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Review

IL-17$^+$Foxp3$^+$ T cells: an intermediate differentiation stage between Th17 cells and regulatory T cells

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ABSTRACT

Foxp3$^+$ T_{regs} have been known as a major regulator of immune homeostasis through their immunosuppressive function. Th17 lineage is a CD4$^+$ T cell subset that exerts its function by secreting proinflammatory cytokines and protecting host against microbial infections. The altered ratio between Foxp3$^+$ T_{regs} and Th17 cells plays an important role in the pathogenesis of immune-related diseases. Recent mice and human studies have demonstrated that T_{regs} can be reprogrammed into a novel population, IL-17$^+$Foxp3$^+$ T cells, phenotypically and functionally resembling Th17 cells under the complicated cytokine stimulation. The identification of IL-17$^+$Foxp3$^+$ T cells may provide a new understanding of therapy targeting T_{regs} and Th17 cells in autoimmune diseases and cancer. Here, we highlight significant data regarding the phenotype profile, origination, differentiation, and the pleiotropic functions of IL-17$^+$Foxp3$^+$ T cells and the reciprocal relationships of these cells to T_{regs} and Th17 cells. Furthermore, the role of IL-17$^+$Foxp3$^+$ T cells in tumorigenesis and clinical implications in cancer therapy are discussed in this review. *J. Leukoc. Biol.* 96: 39–48; 2014.

CD4$^+$ T_{regs}, the development of which is critically dependent on X-linked transcription factor Foxp3, are in charge of suppressing activities of Th cells. One significant finding from recent studies is that Foxp3$^+$ T_{regs} could be reprogrammed into a phenotype resembling Th cells under certain stimuli [1–3]. Despite the high frequency of CD4$^+$ T cells with a regulatory phenotype in inflammatory tissues, inflammation persists. One possible explanation for this evidence is that T_{regs} possess the propensity to be converted into IL-17-producing cells and thereby, lose their suppressive function. After the establishment of Th17 cells in 2005 [4, 5], one mice study in 2007 showed that activated Foxp3$^+$ T_{regs} had the potential to stimulate CD4$^+$CD25$^-$Foxp3$^-$T cells or to be self-induced to differentiate toward the Th17 phenotype in the presence of IL-6 without exogenous TGF-β [6]. After this finding, extensive mice and human studies have been performed on this novel subset of T cells with the appearance of complicated characteristics. In this article, we focus on discussion of the phenotype features and differentiation of IL-17$^+$Foxp3$^+$ T cells and their biological functions and relationship with T_{regs} and Th17 cells. Furthermore, the role of IL-17$^+$Foxp3$^+$ T cells in tumorigenesis is highlighted.

THE PHENOTYPIC PROPERTIES OF IL-17$^+$FOXP3$^+$ T CELLS

The status of this newly identified population may be somewhat embarrassing by its overlapping phenotype with T_{regs} and Th17 cells [7, 8]. nT_{regs} are identified originally by constitutive expression of CD25 [9, 10] and are currently recognized specifically by expression of the transcription factor Foxp3 [11, 12]. Additional markers of T_{regs}, such as CTLA-4, TNFR2, and GITR, are also detected [13, 14], making it more convenient to discern T_{regs}. Th17 cells, different from Th1 and Th2, are characterized by secreting proinflammatory cytokine IL-17 and expressing RORγt [4, 15], whereas it has been reported that Th17 cells in human tonsils express receptors that are present in Th1 and Th2 cells and Foxp3$^+$ T_{regs} [16].

IL-17$^+$Foxp3$^+$ T cells share similar phenotype features with T_{regs} and effector Th cells (**Tables 1** and **2**). Expression of CTLA-4 and GITR on mice IL-17$^+$Foxp3$^+$ T cells is comparable with IL-17$^-$$T_{regs}$ after stimulation with anti-CD3/CD28 mAb and IL-1β and IL-2 in vitro [8], whereas the expression of ICOS on IL-17$^+$Foxp3$^+$ T cells is higher than IL-17$^-$$T_{regs}$

Abbreviations: CRC=colorectal cancer, EAE=experimental autoimmune encephalomyelitis, Foxp3=forkhead box p3, GITR=glucocorticoid-induced TNFR, HDACi=inhibitor of histone/protein deacetylase, HIF-1=hypoxia-induced factor 1, IRF4=IFN regulatory factor 4, iT_{reg}=induced regulatory T cell, mTOR=mammalian target of rapamycin, NnT_{reg}=natural, naive regulatory T cell, nT_{reg}=naturally occurring regulatory T cell, pDC=plasmacytoid dendritic cell, RORγt=retinoic acid receptor-related orphan receptor γ t, SOCS1=suppressor of cytokine signaling 1, T_{reg}=regulatory T cell, Ubc13=ubiquitin-conjugating enzyme

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TABLE 1. IL-17$^+$Foxp3$^+$ T Cells Express Functional Phenotypes of T_{regs}

Subsets	Foxp3	CD25	GITR	CTLA-4	ICOS	CCR4	CD39	TGF-β
IL-17$^-$ T_{regs}	+++	++	+++	+++	+	++	++	++
IL-17$^+$Foxp3$^+$ T cells	+/+++	++	+++	+++	+++	++	++	++

