

## REVIEW

# Regulatory T cells: immune suppression and beyond

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**Foxp3-expressing regulatory T cells (Tregs) were originally identified as critical in maintaining self-tolerance and immune homeostasis. The immunosuppressive functions of Tregs are widely acknowledged and have been extensively studied. Recent studies have revealed many diverse roles of Tregs in shaping the immune system and the inflammatory response. This review will discuss our efforts as well as the efforts of others towards understanding the multifaceted function of Tregs in immune regulation.**

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## INTRODUCTION

Mammals have evolved complex immune strategies to fight foreign pathogens and to maintain health. Adaptive and innate immunity are the two fundamental components of the immune response. Together, they form a defensive front against pathogens. Innate immunity is mediated mainly by macrophages and dendritic cells (DCs), which recognize classes of microorganisms. T and B cells comprise the major task force carrying out the adaptive immune response, and they have highly antigen-specific surface receptors. Most T cells recognize specific antigens and then differentiate into effector T cells to promote immune responses. By using quasi-random recombination mechanisms, thymus-derived T cells can potentially generate infinite numbers of specificities that are reactive to foreign and self-antigens. Self-reactive T cells can cause autoimmune diseases in the host if they are not tightly controlled. Multiple processes therefore are in place to suppress the generation or the function of self-reactive T cells. Self-reactive T cells can be deleted in the thymus as well as in the periphery. Such elimination processes are nevertheless incomplete, resulting in small populations of mostly low-affinity self-reactive T cells in the periphery that can potentially initiate an autoimmune response. Fortunately, active immunosuppressive mechanisms exist to suppress these autoreactive T cells. Great progress has been made in understanding the cellular and molecular components of immunosuppression. Active immunosuppression is mediated mostly through either cytokines or specialized cells. The immunosuppressive cells, previously called suppressor cells<sup>1</sup> and now usually termed regulatory T cells (Tregs), play critical roles in suppressing the immune response. For years, much effort has been devoted to investigating the immunosuppressive functions of Tregs under normal and immunopathological conditions. Recently, increasing evidence suggests that Tregs might also turn into effector T cells that may promote immune responses. Thus, while Tregs are mainly viewed as critical mediators for immunosuppression, they in fact possess many diverse functions in immune regulation. Here we will review the evolution of our views on the functions of Tregs.

## Tregs AND DOMINANT IMMUNOSUPPRESSION

The concept of immunosuppression was proposed by Gershon *et al.*<sup>1–3</sup> in the early 1970s. The inability to identify the cell types performing immunosuppression impeded the validation of this concept and hindered development in this research area. It was not until a study performed by Sakaguchi *et al.* in 1995 that a subset of T cells with markedly increased expression of CD25 was discovered, named suppressor T cells, and later referred to as regulatory T cells (Tregs).<sup>4</sup> In recent years, substantial progress has been made in identifying different types of Tregs as well as in understanding how these cells are generated and function. Based on cell surface markers or cytokine secretion profiles, Tregs can be generally grouped into two categories: naturally occurring Tregs (nTregs) and induced Tregs (iTregs).

nTregs comprise a subset of CD4 T cells that develop in the thymus and constitutively express cell surface IL-2 receptor- $\alpha$  chain (CD25). CD4<sup>+</sup>CD25<sup>+</sup> nTregs comprise approximately 10–20% of peripheral CD4 T cells in mice and in humans. nTregs are critical for maintaining self-tolerance, as disruption of thymic development or peripheral maintenance of these cells invariably results in the development of autoimmunity. A cluster of cell surface molecules besides CD25, such as cytotoxic T lymphocyte antigen-4 (CTLA-4), glucocorticoid-induced tumor-necrosis factor receptor family-related gene and lymphocyte activation antigen-3, have also been used to differentiate nTregs from conventional T cells.<sup>5</sup> Transforming growth factor (TGF)- $\beta$  is expressed in nTregs at high levels as a cell surface-bound form.<sup>6,7</sup> nTregs suppress immune responses in an antigen-independent fashion *in vitro* and *in vivo*.<sup>5,8–10</sup> Foxp3, an X-linked transcription factor belonging to the fork-head family, is specifically and highly expressed in nTregs.<sup>11</sup> Thus, Foxp3 is currently used as the most reliable molecular marker for nTregs.

