory disorders. Janus kinase (JAK) inhibitors are expected to be candidates for molecular targeting therapy for rheumatoid arthritis and IBD.

7. Conclusion

We have reviewed the immunological aspects in IBD pathogenesis. Recently identified susceptibility genes strongly suggest a contribution of abnormal immune response to IBD pathogenesis. Advances in research on the microbiota in mice suggest that commensal microbiota can regulate the host gut immune system. Abnormal immune responses to commensal bacteria and/or food antigens may be a central part of IBD pathogenesis. To regulate excess immune responses, immune regulatory therapies have been applied. As a result of newly discovered knowledge about IBD pathogenesis, molecular targeting therapies such as anti-TNFαmAbshave been developed.

As we have described, the pathogenesis seen in patients with IBD varies, and can be very different between individuals. In the near future, it should be possible to establish tailor-made medicine in the field of IBD therapeutics. For example, a combination of a panel of susceptibility genes with analysis of the human gut microbiome may be useful to select the most adequate treatment that can adjust to an individual's pathogenic status. There is still a long way to go, but we confidently expect that recent advances in our understanding of mucosal immunology will contribute to understanding the etiology of IBD, and therefore the development of new therapeutics.

"The authors declare that there are no conflicts of interest."

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T-helper 17 and Interleukin-17–Producing Lymphoid Tissue Inducer-Like Cells Make Different Contributions to Colitis in Mice

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BACKGROUND & AIMS: T helper (Th) 17 cells that express the retinoid-related orphan receptor (ROR) γt contribute to the development of colitis in mice, yet are found in normal and inflamed intestine. We investigated their development and functions in intestines of mice. **METHODS:** We analyzed intestinal Th17 cells in healthy and inflamed intestinal tissues of mice. We analyzed expression of lymphotoxin (LT)α by Th17 cells and lymphoid tissue inducer-like cells. **RESULTS:** $LT\alpha^{-/-}$ and $ROR\gamma t^{-/-}$ mice had significantly lower percentages of naturally occurring Th17 cells in the small intestine than wild-type mice. Numbers of $CD3^-CD4^{+/-}$interleukin-$7R\alpha^+$c-kit$^+$CCR6$^+$NKp46$^-$ lymphoid tissue inducer-like cells that produce interleukin-17A were increased in $LT\alpha^{-/-}$ and $LT\alpha^{-/-}\times$recombination activating gene $(RAG)-2^{-/-}$ mice, compared with wild-type mice, but were absent from $ROR\gamma t^{-/-}$ mice. Parabiosis of wild-type and $LT\alpha^{-/-}$ mice and bone marrow transplant experiments revealed that LTα-dependent gut-associated lymphoid tissue structures are required for generation of naturally occurring Th17 cells. However, when wild-type or $LT\alpha^{-/-}$ $CD4^+CD45RB^{high}$ T cells were transferred to $RAG\text{-}2^{-/-}$ or $LT\alpha^{-/-}\times RAG\text{-}2^{-/-}$ mice, all groups, irrespective of the presence or absence of LTα on the donor or recipient cells, developed colitis and generated Th1, Th17, and Th17/Th1 cells. $RAG\text{-}2^{-/-}$ mice that received a second round of transplantation, with colitogenic but not naturally occurring Th17 cells, developed intestinal inflammation. The presence of naturally occurring Th17 cells in the colons of mice inhibited development of colitis after transfer of $CD4^+CD45RB^{high}$ T cells and increased the numbers of Foxp3$^+$ cells derived from $CD4^+CD45RB^{high}$ T cells. **CONCLUSIONS: Gut-associated lymphoid tissue structures are required to generate naturally occurring Th17 cells that have regulatory activities in normal intestines of mice, but not for colitogenic Th17 and Th17/Th1 cells during inflammation.**

Keywords: Immune Regulation; T-Cell Development; Mouse Model; Inflammatory Bowel Disease.

Naturally occurring T helper (Th) 17 cells compose a considerable proportion of lamina propria (LP) $CD4^+$ T cells in the small intestine (SI) of healthy mice.[1–3] Retinoid-related orphan receptor (ROR) γt is a key transcription factor for the differentiation program of Th17 cells.[1] Although Th17 cells are absent in $ROR\gamma t^{-/-}$ mice that lack gut-associated lymphoid tissues (GALT), such as Peyer's patches (PP) and cryptopatches,[1] $ROR\gamma t^{-/-}$ mice lack lymphoid tissue inducer (LTi) cells,[4] which are essential for the formation of GALT during ontogeny.[5] $ROR\gamma t^+$ lineage (Lin)$^-$CD4$^{+/-}$interleukin (IL)-7Rα^+ LTi-like cells with a similar phenotype to that of embryonic LTi cells were identified in the adult intestine.[6] In addition to the lymphoid organogenesis, another aspect of LTi-like cells in mucosal immune function has attracted attention. LTi-like cells are a subset of innate lymphoid cells (ILCs)[7] and are also an innate source of IL-22, IL-17A, and interferon (IFN)-γ. Of note, Thy1^{high}Sca-1$^+$ IFN-γ–producing LTi-like cells seem to be involved in the induction of innate colitis induced by *Helicobacter hepaticus* infection and the administration of anti-CD40 monoclonal antibody to recombination activating gene (RAG)-$2^{-/-}$ mice.[8,9] Furthermore, a subset of IL-22-producing ILC co-expressing RORγt and natural killer cell receptors has been described as NK22 (natural killer cell receptors–LTi, NCR-22, natural killer cell receptors$^+$RORγt$^+$ ILC, and ILC22) cells.[10]

However, the direct relationship between RORγt-expressing Th17/LTi-like cells and the existence of GALT has not been investigated. Based on previous studies, we used $LT\alpha^{-/-}$ mice, which lack GALT but retain the RORγt function,[11] to investigate the relationship between LTi-like cells and Th17 cells in steady and inflammatory conditions.

Abbreviations used in this paper: BM, bone marrow; GALT, gut-associated lymphoid tissue; GFP, green fluorescent protein; GITR, glucocorticoid-induced tumour necrosis factor receptor; IFN, interferon; IL, interleukin; ILC, innate lymphoid cells; LP, lamina propria; LTα, lymphotoxin α; LTβR, lymphotoxin β receptor; LTi, lymphoid tissue inducer; PP, Peyer's patches; RAG, recombination activating gene; RORγt, retinoid-related orphan receptor gamma t; Rorc, RAR-related orphan receptor C; SI, small intestine; TCR, T-cell receptor; TGF, transforming growth factor; Th, T helper; WT, wild type.

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BASIC AND

Materials and Methods

See the Supplementary Materials and Methods section for full details.

Animals

C57BL/6J-Ly5.2 mice were obtained from CLEA Japan, Inc (Tokyo, Japan). C57BL/6-Ly5.1 mice and C57BL/6-Ly5.2-RAG-$2^{-/-}$ mice were obtained from Taconic Laboratory (Hudson, NY) and Central Laboratories for Experimental Animals (Kawasaki, Japan). Ly5.2-background lymphotoxin α-deficient ($LT\alpha^{-/-}$) mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice with a green fluorescent protein (GFP) reporter complementary DNA knocked-in at the site for initiation of RORγt translation on the C57BL/6 background (RAR-related orphan receptor C [*Rorc*]$(\gamma t)^{gfp/+}$ reporter mice, and *Rorc*$(\gamma t)^{gfp/gfp}$ mice [hereafter called ROR$\gamma t^{-/-}$ mice]) were kindly provided by Dr Littman.[11] $LT\alpha^{-/-}$ mice were intercrossed into RAG-$2^{-/-}$ mice to generate $LT\alpha^{-/-}\times$RAG-$2^{-/-}$ mice. Mice were maintained after Caesarean surgery under specific pathogen-free conditions in our Animal Care Facility. All experiments were approved by the regional animal study committees.

Statistical Analysis

The results are expressed as mean ± standard error of the mean. Groups of data were compared by the Student *t* test. *P* values less than .05 were considered statistically significant.