[8]. Surprisingly, IL-17$^+$Foxp3$^+$ T cells have been found to express Ikaros family transcription factor Helios [8], a newly identified marker for a novel subset of T_{regs} for distinguishing thymic-derived and peripherally induced Foxp3$^+$ T_{regs} [17, 18]. In human CRC, IL-17$^+$Foxp3$^+$ T cells were observed to express high levels of TGF-β [19, 20], an immunomodulatory cytokine with an anti-inflammatory role and indirect function of promoting inflammation by enhancing the differentiation of Th17 cells in CRC [21–23]. Intriguingly, it was reported that expression of CD39, which participated in immunosuppressive adenosine production, was comparable between IL-17$^+$Foxp3$^+$ T cells and IL-17$^-$$T_{regs}$ in patients with ulcerative colitis [7]. In the environment of chronic colitis, IL-17$^+$Foxp3$^+$ T cells and conventional T_{regs} expressed similar levels of CD25 and CCR4, while at the same time, IL-17$^+$Foxp3$^+$ T cells expressed high levels of CD161 and CD49d, as well as a relatively substantial amount of effector cytokines IFN-γ and IL-2 [7]. Moreover, a small population of IL-17$^+$Foxp3$^+$ T cells in CRC was CCR6- and IL-6-positive, and very few cells expressed functional cytokines IFN-γ, IL-4, and TNF-α [19]. Human memory CD25$^+$CD127^{low}Foxp3$^+$ T_{regs} were detected to express IL-1R1, which was associated with IL-17 secretion and RORγt expression in vitro, and IL-1R1 expression could identify central memory Foxp3$^+$IL-17$^+$ cells, which were CCR7-positive from NnT_{regs} [24]. Expression of moderate levels of effector cytokines suggests that IL-17$^+$Foxp3$^+$ T cells are not a typical T_{reg} subset and may play a distinct role in immune response. In the following, we will discuss the markers, such as Foxp3, RORγt, IL-17, and CCR6, which are associated with biological functions of IL-17$^+$Foxp3$^+$ T cells.

Foxp3

In human studies, the conversion of T_{regs} into IL-17$^+$Foxp3$^+$ T cells retained expression of Foxp3, even though this process was accompanied by gradually reduced Foxp3 expression [25, 26]. When cultured with bone marrow stromal cells, the significantly reduced, suppressive function of T_{regs} was not accompanied by a remarkably reduced Foxp3 expression [27]. This may be in concert with recent studies that Foxp3 was not the only factor that determines T_{reg}-suppressive function [28]. Foxp3 is not only the most specific marker of T_{regs} discriminating T_{regs} and effector T cells, it is also a conclusive transcription factor mediating the development and suppressive function of T_{regs} [11, 12]. However, it was suggested that functional T_{regs} could develop in the absence of Foxp3 [28–30]. Previous studies have indicated that T_{regs} lost Foxp3 expression as soon as transferred into a lymphopenic host or in inflammatory conditions, obtaining an activated memory phenotype and producing proinflammatory cytokines [31, 32]. The stability of Foxp3 expression is sustained by epigenetic modification, depending on that hindrance of DNA methylation-stabilized Foxp3 expression [33, 34]. In addition, the HDACi increased Foxp3 expression and simultaneously maintained the suppressive function of T_{regs} [35]. Accordingly, HDACi could impede the conversion of T_{regs} into IL-17-producing cells by sustaining Foxp3 expression [25]. However, a recent mice study showed that the class I HDACi suppressed Foxp3 gene expression at a transcriptional or post-transcriptional level, breaking immune tolerance without reducing the number of peripheral T_{regs} [36].

RORγt

In addition to T_{regs} regulatory factor Foxp3, RORγt, specific for Th17 cells, is concomitantly expressed in human IL-17$^+$Foxp3$^+$ T cells as well [37, 38], indicating that the expression of Foxp3 and RORγt is not always incompatible. The orphan nuclear receptor, RORγt, a key transcription factor regulating Th17 differentiation, is essential for constitutive expression of IL-17 [22, 39]. Upon in vitro stimulation, human memory T cells rather than naive T cells showed RORC DNA demethylation [40]. Compared with IL-17$^-$$T_{regs}$ and conventional T cells, IL-17$^+$ T_{regs} expressed higher levels of RORγt after coculture of T_{regs} with conventional T cells and APCs [41], and the RORγt expression in human IL-17$^+$$T_{regs}$ is responsible for their capacity to secrete IL-17 [42]. RORγt expression functioned as a phenotypic marker for the identification of IL-17$^+$Foxp3$^+$ T cells and participated in the process of differentiation [43]. Recent studies suggested that Foxp3 interacts with RORγt to inhibit Th17 differentiation in naive CD4$^+$ T cells [44]. How these transcription factors interact with each other in IL-17$^+$Foxp3$^+$ T cells is still unknown.

TABLE 2. IL-17$^+$Foxp3$^+$ T Cells Express Th17-Related Molecules and Secrete Effector Cytokines

Subsets	RORγt	IL-17	CD161	CD49d	CCR6	IL-2	IFN-γ
IL-17$^-$ T_{regs}	+	−	+	++	+	−/+	−/+
IL-17$^+$Foxp3$^+$ T cells	+++	++/+++	+++	+++	++	++	++
Th17 cells	+++	+++	+++	+++	++	+++	+++

IL-17

IL-17 (also designated as IL-17A) is a pleiotropic cytokine, the majority of which can stimulate the secretion of many proinflammatory cytokines (such as IL-6 and TNF-α) and chemokines participating in the recruitment and activation of neutrophils and macrophages [45, 46]. Under appropriate circumstances, T_{reg} lost its stability and skewed toward the Th17 phenotype, characterized by abundant expression of IL-17, which was closely associated with accompanied but reversible deprivation of immunosuppressive function [42]. In human studies, after blockage of IL-17 expression, nT_{regs} failed to obtain proinflammatory properties in the presence of IL-6 and IL-1β and became phenotypically stable [47]. Furthermore, the expression of IL-17F, which was with high homology to IL-17A, was also observed in human and mice when T_{regs} become IL-17 producers [26, 43]. Recently, it was reported that IL-17F exerts a protective role against colon tumorigenesis through inhibiting angiogenesis with decreased vascular endothelial growth factor expression [48]. The contradiction with previous reports that IL-17 promotes tumor angiogenesis [48, 49] suggested a more functional complexity in IL-17-producing cells, including Th17 cells and IL-17$^+$Foxp3$^+$ T cells.