Conventional T cells are able to gain immunosuppressive activities and thus become iTregs. In the presence of exogenous TGF- $\beta$ , naive CD4 T cells upregulate Foxp3 expression through *de novo* mechanisms and become immunosuppressive.<sup>12,13</sup> Similar to nTregs,

Foxp3-expressing iTregs also express higher levels of CD25.<sup>12</sup> In addition, TGF- $\beta$  and IL-10 expression as well as secretion were found to be substantially upregulated in iTregs. iTregs not only are able to be generated *in vitro* but also can be induced *in vivo*.<sup>14</sup> As a transcription factor specifically expressed in Tregs, Foxp3 has served as a surrogate marker for these cells. Therefore, to study the function and regulation of Tregs, different mouse models have been developed by various groups to mark the Foxp3-expressing T cells with fluorescent proteins—enhanced green fluorescent proteins (EGFP) or red fluorescent protein, through genetic manipulations. By purifying CD4 T cells which express these fluorescent markers, nTregs and iTregs generated *in vivo* can be isolated.

For the last decade, the research on Tregs has focused on their immunosuppressive function. The essential role of Tregs in immunosuppression is now indisputable. Yet the mechanisms by which Tregs carry out their function remain ill defined. Nevertheless, it is generally agreed that Tregs suppress immune responses through multiple mechanisms including cell contact-dependent and -independent mechanisms. Several surface molecules preferentially expressed by Tregs are proposed to be important for their function. For example, CD25, a high-affinity IL-2-binding receptor, is highly expressed by the nTreg. The peripheral maintenance of nTregs appears to be dependent on IL-2 signaling,<sup>15–17</sup> which is also important for the proliferation and survival of activated effector T cells. It is therefore hypothesized that one mechanism of nTreg suppression of conventional T-cell activation is through competition for IL-2 consumption.<sup>18–20</sup> However, studies showing that CD25-deficient nTregs possess intact suppressive activity question the validity of this hypothesis.<sup>16</sup> CTLA-4, another surface molecule preferentially expressed by nTregs,<sup>5</sup> is important for inhibiting immune activation by competing for costimulatory receptor ligands expressed on innate cells<sup>21</sup> as well as inhibiting the function of antigen-presenting cells.<sup>22,23</sup> Thus, it is suggested that CTLA-4 is important for nTreg-mediated immunosuppression. However, there is earlier genetic evidence against the critical roles for CTLA-4 in nTreg function, as the function of nTregs deficient in CTLA-4 appeared to be normal *in vitro* and *in vivo*.<sup>24,25</sup> Antibody-mediated blockage of CTLA-4 was shown to abrogate nTreg function. It however remains unknown if this was due to a non-specific effect or a simultaneous blockage of CTLA-4 and its potential homolog(s) with redundant functions. Nevertheless, a recent study in which CTLA-4 is specifically deleted in Tregs definitively showed that CTLA-4 expression in Tregs is critical in mediating Treg function. CTLA-4-deficient Tregs lost suppressive activity and failed to prevent the onset of autoimmunity and systemic inflammation *in vivo*.<sup>26</sup> Recent studies revealed unique roles for T helper (Th)-specifying factors in controlling Treg function and immune responses. When interferon (IFN) regulatory factor-4, a Th2-specifying factor, was specifically deleted in Tregs, enhanced Th2 immunity was observed.<sup>27</sup> In addition, when STAT3, a factor important for Th17 differentiation, was specifically deleted in Tregs, enhanced Th17 immunity was observed.<sup>28</sup> Moreover, T-bet, a Th1-specifying factor, was found to be upregulated in Tregs upon IFN- $\gamma$  stimulation. Such upregulation appeared to be important for the expression of CXCR3 and the survival of Tregs.<sup>29</sup> This result thus suggests that Th-specifying factors are expressed by Tregs under certain conditions to promote immunosuppression in a manner specific to ongoing immune responses.

