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FOXP3 and RORγt: Transcriptional regulation of Treg and Th17

Zuojia Chen, Fang Lin, Yayi Gao, Zhiyuan Li, Jing Zhang, Yue Xing, Zihou Deng, Zhengju Yao, Andy Tsun, Bin Li *

Key Laboratory of Molecular Virology and Immunology, Institut Pasteur of Shanghai, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200025, P.R. China

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ABSTRACT

FOXP3$^+$CD4$^+$CD25$^+$ Regulatory T (Treg) cells and IL-17 producing helper T cells (Th17) are critical subsets of T cells which play essential roles in immune homeostasis. The Forkhead family transcription factor FOXP3 is predominantly expressed in Treg cells, where the FOXP3 ensemble is essential for Treg cell development and function. As FOXP3 is to Treg cells, the orphan retinoic acid nuclear receptor (ROR) family transcription factor RORγt is essential for Th17 development and function. In this review, we summarize recent progress of our understanding towards the molecular mechanisms underlying the differentiation and function of FOXP3$^+$ Treg cells and RORγt expressing Th17 cells. These may provide new insights into therapeutic intervention and targeting of human immune-deficient diseases.

1. Introduction

Antigen-activated naive CD4$^+$ T cells may differentiate into various lineages of effector T cells including T helper 1 (Th1), T helper 2 (Th2), T follicular helper (Tfh), T helper 9 (Th9), T helper 17 (Th17) cells as well as multiple subsets of regulatory T cells including natural FOXP3$^+$CD4$^+$CD25$^+$ regulatory T cells (nTreg), induced FOXP3$^+$ or FOXP3$^-$ regulatory T cells (iTreg)—including T regulatory type 1 cells (Tr1) and T helper type 3 cells (Th3). In the complex immune system, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) expressing Th1 cells are responsible for immune responses against intracellular pathogens, whereas interleukin (IL)-4 and IL-13 expressing Th2 cells mediate humoral immunity and are important to protect hosts against allergic responses and extracellular parasites. Tfh cells locate to B-cell follicles and may help B-cells differentiate into antibody-producing plasma cells, while IL-17 producing Th17 cells are involved in various autoimmune diseases and mucosal immunity. nTreg and iTreg cells differentiate in the thymus and periphery, respectively, and play an important role in suppressive control of both innate and adaptive immunity *in vivo*.

Genetic mutations of the forkhead box transcription factor *FOXP3* leads to lethal X-linked syndrome "Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-Linked Syndrome" (IPEX) in human, and the scurfy (sf) phenotype in mice, which is usually accompanied by multiple immunopathologies including type I diabetes, inflammatory bowel disease as well as lymphadenopathy, splenomegaly, hepatomegaly and massive lymphocytic infiltrates. Experimental evidence shows that Treg cells function as immune suppressors in regulating other effector T cells via multiple non-overlapping molecular mechanisms.

The Th17 lineage is a recently identified subset, which express high levels of IL-17 under the transcriptional regulation of RORγt. Th17 cells participate in the progression of many autoimmune diseases through the secretion of multiple inflammatory cytokines including IL-17A, IL-17F, IL-21, and IL-22. Th17 cells can mediate inflammatory responses and functionally oppose Treg cells. The differentiation of Th17 and iTreg from naive T cells depends on the level of transforming growth factor-β (TGF-β) expression (Fig. 1). A low level of TGF-β, IL23 or IL6 induces naive T cells to develop into Th17 cells [1]. High levels of TGF-β in the tissue microenvironment may lead to the development of iTreg [2]. Loss of the balance between Th17 and Treg cells in favor of the former will break immune homeostasis in the host and lead to the development of autoimmune diseases (Table 1). Moreover, FOXP3$^+$ Treg cells are not terminally differentiated cells, and can also redifferentiate into a variety of Teff cells including Th17 cells [1], Tfh cells [3], as well as autoaggressive and pathogenic memory T cells *in vivo* [4] after losing FOXP3 expression under certain inflammatory conditions.

