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## Th17 cell heterogeneity and its role in tissue inflammation

Alexandra Schnell<sup>1,2</sup>, Dan R. Littman<sup>3,4,\*</sup>, Vijay K. Kuchroo<sup>1,2,\*</sup>

<sup>1</sup>Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA.

<sup>2</sup>Klarman Cell Observatory, Broad Institute of MIT and Harvard, Cambridge, MA, USA.

<sup>3</sup>The Kimmel Center for Biology and Medicine of the Skirball Institute, New York University School of Medicine, New York, NY, USA.

<sup>4</sup>Howard Hughes Medical Institute, New York, NY, USA.

### Abstract

Since their discovery almost two decades ago, IL-17-producing CD4<sup>+</sup> T cells (Th17 cells) have been implicated in the pathogenesis of multiple autoimmune and inflammatory disorders. In addition, Th17 cells have been found to play an important role in tissue homeostasis, especially in the intestinal mucosa. Recently, the use of single-cell technologies along with fate-mapping and various mutant mouse models has led to substantial progress in the understanding of Th17 cell heterogeneity in tissues and of Th17 cell plasticity leading to alternative T cell states and differing functions. In this review, we discuss the heterogeneity of Th17 cells and the role of this heterogeneity in diverse functions of Th17 cells from homeostasis to tissue inflammation. In addition, we discuss Th17 cell plasticity and its incorporation into the current understanding of T cell subsets and alternative views as to the role of Th17 cells in autoimmune and inflammatory diseases.

### Introduction

CD4<sup>+</sup> T helper cells are essential regulators of adaptive immune responses. In the 1980s, CD4<sup>+</sup> T helper cells were classified into Th1 and Th2 subsets based on their distinct expression of cytokines and effector functions [1]. Th1 cells are differentiated with IL-12, produce IFN $\gamma$  and control intracellular pathogens, whereas Th2 cells are differentiated with IL-4 and IL-2, express IL-4, IL-5, and IL-13, and protect from extracellular pathogens, including parasites, but also promote development of allergic diseases. Th1 cells were originally thought to be the main T-cell population driving organ-specific autoimmunity. However, findings that IFN $\gamma$ -, IFN $\gamma$ -receptor-, IL-12p35-, and IL-12 receptor  $\beta$ 2 -deficient mice developed exacerbated experimental autoimmune encephalomyelitis (EAE) challenged

\*Correspondence to Dan R. Littman (Dan.Littman@nyulangone.org) and Vijay K. Kuchroo (vkuchroo@rics.bwh.harvard.edu).

#### Competing interests

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the concept of Th1 cells as the pathogenic population in autoimmunity [2–5]. In the early 2000s, after the discovery of the novel cytokine IL-23 [6], Cua and colleagues demonstrated that IL-23, and not IL-12, is the cytokine required for the induction of EAE [7]. In a follow-up study in 2005, Cua and colleagues suggested that IL-23 promotes expansion of a novel IL-17-producing T-cell population capable of inducing autoimmunity upon adoptive transfer [8]. In 2005, we proposed that indeed IL-17 producing T cells represented an independent T cell subset, but that IL-23 was not the differentiating factor that induces Th17 cells and instead acts on previously differentiated Th17 cells, stabilizing their effector functions [9]. While IL-23 induced IL-17 expression in activated T cells, this required prior induction of IL-23R through STAT3-mediated signaling initiated by IL-6 or IL-21 and amplified by IL-23 itself. Th17 cells were established as an independent T helper cell subset with the discovery of signature cytokines IL-17A, IL-17F, and IL-22, lineage-specific differentiation factors, and dedicated or “master” transcription factors [10–16]. In 2006, the cytokines TGF- $\beta$  and IL-6 were described as differentiation factors required to polarize naïve T cells into Th17 cells [13–15]. Discovery that the orphan nuclear receptor ROR $\gamma$ t was expressed in a subset of intestinal CD4<sup>+</sup> T cells and that it was required for expression of IL-17 by both these cells and *in vitro* differentiated Th17 cells cemented its role as the “master transcription factor” for the Th17 lineage [16]. Although ROR $\gamma$ t was both necessary and sufficient for at least part of the Th17 gene expression program, multiple additional transcription factors were shown to be required for the expression of IL-17, including STAT3, IRF4, and BATF [17–20].

It is well-established that T helper cells have diverse capabilities beyond their conventionally assigned functions. This has only been reinforced by the recent application of single cell genomics to CD4<sup>+</sup> T cells in homeostatic and proinflammatory microenvironments, which has revealed a previously unappreciated complexity of the T helper cell subsets [21]. It is therefore difficult to reconcile such functional diversity with a recently proposed reclassification of T helper cell subsets based on function: providing help to phagocytes (cellular immunity- Th1 cells), to class switching and antibody production by B cells (humoral immunity- Th2 cells), and regulating non-immune cells at barrier sites (mucosal immunity- Th17 cells) [22]. Indeed, Th1 cells are well-known to also regulate B cells and humoral immunity [23, 24], Th2 cells regulate multiple non-immune tissue cells at mucosal sites [25], and Th17 cells may contribute to production of high-affinity IgA antibodies and to IgG2a and IgG3 class switching [26, 27]. As our appreciation of the multiple capabilities of T helper cells continues to grow, it may be preferable to adopt a more nuanced classification of the cells, based on expression of gene modules that distinguish between various T cell subsets, in place of the Th1, Th2 or Th17 nomenclature. Indeed, the distinction of “homeostatic” versus “pathogenic” Th17 cells, discussed in detail below, may be only one example of the important functional diversity within a single CD4<sup>+</sup> T cell subset.

The discovery of Th17 cells in mouse models signaled an important shift in studies of T-cell-mediated inflammatory diseases. It was rapidly appreciated that Th17 differentiation and function are evolutionarily conserved, which led to several successful efforts to target the pathway and to multiple new therapies. In particular, anti-IL-17 pathway antibodies are effective in the treatment of inflammatory conditions, including plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis [28]. Initial double-blind clinical trials showed that

anti-IL-17 antibody secukinumab was also effective in reducing new magnetic resonance imaging (MRI) lesions, cumulative lesion load, and expanded disability status scale (EDSS) scores in multiple sclerosis (MS) patients [29, 30]. However, some animal model studies proposed that IL-17 and Th17 cells are not required to drive autoimmune tissue inflammation [31, 32], and that other T cells, particularly GM-CSF-producing T cells, are the primary inducers of autoimmune tissue inflammation [33–36]. Moreover, analysis of Th17 cells in different tissue sites has revealed that Th17 cells may exhibit distinct transcriptional profiles associated with either homeostatic or pathogenic functions [37–43]. Thus, Th17 cells, besides being highly pathogenic and able to induce autoimmunity, have also been implicated in mediating tissue homeostasis, regulating tissue integrity by protecting mucosal barriers from potentially invasive microbes, particularly extracellular bacteria and fungi, influencing the composition of the commensal microbiota at body surfaces, and modulating epithelial permeability to diverse nutrients and metabolites [44–48]. This homeostatic function of Th17 cells likely underlies the finding that blockade of IL-17 in Crohn's disease is not only ineffective but can exacerbate colitis [28, 49]. Additionally, animal model studies have suggested that distinct Th17-inducing commensal microbes contribute to exacerbated autoimmune disease at extra-intestinal sites, potentially mediated by the mucosa-derived Th17 cells [50–52]. Overall, the mechanisms by which tissue Th17 cells mediate divergent functions, *i.e.*, promoting tissue homeostasis while contributing to the development of autoimmune tissue inflammation, and the roles of the IL-17 cytokines and Th17 cell plasticity in autoimmunity remain unclear.

In this review, we discuss the *in vitro* and *in vivo* heterogeneity of Th17 cells and the role of this heterogeneity in the function of Th17 cells in homeostasis and autoimmunity. In addition, we review and discuss divergent views as to the role of Th17 cells in autoimmune disease and specifically plasticity and diversity of Th17 cells and their relationship to other T cell states.

