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Review

Induction of regulatory Tr1 cells and inhibition of T_H17 cells by IL-27

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ABSTRACT

Accumulating evidence indicates that IL-27, a member of the IL-12 family of cytokines, alleviates the severity of autoimmune diseases in both mice and men. The IL-27-induced activation of signal transducer and activator of transcription (Stat)1 and Stat3 promotes the generation of IL-10- producing type 1 regulatory T (Tr1) cells that inhibit effector T cells. In addition, IL-27 also suppresses the development of pathogenic IL-17-producing CD4$^+$ T cells (T_H17) cells suggesting that pharmacological manipulations of IL-27 signaling pathway could be exploited therapeutically in regulating tissue inflammation. Here, we review how IL-27 controls inflammation through the regulation of Tr1 and T_H17 responses.

1. Introduction

Since the original classification by Mosmann and Coffman of CD4$^+$ helper T (T_H) lymphocytes into T_H1 and T_H2 subsets [1], the repertoire of T_H subsets has expanded to include additional effector and regulatory T cell subsets such as T_H17 cells and regulatory T cells (Foxp3$^+$Tregs and Tr1 cells). T_H1 cells, which predominantly produce interferon (IFN)-γ and lymphotoxin, are essential for eliminating intracellular pathogens, but were also regarded as the major effector T cells in inducing tissue inflammation in organ-specific autoimmunity. However, mice lacking the component of T_H1-IFN-γ pathway (*Il12*$^{-/-}$, *Ifng*$^{-/-}$, *Ifngr1*$^{-/-}$, *Il12rb2*$^{-/-}$) were not protected but overly susceptible to autoimmune diseases including Experimental Autoimmune Encephalomyelitis (EAE) [2], Experimental Autoimmune Uveitis (EAU) [3] and collagen-induced arthritis (CIA) [4]. Subsequent studies revealed that T_H17 cells, instead of T_H1 cells, induce tissue inflammation in autoimmune diseases. Although T_H17 cells are essential for eliminating extracellular pathogens [5,6], exaggerated T_H17 response promotes autoimmunity. Elevated amounts of IL-17A and IL-17F are detected in several autoimmune diseases including multiple sclerosis (MS) [7], rheumatoid arthritis (RA) [8] and psoriasis [9]. The involvement of T_H17 cells in tissue inflammation was confirmed in mouse models such as EAE where IL-17-neutralizing antibodies ameliorate clinical scores [10] or CIA where IL-17-deficient animals develop attenuated disease [11]. The differentiation factors for both mouse and human T_H17 cells were found to be a combination of TGF-β1 and IL-6 or TGF-β1 and IL-21 [12]. The activation of signal transducer and activator of transcription (Stat)3 by IL-6 or IL-21 is critical for inducing the expression of the T_H17 cell master transcription factors retinoid-related orphan receptor (ROR)γt, encoded by the gene Rorc, and RORα (Rora) [13–15]. Rorc$^{-/-}$ and Rora$^{-/-}$ mice show defective T_H17 cell generation [15]. In addition, Chip-Sequencing analysis revealed Stat3 binding sites in the promoters regions of il17a and il17f genes [12]. Furthermore RORγt drives the expression of GM-CSF that is essential for inducing pathogenic T_H17 cells, and mice deficient in making GM-CSF are resistant to develop EAE [16]. These observations indicate that RORγt is essential for the development of T_H17 cells. Indeed T_H17 cell generation can be inhibited by directly targeting RORγt using small chemical compounds such as digoxin and SR1001 [17]. While IL-23 is not required for the induction of T_H17 cell differentiation, IL-23 has a prominent role in expansion and stabilization of pathogenic T_H17 cells [18–20]. Both IL-12p19$^{-/-}$ and IL-23R$^{-/-}$ mice are resistant to EAE, and few T_H17 cells are found in the central nervous system (CNS) of those mice [21–23]. The IL-23-T_H17 pathway has been shown to be critical in many autoimmune diseases, which is consistent with the fact that IL-23R polymorphisms have been genetically associated with a number of human autoimmune diseases including psoriasis, inflammatory bowel diseases (IBD) and ankylosing spondylitis [24]. More recent studies suggested that T_H17 cells could also be induced with the combination of IL-1β, IL-6

Abbreviations: Tr1 cells, type 1 regulatory T cells; T_H17, T helper 17; Stat, signal transducer and activator of transcription; Maf, transcription factor Maf; Ahr, Aryl hydrocarbon receptor.

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and IL-23 in the absence of TGF-β1, suggesting that T_H17 cells might actually represent a heterogeneous population of proinflammatory cells that are highly pathogenic and can be induced by multiple different ways.