Results

$LT\alpha^{-/-}$ Mice Lack Naturally Occurring Th17 Cells but Retain $CD3^-$ IL-17A–Expressing Cells in the SI

We first assessed the generation of naturally occurring Th17 cells in the SI of wild-type (WT), ROR$\gamma t^{-/-}$, and $LT\alpha^{-/-}$ mice at 18–23 weeks of age. We used fully matured mice because the emergence of intestinal naturally occurring Th17 cells is dependent on the presence of commensal microbiota, and the ratio of those cells increases over time after birth.[2] Naturally occurring IL-$17A^+$IFN-γ^- Th17 cells constitute a considerable proportion in the $CD3^+CD4^+$-gated population as well as IL-$17A^-$IFN-γ^+ Th1 cells in the LP of SI in WT mice (Figure 1*A*). ROR$\gamma t^{-/-}$ mice mostly lacked Th17 cells, but retained a normal proportion of Th1 cells (Figure 1*A*). $LT\alpha^{-/-}$ mice also lacked Th17 cells with a normal proportion of Th1 cells (Figure 1*A*). Although the total LP $CD4^+$ T-cell numbers of all 3 groups were comparable (Figure 1*Bi*), the proportion of Th17 cells of ROR$\gamma t^{-/-}$ and LT $\alpha^{-/-}$ mice was reduced significantly compared with that of WT mice (Figure 1*Bii*), whereas that of Th1 cells in all the groups was comparable (Figure 1*Biii*). Consistently, the proportion of IL-$17A^-$IFN-γ^- $CD4^+$ T cells from ROR$\gamma t^{-/-}$ and LT $\alpha^{-/-}$ mice was increased significantly (Figure 1*Biv*). It was notable that IL-$17A^+$IFN-γ^+ Th17/Th1 cells were not detected in all mice in the steady-state condition (Figure 1*A*).

These results suggest that the $LT\alpha$-dependent pathway may be required for the generation of naturally occurring Th17 cells. However, when looking at the CD3/IL-17A fluorescence-activated cell sorter profile in the lymphocyte-gated population, we noticed that a substantial pro-

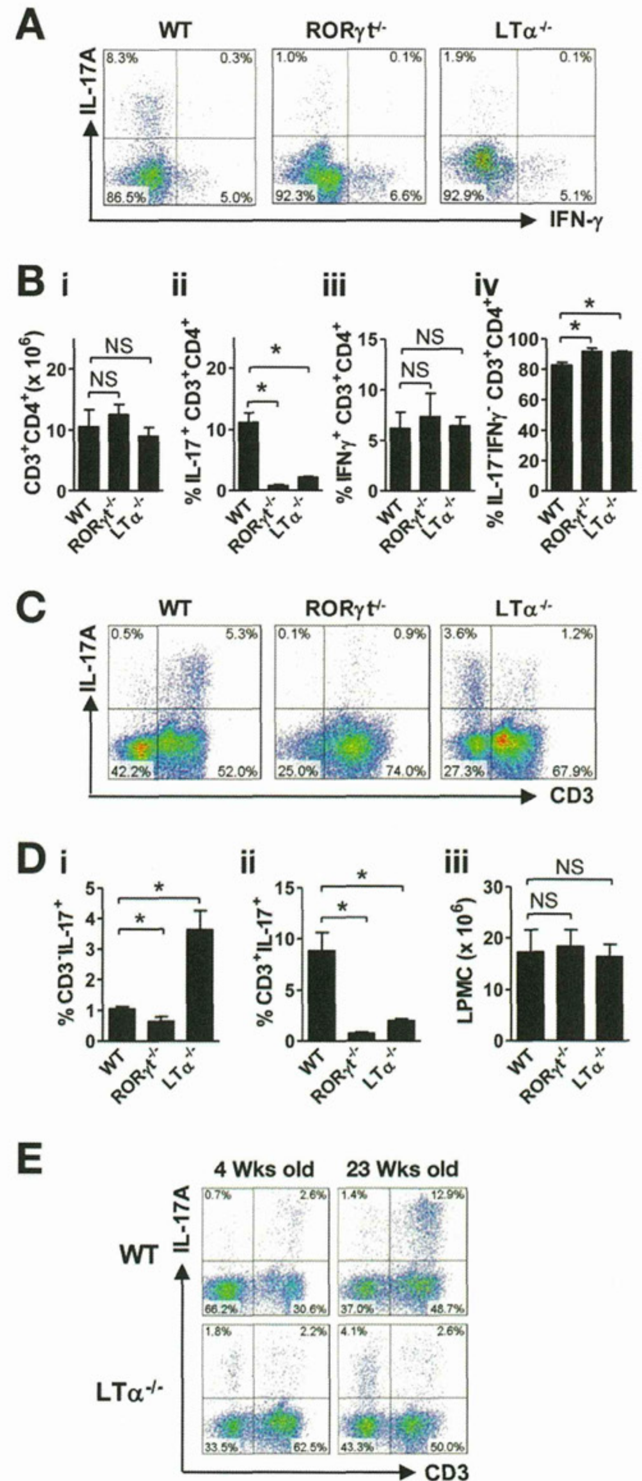

Figure 1. $LT\alpha^{-/-}$ mice lack naturally occurring Th17 cells but retain $CD3^-$ IL-17A–expressing cells in the SI. (*A*) Expression of IL-17A and IFN-γ in SI LP $CD3^+CD4^+$ T cells of the indicated mice. (*B*) Absolute cell number of $CD3^+CD4^+$ T cells (*i*). Mean percentages of IL-$17A^+$ (*ii*), IFN-γ^+ (*iii*), or IL-$17A^-$IFN-γ^- (*iv*) cells in $CD3^+CD4^+$ T cells. (*C*) Expression of IL-17A and CD3 in LP mononuclear cells of the indicated mice. (*D*) Mean percentages of IL-$17A^+CD3^-$ (*i*) or IL-$17A^+CD3^+$ (*ii*) cells in SI LP mononuclear cells. Absolute cell number of LP mononuclear cells of the indicated mice (*iii*). (*E*) Expression of IL-17A and CD3 in LP mononuclear cells of the indicated mice at 4 or 23 weeks old. (*B* and *D*) Data show mean ± standard error of the mean (n = 8/group). *$P < .05$.

