

## Th17 cells and T<sub>regs</sub>: unlikely allies

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### ABSTRACT

Identification of CD4<sup>+</sup>Foxp3<sup>+</sup> T<sub>regs</sub> and Th17 modified the historical Th1–Th2 paradigm. Currently, the Th17–T<sub>regs</sub> dichotomy provides a dominant conceptual framework for the comprehension of immunity/inflammation and tolerance/immunosuppression in an increasing number of diseases. Targeting proinflammatory Th17 cells or immunosuppressive T<sub>regs</sub> has been widely considered as a promising therapeutic strategy in the treatment of major human diseases, including autoimmunity and cancer. The efficacy and safety of such therapy rely on a thorough understanding of immunobiology and interaction of these two subsets of Th cells. In this article, we review recent progress concerning complicated interplay of Th17 cells and T<sub>regs</sub>. There is compelling evidence that T<sub>regs</sub> potentially inhibit Th1 and Th2 responses; however, the inhibitory effect of T<sub>regs</sub> on Th17 responses is a controversial subject. There is increasing evidence showing that T<sub>regs</sub> actually promote the differentiation of Th17 cells in vitro and in vivo and consequently, enhanced the functional consequences of Th17 cells, including the protective effect in host defense, as well as detrimental effect in inflammation and in the support of tumor growth. On the other hand, Th17 cells were also the most potent Th subset in the stimulation and support of expansion and phenotypic stability of T<sub>regs</sub> in vivo. These results indicate that these two subsets of Th cells reciprocally stimulate each other. This bidirectional crosstalk is largely dependent on the TNF–TNFR2 pathway. These mutual stimulatory effects should be considered in devising future Th17 cell- and T<sub>reg</sub>-targeting therapy. *J. Leukoc. Biol.* 95: 723–731; 2014.

### Introduction

Th cells play central roles in orchestrating innate as well as adaptive immune responses [1]. The Th1–Th2 paradigm, proposed more than 25 years ago [2], has evolved with the identification of distinct additional lineages of immunosuppressive

CD4<sup>+</sup>Foxp3<sup>+</sup> T<sub>regs</sub> and proinflammatory Th17 cells. T<sub>regs</sub> are crucial for immunological homeostasis and play an important role in preventing immune responses to autoantigens [3], while they also dampen host immune responses against pathogens [4] and tumor antigens [5]. Up- or down-regulation of T<sub>reg</sub> activity has clear, beneficial effects in various animal models, including autoimmune disorder [6] and cancer [7], suggesting a considerable potential for therapeutically targeting T<sub>regs</sub> in the treatment of major human diseases. Indeed, ex vivo-expanded human T<sub>regs</sub> have the capacity to inhibit the rejection of skin and islet allografts in humanized mouse models [8–10]. Ex vivo-expanded antigen-specific human T<sub>regs</sub> hold the promise to prevent GvHD in the treatment of leukemia, while maintaining graft-versus-malignancy capacity [11–14]. On the other hand, immunotherapy by targeting T<sub>regs</sub>, including depletion of T<sub>regs</sub>, is currently being tested in tumor patients [15].

In contrast to T<sub>regs</sub>, Th17 cells have been found to be major stimulatory participants in the pathogenesis of autoimmune disease [16, 17]. Increasing numbers of chronic inflammatory disorders, which were previously attributed to Th1 cells, were found to be caused by Th17 cells [18]. Although Th17 cytokines may provide proinflammatory support of tumor development [19], Th17 cells also contribute to anti-tumor immune responses [19, 20]. Consequently, Th17 cells have been considered to be a promising target in the treatment of autoimmune disorders [21, 22], as well as cancer [19, 20]. For example, treatment with secukinumab, a highly selective human anti-IL-17A mAb, resulted in rapid and sustained reductions of symptoms in patients with psoriasis, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis [23]. In contrast to its pathogenic role in autoimmune disorders, the contribution of Th17 cells to anti-tumor immunity was recapitulated by a recent report showing that the anti-tumor effect of cyclophosphamide critically depended on Th17 cells, which were induced by intestinal microbiota [24]. In mouse tumor models, infusion of in vitro-differentiated Th17 cells achieved a marked anti-tumor effect, which was even superior to the infusion of

Abbreviations: AHR=aryl hydrocarbon receptor, EAE=experimental autoimmune encephalomyelitis, Foxp3=forkhead box p3, GvHD=graft-versus-host disease, Rag<sup>−/−</sup>=RAG-deficient, RORγ=RAR-related orphan receptor γ, T<sub>eff</sub>=effector T cell, Th cell=CD4<sup>+</sup> Th cell, Th17=IL-17-producing CD4<sup>+</sup> Th cell, T<sub>reg</sub>=regulatory T cell

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in vitro-differentiated Th17 cells [25, 26]. Thus, the targeting of Th17 cells holds promise for the treatment of human cancers.