Cytokines also play important roles in nTreg function. TGF- $\beta$  appears to be critical in mediating nTreg function, as T cells from CD4-dnT $\beta$ RII mice that are unresponsive to TGF- $\beta$  are refractory to Treg-mediated suppression *in vitro* and *in vivo*.<sup>6,30,31</sup> Despite the

fact that TGF- $\beta$  mRNA is not elevated in nTregs, it is suggested that the membrane-bound form of TGF- $\beta$  is increased in nTregs and is important for their function.<sup>6,7</sup> IL-10 is another immunosuppressive cytokine preferentially expressed in Tregs.<sup>32,33</sup> and is important in mediating the functions of these cells.<sup>32,34</sup> Besides suppressing effector T-cell function directly, Tregs dampen immune responses through regulating innate components, such as DCs. nTregs can potentially induce tolerogenic DCs through CTLA-4 engagement-induced tryptophan catabolism.<sup>35</sup> In addition, it appears that Tregs destabilize the interaction between antigenic DCs and conventional T cells to prevent the activation of T cells.<sup>36</sup> It is therefore clear now that multiple mechanisms are involved in Treg-mediated immunosuppression. Much work is needed in the future to identify cell contact-dependent and -independent mechanisms critical for controlling Treg function.

### DIVERSE FUNCTIONS OF Tregs BEYOND IMMUNOSUPPRESSION

While we are still at the tip of the iceberg of understanding how Tregs control immunosuppression, emerging evidence suggests that Tregs are not only immunosuppressive but also able to become other types of effector Th cells to promote but not to suppress immune responses. To comprehend this idea, we will have to first clarify the definition of Tregs. Tregs have been traditionally defined by immunosuppressive activity since their discovery. However, because Foxp3 expression is accepted as a surrogate marker for identifying Treg lineage, Tregs have been redefined as Foxp3-expressing cells. Thus, Foxp3-expressing cells are now dubbed Treg lineage cells instead of functional immunosuppressing cells. By accepting this new definition, we start to realize that cells of Treg lineage, or Foxp3-expressing cells, play much more diverse roles than suppression in controlling immune responses. Before we explore the conversion of Tregs into other types of Th cells, we shall review what other types of Th cells have been identified and their characteristics, function and generation.

Th1 cells produce cytokine IFN- $\gamma$ , TNF- $\alpha$  and TNF- $\beta$  to stimulate innate and T-cell immune responses. The prominent function for Th1 cells is to promote cell-mediated immunity characterized by cellular cytolytic activity. Th1 cells are important to protect the host from obligate intracellular pathogens, such as the protozoal parasite *Leishmania major*,<sup>37–39</sup> the bacteria *Mycobacterium avium*<sup>40</sup> and *Salmonella typhimurium*<sup>41</sup> as well as viruses herpes simplex virus,<sup>42</sup> influenza A virus<sup>43</sup> and vaccinia virus.<sup>44</sup> Aberrant proinflammatory properties of Th1 cells can cause tissue damage and elicit unwanted inflammatory disease as well as self-reactivity including inflammatory bowel disease,<sup>45,46</sup> graft-versus-host disease<sup>47</sup> and autoimmune disorders, such as insulin-dependent diabetes mellitus<sup>48,49</sup> and rheumatoid arthritis.<sup>50</sup> IL-12 produced by activated innate immunity is a potent inducer for Th1 differentiation.<sup>51–54</sup> Th1 differentiation and function are controlled through Th1-specific transcription factors. T-bet, also known as Tbx21,<sup>55</sup> belongs to the T-box family of transcription factors and is the only known T-box gene specifically expressed in the lymphoid system. T-bet is rapidly and specifically induced in developing Th1 cells and is critically involved in initiating Th1 development, and thus, T-bet is recognized as a master regulator for Th1 differentiation. STAT4 is a transcription factor critical for IL-12 signaling and thus the full commitment of Th1 cells.<sup>56–58</sup>

