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Origin and functions of pro-inflammatory cytokine producing Foxp3⁺ regulatory T cells

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Abstract

CD4⁺CD25⁺Foxp3⁺ regulatory cells (T_{regs}) are a special lineage of cells central in the maintenance of immune homeostasis, and are targeted for human immunotherapy. They are conventionally associated with the production of classical anti-inflammatory cytokines such as IL-10, TGF- β and IL-35, consistent to their anti-inflammatory functions. However, emerging evidence show that they also express effector cytokines such as IFN- γ and IL-17A under inflammatory conditions. While some studies reveal that these pro-inflammatory cytokine producing Foxp3⁺ regulatory cells retain their suppressive ability, others believe that these cells are dys-regulated and are associated with perpetuation of immunopathology. Therefore the development of these cells may challenge the efficacy of human T_{reg} therapy. Mechanistically, toll-like receptor (TLR) ligands and the pro-inflammatory cytokine milieu have been shown to play important roles in the induction of effector cytokines in T_{regs}. Here we review the mechanisms of development and the possible functions of pro-inflammatory cytokine producing Foxp3⁺ T_{regs}.

Keywords

T_{reg}; Th17; IL-17; Foxp3; Suppression; Inflammation; Cytokine; Reprogramming

1. Introduction

As CD4⁺Foxp3⁺ T_{regs} are central to immune tolerance [1–7], the potential of these cells for the treatment of T-cell-mediated diseases in humans has gained momentum in recent years. Therefore it is essential to understand precisely how they respond under steady state conditions and inflammatory conditions. Although T_{regs} are conventionally known to be non-producers of pro-inflammatory cytokines, emerging evidence shows that Foxp3⁺ T_{regs} are reprogrammed into T helper (Th) like cells producing pro-inflammatory cytokines in mice as well as in various human diseases [8–17] (Fig. 1). Zhou et al. first described the

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Conflicts of interest

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potential pathogenicity of reprogrammed T_{regs} that lose Foxp3 in murine model of Type 1 Diabetes [18]. Besides reprogrammed T_{regs} that lose Foxp3, T helper (Th) like T_{regs} that still express Foxp3 have also been reported to produce inflammatory cytokines. Th1 like T_{regs} co-express Foxp3, T-cell-specific T-box transcription factor (T-bet) and CXCR3 [19]. Th17 like T_{regs} express Foxp3, retinoic acid-related orphan receptor (ROR)- γ t and IL-17A [11]. Such effector like Foxp3⁺ T_{reg} cells producing pro-inflammatory cytokines are readily found at very low levels in tissues, but their presence is exaggerated during inflammation [11,20–23]. Th effector like T_{regs} share phenotypic and functional features with conventional Th cells, such as the expression of tissue homing receptors such as CCR4, CXCR5, CCR6 and CXCR3 [24,25]. These T_{regs}, with a possibility of losing Foxp3 and transforming into pathogenic cells under inflammatory conditions *in vivo*, temper the interests of using T_{regs} as cellular therapeutics, at least in the context of certain diseases. Since T_{reg} cells are enriched for autoreactive TCR specificities, the possibility of them converting into pathogenic effector T cells could be a critical threat to the host in the context of autoimmunity [18]. While Th17 like T_{regs} are shown by some studies to retain suppressive function [8,11], some others suggest that they may contribute to immunopathology [20,26]. Human studies on whether these pro-inflammatory cytokine producing T_{regs} lose their suppressive functions and cause autoimmunity are only beginning to emerge. Although these cells are prominently seen during inflammatory conditions, under steady state conditions, the development of Th effector like T_{regs} must be tightly regulated on several levels. Therefore studies investigating (1) how T_{regs} are dys-regulated or reprogrammed, (2) what contributes to their pathogenic cytokine production and, (3) if these reprogrammed cells contribute to, or control immunopathology are essential, so that we can gain a complete understanding of this process in order to account for and regulate it when considering employing T_{regs} as treatment. In this review, we will focus on the recent studies that provide varying answers, and unresolved questions about the development and possible functions of Foxp3⁺ cells co-expressing inflammatory cytokines [11,27]. Especially, we will focus on the markers and mechanisms of development of these cells in the context of various diseases.

