EXPERT REVIEWS

The balance of intestinal Foxp3$^+$ regulatory T cells and Th17 cells and its biological significance

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Balanced mucosal immunity in the gut is critical for host homeostasis and defense. Th17 cells are a subset of IL-17-producing CD4$^+$ T cells, which play a crucial role in clearing pathogens during host defense reactions and in inducing tissue inflammation in autoimmune diseases. CD4$^+$CD25$^+$ Foxp3$^+$ regulatory T cells (Tregs) are recognized as one of the major regulatory factors in immune tolerance and inflammatory responses. Since both Tregs and Th17 cells pertain to the gut immune system, their inter-regulation and balance represent a novel mechanism for maintaining the intestinal immune and inflammatory homeostasis. Accordingly, the imbalance and dysregulation of Tregs and Th17 cells in the intestine is closely associated with intestinal autoimmune disorders like the inflammatory bowel diseases. In this review, we discuss the characteristics of gut Tregs and Th17 cells and their role in gut diseases.

KEYWORDS: immune regulation • intestine • mucosal immunity • Treg • Th17

The gut is an important organ with multiple roles. It is a digestive organ that comes in direct contact with food proteins, commensal bacteria and exogenous pathogenic microorganisms. It also acts as a lymphoid organ by promoting immune tolerance to food, self-antigens and parasites, and by mounting immune responses against exogenous pathogenic microorganisms [1,2]. In general, IFN-γ^+/T-bet$^+$ Th1 cells are recognized as the prominent cells in defending against the invasion of intracellular pathogens. On the other hand, IL-17-producing CD4$^+$RORγt$^+$ Th17 cells, which exhibit effector functions distinct from Th1 and Th2 cells, play important roles in the clearance of extracellular pathogens and fungi in the intestine [3]. CD4$^+$CD25$^+$Foxp3$^+$ Treg, also found in the gut, are capable of suppressing the functions of Th1 and Th17 cells, thereby downregulating excessive immune responses and establishing oral tolerance [4]. The dysregulation of T-cell subsets is markedly related to the pathogenesis of the intestine in inflammatory diseases and cancer. This review will focus on the regulation of intestinal Th17 cells and Treg and discuss their biological significance in inflammatory diseases and cancer.

The characteristics of Treg & Th17 cells in the intestine

Treg are a subset of CD4$^+$ T cells that highly express IL-2R α chain (CD25) and Foxp3, and are commonly categorized into thymus-derived natural Treg (nTreg) and induced Treg (iTreg). Naïve CD4$^+$ T cells in peripheral lymph nodes (LNs), spleen and the intestine can also differentiate into iTreg via the stimulation of T-cell antigen receptor in the presence of TGF-β and IL-2 *in vitro* and *in vivo* (FIGURE 1) [5]. The majority of Treg present in most sites are nTreg, while the intestines contain local factors such as TGF-β and chronic antigen exposure that preferentially promote iTreg differentiation [6]. Previous studies suggested that intestinal homeostasis depends on microbiota-specific induced Treg [7], while recent studies found that thymus-derived nTreg constitute most Treg in intestinal organs and play a crucial role in intestinal homeostasis [8]. Further studies are needed to shed light on this issue. Under steady state, Treg migrate to the small intestine lamina propria (SI-LP) to exert their immunoregulatory function via their expression of CCR9 and α4β7 [6]. By contrast, the large intestine uses