The differentiation programs of  $T_{\text{regs}}$  and Th17 cells are reciprocally interconnected and are probably competitive in using naive CD4 T cells as precursors and TGF- $\beta$  as a differentiation factor [27–29]. Currently, the Th17– $T_{\text{reg}}$  dichotomy tends to become a dominant conceptual framework for comprehending the relationship of immunity/inflammation and tolerance/immunosuppression in a wide spectrum of diseases [30–36]. However, recent studies reveal that Th17 cells and  $T_{\text{regs}}$  do not always antagonize each other. Abundant numbers of Th17 cells and  $T_{\text{regs}}$  frequently colocalize in the same compartments. Importantly, these two populations of Th cells, although functionally opposite, can actually positively stimulate and support each other. The interplay of Th17 cells and  $T_{\text{regs}}$  can be complicated further by their phenotypic plasticity, which has been well-reviewed previously [37] and will not be discussed further in this article. In addition to Foxp3-expressing CD4  $T_{\text{regs}}$ , it is known that other subsets of T cells with immunosuppressive capacity are differentiated from naive CD4 cells during immune responses, including IL-10-producing type 1  $T_{\text{regs}}$  [38] and TGF- $\beta$ -producing Th3 cells [39]. We will focus our discussion in this article on naturally occurring, thymic-derived, suppressive CD4<sup>+</sup>Foxp3<sup>+</sup>  $T_{\text{regs}}$ .

## TH17 CELLS AND $T_{\text{REGS}}$ FREQUENTLY COLOCALIZE IN THE SAME ANATOMIC COMPARTMENTS

Most Th17 cells in the body reside in the barrier tissues, including intestinal tracts and skin [18]. Interestingly,  $T_{\text{regs}}$  are also abundant in these interfaces of host and microbiota [40, 41]. It has been reported that the members of commensal microbiota can induce the generation of  $T_{\text{regs}}$  and Th17 cells [42–46]. Interestingly, a recent study showed that the up-regulation of Foxp3 expression by  $T_{\text{regs}}$  and increase of Th17 cells simultaneously occurred in NOD mice fed acidic water, which was associated with an alteration in the gut microbiome and a decreased risk of diabetes [47]. However, Th17 cells and  $T_{\text{regs}}$  are also frequently most abundant in other anatomical compartments, such as inflamed synovial fluids [48], and in cancerous tissues, such as aggressively growing breast cancer [49], uterine cervical cancer [50], colorectal cancer [51], gastric cancer [52], and malignant pleural effusion [53] in humans, as well as a number of mouse tumor models, including gliomas [54]. Therefore, the physiological relevance of colocalization of these two subsets of CD4 cells likely does more than maintain defense against invading pathogen and tolerance to self-tissues.

Th17 and  $T_{\text{regs}}$  intriguingly share many similarities, which may contribute to their colocalization. Th cells, in addition to their distinct cytokine profiles, express characteristic chemokine receptors that direct them to traffic to lymphoid or peripheral tissues. For example, naive Th cells express CCR7, whereas Th1 cells express CXCR3 and CCR5, and Th2 cells express CCR3 and CCR8 [55]. CCR6 is the predominant chemokine receptor shared by Th17 cells and  $T_{\text{regs}}$  [56]. Expression of this chemokine receptor was reported to recruit

Th17 cells and  $T_{\text{regs}}$  simultaneously to inflammatory sites [57] or barrier tissues [58].

It was also clearly demonstrated that the differentiation of Th17 and  $T_{\text{regs}}$  requires TGF- $\beta$  [27–29]. Th17 and  $T_{\text{regs}}$  highly express the AHR, which can promote the generation of both cell types by integrating environmental stimuli [59, 60]. Thus, it is likely that Th17 and  $T_{\text{regs}}$  could be generated at the sites enriched in TGF- $\beta$  and respective AHR ligands. However, emerging evidence also favors the idea that these two subsets of Th cells mutually promote each other's generation and expansion, which would contribute to the colocalization of abundant Th17 and  $T_{\text{regs}}$  in the same anatomic compartments.