When stimulated by IL-1β/IL-6, DR$^-$$T_{regs}$, which do not express the MHC class II dimer HLA-DR, secreted high levels of IL-17 [42]. The secretion of IL-17 was preceded by increased RORC mRNA expression, the human homologue for the Th17-associated transcription factor RORγt [42]. As a complementation, Yurchenko et al. [50] demonstrated that secretion of IL-17 occurred earlier than Foxp3 down-regulation in the process of the differentiation in mesenteric sites in mice models. Then, we can deduce that the sequence occurred during the process that under circumstances of proinflammatory cytokines, T_{regs} expressed Th17-associated transcription factor RORγt, resulting in IL-17 secretion, which is directed by RORγt. Afterward, IL-17 is responsible for the deficiency of Foxp3 expression, as well as immunosuppression in the end, although expression of IL-17 does not completely rely on expression of RORγt, in that in the RORγt-deficient mice, serum levels of IL-17 did not significantly decrease compared with tissue lysate [51]. Maybe the loss of RORγt hindered excessive IL-17 production but did not eliminate it completely.

In the differentiation of human IL-17$^+$Foxp3$^+$ T cells, increased IL-17 excretion correlated with the presence of APCs, to a large extent, which serves as a precondition in this process [7, 25, 42]. IL-17 production may be STAT3 signaling-dependent, as hyper-IgE syndrome patients with defective STAT3 proteins failed to undergo IL-17 induction [52]. However, another group found no coexpression of IL-17 and Foxp3 in RORγt$^+$ T cells in mice studies, and the Foxp3$^+$RORγt$^+$ T cells expressed IL-10 instead [53].

CCR6

Chemokine receptors have been instrumental in the characterization of effector/memory subset of T_{regs} [54]. Researchers found that T_{regs} that produced IL-17 coexpressed CCR6 [37], and human IL-17$^+$Foxp3$^+$ T cells are mainly originated from CCR6$^+$ populations [7, 25, 42]. These results suggest that CCR6 may have the capacity to discriminate human IL-17$^+$ T_{regs} from other T_{regs} subsets [25]. However, CCR6 alone is not sufficient to function as a marker with minimal contamination with other lineages, as a result of the fact that a small part of human IL-17$^-$$T_{regs}$ expressed CCR6 as well [42]. Presumably, this identification can be realized together with CCR4, according to the report that CCR6 and CCR4 cooperatively identified IL-17-producing T cells rather than IFN-γ-producing Th17 memory cells in human studies [55]. Apart from CCR6, other chemokine receptors, such as CCR4 [7, 20] and CXCR3 [20], were detected in tissues of CRC patients. Expression of chemokine receptors and adhesion molecules may confer IL-17$^+$Foxp3$^+$ T cells with specific migratory capacities that can reduce the number of circulating cells.

THE ORIGIN OF IL-17$^+$FOXP3$^+$ T CELLS

It has been well-investigated that there are two independent subpopulations of T_{regs}: nT_{regs}, derived from thymus via a separate thymic developmental pathway, distinct from positive and negative selection [56], and iT_{regs}, originated from CD25$^-$ T cells in the periphery when exposed to antigens and extrathymic signals, possessing great destabilization and plasticity [57, 58]. With regard to the origination of IL-17$^+$Foxp3$^+$ T cells, there are three major perspectives: (1) a stable T cell subset in the periphery, (2) a transient differentiation stage between T_{regs} and Th17 cells, and (3) the demonstration of T_{regs} plasticity facing up with pathogens or specific antigens. The doubt of whether IL-17$^+$Foxp3$^+$ T cells develop in the thymus or in the periphery was addressed by Voo et al. [38]—that human T_{regs} acquire the ability to produce IL-17 in the periphery. Another mice study also showed that thymus-derived T_{regs} produced little IL-17 under polarizing conditions [59]. After stimulation with IL-6, IL-2, and TGF-β, mice iT_{regs} were resistant to Th17 conversion, and Foxp3 expression was not affected compared with nT_{regs} [60]. Irrespective of the fact that T_{regs} and Th17 cells are from naive T cells, previous human studies indicated that IL-17$^+$Foxp3$^+$ T cells originated efficiently from memory T cells, including memory T_{regs} and conventional memory T cells [7, 40]. Similar to the origin of Th17 cells, IL-17$^+$Foxp3$^+$ T cells are preferentially stemmed from CCR6$^+$ but not CCR6$^-$ T cells [7].

THE DIFFERENTIATION AND DEVELOPMENT OF IL-17$^+$FOXP3$^+$ T CELLS

In the development of thymic-derived nT_{regs}, TCR, costimulatory, and IL-2 signals are required. Upon TCR stimulation, naive T cells can be driven to express Foxp3 and become T_{regs} in the presence of IL-2 and TGF-β. The generation of iT_{regs} can be modulated further by other stimuli, particularly through retinoic acid [61] and the aryl hydrocarbon receptor [62]. In the differentiation of Th17 cells from naive T cells, effector cytokines IL-21 [63, 64], IL-23 [65], and IL-1 [66] play a promoting role, and the process is negatively regulated by IL-2 [67]. Regarding the provenance of IL-17$^+$Foxp3$^+$ T cells and

the differentiation mechanisms, cumulative studies have provided evidence that offers clues to the potential pathway by which IL-17$^+$Foxp3$^+$ T cells develop.