2. Regulatory T cells in health and disease

FOXP3$^+$CD25$^+$CD4$^+$ Treg cells have the ability to suppress the proliferation and function of other immune and non-immune cells including Teff cells, B cells, macrophages, dendritic cells (DCs) and osteoblasts. So far, at least two types of FOXP3$^+$ Treg cells, including nTreg and iTreg have been identified. Thymic-derived nTreg cells are constant expressers of FOXP3, and comprise nearly 10% of total peripheral CD4$^+$ T cells in mice and humans. nTreg cells function as

* Corresponding author. 411 Hefei Road, Institut Pasteur of Shanghai, Chinese Academy of Sciences, Shanghai, 200025, P.R. China.
E-mail address: binli@sibs.ac.cn (B. Li).

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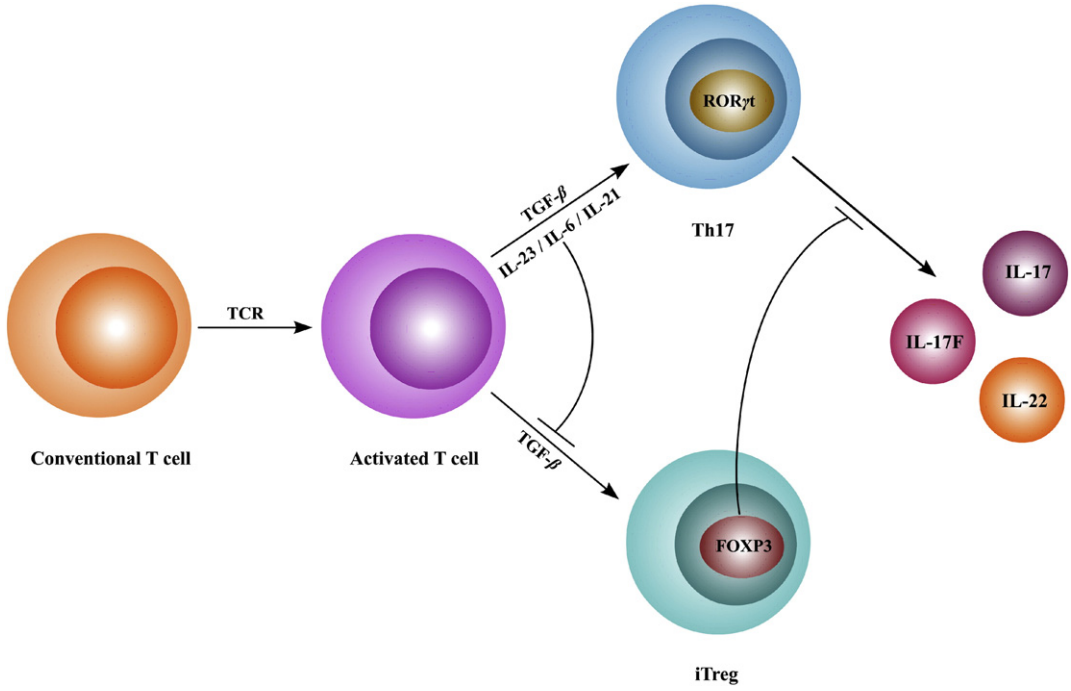

Fig. 1. TGF-β mediated regulation of the differentiation and function of Th17 and iTreg cells. TGF-β orchestrates Th17 and Treg cell differentiation in a content and concentration dependent manner. Low concentration of TGF-β stimulation in cooperation with IL-6 and IL-21 favor the differentiation of Th17, whereas it is favorable towards the differentiation of Treg cells under high concentration of TGF-β that represses IL-23R expression. FOXP3 may repress RORγt-mediated transcriptional induction of IL-17, IL-17F and IL-21. However, a recent paper describes the pathogenic potential of T-bet^{+}RORγt^{+} Th17 cells which do not require TGF-β for their development {Ghoreschi, 2010 #94}, highlighting further complexity to an already complicated and fascinating system.

immune suppressors and express high levels of FOXP3, which play a crucial role in the maintenance of self-tolerance and immune homeostasis.