## $T_{\text{REGS}}$ PROMOTE THE DIFFERENTIATION OF TH17 CELLS

$T_{\text{regs}}$  have clearly been shown to inhibit the activation of Th1 and Th2 cells [61, 62]. Although a certain subset of CD39<sup>+</sup>Foxp3<sup>+</sup>  $T_{\text{regs}}$  was reported to inhibit Th17 cells [63], the susceptibility of Th17 cells to  $T_{\text{reg}}$ -mediated inhibition is still controversial. For example, in experimental mouse gastritis,  $T_{\text{regs}}$  markedly inhibited Th1 cell- and Th2 cell-mediated pathogenesis but had no effect on Th17 cell-mediated pathogenesis [64]. In contrast, the majority of studies shows that  $T_{\text{regs}}$  actually promote the differentiation as well as function of Th17 cells, as discussed below.

### $T_{\text{regs}}$ promote differentiation of Th17 cells in vitro

The first evidence showing the stimulatory effect of  $T_{\text{regs}}$  on Th17 cells was actually a study revealing the biological signals for de novo generation of Th17 cells. In this study, Veldhoen and colleagues [29] showed that IL-17-producing T cells were differentiated from naive CD4 cells when cocultured with  $T_{\text{regs}}$  in the presence of TLR3, TLR4, or TLR9 stimuli. This led to the finding that TGF- $\beta$ 1, which could be a substitute for  $T_{\text{regs}}$ , and IL-6, which was produced by TLR stimuli, were able to induce the differentiation of Th17 cells. Subsequently, Xu and colleagues [65] reported that in the presence of IL-6,  $T_{\text{regs}}$  not only induced IL-17 expression by  $T_{\text{effs}}$  but themselves also expressed IL-17. Coculturing of  $T_{\text{effs}}$  with  $T_{\text{regs}}$  inhibited production of the Th1 cytokine (IFN- $\gamma$ ) and Th2 cytokine (IL-4), whereas IL-17 family cytokines, including IL-17A, IL-17F, IL-21, and IL-22, were markedly enhanced [66, 67]. This in vitro effect of  $T_{\text{regs}}$  in promoting Th17 differentiation was reportedly mediated by providing TGF- $\beta$  [65] and consumption of IL-2 [67].

### Cotransfer of $T_{\text{regs}}$ promotes differentiation of Th17 cells and inhibits colitis in lymphopenic mice

The critical role of functional  $T_{\text{regs}}$  in immune homeostasis can be demonstrated in a mouse colitis model induced by transfer of naive CD4 T cells into Rag1<sup>−/−</sup> mice [68]. After transfer into Rag1<sup>−/−</sup> mice, naive CD4 cells can be differentiated into Th1 and Th17 cells in the colon [69]. Thus, this model provides a system to evaluate the in vivo effect of  $T_{\text{regs}}$  on the differentiation of Th17 and Th1 cells. Sujino and colleagues [70] found three types of Th1 (IL-17A<sup>−</sup>IFN- $\gamma$ <sup>+</sup>) cells

to be generated in this system: ROR $\gamma$ <sup>−</sup> classic Th1 cells that were differentiated directly from naive cells; ROR $\gamma$ <sup>+</sup> Th1-like cells and ROR $\gamma$ <sup>−</sup> alternative Th1 cells that were terminally differentiated from ROR $\gamma$ <sup>+</sup> cells via Th17 (IL-17A<sup>+</sup>IFN- $\gamma$ <sup>−</sup>); and Th17/Th1 (IL-17A<sup>+</sup>IFN- $\gamma$ <sup>+</sup>) or Th1-like (IL-17A<sup>−</sup>IFN- $\gamma$ <sup>+</sup>) cells. T<sub>regs</sub> not only suppressed Th1 cells but also suppressed the transition of Th17/Th1 cells into alternative Th1 cells, resulting in an accumulation of Th17 and Th17/Th1 cells in mice with inhibition of colitis [70].