Exaggerated inflammatory responses are prevented by regulatory T cell subsets that suppress activation of effector T cells. $CD4^+$ regulatory T cells comprise $Foxp3^+$ regulatory T-cells (Tregs) and IL-10-producing regulatory type I (Tr1) cells [25]. $Foxp3^+$Tregs are important to maintain self-tolerance as illustrated by the severe autoimmune inflammation observed in mice deficient in Foxp3 [26] or in patients with dysfunctional FOXP3 protein [27]. Although $Foxp3^+$Tregs inhibit effector T cell responses, they lose their suppressive functions in inflammatory conditions [28]. Therefore, IL-10-producing Tr1 cells might be crucial in controlling tissue inflammation. In humans, Tr1 cells were first described in severe combined immunodeficient (SCID) patients who had developed long-term tolerance to stem cell allografts, supporting the existence of these cells in humans and suggesting that they may play a role in mediating T cell tolerance [29]. Tr1 cells mediate immune suppression by secreting the suppressive cytokine IL-10 and by killing effector cells via Granzyme-B and Perforin [30,31]. While IL-10 was initially described to be the differentiation factor for Tr1 cells, these T cells could not expand in the presence of IL-10. Therefore there was an emphasis on identifying growth/differentiation factors for Tr1 cells. Recent identification of IL-27 as a differentiation/growth factor for Tr1 cells has revived the interest in examining their role in tissue inflammation [32–34].

2. IL-27 dampens autoimmune inflammation

IL-27, an heterodimeric cytokine composed by the subunit p28 (IL-27p28) and the Epstein–Barr virus-induced gene 3 (EBI3), is mainly produced by activated antigen-presenting cells APCs [35]. IL-27 signals through a receptor complex consisting of the common IL-6 receptor chain, gp130, and the unique IL-27 receptor alpha chain (IL-27Ra or WSX-1) that is homologous to IL-12Rβ2 of IL-12 receptor [35,36]. Based on the structural homology between IL-12 and IL-27 and their receptors, IL-27 was initially described as a proinflammatory cytokine that could induce T_H1 differentiation, which was consistent with the ability of IL-27 to induce T-bet (Tbx21), the master transcription factor for the generation of T_H1 cells. Subsequent work, using both T_H1 and T_H2 associated pathogens, established that IL-27 suppresses T_H cells (T_H1, T_H2 and T_H17 cells) functions *in vivo*, as *Il27ra*$^{-/-}$ mice showed enhanced T cell functions (reviewed in [37]). However, the mechanism by which IL-27-induced inhibition of T cell functions was not understood until the discovery that IL-27 can induce IL-10 production from $CD4^+$ T cells.

3. IL-27 controls T cell responses

3.1. Regulation of T_H1 and T_H2 differentiation

While IL-27 induces T-bet and expression of IL-12Rβ2 in naïve $CD4^+$ T cells, IL-27 signaling is not mandatory for T_H1 differentiation as illustrated by mice lacking the IL-27R subunit (*Il27ra*$^{-/-}$) that can mount adequate T_H1 responses to eliminate intracellular pathogens [38–40]. Moreover, *Il27ra*$^{-/-}$ mice die due to uncontrolled immunopathology and severe tissue inflammation associated with exaggerated T cell responses and enhanced production of IFN-γ and TNF-α [38–40]. IL-27 was also reported to control the generation of T_H2 cells. IL-27 treatment during *Strongyloides venezuelensis* infection decreases T_H2 responses against the parasite and treated mice failed to develop intestinal mastocytosis and exhibited a marked delay in parasite expulsion [41]. Furthermore,