## Heterogeneity of *in vitro* and *in vivo* differentiated Th17 cells

The concept of Th17 cell heterogeneity came originally from analyses in which different cytokine combinations that induce bona fide IL-17-producing Th17 cells in *in vitro* cultures had diverse abilities to induce autoimmune disease following adoptive transfer [39, 40, 53]. *In vitro* polarization of naïve CD4<sup>+</sup> T helper cells with IL-6 and TGF- $\beta$  generates Th17 cells that produce IL-17 but induce little or no tissue inflammation or autoimmunity (non-pathogenic Th17 cells), whereas IL-6, IL-1 $\beta$  and IL-23 or IL-6, TGF- $\beta$ , and IL-23 differentiate Th17 cells that are highly potent in transferring disease (pathogenic Th17 cells) [39, 53]. In subsequent studies, the transcriptional signatures distinguishing *in vitro*-differentiated non-pathogenic Th17 cells and pathogenic Th17 cells were defined [53, 54]. Although both subtypes expressed IL-17, non-pathogenic Th17 cells additionally expressed the immunoregulatory genes *Il10*, *Il9*, *Maf*, and *Ahr*, whereas pathogenic Th17 cells were characterized by the expression of proinflammatory genes including *Csf2* (gene encoding GM-CSF), *Ifng*, *Tbx21*, *Il23r* and *Gzmb*. These analyses led to the discovery and successful validation of multiple novel Th17 cell regulators [55–58]. By undertaking high density, temporal expression analysis of differentiating Th17 cells, regulatory networks for Th17 cells were constructed, which confirmed the central role

of ROR $\gamma$ t in differentiating Th17 cells [20, 59], but also identified a number of novel regulators that formed mutually antagonistic modules, which, while promoting Th17 cell fate, suppress differentiation to other T cell states [20]. Furthermore, by integrating single-cell ATAC-seq and single-cell RNA-seq, we generated a regulatory network across npTh17, pTh17, and Th1 cells to reveal the underlying regulators and mechanisms that drive the non-pathogenic and pathogenic Th17 subsets [60]. The network predicted the pathogenic Th17- and Th1- program to be regulated by the transcription factors ATF3, BHLHE40, FOS, NR4A1, EOMES, and T-BET. Predicted regulators of the non-pathogenic- and general-Th17 program included ROR $\gamma$ t, ETS1, BACH2, FOSL2, and RBPJ. We validated BACH2 as a novel regulator of Th17 pathogenicity that promoted immunomodulatory programs and restrained pro-inflammatory Th1-like programs in Th17 cells. In a study analyzing the transcriptional programs of human Th17-IL-10<sup>+</sup> and Th17-IL-10<sup>-</sup> cells, BACH2 was predicted to regulate the transcriptional program of the Th17-IL-10<sup>+</sup> subset [61]. Moreover, multiple single-nucleotide polymorphisms (SNPs) in the *BACH2* locus are associated with human autoimmune diseases and a protective allele for MS disease correlates with an increase of *BACH2* expression in CD4<sup>+</sup> T cells [60, 62]. Hence, in humans BACH2 might be an important regulator of homeostatic Th17 cells that could be exploited for the treatment of human autoimmune diseases.

The divergent transcriptomic profiles of *in vitro* differentiated pathogenic and non-pathogenic Th17 cells have also been observed in differentiated T cells induced by diverse intestinal commensal microbes. Thus, intestinal non-pathogenic/homeostatic Th17 cells specific for segmented filamentous bacteria (SFB) have a transcriptome that differs markedly from the pro-inflammatory Th17 cells elicited by *Helicobacter hepaticus* (*H. hepaticus*) in mice defective for induced intestinal Treg cells [43]. The gene expression profiles of these cells resemble those described for the *in vitro* differentiated pathogenic Th17 cells, suggesting that genes identified in the *in vitro* studies could serve as good therapeutic targets [53, 54, 63].

Interestingly, human T cell subsets have been identified that are transcriptionally similar to murine *in vitro*-differentiated non-pathogenic and pathogenic Th17 cells. While in mice evidence for distinct types of Th17 cells came from studies of microbiota-specific T cells and inflammatory disease models, human Th17 cell heterogeneity was identified based on the roles of different Th17 populations in immunity against fungi and bacteria [64, 65]. Th17 memory cells (initially defined as CCR4<sup>+</sup>CCR6<sup>+</sup>) specific for *Candida albicans* (*C. albicans*) produced IL-17 and IFN $\gamma$  and no IL-10, and thus resemble pathogenic Th17 cells. In contrast, *Staphylococcus aureus* (*S. aureus*)-specific Th17 cells produced IL-17 and IL-10, but no IFN $\gamma$ , and thus resemble non-pathogenic/homeostatic Th17 cells [66]. Analysis of CXCR3<sup>+</sup> cells identified two T-BET-expressing populations, one that is CCR6<sup>-</sup>, and represents Th1 cells, and another that is also CCR6<sup>+</sup>, and is dependent on expression of ROR $\gamma$ t [64, 65]. It is now appreciated that the CXCR3<sup>+</sup>CCR6<sup>+</sup> cells, named Th1\* cells, resemble pathogenic Th17 cells and are required to control infection with both *C. albicans* and mycobacteria, including the attenuated Bacillus Calmette-Guerin (BCG) [64, 67]. Indeed, genetic defects in humans in the IL-17/IL-17-receptor axis, STAT3, and ROR $\gamma$ t, resulting in dysfunctional Th17 cells, are associated with chronic mucocutaneous candidiasis and severe disease following BCG immunization [67–69]. Patients with mucocutaneous

candidiasis also show increased susceptibility to *S. aureus* infections. Hence, the non-pathogenic/homeostatic and pathogenic Th17 cell phenotypes studied in the mouse models may mirror the physiological roles of human Th17 cell subsets that are important for immunity against distinct bacteria and fungi.

## Tissue Th17 cells during homeostasis

### Environmental influence on tissue Th17 cells

Although Th17 cells were originally described as drivers of tissue inflammation, at steady-state Th17 cells are most abundant at mucosal barriers such as the intestinal lamina propria, where they play important roles in mediating tissue homeostasis and in preventing microbial invasion and controlling infections at barrier sites. The Th17 cell-signature cytokines IL-17A, IL-17F, and IL-22 induce the expression of antimicrobial proteins and the formation of tight junctions by intestinal epithelial cells and have been implicated in regulation of IgA responses [70, 71]. The constant exposure of the intestine to environmental agents including microbiota, diet, and metabolites has a significant influence on the intestinal Th17 population (Figure 1). A number of seminal studies have established the ability of specific microbes to induce intestinal Th17 cells. Colonization of germ-free mice with SFB, but not other *Clostridium* or *Bacteroides* species, induced SFB-specific Th17 cells, largely in the terminal ileum, where SFB binds to the intestinal epithelium [72]. Adhesion to the epithelium is thought to be essential for the induction of Th17 cells, as rat-derived SFB, that does not adhere to the small intestinal epithelium in mouse hosts, failed to induce intestinal Th17 cells [73]. In addition, with the use of adhesion-defective mutants, the induction of intestinal Th17 cells by *Citrobacter rodentium* (*C. rodentium*) and *Escherichia coli* O157 was shown to be dependent on their physical interaction with the intestinal epithelium. SFB antigen delivery across the intestinal epithelium involves a novel vesicular compartment and the GTPase CDC42 [74], and results in induction of the homeostatic Th17 cell program in naïve antigen-specific CD4<sup>+</sup> T cells in the draining mesenteric lymph nodes. These Th17 cells distributed throughout the length of the intestine, but IL-17 cytokine production was largely confined to ileal Th17 cells, in part in response to epithelium-derived serum amyloid A proteins 1 and 2 (SAA1/2) [75]. SAA induction is one component of an intestinal epithelial cell program induced locally by IL-22 derived from SFB-activated ILC3s, thus explaining the basis of the localized effector response. Interestingly, the induction of Th17 cells by SFB in the small intestine does not induce a proinflammatory reaction in the intestine, but instead induces a homeostatic Th17 population that supports intestinal barrier function [37, 43]. The barrier-protective function of Th17 cells is mainly mediated by the expression of their signature cytokines IL-17 and IL-22, that are also expressed by intestinal  $\gamma\delta$  T cells and ILC3s, making it difficult to distinguish the contribution of the different immune cell populations to tissue homeostasis. Transcriptomic and fate-mapping studies indicate that at homeostasis the main source of IL-17A is Th17 cells, while most IL-22 is derived from ILC3s [76, 77]. However, during *C. rodentium* infection, while early phase defense is mediated by ILC3-derived IL-22, protection from systemic dissemination and survival require IL-22 production by T cells [78].

To analyze the immunomodulatory effects of human gut microbes, mice were mono-colonized with human bacterial species and analyzed for effects on the host immune system. These analyses identified multiple human bacterial species, including *Bifidobacterium adolescentis* and *Staphylococcus saprophyticus*, that induced intestinal Th17 cells [79, 80]. In addition to the microbiota, intestinal mycobiota and protists, including *Tritrichomonas musculus*, were shown to induce intestinal Th17 cells that likely promote homeostatic functions [81–83].