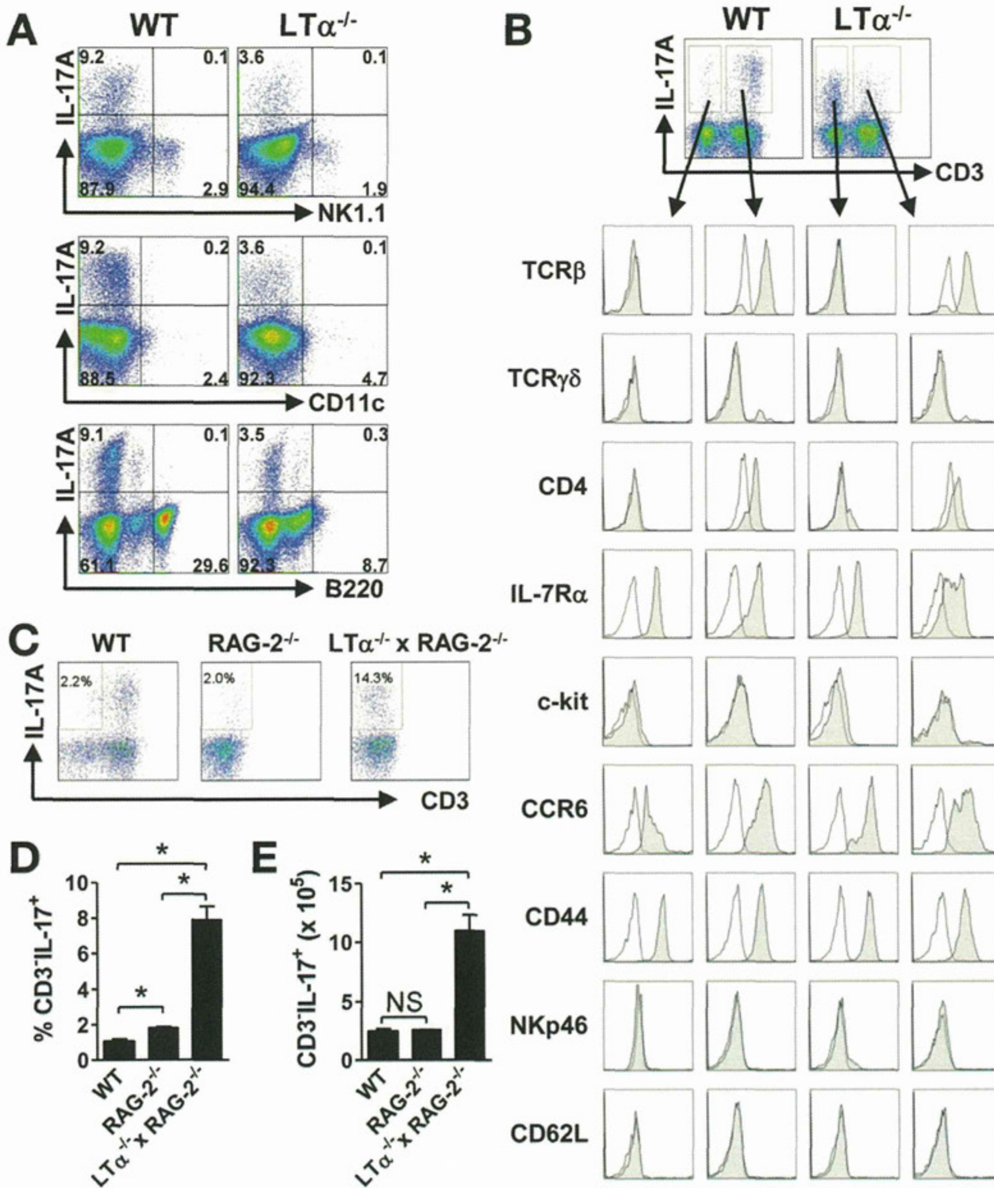

Figure 2. IL-17–expressing CD3$^-$ cells in the SI of LT$\alpha^{-/-}$ mice are LTi-like cells. (*A*) IL-17–producing cells in LT $\alpha^{-/-}$ mice are neither T, natural killer, dendritic, nor B cells. Expression of IL-17A and NK1.1, CD11c, or B220 on LP mononuclear cells from the indicated mice. Data are representative of 6 mice in each group. (*B*) Flow cytometry analysis of LP mononuclear cells from WT or LT$\alpha^{-/-}$ mice. Cells were stimulated in vitro with phorbol myristate acetate/ionomycin and subjected to intracellular cellular staining for IL-17A co-stained with CD3 and NK1.1, CD11c, B220, CD4, IL-7Rα, c-kit, CCR6, CD44, NKp46, CD62L, TCRβ, TCR$\gamma\delta$ (*gray histograms*), or isotype control antibodies (*open histograms*). Data are representative of 6 mice in each group. (*C*) Expression of IL-17A and CD3 in LP mononuclear cells of the indicated mice. (*D*) Mean percentages of CD3$^-$IL-17A$^+$ cells in LP mononuclear cells. (*E*) Absolute cell number of CD3$^-$IL-17A$^+$ cells in SI. (*D* and *E*) Data show mean ± standard error of the mean (n = 3/group). **P* < .05.

portion of IL-17–expressing cells in LT$\alpha^{-/-}$ mice resided in the CD3-negative subpopulation, whereas IL-17–expressing cells in WT mice resided in the CD3-positive subpopulation (Figure 1*C*). RORγt$^{-/-}$ mice mostly lack both IL-17–expressing subpopulations (Figure 1*C*). This was confirmed by statistical analysis (Figure 1*Di* and *ii*). Furthermore, the absolute cell numbers of LP mononuclear cells were comparable among all 3 groups (Figure 1*Diii*). Intriguingly, the ratio of IL-17–expressing CD3$^-$ cells in LT$\alpha^{-/-}$ mice was increased with age in parallel with that of IL-17–expressing CD3$^+$ Th17 cells in WT mice (Figure 1*E*).

In the spleen, the proportion of CD3$^-$ and CD3$^+$ subpopulations of IL-17–expressing cells was very small, but comparable between WT and LT$\alpha^{-/-}$ mice. In the colon, the proportion of CD3$^+$IL-17$^+$ Th17 cells in LT$\alpha^{-/-}$ mice was reduced significantly compared with that in WT mice, whereas the proportion of CD3$^-$IL-17$^+$ cells was comparable between WT and LT$\alpha^{-/-}$ mice (Supplementary Figure 1).

To exclude the possible contribution of distinct pathogen colonization in WT and LT$\alpha^{-/-}$ mice to the balance of IL-17–expressing CD3$^-$ and CD3$^+$ subpopulations, we prepared 20-week-old WT and LT$\alpha^{-/-}$ mice co-housed for 16 weeks in the same cages. The findings were the same as observed in Figure 1 (Supplementary Figure 2).

IL-17–Expressing CD3$^-$ Cells in LT$\alpha^{-/-}$ Mice Are LTi-Like Cells

To delineate the population of IL-17–expressing CD3$^-$ cells in LT$\alpha^{-/-}$ mice, we assessed the expression of lineage markers. First, IL-17–expressing cells in the SI of LT$\alpha^{-/-}$ or WT mice did not express NK1.1, CD11c, or B220 (Figure 2*A*). Furthermore, most IL-17A$^+$CD3$^+$ cells in LT$\alpha^{-/-}$ or WT mice expressed T-cell receptor (TCR)β, but a small proportion of those cells expressed TCR$\gamma\delta$, whereas most IL-17A$^+$CD3$^-$ cells in LT$\alpha^{-/-}$ or WT mice did not express TCRβ (Figure 2*B*). Also, most IL-

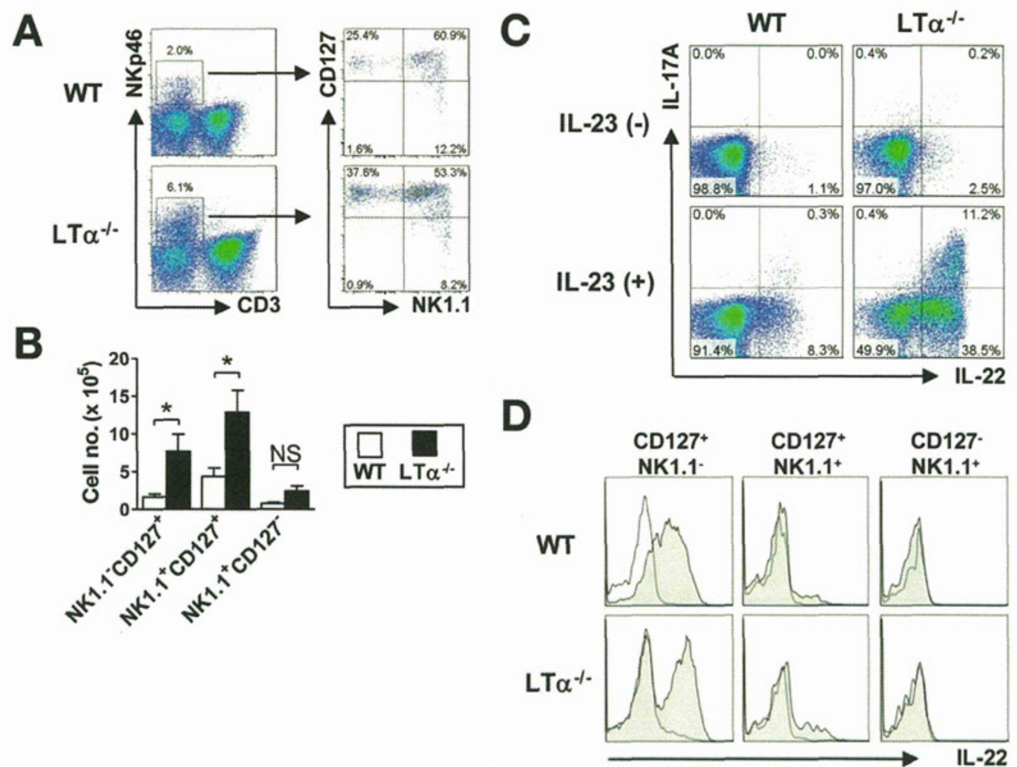

Figure 3. Numbers of intestinal NKp46$^+$ NK22 cells increase in the SI of LT$\alpha^{-/-}$ mice. (*A*) Expression of CD3 and NKp46 cells in SI neither T, natural killer, dendritic, nor B cells of the indicated mice (*left*). Expression of NK1.1 and CD127 gated on CD3$^-$NKp46$^+$ cells (*right*). Data are representative of 6 mice in each group. (*B*) Absolute cell number of CD3$^-$NKp46$^+$CD127$^+$NK1.1$^-$, NK1.1$^+$, and CD3$^-$NKp46$^+$CD127$^-$NK1.1$^+$ cells. *White bar*, WT mice; *black bar*, LT$\alpha^{-/-}$ mice. Data show mean ± standard error of the mean (n = 4/group). **P* < .05. (*C*) Expression of IL-17A and IL-22 in SI LP CD3$^-$ cells of the indicated mice. Cells were cultured in vitro with or without IL-23. (*D*) Expression of IL-22 in SI LP CD3$^-$NKp46$^+$ cells of the indicated population. Cells were cultured in vitro with or without IL-23. *Gray histograms* indicate the subset cultured with IL-23. *Open histograms* indicate the subset cultured without IL-23.