FOXP3 expressing iTreg are induced in the periphery by immune suppressive cytokines such as TGF-β after TcR stimulation [5]. Other iTregs include Tr1 [6] and Th3 cells [7]. Tr1 cells often locate in the intestinal mucosa and presently no specific surface marker for Tr1 cells has been identified; Foxp3 gene expression cannot be constitutively induced, and they highly express immune suppressive cytokines including interleukin-10 (IL-10) and TGF-β, showing that Tr1 cells are a subset of Treg rather dissimilar from nTreg. Th3 cells are induced primarily from naive CD^{4+} T cells and have regulatory function in oral tolerance [7]. Th3 cells highly express TGF-β, and may also express Foxp3, so they cannot be identified as a distinct subpopulation of iTreg or as an activated nTreg cells. Both nTreg and iTreg cells have the ability to suppress the function of Teff cells and in maintaining host immune homeostasis. Recently, a CD8^{+} regulatory T cell lineage was reported to be essential for self-tolerance. These CD8^{+} Treg cells target Qa-1^{+} follicular T helper cells and suppress lethal systemic-lupus-erythematosus-like autoimmune disease [8].

Multiple processes take charge in complex immune systems to maintain the balance between self-reactive T cells and regulatory T cells. Dysregulation of auto-reactive T cells without the control of Treg cells will lead to the development of autoimmune diseases. In order to exploit new therapeutic methods in treating autoimmune diseases, it is important to enhance suppressive function or promote Treg proliferation *in vivo* after activation through antigen stimulation, or signal pathways regulated by immunomodulating cytokines, small molecular inhibitors or therapeutic antibodies.

Table 1
Comparison of Treg and Th17 cells in autoimmune diseases.

	Treg cells	Th17 cells
Key transcription factor	FOXP3	RORγt
Cytokines involved	IL-10, TGF-β	IL-17, IL-23
Function in disease	Inhibition and suppression	Induction and propagation
Outcome	Self-tolerance	Tissue inflammation
Application in therapy	Activation by antigen stimulation	Intervention by neutralization antibody

Allergen-specific immunotherapy (SIT) which consists of repeated subcutaneous or sublingual administration of allergen has been used for almost a century and has been found to manage allergic responses effectively [9]. The SIT approach may involve both activation and expansion of nTreg cells and iTreg cells, such as Tr1 cells [10]. Therefore, administered antigen through the oral or sublingual routes with low dose auto-antigen peptides or modified peptides together with the inhibitor of the activation of effector T cells may suppress autoimmune diseases by inducing antigen-specific tolerance [11].

Multiple cell surface receptor signaling pathways on T cells have been extensively studied for their ability to control immune activation of Treg cells to downregulate the activation and function of effector T cells. Anti-CD3 mAb therapy has been employed to treat type 1 diabetes in non-obese diabetic (NOD) mice [12], as well as a mouse model of autoimmune encephalomyelitis (EAE) [13]. Lee and colleagues [14] found that mice treated with agonistic anti-GITR monoclonal antibody (mAb) could not only neutralize the suppressive effect of Treg cells, but also augment the activation, proliferation and cytokine production of effector T cells. According to aforementioned results, methods to inactivate the GITR pathway may provide therapeutic potential for the treatment of chronic inflammatory bowel disease. Furthermore, different levels of vitamin A was found to be able to induce Treg cells and dampen intestinal inflammation in SAMP1/YP mice, an *in vivo* model of Crohn's disease [15].

Besides the use of small molecules or peptides, the pathologic and physiologic mechanism of autoimmune diseases, such as diabetes, has been extensively studied in order to find potential therapeutic pathways. Winer et al. [16] found that CD4^{+}Foxp3^{+} cells primarily regulate insulin resistance and glucose tolerance that are responsible for the progression of type 2 diabetes. Mathis and her colleagues [17]

saw that the percentage of Treg cells in abdominal adipose tissue in mouse models of obesity is less than that in abdominal adipose tissue of the lean mouse. Conventional T cells in obese tissue prefer to differentiate into Th1 cells, while Treg cells developed a down-regulated suppressive ability towards the proliferation of macrophages and adipocytes. Bluestone and his colleagues [4] found a cluster of iTreg cells, in which the expression of FoxP3 is instable, can be induced to pathogenic memory T cells and lead to the onset of diabetes. This study can particularly explain *in vivo* mechanisms triggering autoimmune diseases and implicate potential therapeutical methods for intervention [4].