Diet and metabolism have also been shown to influence the phenotype of intestinal Th17 cells. A high-salt diet increases the number of Th17 cells in the intestine and exacerbates Th17-dependent autoimmunity [84, 85]. The increase in salt concentration induces the expression of serum/glucocorticoid-regulated kinase 1 (SGK1) that deactivates FOXO1 and thereby promotes the expression of IL-23R and the generation of pathogenic Th17 cells [84]. In addition, high levels of salt in the diet have been shown to have profound effects on microbiota which in turn may indirectly affect the development of Th17 cells [86]. In a recent study by *Kawano et al.*, sugar in mouse diets was found to induce metabolic disease by disrupting Th17-inducing microbiota leading to a loss of intestinal Th17 cells [87]. Intestinal Th17 cells protected against metabolic disease by regulating epithelial lipid absorption. Lipids have also been shown to regulate the differentiation and phenotype of Th17 cells. Long-chain fatty acids (LCFAs) promote the differentiation of Th17 cells and enhance the severity of EAE, whereas dietary short-chain fatty acids (SCFAs) induce intestinal Treg cells and ameliorate EAE [88]. Th17 cells utilize acetyl-CoA carboxylase 1 (ACC1)-mediated de novo fatty acid synthesis to synthesize phospholipids for cellular membranes [89]. ACC1-deletion in mice results in inhibition of Th17 cell-driven autoimmune disease. CD5 antigen-like (CD5L), a regulator of lipid metabolism, was selectively expressed by Th17 cells differentiated *in vitro* under non-pathogenic conditions, and was shown to inhibit Th17 cells to attain a pathogenic Th17 cell phenotype [57]. In addition, bile acid metabolites were shown to regulate the differentiation of Th17 cells and Treg cells, and may control their balance in the intestine. While the secondary bile acids 3-oxo-lithocholic acid (LCA) and iso-LCA inhibit the differentiation of intestinal Th17 cells by direct binding to ROR $\gamma$ t, isoalloLCA enhances the differentiation of intestinal Treg cells [90]. Defined human gut microbes were found to produce 3-oxoLCA and iso-LCA that inhibit Th17 differentiation, thereby potentially directly regulating inflammatory disorders in the intestine including IBD [91]. Interestingly, centenarians, who have decreased susceptibility to chronic inflammation and infectious diseases, were found to have distinct gut microbiomes enriched for microorganisms that produce these secondary bile acids, further supporting a potential beneficial role of the bile-acid producing microbes [92]. A recent publication by *Chen et al.* showed that the nuclear xenobiotic receptor constitutive androstane receptor (CAR) induces the expression of bile acid detoxifying enzymes/transporters and the anti-inflammatory cytokine IL-10 in T cells specifically in the small intestine lamina propria [93]. These data suggest a direct role of CAR in detoxifying bile acids and preventing inflammation in the small intestine, resulting in the induction of nonpathogenic/homeostatic Th17 cells and offering a potential novel approach in the treatment of Crohn's disease.

The metabolic states of immune cells were recently shown to regulate cell phenotype and function. Multiple studies on Th17 cells have highlighted the distinct metabolic mechanisms underlying different Th17 cell phenotypes. Intestinal SFB-induced homeostatic Th17 cells are characterized by a resting aerobic profile primarily dependent on OXPHOS metabolism, which is typical for quiescent T cells [42]. Differentiation of these cells proceeds even if they lack expression of GPII and, hence, have reduced glycolytic flux [94]. In contrast, *C. rodentium* infection-induced Th17 cells with a pro-inflammatory phenotype show enrichment in aerobic glycolysis and oxidative phosphorylation. Consistent with this, the differentiation of proinflammatory Th17 cells in both colitis and EAE models was dependent on intact glycolytic flux due to the relatively hypoxic environment in the setting of inflammation.

The existence of metabolically distinct Th17 cell populations was further substantiated by recent studies with the EAE model. A Th17 cell subset with stem-like features, resembling *in vitro* differentiated, non-pathogenic Th17 cells, was found to have low anabolic metabolism, while another subset with Th1-like features had high metabolic activity [95]. Disruption of mTORC1 signaling or anabolic metabolism inhibited differentiation of the Th1-like phenotype cells and ameliorated autoimmunity. This may be due to an inability of the stem-like cells to trans-differentiate towards the more pro-inflammatory phenotype. To study cellular metabolism based on single-cell transcriptomes and flux balance analysis, we and others developed a novel algorithm called Compass [96]. Using Compass analysis, polyamine metabolism was identified as a major regulator of T helper cell fate direction, epigenetic programming, and pathogenic potential. Increased glycolysis and polyamine biosynthesis was detected in *in vitro* differentiated pathogenic Th17 cells, whereas non-pathogenic Th17 cells exhibited enhanced fatty acid oxidation (FAO). Blockade of the polyamine pathway by perturbation of ornithine decarboxylase 1 (ODC1) or spermidine/spermine N1 acetyltransferase 1 (SAT1), rate-limiting enzymes of biosynthesis and catabolism in the polyamine pathway, respectively, alleviated EAE disease. This weakened pathogenic potential of Th17 cells was accompanied by a remodeled transcriptome and epigenome toward a Treg-like program, highlighting the importance of the polyamine pathway in Th17 pathogenicity and in Th17 cell lineage fidelity. In a parallel study performed by the laboratory of Erika Pearce, the polyamine pathway was further identified as generally important for T helper cell polarization [97]. ODC1 deficiency resulted in the failure of CD4<sup>+</sup> T cells to acquire subset-specific cytokine and transcription factor expression. Furthermore, perturbation of the polyamine-hypusine axis led to changes in histone acetylation, inhibiting accurate CD4<sup>+</sup> T cell polarization. Collectively, the two studies demonstrate an essential function of polyamine metabolism in regulating CD4<sup>+</sup> T cell fate decisions. Interestingly, in pathogenic settings *in vivo*, Th17 cells can acquire features of Th1 cells [76] and Treg cells have been suggested to convert to Th17 cells [98]. Hence, since the polyamine pathway is critical for T cell subset fidelity, limited polyamine availability and inhibited polyamine biosynthesis in pathogenic settings may promote the trans-differentiation of Th17 cells to other T cell subsets that have reduced potential for pathogenesis.

Changes in physiological conditions including hypoxia and temperature also regulate Th17 phenotype. Immune cells in lymphoid organs and in tissues are exposed to different levels of

oxygen, which can directly influence their phenotype and function. The transcription factor hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) plays an essential role in the response to hypoxia and is required for the differentiation of Th17 cells [99, 100]. At the same time, HIF1 $\alpha$  mediates glycolytic activity and FOXP3 degradation, thereby preventing Treg differentiation. Hence, low oxygen availability is a direct regulator of the Th17/Treg balance. In a recent study, temperature was shown to have an influence on the differentiation and phenotype of Th17 cells [101]. Febrile temperature enhanced the production of cytokines by Th17 cells *in vitro* and promoted pathogenic gene expression and pro-inflammatory function *in vivo*. In contrast, cold exposure was found to lead to ameliorated neuroinflammation in EAE with decreased frequencies of Th17 cells in the CNS, likely due to reduced priming by monocytes [102]. These results imply that temperature plays an important role in regulating the proinflammatory program of Th17 cells, with implications for infections and autoimmune diseases.

### Heterogeneity of tissue Th17 cells during homeostasis

Several studies have reported evidence of Th17 cell plasticity under physiological conditions *in vivo*. Fate-mapping studies using IL-17-fate reporter mice showed IL-23R-dependent differentiation of IL-17-producing cells into encephalitogenic IFN $\gamma$ -producing Th1/Th17 cells [37, 76]. However, it is unclear whether this represents plasticity of homeostatic Th17 cells differentiating into pathogenic Th1-like cells upon exposure to IL-23 or if there is a distinct Th17 cell population with potential to become pathogenic in the presence of IL-23 (see further discussion in later sections). Peyer's patch Th17 cells were also proposed to trans-differentiate into T follicular helper (Tfh) cells that promote IgA production by germinal center B cells [26]. However, a more recent study reported normal Peyer's patch IgA production to oral immunization even when Th17 cells were abrogated, and suggested that the earlier study reflected sustained IgA plasma cell function following SFB-induced Th17 cell expansion [103]. Th17 cells were additionally shown to upregulate expression of the anti-inflammatory cytokine IL-10 and acquire a T regulatory type 1 (Tr1) cell phenotype during the resolution of intestinal inflammation and re-establishment of homeostasis [104, 105]. This phenotypic transition may thus reflect trans-differentiation of pathogenic Th17 cells to cells with a regulatory or anti-inflammatory function.

