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Balancing Pro- and Anti-Inflammatory CD4⁺ T Helper Cells in the Intestine

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1. Introduction

The intestinal mucosal surface represents a huge border where different pathogenic particles, such as bacteria, fungi, viruses or parasites can potentially invade and harm the body. One crucial task of the immune system in the intestine is to maintain this epithelial barrier, in order to prohibit or defeat a microbial invasion. Pro-inflammatory effector CD4⁺ T helper cells play a crucial role during this task. These effector T helper cells can be subdivided into different subsets (Figure 1), which are characterized by a master transcriptional regulator and a unique cytokine profile: Th17 cells express ROR γ t that in turn promotes the transcription of *Il17a*, *Il17f*, *Il21* and *Il22*. Th1 cells express T-bet and produce IFN- γ , IL-2 and TNF- α . Th2 cells express GATA-3 and secrete IL-4, IL-5 and IL-13 [1-5]. The intestine also contains numerous non-pathogenic bacteria (commensal bacteria), which are beneficial to the host, as well as food antigens. This vast collection of non-self antigens can also promote the activation of T helper cells, and in turn cause immune-pathology. Therefore it is important for the immune system to control effector T helper cells. Indeed different types of regulatory T cells with anti-inflammatory properties team up in order to control effector T cells. The two most studied regulatory T cell subtypes are Foxp3⁺ regulatory T cells, which can be generated either in the thymus (nTreg) or induced in peripheral lymphoid organs (iTregs) and type 1 regulatory T cells (Tr1), which are induced in the periphery (Figure 1).

2. Differentiation of naïve CD4⁺ T cells into effector T helper cells

Naïve T cells, which are functionally immature, can be differentiated into different subsets of effector T cells upon activation. The fate of naïve T cell is directed by cytokines. These cytokines signal via different members of the STAT family, which induce master transcriptional regulators. Most of these transcriptional factors bind then to the effector

cytokine gene thereby inducing gene activation, repression or epigenetic modification [6] (Figure 1). It should be noted that there is a certain amount of T helper cell heterogeneity and plasticity regarding cytokine production and expression of the master transcriptional factor of each T helper cell subset. This fact is currently also one of the most intriguing aspect of the ongoing research in Immunobiology. However, the model of different T helper cell lineages as first proposed by Mosmann and Coffman [7] is still the most useful one in order to understand the function and differentiation of T helper cells.

Figure 1. Differentiation of naïve T cells into different effector and regulatory T cells. A specific combination of cytokine signals leads to the differentiation of naïve T cells into different T helper cell subsets. Each T helper cell subset is characterized by the production of a combination of cytokines and exerts specific functions.

2.1. Differentiation and function of Th1 cells

Th1 cells produce IFN- γ as their signature cytokine. Th1 cells secrete also IL-2 and/ or TNF- α . Naive T cells upon TCR stimulation in the presence of IL-12 differentiate in Th1 cells [8].

IL-12 signals via STAT4 promoting the expression of T-bet, which transcribes the *Ifng* gene [9, 10]. T-bet is the master transcriptional regulator of Th1 cells, which is essential for the IFN- γ production [5]. Accordingly T-bet deficient mice have a defective Th1 differentiation [11]. One other important function of T-bet is the inhibition of GATA-3 expression, the master transcriptional regulator of Th2 cells [9].

Th1 cells are particularly important for the defense against intra-cellular bacteria. Some microorganisms such as mycobacteria, like *Mycobacterium tuberculosis* or *Mycobacterium lepromatosis*, are examples for these intracellular pathogens. These bacteria grow primarily in phagolysosomes of macrophages. Because of this feature these microorganisms are protected from the effects of antibodies and cytotoxic T cells. These bacteria can inhibit the fusion of lysosomes to the phagosomes, in which they grow and prevent the activation of the lysosomal proteases. The defense against these microorganisms is the important task of Th1 effector cells because they can activate macrophages, which are then able to kill ingested pathogens. Accordingly, deficiency in Th1 cells increases the susceptibility to infections with intracellular pathogens in humans [12]. These patients suffer from infections with mycobacteria, particularly *Mycobacterium tuberculosis*, but also with *Salmonella*. Of note both of these bacteria strains can typically infect the gastrointestinal system.