TGF-β induces IL-17 production in T_{regs}

Although TGF-β participates in the expansion of T_{regs} and Th17, and its role has been probed [21, 68, 69], the explicit function that TGF-β exhibits in the differentiation of IL-17$^+$Foxp3$^+$ T cells has not reached a consensus. What maintains mainstream is that the generation of IL-17 by T_{regs} is TGF-β-dependent [70], and the use of a TGF-β inhibitor reduced but did not eliminate IL-17 production completely [6, 41, 71]. It is reported that TGF-β and IL-2 were the optimal combination to maximize the levels of human IL-17$^+$Foxp3$^+$ T cells in the presence of APCs [7]. As Xu et al. [6] have addressed in mice models, with addition of IL-6, T_{regs} could produce a sufficient amount of TGF-β, which enabled T_{regs} to be self-induced to IL-17-producing cells without exogenous TGF-β. Moreover, T_{regs} may be even more efficient inducers than exogenous TGF-β in stimulating IL-17 production [6]. The role of endogenous TGF-β may account for the phenomenon that a high concentration of TGF-β-neutralizing antibody was not able to inhibit IL-17 production completely. However, in contradiction with this study is the report that the secretion of IL-17 by human DR$^-$$T_{regs}$ can be blocked by TGF-β [42]. This report considers it an additional mechanism by which TGF-β can promote T_{reg} function: TGF-β can inhibit IL-17 production by T_{regs}. Another human study observed no promoting effect in IL-17 production when TGF-β was added to the culture [25]. One of the reasons for the pleiotropy of TGF-β orchestrating the process may a result of the fact that high concentration of exogenous TGF-β may impede IL-17 production by favoring Foxp3 expression [44]. Another possible reason is that the conversion of T_{regs} into IL-17-producing cells is influenced by other dominant factors other than TGF-β.

IL-6 assists in the development of IL-17$^+$Foxp3$^+$ T cells

Proinflammatory cytokine IL-6, which plays an important role in the differentiation of Th17 cells [72], induces the production of IL-17 in T_{regs} likewise [6, 42, 71]. In human CRC tissue, hypoxia was found to enhance Foxp3$^+$ T_{regs} to express IL-17 by inducing IL-6 expression in CD68$^+$ cells [20]. Recently, it was reported that because of the reduced expression of CD126 (the IL-6R α chain) and the signaling chain gp130, T_{regs} from the mouse-inflamed nervous system during EAE are resistant to conversion to IL-17-producing cells [73]. After coculture of CD68$^+$ cells with CD4$^+$CD25^{high} T cells from PBMC of esophageal cancer patients, anti-IL-6 antibody was added under an hypoxia environment; consequently, the production of IL-17 decreased sharply [71]. Recent research in a mice arthritis model showed that synovial fibroblast-derived IL-6 plays an important role in the conversion of CD4$^+$Foxp3$^+$ T cells to Th17 cells [74]. Neutralizing antibody against IL-6, inhibited the generation of Th17 cells after coculture of CD4$^+$Foxp3$^+$ T cells with synovial fibroblasts. All of these mice and human findings illustrate the vital function of IL-6 in the differentiation of IL-17$^+$Foxp3$^+$ T cells. Even so, IL-6 alone showed poor efficacy in IL-17 production [25], and IL-6 was not a leading factor in this course, further implicating the intricate cytokine milieu needed for the differentiation of IL-17$^+$Foxp3$^+$ T cells.

Other molecules and transcription factors promote the differentiation of IL-17$^+$Foxp3$^+$ T cells

The biological function of the IL-1/IL-1R antagonist (IL-1/IL-1Ra) control system has been explicit in mice and humans. Abnormal T cell activation caused by the imbalance of the IL-1/IL-1Ra system was responsible for the development of EAE [75]. In the presence of exogenous IL-2/IL-15, a prerequisite to induce the differentiation, the IL-1/IL-1Ra system probably had the capacity to control human T_{regs} reprogramming into IL-17-producing cells [25]. Based on what Li et al. [41] have reported in a mice model, IL-1β efficiently promoted conversion of T_{regs} but not conventional T cells into IL-17-producing cells, and IL-1β-mediated activation of p38 and JNK signals are involved in the differentiation.

The TLRs signals play a critical role in the host immune system against infectious pathogens and modulate functions of T_{regs} [76, 77]. Recent human study indicate that activation of TLR2 contributed to increased levels of IL-6 and IL-17 and reduced Foxp3 expression, promoting naive and effector T_{regs} into a Th17-like phenotype [26]. Another human study showed that after coculture with TLR4- and TLR9-stimulated B cells, T_{regs} develop into RORC$^+$IL-17$^+$ T cells [78].

It was reported that mTOR, which is a conserved, serine-threonine protein kinase, could influence the stability of Foxp3$^+$$T_{regs}$. During inflammation-driven reprogramming of mice T_{regs} into IL-17$^+$Foxp3$^+$ T cells in vivo, inhibition of mTOR signaling stabilized Foxp3 expression in T_{regs} and simultaneously inhibited IL-17 but not RORγt expression [50]. Thus, T_{regs} differentiate into IL-17-producing cells in response to various stimuli.

Furthermore, mice IL-17$^+$Foxp3$^+$ T cells expressed increased levels of Runx1 [8], a transcription factor important for the differentiation and function of T_{regs} and Th17 cells. The silencing of Runx1 with small interfering RNA strikingly reduced the production of IL-17, as well as the expression of Rorc and Foxp3 mRNA, suggesting that Runx1 is essential for the generation of IL-17$^+$Foxp3$^+$ T cells by regulating the expression of Rorc and Foxp3 [8]. Expect for plentiful expression of the transcription factor RORγt in IL-17-producing T_{regs}. RORγt is also required for the differentiation of mice IL-17$^+$Foxp3$^+$ T cells, in that the secretion of IL-17 is abundant in Foxp3$^+$RORγt$^+$ T cells compared with the negligible production in Foxp3$^+$ RORγt$^-$ T cells [43]. In a human study, the expression of RORγt, which is responsible for the generation of IL-17, is initiated by the strong TCR signals [42].