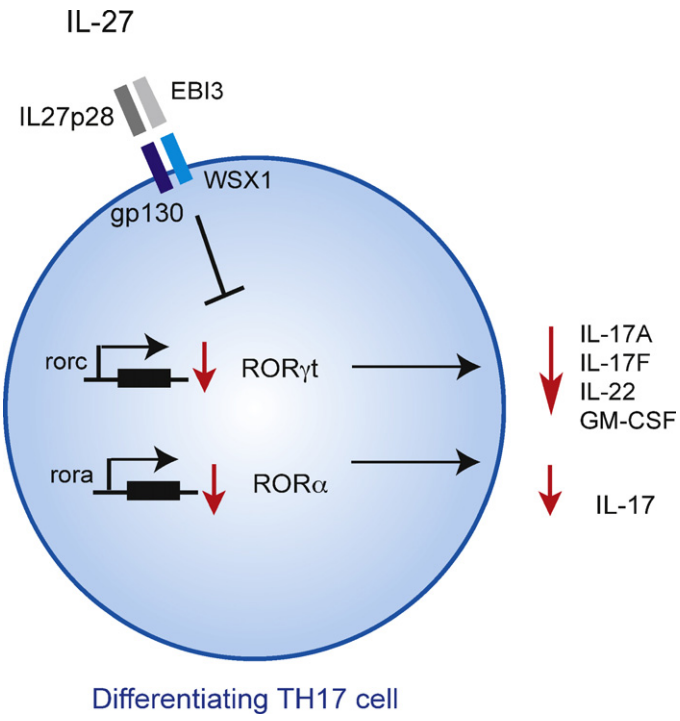

Fig. 1. IL-27 inhibition of differentiating T_H17 cells. On differentiating T_H17 cells, IL-27 inhibits the expression of transcription factors Rorγt and Rorα, thereby impairing the secretion the T_H17-related cytokines, IL-17A, IL-17F, IL-22 and GM-CSF.

intranasal administration of IL-27 inhibits OVA-induced airway hyperresponsiveness and inflammation in OVA-sensitized animals [41]. At the transcriptional level, IL-27 has been shown to suppress the master T_H2 transcription factor GATA-3 [41]. Recently, genome-wide association study (GWAS) has shown that a single nucleotide polymorphism (SNP) in the *IL-27p28* gene was associated with an increased susceptibility to asthma [42] or COPD [43] and IL-27 has been proposed as a potential treatment for bronchial asthma.

3.2. Inhibition of T_H17 cell differentiation

In addition to inhibiting both T_H1 and T_H2 development, IL-27 prevents the development of T_H17 cells *in vitro* and *in vivo*. *Il27ra*$^{-/-}$ mice are overly susceptible to EAE compared to wild-type mice and present an increased accumulation of T_H17 cells in the draining lymph nodes and in the CNS [44]. In this model, neutralization of IL-17 in *Il27ra*$^{-/-}$ mice during EAE disease course attenuated their disease phenotype [44]. Accordingly, recombinant IL-27 treatment decreases the disease incidence and severity in EAE with the inhibition of development of T_H17 cells [45]. Similarly, *Il27ra*$^{-/-}$ mice chronically infected with *Toxoplasma gondii* developed severe neuropathology mediated by $CD4^+$ T cells, associated with increased T_H17 cell development. IL-27 inhibits the production of IL-17 by BMNCs from chronically infected mice stimulated with IL-23 [46]. Finally in the absence of IL-27 during murine flu infection, flu-specific T cell responses are skewed towards T_H17 [47].

Above observations clearly indicated that IL-27 is negative regulator of development of T_H17 cells. However, the mechanism by which IL-27 inhibits the development of T_H17 cells is not clearly understood. Accumulating data suggest that IL-27 utilizes multiple mechanisms to inhibit the development of T_H17 cells (Figs. 1 and 2). During T_H17 cell differentiation, IL-27 directly suppresses the expression of both RORγt, the master transcription factor of T_H17 cells [48] and RORα [49] (Fig. 1). IL-27 inhibits expression of RORγt in T_H17 cells both in mouse and man [48]. Interestingly, IL-27 decreases the expression of GM-CSF and thereby dampens the