Systemic autoimmune disease can be generated in this model by transferring CD4 T cells from the DO11.10 TCR transgenic mouse, specific for the OVA<sub>323–339</sub> peptide, into a Rag<sup>−/−</sup> host, expressing OVA as a secreted antigen [71]. With the use of this model, Lohr and colleagues [71] showed that transfer of OVA-specific T<sub>regs</sub> prevented weight loss and skin inflammation, which were associated with the inhibition of T cell accumulation, as well as complete suppression of the proportion of Th1 cells. In contrast, T<sub>regs</sub> increased the proportion of IL-17-expressing cells.

In the polyclonal system, e.g., transfer of WT naive CD4 T cells and WT T<sub>regs</sub> into Rag<sup>−/−</sup> mice, we also found that T<sub>regs</sub> markedly inhibited development of colitis, accompanied by an inhibition of IFN- $\gamma$ -producing cells, while increasing IL-17A-producing cells [69]. Th17 cells exhibited a tissue-protective and immunosuppressive effect and consequently, suppressed colon inflammation by multiple mechanisms, including inhibition of pathogenic Th1 responses [72], increase of barrier function of intestinal epithelial cells [73], up-regulation of polymeric IgR and intestinal IgA [74, 75], and production of protective IL-22 [76]. Thus, the Th17-promoting effect of T<sub>regs</sub> may contribute to the inhibitory effect on the development of colitis and other inflammatory responses in this model.

### Depletion of T<sub>regs</sub> inhibits generation of antigen-specific Th17 cells

Chen and colleagues [77] examined the role of T<sub>regs</sub> in the in vivo development of antigen-specific Th17 cells. They transferred OT-II T cells into *Foxp3*.lucDTR4 mice, which were immunized with OVA peptide and CFA. T<sub>regs</sub> were depleted by administering diphtheria toxin. Depletion of T<sub>regs</sub> at the time of immunization, but not at later time-points, reduced the number of antigen-specific IL-17-producing cells and consequently, reduced inflammatory skin responses [77]. This Th17-promoting effect of T<sub>regs</sub> was found to be mediated by consumption of IL-2 [77], a cytokine critical for the maintenance and expansion of T<sub>regs</sub> [78] and for the inhibition of differentiation of Th17 cells [79].

### T<sub>regs</sub> promote beneficial host defense function of Th17 cells against fungal infections

Th17 cells and IL-17 cytokines are critical for oral fungicidal immune responses, based on the recruitment of neutrophils to the oral mucosa and induction of salivary antimicrobial factors [80]. Patients with impaired Th17 responses [81] or lacking T<sub>regs</sub> [82] were more susceptible to *Candida albicans* infection. Pandiyan and colleagues [67] reported that T<sub>regs</sub> potently pro-

moted the differentiation of naive CD4 cells into Th17 cells capable of producing the full suite of characteristic cytokines in vitro and in vivo. T<sub>regs</sub> did not suppress but actually promoted IL-17A-dependent clearance of fungi during acute *C. albicans* infection. This is demonstrated by the fact that depletion of T<sub>regs</sub> in WT B6 mice resulted in a reduced level of Th17 cells and increased the fungal burden. In addition, in the Rag<sup>−/−</sup> mice cotransfer of T<sub>regs</sub> with T<sub>effs</sub> resulted in an increase in Th17 cells and enhanced fungal clearance and recovery from *C. albicans* infection [67]. Therefore, in addition to maintaining immune homeostasis and preventing autoimmunity, T<sub>regs</sub> play a positive role in host defense and in clearance of fungal infections, by promoting Th17 responses. T<sub>regs</sub> have also been shown to confer protection against viral infections [83, 84]. Whether this effect of T<sub>regs</sub> was achieved by collaboration with Th17 cells should be clarified further.

### T<sub>regs</sub> enhance Th17 cell-mediated immunopathogenesis during intracellular bacterial infections

More recently, it has been shown that upon intracellular *Chlamydia muridarum* infection, T<sub>regs</sub> not only promoted Th17 differentiation from conventional CD4<sup>+</sup> T cells but also themselves converted into proinflammatory Th17 cells in vitro and in vivo settings [66]. Intriguingly, partial depletion of T<sub>regs</sub> markedly reduced the Th17 responses, as shown by the attenuated neutrophil infiltration and reduced severity of oviduct inflammation after *C. muridarum* genital infection [66]. Thus, T<sub>regs</sub> play a critical role in the immunopathogenesis in this model, which is completely contradictory to their well-documented immunosuppressive activity. It is worth noting that Th17 responses, enhanced by T<sub>regs</sub>, strengthen host resistance to *C. albicans* infection [67], whereas the same action causes the immunopathology in *C. muridarum* infection [66], suggesting that the biological outcome of interplay of T<sub>regs</sub> and Th17 may be dependent on the specific pathogen.