17A$^+$CD3$^+$ cells in LT$\alpha^{-/-}$ or WT mice expressed CD4, CD44, CCR6, and IL-7Rα, but not CD62L, whereas IL-17A$^+$CD3$^-$ cells in LT$\alpha^{-/-}$ or WT mice expressed CD44, CCR6, and IL-7Rα, but most of those cells in WT mice did not express CD4 and only 10% of those cells in LT$\alpha^{-/-}$ mice expressed CD4 (Figure 2*B*). WT or LT$\alpha^{-/-}$ IL-17A$^+$CD3$^-$ cells, but not IL-17A$^+$CD3$^+$ cells, expressed c-kit, whereas only IL-17A$^+$CD3$^-$ subpopulations in LT$\alpha^{-/-}$ mice, but not those cells in WT mice and IL-17A$^+$CD3$^+$ Th17 cells in both mice, retained a small proportion of NKp46 (Figure 2*B*). These results suggest that IL-17A$^+$CD3$^+$ cells are categorized as Th17 cells or IL-17A-producing TCR$\gamma\delta^+$ T cells, whereas IL-17A$^+$CD3$^-$ cells are adult LTi-like cells.

Because it still was possible that the increased IL-17A$^+$CD3$^-$ cells in LT$\alpha^{-/-}$ mice are T lymphocytes that have down-modulated the expression of CD3 and TCRβ, we assessed if IL-17A$^+$CD3$^-$ cells reside in the SI of RAG-2$^{-/-}$ and LT$\alpha^{-/-}$×RAG-2$^{-/-}$ mice in the absence of T cells. Although IL-17A$^+$CD3$^+$ Th17 cells disappeared in both LT$\alpha^{-/-}$×RAG-2$^{-/-}$ and RAG-2$^{-/-}$ mice, IL-17A$^+$CD3$^-$ cells were retained in the SI of both mice (Figure 2*C*), and the proportion (Figure 2*D*) and absolute number of those cells (Figure 2*E*) in LT$\alpha^{-/-}$×RAG-2$^{-/-}$ mice were increased significantly compared with those in RAG-2$^{-/-}$ mice. This suggested that the increased numbers of IL-17A$^+$CD3$^-$ cells in LT$\alpha^{-/-}$×RAG-2$^{-/-}$ mice were LTi-like cells, and were independent of the presence of Th17 cells.

Intestinal NK22 Cells Also Increase in LT$\alpha^{-/-}$ Mice

Recently, NK22 cells co-expressing RORγt have gained attention,[10] although controversially recent articles reported that NK22 cells are the progeny of LTi-like cells,[12] although other articles have disagreed.[13] Intriguingly, both CD3$^-$NKp46$^+$IL-7Rα^+NK1.1$^-$ and NK1.1$^+$ subsets in the SI of LT$\alpha^{-/-}$ mice were increased significantly compared with those in WT mice (Figure 3*A* and *B*). Furthermore, in vitro stimulation by IL-23 strongly induced IL-22$^+$IL-17A$^-$ cells and IL-22$^+$IL-17A$^+$ cells in the CD3-negative population from WT and LT$\alpha^{-/-}$ mice (Figure 3*C*). Furthermore, IL-22–expressing CD3$^-$NKp46$^+$ cells were CD127$^+$NK1.1$^-$ irrespective of whether they were isolated from WT or LT$\alpha^{-/-}$ mice (Figure 3*D*). This suggested the following: (1) the CD3$^-$ population contained IL-23R–expressing cells; (2) the increase of IL-23–responding LTi-like cells in the SI of LT$\alpha^{-/-}$ mice resulted in the development of NK22 cells; and (3) LTα expression on LTi-like cells was dispensable for the differentiation of NK22 cells.

LTα Molecules on CD4$^+$ T Cells Are Not Required for the Development of Naturally Occurring Th17 Cells

We further investigated whether LTα molecules on CD4$^+$ T cells and LTi-like cells are required for the development of naturally occurring Th17 cells. To this end, we used a parabiosis system that joined Ly5.1$^+$ WT and Ly5.2$^+$ LT$\alpha^{-/-}$ mice (Figure 4*A*). Four weeks after parabiosis surgery, SI Th17 cells in LT$\alpha^{-/-}$ mice in the parabionts were significantly restored as compared with those in LT$\alpha^{-/-}$ mice without parabiosis (Figure 4*B*), whereas SI Th1 cells were comparable among all mice (Figure 4*C*). Furthermore, a substantial number of Th17 and Th1 cells in both mice emerged on opposite sides of the parabionts (Figure 4*B* and *C*). In contrast, it was notable that the LTi-like cell subset was restricted to the original SI (Figure

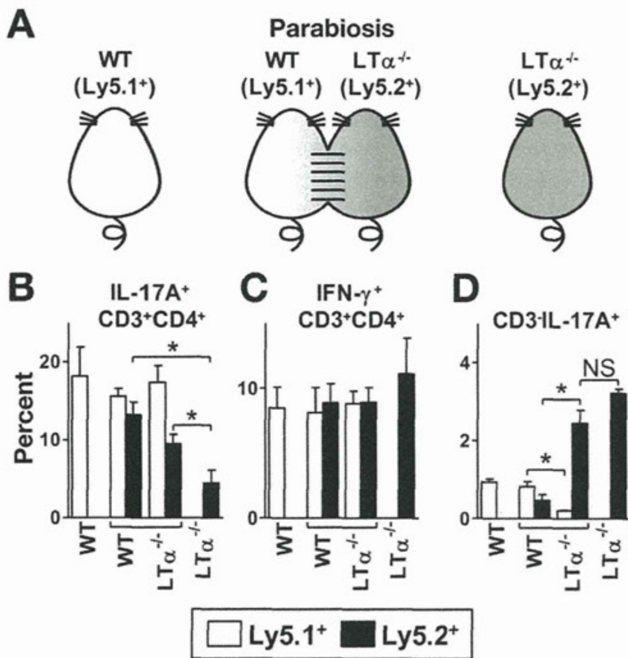

Figure 4. LTα expression on CD4$^+$ T cells is not required for the development of intestinal naturally occurring Th17 cells. (*A*) Scheme of the parabiosis experiment. Ly5.2$^+$ LTα$^{-/-}$ mice were joined with age-matched Ly5.1$^+$ WT mice (n = 7). Age-matched Ly5.1$^+$ WT mice and Ly5.2$^+$ LTα$^{-/-}$ mice without parabiosis surgery were used as controls (n = 4/group). (*B*) Mean percentages of Th17 cells in SI LP CD3$^+$CD4$^+$ cells. (*C*) Mean percentages of Th1 cells in SI LP CD3$^+$CD4$^+$ cells. (*D*) Mean percentages of IL-17A$^+$ cells in SI LP CD3$^-$ cells. *White bar*, Ly5.1$^+$ cells; *black bar*, Ly5.2$^+$ cells. (*B–D*) Data show mean ± standard error of the mean (n = 4 or 7/group). *$P < .05$.

4*D*), suggesting that LTi-like cells are resident cells, whereas Th17 cells are well mixed by the blood circulation or that progenitor-naive CD4$^+$ T cells migrate to both SI sites for the generation of Th17 cells. Therefore, these results suggest that naive LTα$^{-/-}$ CD4$^+$ T cells reach the SI of WT mice, and differentiate into Th17 cells at the site where normal LTi-like cells reside. Furthermore, this parabiosis experiment indicates that LTα expression on CD4$^+$ T cells is not required for Th17 cell differentiation.

Given that LTα expression on CD4$^+$ T cells is not required for naturally occurring Th17 cell differentiation, we directly assessed whether the lack of LTα on CD4$^+$ cells impaired the development of in vitro-manipulated Th17 cells. Consistent with the parabiosis experiment, WT and LTα$^{-/-}$-naive CD4$^+$ T cells differentiated into Th17 cells at a comparable level after transforming growth factor (TGF)-β and IL-6 stimulation (Supplementary Figure 3).