2.2 Differentiation and function of Th2 cells

The signature cytokines of Th2 cells are IL-4, IL-5, and IL-13. Some Th2 cells also produce TNF- α and/ or IL-9. Additionally, some Th2 cells can secrete small amounts of IL-2. The cytokines leading to Th2 differentiation are IL-2 and IL-4. Therefore the signature cytokine of Th2 cells, IL-4 also promotes the differentiation of Th2 cells [13-15]. STAT6 is the major signaling pathway of IL-4 mediated Th2 differentiation, and induces GATA-3 expression [16-20]. GATA-3 is the master transcriptional regulator of Th2 cells [3, 21] and the differentiation of these cells is indeed dependent on the induction of this master transcriptional regulator [22]. GATA-3 binds to the promoters of *Il5* and *Il13*, and the enhancer of *Il4* thereby promoting their transcription [6]. Additionally STAT5, which can be activated by IL-2, is important for Th2 differentiation and for the maintenance of GATA-3 expression [23].

Th2 cells and their effector cytokines IL-4, IL-5, and IL-13 are essential to control helminth infections in the intestine. In line with this, mice deficient in IL-4 receptor α -chain (IL-4R α), STAT6 or GATA-3 show highly compromised anti-helminth immunity [24]. One of the most unique tasks of Th2 cells is the induction of B-cell immunoglobulin class switching. Through CD40-CD40L interaction, Th2 cells promote B cells to secrete IgG1, IgE and (in humans) IgG4 isotype antibodies. These antibodies are again important for mediating protection against helminth infections. The Th2-immune response involves also eosinophils, basophils and mast cells, which all together mount the immune response controlling helminth infection. The release of IL-4 and IL-13 is key for eliciting the alternative activation of macrophage, which is crucial in order to trap the intestinal parasite [25, 26]. Th2-cytokines, in particular

IL-4 and IL-13, promote the goblet cell differentiation, the enhancement of mucus secretion and the production of resistin-like molecule- β (RELM β), an innate protein with direct anti-helminth activity [27-29]. Moreover IL-4 stimulates intestinal muscle hyper-contractility and accelerates epithelial turnover to promote the 'epithelial escalator', which functions together with epithelial secretions to dislodge resident parasites [30, 31]. Another Th2 associated cytokine, namely IL-9, promotes the release of mast cell protease that can depredate tight junctions and in turn increase the fluid flow in the intestine. All together these mechanisms are part of the "weep and sweep" response, which is key for the control of a helminth invasion.

2.3. Differentiation and function of Th17 cells

The signature cytokines of Th17 cells are IL-17A and IL-17F. Th17 cells produce also, IL-22 and TNF- α . TGF- β , IL-1 β , IL-6, and IL-23 are the cytokines, which are important for Th17 cell differentiation. IL-6 can activate STAT3, which induces IL-23R and ROR γ t [32-34], the master transcriptional regulator of Th17 cells. This master transcriptional regulator leads to the production of IL-17A and IL-17F [1, 4, 35, 36]. IL-6 also promotes the release of IL-21 [33], which synergizes with TGF- β , IL-6, and IL-1 β , for the induction of IL-23 receptor expression [37]. In the presence of IL-23, CD4⁺ ROR γ t⁺ IL-17A⁺ T cells can expand and fully mature in Th17 cells [38, 39].

Human and mouse Th17 cells are rare in a non-pathological state [2, 40]. A specific member of commensal microbiota, known as segmented filamentous bacteria (SFB), attracts Th17 cells in the terminal ileum of mice [41]. Therefore in steady state condition most of the few Th17 cells accumulate mainly in the intestine. The commensal microbiota promotes the release of serum amyloid A [41] and adenosine 5'-triphosphate (ATP), which activates lamina propria mononuclear phagocytes. These phagocytes in turn promote Th17 cell differentiation [42]. Among all cytokines known to induce the differentiation of Th17 cells, the presence of IL-1 β rather than IL-6 is essential in the intestine [43]. TGF- β 1 is also not essential for the differentiation of Th17 cells in the intestine, but may influence the phenotype of Th17 cells together with IL-1 β [44]. Th17 cells, which have been differentiated in the presence of TGF- β 1 are less pathogenic and produce more IL-10 compared to Th17 cell differentiated in the presence of IL-1 β [45, 46].