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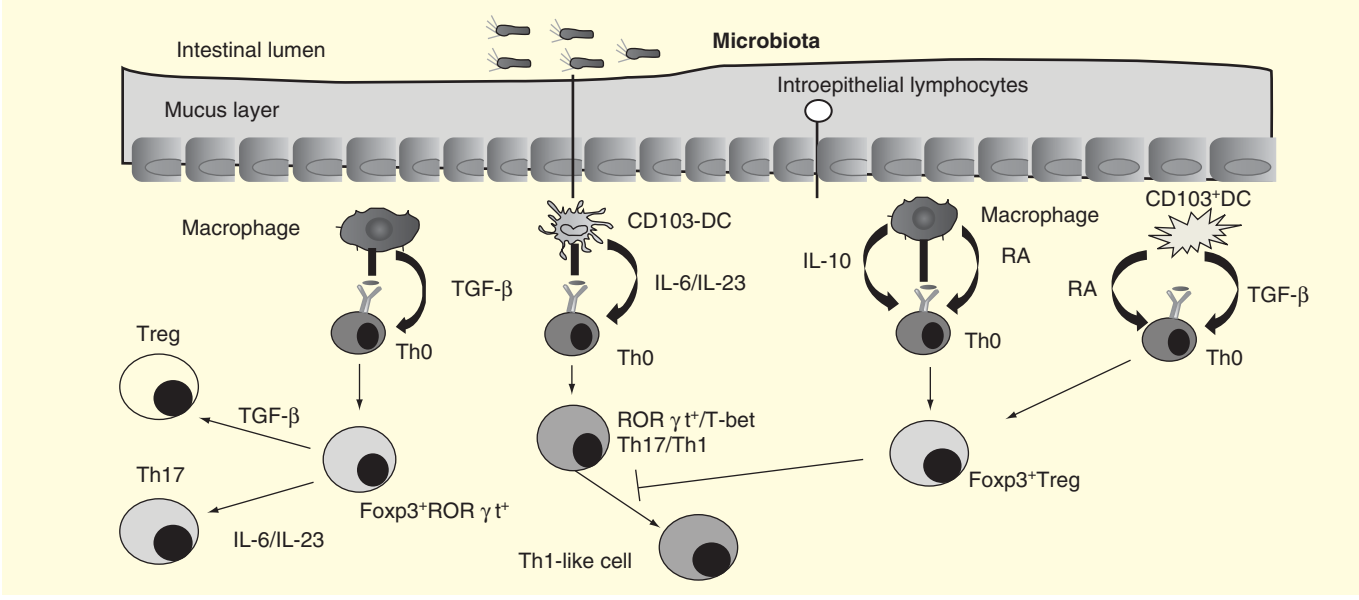

Figure 1. Interactions between Th17 and Treg cells in the gut. Treg cells are mainly induced by macrophages and CD103$^+$ DCs in the lamina propria of the gut via the production of retinoic acid and TGF-β. Treg cells inhibit the function of Th1-like cells and regulate the differentiation of Th1-like cells from Th17/Th1 cells in the inflamed gut. There is an abundance of T cells expressing both Foxp3 and RORγt in the gut of healthy individuals. These cells can further differentiate into Treg cells or Th17 cells through different cytokine conditions such as TGF-β or IL-6/IL-23.
RA: Retinoic acid.

different homing cues coupled to different homing receptors for Treg. GPR15, an orphan heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptor, was identified to control the homing of Treg to the large intestinal LP [9].

Th17 cells are a lineage of CD4$^+$ T cells distinct from Th1 and Th2 cells, and have the ability to secret proinflammatory cytokines such as IL-17A, IL-17F, GM-CSF and IL-22 [10–12]. They mainly exist in the intestine and protect vertebrates from exogenous pathogenic microorganisms through the secretion of IL-17A and IL-17F used to recruit macrophages and neutrophils to infected tissues [13,14]. They also promote epithelia cell proliferation and survival, and tissue repair in the intestine via the secretion of IL-22 [15]. RAR-related orphan receptor γ t (RORγt) and RAR-related orphan receptor α (RORα) are two key transcription factors in mice Th17 cells and RAR-related orphan receptor C in humans. Other transcription factors such as IRF4 [16], Runx1 [17], BATF [18], STAT3, c-Maf [19] and AHR [20] may also be important for the induction of Th17 cells. The differentiation of Th17 cells from naïve CD4$^+$ T cells *in vitro* required TGF-β1 and IL-6 via the STAT3 signal transduction pathway, whereas IL-23, IL-1β and/or IL-6 were suggested to induce Th17 differentiation in the intestine [21–23]. Like Treg in the LP, Th17 cells also migrate to the surface of the intestinal mucosa through the action of CCR6 and CCL20 on the surface of the intestinal epithelial cells (IECs) [24,25].

The balance between Th17 & Treg in the gut

The requirement for a homeostatic balance between immunity and tolerance in the intestine is of great importance to the mucosal immune system. Since immune cells are distributed throughout the gut-associated lymphoid tissues (GALTs), they must remain immunologically hyporesponsive to commensal bacteria while retaining their capacity to respond to a pathogenic challenge. Simultaneously, immune cells like dendritic cells (DCs) and macrophages, non-immune cells such as IECs and local environmental factors regulate the balance between Th17 and Treg in the intestine [26,27]. We will highlight these issues.