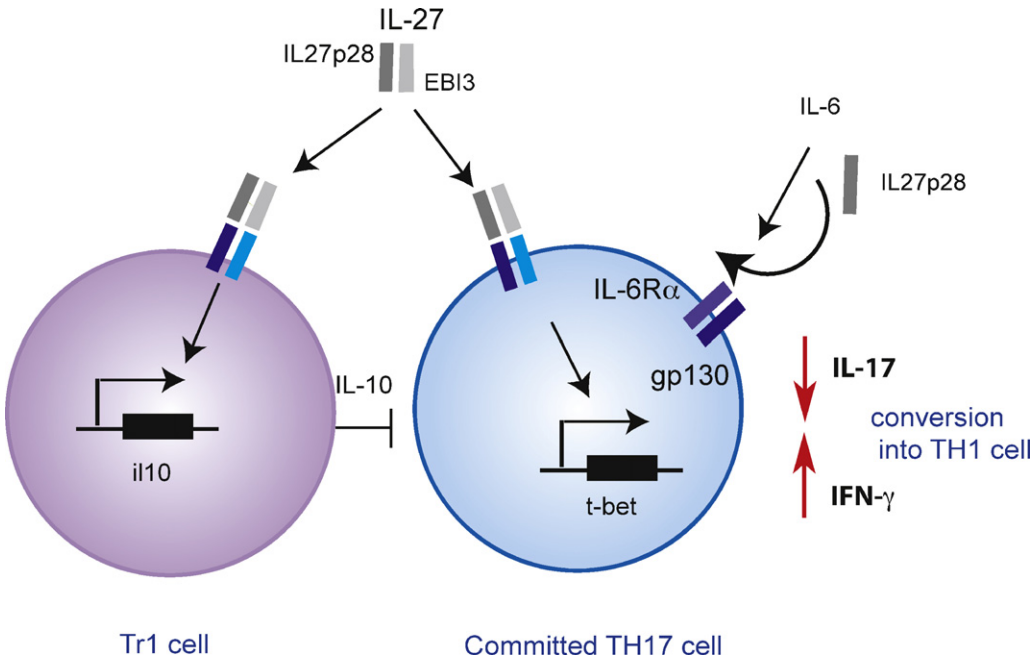

Fig. 2. IL-27 inhibition of committed T_H17 cells. IL-27 induces the differentiation of Tr1 cells that inhibit T_H17 cells in an IL-10-dependent manner. IL-27p28 monomers interfere with IL-6 cytokine signaling through gp130 and thereby inhibit the maintenance of T_H17 cells and their IL-17 secretion. IL-27 further induces T-bet expression that drives IFN-γ production and promotes the conversion of T_H17 cells into T_H1 cells.

pathogenicity of T_H17 cells [16]. By blocking GM-CSF secretion and inhibiting both RORα and RORγt expression, IL-27 interferes with T_H17 cell differentiation at several levels, explaining its potent ability to suppress the induction of T_H17 cells.

Whether IL-27 can directly suppress effector/memory T_H17 cells or fully differentiated T_H17 cells is still debated. Indeed, T_H17 maintained in culture for at least two rounds become unresponsive to IL-27 as IL-27 fails to inhibit the expression of RORα and RORγt in these cells [49]. However, IL-27 could modulate effector/memory T_H17 cells using different strategies. Among the two IL-27 cytokine subunits, EBI3 is constitutively expressed but IL-27p28 secretion is transcriptionally regulated. IL-27p28 monomers can interfere with the IL-6-mediated production of IL-17 by preventing IL-6 signaling through gp130, suggesting that IL-27p28 monomers could also be exploited in regulating T cell responses [50]. IL-27p28 thus limits the generation and maintenance of T_H17 cells *in vivo* without directly interfering with T_H17 transcriptional program (Fig. 2). Furthermore, it has been proposed that T_H17 could be converted into T_H1 cells that are presumably less pathogenic [51,52]. One putative mechanism by which IL-27 could converts T_H17 into T_H1 cells may be by inducing the expression of T-bet that drives IFN-γ expression and reduces the expression of IL-17 (Fig. 2). However, this hypothesis by which IL-27 may increase T_H17 plasticity has not been proven experimentally.

3.3. Induction of Tr1 cells

IL-27, while inhibiting TGF-β-induced Foxp-3$^+$ Tregs, induces IL-10$^+$, IFNγ$^+$ T cells that are immunosuppressive, a phenotype in line with the previously described Tr1 cells [32–34,53,54]. The role of IL-27 in generation of IL-10-producing Tr1 cells was further emphasized *in vivo*. IL-27 treated MOG-specific splenocytes lose their ability to transfer EAE in an IL-10 dependent manner [33]. Furthermore, during flu infection, IL-27 generates regulatory T cells that inhibit T_H17 cells by secreting IL-10 and IFN-γ. In the absence of IL-10, flu-specific T cell responses developed a stronger T_H17 component [47]. Furthermore, it has been shown that Tr1 cells can inhibit T_H17 cells *in vivo* in an IL-10 dependent manner during murine colitis [55] (Fig. 2). Akin to what has been observed in murine T cells, activation of naïve human T cells in the presence of IL-27 similarly induces Tr1 cells that produce both IFN-γ and IL-10 [56].

4. Molecular pathways involved in IL-27 biology

Similar to other type 1 cytokine receptors, IL-27 also induces the activation of Janus kinase/Stat pathway. IL-27 predominantly induces the phosphorylation of Stat1 and Stat3. Here we will discuss the IL-27-induced signaling events following the activation of the Stats and analyze their roles in inhibiting T_H17 cell and in inducing Tr1 cell differentiation.

4.1. IL-27 and Stat1 activation

4.1.1. Stat1 activation by IL-27 represses T_H17 differentiation and induces Tr1 cells

The activation of the IL-27 specific subunit WSX-1 drives the tyrosine phosphorylation of JAK1 that further activates Stat1. Indeed, JAK1, but not other JAKs, coprecipitates with the WSX1 subunit [57].