To generate an atlas reflecting the diversity of Th17 cells within and across tissues at homeostasis, we profiled Th17 cells from multiple tissues of naïve IL-17-fate reporter mice by single-cell RNA-seq [38]. Similar to tissue Treg cells [106] and tissue macrophages [107], tissue Th17 cells were found to exhibit tissue-specific transcriptomic signatures. These distinct tissue signatures could indicate distinct environmental cues in the tissue microenvironment that drive the adaptation of the transcriptome and thereby the cellular function of immune cells in various tissue microenvironments (Figure 2). In recent years, immunometabolism has emerged as a field describing how metabolic states regulate phenotype and function of immune cells [108]. Hence, in tissues, we propose that available nutrients and metabolites are likely to have a strong impact on immune cell transcriptome and functional phenotype. In addition, tissue Th17 cell signatures are likely influenced by growth factors, oxygen levels, and cellular interactions that potentially affect their transcriptome and function. Tissue Th17 signatures in the spleen and lymphoid tissues



included genes associated with a stem-like T-cell state (*Tcf7*, *Il7r*, *Ccr7* etc.), whereas genes associated with activation of Th17 cells (*Cd44*, *Cd69*, *Il17a* etc.) were upregulated in intestinal tissues. Analysis of the variation among Th17 cells within each tissue revealed distinct functional clusters of Th17 cells that, interestingly, were transcriptionally similar to clusters in other tissues, indicating the presence of similar Th17 heterogeneity even in different tissue sites. In particular, Tfh-like clusters, effector-like clusters, stem-like clusters, proliferating clusters, interferon-stimulated genes (ISGs)-expressing clusters and Treg clusters were identified (Figure 1). While previous studies described the presence of ROR $\gamma$ <sup>+</sup> Treg cells at homeostasis [109, 110], the relationship of these Th17-like Treg cells to other Treg and Th17 cells remained unclear. Using TCR clonality analysis, the Th17-like Treg clusters were found to be clonally distinct from those of non-Treg Th17 clusters, indicating that, at homeostasis, Th17-like Treg cells likely differentiate from other Treg cells or naïve CD4<sup>+</sup> T cells, and not from Th17 cells. This is likely explained by the specific induction of distinct intestinal Th17 populations and Treg cells by gut microbiota [71, 72]. In contrast to the Treg cells, tissue Th17 cells were highly clonally related within a tissue, suggesting intra-tissue plasticity and transitions between subpopulations [38].

## The role of Th17 cells in autoimmunity and tissue inflammation

### Th17 cells in animal models of autoimmune disease

The discovery of IL-23 as an essential cytokine for induction of EAE and programming of IL-17-producing T cells that induce autoimmunity upon adoptive transfer [8] paved the way for studying the role of Th17 cells in autoimmunity. Multiple follow-up studies in mouse models supported the role of Th17 cells as important drivers of autoimmunity. In EAE, a mouse model for MS, Th17 cells were shown to be potent inducers of autoimmunity. IL-17A-deficient mice exhibit significantly attenuated EAE disease, whereas IL-17F-deficient mice show marginally reduced EAE [111, 112]. Therapeutic neutralization of IL-17A ameliorates disease in EAE [113]. In addition, the transfer of in *vitro* differentiated pathogenic Th17 cells expressing a transgenic TCR specific for the MOG<sub>35–55</sub> peptide (2D2 cells [114]) is sufficient to induce severe disease in recipient mice [115]. Some reports have suggested that the expression of IL-17A and F by T cells was not required for the development of EAE [31], which was based on studies in which IL-17A was neutralized with an antibody in IL-17F-deficient mice. However, when using genetically deficient IL-17A and IL-17F mice, our own unpublished studies and those of others have shown that IL-17A/F double-deficient mice were highly resistant to the development of EAE [116]. To explain this discrepancy, the Waisman group suggested that the disease resistance was due to changes in the microbiota mediated by loss of IL-17A/F in the mice [116]. In the study they showed that overexpression of IL-17A by intestinal epithelial cells was sufficient to reestablish EAE susceptibility in IL-17A/F-knockout mice and therefore IL-17 production by T cells was not pre-requisite for the development of EAE. However, in this study it was unclear whether the IL-17A overexpression in the intestine remained local or led to systemic elevation of IL-17A levels, thereby impacting blood-brain-barrier (BBB) function and affecting trafficking and the phenotype of immune cells that promote encephalitogenicity. Moreover, in a recent study from one of our labs (unpublished), we found that IL-17A/F-deficient MOG-TCR transgenic 2D2 cells lacked the ability to induce

disease in *Rag1*-deficient recipient mice, whereas 2D2 T cells from wildtype mice induced severe disease, which supports the idea that IL-17 produced by T cells is critical for the induction of EAE, independently of changes in the microbiota. These results highlight the important role of IL-17 expression in Th17 cells for their pathogenic effector functions. Hence, additional analysis will be required to understand the role of IL-17 expression in T cells and in the intestine in the development of autoimmune diseases.

In experimental models of psoriasis, Th17 cells are thought to be the main pathogenic T cell population driving disease. The Th17-associated cytokines IL-23, IL-22 and IL-17A have been shown to play essential roles in these models and in the pathogenesis of psoriasis [117–119]. IL-23, produced by macrophages and dendritic cells, drives the differentiation and expansion of IL-22<sup>+</sup> and IL-17A<sup>+</sup> Th17 cells that activate the surrounding tissue cells including keratinocytes and fibroblasts, thereby mediating tissue inflammation. In addition to Th17 cells, dermal  $\gamma\delta$  T cells respond to IL-23 and are a major source of IL-17A, IL-17F, and IL-22 cytokines in psoriatic lesions, making them additional contributors to disease pathogenesis [120, 121].

IL-17A has been shown to have a key pathogenic role in murine arthritis models. IL-17-deficient mice are protected against collagen-induced arthritis (CIA) [122] and from spontaneous arthritis in IL-1R $\alpha$ <sup>-/-</sup> mice [123]. Blockade of IL-17 or the IL-17 receptor inhibits the development of arthritis in mice [124].

The role of Th17 cells in mouse models of inflammatory bowel disease (IBD) is controversial, which is likely due to the dichotomous nature of intestinal Th17 cells, as they are mediators of tissue homeostasis yet can become drivers of tissue inflammation [37]. In the T cell transfer model of colitis, disease induction is not dependent on IL-17A-expression by T cells [125, 126], although loss of both IL-17A and IL-17F abrogated T cell pathogenicity [127]. In fact, some studies show a protective function for IL-17A in colitis [128, 129], possibly due to the role of IL-17A in promoting intestinal epithelial barrier integrity [130]. However, multiple studies have shown that IL-23 and ROR $\gamma$ t, which are essential mediators of Th17 differentiation, are required for colitis [127, 131, 132]. In the *Il10*<sup>-/-</sup> model of colitis, in which IL-10-deficient mice develop spontaneous microbiota-dependent colitis, Th17 cells were identified as the main immune population mediating the inflammatory immune response [43, 133]. Collectively, these data suggest that Th17 cells are major disease drivers in the transfer model and *Il10*<sup>-/-</sup> model of colitis.

### Th17 cells in human autoimmune diseases

The importance of Th17 cells in autoimmunity, initially demonstrated in multiple mouse models, has been validated by targeting the pathway in human inflammatory and autoimmune diseases. The most striking efficacy of such therapies is in psoriasis, for which multiple agents targeting this pathway are now in the clinic [28, 29]. In psoriatic skin lesions, the majority of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and  $\gamma\delta$  T cells, express IL-17 [134], and elevated IL-17A, IL-22 and IL-23 levels were found in patients with active psoriasis [135]. After the identification of the IL-23/IL-17 axis as an important mediator of pathogenesis in preclinical models, ustekinumab, an inhibitor of p40 targeting both IL-23 and IL-12, was first shown to be efficacious in treating psoriasis [136]. More recently, selective

IL-23 inhibitors (tildrakizumab, risankizumab, and guselkumab) that specifically target the IL-23 p19 subunit were found to be more effective than TNF inhibitors [137] and IL-23/IL-12 inhibitors [28, 138]. IL-17A and IL-17RA inhibitors (secukinumab, ixekizumab, and brodalumab) are approved for the treatment of moderate to severe plaque psoriasis and show superior efficacy to previously approved therapies for psoriasis, including TNF inhibitors and IL-23/IL-12 inhibitors, resulting in disease-free skin in up to 42% of patients [28, 29, 139, 140].

Psoriatic arthritis (PsA), which shares clinical features with psoriasis and ankylosing spondylitis, is characterized by skin and joint inflammation. Similar to psoriasis, IL-17 pathway inhibitors (secukinumab and ixekizumab) have been shown to be effective in the treatment of PsA [141, 142]. Moreover, secukinumab has also been approved for treatment of ankylosing spondylitis [143], demonstrating a role of IL-17 in the pathology of multiple human autoimmune and inflammatory diseases.