The role of antigen-presenting cells in the balance between Th17 & Treg in the gut

DCs and macrophages are situated in the GALT and LP, and they encounter the majority of microbial antigens at mucosal surfaces under homeostatic conditions [24]. There are several subsets of intestinal antigen-presenting cells (APCs), which are characterized by their varying levels of cell surface markers such as CD11c, CD11b, F4/80, CD8α and CD4. The nature and function of DCs and macrophages occupying the intestinal LP were previously reviewed [28]. We will discuss the specific subset of DCs and macrophages responsible for the differentiation of Th17 cells and/or Treg, and their function on Th17 and/or Treg.

CD103$^+$ DCs are normally dispersed throughout the LP and migrate to mesenteric lymph nodes (mLNs) in a CCR7-dependent manner [6]. They promote the differentiation of Treg via the production of retinoic acid (RA) and IL-10 in the presence of TGF-β1 in the mLNs [29]. A study reported that the expansion and maintenance of Treg in the LP are directed by CX3CR1$^+$ LP macrophages through their secretion of IL-10 [30]. Recent studies, however, discovered that all DC

subsets could do the same and particularly at the higher T:APC ratios *in vitro* [24], thus challenging the unique role of $CD103^+$ DCs and $CX3CR1^+$ LP macrophages in the induction of Treg in the intestine. It was reported that mLNs rather than intestinal tissues are essential for generating intestinal Th17 cells [31]. $CD103^-CD11b^+$ DCs but not $CD103^+CD11b^-$ DCs in mLNs induce Th17 cell differentiation possibly due to their high expression of IL-23 or IL-6 [24,32,33]. LP $CD11c^-F4/80^+$ macrophages in the small intestine, which sample the commensal microbiota, also play critical roles in the induction and possibly in the maintenance of Th17 cells in an IL-1β-dependent manner [34,35]. Since $CD103^+CD11b^+$ DCs isolated from steady-state intestinal lymph were ineffective in inducing Th17 cell differentiation [33], inflammatory signals from $CD103^+CD11b^+$ DCs may be important to promote an efficient Th17 cell-polarizing capacity. LP $CD103^+CD11b^+$ DCs in the small intestine were consistently shown to rapidly produce IL-23 after intraperitoneal administration of flagellin [36]. In conclusion, subsets of DCs and macrophages in the intestine drive the differentiation of and balance between Treg and Th17 cells (Figure 1).

The role of IECs in the balance between Th17 & Treg in the gut

The intestinal epithelium is composed of a single layer of IECs, which acts as a physical barrier that separates the host connective tissue from the external environment. Intercellular tight junctions, which are coupled with actin-rich microvillar extensions, create a brush border on the apical surface of the epithelium that impedes microbial attachment and invasion [37]. Recent studies found that the intestinal epithelial goblet cells can also capture soluble, low molecular weight molecules and then present them to LP $CD103^+$ DCs in the intestines [38]. In addition to providing a physical barrier and secreting antimicrobial peptides against pathogenic and commensal bacteria, they can also influence the function of APCs and lymphocytes in the intestinal microenvironments [37].

Under steady-state conditions, IECs maintain a hyporesponsive state to the commensal flora and secret thymic stromal lymphopoietin, TGF-β and RA. IECs-derived thymic stromal lymphopoietin was found to activate $CD8\alpha^+$ DCs that can promote the differentiation of T cells with a regulatory capacity [39], and it could also influence the Th1-cell differentiation by inhibiting the expression of IL-12 by DCs *in vitro* [37]. IECs-derived TGF-β and RA were found to drive the differentiation of Treg-inducing DCs via upregulating the expression of CD103 and the induction of *aldh1a2* [40]. Thus, all three of the molecules drive DCs in the intestine to acquire a tolerogenic phenotype in the steady state. Furthermore, there is increasing evidence that IECs can regulate intestinal lymphocytes via direct and/or indirect pathways. RA can be produced by both IECs and $CD103^+$ DCs. During chronic ileitis, there is a decreasing number of DCs in the intestine as well as a lower concentration of RA [41]. IECs responded to reduced levels of RA by upregulating RALDH3 *in vivo* and *in vitro* [41]. Although IECs could not completely rescue the lower levels of RA, their synthesis of RA help to maintain local RA levels, and thus possibly ameliorate disease progression as adequate RA supplementation was proven to significantly attenuate disease progression by enhancing the number and function of $CD103^+$ DCs and Treg while suppressing Th17 cells [41]. Thus, IECs regulate the balance between Th17 and Treg by producing RA. The IECs-intrinsic Notch signaling was also found to function on Th17 cells possibly via an indirect pathway, as loss of Notch signaling in IECs resulted in chronic colitis characterized by the accumulation of Th17 cells in the colonic LP [42]. Together, these studies highlight that IECs-derived signals control innate and adaptive immune-cell functions. Elucidating the recognition of commensal bacteria by IECs and the specific mechanisms IECs use to regulate the functions of APCs and lymphocytes offer future challenges and therapeutic prospects.