The LTα-Dependent GALT Structures Are Required for the Generation of Intestinal Naturally Occurring Th17 Cells

We further investigated whether LTα expression on bone marrow (BM)-derived cells was required for the development of naturally occurring Th17 and LTi-like cells. To this end, irradiated WT or LTα$^{-/-}$ mice were reconstituted with BM from WT or LTα$^{-/-}$ mice to set up 4 experimental groups as depicted in Supplementary Figure 4*A*. Naturally occurring Th17 cells developed in group 3 (LTα$^{-/-}$→WT) and group 1 (WT→WT) in similar numbers, but were not observed in group 2 (WT→LTα$^{-/-}$) or group 4 (LTα$^{-/-}$→LTα$^{-/-}$) (Supplementary Figure 4*B* and *C*). In groups 2 and 4 (LTα$^{-/-}$ recipients), IL-17A$^+$CD3$^-$ subpopulations were increased compared with groups 1 and 3 (WT recipients) (Supplementary Figure 4*D* and *E*).

To further confirm this observation, freshly isolated Lin$^-$CD45$^+$NKp46$^-$ GFP (RORγt)^{high} LTi-like cells from the SI of *Rorc(γt)*$^{gfp/+}$ mice were transferred into LTα$^{-/-}$ mice (Supplementary Figure 5*A*). Consistent with the BM chimera experiment, the proportion of naturally occurring Th17 cells in the SI of LTα$^{-/-}$ mice with LTi-like cell transfer and LTα$^{-/-}$ mice without transfer was comparable and significantly lower than that in the SI of WT mice (Supplementary Figure 5*B* and *C*). Collectively, LTα-expressing BM-derived cells including LTi-like cells were not essential for the development of naturally occurring Th17 cells, but rather the GALT structure is essential as the induction site of naturally occurring Th17 cells.

LTα Molecules on LTi-Like and CD4$^+$ T Cells Are Not Required for the Development of Colitogenic Th17 and Th17/Th1 Cells

We next investigated if this is a case with colitogenic Th17 cells developed under colitic conditions. To this end, we created 4 groups in an adoptive transfer colitis model as depicted in Figure 5*A*. Six weeks after transfer, all groups of mice developed colitis (Figure 5*B*) as confirmed by histologic scores (Figure 5*C*), although LTα$^{-/-}$×RAG-2$^{-/-}$ recipients transferred with WT or LTα$^{-/-}$ donor CD4$^+$CD45RB^{high} T cells (group 2 or 4 mice) had significantly milder colitis than the paired RAG-2$^{-/-}$ recipients (group 1 or 3 mice) (Figure 5*C*). The clinical scores of mice confirmed that all groups of mice developed colitis, but the lack of LTα on CD4$^+$CD45RB^{high} T cells did not affect the clinical scores, which were consistent between groups 1 and 3 RAG-2$^{-/-}$ and between groups 2 and 4 LTα$^{-/-}$×RAG-2$^{-/-}$ mice, respectively (Figure 5*B*). Consistently, the absolute cell numbers of colonic LP CD3$^+$CD4$^+$ from all 4 groups were significantly higher than in control WT mice, but colonic LP CD3$^+$CD4$^+$ cells from all groups 2 and 4 mice were significantly lower than in groups 1 and 3 mice, respectively.

In sharp contrast to the finding that there is a lack of naturally occurring Th17 cells in the SI LP of LTα$^{-/-}$ mice in the steady state (Figure 1*A*), surprisingly, not only a comparable proportion of Th17 cells emerged in colitic LP of all 4 groups, but also a comparable proportion of IL-17A$^+$IFN-γ$^+$ Th17/Th1 cells (Figure 5*Fi* and *ii*). Likewise, the proportion of colitic IL-17A$^-$IFN-γ$^+$ Th1 cells was comparable among all 4 groups (Figure 5*Fi* and *ii*). These results indicate that not only the existence of GALT but also LTα expression on CD4$^+$ and LTi-like cells are not essential for the development of colitogenic LP Th17 and Th17/Th1 cells, unlike in the steady conditions.

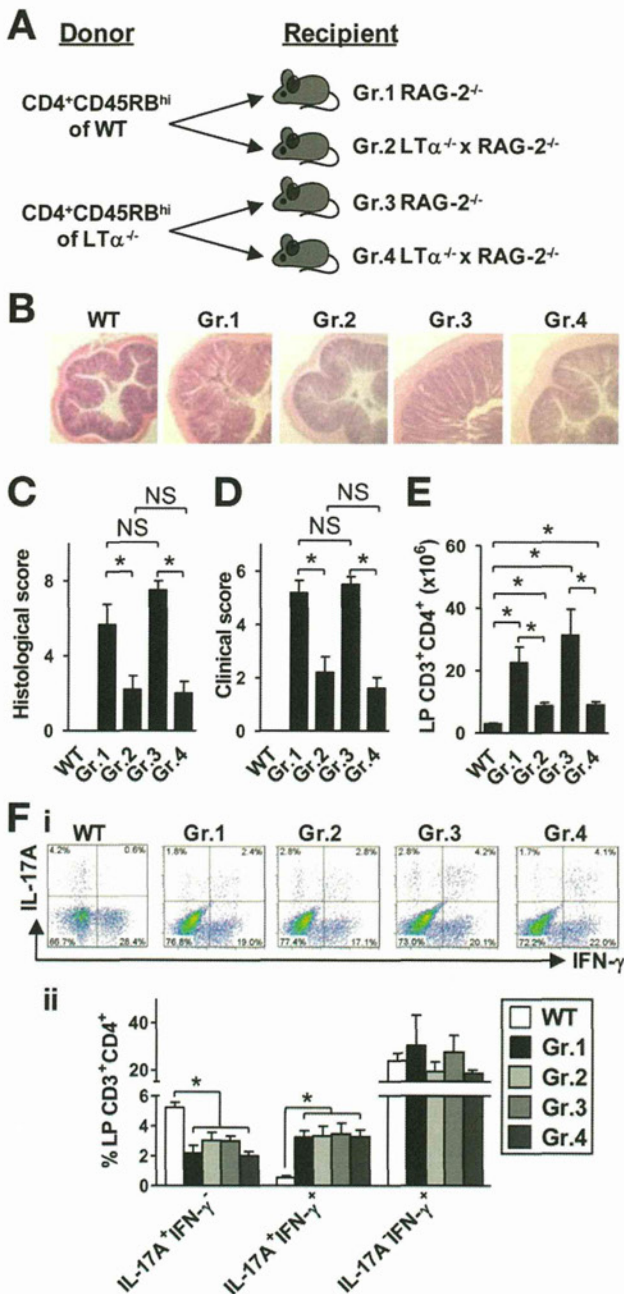

Figure 5. LTα expression is not required for the development of colitogenic Th17 and Th17/Th1 cells. (*A*) Scheme of the adoptive transfer experiment. (*B*) Histopathology of distal colon at 6 weeks after transfer. Original magnification, ×40. (*C*) Histologic scores of distal colon. (*D*) Clinical score at 6 weeks after transfer. (*E*) Absolute cell number of LP CD3$^+$CD4$^+$ T cells from colon at 6 weeks after transfer. (*F*) Expression of IL-17A and IFN-γ on colonic LP CD3$^+$CD4$^+$ T cells of the indicated mice (*i*). Mean percentages of Th17, Th17/Th1, and Th1 cells in LP CD3$^+$CD4$^+$ T cells from each group (*ii*). (*C*, *D*, *E*, and *Fii*) Data show mean ± standard error of the mean (n = 5/group). *$P < .05$. Gr, group.

Intestinal Naturally Occurring Th17 Cells Are Not Enterocolitogenic Cells

Because it is well known that naturally occurring Th17 cells reside in the RORγt (GFP)^{intermediate} subpopulation, whereas LTi-like cells reside in the RORγt (GFP)^{high} subpopulation,[1] we strictly isolated CD3$^+$CD4$^+$GFP^{intermediate} cells from the SI of *Rorc(γt)*$^{gfp/+}$ mice to exclude LTi-like cells for further study (Supplementary Figure 6*A* and *B*). We previously showed that almost all the LTi-like cells did not express CD4 (Figure 2*B*). However, to further exclude the possibility that the isolated naturally occurring GFP$^+$ Th17 cells contaminate a small number of LTi-like cells that can respond to IL-23, we stimulated the sorted GFP$^+$ SI LP CD4$^+$ T cells from *Rorc(γt)*$^{gfp/+}$ mice with IL-23. As depicted in Supplementary Figure 6*C*, although the ratio of IFN-γ expression in colitic LP CD3$^+$CD4$^+$ T cells without IL-23 stimulation was significantly higher than that in isolated GFP$^+$ CD4$^+$ T cells, GFP$^+$ CD4$^+$ T cells as well as colitic CD3$^+$CD4$^+$ T cells did not up-regulate the expression of IFN-γ in response to IL-23 as compared with the paired cells without IL-23. Collectively, we concluded that GFP^{intermediate} cells were CD3$^+$CD4$^+$ Th17 cells (hereafter called *naturally occurring Th17 cells*), whereas GFP^{high} cells were CD3$^-$ LTi-like cells.