Several scientific obstacles need to be solved before we can use Treg cells to treat autoimmune disease in the clinic. Zingg et al. [18] described that the best schedule for administration of Treg cells in treatment of autoimmune diseases or organ transplantation is before disease onset or organ transplantation, based on the spatial and temporal modulated mechanisms of Treg cell. It is necessary to identify the molecular basis of Treg activation, proliferation and differentiation as well as an effective method of separating purified Treg cells, which could then be used as a new approach to treating autoimmune diseases.

3. Th17 cells in health and disease

The Th17 subset exhibits effector T cell function distinct from Th1 and Th2 cells and was recently identified as an important mediator of autoimmune disorders [19,20]. Th17 cells were characterized by them producing the cytokine IL-17. The IL-17 cytokine family contains six members, which include IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (i.e. IL-25) and IL-17F. IL-17 cytokines promote tissue inflammation via the induction of other pro-inflammatory cytokines and chemokines [21]. Increased IL-17 levels have been detected in multiple autoimmune models in animals, as well as in human patients with various autoimmune disease syndromes, such as systemic lupus erythematosus, multiple sclerosis, inflammatory colitis, rheumatoid arthritis and psoriasis. In rheumatoid arthritis, Th17 cells may induce matrix metalloproteases and stimulate osteoclasts, which leads to the development of cartilage and bone destruction. IL-17 deficient mice or mice treated with an IL-17 receptor antagonist are resistant to the development of arthritis. Similarly, IL-17 deficient mice display delayed onset and reduced severity of experimental autoimmune encephalomyelitis (EAE) [22]. Evidence also showed that Th17 cells were involved in the pathogenesis of colitis and are capable of inducing EAE when adoptively transferred into naive wild-type mice. In chronic inflammatory bowel disease, Th17 cells seem to be essential for inducing the breakdown of intestinal epithelial barriers. Once induced, Th17 cells drive immunopathology and tissue inflammation *in vivo* [23]. These data support the notion that IL-17 producing Th17 cells play an important role in the induction and propagation of autoimmune diseases.

The Th17 subset was identified during previous studies on autoimmune disease. More than 20 years ago, the Th1/Th2 paradigm, introduced by Mossman and Coffman, was applied to explain the differentiation and function of T helper cells [24]. Th1 and Th2 cells could develop mutually and exclusively. Th1 cells are mainly in charge of the clearance of intracellular pathogens; while Th2 cells are key regulators in helping B cells control parasite infection. However, this paradigm was challenged and expanded during more recent studies on autoimmune diseases. Th1 cells are highly proinflammatory and were thought to be involved in organ-specific autoimmune diseases. The induction of IFN-γ, one of the major effector T cell cytokines produced by Th1 cells in target tissues, is correlated with clinical signs in EAE and collagen-induced arthritis (CIA). Interestingly, the severity of EAE is reduced after IFN-γ treatment in animal models. Moreover, mice deficient in IFN-γ signaling, IFN-γ or IFN-γ receptor do not develop any resistance to autoimmunity. On the contrary, IFN-γ or IFN-γ receptor deficient mice are more susceptible to autoimmunity. Nearly one decade ago, the discovery of cytokine IL-23 helped to shed light on this paradox. As a member of the IL-12 cytokine family, IL-23 is a heterodimer composed of a unique p19 subunit and a shared p40 subunit with IL-12. In the study of IL-23p19 deficient mice, similar levels of IFN-γ were detected but the expression level of IL-17 was dramatically decreased. IL-23p19 deficient mice were found to be resistant to the induction and development of EAE and CIA [25]. Thus, in contrast to IL-12, IL-23 does not promote the development of IFN-γ expressing Th1 cells, but is required for the expansion of a distinct IL-17 producing T cell, which was subsequently identified as Th17 cells [26].