Th2 cells are defined as producers of IL-4, IL-5, IL-9, IL-10 and IL-13. The Th2 response is often associated with humoral responses and is important to resist extracellular pathogens, such as helminths and nematodes.<sup>59,60</sup> Th2 cells are also important for mucosal immunity in the lung. An overstimulated Th2 response often leads to chronic

inflammatory airway diseases, such as atopic asthma and allergy.<sup>61–67</sup> IL-4 is the most prominent, if not the only, cytokine known to have the greatest influence in directing Th2 differentiation.<sup>68,69</sup> The presence of IL-4, even if endogenously derived, is essential for Th2 differentiation.<sup>70–73</sup> GATA3, a member of the GATA family of transcription factors, is sufficient and required for Th2 differentiation.<sup>74–76</sup> Therefore, GATA3 is regarded as the master regulator for Th2 differentiation. STAT6 activated by IL-4 stimulation is the major signal transducer in IL-4-mediated Th2 differentiation.<sup>77,78</sup> One of the mechanisms by which STAT6 promotes Th2 differentiation is through inducing high levels of transcription factor GATA3.<sup>79,80</sup>

Th17 cells are a newly identified class of effector T cell, which produces IL-17A and IL-17F. Th17 cells have been suggested to contribute to resistance to *Listeria*, *Salmonella*, *Toxoplasma*, *Cryptococcus*, *Leishmania* and *Francisella*.<sup>81–83</sup> In addition to controlling infection, Th17 cells play a critical role in the induction and propagation of autoimmunity. IL-17 expression has been associated with autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis and inflammatory bowel disease as well as allergic responses.<sup>84,85</sup> TGF- $\beta$  and IL-6 act cooperatively and non-redundantly to promote Th17 commitment.<sup>86–88</sup> IL-21, an IL-2 family member recently found to be highly produced by Th17 cells, can substitute for IL-6 to induce Th17 cells along with TGF- $\beta$ .<sup>89–91</sup> Retinoic acid-related orphan receptors (RORs) are the key transcription factors in Th17 differentiation. ROR- $\alpha$  and ROR- $\gamma$ t are both critical and somewhat redundant in promoting Th17 differentiation.<sup>92,93</sup>

Follicular helper T (T<sub>fh</sub>) cells express high levels of CXCR5. B cell-promoting cytokines IL-10 and IL-21 are highly produced by T<sub>fh</sub> cells, which are not typically associated with other types of Th cells.<sup>94–97</sup> IL-21 has been found to be critical for T<sub>fh</sub> differentiation.<sup>95,97</sup> Bcl-6 was recently reported to be a transcription factor that can be upregulated by IL-21 signaling and is critical for T<sub>fh</sub> generation.<sup>98</sup>

### Plasticity of Tregs *in vitro*

**Treg to Th17 conversion.** Under culture conditions, Treg to Th17 conversion has been extensively studied. The earlier observation that IL-6 was able to abrogate Treg-suppressive activity<sup>99</sup> was intriguing. The activation of innate cells, such as DCs, was able to abrogate the suppressive function of Tregs. This effect was largely due to the secretion of proinflammatory cytokine IL-6 by DCs because addition of exogenous IL-6 alone could abrogate Treg suppression. In addition, IL-6 production by the DCs is required for abolishing Treg suppressive activity, as IL-6 deficient DCs or antibody neutralization of IL-6 in the DC/T cell coculture restored Treg suppression. In the ensuing years, IL-6 was found to be important to suppress Treg generation and to promote Th17 differentiation. TGF- $\beta$  promotes the generation of Foxp3-expressing Tregs. However, IL-6 antagonizes TGF- $\beta$ -induced Treg generation and promotes Th17 differentiation.<sup>86,87</sup> These reports further confirmed the notion that naive T cells can be directed into different types of effector T cells under different cytokine milieu. It nevertheless raises the possibility that Tregs may be able to convert into Th17 upon IL-6 stimulation due to the fact that TGF- $\beta$  is highly expressed by Tregs. Indeed, freshly isolated nTregs produced a large amount of TGF- $\beta$  upon stimulation *in vitro*. In the presence of IL-6, Foxp3-expressing cells expressed IL-17, suggesting that nTregs can be converted into Th17-like cells after commitment to the Treg program.<sup>100,101</sup> Not only are nTregs able to be converted into Th17 cells, but also TGF- $\beta$ -induced iTregs have been shown to turn into IL17-producing cells upon IL-6 stimulation.<sup>101</sup> In these reports, Treg to Th17 conversion was concomitant with downregulation of Foxp3,