IL-17 was also found to be highly expressed in active MS lesions [144] and Th17 cells mediate blood-brain barrier disruption by the expression of IL-17 and IL-22 [145]. In therapeutic interventions the inhibitor of IL-12+23, ustekinumab, showed no efficacy in relapsing remitting MS (RRMS) [146]. However, this antibody inhibits the generation of both Th1 and Th17 cells and therefore cannot be equated with pure Th17 blockade. On the other hand, the IL-17A inhibitor secukinumab was found to be effective in reducing active lesions in patients with RRMS in a double-blind clinical trial ([NCT01051817](#)), but was not pursued further because of other competing orally available drugs [29, 30].

Multiple studies found an association of IL-17 with rheumatoid arthritis (RA). Elevated levels of IL-17 and IL-17R were detected in tissues and synovial fluids from RA patients and synovial IL-17 levels were found to correlate with disease progression [147–149]. However, despite the rationale from preclinical mouse and human studies, targeting IL-17 in RA was found to be only modestly effective. IL-17A inhibition was superior to placebo and improved symptoms in patients non-responsive to TNF targeting, suggesting a pathogenic role for IL-17A in RA [150–152]. However, in this study, TNF-targeting abatacept was found to be superior to secukinumab in efficacy.

In IBD, increased levels of IL-17 and IL-23 were found in colon biopsies from patients and multiple single nucleotide polymorphisms in *IL23R* were associated with susceptibility to IBD [153, 154]. However, blockade of IL-17A in Crohn's patients was found to be ineffective and instead resulted in exacerbated colitis leading to study termination [29, 49]. Recent clinical trials testing selective inhibition of IL-23 in Crohn's disease have shown promising results [155, 156]. These clinical results suggest that IL-23-specific inhibition may prevent the development of pathogenic T cells, while allowing the IL-23 independent production of IL-17A, potentially from homeostatic Th17 cells, to maintain intestinal epithelial barrier integrity [130, 157].

## Tissue Th17 cell heterogeneity during autoimmunity

### Th17 cell plasticity during autoimmunity

Under inflammatory conditions, tissue Th17 cells have been reported to exhibit plastic changes in their effector programs. Use of cell fate reporter mice showed that T cells that initially express IL-17 (ex-Th17 cells) can subsequently express proinflammatory cytokines, including IFN $\gamma$  and GM-CSF [38, 76]. Especially the trans-differentiation of Th17 cells (IL-17A<sup>+</sup> IFN $\gamma$ <sup>-</sup>) to ex-Th17 Th1 cells that lose ability to produce IL-17 but continue to express IFN $\gamma$  (IL-17A<sup>-</sup> IFN $\gamma$ <sup>+</sup>) have been implicated in pathogenicity of autoimmune diseases. In the T cell transfer model of colitis, the trans-differentiation of Th17 cells to Th1-like cells has been shown to be required for their ability to induce disease [158, 159]. In human autoimmune diseases, IL-17A<sup>-</sup> and IFN $\gamma$ -expressing T cells are preferentially found at sites of inflammation and correlate with pathogenicity [160–165].

A recent study from one of our laboratories, using fate reporter mice, provides strong *in vivo* support for the notion that homeostatic Th17 cells differentiate into inflammatory Th17 cells that mediate autoimmune disease. Using single cell genomics, we identified a homeostatic IL-17A/F<sup>+</sup> population, marked by SLAMF6, that gives rise to a pathogenic IFN $\gamma$ <sup>+</sup> GM-CSF<sup>+</sup> population marked by CXCR6 during EAE [38], suggesting a direct *in vivo* relationship between these cells. In the CNS, the pathogenic CXCR6 population gives rise to multiple different, clonally related Th17 subpopulations, including quiescent-, migratory-, proliferating-, proinflammatory- and CD8-like cells, revealing intra-tissue plasticity and/or transitions between subpopulations of Th17 cells. Interestingly, the CD8-like cells expressed both *Cd4* and CD8<sup>+</sup> T cell markers and were also recently reported in human IBD, and may thus represent a common phenomenon in T-cell-driven autoimmune tissue inflammation [166, 167].

A key driver of the Th17 cell transition towards a pathogenic phenotype is the cytokine IL-23. In the absence of IL-23, Th17 cells lose the ability to up-regulate IFN $\gamma$  and GM-CSF [76]. IL-23 was shown to induce PRDM1, that, together with ROR $\gamma$ t and STAT3, enhances the expression of pro-inflammatory molecules including IL-23R, IL17A, and GM-CSF [168]. We identified IL-23-signaling as a main driver of the conversion of homeostatic IL-17A/F<sup>+</sup> SLAMF6<sup>+</sup> cells to pathogenic IFN $\gamma$ <sup>+</sup> GM-CSF<sup>+</sup> CXCR6<sup>+</sup> cells in EAE [38]. Thus, multiple studies support IL-23 as a key mediator of the generation of pathogenic IFN $\gamma$ <sup>+</sup> GM-CSF<sup>+</sup> Th17 cells. In addition to its effect on Th17 cells, IL-23 induces expression of cytokines that can have protective or proinflammatory functions in multiple cell types, including  $\gamma\delta$  T cells, ILC3s, and MAIT cells [169–172]. In a recent study, we found a small fraction of Th1 cells to express IL-23R. When differentiated with IL-12 and IL-21, Th1 cells express IL-23R [173]. IL-23 signaling in Th1 cells is critical for their ability to become colitogenic, with upregulation of several effector molecules that are not induced by IL-23 in Th17 cells. This suggests that IL-23R signaling is more broadly relevant than previously thought and that it may also provide an important target for inhibition of the pathogenicity of multiple effector cells that mediate autoimmunity.

Despite many reports showing a direct relationship between Th17 cells and GM-CSF<sup>+</sup> T cells, some publications have proposed the existence of an independent GM-CSF-expressing

T helper cell subset, termed Th<sub>GM</sub> cells [22, 33, 174, 175]. Multiple studies have described an important pathogenic function of GM-CSF in neuroinflammation. Similar to IL-17-deficient mice, GM-CSF-deficient mice are resistant to EAE [176] and GM-CSF<sup>+</sup> T cells are enriched in patients with MS [36]. Specific ablation of GM-CSF expression in T cells during EAE leads to unchanged invasion of T cells into the CNS, but the infiltrating cells induce a significantly milder disease with reduced numbers of CNS-invading monocyte-derived cells [33]. However, in the 2D2 T cell transfer EAE model, *Csf2*<sup>-/-</sup> 2D2 cells transfer similar disease to wildtype 2D2 cells, whereas deletion of *Bhlhe40* (required for expression of *Csf2*) leads to complete loss of disease (unpublished). Moreover, minimal evidence exists that Th<sub>GM</sub> cells exhibit a novel T helper program that is independent of other T helper cell subsets. To date, no master transcription factor for GM-CSF<sup>+</sup> T cells has been identified and no distinct mechanisms or differentiation conditions have been identified that specifically induce GM-CSF in naïve CD4<sup>+</sup> T cells. Most importantly, fate-mapping studies need to be performed to show that encephalitogenic GM-CSF-producing T cells do not reflect induction of the cytokine in other T helper cell subsets. In fact, during EAE, ~80% of CNS-infiltrating CD4<sup>+</sup> T cells were found to originate from the Th17 lineage and ~40% of these cells expressed IFN $\gamma$  and ~40% expressed GM-CSF, indicating that a great proportion of GM-CSF<sup>+</sup> T cells during EAE are derived from IL-17A-expressing cells [76]. Moreover, our recent *in vivo* fate-mapping studies show that in EAE there is significant TCR clonal sharing between homeostatic IL-17A/F<sup>+</sup> Th17 cells (SLAMF6<sup>+</sup>) and pathogenic GM-CSF<sup>+</sup> cells (CXCR6<sup>+</sup>). There was also *in vivo* conversion of SLAMF6<sup>+</sup> cells to CXCR6<sup>+</sup> cells upon adoptive transfer, suggesting that, during EAE, pathogenic Th17 cells arise by the conversion of homeostatic Th17 cells to pathogenic Th17 cells [38]. Together, these data suggest that under pro-inflammatory conditions (IL-23, IL-1 $\beta$ , etc.) GM-CSF is generally up-regulated in T cells. This conclusion is further supported by the findings that GM-CSF<sup>+</sup> T cells co-express a great variety of other cytokines including IL-2, IL-3, IL-4, IL-6, IL-13, IL-17A, IL-22, IFN $\gamma$ , and TNF [33, 36, 76, 175]. Therefore, GM-CSF does not seem to mark T cells with a unique program that is distinct from programs directed by the key master transcription factors for Th1 (T-BET), Th2 (GATA3), and Th17 (ROR $\gamma$ t) cells. Hence, we propose GM-CSF expression as part of a general pathogenic program together with other pro-inflammatory molecules such as BHLHE40, IFN $\gamma$ , and CXCR6 that can be induced across multiple T helper cell subsets [33, 38, 177]. (Figure 3). Conversely, expression of IL-10 and co-inhibitory molecules marks a general anti-inflammatory module that can be induced by IL-27 in any T cell subset [105, 178–180].