The role of microorganisms in the balance between Th17 & Treg in the gut

Numerous microorganisms such as commensal bacteria exist in the intestine [43,44]. Since germ-free (GF) animals do not develop experimental colitis and the gut microbiota is substantially altered in inflammatory bowel disease (IBD) patients [45], maintaining the homeostasis of the intestinal microbial milieu is essential to good health [46]. There are immune cells producing IL-17 in the small intestine of newborn mice [47], but they are not Th17 cells and the number of Th17 cells increases gradually with age [44]. Notably, the number of Th17 cells in the gut is much lower in GF or antibiotic-treated mice [47]. Moreover, the level of IL-17 mRNA was lower than that of specific pathogen-free (SPF) mice at the same age, while the ratio of Treg greatly increased [47]. After transferring the fecal matter of SPF mice into GF mice, the number of Th17 cells and the level of IL-17 mRNA increased to the same level as SPF mice, while the ratio of Treg decreased gradually [47]. Thus, the induction of Th17 cells in the gut also requires the participation of the commensal microbiota. Several specific bacterial taxons were found to promote Th17 differentiation and the mechanisms that intestinal commensal bacteria used to induce Th17 differentiation included toll-like receptor (TLR) and ATP signaling [48]. Studies have also shown that GF animals have altered Treg proportions in the gut, suggesting that the commensal microbiota plays a key role in the development of both pro- and anti-inflammatory T-cell subpopulations (Figure 1) [49].

Ivanov *et al.* discovered that segmented filamentous bacterium could induce the differentiation of Th17 cells and block the invasion of *Citrobacter* species by adhering to Th17 cells residing on the surface of IECs [50]. Other floras such as the *Clostridium* species [48] and *Bacteroides fragilis* [51] are also able to induce the differentiation of Treg. Mazmanian *et al.* did a series of work on polysaccharide A (PSA), which is produced by the human-symbiont *B. fragilis*. They reported that PSA was able to protect animals from experimental colitis caused by *Helicobacter hepaticus*, possibly by suppressing IL-17 production and enhancing IL-10 production [52]. Further results revealed that besides suppressing IL-17 production, PSA also mediated

the local expansion of Treg in the gut during commensal bacteria colonization [49]. *B. fragilis* lacking PSA did not exhibit the same effect, and oral administration of PSA in mice resulted in an increased number of Treg in the gut [49]. In addition, Treg from PSA-treated mice showed a PSA-specific profile with an enhanced capacity of suppressing effector T cells *in vitro* and expressed higher levels of IL-10, TGF-β2, granzyme B and CCR6 mRNA [51]. The development of Treg in response to PSA required the expression of TLR2 [49]. The engagement of TLR2 signaling directly on Treg also contributed to suppressing mucosal Th17 immunity since TLR2-deficient Treg were unable to reduce Th17 responses by PSA [51]. These results provide a molecular mechanism for PSA's anti-inflammatory properties through regulating the balance between Th17 cells and Treg [51].