Th17 cells also produce several other cytokines besides IL-17A and IL-17F. Cytokine production by Th17 cells is also modulated by environmental factors in the intestine. For example, the activation of the environmental chemical receptor and transcription factor aryl hydrocarbon receptor in Th17 cells is important for the production of IL-22 [47-49]. IL-22 is a critical cytokine for antimicrobial immunity exerted by Th17 cells [50]. On the other hand, the induction of c-maf upon stimulation with IL-27, promotes the release of IL-10 from Th17 cells [51], and these IL-10 producing Th17 cells are also particularly induced in the intestine [40]. Therefore Th17 cells can have different cytokine profiles depending on environment factors.

In the absence of pathology, Th17 cells are very rare. However pathogenic infections, such as fungi infection with *Candida albicans*, or bacterial infection with gram positive or gram negative extracellular bacteria, such as *Citrobacter rodentium* or *Klebsiella pneumoniae* lead to a dramatic increase of the number of Th17 cells [52-56]. Viral infection also promotes a Th17-cell mediated immune response [40]. In line with this, Th17 cells and their effector cytokines IL-17A, IL-17F, and IL-22 are critical for proper host defense against various infections, especially against extracellular bacteria and fungi. The receptors for IL-17A, IL-17F and IL-22 are broadly expressed throughout the intestinal epithelial tissue. Therefore Th17 cells can provide crosstalk between immune system and tissues [2, 57].

IL-17A and IL-17F strongly induce the recruitment of neutrophils to the inflammatory site. The subsequent induction of the chemokine CCL20 attracts even more Th17 cells via CCR6, the chemokine receptor of CCL20, which is highly expressed by Th17 cells [58]. Additionally, both IL-17A and IL-17F promote β -defensin production [56, 59, 60]. β -defensins play an important role in the immune responses against bacterial infections. Interestingly, IL-17A and IL-17F can compensate each other during the host defense against *S. aureus* [56]. However during other infections, such as *Citrobacter rodentium*, IL-17A and IL-17F are both required in order to control the bacterial dissemination [56].

At the mucosal surface IL-22 has a crucial function for host defence and tissue homeostasis. IL-22 induces the expression of antimicrobial peptides from epithelial cells and limits bacterial replication and dissemination during *Citrobacter rodentium* infection [57, 61]. Furthermore IL-22 can promote epithelial cell proliferation, survival, and tissue repair in the intestine [62-64].

However it should be noted that several other immune cells besides Th17 cells can produce IL-17A and IL-22, thereby also contributing to the defense against pathogens (for review see [50]).

2.4. Differentiation and function of Foxp3⁺ Treg cells

In 1995 Sakaguchi et al. first described a subpopulation of regulatory T cells characterized by the constitutive expression of the IL-2 receptor α -chain (CD25). These regulatory T cells were called CD4⁺CD25⁺ Treg [65]. Foxp3 was identified later on as the master transcriptional regulator of CD4⁺CD25⁺ Treg cells, which have been called Foxp3⁺ Treg cells thereafter [66, 67]. Foxp3⁺ Treg cells can be generated within the thymus (tTreg) [65]. However, Foxp3⁺ Treg cell numbers are also regulated in peripheral lymphoid organs both by expansion of pre-existing Foxp3⁺ Treg and by de novo generation of induced regulatory T cells (iTreg). The combination of the cytokines IL-2 and TGF- β 1 are key for the differentiation of naïve T cells into Foxp3⁺ iTreg cells [68-73]. Foxp3⁺ Treg cells are essential to control auto-reactive T cells, which can react to self-antigens and cause damage to the host. The key role of Foxp3⁺ Treg cells in the peripheral immune response is evident in murine models [74] and in humans [75]: scurfy mice [74] and IPEX

(immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) patients [75] lacking the master transcriptional factor of regulatory T cells - Foxp3 - consequently develop strong autoimmune disorders. Importantly, a severe form of autoimmune enteropathy is characteristic for scurfy mice and IPEX patients [75]. This underlines the importance of Foxp3⁺ Treg cells for controlling the immune response in the intestine. Foxp3⁺ Treg cells have different mechanism to suppress effector T cells. Some of these are mediated via soluble factors (i.e. IL-10, TGF-β1 [76, 77]) and others are cell contact dependent (i.e. CTLA-4, cAMP [78, 79]). Recent studies have demonstrated that Foxp3⁺ Treg cells can acquire some features of effector T helper cells in order to better control them (see paragraph 4.3).