2. Conventional view on T_{regs}

CD4⁺CD25⁺Foxp3⁺ T_{regs} that constitute about 5–10% of peripheral CD4 T cells are endowed with potent suppressive activity [28]. These cells are identified by markers that include CTLA-4, GITR, Neuropilin (Nrp)-1, besides CD25 and Foxp3. In humans, T_{regs} are identified also by CD127^{low} expression. The use of Helios as a marker for natural T_{regs} is controversial, especially in humans [29]. T_{regs} are considered “anergic” because they proliferate poorly *in vitro*, when stimulated through T cell receptor in the absence of γ -chain cytokine, and do not express the CD4 cell signature cytokine IL-2 [30,31]. Their anergic state is attributed to the expression of Foxp3 itself, which is a repressive forkhead or winged-helix family transcription factor. On the one hand, Foxp3 directly binds to the regulatory elements of IL-2 and IFN- γ genes and induces active deacetylation of histone H3, thereby inhibiting chromatin remodeling and controlling gene transcription [32–35]. On the other, Foxp3 binds to *GITR*, *CD25*, and *CTLA-4* genes increases histone acetylation and contributes to increased expression of these proteins in T_{regs}. Foxp3 also interacts and/or collaborates with dozens of other transcription factors, including NFAT, Runx1, Eos and

Blimp1 in promoting classical T_{reg} functions, as well as repressing the transcription of Th effector genes [36]. Thus Foxp3 is the master regulator for T_{reg} generation and their suppressive functions. Mutations of the *FOXP3* gene in humans result in T_{reg} deficiencies, and are responsible for immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome [37,38]. In mice, disruption of the *Foxp3* gene, including *Scurfy* mutation, also results in the loss of T_{regs} leading to fatal systemic autoimmune disease [39]. Similar symptoms of autoimmunity both in mice and humans highlight the parallelism between mice and humans in terms of functional importance of T_{regs} in suppressing severe autoimmune reactions [38].

Whereas Foxp3 is essential for T_{reg} cell development and function, most recent studies show that some T_{reg} cell signature genes can be expressed in the absence of functional Foxp3 [40–42]. Besides Foxp3, Foxo family members are also involved in classical T_{reg} induction by binding to and remodeling many Foxp3-binding sites even before Foxp3 actually binds to these sites [43,44]. Thus Foxp3 by largely exploiting the preformed enhancer network, defines the T_{reg} landscape and functionality. Moreover, it was recently shown the demethylation profile (nT_{reg}-Me) of nT_{reg} signature genes such as *Foxp3*, *Ctla-4*, *Ikzf4* (encoding Eos) and *Tnfrsf18* (encoding GITR), and not the Foxp3 expression alone, is a crucial component of T_{reg} identity. As this hypomethylation signature is independent of Foxp3, it is clear that though Foxp3 is required, it is not sufficient for the establishment of a robust and stable T_{reg} cell program [45]. Thus, although Foxp3 is the most reliable marker for T_{regs} suppression to date, hypomethylation status of T_{reg} signature genes should also be taken into consideration in studies that identify changes in number and frequency of T_{regs} *in vivo*.

2.1. Natural Foxp3⁺ cells vs Induced Foxp3⁺ cells

Foxp3⁺ T_{reg} cells include both natural or thymus-derived T_{reg} (nT_{reg} or tT_{reg}) cells and peripherally induced T_{reg} (pT_{reg}) cells [46–49]. nT_{reg} cells develop in the thymus and constitute the majority of the peripheral T_{reg} cell pool in naïve mice and in human cord blood. In contrast, pT_{reg} cells arise predominantly at sites of inflammation and environmental interfaces, such as the gut. They can also be generated *in vitro* (induced T_{reg} (iT_{reg})) after T-cell-receptor (TCR) stimulation of conventional CD4⁺ T cells in the presence of transforming growth factor- β (TGF β) and interleukin-2 (IL-2). Both T_{regs} require Foxp3 for their development and function. In terms of Foxp3 stability and suppressive functions, nT_{regs} constitute a relatively more stable population than pT_{regs} [50–52].