whose expression has been shown to suppress ROR- $\gamma$ t expression and to shut down the Th17 program.<sup>100,102</sup> Therefore, the downregulation of Foxp3 seems to be important to license Treg to Th17 differentiation. IL-6-dependent signaling is sufficient in both suppressing Foxp3 expression and promoting IL-17 expression. It is specifically required for the downregulation of Foxp3, as STAT3-deficient mice failed to repress Foxp3 following IL-6 stimulation. Thus, IL-6 signaling appears to be the molecular switch between Tregs and Th17. Note that, although IL-17 expression in Tregs coincides with Foxp3 downregulation, the total abolishment of Foxp3 is not required for Th17 conversion of Tregs because cells expressing both Foxp3 and IL-17 were found.<sup>100, 101</sup> Thus, Foxp3 expression and IL-17 expression are not mutually exclusive.

**Treg conversion to other Th cells.** Unlike Treg to Th17 conversion, few studies have addressed the potential of Foxp3-expressing cells to produce IFN- $\gamma$ , and thus to undergo Th1 conversion *in vitro*, with somewhat controversial findings. In one study, Tregs were not found to be able to produce IFN- $\gamma$ , even under Th1-polarizing conditions.<sup>101</sup> However, in the other study, Foxp3-expressing cells were found to produce IFN- $\gamma$  under Th1 conditions in Foxp3-expressing and Foxp3-non-expressing populations<sup>103,104</sup> (our unpublished observation). Such discrepancy in the results could be due to different experimental conditions. Without addition of a large amount of exogenous IL-2, Foxp3-expressing cells display an anergic phenotype and can not be activated. This might account for the lack of cytokine production observed in the first report. However, when a high dose of exogenous IL-2 is provided in the culture to force Tregs to exit the anergic state, as described in the second report, Tregs generate substantial amounts of IFN- $\gamma$  to become Th1 cells<sup>103,104</sup> (our unpublished observation). These findings suggest that Tregs are able to convert into Th1 cells *in vitro* under certain conditions. While Tregs possess great plasticity to convert into Th1 and Th17 cells, no evidence has been presented to show that nTregs can be converted into Th2 cells under culture conditions. When CD4 T cells were stimulated in the presence of IL-4 and TGF- $\beta$ , TGF- $\beta$ -induced Foxp3 expression was decreased.<sup>105</sup> However, activated T cells did not become IL-4-producing T cells. Instead, they produced IL-9.<sup>106,107</sup> Therefore, whether Tregs are able to become IL-4-producing cells *in vitro* remains unknown.

### Plasticity of Tregs *in vivo*

The aforementioned studies' efforts were devoted to investigating cytokine-driven conversion of Tregs. Most of these studies focused on converting TGF- $\beta$ -induced iTregs into other types of Th cells under culture conditions. One of the important questions is whether Treg can be converted into other types of Th cells under physiological conditions. In addition, if such conversions indeed occur *in vivo*, what might be the biological significance of such a conversion?

Accumulating evidence has been presented to show that Tregs can convert into effector T cells *in vivo*. When an EGFP expression cassette was knocked into the first exon of the endogenous *foxp3* gene, cells that lacked functional Foxp3 protein but had active Foxp3 promoter activities were thus marked with green fluorescence protein (GFP). In such a mouse model, loss of functional Foxp3 resulted in a Scurfy-like phenotype in the mice. However, in these mice, GFP-expressing cells were readily detected and were at an elevated percentage compared to that in the healthy mice. These GFP-expressing cells bore Treg signatures at reduced levels. Although these cells could not suppress the immune response, they were anergic to T-cell receptor stimulation.