The IL-23/Th17 axis has a strong impact on the pathogenicity of autoimmune diseases. IL-23 was shown to promote the production of IL-17 by activated T cells and IL-23 expanded T cells are able to transfer EAE and CIA [27]. Th17 cells cannot maintain their immune features without IL-23 *in vivo* and *in vitro*. IL-23 may not be involved in the initial steps of driving the differentiation of naive T cells into Th17 cells [28]. Similar to IL-12 for Th1 cells, IL-23 plays a fundamental role in stabilizing the Th17 lineage and expanding Th17 responses. IL-23 also induces proinflammatory effector cytokines in Th17 cells. Therapeutic neutralization of IL-23 can prevent EAE relapses and decrease the expression level of IL-17 in the central nervous system [29]. Thus, IL-23 is essential for full differentiation and exhibiting of effector function by Th17 cells.

Th17 cells are now identified as potent inducers of autoimmunity, while Th1 cells have been well studied in induction and development of organ-specific autoimmune diseases. Th1 cells commonly present along with Th17 cells in inflamed tissues, but the interaction between Th1 and Th17 cells during autoimmune disease is still unclear. Recent work indicated that Th17 cells might cross-regulate Th1 development in intestinal inflammation, in which IL-17 cytokine could mediate a protective effect [30]. These researchers demonstrate that IL-17 could suppress the expression of T-bet, osteopontin as well as the IL-12β2 receptor to inhibit the development of Th1 cells. The mechanism underlying how IL-17 and Th17 cells cross-regulate other T cell subsets remains unclear.

4. Transcriptional regulation of Treg development and function

The subpopulations of Treg cells are heterogeneous and plastic in the peripheral immune system, and may change their immunosuppressive abilities under certain physiological conditions. A significant number of $CD4^+FOXP3^+$ Treg cells in human peripheral blood and lymphoid tissue which express CCR6 (chemokine (C–C motif) receptor 6) can develop into Th17 cells with the capacity to produce IL-17 on mucosal sites during inflammation [31]. Bluestone and colleagues [4] also found that a subset of Treg cells that lost Foxp3 expression and suppression function, named as: “exTreg”, could produce IL2 and IFN-γ instead of CD25, GITR, and CTLA-4; consequently, these “exTreg” cells act as effector T cells rather than the conventional suppressor.

As aforementioned, the crucial function of Foxp3 in scurfy mutant mice and human IPEX patients, and the mutations of the Foxp3 gene lead to the deficiency or malfunction of natural Treg cells—and, consequently, the development of a similar autoimmune and/or inflammatory disorder in mice and humans. Foxp3 has been defined as a determinant role of functional differentiation and/or maintenance of nTreg cells.

FOXP3 harbors four main subdomains, including an N-terminal proline-rich region for co-repressor interaction, a zinc finger domain with unknown function, a leucine zipper domain for homo-/hetero-oligomerization and a C-terminal forkhead domain which is essential for DNA binding and nuclear subcellular localization. Recent studies have shown that the transcription of up to seven hundreds genes were controlled by Foxp3 directly or indirectly in mice and most of the

underlying mechanisms of how FOXP3 mediated transcriptional regulation on site-specific promoters are still unclear [32–34]. The Foxp3 protein itself can form a homo-/or hetero-oligomer with other FOXP subfamily members such as FOXP1 [35], and interact with other transcription factors including NFAT [36], AML1/Runx1 [37], IRF4 [38], phosphorylated Stat3 [39], phosphorylated AP-1 [40], Eos [41], NF-κB [42] and transcriptional corepressors such as the HAT/ HDAC complex [43], to orchestrate certain cellular and molecular programs involved in Treg function.