### Allograft rejection triggered by Th17 cells is fueled by T<sub>regs</sub>

T<sub>regs</sub> are considered as a therapy to induce immune tolerance in clinical transplantation [3]; thus, their interaction with rejection-inducing Th cells should be clarified. Vokaer and colleagues [85] reported that T cell-derived IL-17 was critical for the neutrophil infiltration and rejection of minor antigen-mismatched skin grafts. In this model, depletion of T<sub>regs</sub> resulted in a marked reduction of IL-17A mRNA within the grafts and draining LNs, with a marginal increase of IFN- $\gamma$  mRNA, consistent with the results of a study on silica-induced lung fibrosis [86]. Furthermore, cotransfer of T<sub>regs</sub> together with anti-donor naive T cells into Rag<sup>−/−</sup> mice not only enhanced Th17 differentiation by T<sub>effs</sub>, but a considerable number of T<sub>regs</sub> by themselves also became IL-17 producers [85]. Thus, the potential of T<sub>regs</sub> to promote Th17-mediated, neutrophil-dependent rejection of graft should be considered in T<sub>reg</sub>-based therapy in bone marrow transplantation and solid organ transplantation.



## **T<sub>regs</sub> increase inflammatory support of tumor growth by Th17 cells**

Th17 cells have been reported to play dual roles in tumors: they promote inflammatory support of tumor growth and contribute to the immune surveillance against tumor [19]. In the mouse glioma model, IL-10-producing Th17 cells appeared to support tumor growth [54]. In this model, an elevated number of T<sub>regs</sub> promoted the generation of IL-10-producing Th17 cells, while inhibiting IFN- $\gamma$ -producing Th17 cells [54]. Therefore, multiple mechanisms may be attributed to T<sub>regs</sub> in promoting cancer immune evasion, including direct inhibition of anti-tumor Th1 responses and stimulation of tumor-supporting Th17 responses.

Taken together, recent studies do not support the view that Th17 cells are a cellular target of T<sub>reg</sub>-mediated inhibition. Instead, there is clear in vitro and in vivo evidence that T<sub>regs</sub> actually promote the differentiation of Th17 cells and consequently, enhance the beneficial as well as detrimental functions of Th17 cells.

## **TH17 CELLS PROMOTE THE EXPANSION AND PHENOTYPE STABILITY OF T<sub>REGS</sub>**

In the past decade, extensive study of T<sub>regs</sub> greatly improved our understanding of the effect of T<sub>regs</sub> on T<sub>effs</sub>; however, much less is known about the effect of T<sub>effs</sub> on T<sub>regs</sub>. Accumulating evidence indicates that in addition to being the cellular target for suppression by T<sub>regs</sub>, T<sub>effs</sub> can have a marked impact on T<sub>regs</sub>. More specifically, T<sub>effs</sub> are important in support of sustained suppressive function of T<sub>regs</sub>. Among Th subsets, Th17 cells are the most potent stimulators of T<sub>regs</sub>, as shown by our recent results. The interactions of Th17 cells and T<sub>regs</sub> are bidirectional, and Th17 cells have a considerable effect on T<sub>regs</sub>.

### **Activation of T<sub>regs</sub> requires stimulation by T<sub>effs</sub>**

T<sub>regs</sub> constitutively express high levels of functional cytokine receptors, such as CD25 [87, 88] and TNFR2 [89–91], but do not have the capacity to produce ligands for these receptors. This suggests that T<sub>regs</sub> may rely on the cytokine producers, such as activated T<sub>effs</sub>, for the maintenance of their function.