In addition to *B. fragilis*, *Clostridium* species belonging to clusters XIVa and IV are also thought to be critical in inducing the differentiation of Treg in the gut. Matrix metalloproteinases were identified to mediate the conversion of TGF-β from the latent to the active form in the gut [53]. These *Clostridium* bacteria upregulate the expression of indoleamine 2,3-dioxygenase (IDO) and matrix metalloproteinases in colonic epithelia cells to promote an environment rich in TGF-β, thereby affecting Tregs number [54]. Tregs induced by *Clostridium* bacteria also express high levels of IL-10 [54]. Further isolation of *Clostridium* species showed that 17 species fall within clusters IV, XIVa and XVIII, which lack prominent toxins and virulence factors, help in the proliferation and differentiation of Tregs [55]. Oral administration of the 17 strains combined was proven to be effective in treating mice in the models of colitis and allergic diarrhea [55]. Thus, using the isolated strains may provide promising therapies for treating human immune disorders [55]. In general, microorganisms in the gut regulate the homeostasis through influencing the induction of Th17 cells and/or Treg. The molecular mechanisms underlying the microbiota-mediated development of Th17 cells and Treg are still unclear and further studies may shed light on this matter by investigating the microbial composition and their metabolites.

The role of selected key molecules in the balance between Th17 & Treg in the gut

TGF-β

TGF-β, relatively abundant in the intestine, is produced by numerous subsets of cells including IECs and APCs. TGF-β1 alone can induce the expression of Foxp3 in naïve $CD4^+$ T cells, and 3–4 days of continuous stimulation resulted in $CD4^+$ T cells expressing both RORγt and Foxp3 *in vitro* [56]. Foxp3 could directly bind RORγt and RORα to inhibit the association of RORγt with the *Il17* promoter and eventually decrease the expression of IL-17 and the induction of Th17 cells [57]. Hence, these $ROR\gamma t^+$ $Foxp3^+$ T cells, which co-express two transcription factors, do not express Th17-related cytokines such as IL-17A and/or IL-22, and display an immunosuppressive function. Proinflammatory signals such as IL-6 and IL-1β can reverse this effect through STAT3 signaling and promote the differentiation of Th17 cells from $ROR\gamma t^+$ $Foxp3^+$ T cells [58,59].

Does TGF-β1 have an indispensible role in the induction of Th17 cells and Treg in the intestine? Studies suggested that TGF-β1 downregulated the expression of T-bet, GATA-3 and Gif1 [60], which play a critical role in enhancing Th2 cell expansion, and further suppressed the induction of Th1 and Th2 cells as well as their associated cytokines like IFN-γ and IL-4. IL-6 alone in the absence of T-bet and STAT6 can induce Th17 cells without the participation of TGF-β1 [61], and Th17 cells also exist in the gut of TGF-βR knock-out mice [21]. It is also apparent that some Th17-cell-specific genes such as *Il22* and *Il21* are regulated via a TGF-β1-independent pathway [62–64]. Therefore, TGF-β may be dispensable in suppressing the differentiation of naïve T cells into Th1 and Th2 cells.

The level of TGF-β1 in the small intestine influences the balance between Th17 cells and Treg. Low level of TGF-β1 allows increased expression of IL-23R mRNA in naïve T cells, subsequently promoting more efficient differentiation of Th17 cells [65]. By contrast, high levels of TGF-β1 enhance Foxp3 expression and repress IL-23R transcription, consequently moving T-cell-differentiation away from the Th17 transcriptional program [66].

Recently, Lee *et al*. found that TGF-β1-induced Th17 cells are less reactive, and after exposure to IL-23, these cells became extremely reactive and produced TGF-β3 autonomously [67]. These TGF-β3 can further maintain and stabilize Th17 cells as TGF-β3 together with IL-6 can generate reactive Th17 cells without the need of IL-23 or TGF-β1 [67]. Thus, both TGF-β1 and TGF-β3 are capable of inducing Th17 cells, while the two Th17 cell subsets may differ significantly. A thorough understanding of the mechanisms of TGF-β1 and TGF-β3 in regulating immunity and tolerance may provide clues for promising therapies in treating intestinal diseases.

Retinoic acid

RA, a metabolite of vitamin A, is produced by APCs in the intestine [68]. $CD103^+$ DCs in the LP, unlike other DC subsets, have a strong RA-producing capacity by expressing high levels of *Aldh1a1* and *Aldh1a2*, which are involved in the metabolism of vitamin A to RA [68]. RA promotes the development of Treg in the presence of TGF-β1; the induction of Treg was significantly blocked by RA inhibitor and RARi [69]. On the other hand, RA could also suppress the development of Th17 cells and induce the differentiation of Treg by downregulating the expression of IL-6 mRNA and IL-6α [70]. This effect of RA is dependent on the expression of RARα in naïve T cells [71]. These results collaboratively imply that RA may be a crucial co-stimulatory factor for the induction of Tregs [70]. The complete mechanism of RA in the development of Tregs and Th17 cells are still in debate. The identification of a potential RAR binding site on conserved non-coding sequence 1 partly explained the mechanism of RA in enhancing Smad3 activity [68]. Recent studies also suggested that RA could act on $CD103^+$ DCs to promote the expression of Arg1 to indirectly regulate Treg differentiation [72].