The forkhead domain of FOXP3 is critical for both DNA binding and for its nuclear localization. NFAT interacts with Foxp3 on its forkhead domain [36]. Upon T cell activation, FOXP3 forms a complex with NFAT/AP-1 and induce certain inflammatory factors expressed in conventional T cells by binding to the promoter regions of FOXP3 target genes, thus contributing to the activation of T cells and their differentiation into effector T cells. Once interacted with NFAT, FOXP3 can repress the expression of IL-2 and confer suppressive activity in normal T cells. Although four known types of NFAT may function redundantly, mice deficient in both NFATc2 and NFATc3 disrupt the function of FOXP3 and consequently impair the function of Treg cells. Transcription factor AML1/Runx1, which can bind to FOXP3 at the linker region between the leucine zipper and Forkhead domains, is crucial for normal haematopoiesis including thymic T-cell development. AML1/Runx1 binds to the promoter region of IL-2 which may enhance IL-2 expression [37]. FOXP3 can reverse the function of AML1/Runx1 on IL-2 expression through the direct interaction between FOXP3 and AML1/Runx1, and consequently repress the expression of IL-2 [37]. AML1/Runx1 also participates in modulating the functions of FOXP3 and RORγt in T cells and maintains immune system homeostasis. On one hand, AML1/Runx1 can work as a transcriptional coactivator via the binding to RORγt and associate to the enhancer and promoter regions of the *Il17* gene, to then upregulate the transcription of *Il17*. On the other hand, AML1/Runx1 can also act as a corepressor to repress the transcription of *Il17* in the presence of FOXP3 [44]. Moreover, the exon2 encoding region of FOXP3 protein binds to RORγt and RORα, which may repress RORγt and RORα mediated induction of IL-17 production and thereby inhibit Th17 differentiation. Furthermore, FOXP3 can be acetylated by its N-terminal proline-rich domain mediated interaction with the histone acetyltransferase, TIP60, which may increase its stability and enhance its binding to the IL-2 promoter and subsequently strengthen the repression of IL-2 [45].

In addition to its protein regulation, the *FOXP3* gene cluster also plays an important role in the function of Treg cells. Zheng et al. [46] described three conserved non-coding DNA sequence (CNS) elements at the *Foxp3* locus critical in the determination of Treg cell fate in mice. CNS1-3 encodes the information about the size, composition and stability of the Treg population with their specific response element. Several small non-coding RNA known as microRNAs (miRNAs) serve as critical regulators of Treg cell homeostasis and function [47]. miR-155, a well-known onco-miR (cancer-associated micro-RNA), is highly expressed in Treg cells. miR-155 expression is dependent on FOXP3 and increases the responsiveness of Treg cells to IL-2 signaling, thereby maintaining Treg cell survival. However, miR-155 is dispensable for Treg suppressor function [48]. Recently, another miRNA, miR-146a, was found highly expressed in Treg cells and is necessary for the ability of Treg to inhibit the Th1 response. miR-146a mediates the downregulation of Stat1, a key transcription factor in Th1 cells, and restrains IFN-γ-mediated pathogenic Th1 responses and associated inflammation [49].

The function and stability of FOXP3 may be regulated by various positive and negative regulators of FOXP3 interacting partners *in vivo*. Comprehensive characterization of FOXP3 interacting proteins will provide molecular insight into FOXP3$^+$ Treg function under the microenvironment of immunosuppression or inflammation. In addition to FOXP3, another transcription factor, Hopx homeodomain-only protein, was recently reported to be required for iTreg cells, but not nTreg cells. Although Hopx has no effect on FOXP3 expression, it serves a critical role in the function of iTreg cells generated by DCs, by promoting DC-mediated T cell unresponsiveness *in vivo*. In Treg cells induced by DCs, Hopx downregulated proliferation-promoting genes with antigen rechallenge. Hopx is necessary to maintain suppressive function by iTreg cells during acute immunogenic responses. Whether Hopx is affected by FOXP3 and how Hopx is regulated requires further study [50].

5. Transcriptional regulation of Th17 cells development and function

As an independent subset of T helper cells, Th17 cells differentiate distinctively from Th1 or Th2 cells. The steroid receptor-type nuclear receptor RORγt, a splice variant of ROR, has been recently found to be an essential factor for the differentiation of Th17 cells. The comparison of gene expression profiles of Th17 and Th1 cells indicate a high expression of Rorc gene, which encodes RORγt in Th17 cells, while Th1 cells highly express transcription factor T-bet. Retroviral vector mediated transduction of RORγt into naive T cells promoted the development of Th17 cells. Moreover, using RORγt linked expression with GFP in mice revealed that cells expressing GFP were those expressing IL-17, suggesting a clear association between RORγt and IL-17 expression [21,51]. TGF-β and IL-6 together can induce the expression of RORγt, which can promote Th17 development and suppress Th1 and Th2 differentiation. Accordingly, in RORγt deficient mice, naive T cells were unable to differentiate into Th17 cells and showed no response to IL-23 induction. Such mice were also resistant to EAE. These findings demonstrate that RORγt is required for Th17 cells and sufficient to induce the differentiation of Th17 cells. The target genes of RORγt have not been fully characterized and the mechanism of IL-17 production by RORγt remains unknown.