Certain factors prevent conversion of T_{regs} into IL-17$^+$Foxp3$^+$ T cells

Although the differentiation of T_{regs} into IL-17-producing cells can be enhanced under complex circumstances, this pathway is negatively regulated at the same time (**Fig. 1**). In a mice

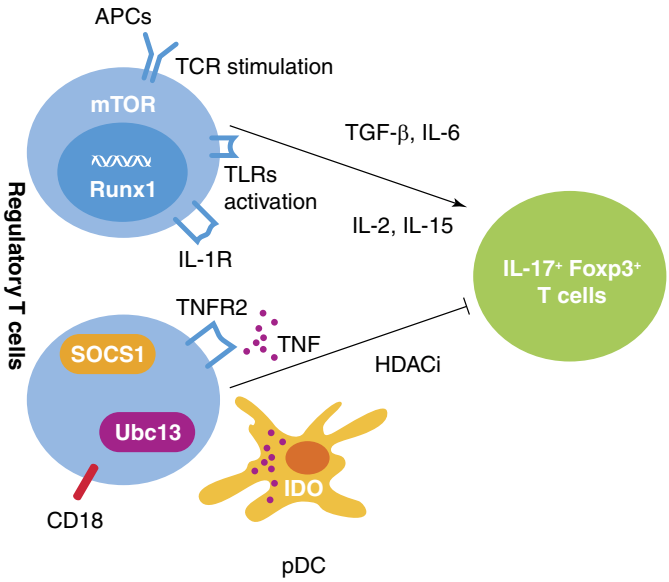

Figure 1. The differentiation of T_{regs} into IL-17$^+$Foxp3$^+$ T cells is closely regulated and controlled. Under TCR stimulation, cytokines TGF-β, IL-6, and IL-2/IL-15 promote T_{regs} to convert into IL-17$^+$Foxp3$^+$ T cells. The activation of TLRs, mTOR, and IL-1R also plays a promoting role. In contrast, IDO, expressed by induced pDCs, cytoplasm protein SOCS1, and Ubc13, could impede differentiation of T_{regs} into IL-17$^+$Foxp3$^+$ T cells by maintaining the stability and suppressive function of T_{regs} in response to inflammatory stimuli. Proinflammatory cytokine TNF also stabilizes the expression of Foxp3 and inhibits the expression of IL-17 in T_{regs} through the TNF-TNFR2 pathway.

model, Baban et al. [79] found that CpG-induced IDO served as an important molecular switch, determining the different destiny of T_{regs}. After high-dose CpG treatment following TLR9 ligation, pDCs were stimulated to express IDO, and at the same time, the production of IL-6, which was essential for the conversion of T_{regs} into IL-17$^+$Foxp3$^+$ T cells, was blocked [79]. In contrast, in the absence of IDO, CpG treatment triggers T_{regs} to acquire a proinflammatory, Th17-like phenotype by inducing IL-6 production [79]. Nearly meanwhile, Sharma et al. [80] showed that in the model of established mouse B16 melanoma, a combination of antitumor vaccination and an IDO-inhibitor drug demonstrated a dramatically synergistic antitumor effect, with increased IL-6 expression and in situ reprogramming of T_{regs} without the need for physical depletion [80].

Another guardian of T_{regs} was SOCS1, which maintained the stability and suppressive function of mice T_{regs}, and SOCS1-deficient T_{regs} were inclined to convert into Th1- or Th17-like cells [81]. In addition, Ubc13, which regulated expression of SOCS1 and IL-10 in T_{regs}, was essential for mice T_{reg} function in vitro and in vivo via a Ubc13-IκB kinase signaling axis, and Ubc13 could inhibit T_{regs} to acquire proinflammatory phenotypes resembling Th1 and Th17 cells [82]. Furthermore, it was addressed that HDACi inhibited T_{regs} to produce IL-17, demonstrating that the process was also regulated by epigenetic modification [25]. As leukocyte adhesion molecules regulating cell–cell contacts, CD18 heterodimeric molecules are essential for function of T_{regs} and deficiency of CD18-facilitated differentiation of T_{regs} into IL-17-producing cells [83]. Recently, in mice studies, TNF was found to stabilize Foxp3 expression of T_{regs} through the TNF-TNFR2 pathway, and TNF can reduce IL-17-producing cells in T_{regs} under TCR stimulation [84]. Up until now, the negative regulation of IL-17$^+$Foxp3$^+$ T cells was studied mainly in mice models, and more human researches are needed, as the conversion of T_{regs} into Th17-like cells may aggravate disease progression in autoimmune diseases.

DUAL FUNCTIONS OF IL-17$^+$FOXP3$^+$ T CELLS IN THE IMMUNE SYSTEM

Because of the partially overlapping immunophenotype and differentiation of IL-17$^+$Foxp3$^+$ T cells with T_{regs} and Th17 cells, their functions in the immune system may possess intersection as well. The immunosuppressive function of T_{regs}, previously termed suppressor T cells, has been studied deeply since early 1970s, exerting their function through maintaining self-immune tolerance and regulating immune responses. In contrast, Th17 cells play an indirect but magnifying role in inflammation response by secreting proinflammatory cytokines, participating in the development of a wide spectrum of diseases. Despite the diametrically opposite function of T_{regs} and Th17 cells, both of them orchestrate the immune system in harmony, and the maladjustment of them is closely related to the autoimmune diseases, pathogenic infections, transplant rejection reaction, and formation of cancer. Considering the already elucidated and complicated role of T_{regs} and Th17 cells in normal physiological homeostasis and disease state, what about the functions of IL-17$^+$Foxp3$^+$ T cells?