Recently, the anti-inflammatory effects of RA on immune responses have been challenged. Uematsu *et al.* suggested that induced innate stimuli contrast the effects of RA on either DCs or T cells as they found that production of RA by $CD11c^{hi}CD11b^{hi}$ LP DCs, when stimulated by the TLR5-ligand flagellin, prompted a differentiation of antigen-specific Th17 and Th1 cells [73]. DePaolo *et al.* demonstrated that RA led to enhanced pathogen responses and celiac disease-like inflammation by promoting the differentiation and enhancing the function of inflammatory DCs in the presence of IL-15 [74]. In addition, Hall *et al.* recently suggested that RARα signaling is indeed required for developing efficient Th1/Th17 intestinal immune responses by using a model of *Toxoplasma gondii* infection [75]. Hence, pathogenic signals may switch off the tolerogenic functions of RA when an immune response is needed to maintain the homeostasis of the intestinal immune system. It was also notable that Th17 cells were ablated in the GALT of mice reared on a vitamin A-deficient diet during steady state [76]. The ability of RA to support Th17 cell differentiation was dependent on the co-operative action of DCs and T cells because addition of RA to Th17 cell-polarizing conditions with fewer APCs did not enhance Th17 cell differentiation [76]. In summary, the RA pathway plays diverse roles in the adaptive immune response and their functions may be environment-dependent.

Surprisingly, trans RA (the geometric isomer of RA) inhibits the TGF-β1-mediated induction of *Il10* transcription in $CD4^+$ T cells even though Treg are resistant to this, while changes to the posttranscriptional regulation of *Il10* were undetectable [69]. It was proven that Th17 cells express IL-10R and are controlled by Treg and Tr1 cells partly in an IL-10-dependent manner [77,78]. IL-10 blockade by macrophages in the LP increased the ratio of Th17 cells to Th17/Th1 cells [79,80]. Therefore, RA may also regulate the differentiation of Th17 cells by indirectly inhibiting the transcription of *Il10* and the effect may be more pronounced in Tr1 cells, which play prominent roles in the gut immunity.

Involvement of Treg & Th17 cells in intestinal diseases

Animal models of IBD

Deletion of or mutations in the gene encoding Foxp3 result in a fatal inflammatory disease in mice, and X-linked syndrome in humans is accompanied by severe intestinal inflammation [81]. Mice with a *Stat3* mutation in $Foxp3^+$ Treg develop severe colitis due to elevated numbers of Th17 cell infiltration in the intestine [82]. The underlying mechanisms involve decreased chemokine receptor CCR6 expression on Treg, which prevent migration into the intestine, and increased IL-6 and TGF-β1 expression, which promote the differentiation of Th17 cells [82].