Thornton and colleagues [92] found that T<sub>regs</sub> did not suppress the initial activation of T<sub>effs</sub> but exerted their suppressive effects only after production of IL-2 by T<sub>effs</sub>, resulting in the expansion of T<sub>regs</sub> and the induction of their suppressor function. Early evidence of the in vivo-supportive effect of T<sub>effs</sub> for T<sub>regs</sub> was shown by Curotto de Lafaille and colleagues [93]. They found that CD25 expression on donor T<sub>regs</sub>, defined by CD4<sup>+</sup>CD25<sup>+</sup>, was not stable after transfer into recipient mice. However, cotransferred T<sub>effs</sub>, by producing IL-2, could maintain the expression of CD25 on T<sub>regs</sub> [93]. Furthermore, the same group also showed that IL-2 from T<sub>effs</sub> was required for the function of T<sub>regs</sub> in the inhibition of EAE [94].

We found previously that the number of T<sub>regs</sub> was reduced in mice with EAE, and PTX, contained in the immunogen used to induce EAE, was responsible for the reduction of T<sub>regs</sub> [95]. Injection of PTX into a WT mouse resulted in a

marked reduction of T<sub>regs</sub> [95, 96]. To our surprise, in the absence of IL-6, PTX treatment had the opposite effect and expanded T<sub>regs</sub> in vitro [97] and in vivo (data not shown). We therefore tested the effect of proinflammatory cytokines elicited by PTX on T<sub>regs</sub>. This led us to find that TNF, though TNFR2, preferentially activated, expanded, and promoted the phenotypic stability of T<sub>regs</sub> [69, 89, 98]. Although counterintuitive and contradictory to a previous report [99], our observation is supported by increasing evidence from other investigators [100–103].

TNF–TNFR2 pathways actually also enable T<sub>effs</sub> to activate T<sub>regs</sub> in vivo, as shown by an elegant study performed by Grinberg-Bleyer and colleagues [104]. They initially found that T<sub>regs</sub> proliferated significantly more when coinjected into mice with activated T cells. When islet-specific T<sub>effs</sub> were transferred alone, they induced diabetes, whereas mice injected with T<sub>regs</sub> alone or T<sub>regs</sub> plus T<sub>effs</sub> did not develop diabetes. However, upon a second injection of activated T<sub>effs</sub>, 3 weeks after the initial injection, mice that had received a first injection of T<sub>regs</sub> alone developed diabetes, whereas mice that originally had been injected simultaneously with T<sub>regs</sub> and T<sub>effs</sub> were protected from diabetes. Thus, the T<sub>effs</sub> were indirectly protective by stimulating T<sub>reg</sub> expansion and suppressive activity. The results of the RNA microarray indicated that TNF/TNFR2 but not IL-2/CD25 was the pathway responsible for the T<sub>reg</sub>-stimulatory effect of T<sub>effs</sub> [104].

### **Th17 cells produce high levels of TNF: perhaps they should be termed Th-TNF**

We determined the identity of the subset of T<sub>effs</sub> that was a major TNF producer. We compared Th0, Th1, Th2, and Th17 subsets in vitro, differentiated from naive CD4 cells under standard respective polarized culture conditions. Th17 cells expressed the highest level of TNF (46%), followed by Th1 cells (26%) and Th0 (14%), whereas Th2 cells (6%) expressed the lowest levels of TNF. The TNF expression by Th subsets was stable and remained largely unchanged 5 weeks after transfer into Rag<sup>−/−</sup> mice [105]. Interestingly, although named for the production of IL-17, Th17 cells also expressed similar or even higher levels of TNF than IL-17A in vitro and in vivo [105]. In addition to being expressed by in vitro-differentiated mouse Th17 cells [106], TNF is expressed by natural Th17 cells present in human patients [107, 108]. Recently, it was reported that the pathogenic effect of Th17 cells was actually mediated by its TNF expression [109]. Thus, this subset of Th cells could also be renamed “Th-TNF”.

### **Th17 cells stimulate expansion and promote stability of the T<sub>reg</sub> phenotype, mainly depending on the TNF–TNFR2 pathway**

As Th17 cells expressed the highest levels of TNF among Th subsets, they have the potential to stimulate T<sub>regs</sub> in vivo. To test this idea, freshly isolated, highly purified T<sub>regs</sub> (CD4<sup>+</sup>Foxp3<sup>+</sup>/gfp<sup>+</sup> cells) were transferred alone or cotransferred with Th subsets into Rag<sup>−/−</sup> mice. After 5 weeks, the number and level of Foxp3 expression by T<sub>regs</sub> were determined. The results showed that, when transferred alone, T<sub>regs</sub>