Thus, these cells were Treg lineage but with abrogated immunosuppressive function. These Tregs were shown to become effector T cells *in vivo* by producing IFN- $\gamma$ , IL-4 and IL-17. Therefore, with the loss of Foxp3 expression, Tregs could be generated but functionally dysregulated by losing suppressive activity and gaining Th1, Th2 and Th17 effector function.<sup>108</sup> Without artificially ablating Foxp3 function, Tregs were also shown to downregulate Foxp3 expression and convert into effector T cells *in vivo*. In one study, Zhou *et al.* generated a bacterial artificial chromosome transgenic mouse model where Cre and EGFP were expressed in Foxp3-expressing Tregs<sup>109</sup>. By crossing such a mouse with a yellow fluorescent protein (YFP) Cre-reporter mouse strain, they tracked all the T cells that were currently expressing and had previously expressed Foxp3. They found that, *in vivo*, a small percentage of CD4 T cells expressed YFP but not GFP, indicating that these T cells used to express Foxp3 but had shut down the Foxp3 expression at the time of assay. Therefore, downregulation of Foxp3 expression in Tregs normally occurs *in vivo*. They referred to these YFP positive and GFP negative cells as exFoxp3 Tregs. In addition, they found that exFoxp3 Tregs expressed IFN- $\gamma$  and IL-17, suggesting their conversion into Th1 and Th17 cells. Moreover, exFoxp3 Tregs are able to promote inflammation and to contribute to the onset of type 1 diabetes upon transfer. Therefore, Treg to Th conversion could be important for directing inflammatory responses. Following transfer into the recipients, exFoxp3 Tregs were not able to regain Foxp3 expression, suggesting that conversion of Treg into Th cells is an irreversible process.<sup>109</sup> Th1 conversion of Tregs was also reported during lethal parasitic infection.<sup>110</sup> When mice were infected with a lethal dose of *Toxoplasma gondii*, Foxp3-expressing Tregs became IFN- $\gamma$ -producing Th1 cells. Interestingly, a decrease of Foxp3 expression was not associated with Treg to Th1 conversion in this study. Evidence for Treg to Tfh conversion *in vivo* is also available. Upon transfer into CD3<sup>-/-</sup> mice where T cells were absent, Foxp3-expressing Tregs downregulate Foxp3 expression in Peyer's patches. The transferred Foxp3-non-expressing Tregs but not Foxp3-expressing Tregs clustered around germinal centers, expressing CXCR5, IL21 and Bcl6, a phenotype resembling Tfh.<sup>111</sup>

In the studies mentioned above, Treg to Th conversion invariably concurred with the loss of Foxp3 expression, suggesting that conversion and Foxp3 expression might be mutually exclusive, and questioning the intrinsic ability of Tregs to become other types of Th cells *in vivo*. By knocking a luciferase and EGFP-expressing cassette into the 3'-untranslated region of the endogenous Foxp3 gene locus, we serendipitously generated a mouse model whose Tregs expressed Foxp3 but with reduced levels.<sup>112</sup> Reduction of Foxp3 expression resulted in a systemic autoimmune syndrome similar to what has been observed in Scurfy mice. Further analysis revealed that Foxp3-expressing T cells were generated in these mice, but without suppressive activities, although they remained anergic to T-cell receptor stimulation. Interestingly, reduction of Foxp3 led to a preferential conversion of Tregs into IL-4-producing Th2 cells.<sup>112</sup> Th2 conversion of Tregs might be essential for directing Th2 responses, as reduced Foxp3 expression in Tregs has been associated with Th2-type immune disorders in different mouse models. Thus, *in vivo*, Foxp3 expression and Treg to Th conversion are not mutually exclusive. Additionally, the expression of Foxp3 seems to limit the potential of Tregs to be converted into all types of Th cells. Whether Tregs with reduced Foxp3 expression are able to convert into other types of Th cells remains to be addressed. More importantly, it appears that the expression levels of Foxp3 control Treg function: high levels of Foxp3 expression endow T cells with suppressive activity; reduced levels lead to Th2 conversion and loss of