The Stat1 signaling pathway is necessary for IL-27-induced T-bet expression [58]. T-bet not only drives the expression of IFN-γ but also plays an important role in the inhibition of T_H17 cytokines, independently of IFN-γ. T-bet can reprogram committed T_H17 cells by repressing T_H17 gene program, which results in fewer transcripts of *Rorc*, *il17a*, *il17f*, *il23r* [59]. These finding were supported by studies showing that T-bet utilizes Runt-related transcription factor 1 (Runx1), a transcriptional activator that sequesters *Rorc* away from the regulatory regions on *Rorc* promoter [59]. Indeed Runx1 binding site is located upstream of T-bet binding site on *Rorc* promoter. By sequestering Runx1, T-bet inhibits the expression of RORγt, resulting impaired development of T_H17 cell [59] (Fig. 3).

Stat1$^{-/-}$ and T-bet$^{-/-}$ mice exhibit an increased number of T_H17 cells both during systemic inflammation *in vivo* or during T_H17 cells differentiation *in vitro*. IL-17 production is greater in the absence of T-bet compared to the absence of Stat1 [60]. This

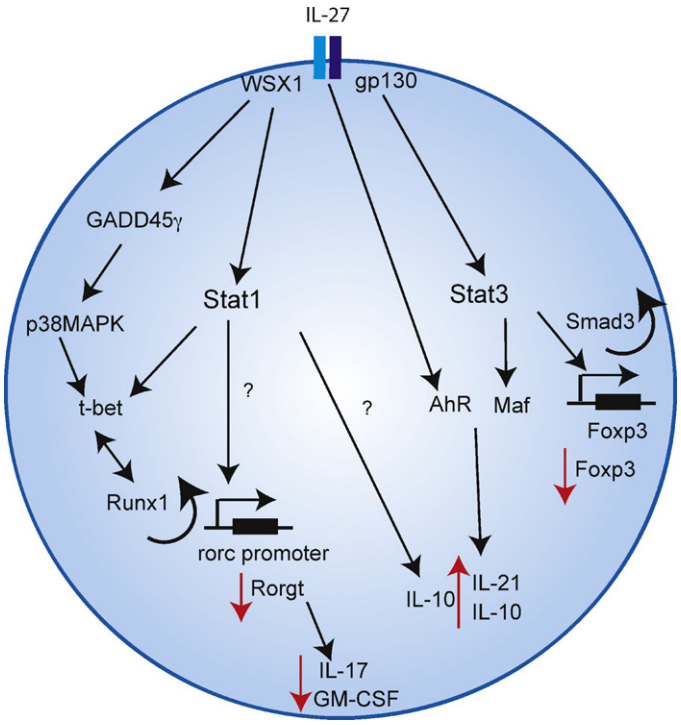

Fig. 3. Reciprocal regulation of T_H17 and Tr1 cells by IL-27. The molecular mechanisms by which IL-27 promotes Foxp3$^-$IL-10$^+$Tr1 cell differentiation and represses T_H17 cell development through activation of Stat1 and Stat3 activation are shown. IL-27 activates Stat1 through the subunit WSX1 that inhibits Rorγt expression through T-bet-dependent as well as T-bet-independent pathways. Alternatively, IL-27 can promote T-bet expression in a Stat1 independent pathway via GADD45γ. In addition, IL-27 activates Stat3 signaling through gp130. Stat3 induction then drives Maf transcription. Maf together with Ahr transactivates *il21* and *il10* promoters. On the other hand, IL-27 inhibits Foxp3 transcription in a Stat3/Smad3 dependent manner.

may be related to the fact that T-bet might also be induced in a Stat1 independent manner. In this vein, Owaki et al. have shown that IL-27 induces a Stat1 independent T-bet expression [61]. Indeed IL-27 induces the expression of GADD45γ that further drives the phosphorylation of p38 MAPK leading to T-bet expression (Fig. 3).

It has been further proposed that Stat1 could inhibit RORα and RORγt expression in differentiating T_H17 cells in a T-bet independent manner (Fig. 3). While a direct inhibitory effect of Stat1 on RORα and RORγt expression has not been ruled out, Stats could also indirectly affect T_H17 responses by promoting the function of auxiliary inhibitory T_H17 factors. Different repressors of T_H17 cells differentiation have been identified, including Ets-1, which negatively regulates T_H17 cell differentiation [62]. Stat1 and Ets-1 have been shown to bind together [63] and might cooperate to inhibit T_H17 cell differentiation by directly or indirectly interfering with RORγt function in T_H17 cells.

IL-27 has been shown to induce IL-10 expression from CD4$^+$ T cells using both Stat1 and Stat3 pathways (Fig. 3). Indeed, in the absence of Stat1 signaling, IL-27 driven IL-10 production is decreased. While it is clear that the Stat1 driven IL-10 secretion is independent of T-bet signaling, the underlying mechanisms still remain unclear [34].