IL-17$^+$Foxp3$^+$ T cells retained immunosuppressive function

Similar to IL-17$^-$ T_{regs}, IL-17$^+$Foxp3$^+$ T cells are committed to immunosuppressive function. Recently, human studies showed that only CD4$^+$CD25^{hi}CD127^{lo}CD45RA$^-$ T_{regs}, expressing the NK cell marker CD161, can produce IL-17, which is not accompanied by loss of regulatory function [52]. Based on experiments in vitro, the regulatory ability of this unique cell group in human as a potent suppressor of T cell proliferation is still maintained [7, 38, 42], albeit the suppression ability is inversely proportional to the IL-17 production of DR$^-$$T_{regs}$ under strong TCR signals in the presence of APCs. Based on the suppression assay, IL-17$^+$Foxp3$^+$ T cells showed more effective inhibition ability than IL-17$^-$ T_{regs} [8]. However, on condition that the stimulation is strong enough to secrete adequate IL-17, the suppressive properties may be abrogated [42]. Moreover, TCR signals [42] and the presence of APCs [7, 25] are essential for the production of IL-17, as well as the loss of suppression, which may be in concert with the report that the T_{reg} function can be perturbed with strengthened TCR signal [85]. Expression of RORγt rather than IL-17 is a fundamental factor for the anti-proinflammatory properties of RORγt$^+$ T_{regs} [51]. What interests us is the reversibility of the dropped, suppressive function in IL-17$^+$Foxp3$^+$ clones when returning to a non-IL-17-producing state [42].

Regulatory mechanisms of IL-17$^+$Foxp3$^+$ T cells

Several mechanisms, by which classic IL-17$^-$ T_{regs} suppress functional effector cells, have been explored: (1) cell contact, mediated by membrane-bound molecular, (2) secretion of cytokines with regulatory function, such as IL-10, TGF-β, and IL-35, (3) consumption of IL-2, and (4) cytolysis of targeted cells. As for human IL-17$^+$Foxp3$^+$ T cells, Voo et al. [38] reported that TGF-β had nothing to do with the suppression function by adding a TGF-β inhibitor, whereas their transwell assay suggested that the suppression depended highly on cell–cell contact. Recently, a mice finding uncovered that IL-17$^+$Foxp3$^+$ T cells manifested the capacity of degranulation and cytolysis by expressing granzyme B and perforin, whose expression was regulated by the transcription factor Runx3, which could be detected at a significantly high level in IL-17$^+$Foxp3$^+$ T cells [8]. The report indicated that IL-17$^+$Foxp3$^+$ T cells may function as cytotoxic cells and directly kill effector cells in a manner similar to CD8$^+$ T cells. Yet, the mechanisms of how IL-17$^+$Foxp3$^+$ T cells exert their immunosuppressive function are to be elucidated further, although we can presumably draw a conclusion that IL-17$^+$Foxp3$^+$ T cells are functionally T_{regs}.

IL-17$^+$Foxp3$^+$ T cells acquire the effector ability

Different from IL-17$^-$ T_{regs}, IL-17$^+$Foxp3$^+$ T cells may be an atypical effector T cell population or proinflammatory T_{regs}, expressing not only proinflammatory cytokines IL-17 but also moderate levels of polyfunctional cytokines, such as IL-2, IFN-γ [7], TNF-α, and so on. The conversion of T_{regs} into IL-17-producing cells potentially aggravates an inflammation response, resulting in the deterioration of autoimmune diseases. In the human chronic colitis tissue, high levels of CD161 and CD49d (the VLA-4 α chain), expressed by IL-17$^+$Foxp3$^+$ T cells, were detected, mediating immune adhesion [7]. Furthermore, the expression of the Th17-associated chemokine receptors CCR6 and CCR4 [20, 42] confers human IL-17$^+$Foxp3$^+$ T cells with chemotactic capacities. Such modification of T_{reg} phenotype and function may offer more effective protection against pathogenic agents but at the same time, may lead to increased susceptibility to autoimmune responses.

THE RELATIONSHIP AMONG T_{regs}, TH17, AND IL-17$^+$FOXP3$^+$ T CELLS

Recent research made the relationship between T_{regs} and Th17 cells more complex. considering the plasticity between the two T cell subsets and the identification of IL-17$^+$Foxp3$^+$ T cells. It has clearly been shown that T_{regs} can inhibit the responses of Th1 and Th2; however, the susceptibility of Th17 cells to T_{reg}-mediated suppression is still controversial. Although T_{regs} were reported to inhibit Th17 cells, nevertheless, T_{regs} were also able to promote the differentiation of Th17 cells by providing TGF-β and inhibiting Th1 and Th2 activities. A study by Valmori et al. [86] documented that human Th17 cells are likely to originate from NnT_{regs} rather than from conventional T cells. In their subsequent study, IL-1R$^+$ central memory T_{regs}, generating IL-17 and coexpressing RORγt, were identified as an early intermediate stage in the differentiation from NnT_{regs} into Th17 cells controlled by the IL-1-IL-1R pathway [24]. What is interesting is that after repeated in vitro TCR stimulation and expansion, human Th17 cells can convert into Foxp3$^+$ T_{regs}, secreting moderate levels of IL-10 and TGF-β and acquire potent suppressive function [87]. These Th17-derived T_{regs} are more stable, as they are resistant to Th17 reconversion under Th17 differentiation cytokines, such as IL-1β, IL-6, and IL-23. The underlying mechanisms of mutual conversion between T_{regs} and Th17 cells remain unknown. Recently, it was reported that Th17 cells and T_{regs} can stimulate each other through TNF-TNFR2 pathways [88]. In mice models, Th17 cells promoted expansion of T_{regs} and stabilized Foxp3 expression of T_{regs} by producing high levels of TNF, and this stimulating effect was abolished when TNFR2 was deficient [88]. In addition, T_{regs}, deficient in TNFR2, also resulted in a much lower level of IL-17A production by Th17 cells [88].