did not proliferate well in a lymphopenic environment and a majority of them lost Foxp3 expression, which is consistent with our previous report [69]. All types of Th subsets, including Th0 cells, were able to support expansion and phenotypic stability of T<sub>regs</sub> in vivo. Among tested Th subsets, Th17 cells were the most potent stimulators of T<sub>regs</sub>, resulting in markedly more T<sub>regs</sub> with the highest levels of Foxp3 expression. This was followed, in order, by Th1, Th2, and Th0 cells [105]. The potency of the T<sub>reg</sub>-stimulatory effect of Th subsets (Th17>Th1>Th0) correlated with their capacity to express TNF (Th17>Th1>Th0) but not with their capacity to express IL-2, as Th17 cells are poor producers of IL-2 [110]. Nevertheless, IL-2 also played a role, as the neutralizing antibody against IL-2 partially blocked the stimulatory effect of Th17 cells on T<sub>regs</sub>. Other cytokines, in addition to TNF and IL-2, may also contribute to this effect, as Th2 cells expressed relatively lower levels of TNF and IL-2, while having more potent T<sub>reg</sub>-stimulatory activity than Th0 cells. However, the TNF–TNFR2 pathway appears to be dominant, as the effect of Th17 cells on T<sub>regs</sub> was largely abolished when cotransferred with TNFR2-deficient T<sub>regs</sub>. Intriguingly, expression of IL-17A and TNF by WT Th17 cells was reduced by up to 80% when cotransferred with T<sub>regs</sub> deficient in TNFR2 [105]. Based on these observations, we favor the idea that the TNF–TNFR2 signal is crucial for the reciprocal stimulatory effect of proinflammatory Th17 cells and immunosuppressive T<sub>regs</sub>.

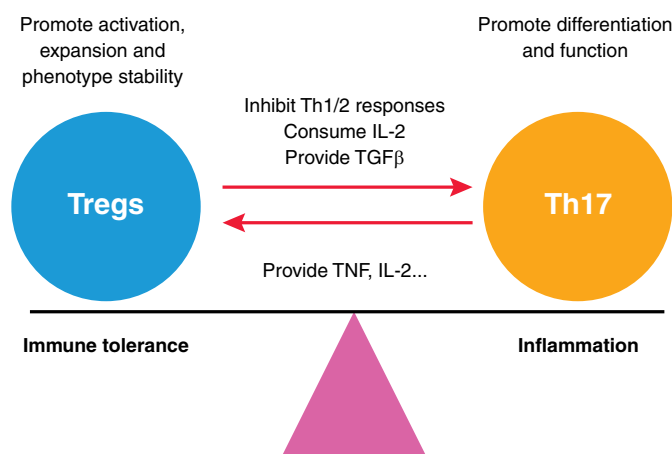
### Why are Th17 cells most able to stimulate T<sub>regs</sub>?

Distinct effector functions of Th subsets have evolved presumably to protect the host efficiently against myriad offending pathogens by mounting a robust immune response. However, excessive and prolonged activation of Th cells can result in the severe inflammation and collateral damage to normal tissues. The capacity of activated T<sub>effs</sub> to stimulate T<sub>regs</sub> is likely to represent a major mechanism by which a dynamic equilibrium is established between T<sub>effs</sub> and T<sub>regs</sub> in an ongoing immune or inflammatory response to avoid damage to self. Th17 cells have stem cell-like features [26, 111] and can mediate sustained autoimmune inflammation [112]. It is reasonable to hypothesize that the potent T<sub>reg</sub>-stimulatory activity of Th17 cells coevolved to counter their robust and prolonged proinflammatory effector function. Th17 cells not only produce TNF by themselves, but the IL-17 cytokine is also able to stimulate the production of TNF from macrophages [113], which can further amplify the T<sub>reg</sub>-stimulatory effect. Therefore, Th17 cells exert critical functions in host defense, immune responses, and inflammation and together with T<sub>regs</sub>, orchestrate the maintenance of immunological homeostasis. Presumably, expanded and activated T<sub>regs</sub> are responsible to attenuate and eventually quench the inflammatory cascade induced by Th17 cytokines. For example, activation of neutrophils by Th17 cells [114] could be inhibited by T<sub>regs</sub> [115].