4.2. IL-27 and Stat3 activation

4.2.1. Stat3 activation by IL-27 does not enhance T_H17 cell differentiation

IL-27 utilizes gp130 subunit of IL-6 receptor complex, which results in activation of Stat3 signaling. A genetic defect in Stat3 signaling in humans, in hyperIgE syndrome, results in defective T_H17 cells and in unrelenting fungal infections, supporting the critical role of Stat3 in the generation of T_H17 cells [64]. At the first glance, it is puzzling that IL-6 and IL-27, which both activate Stat3 pathways, have antagonistic properties. It has been proposed that IL-6 leads to a faster and more persistent pattern of Stat3 phosphorylation that is crucial to drive pro-inflammatory signals downstream Stat3. pStat-3 directly binds to *il17a* and *il17f* promoters and transactivate these genes by collaborating with other transcription factors like IRF-4 and RORγt. Furthermore, the formation of Stat1–Stat3 heterodimers in response to IL-27 rather than the formation of mainly Stat3 homodimers in response to IL-6 or IL-21 may play a role in the difference between IL-6 and IL-27 signaling. Indeed preliminary data from our laboratory supports this hypothesis. In addition, IL-6 activation rapidly induces Stat3 repressor SOCS3 [65]. SOCS3 is an essential negative regulator of Stat3 phosphorylation and constrains T_H17 cell differentiation [66,67]. While IL-27 induces expression of SOCS3, IL-27-mediated inhibition of IL-17 production is independent of SOCS3 [46]. It therefore seems unlikely that IL-27-induced SOCS3 contributes to the inhibition of T_H17 cells. Instead, the inhibition of T_H17 differentiation might mainly be mediated through Stat1 and T-bet as discussed above.

4.2.2. Stat3 activation by IL-27 promotes Tr1 cell differentiation

IL-27-induced Stat3 phosphorylation is essential for the anti-inflammatory role of IL-27, as it triggers IL-10 secretion from CD4$^+$ T cells [34] (Fig. 3). Sustained activation of Stat3 leads to the induction of the transcription factor Maf [68]. We and others have recently shown that Maf is essential for IL-10 production induced by IL-27 [53]. Similarly to Stat3 deficient CD4$^+$ T cells, Maf deficient CD4$^+$ T cells cannot produce IL-10 in response to IL-27. It has been further shown that Maf directly transactivates *il10* and *il21* promoters [53]. In addition to Maf, IL-10 production by IL-27 is regulated by the ligand activated transcription factor Aryl hydrocarbon receptor (AhR) that binds to Maf resulting in a complex that induces both *il10* and *il21* transcription [69]. The finding of AhR involvement in IL-10 production is significant as it provides impetus to design AhR ligands that can modulate the anti-inflammatory properties of Tr1 cells both *in vitro* and *in vivo* (reviewed in [31]). The expression of the cytokine IL-21 is further essential for IL-27-induced-IL-10 production [53] (reviewed in [37]). In the absence of IL-21, IL-10 production is reduced in Tr1 cells. IL-21 secretion can be further amplified by AhR activation [69].

4.2.3. Stat3 activation by IL-27 and inhibition of Foxp3

IL-27 inhibits the generation of Foxp3$^+$Tregs [70]. The fact that Foxp3$^+$Tregs express IL-27R strongly suggested that IL-27 might block the development of those regulatory cells *in vitro* [71]. IL-27 indeed leads to a decreased expression of Foxp3 through a mechanism that is at least partially dependent on Stat3 [70]. Smad3 binding to Foxp3 promoter is implicated in Foxp3 transcription. It has been proposed that IL-27-induced pStat3 binds to a gene silencer region (enhancer II) in a conserved region of Foxp3 gene that reduces the acetylation in the region of Smad3 binding site and decreases the binding of pSmad3 to Foxp3 promoter [72]. This results in a decreased accessibility and binding of Smad3 to Foxp3 promoter and thereby decreases *Foxp3* transcription (Fig. 3). IL-27 impacts Foxp3$^+$Treg development and function *in vivo*. Indeed mice that overexpress both IL-27 subunits, IL-27p28 and EBI3, have decreased number of Foxp3$^+$Tregs and developed spontaneous inflammation similar to mice that lack Foxp3$^+$Tregs such as the scurfy Foxp3 mutant mice or IL-2$^{-/-}$ mice [73]. Interestingly, IL-27 transgenic mice are deficient in IL-2. Those results are in accordance with another recent study showing that IL-27 inhibits Foxp3$^+$Treg *in vivo* in a murine T cell transfer colitis model.