To clarify the difference between naturally occurring Th17 cells and colitogenic LP RORγt$^+$ (GFP$^+$) CD4$^+$ T cells obtained from colitic RAG-2$^{-/-}$ mice previously transferred with *Rorc(γt)*$^{gfp/+}$ CD4$^+$CD45RB^{high} T cells (colitogenic Th17 cells), we assessed phenotypical differences in parallel with in vitro-stimulated RORγt$^+$ Th17 cells differentiated with TGF-β and IL-6 (in vitro-stimulated Th17 cells) (Figure 6*A*). Naturally occurring RORγt$^+$ cells and in vitro-stimulated RORγt$^+$ cells expressed IL-17A, but not IFN-γ, whereas colitogenic Th17 cells included mainly IL-17A$^-$IFN-γ$^+$ Th1-like cells, and some IL-17A$^+$IFN-γ$^+$ Th17/Th1 and IL-17A$^+$IFN-γ$^-$ Th17 cells as previously described.[14] Colitogenic Th17 cells did not express IL-17F, but a very small portion of naturally occurring Th17 cells expressed IL-17F as only IL-17A$^+$IL-17F$^+$ cells. In contrast, approximately one-third of in vitro-stimulated Th17 cells expressed IL-17F as IL-17A$^+$IL-17F$^+$ or IL-17A$^-$IL-17F$^+$ cells. IL-22-expressing cells were detected only in colitogenic Th17 cells as IL-17A$^-$IL-22$^+$ cells, but not in the other 2 RORγt$^+$ populations. Distinct expression of TNF-α among the 3 groups was intriguing because only colitogenic Th17 cells abundantly included an IL-17A$^-$TNF-α$^+$ subpopulation, although all 3 RORγt$^+$ populations equally included an IL-17A$^+$TNF-α$^+$ subpopulation.

Regarding regulatory molecules, Foxp3-expressing cells were detected in naturally occurring Th17 cells and in vitro-stimulated Th17 cells, but not in colitogenic Th17 cells, as IL-17A$^-$Foxp3$^+$ cells. Intracellular CTLA-4[15] was expressed in naturally occurring Th17 cells, whereas it was expressed at a low level in colitogenic Th17 cells and in vitro-stimulated Th17 cells as IL-17A$^+$CTLA-4$^+$ or IL-17A$^-$CTLA-4$^+$ cells. Glucocorticoid-induced tumour necrosis factor receptor (GITR), another regulatory molecule,[16] was expressed exclusively on colitogenic Th17 cells and in vitro-stimulated Th17 cells as IL-17A$^+$GITR$^+$ or IL-17A$^-$GITR$^+$ cells, but very slightly expressed on naturally occurring Th17 cells. Last, we addressed homing receptors: Th17 cell-associated CCR6[17] and gut homing-associated CD103.

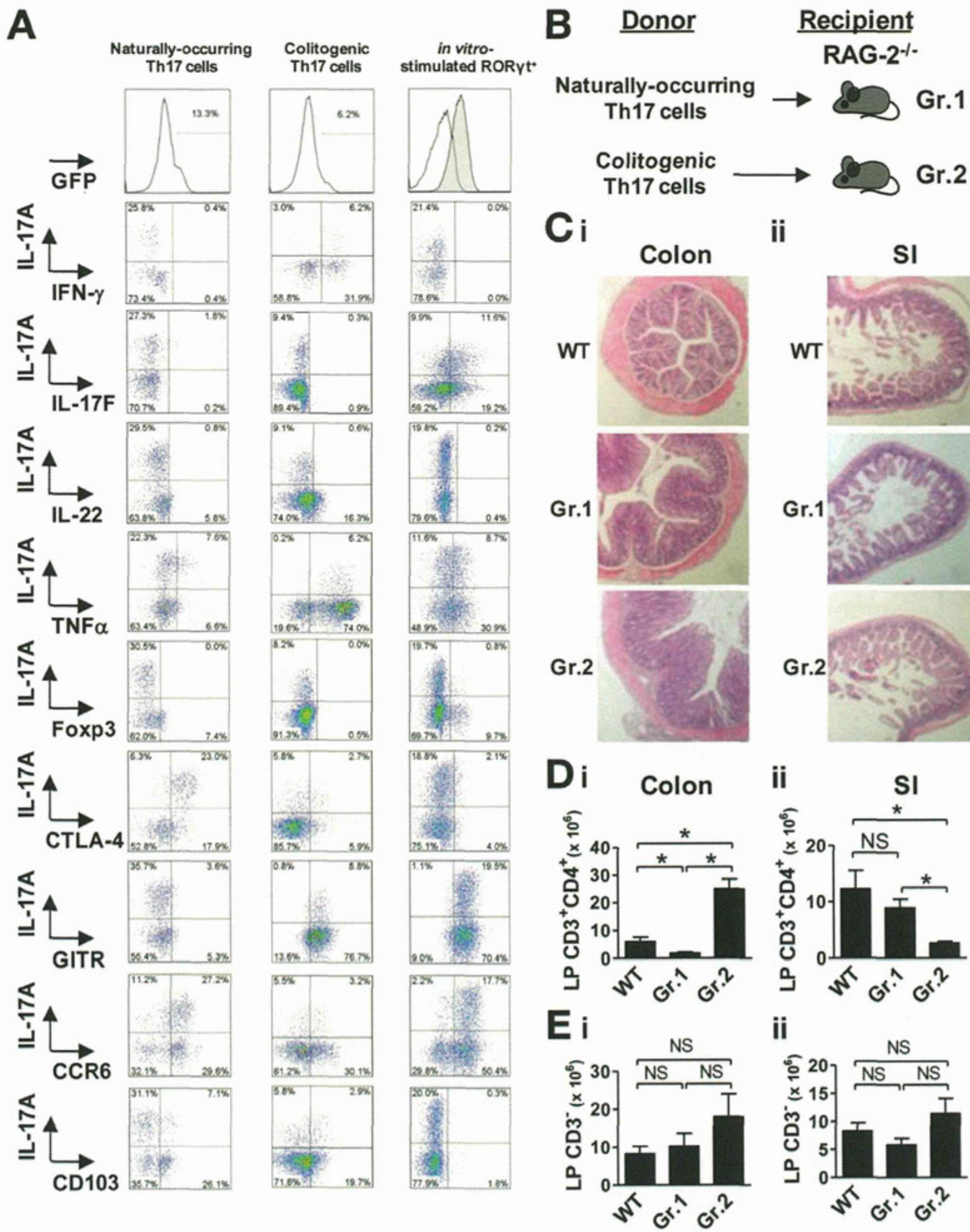

Figure 6. Naturally occurring RORγt$^+$ Th17 cells are not enterocolitogenic cells. (*A*) Expression of Th17-associated cytokines, regulatory molecules, and homing receptors on/in naturally occurring RORγt$^+$ Th17 cells, colitogenic RORγt$^+$ CD4$^+$ T cells, and in vitro–stimulated RORγt$^+$ Th17 cells. (*B*) Scheme of the adoptive transfer experiment. (*C and D*) Histopathology of (*C*) distal colon and (*D*) terminal ileum of the indicated mice 8 weeks after transfer. Original magnification, ×40. (*E and F*) Absolute cell number of (*E*) colonic and (*F*) SI LP CD3$^+$CD4$^+$ T cells of the indicated mice at 6 weeks after transfer. (*D and E*) Data are indicated as the mean ± standard error of the mean (n = 5/group). *$P < .05$. Gr, group.