The relative abundance of Th17 cells at mucosal sites and the increased levels of Th17 cytokines in the inflamed gut have fuelled interests in elucidating the role of Th17 cells in IBD pathogenesis [83]. This was confirmed in the subsequent studies. Muc1 is a membrane-bound mucin upregulated by Th17 cell signaling and is involved in a negative feedback loop that prevents excessive Th17 cell responses in inflamed mouse colons. Loss of Muc1 led to the development of more severe colitis with increased number of Th17 cells in mice [84]. The transfer of naïve $CD4^+$ T cells from mice deficient in the transcription factor RORγt into $RAG^{-/-}$ mice failed to induce colitis, whereas the transfer of Th17 cells into $RAG^{-/-}$ mice induced severe intestinal inflammation [85]. Interestingly, the two major Th17 cell-secreted cytokines, IL-17A and IL-17F, contribute differently to the pathogenesis of colitis. IL-17A suppresses mouse colitis by blocking the development of Th1 cells via IL-17R on naïve T cells, while IL-17F promotes the differentiation of Th1 cells during the late stage of colitis [86]. Although Th17 cells can induce colitis alone, their competition with Th1 cells is also important in the pathogenesis of colitis in mice [85]. $RAG1^{-/-}$ mice transferred with naïve T cells from $T\text{-}bet^{-/-}$ or $ROR\gamma t^{-/-}$ mice did not develop colitis [87]. Transfer of naïve T cells into $RAG^{-/-}$ mice can induce $ROR\gamma t^+$ T cells and $ROR\gamma t^-$ T cells. $ROR\gamma t^+$ T cells consist of $IL\text{-}17A^-IFN\text{-}\gamma^+$ Th1 cells, $IL\text{-}17A^+IFN\text{-}\gamma^+$ Th17/Th1 cells and $IL\text{-}17A^+IFN\text{-}\gamma^-$ Th17 cells, whereas $ROR\gamma t^-$ T cells consist of $IL\text{-}17A^-IFN\text{-}\gamma^+$ Th1 cells [88]. After retransferring $ROR\gamma t^+$ T cells or $ROR\gamma t^-$ T cells into $RAG2^{-/-}$ mice, $ROR\gamma t^-$ T cells remained $ROR\gamma t^-$, which only produce IFN-γ, and most $ROR\gamma t^+$ T cells became Th1 cells or Th17/Th1 cells [88]. Thus, there is a distinct development map from Th17 to Th1, possibly via Th17/Th1- and Th1-like cells during the induction of colitis *in vivo*. Nevertheless, it remains unclear whether or not these $ROR\gamma t^-$ T cells (Th1 cells) are generated in a RORγt-dependent manner and whether or not this pathway can occur under lymphosufficient conditions, as the lymphopenic microenvironment may favor the transformation of Th17 cells to alternative Th1 cells [88]. Additional studies are also required to reveal the possible relationship between T-bet and RORγt in influencing the pathogenesis of colitis.

Today, there is a plethora of animal IBD models in which IBD is induced by experimental manipulations or spontaneously developed to reflect distinct human IBD features. It is, however, extremely difficult to perfect a model to reproduce all pathological features of human IBD. A recent study showed that around 100 distinct genetic loci may contribute to IBD susceptibility [83]. Moreover, the gut microbiota, a key contributor to human IBD development, is unique to each individual [89]. Thus, further work is necessary to uncover the pathogenesis of IBD and the factors that influence the balance between Th17 cells and Treg in humans.

Human IBD

Crohn's disease (CD) and ulcerative colitis (UC) were thought to be mediated by IFN-γ producing Th1 cells and IL-13/IL-4 producing Th2 cells for years until the recent discovery of Th17 cells, which expand in response to the proinflammatory cytokine IL-23 [90]. The treatment using Abs to block IFN-γ or IL-12/p40 showed disappointing results in CD patients [91,92] and the gut of UC patients contains more Th17 cells compared

Table 1. Th17-related intestinal diseases in animal models and humans.

Th17-related cytokines	Role in intestinal diseases	
	Protective	*Causative*
IL-17A	Mouse colitis [117]	Mouse colitis [118,119] CAC [110] CD [94] UC [93]
IL-17F	Colorectal cancer [113]	Mouse colitis [86] CD [120]
IL-21		Mouse colitis [121] CAC [111]
IL-22	Mouse colitis [122]	CD [94] Colon cancer [112]
IL-23	Mouse colitis [123] Mouse colon carcinoma [124]	Mouse colitis [125] CD [126] UC [107] CAC [114] Colorectal carcinoma [127]

CAC: Colitis-associated cancer; CD: Crohn's disease; UC: Ulcerative colitis.

with healthy controls or even CD patients [93]. Further studies revealed that an increased number of $CD73^+$ T cells, which showed a memory Th17 cell phenotype with the ability to secret IL-17, IL-22 and TNF-α, was also present in the intestine of patients with active CD [94–96]. Thus, Th17 cells play critical roles in both forms of human IBD (Table 1). It is postulated that in mice with CD-like colitis, Th1 cells facilitate the initial IBD phases while Th17 cells dominate the later phases [97,98]. Indeed, a treatment involving both Th17 and Th1 cells and their respective cytokines may be promising.