### CONCLUDING REMARKS

Extensive study of T<sub>regs</sub> and Th17 cells has established a critical role of these two subsets of Th cells in the pathogenesis of

major human diseases. Consequently, targeting Th17 cells and T<sub>regs</sub>, by up-regulating or down-regulating their respective function, can be considered a promising means of treating autoimmune disorders and tumors [21, 22, 116]. However, recent evidence shows that Th17 cells and T<sub>regs</sub> do not simply antagonize each other, but instead, they stimulate each other reciprocally (Fig. 1). It is likely that therapeutic manipulation of one of these subsets would have an impact on the other. The complexity of their interactions should be considered carefully when designing therapeutic strategies. For example, increasing T<sub>reg</sub> activity, by stimulating in vivo expansion of T<sub>regs</sub> or adoptive transfer of ex vivo-expanded T<sub>regs</sub>, has become a strategy in the treatment of autoimmunity, allograft rejection, and GvHD [116]. Although current data from studies on humanized mice and human patients suggest that such an approach is effective and safe [117, 118], the potential risk of developing acute and chronic Th17-mediated inflammation by such T<sub>reg</sub>-based treatment, as has occurred in mouse models [85, 86], should be closely monitored. Another possibility—that Th17 cells generated by T<sub>regs</sub> can actually promote T<sub>reg</sub> activity further and that Th17 and T<sub>regs</sub> may collaboratively inhibit autoimmunity, allograft rejection, and GvHD—should also be investigated. On the other hand, caution should be used when targeting Th17 cells in the treatment of autoimmunity, as it may reduce immunosuppressive T<sub>reg</sub> activity as a side-effect. However, the interplay of Th17 and T<sub>regs</sub> may also provide new avenues to up- or down-regulate their function therapeutically. For example, although the beneficial effect of T<sub>reg</sub> depletion in experimental tumor models has been clearly shown [119], complete depletion of T<sub>regs</sub> is difficult and risks



**Figure 1. Reciprocal stimulation of T<sub>regs</sub> and Th17 cells.** T<sub>regs</sub> are able to promote the differentiation and function of Th17 cells, through a mechanism involving the supply of TGF-β, which is required for Th17 differentiation, or inhibition of Th1 and Th2 cytokines and IL-2, which are known to block Th17 differentiation. Induced and natural Th17 cells also stimulate the activation and expansion and promote Foxp3 expression of T<sub>regs</sub> by producing TNF, IL-2, or other mediators required for the survival and activation of T<sub>regs</sub>. Therefore, the immunosuppressive T<sub>regs</sub> and proinflammatory Th17 cells reciprocally stimulate and temper each other, resulting in a dynamic equilibrium in an ongoing immune/inflammatory response.

the development of autoimmune responses in cancer patients. Tumor-infiltrating  $T_{\text{regs}}$  expressed markedly higher levels of TNFR2 [90], and abundant  $T_{\text{regs}}$  and Th17 cells frequently colocalized in tumors [49–54]; thus, therapeutically targeting Th17 cells may reduce the suppressive activity of tumor-associated  $T_{\text{regs}}$  and mitigate the autoimmune risk. Furthermore, Th17 cell-derived TNF may be considered a means to activate and expand  $T_{\text{regs}}$ . For example,  $T_{\text{reg}}$  expansion is usually achieved through agents, such as IL-2 and rapamycin [120–123]. Recently, Okubo and colleagues [100] reported that a TNFR2 agonist had a superb effect on causing homogenous expansion of human  $T_{\text{regs}}$  with potent suppressive capacity. Thus, it is worthwhile—using a TNF–TNFR2 pathway-based Th17– $T_{\text{reg}}$  interaction or in conjunction with additional  $T_{\text{reg}}$ -activating agents—in the treatment of autoimmunity, GvHD, and allograft rejection by up-regulation of  $T_{\text{reg}}$  activity. It is known that Th17 cells and  $T_{\text{regs}}$  are not homogenous. For example, a subset of Th17 cells is immunosuppressive [124, 125], whereas a subset of  $T_{\text{regs}}$  has the capacity to produce proinflammatory cytokines [126]. Therefore, the interactions between Th17 subsets and  $T_{\text{reg}}$  subsets merit future investigation. Furthermore, the role of antigenic DCs, which have a major impact on the differentiation, expansion, and function of  $T_{\text{regs}}$  [127, 128] and Th17 cells [129, 130] in the reciprocal stimulation of these two Th subsets, also needs to be defined further.

## AUTHORSHIP

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## DISCLOSURES

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## KEY WORDS:

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