Naturally occurring Th17 cells and in vitro-stimulated Th17 cells included substantial CCR6$^+$ cells as IL-17A$^+$CCR6$^+$ or IL-17A$^-$CCR6$^+$ cells, although almost all CCR6$^+$ cells in colitogenic Th17 cells resided in the IL-17A$^-$CCR6$^+$ subpopulation. In contrast, almost all in vitro-stimulated Th17 cells did not express CD103, but naturally occurring Th17 cells and colitogenic Th17 cells substantially expressed CD103 as IL-17A$^-$CD103$^+$ cells, and some IL-17A$^+$CD103$^+$ cells. Together, these 3 types of RORγt$^+$ cells were quite distinct at least in view of phenotypical characterization; naturally occurring Th17 cells, resting Th17 cells; colitogenic Th17 (RORγt$^+$) cells, a mixture of Th17, Th17/Th1, and Th1-like cells with activation; and in vitro-stimulated Th17 cells, activated Th17 cells.

We next assessed whether naturally occurring Th17 cells in the SI and colitogenic Th17 cells induced enteritis and/or colitis if transferred into RAG-2$^{-/-}$ mice. RAG-2$^{-/-}$ mice were transferred with naturally occurring Th17 cells (group 1) or colitogenic Th17 cells (group 2) (Figure 6*B*). Group 2, but not group 1, mice developed severe colitis 6 weeks after transfer, whereas neither group 1 nor group 2 mice developed ileitis (Figure 6*Ci* and *ii*). Consistently, the absolute cell number of colonic LP CD4$^+$ T cells obtained from group 2 mice was significantly higher than group 1 mice and WT mice, whereas the SI LP CD4$^+$ T cells obtained from group 1 mice was comparable with WT mice (Figure 6*Di* and *ii*). The number of SI LP CD4$^+$ T cells obtained from group 2 mice was significantly lower than from WT mice (Figure 6*Dii*), suggesting that colitogenic Th17 cells, but not naturally occurring Th17 cells, are pathogenic.

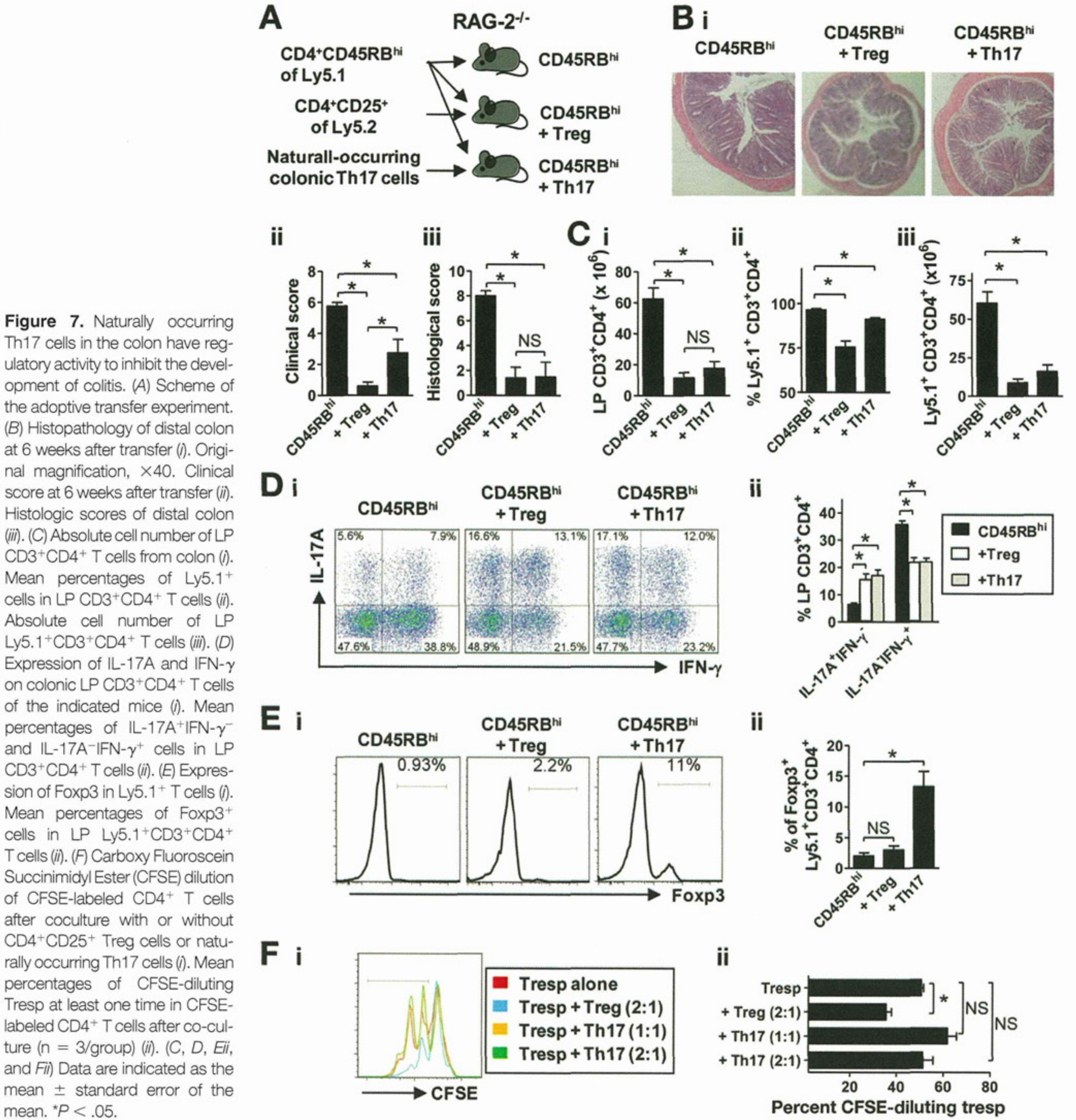

Figure 7. Naturally occurring Th17 cells in the colon have regulatory activity to inhibit the development of colitis. (*A*) Scheme of the adoptive transfer experiment. (*B*) Histopathology of distal colon at 6 weeks after transfer (*i*). Original magnification, ×40. Clinical score at 6 weeks after transfer (*ii*). Histologic scores of distal colon (*iii*). (*C*) Absolute cell number of LP CD3$^+$CD4$^+$ T cells from colon (*i*). Mean percentages of Ly5.1$^+$ cells in LP CD3$^+$CD4$^+$ T cells (*ii*). Absolute cell number of LP Ly5.1$^+$CD3$^+$CD4$^+$ T cells (*iii*). (*D*) Expression of IL-17A and IFN-γ on colonic LP CD3$^+$CD4$^+$ T cells of the indicated mice (*i*). Mean percentages of IL-17A$^+$IFN-γ$^-$ and IL-17A$^-$IFN-γ$^+$ cells in LP CD3$^+$CD4$^+$ T cells (*ii*). (*E*) Expression of Foxp3 in Ly5.1$^+$ T cells (*i*). Mean percentages of Foxp3$^+$ cells in LP Ly5.1$^+$CD3$^+$CD4$^+$ T cells (*ii*). (*F*) Carboxy Fluoroscein Succinimidyl Ester (CFSE) dilution of CFSE-labeled CD4$^+$ T cells after coculture with or without CD4$^+$CD25$^+$ Treg cells or naturally occurring Th17 cells (*i*). Mean percentages of CFSE-diluting Tresp at least one time in CFSE-labeled CD4$^+$ T cells after co-culture (n = 3/group) (*ii*). (*C, D, Eii,* and *Fii*) Data are indicated as the mean ± standard error of the mean. *$P < .05$.