Foxp3 licenses Tregs to Th1, Th2, Th17 and Tfh conversion. Thus, Foxp3 serves as a rheostat controlling the diverse function of Tregs *in vivo*. The mechanisms underlying such phenomena are important questions deserving to be addressed in the future. In addition, as Foxp3 downregulation is a critical trigger for Treg to Th conversion *in vivo*, what triggers Foxp3 downregulation, or alternatively, what maintains high levels of Foxp3 expression, are questions that need to be addressed. Furthermore, the biological functions of Treg conversion during normal and aberrant immune responses still wait to be revealed.

While the signal transduction network controlling Treg to Th conversion waits to be unveiled, studies on epigenetic control of Treg and Th differentiation have provided important mechanistic insights. T-lineage specification is accompanied by epigenetic modifications of key cytokine and transcription factor gene loci. Such epigenetic change provides a basis for the heritability of gene expression patterns acquired by differentiating T cells.<sup>113,114</sup> Histone methylation or acetylation, DNA methylation and higher order chromatin structure each contribute to regulation of the accessibility of *cis* elements that bind lineage-specifying transcription factors and ultimately the expression of lineage-specific genes. The growing recognition of instability in the functional T-cell repertoire, as demonstrated in particular by plasticity of Treg programs, implies greater malleability in T-cell epigenetic modifications than previously thought, and suggests that conversion of Tregs is likely reflected in the reversal of epigenetic modifications induced during initial differentiation of these cells.

A global genome analysis of permissive and repressive histone methylation marks of naive, Th1, Th2, Th17 cells, nTregs and iTregs was recently reported, providing a mechanistic basis for aspects of Treg conversion.<sup>104</sup> Trimethylations of histone H3 were identified at the proximal promoters of key T-lineage transcription factors or cytokine genes. H3K4me3 and H3K27me3 were used to mark the genes that are expressed or repressed, respectively. The promoters for the genes highly expressed in certain Th lineages correlated with permissive H3K4me3 configuration. However, the promoters for the genes not expressed were not necessarily associated with high amounts of repressive H3K27me3 configuration. While only a small number of lineage-specific H3-methylation islands were found for Th cells, a large number of the unique H3K4me3 and H3K27me3 islands were found in nTregs, underlying their greater potential for diverse functions. Indeed, although the promoters for IFN- $\gamma$ , IL-4 and IL-17A were not marked with H3K4me3 for active expression, they were not marked with H3K27me3 for active suppression either. In addition, transcription factors T-bet, GATA3 and ROR- $\gamma$ t that master Th1, Th2 and Th17 differentiation, respectively, were not marked with the suppressive configuration in Tregs. Thus, at the epigenetic level, Tregs did not shut down the genetic program for other Th cells and remained permissive for their differentiation.<sup>104</sup>

## FINAL REMARKS

Being initially identified as suppressor T cells, Foxp3-expressing cells are critical in maintaining self-tolerance and immune homeostasis. With collective efforts over many years, we have achieved tremendous progress in understanding the immunological function of Tregs as well as how this type of T cells is regulated during normal and pathological immune responses. Recently, evidence has suggested that immunosuppression might not be the only function of Foxp3-expressing cells. They may convert into proinflammatory cells to promote immune responses. Such findings have important biological implications, as T-cell receptors of Tregs tend to be modestly self-reactive. The



aberrant function of Tregs and Treg to Th conversion could be critical in instigating and directing unwanted immunopathologies. Therefore, understanding how Treg to Th conversion occurs should enhance our understanding of the etiology of autoimmunity and inflammatory diseases. By generating reporter mice for Tregs and different subtypes of Th cells, we have started to unravel the diverse function of Tregs *in vitro* and *in vivo*. Future studies to investigate the molecular mechanisms of Treg to Th conversion and to address what impact such converted Tregs might have on various immune disorders are warranted.

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