All of the T_{regs}, Th17, and IL-17$^+$Foxp3$^+$ T cells are indispensable subsets of T cells, exerting crucial roles in the maintenance of immune homeostasis. Cytokine milieu, especially TGF-β, is the key player that connects the three populations together (**Fig. 2**). Upon TCR stimulation, naive T cells can be driven to express Foxp3 and become T_{regs} in the presence of TGF-β [68], whereas when TGF-β collaborates with IL-6, the

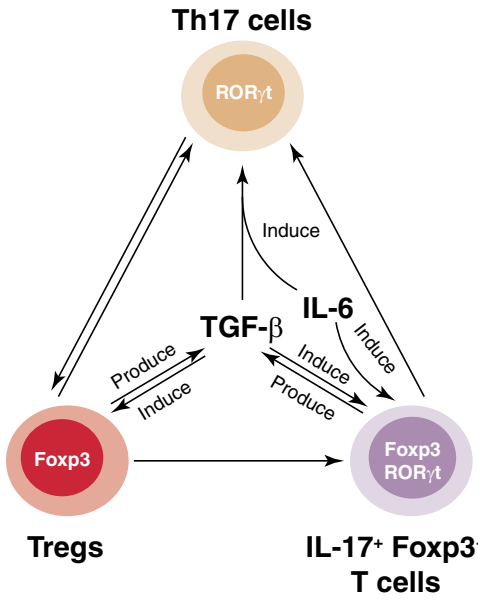

Figure 2. T_{regs}, Th17, and IL-17$^+$Foxp3$^+$ T cells interact with each other. IL-17$^+$Foxp3$^+$ T cells may be an intermediate differentiation stage between T_{regs} and Th17 cells. Under appropriate conditions, T_{regs} can convert into IL-17$^+$Foxp3$^+$ T cells, whereas T_{regs} and Th17 cells can finish their reciprocal transformation. Cytokine milieu, especially TGF-β and IL-6, connect the three populations together. Upon TCR stimulation, naive T cells can be driven to express Foxp3 and become T_{regs} in the presence of TGF-β. When TGF-β collaborates with IL-6, naive T cells develop into Th17 cells. TGF-β and IL-6 also participate in the differentiation of IL-17$^+$Foxp3$^+$ T cells. T_{regs} and IL-17$^+$Foxp3$^+$ T cells secrete TGF-β, in turn, acting on the differentiation of the three populations.

T_{reg} pathway is reserved, and instead, naive T cells develop into Th17 cells [64, 89]. Likewise, TGF-β is involved in the differentiation of IL-17$^+$Foxp3$^+$ T cells, even though the specific role remains controversial [6, 7, 25, 42]. Apart from the pivotal role of TGF-β on differentiation, TGF-β also served as a biological outcome of T_{regs} and IL-17$^+$Foxp3$^+$ T cells, in turn, promoting the differentiation of Th17 cells. Another cytokine correlating the three populations is IL-6, which promotes differentiation of Th17 cells and IL-17$^+$Foxp3$^+$ T cells. However, IL-6 plays a negative role in T_{reg} differentiation.

Accordingly, all of the T_{regs}, Th17, and IL-17$^+$Foxp3$^+$ T cells appear to depend on TGF-β for their differentiation and maintenance, and additional cytokines, such as IL-6, may determine whether they become T_{regs}, Th17, or dual-function effector cells. The aforementioned data suggest that the therapeutic methods targeting T_{regs} and Th17 cells may need more attention, considering their plasticity under certain stimulus. A better understanding of the relationship among T_{regs}, Th17 cells, and IL-17$^+$Foxp3$^+$ T cells may result in novel approaches for control of immune-related diseases.

IL-17$^+$FOXP3$^+$ T CELLS IN THE TUMOR MICROENVIRONMENT

Aberrant T cell homeostasis is a crucial event in tumor pathogenesis, and Th17/T_{reg} imbalance appears to be an important key player. Although accumulating data suggest an important role of T_{regs} and Th17 cells in tumor immunity, whether they exert the tumor-promoting effects or antitumor activity remains controversial. Recently, IL-17$^+$Foxp3$^+$ T cells, which had a close relationship with T_{regs} and Th17 cells, were identified in human cancer. IL-17$^+$Foxp3$^+$ T cells were cardinally identified in human CRC [7, 19, 20] and in human esophageal cancer [71] but not in other types of cancer, including ovarian cancer, melanoma, and renal cell carcinoma [7]. The reasons may be that chronic colitis plays an important role in the CRC-associated immune responses. A great quantity of IL-17$^+$Foxp3$^+$ T cells was detected exclusively in the mucosa of chronic colitis tissues and CRC tissues rather than in the adjacent tissues [7]. In peripheral blood of chronic colitis and CRC patients, finite IL-17$^+$Foxp3$^+$ T cells were observed, sharing the similar phenotype of those in the pathological colonic tissue [7].

The presence of IL-17$^+$Foxp3$^+$ T cells in the tumor microenvironment may have a close relationship with tumor progression. In patients with chronic inflammation and cancer of colon tissue, cells coexpressing IL-17 and Foxp3 are detected [20], with the capacity to enhance the expression of CRC-relevant markers (CD133, CD44s, CD166, epithelial cell adhesion molecule, and aldehyde dehydrogenase-1) in bone marrow-derived mononuclear cell, demonstrating that IL-17$^+$Foxp3$^+$ T cells can promote development of cancer-initiating cells. Moreover, IL-17$^+$Foxp3$^+$ T cells can increase significantly the phosphorylated Akt and p38 of cancer-initiation cells, exerting an important role in cell proliferation and differentiation and participating in tumorigenesis [20]. The cellular, molecular, and genetic relationship between inflammation and cancer [90–92] may provide persuasive evidence that the special "inflammatory" T_{regs} may be involved in the development of tumor as well as tumor-associated immune response [7, 20].