Naturally Occurring Th17 Cells in the Colon Have Regulatory Activity to Inhibit the Development of CD4$^+$CD45RB^{high} T-Cell–Induced Colitis

Given that naturally occurring SI Th17 cells were not colitogenic, we lastly addressed the possibility that naturally occurring Th17 cells could suppress the development of colitis because a substantial subpopulation of these cells expressed CTLA-4 (Figure 6*A*). In this setting, we used colonic naturally occurring Th17 cells to inhibit the development of colitis because colonic, but not SI, naturally occurring Th17 cells would preferentially migrate to the colon because of their tissue-specific trafficking properties. Therefore, we divided the mice into 3 groups as depicted in Figure 7*A*. The results clearly showed that control of intestinal inflammation was caused by the colonic naturally occurring Th17 cells (Figure 7*B*), although the clinical scores of mice co-transferred with CD4$^+$CD45RB^{high} T cells and colonic naturally occurring Th17 cells was more than that of mice co-transferred with CD4$^+$CD45RB^{high} T cells and CD4$^+$CD25$^+$ Treg cells (Figure 7*Bii*). Consistently, co-transfer in RAG-2$^{-/-}$ mice significantly inhibited the development of colitis (Figure 7*Biii*) as compared with mice transferred with

$CD4^+CD45RB^{high}$ T cells alone. We consistently observed significantly fewer numbers of colonic $CD4^+$ T cells in the mice co-transferred with $CD4^+CD45RB^{high}$ T cells and Treg cells or naturally occurring Th17 cells compared with mice transferred with $CD4^+CD45RB^{high}$ T cells alone (Figure *7Ci*). Although it was possible that naturally occurring Th17 cells expanded and thereby prevented colitis, this was not the case because a major population in co-transferred mice was cells derived from $Ly5.1^+$ $CD4^+CD45RB^{high}$ T cells (Figure *7Cii* and *iii*). Furthermore, the proportion of IFN-γ^+IL-17A$^-$ Th1 cells in the colon of mice transferred with $CD4^+CD45RB^{high}$ and Treg cells or naturally occurring RORγt$^+$ cells was reduced significantly as compared with that in mice reconstituted with $CD4^+CD45RB^{high}$ alone. The percentage of IFN-γ^-IL-17A$^+$ Th17 cells in mice reconstituted with $CD4^+CD45RB^{high}$ and Treg cells or naturally occurring Th17 cells was increased significantly as compared with that in mice reconstituted with $CD4^+CD45RB^{high}$ alone (Figure *7Di* and *ii*). Of note, cotransfer of naturally occurring Th17 cells, but not Treg cells, resulted in the significant increase of $Foxp3^+$ cells derived from $Ly5.1^+$ $CD4^+CD45RB^{high}$ T cells (Figure *7Ei* and *ii*). However, unlike Treg cells, naturally occurring Th17 cells did not suppress the proliferation of naive $CD4^+$ T cells (Figure *7Fi* and *ii*). These data suggest that naturally occurring Th17 cells function as another type of Treg cells in the colon.

Discussion

In the current study, we showed that the existence of an LTα-dependent GALT structure is essential for the generation of intestinal naturally occurring Th17 cells in the steady state, but the lack of LTα molecules results in a marked increase in IL-17–producing LTi-like cells. In sharp contrast to physiological conditions, the generation of colitogenic Th17 cells during inflammation is not dependent on LTα. Consistent with the distinct developmental mechanisms of naturally occurring and colitogenic Th17 cells, naturally occurring Th17 cells have regulatory activity to suppress the development of colitis. Our current immunologic scenario is depicted in Supplementary Figure 7.

Th17 cell differentiation in vitro from naive T cells requires the coordinated action of multiple cytokines.[18–20] In vitro stimulation with TGF-β and IL-6 can induce Th17 differentiation dependent on RORγt and STAT3.[19] Recently, however, it has been shown that Th17 differentiation can occur in the absence of TGF-β signaling.[21] Th17 cells in vitro generated with IL-6 and IL-23 but in the absence of TGF-β were encephalitogenic, whereas Th17 cells generated with IL-6 in the presence of TGF-β were not.[21] Here, we showed that the proportion of naturally occurring Th17 cells markedly decreased in the SI of LT$\alpha^{-/-}$ mice despite their intact RORγt gene, although in vitro–manipulated Th17 cells normally develop from LT$\alpha^{-/-}$ naive T cells.

Knowledge of the origin and relationship among various innate lymphocyte populations is admittedly incomplete at present. Because LTi-like cells differentiate into $NKp46^+$ cells at a point when RORγt levels have decreased, and begin to produce IFN-γ to have a colitogenic potential, LTi-like cells do express NKp46 at a certain point.[9] However, a contrary theory has been put forward by Sawa et al[13]; LTi cells and $NKp46^+$ (IL-22–producing) innate NK22 cells are derived from separate lineages so that the latter cells are not LTi-like cells. Given the fact that the IL-17–producing $CD3^-$ cells arising in mice lacking LTα do not bear NKp46, we would conclude that our IL-17–producing $CD3^-$ cells are LTi-like cells, but are nevertheless distinct from those LTi-like cells that have been implicated as colitogenic cells by other groups.[8,9] Furthermore, the present study showed that the proportion of NK22 cells also was increased in the SI of LT$\alpha^{-/-}$ mice in parallel with the increase of LTi-like cells, suggesting the sequential development of NK22 cells from IL-17–producing LTi-like cells even in the absence of LTα/lymphotoxin β receptor (LTβR) signaling.

The possible role of LTi-like cells on the pathogenesis of colitis should be discussed. We showed that IL-17A–expressing LTi-like cells were increased significantly in LT$\alpha^{-/-}$ mice, and those cells produced a large amount of IL-22. Furthermore, LT$\alpha^{-/-}$×RAG-2$^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells did develop colitis, but the severity of those mice was significantly less than that of the control RAG-2$^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells. Although it is possible that the decreased severity in LT$\alpha^{-/-}$×RAG-2$^{-/-}$ mice transferred with $CD4^+CD45RB^{high}$ T cells is the result of another possibility, that LT$\alpha^{-/-}$×RAG-2$^{-/-}$ mice lack GALT including mesenteric lymph nodes, LTi-like cells may function as protective cells that produce IL-22. Further studies are needed to address this issue.

To investigate whether LTα expression on $CD4^+$ T cells is required for the development of Th17 cells, we adopted a parabiosis system between $Ly5.1^+$ WT and $Ly5.2^+$ LT$\alpha^{-/-}$ mice. Our data showed that LTα expression on $CD4^+$ T cells is dispensable for the development of Th17 cells in the steady state. In contrast, LTi-like cells were restricted to the original SI as resident cells of the SI even after parabiosis surgery. These results clearly show that LT$\alpha^{-/-}$ $CD4^+$ T cells migrate to the SI of WT mice that retain normal GALT residing LTα-expressing LTi-like cells, and thereafter become Th17 cells in the GALT. By using BM chimeras, we observed the expression of LTα on BM-derived cells was dispensable for the development of naturally occurring Th17 cells, but that it was dependent on LTα expression in recipient mice. This suggested the development of naturally occurring Th17 cells required the existence of GALT as the induction site. In this regard, Atarashi et al[2] showed that naturally occurring Th17 cells develop normally in PP-null mice that are generated by injection with anti–IL-7Rα monoclonal antibody (A7R34) during the gestational period. Importantly, because PP-null mice develop normal cryptopatches and isolated lymphoid follicles after birth, we concluded that naturally occurring Th17 cells are generated in the GALTs, except for PP.

To investigate whether the LTα1β2/LTβR signaling pathway was essential for the development of colitogenic Th17 cells during inflammation, we performed adoptive

transfer using a colitis model induced by transfer of $CD4^+CD45RB^{high}$ T cells. We found that $LT\alpha^{-/-}\times RAG\text{-}2^{-/-}$ recipients developed colitis 6 weeks after transfer, despite the lack of GALT, although the clinical and histologic scores in the groups using $LT\alpha^{-/-}\times RAG\text{-}2^{-/-}$ recipients was significantly milder than those in the groups using $RAG\text{-}2^{-/-}$ recipients. This is not surprising because this difference may be caused by the absence of GALT, including mesenteric lymph nodes that are the main places for priming naive $CD4^+$ T cells in $LT\alpha^{-/-}\times RAG\text{-}2^{-/-}$ recipients.[15] Notably, comparable proportions of Th17 and Th17/Th1 cells emerged in colonic LP of all 4 groups whether in $RAG\text{-}2^{-/-}$ or $LT\alpha^{-/-}\times RAG\text{-}2^{-/-}$ recipients. These results indicated that the $LT\alpha/LT\beta R$ signaling pathway is not required for the development of colitogenic Th17 and Th17/Th1 cells that were hardly detected in physiological conditions. This may be consistent with a recent report showing that LTi-like cells in inflammatory conditions lose the expression of RORγt with an ability to produce IFN-γ production by those cells.[9] Notably, these inflammatory RORγt-losing LTi-like cells down-modulated the expression of surface LTα molecules, suggesting a possibility that other molecules rather than LTα may be involved in the induction of pathologic (colitogenic) Th17 and Th17/Th1 cells in inflammatory conditions.

This study indicates that LTα are essential for the development of naturally occurring Th17 cells in the steady-state condition, but colitogenic Th17 and Th17/Th1 cells develop by different mechanisms independent of GALT and LTα-expressing LTi-like cells, suggesting a link between the current topics regarding protective and pathogenic Th17 cells in vivo.

Supplementary Materials

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2012.07.108.

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Conflicts of interest

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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