2.5. Differentiation and function of Tr1 cells

In 1994, T regulatory type 1 (Tr1) cells were isolated from severe combined immuno deficiency (SCID) patients transplanted with allogeneic haematopoietic stem cells (HSCT). Subsequently it was possible to test the regulatory capacity of this new type of T cells directly in murine IBD models. To date Tr1 cells lack a defined cell surface signature, and their identification relies therefore on their unique cytokine profile. Tr1 cells secrete high levels of IL-10 as compared to IL-4 and IL-17A, the hallmark cytokines of Th2 and Th17 cells respectively. Depending on the milieu Tr1 cells can produce variable levels of IFN-γ, the key cytokine produced by Th1 cells [80]. However, Tr1 cells possess the capacity to suppress inflammatory T cell responses and, therefore are distinct from bona fide Th1, Th2 and Th17 cells that largely promote rather than suppress the inflammatory responses.

Tr1 cells are induced in the periphery, and they respond selectively to persistent foreign and self-antigens under steady-state conditions [81].

After the discovery of Foxp3 as the master transcriptional regulator of Foxp3⁺ Treg cells, it became a key point, if also Tr1 cells express a master regulator. The double reporter mouse model for IL-10 and Foxp3 was instrumental in order to demonstrate that these two types of regulatory T cells are distinct. Indeed, Tr1 cells do not constitutively express Foxp3 [82, 83] and can be induced from IPEX patients who lack Foxp3 [84]. However the master transcriptional regulator for Tr1 cells has not been identified so far.

IL-10 has been considered to be the driving force for Tr1-cell generation on the basis of experiments in which antigen-specific Tr1 cells are induced *in vitro* by repeated TCR stimulation in the presence of high doses of IL-10 [85, 86]. However, the frequency of Tr1 cells in IL-10 deficient mice is not altered. Several recent publications have demonstrated a key role of IL-27, which can even synergize with TGF-β, in the induction of Tr1 cells. During the induction of Tr1 cells by IL-27, the ligand-activated transcription factor hydrocarbon receptor (AhR) physically associates with c-avian musculoaponeurotic fibrosarcoma (c-Maf) and transactivates the *Il10* and *Il21* promoters. The secretion of IL-21 acts as an autocrine growth factor for Tr1 cells (Reviewed in [87]).

Tr1 cells can control Th1, Th2 and Th17 cell, and regulate immune responses mainly through the secretion of the immunosuppressive cytokines IL-10 and TGF- β 1 [88]. The antigen-specific activation of Tr1 cells is important to potentiate their regulatory function [86]. IL-10 acts by limiting the magnitude of immune responses, as proved by mice that lack IL-10 and that exhibit spontaneous enterocolitis. IL-10 down-regulates the expression of co-stimulatory molecules, such as CD80, CD86, and MHC Class II, and pro-inflammatory cytokine production by APCs and inhibits the secretion of IL-2, TNF- α and IL-17 by effector T cells [89]. In particular, Tr1-cell supernatant diminishes the capacity of monocytes to stimulate Th1-cell responses and blocks the differentiation and maturation of DCs *via* IL-10 [90]. TGF- β down-regulates the functions of APCs [91] and inhibits the proliferation and cytokine production by T cells [92]. Therefore, the suppressive effects of Tr1 cells are reversed by the addition of anti-IL10 and anti-TGF- β neutralizing antibodies [85, 93, 94], but additional mechanisms may also contribute. Human Tr1 cells generated *in vitro* by crosslinking CD3 with CD46 can kill target cells through a granzyme B/ perforin dependent mechanism [95] [96]. Accordingly human Tr1 cells selectively kill myeloid cells (i.e., DC and monocytes) through granzyme B/ perforin [97]. This selective cell-killing is mediated by CD226, which is expressed on Tr1 cells. Only myeloid cells express the CD226-ligand (CD155). Thus, this type of regulatory mechanism by Tr1 cells requires a cell-cell contact with APCs.