Another member of ROR family, RORα was shown upregulated during Th17 cell differentiation *in vitro*. RORα seemed to be sufficient to induce Th17 cells, but differentiation of these Th17 cells was not affected too noticeably when lacking RORα [52]. The similar function of RORα suggests that RORα may act synergistically with RORγt, or as a compensation factor in Th17 cells. Th17 cell differentiation may be blocked by the lack of both RORα and RORγt. The induction of RORγt is dependent on STAT3. RORγt expression together with STAT3 leads to higher production of IL-17. A study using chromatin immunoprecipitation showed that STAT3 could bind to the *IL17a* promoter directly, which suggests that RORγt and STAT3 may collaboratively regulate the transcriptional profile of Th17 cells [53]. The function of interferon regulatory factor 4 (IRF4) is also associated with Th17 cell differentiation. IRF4 deficient mice are resistant to EAE and IRF4$^{-/-}$ T cells fail to upregulate RORγt expression and differentiate into Th17 cells. Overexpression of RORγt in such T cells could not restore IL-17 production [54]. RORγt was also shown to interact with transcription factor RUNX1. Binding of the RORγt-RUNX1 complex to the *IL17a* promoter leads to increased IL-17 production [37]. Aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor. It has also been reported that AHR is involved in the differentiation of Th17 cells [55]. The identification of many binding and functional partners of RORγt details the complexity in the transcriptional regulation of Th17 cells.

FOXP3 may act as a negative transcription regulator of Th17 cells *in vivo*. For instance, FOXP3 is able to suppress Th17 cell differentiation through the antagonism of RORγt activity [56]. The interaction between RORγt and FOXP3 has been confirmed by several groups, but it remains unclear if it is direct or in the context of a larger complex [57]. Furthermore, FOXP3 can interact and inhibit RUNX1. RUNX1 may participate in mediating the activation of proinflammatory cytokine signals [37,44]. IRF4-deficient T cells also showed increased Foxp3 expression [54]. Moreover, different AHR ligands have opposite effects

on Th17 and Treg cell differentiation, suggesting the role of AHR in controlling the balance between Th17 and Treg cells [55].

In Th17 cells, a complex transcription regulatory network may exist. RORγt plays a center role by interacting with other important partners on site-specific chromatin, either positively or negatively, to coordinate the development and function of Th17 cells. It is important to disclose the molecular mechanism and regulatory factors in the reciprocal interaction between Foxp3 and RORγt.

6. Plasticity of Treg and Th17 cell lineage differentiation

Epigenetic regulation plays an essential role in eukaryotic gene regulation and epigenetic abnormalities may led to severe autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), multiple sclerosis (MS) and Type 1 diabetes (T1DM) [58]. The differentiation of Treg and Th17 cells are also accompanied by epigenetic events, including the change in chromatin structure, histone and DNA modifications [59,60]. We previously reported that FOXP3 may function in two distinct complexes: one is a lower molecular weight complex and the other a higher molecular weight complex [45]. In the lower molecular weight complex FOXP3 recruits transcriptional factors and regulators such as FOXP1, NFATc2, TIP60, HDAC7 and HDAC9; and in the higher molecular weight complex FOXP3 associates with many chromatin remodeling factors including BRG1, Ku70/Ku80 and MBD3. In general, histone acetylation promotes permissive remodeling by decreasing net basic charge, leading to the induction of gene expression, whereas histone deacetylation functions in the opposite manner [61]. Consistently, TCR stimulation promotes FOXP3 complex binding to the IL-2 and IFN-γ gene locus and induced deacetylation of histone H3 and gene repression, whereas binding of the FOXP3 complex to the GITR, CD25, and CTLA-4 genes results in histone acetylation and their expression [62]. Murine studies identified that HDACi administration *in vivo* leads to increased expression of CTLA-4, GITR, and IL-10 to promote thymic Treg production and their functions [62]. These data implicate that the FOXP3 complex can direct chromatin remodeling and regulate gene transcription through the interaction between FOXP3, HATs, HDACs and some other transcriptional regulatory factors.