### Intestinal Th17 cells and autoimmunity

Intestinal Th17 cells are important mediators of intestinal epithelial barrier integrity [130, 157], however, they have also been implicated in driving intestinal inflammation and, interestingly, in regulating autoimmune diseases outside of the intestine (Figure 4). In the intestine, Th17 cytokines IL-17A, IL-17F, and IL-22 were shown to mediate protective functions, but the requirement for IL-23 and ROR $\gamma$ t in mouse models of colitis indicates the potential involvement of Th17 cells in inducing intestinal inflammation as well [127, 131, 132]. In *Helicobacter hepaticus*-dependent colitis, pathobiont-specific inflammatory Th17 cells are major mediators of intestinal inflammation [43]. Expansion of these pro-inflammatory Th17 cells is restrained by *H. hepaticus*-specific ROR $\gamma$ t<sup>+</sup>FOXP3<sup>+</sup> regulatory

T cells (iTreg) that are dependent on the expression of the transcription factor c-MAF. Recent data suggest that the induction of these iTreg cells is mediated by a novel population of ROR $\gamma$ t<sup>+</sup> antigen presenting cells (APCs) [181–183]. Treg-inducing activity of these ROR $\gamma$ t<sup>+</sup> APCs requires their expression of the MHC class II antigen presentation machinery, CCR7, and  $\alpha$ <sub>v</sub> $\beta$ <sub>8</sub> integrin that releases active TGF- $\beta$  from its latent form. The ROR $\gamma$ t<sup>+</sup> cells have been proposed to be a subset of type 3 innate lymphoid cells (ILC3) or a recently described novel bone marrow-derived AIRE<sup>+</sup> cell with features of both dendritic cell and medullary thymic epithelial cells [181–183]. In contrast, induction of the *H. hepaticus*-specific pathogenic Th17 cells is mediated by APCs that do not require CCR7 and do not express CD11c. While the precise identity of these APCs is not yet known, they are distinct from APCs required for the induction of homeostatic Th17 cells by SFB, as those cells are dependent on CCR7 and express CD11c [182] (unpublished).

In addition to pro-inflammatory functions in the intestine, the intestinal Th17 population has been implicated in driving extra-intestinal autoimmune diseases. Multiple studies have highlighted a critical role of the intestinal microbiota in the development of extra-intestinal autoimmune diseases [88, 184–186]. The intestinal microbiota is required for the development of EAE, as germ-free mice are protected against EAE [50, 184]. The transfer of intestinal microbiota from patients with MS into mice exacerbates EAE [186]. In a recent study two intestinal microorganisms were found to act together to promote EAE disease [185]. Erysipelotrichaceae acted as an adjuvant that induced Th17 cells in the small intestine and was synergistic in promoting EAE with *Lactobacillus reuteri*, which possesses peptides that show molecular mimicry with MOG. Mice co-colonized with both strains developed more severe EAE disease than germ-free or mono-colonized mice. Interestingly, the induction of intestinal Th17 cells by gut microbes has been suggested as the mechanism of microbiota-dependent exacerbation of EAE disease in multiple studies [50, 184, 185, 187]. Indeed, in multiple autoimmune disease mouse models, including EAE, autoimmune arthritis, and autoimmune renal disease, the microbiota-specific induction of intestinal Th17 cells was found to be associated with worsened autoimmune disease at extra-intestinal sites [50–52]. However, until recently, the mechanisms by which intestinal Th17 cells influence extra-intestinal autoimmunity remained unclear. One study by *Hiltensperger et al.* [188] performed site-specific *in vivo* labeling of T cells in EAE and identified inguinal lymph node-derived T cells (i-T cells) as exhibiting a distinct transcriptomic signature from mesenteric lymph node-derived T cells (m-T cells). While i-T cells are marked by CXCR6 and infiltrate white matter, m-T cells are marked by P2RX7 and migrate to the gray matter, indicating that the priming-site of T cells regulates their function. In our recent study we combined fate-mapping with single-cell profiling of the transcriptomes and TCR clonotypes of Th17 cells during EAE and identified a homeostatic, stem-like TCF1<sup>+</sup> IL-17<sup>+</sup> SLAMF6<sup>+</sup> Th17 cell population that traffics to the intestine where it is maintained by the microbiota [38]. Interestingly, this intestinal SLAMF6<sup>+</sup> population shares TCRs with encephalitogenic GM-CSF<sup>+</sup> IFN $\gamma$ <sup>+</sup> CXCR6<sup>+</sup> T cells, suggesting that it may provide a reservoir for the IL-23-driven generation of the pathogenic cells. These findings provide a plausible mechanism for how homeostatic intestinal Th17 cells participate in extra-intestinal autoimmune disease. In the future it will be important to identify antigen specificities of the TCRs shared between the SLAMF6 and CXCR6 populations to identify the pathogenic

targets and to explore the possibility of the involvement of cross-reactivity to microbiota in tissue-specific autoimmunity. In addition, it will be interesting to reveal the identity of the APCs inducing the different types of Th17 cells and whether homeostatic Th17 cells with potential to further differentiate into pathogenic Th17 cells are primed by the same or different APC subsets. Our data indicate that APCs that direct the differentiation of SFB-specific homeostatic Th17 cells are distinct from those that direct *H. hepaticus*-specific colitogenic Th17 cells. It is possible that Th17 cells primed by one type of APC can acquire pathogenic functions in the presence of IL-23 upon subsequent stimulation by the same or a different type of APC. It will also be important to understand whether similar mechanisms induce pathogenic Th17 cells in autoimmune inflammation in other tissues besides the CNS. Moreover, if the stem-like, non-pathogenic Th17 cells in the intestine can be further validated as a reservoir for the generation of effector T cells across autoimmune diseases, the population might contribute to the chronicity and relapses observed across many human autoimmune conditions [189]. Hence, the targeting of the conversion of stem-like homeostatic to pathogenic T cells in autoimmunity could yield a novel treatment approach for prevention of relapses. On an additional note, the homeostatic SLAMF6<sup>+</sup> cell population shows multiple parallels in transcriptional phenotype and function to previously described stem-like CD8<sup>+</sup> T cells in tumors and in response to LCMV infection. Interestingly, anti-tumor immunity [190] and responses to check-point blockade therapy [191–195] have recently been shown to be influenced and regulated by the gut microbiota. Hence, the conversion of stem-like intestinal CD8<sup>+</sup> T cells to effector T cells driving anti-tumor immunity could be a mechanism by which gut microbiota influences anti-tumor immunity and response to checkpoint blockade therapy. Future studies will be required to explore these novel directions.

## Conclusion and perspective

Th17 cells are an important cell type that exerts both beneficial or pathogenic functions under different conditions and at multiple tissue sites. In the last few years, the use of single-cell technologies has revealed a significant amount of heterogeneity within the Th17 population *in vivo*, shedding light on mechanisms that underlie the diversity between pathogenic Th17 and homeostatic Th17 states. Furthermore, plasticity has been identified as major characteristic of the Th17 lineage, including the upregulation of discrete effector modules in response to environmental stimuli in different tissues. At homeostasis Th17 cells were found to exhibit strong tissue-specific signatures. In future studies it will be interesting to understand if these tissue-specific signatures represent specialized functional roles acquired by Th17 cells in different tissue microenvironments, similar to tissue Treg cells [106] and tissue macrophages [107].

The role of Th17 cells in autoimmune diseases has been controversial, partly due to the proposal that GM-CSF<sup>+</sup> cells make up a pathogenic T cell subset distinct from Th17 cells. However, fate-mapping studies revealed an *in vivo* relationship of IL-17-expressing Th17 cells and GM-CSF<sup>+</sup> T cells, indicating that GM-CSF<sup>+</sup> cells are either current Th17 cells or ex-Th17 cells. These analyses highlight the importance of fate-mapping studies and/or TCR clonotype tracing in understanding the trajectory of T cell phenotype development. In future studies, it will be interesting to understand the role of the Th17 program in the context of

pathogenic GM-CSF<sup>+</sup> IFN $\gamma$ <sup>+</sup> T cells. Why do these cells go through a Th17 trajectory and is that required for their pathogenic potential? Moreover, in addition to IL-23, what are the main drivers converting homeostatic Th17 cells to pathogenic Th17 cells *in vivo*? Finally, is there a requirement for a stem-like or homeostatic pool of Th17 cells to supply further differentiated pathogenic cells at inception and during the chronic phase of inflammation?