3. The immune homeostasis in the intestine

The immune system has to respond selectively to harmful non-self pathogens and at the same time needs to minimize reactions against self and not-harmful antigens. This highly fine-tuned mechanism is possible due to a strict selection process, which happens in the thymus. Potentially auto-reactive CD4⁺ T cell progenitors, which recognize self-antigens with their T cell receptor (TCR), are either deleted or converted into thymic-derived CD4⁺ regulatory T cells (tTreg) with anti-inflammatory properties. This process, called central tolerance, is essential for the education of CD4⁺ T cells to respond selectively against foreign antigens. However this thymic control appears still to be insufficient. Therefore the immune system developed several other mechanisms to control potentially auto-reactive T cells, which take places in the periphery (peripheral tolerance). Among these mechanisms, the action of CD4⁺ regulatory T cells, which can be either selected in the thymus, (tTreg) or induced in the periphery (iTreg)[98], is one of the most studied. Treg cells are essential to control auto-reactive T cells, which can react to self-antigens and cause damage to the host.

The intestine is not only a source of self-antigens, but also contains a vast collection of non-self antigens, such as commensal bacteria. These antigens can promote the activation of naïve T cells causing immune-pathology such as inflammatory bowel disease (IBD). Therefore the immune system has established a second checkpoint in the intestine where naïve T cells, which are potentially able to respond to non self-antigens, are educated to be tolerant. There are important differences between thymus and intestine in tolerance induction. The driving force for the selection in the thymus is the affinity of TCRs to

MHC, while the flora and cytokines are crucial to determine the fate of naïve T cells in the intestine. Accordingly different commensal bacteria can selectively drive a tolerogenic or pro-inflammatory response. In line with this, the bacterial composition of the intestine has a substantial impact on the balance between pro-inflammatory and regulatory T cells in the intestine, and can also affect other organ specific diseases [41, 99-101]: For example mice lacking an innate sensor, which controls the intestinal micro-flora, are more susceptible to develop colitis [100]. Another interaction between the gut flora and an autoimmune disease was found in EAE (experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis). Multiple sclerosis is caused by an attack by auto-reactive T cells against brain white matter. Interestingly it was shown that the commensal gut flora can trigger these auto-reactive T cells, which then drive the disease. Finally it is known that the bacterial colonization between neonates born vaginally or by cesarean delivery differs, and interestingly these differences have been linked to an increased risk for atopic diseases such as allergic rhinitis and asthma in children born by cesarean delivery [102].

Considering the amount of self- and non self-antigens present in this organ and with it the potential to generate an unwanted immune response, different players are required to control the immune homeostasis in the intestine. The first one is a specialized subset of DC, which through the release of TGF- β and retinoic acid, is able to induce iTreg cells [103]. These iTreg cells represent then the second players. It is also known that naïve T cells migrate to the intestine in order to acquire an iTreg cell phenotype [104, 105]. Interestingly, these iTreg cells have a TCR repertoire, which is specific to an individual's microflora. Based on these results, one could hypothesize that iTreg cells have an advantage over tTreg cells (3th player), which are also present in the intestine, but are obviously non-bacteria specific. However, it was shown that specific commensal bacteria can directly activate tTreg cells bypassing the antigen specificity [106]. Both tTreg and iTreg are able to suppress effector T cells in the intestine, thereby curing or preventing colitis development [68, 98, 107-110]. It seems that tTregs and iTreg can also supplement the function of each other partially by expanding the TCR diversity [111]. Tr1 cells (4th players) are expanded in the absence of iTregs, and can at least partially compensate the absence of iTregs [112, 113]. Consistent with this, Tr1 cells and Treg cells can compensate each other to suppress effector T cells in the intestine [114].

In conclusion, commensal antigens in the intestine play an essential role in the regulation of the immune homeostasis. Naïve T cells, which could be potentially auto reactive, are converted into different types of regulatory T cells, which in turn control other effector T helper cells. The regulatory T cells originated in the thymus (nTregs) also participate in this regulatory environment by expanding the antigen specificity of the immune response.

4. Breakdown of the immune homeostasis in the intestine

Imbalance between pro- and anti-inflammatory T helper cells can cause intestinal pathology, such as IBD in humans. Crohn's disease (CD) and ulcerative colitis (UC) are the two main

forms of IBD. CD can attack any part of the digestive tract. It typically manifests in the ileum, although it can also selectively affect the large intestine. Histological Crohn's disease shows a transmural inflammation. This inflammation is characterized by focal infiltration of neutrophils into the epithelium. These neutrophils, along with mononuclear cells, can infiltrate the crypts, leading to inflammation or abscess. Granulomas, aggregates of macrophage derivatives, known as giant cells, are found in CD and are specific for the disease. Ulceration can also be seen in highly active CD. On the other hand, UC is a disease mainly of the colon that includes ulcerations. UC normally begins in the rectum, and can continuously affect the whole colon and also the terminal ileum. The pathology in ulcerative colitis involves distortion of crypt architecture, inflammation of crypts and hemorrhage. The inflammation is more superficial compared to CD and affects the mucosa and submucosa.