Recently there has been support that the difference in stability of FOXP3 expression between nTreg cells and iTreg cells is due to epigenetic difference at the *FOXP3* locus [63]. The complete demethylation of CpG motifs at the intronic region of *FOXP3* promoter has been confirmed in nTregs, but not completely in murine and human iTregs [64,65]. It has also been found that the inhibition of DNA methylation may stabilize Foxp3 expression [66]. Furthermore, *in vivo*-derived antigen-specific Treg cells have stable FOXP3 expression and demethylated status of their CpG motifs. Thus, the stability of FOXP3 expression must be sustained by epigenetic modification.

The plasticity of $CD4^+$ T Cell lineage differentiation has been comprehensively reviewed recently and actively debated [1]. By using inducible labeling to track Treg cell fate, Rubtsov et al. [67] indentified a remarkably stable Treg cell lineage under an inflammatory physiologic environment. Self- renewal of mature Treg cells may be a major mechanism for the maintenance of this cell lineage in mice. S1P1, a receptor of sphingosine 1-phosphate (S1P), negatively controls the development and activity of Treg cells, dependent on the downstream pathway of Akt and mTOR [68]. S1P1 activates the mTOR pathway and represses Smad3 and TGF-β receptor function to promote Th1 development, thus inhibiting Treg cell differentiation. The S1P1-mTOR axis and TGF-β-Smad3 signaling regulate the reciprocal differentiation of Th1 and Treg cells [69]. Dong and his colleagues [70] found that the molecular antagonism and plasticity of Treg and Th17 cell programs coexist, and are also associated with epigenetic modifications. With ChIP-chip or ChIP-Seq technology, it has been revealed that DNA methylation and histone modification regulates gene expression in $CD4^+$ T cell subsets. Previous genome-wide maps of H3K4me3 and H3K27me3 has confirmed that many transcriptional factors of T cells are regulated by epigenetic modification. The epigenetic mark of H3K27me3 was found at the *IL17a* locus, whereas H3K4me3 was found at the *Rorc* locus in iTreg cells [2,71]. The finding of IL-17 expressing Foxp3+ nTreg cells further indicate that epigenetic modifications may affect Treg and Th17 differentiation. In contrast, iTreg cells readily lose Foxp3 expression and acquire IL-17 expression, which is also connected to differential histone trimethylation. In summary, epigenetic modifications of cytokine genes and key transcriptional regulator genes direct the T helper cell differentiation program. The plasticity and unstable phenotypes of Tregs and Th17 cells will have important biological implications for designing therapeutic regimens to combat infection and control autoimmunity.

7. Conclusions

Th17 and Treg cells are both indispensable subsets of T cells, and have crucial roles in the maintenance of immune homeostasis. FOXP3 activity is the most critical factor for Treg development and function, as well as RORγt for Th17 cells. In addition, differentiation of these two T cells subsets requires TGF-β. During the revision of this manuscript a paper from O'Shea and colleagues published in "Nature" added to our understand of Th17 cell differentiation by driving this lineage without TGF-β—in mice which lack the TGF- β receptor 1 subunit in their T cells [72]. They found that IL-6 and IL-23 in combination with IL-1β is sufficient to *IL17* transcription *in vitro*, and that these Th17 cells induce a higher severity of disease than Th17 cells produced using TGF-β. These IL-23 driven Th17 cells express T-bet and RORγt which are essential for the development of EAE and traffic to the CNS, whereas TGF- β driven Th17 cells preferentially locate to the spleen. Therefore, Th17 cells may actually comprise of a rather heterogeneous population [72]. Further studies on the molecular and cellular basis of development and function of Treg and Th17 cells are crucial to our understanding towards the maintenance of immune homeostasis and the operational relationship between these two cell subsets and their transcriptional markers (FOXP3 and RORγt). Further insight into the function of Treg and Th17 cells may provide new and potential strategies for future therapeutic intervention for the treatment of autoimmune diseases.

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