Il27ra$^{-/-}$ deficient T cells transferred an attenuated disease due to a larger percentage of transferred cells expressing Foxp3 compared to wild-type T cells [74].

5. Therapeutic implications

5.1. IL-27 confers protection against multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system resulting in inflammation, demyelization and axonal loss. It is a common neurological disorder, which attacks young adults. T_H17 cells were shown to contribute to MS development [75]. By contrast, IL-27 protects against autoimmune inflammation in the mouse model EAE as exemplified by *Il27ra*$^{-/-}$ mice which develop an accelerated EAE disease course compared to WT controls and show increased levels of T_H17 cells in the CNS [44]. Furthermore, daily intrathecal treatment with IL-27 during EAE alleviates the disease and decreases both the inflammation in the brain and the number of infiltrating T_H17 cells [45]. Similarly in a T cell adoptive transfer model, pre-treatment of autoreactive CD4$^+$ T cells with IL-27 leads to a reduction of their pathogenicity in an IL-10 dependent manner [33]. Interestingly, IL-27 was also shown to mediate the protective effect of Bone marrow stromal cells (BMSCs) that prevent EAE in mice and suppress IL-17 production [76].

Support for IL-27 in regulating autoimmune tissue inflammation has also been provided in humans. The immunomodulatory drug IFN-β, used in the first line of treatment for MS, has been shown to induce IL-27 production from dendritic cells (DCs). Interferon (IFN)-β, a member of the type I interferon family, is an approved treatment for relapsing remitting MS (RRMS) that reduces the rate of relapses by 30%. While the therapeutic mechanisms of IFN-β remain poorly understood, recent studies indicate that IL-27 contributes to its regulatory properties both in mouse [77] and human [78,79]. One limitation of IFN-β treatment is that 20–50% of patients fail to respond to therapy thus delaying a change in the treatment strategy of those patients. While the presence of neutralizing antibodies (Nabs) against IFN-β in the blood has been proposed to correlate with treatment failure [80], a proportion of non-responder patients do not develop Nabs, limiting the use of Nabs to predict the response to IFN-β therapy [81]. IL-27 secretion from PBMC from RRMS patients has been proposed as a predictive factor of clinical response to IFN-β treatment. Indeed, PBMC isolated from RRMS patients that respond to IFN-β treatment secrete more IL-27 when exposed *in vitro* to IFN-β than PBMC isolated from "non-responder" patients [78]. Finally, other therapies proposed for treating MS, such as Statins, which in addition to their cholesterol-lowering activity have anti-inflammatory properties, were shown to increase *in vitro* IL-27 secretion from human monocytes of MS patients [82].

5.2. IL-27 protects against rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic inflammatory disorder that principally attacks synovial joints. T_H17 cells and IL-17 expression is elevated in RA synovial tissue and fluid macrophages compared to controls [83,84]. Elevated levels of IL-17 have been reported in the animal model of RA, collagen-induced arthritis (CIA), and IL-17 neutralization prevents bone destruction suggesting a pathological role of T_H17 cells in the development of RA [85]. Administration of IL-27 in mice suffering from CIA reduces the severity of the disease, as shown by reduced cellular infiltration in the joints, synovial hyperplasia, and joint erosion [84]. IL-27 treatment further decreases serum levels of IL-6. In addition, lymphocytes isolated from spleen and lymph node of IL-27-treated mice produce significantly reduced amounts of IFN-γ and IL-17 when cultured with type II collagen *in vitro* compared with lymphocytes from control mice. Similar results were obtained when IL-27 was ectopically expressed in the joints [86]. These studies highlight in the therapeutic potential of IL-27 in RA, especially with the feasibility of local, intra-articular, administration of recombinant IL-27.