The aetiology of IBD is still unknown, but it seems that genetic and environmental factors contribute to disease development. Initial studies suggested that CD and UC are mediated by Th1 and Th2 cells respectively. This was based on the cytokine profile seen in CD (IL-12 and IFN- γ) and UC (IL-5, IL-13) [115]. However more recent work has shown that Th17 cells also infiltrate the intestine in CD and UC patients as well [116-118]. Accordingly the signature cytokines of Th17 cells (IL-17A, IL-17F, IL-22) are produced in the intestine of CD and UC patients [116, 118-120]. Additionally, genome wide association studies have linked polymorphism in Th17-related genes, such as *IL-23R* and *STAT3* with IBD [121-124]. In line with these associations murine studies have also shown that Th17 cells are involved in numerous autoimmune and chronic inflammatory diseases [2], and IBD is one of these diseases [125]: ROR γ t deficient mice, which lack Th17 cells, exhibit attenuated experimentally induced autoimmune disease [4]. Adoptive transfer of *in vitro* or *in vivo* differentiated Th17 cells into lymphopenic hosts leads to the development of colitis [114, 126-128]. IL-23, which is important for the maintenance, expansion and pathogenicity of Th17 cells [38, 39], is essential for the induction of colitis in mouse models. All together, these data argue for an important role of Th17 cells in IBD. However, Th17 cells produce several factors. And it is currently not completely understood, which of these is/are responsible for the pathogenicity of Th17 cells in the intestine [129-132].

One key feature of Th17 cells is their plasticity, which might also contribute to the pathogenic potential of Th17 cells. Epigenetic studies have shown that Th17 cells are more plastic compared to Th1 and Th2 cells [56, 133-135]. Th17 cells have bivalent domains of histone modifications in the *Tbx21* locus, which encodes for T-bet, the key transcriptional factor for Th1 cells. On the contrary, Th1 cells have only repressive markers in both *Rorc* and *Il17a* loci. These differences might account for the higher plasticity of Th17 cells relative to Th1 cells [133]. In line with these data, CD4⁺ T cells, which express both the key transcriptional factors and cytokines of Th17 and Th1 cells, have been found in the colon of mouse colitis models and moreover in colon of human IBD patients. They are also suggested to play an important role for the development of chronic disease [39, 114, 118].

Human IBD is characterized by a mixture of effector T cells. Therefore it is difficult to assess the relative contribution of a specific T helper cell subset in patients. However there are mouse IBD models, which are dominated by one specific T helper cells subset: Selective

deficiency in iTreg cells causes Th2 dominated intestinal pathology, which is characterized by gastritis and plasmacytic enteritis with increased frequencies of plasma cells in the intestinal lamina propria [113]. Another mouse model of colitis, which is induced by the transfer of naïve T cells into a lymphopenic host, is dominated by Th1 cells. This colitis model is characterized by IFN- γ dependent mucosal ulceration in the colon [136, 137]. Th17 cell-dominated intestinal pathology is characterized by mucosal hyperplasia but not ulceration [76, 136, 138]. IL-22, a signature cytokine of Th17 cells, can promote epithelial cell survival and proliferation. It is also important for the repair of the intestinal mucosa [63, 136, 139]. Accordingly, IL-22 induces the hyperplasia in the Th17 dominated colitis models [136]. On the contrary IL-22 is beneficial in Th1 dominated colitis models, which are characterized by ulceration [62]. Of note, the histomorphology in these Th1, Th2, or Th17 cell dominated mouse IBD models features only some characteristics of human IBD. But still these models are useful to evaluate the function of specific T helper cell subsets.

5. Control of pro-inflammatory T helper cells in the intestine

There are three ways to control effector T cells. First, inhibition of the differentiation of naïve T cells into effector T cells. Second, endogenous mechanism limiting the pathogenic potential of effector T cells. Third, control of effector T cells through regulatory T cells.