In addition to autoimmunity, Th17 cells have also been implicated in playing a role in cancer. However, current evidence for the role of Th17 cells in cancer is contradictory. While some studies suggest that Th17 cells contribute to carcinogenesis by promoting chronic inflammation, fostering angiogenesis, tumor cell proliferation and migration, other studies suggest an anti-tumor function of Th17 cells by enhancing cytotoxic T cell and NK cell responses and neutrophil infiltration [196–202]. The differences in Th17 cell functions in tumors seem to be tumor context-dependent. The comprehension of the processes controlling anti- and pro-tumor functions of Th17 cells will likely provide important insight for developing effective cancer immunotherapy.

Our understanding of the dichotomy within the Th17 cellular compartment creates opportunities for improved strategies for the treatment of autoimmune and inflammatory diseases. The exacerbation of IBD following IL-17 blockade [29, 49] highlights the need for specific targeting of the pathogenic Th17 population, while leaving Th17 cells that mediate barrier functions and homeostasis intact. Indeed, strategies focused on the transition of homeostatic Th17 cells to pathogenic Th17 cells, beyond IL-23 blockade, could represent a novel approach that would leave homeostatic functions intact while limiting tissue damage mediated by pathogenic Th17 cells.

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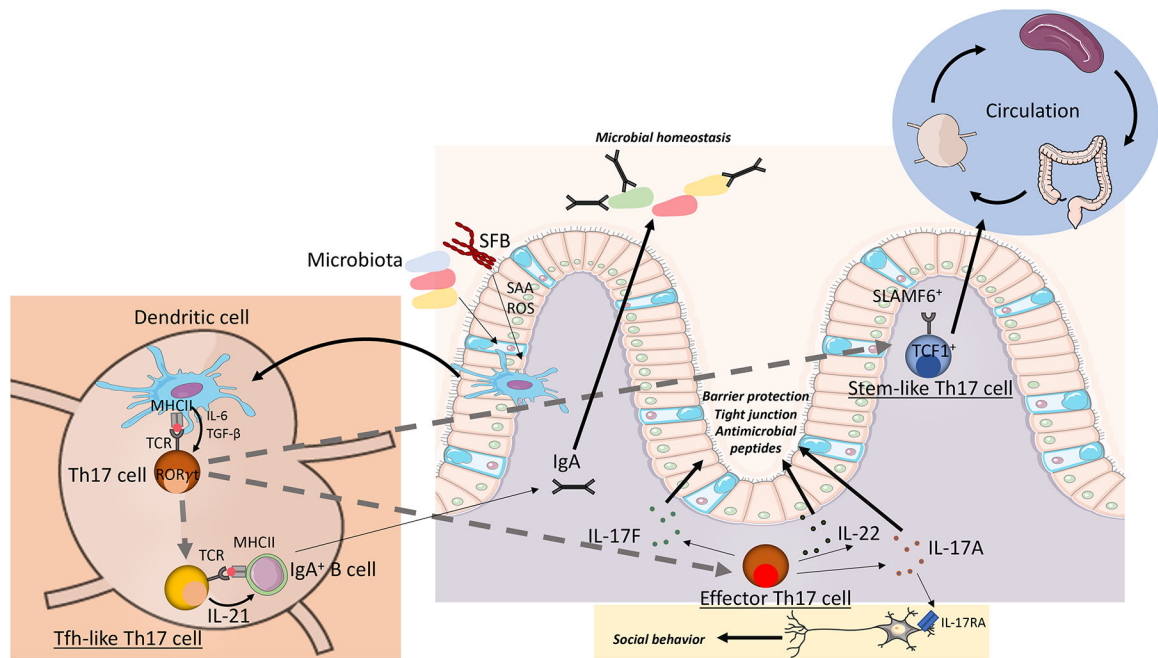
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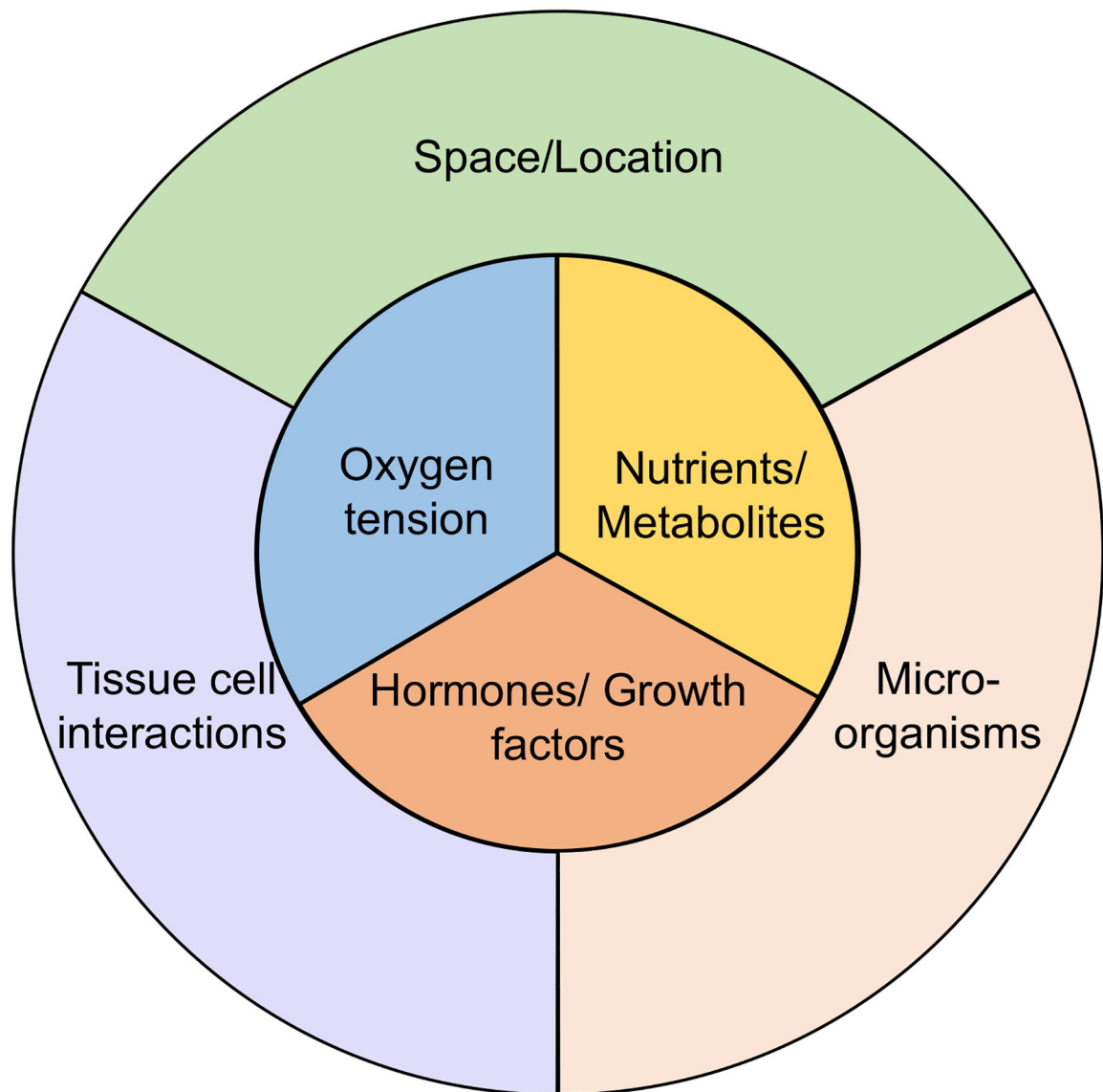