5.3. Controversial role of IL-27 in inflammatory bowel disease

IL-27 is implicated in the pathogenesis of IBD, Crohn's disease and ulcerative colitis. Genome wide studies have identified SNPs in the gene encoding p28 subunit associated with a lower expression of IL-27 and early onset inflammatory bowel disease, which would be consistent with a protective role of IL-27 in IBD [87]. Two other studies have found transcripts for IL-27p28 [88] and Ebi3 [89] to be overexpressed in biopsy samples from IBD patients. The function of IL-27 has been assessed using different murine models of IBD. In the mouse IBD model of acute inflammation, which relies on the presence of dextran sulfate sodium (DSS) to induce inflammation, *Il27ra*$^{-/-}$ mice receiving 5–10% DSS in drinking water were more susceptible to disease [90]. *Il27ra*$^{-/-}$ deficient mice showed a reduction in T_H1 IFNγ-producing cells and an increase in T_H17 cells in gut-associated lymphoid tissue pointing towards an important regulatory role of IL-27 in dampening T_H17 cell function [90]. In the 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced mouse acute colitis model, subcutaneous scIL-27 (EBI3 and p28 subunits generated as a single-chain human IL-27) treatment significantly improved in a dose-dependent manner the extent of the lesions as well as necrosis, ulceration and thickening of mucosal epithelium. scIL-27 suppressed several inflammatory cytokines in inflamed colon, including IL-17 [91]. However, in a T cell transfer colitis model, IL-27 was shown to exert proinflammatory effects as it suppressed induced Treg development *in vivo* [74]. In contrast, in the DSS model, *Il27ra*$^{-/-}$ mice treated with lower doses of DSS (0.5% in drinking water), were protected compared to WT controls [92]. The implication of different pathogenic or regulatory subsets and the heterogenicity of the models may explain the different responses to IL-27 treatment in murine models of colitis. However, in models where T_H17 cells are implicated in the development of the disease, the anti-inflammatory role of IL-27 appears to be dominant. Indeed, T_H17 cells have been shown to be crucial for the development of TNBS-induced colitis as IL-17 receptor A (IL-17RA) knockout mice do not develop TNBS colitis [93] and IL-17F-deficient mice develop more severe DSS colitis than controls [94]. A better understanding of the pathogenesis of IBD should provide additional insight into the role of IL-27 in colitis.

6. Open questions and concluding remarks

While IL-27 promotes Tr1 cells, it inhibits CD4$^+$Foxp3$^+$Tregs induced by TGF-β. These observations are reminiscent of the action of AhR ligands such as FICZ that promotes Tr1 cells but inhibits Foxp3$^+$Tregs. This paradoxical effect on regulatory T cells might stem from different and/or complementary roles of regulatory T cells. Tr1 cells but not Foxp3$^+$Tregs may develop *in situ* in the inflamed tissue as IL-27 can be secreted by resident cells in the target organ, such as in the brain during EAE and MS. Foxp3$^+$Tregs cannot inhibit highly pathogenic effector T cells in the target organ [95] but they induce tolerogenic plasmacytoid dendritic cell (DC) that secrete IL-27 thus promoting Tr1 cell generation [32]. Under inflammatory settings, Foxp3$^+$Tregs can produce cytokines that belong to other lineages [96,97] and we propose that Tr1 cells could be more stable and thereby regulate tissue inflammation at the target site.

IL-27 controls inflammation by inhibiting T_H17 cells and by promoting the development of IL-10-producing regulatory Tr1 cells. Despite their opposite *in vivo* functions, Tr1 and T_H17 cells harbor striking similarities. First, they rely on the transcription factors Maf and AhR for their generation. Second, they require IL-21 for their growth. Third, they produce IL-10. In this regard, Ghoreschi et al. showed that T_H17 differentiated with TGF-β and IL-6 ($T_H17(\beta)$) produced IL-10 and were poorly pathogenic *in vivo* in contrast to T_H17 cells induced by IL-6, IL-1β and IL-23 (T_H17) (23) that did not produce IL-10 and were highly pathogenic. In addition, TGF-β induced T_H17 expressed higher levels of Maf and AhR compared to T_H17 induced with IL-1, IL-6 and IL-23 (23). This observation would thus be in line with a previous work suggesting that the Maf-driven induction of IL-10 in T_H17 cells reduced their pathogenicity [98]. Since we have shown that the expression of Maf and AhR is required for the production of IL-10 and IL-21 in Tr1 cells, it might be interesting to explore whether IL-27 could actually be converting T_H17 to Tr1 cells. We are currently conducting a functional transcriptional analysis of Tr1 (differentiated with IL-27) and T_H17 (IL-6 and TGF-β) cells using a computational approach and a whole genome microarray analysis to address this question.

In the same line, IL-21 has been ascribed a functional role in promoting both T_H17 [99,100] and Tr1 cells [53]. The role of IL-21 during autoimmune disease such as EAE is controversial. While initial studies have proposed that IL-21R$^{-/-}$ mice presented a less severe EAE disease [100], longer observation of EAE disease course showed that IL-21R$^{-/-}$ mice developed a more severe disease [101,102]. Besides being a growth factor for T_H17 cells [103], IL-21 may behave as an anti-inflammatory effect by promoting IL-10 secretion from different T cell subtypes. It remains to be seen whether IL-27 and its downstream cytokine IL-21 can modulate the pathogenicity and stability of different subtypes of T_H17 cells that have been further treated with IL-23. In conclusion, IL-27 not only induces the generation of anti-inflammatory Tr1 cells but broadly controls autoimmune responses by inhibiting effector T cells in various target organs.

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