5.1. Inhibition of the *de novo* differentiation of effector T cells

One possibility is to inhibit the *de novo* differentiation of naïve T cells into effector T cells and generate regulatory T cells in stead. Such a reciprocal development pathway has been described for Th17 cells: High concentrations of TGF- β and/or retinoic acid up-regulate Foxp3 [140, 141], which in turn inhibits the induction of ROR γ t [37], thereby preventing the differentiation of Th17 cells. Moreover, IL-2 together with TGF- β 1 promotes the induction of Foxp3⁺ regulatory T cells (iTregs) instead of Th17 cells [142]. Interestingly, IL-2 blocks Th17 cell differentiation by directly inhibiting *Il17a* transcription. This second mechanism is largely independent of Foxp3 or ROR γ t expression, but dependent on the induction of STAT5, which competes with STAT3 for the common sites across the locus encoding IL-17A [143]. Finally, IL-27 through the activation and interaction of AhR and c-maf promotes the induction of type 1 regulatory T cells (Tr1) and efficiently counteracts the effects of TGF- β and IL-6 on CD4⁺ T cells, resulting in the inhibition of Th17 development in a STAT1-dependent manner (Reviewed in [144]).

5.2. Endogenous control of effector T cells

All effector T cell subsets (Th1, Th2, Th17) have the ability to acquire IL-10 production, thereby limiting their own pathogenicity [145]. This mechanism of self control has been very well described for Th17 cells (Figure 2): During particular bacterial and viral infection, naïve T cells mature in effector Th17 cells and contribute to the eradication of infections. However, if the Th17 response is too strong and potentially life threatening, Th17 cells are redirected to the small intestine in order to be controlled [40]. The reason why mature Th17

cells migrate mainly to the small intestine is because of the high expression of the chemokine receptor CCR6 [146]. The highest concentration of CCL20, the ligand of CCR6, is indeed in the small intestine [40]. Interestingly, IL-17A and IL-17F promote the release of CCL20 from epithelial cells in the duodenum. The recruited Th17 cells also produce CCL20, furthermore amplifying CCL20 production. This suggests that Th17 cells implement through a positive feedback loop the recruitment of other Th17 cells to the small intestine. Once effector Th17 cells migrated to the intestine, two complementary mechanisms occur in order to control them. First, effector Th17 cells are washed out and eliminated via the intestinal lumen due to the strong tissue destruction and diarrhoea. Secondly, Th17 cells are reprogrammed in regulatory Th17 (rTh17) cells. This last mechanism relies on the plasticity of these cells. In the intestine, effector Th17 cells acquire the capacity to produce IL-10 [40] and in parallel express IL-10R α . If Th17 cells cannot respond to IL-10, they acquire a “promiscuous” phenotype co-expressing IFN- γ and promote the inflammation in the small intestine [114].

Figure 2. Endogenous control of Th17 cells in the intestine. A strong Th17 response leads to the redirection of effector Th17 (eTh17) cells to the small intestine. Once eTh17 cells migrated to the intestine, two complementary mechanisms occur in order to control them. First, eTh17 cells are washed out and eliminated via the intestinal lumen due to the strong tissue destruction and diarrhoea. Secondly, Th17 cells are reprogrammed in regulatory Th17 (rTh17) cells.

5.3. Exogenous control of effector T cells via regulatory T cells

Importantly, other control mechanisms, which do not rely on the “sense of responsibility” of effector T helper cells, are also present (Figure 3). Regulatory T cells play an essential role for controlling T helper cells. The two most studied regulatory T cell subsets are Foxp3⁺ Treg and Tr1 cells. Foxp3⁺ Tregs can be induced in the periphery (Foxp3⁺ iTregs) or in the thymus (Foxp3⁺ tTregs). Interestingly, Foxp3⁺ iTregs are induced in the intestine by TCR recognition of commensal antigens [101, 104, 105, 147]. Foxp3⁺ tTregs cells are obviously non-bacteria specific, but nevertheless can be activated by some bacterial species in the intestine [106]. It

is also important that Foxp3⁺ iTregs and Foxp3⁺ tTregs perform complementary functions, in part by expanding the TCR diversity [111].

Figure 3. Control of effector T helper cells in the intestine. Different regulatory T cells can efficiently suppress specific T helper cell subsets in the intestine.