Unlike CD, the etiology of UC is more intricate [99] and it is suggested that inhibition of multiple cytokines maybe be effective in therapy [99,100]. Remarkably, $Foxp3^+$ T cells in inflamed intestinal mucosa from patients with CD were shown to be capable of producing IL-17 as well as low levels of IFN-γ, while retaining their immunosuppressive function *in vitro* [101,102]. By contrast, $Foxp3^+$ T cells in UC patients did not produce IL-17 *in vivo* unless exposed to TGF-β1 *in vitro* [101]. The proportion of $IL\text{-}17^+$ producing T cells was also noticeably higher in the bowel of CD patients than that from UC patients [101]. Elevated levels of TGF-β and IL-6 were measured in CD patients when compared with those with UC patients [101,103,104]. However, other reports observed more Th17 cells in UC patients than in CD patients [105–107]. Hence, the form of IBD with more Th17 cells and more sensitive to Th17 cells is still undetermined. Not to mention, mucosal samples obtained from different parts of the intestines may also affect the results [108]. UC usually affects the colon, which mainly favors the differentiation of Th17 cells *in vivo*, while CD affects the whole intestinal tract and particularly the small intestine [108].

Colorectal cancer

Clinical investigations noticed that IBD patients were more susceptible to colorectal cancer than unaffected individuals [109]. The role of Th17 cells in the protection against the progression of colorectal tumors is controversial. One of Th17 cell-related cytokines, IL-17A, promotes the development of tumorigenesis in an experimental colitis-associated cancer (Table 1) [110]. Another Th17 cell-related cytokine, IL-21, was increased in the gut of patients with UC-associated colon cancer and in mice with colitis-associated cancer induced by azoxymethane and dextran sulfate sodium [111]. Elimination of IL-21 inhibits tumorigenesis through downregulating STAT3 activation in tumors and stromal cells [111]. IL-22 was also found to promote the development of human colon cancer through the activation of STAT3 (Table 1) [112]. Unlike the cytokines mentioned above, IL-17F showed a protective role in colorectal cancer development possibly by inhibiting tumor angiogenesis [113]. The direct evidence for Th17 cells participating in the development of tumors comes from the study which showed that colorectal cancers in both human and mouse models secrete greater amounts of IL-23 produced by myeloid cells in the mucosa through the stimulation of their TLRs by bacterial products derived from the commensal microbiota [114]. IL-23 then acted on lymphocytes to trigger a Th17 response. This study also suggested that the Th17 response led to tumor formation and played a minor role in tumor growth as blockade of IL-17A alone or combined with IL-23R blockade considerably inhibited colon tumor formation while had little effects on the size distribution of the tumors [114]. Antibody-mediated depletion of Th17 cells drastically inhibited tumor formation in an experimental enterotoxigenic *B. fragilis*-induced colon tumor model [115]. Clinical researches also indicated that a molecular signature in human colorectal cancer with a Th17 response dominating over a Th1 response is associated with a poorer prognosis even for stage I or II of the disease [116].

Expert commentary & five-year view

Th17 cells and Treg play important roles in the homeostasis of intestinal immune system. Local APCs, IECs and commensal bacteria coordinately modulate the balance between Treg and Th17 cells in the gut. On the other hand, Treg not only suppress the function of Th17 cells and exaggerated immune response, but also differentiate into Th17 or Th1 cells to induce colitis development under certain conditions. Further analysis of the relationship between Th17 cells and Treg in GALT and LP in different IBD models will give us a more conclusive view of the immune regulation in the intestine and offer novel approaches for treating immune disorders in the intestine.

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Key issues

- Maintaining the homeostasis of the gut immune microenvironments is critical for host immune tolerance to food, self-antigens and parasites, while allowing efficient immune response to exogenous pathogenic microorganisms.
- Treg actively suppress effector cells and contribute to the maintenance of intestinal immune homeostasis. Disturbances in Treg number and function are closely associated with immune disorders in the gut.
- Th17 cells mainly exist in the intestine and protect vertebrates from exogenous pathogenic microorganisms through the recruitment of macrophages and neutrophils to infected tissues. To a lesser extent, Th17 cells also cause numerous intestinal diseases such as colitis and colorectal cancers.
- Retinoic acid cooperates with TGF-β1 to promote the differentiation of Treg in mesenteric lymph nodes and lamina propria. It is also capable of inducing Th17 cells under certain inflammatory conditions.
- Microorganisms in the gut considerably influence the intestinal immune homeostasis through controlling the induction of Th17 cells and/or Treg.

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