Yang et al. [20] reported that in CRC tissue, hypoxia stimulated IL-17 production in Foxp3$^+$ T_{regs}, which are recruited by CRC-derived CD68$^+$ cells, expressing high levels of CXCL11. IL-17$^+$Foxp3$^+$ T cells derived from human CRC express CCR6, TGF-β, and IL-6, and these cells could suppress tumor-responsive CD8$^+$ T cells, which had the potential to fight against tumors in the presence of tumor-associated self-antigen [19]. When pretreated with anti-IL-17 antibody, the suppression of IL-17$^+$Foxp3$^+$ T cells to CD8$^+$ T cells was weakened [19]. In a polyposis-prone mice model, inhibition of RORγt stabilized the function of T_{regs} and probably improved an antitumor response rather than impair antitumor immunity. In contrast, IL-17A-deficient mice developed invasive cancer [51]. Lack of effective research of IL-17$^+$Foxp3$^+$ T cells in vivo restricted its use in clinics, and far more exploration is needed.

CLINICAL IMPLICATIONS IN CANCER THERAPY

T_{regs} are often found at high frequencies in the peripheral blood and tumors of human patients compared with those in healthy donors and normal mucosa or tissue [93], and in many cancers, a high density of T_{regs} correlates with poor disease outcomes [94]. In adoptive cellular immunotherapy in cancer, the functions of immune cells are always inhibited. One of the reasons is T_{reg}-mediated immune tolerance in the tumor microenvironment. In-depth knowledge of IL-17$^+$Foxp3$^+$ T cells may offer new insight into cell therapy toward cancer.

On the one hand, IL-17$^+$Foxp3$^+$ T cells express the effector cytokine IL-17, which contributes to the antimicrobial innate immune defense. The suppressive capacity of T_{regs} partly lost, and T_{regs} obtained effector abilities at the same time. Hence, it may be a promising strategy to reprogram T_{regs} in situ into Th17-like cells, overcoming the immunosuppressive function of T_{regs} and shifting from pathogenic to protective anti-tumor immunity in cancer. In mice models, IDO-inhibitor drug converted T_{regs} to IL-17-producing cells in vivo and enhanced functional antitumor response [80]. In another mice model, CpG treatment directly reprogrammed mice splenic Foxp3$^+$ T_{regs} to express IL-17 in the absence of IDO [79]. Reprogramming the function of T_{regs} without the need for physical depletion of T_{regs} by using anti-CD25 mAb may be feasible in cancer therapy. On the other hand, in inflammation-associated human cancers, such as colon cancer, IL-17$^+$Foxp3$^+$ T cells retained suppressive function of T_{regs} and secreted immunosuppressive molecule TGF-β, controlling excessive inflammation and probably delaying disease progression.

Despite of the great hope, questions still exist because of the dual functions of the new subset, and whether the "Th17 conversion" is beneficial may depend on many factors, including the inflammation reaction in the tumor microenvironment. As T_{regs} will soon be used in clinical trials as an immunotherapy, it is essential that we understand the potential of T_{regs} to convert into IL-17-producing cells and take advantage of this conversion.

CONCLUDING REMARKS

In summary, a number of studies have identified IL-17$^+$Foxp3$^+$ T cells as a unique subset of human T cells that derived from T_{regs} under proinflammatory cytokine stimulation using in vivo and in vitro models. Th17 cells protect against extracellular pathogens, whereas T_{regs} keep the effector population in check and prevent Th17 cell-medicated destruction. However, the excessive activation of T_{regs} may inhibit anti-tumor or anti-pathogenic immunity and consequently, facilitate tumor development and chronic infection. The conversion of T_{regs} to IL-17$^+$Foxp3$^+$ T cells may play a role in regulating the balance between T_{regs} and Th17 cells. In humans, IL-17$^+$Foxp3$^+$ T cells have been identified primarily in inflammatory bowel disease [70] and in colon tumors and other diseases, such as periodontitis [95], psoriasis [96], and rheumatoid arthritis [74]. However, many questions remain to be resolved further in the research field for IL-17$^+$Foxp3$^+$ T cells. Previous studies have been performed on the plasticity of T_{regs} and Th17 cells and have shown that they may not be at the ultimate differentiation stage. Thus, we should demonstrate the specific existence state of IL-17$^+$Foxp3$^+$ T cells. Then, it should be studied whether the differentiation happens in other subclass of T_{regs}, such as CD8$^+$ T_{regs} and CD4$^-$CD8$^-$ T_{regs}. Regarding IL-17$^+$Foxp3$^+$ T cells, which continuously exist in a physical or pathological state, in spite of their small amount, more researches are required to study their transcription program at a molecular level. For example, transcription factor IRF4 is involved in the development and function of Th17 cells and T_{regs} [97, 98]. We can explore whether IRF4 participates in the differentiation of IL-17$^+$Foxp3$^+$ T cells and how IRF4 regulates the process. Furthermore, it was reported that HIF-1 interacted with Foxp3 and RORγt, and HIF-1α-deficient mice developed down-regulation of Th17 responses and up-regulation of T_{reg} numbers [99]. Accordingly, we make the hypothesis that HIF-1 may be associated with the inhibition of a T_{reg}-suppressive function. Finally, considering the potentially protective role or deteriorating role of IL-17$^+$Foxp3$^+$ T cells in distinct pathologic states, it will be most critical to determine whether IL-17$^+$Foxp3$^+$ T cells can aggravate the immune responses in autoimmune diseases or can break immune tolerance in tumor immunity from the theoretical and therapeutic aspects. A more profound understanding of IL-17$^+$Foxp3$^+$ T cells will shed new light on strategies to modulate T_{reg} functions for human immunity-associated diseases.

AUTHORSHIP

H.L. designed the review. R.D. wrote the manuscript. H.Z. and F.Y. participated in the modification of grammar.

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DISCLOSURES

The authors declare no conflict of interest.

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