Although different types of regulatory T cells can partially compensate each other, it seems that regulatory T cells can also have a more specialized function, and suppress specific types of effector T cells more potent than others: mice with a selective deficiency in iTreg cells develop spontaneous intestinal inflammation, which is characterized by an expansion of Th2 cells [113], indicating that iTregs play an important role in controlling Th2 cells in the intestine. Moreover expression of GATA-3 and IRF-4, master regulators of Th2 cells, by Foxp3⁺ Treg cells is important for the control of Th2 cells [148, 149]. Additionally, some

Foxp3⁺ Treg cells can express T-bet the master transcriptional regulator of Th1 cells. These T-bet⁺Foxp3⁺ Tregs express CXCR3, which is also highly expressed by Th1 cells. Thanks to the expression of the same chemokine receptor T-bet⁺Foxp3⁺ Treg cells can better “follow” and in turn suppress Th1 cells [150]. Finally, it was shown that IL-10 can induce IL-10 production by Foxp3⁺ Treg via STAT3 activation, and Foxp3⁺IL-10⁺ Tregs are particularly important to control Th17 cells [76, 138, 151]. In addition to Foxp3⁺ Treg the immune system uses an alternative type of regulatory T cell, which can compensate a possible paucity of Foxp3⁺ Treg in order to avoid immune pathology in the intestine [112]. These cells, Tr1 cells, which are characterized by an abundant production of IL-10 and by the absence of Foxp3 expression, exert an efficient regulation of Th17 cells in the intestine [114]. Interestingly, IL-10 seems to play a non-redundant role in controlling Th17 cells: acting on both Th17 cells and regulatory T cells. Th17 cells are suppressed directly via IL-10, which is produced by Tr1 and Foxp3⁺Treg cells [114, 136]. Additionally, IL-10 acts on Foxp3⁺Treg. It activates STAT3 in Foxp3⁺Tregs, which is crucial to enable them to suppress Th17 cells [138, 151]. Moreover IL-10 signalling in Foxp3⁺Treg cells is required to promote IL-10 production [138] (Figure 3).

In conclusion Foxp3⁺ Treg cells can have different phenotypes. This feature allows Foxp3⁺ Tregs to suppress specific effector T cells more efficiently. Additionally Foxp3⁺ Tregs can team up with Tr1 cells to maintain the immune homeostasis in the intestine.

However, regulatory T cells do not only suppress effector T cells but can also promote effector T cell function in some settings [152, 153], indicating that the immune system aims to maintain a proper balance between regulatory and effector T cells rather than uncontrolled suppression of effector T cells.

6. Conclusions

CD4⁺ T helper cells have important physiological functions at the large intestinal mucosal surface: they secrete cytokines thereby attracting other immune cells, inducing anti-microbial peptides, and promoting tissue repair. Therefore effector CD4⁺ T helper cells play an important ‘border patrol’ function, and protect the body against infections. Thymic derived naïve CD4⁺ T cells express bacterial antigen specific TCRs. Encounter with these bacterial derived foreign antigens in the colon can drive the differentiation of regulatory T cells or pro-inflammatory effector T cells dependent on the bacteria and the environmental milieu. If effector CD4⁺ T helper cells are uncontrolled, they can elicit tissue damage and induce disease such as IBD. Therefore the immune system has established several mechanisms in order to control pro-inflammatory T helper cells. These mechanisms are primarily important to avoid immune pathology and in turn to maintain tolerance in the intestine. However a growing body of evidence suggests that these mechanisms can also be used to suppress other organ specific diseases. One example for this interaction between the intestine and another organ is that the commensal gut flora can trigger a T-cell mediated immune response, which leads to autoimmune disease in the brain [99]. Therefore one

possible strategy for the treatment of autoimmune diseases in future would be to specifically target the gut flora. Although the mechanisms controlling effector T cells in the intestine work well in most humans, the frequency of autoimmune and chronic inflammatory disease is steadily increasing. Unfortunately, there are currently no curative treatments for these diseases available. Therefore the patients suffer from the side effects of the drugs and from the relapses of their disease. The the challenge will be to better understand the mechanisms controlling effector T cells in order to establish new and potentially curative treatments for autoimmune and chronic inflammatory diseases. The intestine has the potential to serve as the key target organ of these therapies.

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