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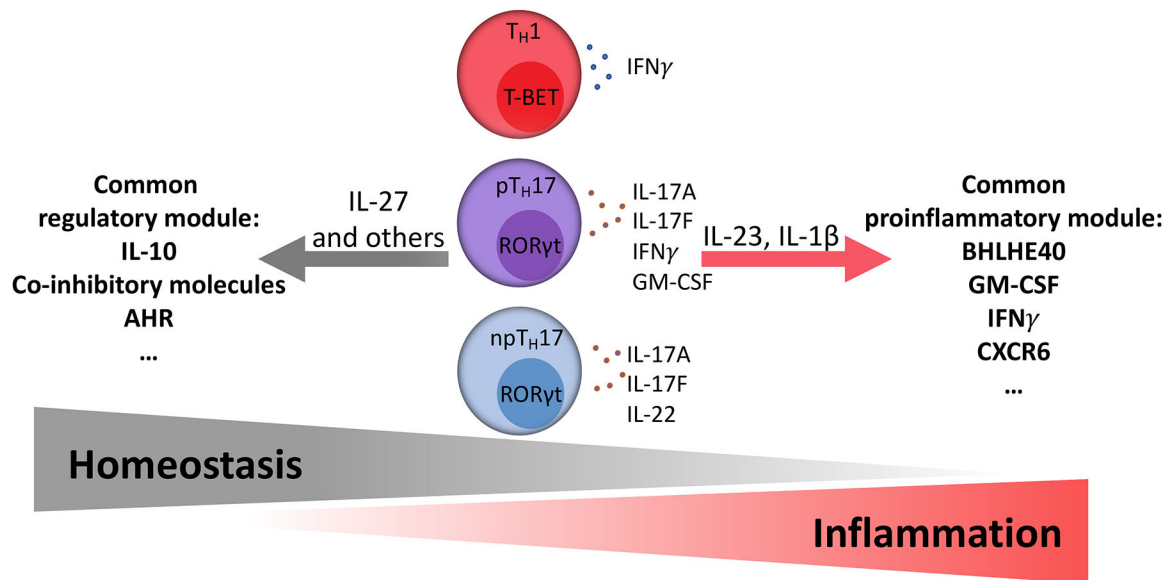
**Figure 1: Th17 cell heterogeneity in intestinal homeostasis**

Schematic depicting the role of intestinal Th17 cells in intestinal homeostasis. Specific microbiota, including epithelial-adhering bacteria, induce the differentiation of antigen-specific, naïve CD4<sup>+</sup> T cells to Th17 cells [71]. By differences in the APC or intra-tissue plasticity, the Th17-polarized T cell clones can give rise to different Th17 subpopulations. One subpopulation includes SLAMF6<sup>+</sup> stem-like Th17 cells that circulate through lymphoid organs and intestinal tissues and are maintained by the gut microbiota [38]. Effector Th17 cells express high levels of IL-17A, IL-17F, and IL-22 which directly support the barrier function of the intestinal epithelium by enhancing the expression of tight junction proteins and antimicrobial peptides [70, 71]. In addition, intestinal IL-17A has been shown to support social behavior by directly signaling through neurons [82]. Tfh-like Th17 cells induce IgA-production by B cells in mesenteric lymph nodes and Peyer's patches, thereby supporting microbial homeostasis [26, 38].



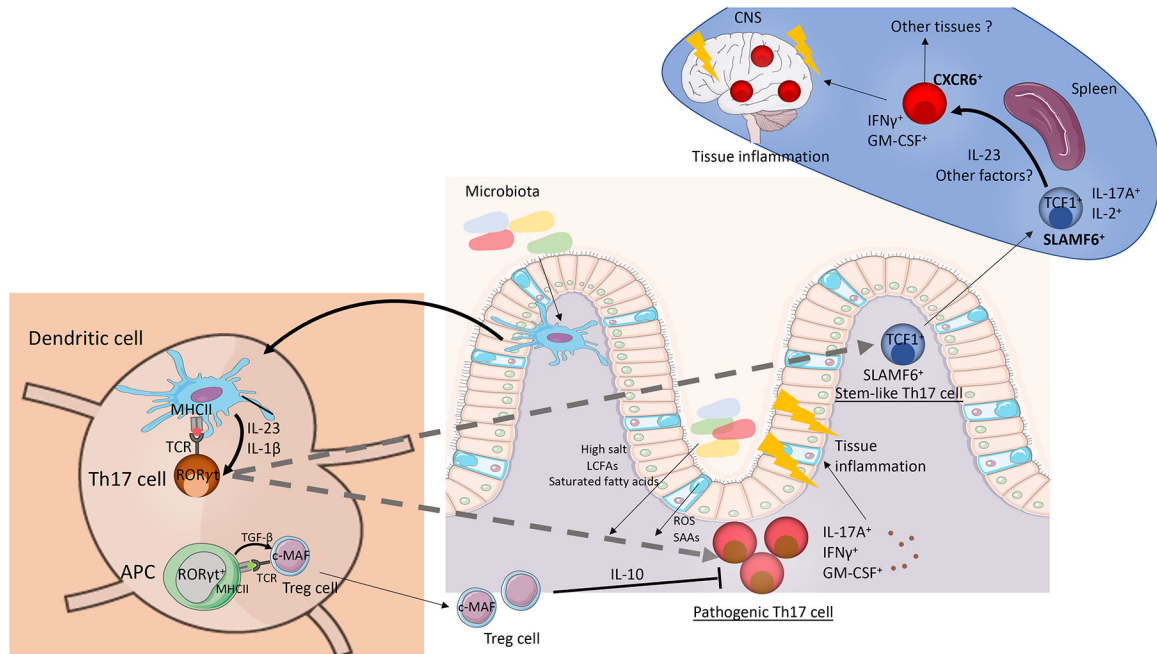
**Figure 2: Drivers of tissue-specific signatures of immune cells**

Tissue immune cells, including macrophages [107], Treg cells [106] and Th17 cells [38], acquire specific transcriptomic signatures depending on the tissue they are resident in. Indirect (outer circle) and direct (inner circle) factors likely shape these tissue-specific signatures of immune cells. Indirect factors include the space and location of the cell in the tissue, the interactions with tissue cells, and the presence of microorganisms. Direct factors influencing the tissue signatures are oxygen availability in the tissue, present nutrients and metabolites shaping the cell metabolism, and hormones and growth factors that directly bind and signal through their receptors on the immune cells.



### Figure 3: Th1 and Th17 cell subsets in tolerance and inflammation

CD4<sup>+</sup> T helper cell subsets, including Th1 cells, Th2 cells, and Th17 cells, are characterized by the expression of signature cytokines and master transcription factors. In addition, during inflammation or resolution of inflammation common pro-inflammatory or regulatory modules can be induced in the cells, as shown for Th1 [173] and Th17 cells [53]. IL-23 and IL-1 $\beta$  induce a common pro-inflammatory module including Bhlhe40, GM-CSF, IFN $\gamma$ , and CXCR6 [33, 38, 177]. In contrast, IL-27 induces the expression of a common regulatory module that includes anti-inflammatory molecules such as IL-10 and co-inhibitory molecules [105, 180]. The color coding and positioning of the cell types represents pTh17 cells as an intermediate cell type between npTh17 cells and Th1 cells [60]. pTh17= pathogenic Th17 cell, npTh17= non-pathogenic Th17 cell.



#### Figure 4: Th17 cells in intestinal and extra-intestinal autoimmunity

Schematic of the role of intestinal Th17 cells in autoimmune tissue inflammation in the intestine and in extra-intestinal organs. Specific microbiota induce the generation of intestinal Th17 cells [71]. Some of the Th17 cells can give rise to the SLAMF6<sup>+</sup> stem-like Th17 subpopulation that circulates through lymphoid organs and intestinal tissues [38]. Under the presence of IL-23 signaling and presentation of auto-antigens by APCs in the spleen, the SLAMF6<sup>+</sup> population can give rise to CXCR6<sup>+</sup> pathogenic Th17 cells that express high levels of GM-CSF and IFN $\gamma$ . This CXCR6<sup>+</sup> pathogenic Th17 population migrates directly to the inflamed tissue where it mediates autoimmune tissue inflammation. In the intestine diverse factors promote the generation of pathogenic Th17 cells that express high levels of IL-17A, GM-CSF, and IFN $\gamma$  and drive tissue inflammation. High salt, LCFAs, and saturated fatty acids from the diet and the microbiota and the expression and secretion of SAAAs and reactive oxygen species (ROS) by intestinal epithelial cells favor a pathogenic phenotype in Th17 cells [37, 71]. In addition, the secretion of pro-inflammatory cytokines IL-1 $\beta$  and IL-23 by myeloid cells differentiates pathogenic Th17 cells. The pathogenic Th17 population is regulated by a pool of intestinal Treg cells that are dependent on the transcription factor c-MAF [43]. In *Helicobacter hepaticus*-dependent intestinal colitis, the induction of the *H. hepaticus*-specific Treg cells is mediated by a recently identified population of ROR $\gamma$ t<sup>+</sup> APCs, that are equipped to release TGF- $\beta$  [181–183].