2.2. T_{reg}'s suppressive functions and their anti-inflammatory cytokine production

T_{reg} cells exert their immunoregulatory functions through various mechanisms requiring CTLA-4, direct killing of antigen presenting cells or T cells, consumption of IL-2, and the production of immunosuppressive cytokines such as IL-10, transforming growth factor (TGF)- β , IL-35 and galectin-1 [53]. Although a myriad of suppressive mechanisms have been proposed for T_{regs}, two basic modes of suppression, (1) deprivation mode under non-inflammatory and steady state conditions, and (2) active suppression mode during inflammatory reactions, have been established [1,54]. In the context of non-inflammatory conditions or at minimal levels of IL-2 and other inflammatory cytokines, natural T_{reg} cells

through CD25 and CTLA-4, deprive IL-2 and CD28 signals from antigen-reactive T cells resulting in blockade of progression of naïve T cells into effector states, and apoptosis of the differentiating T effectors. In highly inflammatory environments, for example, during a microbial infection, these deprivation mechanisms are transiently abrogated allowing T cells to respond to antigen. With excess of IL-2 and other cytokines in the milieu, T_{regs} also get activated acquire an armor of suppressive arsenal to kill or inactivate effector T cells and antigen-presenting cells through (1) granzyme/perforin pathways [55] and CD39 mediated ATP hydrolysis (2) IL-10 secretion, and (3) infectious tolerance mediated by TGF- β and IL-35. These mechanisms together constitute dampening of excessive immune responses. Disruption of one of these mechanisms alone cannot abrogate suppressive capacity of T_{regs}, as shown in earlier studies [56–59]. Currently there seems to be a consensus in the literature that T_{reg} mechanisms differ depending on the disease setting, inflammatory status of the local environment, and their anatomical localization [1,2,60]. Thus it is well known that anti-inflammatory cytokines such as IL-10, TGF- and IL-35 are produced by T_{reg} cells, and are critical for some of their suppressive functions. However, T_{reg}'s ability to undergo reprogramming into helper-like cells and produce inflammatory cytokines without the loss of Foxp3 was discovered only recently.

3. Pro-inflammatory cytokine production in Foxp3⁺ T_{regs} is associated with human diseases

3.1. Psoriasis

Psoriasis is an autoimmune chronic inflammatory skin disease that is associated with hyperactive IL-23 signaling and Th17 effector cytokines [61]. In this disease T_{regs} are known to be dysfunctional and lacking suppressive capacities [23]. Although IL-17A producing T_{reg} cells are readily found in small numbers in the tissues such as tonsils and skin, the association of Th17-like T_{regs} with an human inflammatory condition was first identified in psoriasis patients [11,20,22,62]. The qualitative defect in T_{regs} is believed to be an underlying mechanism for unrestrained pathogenic effector T cell proliferation resulting in skin inflammation [20,62]. In human psoriatic skin the Foxp3⁺ cells have a memory phenotype expressing CD45RO, showing high capacity to proliferate and produce IL-17A [20,22]. However, they are *bona fide* T_{regs} expressing Foxp3 and CD25 and low levels of CD127, with no expression of IL-2 and IFN- γ [20,26]. TCR β sequences of these T_{regs} show a very little overlap with those of effector T cells from the skin, suggesting that IL-17A⁺Foxp3⁺ cells do not arise from T effector cells transiently expressing Foxp3 [22]. It is believed that these dys-regulated cells may represent Foxp3⁺T_{regs} that pass through a transient Foxp3⁺IL-17A⁺ stage and may progressively lose Foxp3 expression to become Ex-T_{regs} [18,20]. Examining TCR repertoires and nT_{reg}-Me signature of specifically the Foxp3⁺IL-17A⁺ cells in psoriasis human tissue samples will offer more information about their origin and functions.

3.2. GVHD

Graft-versus-host disease (GVHD) is a major complication of allogeneic hematopoietic stem cell transplantation in which the transplanted immune system attacks recipient tissues as

non-self. Acute GVHD is predominantly related to Th1 responses, where migration of T-bet⁺ and CXCR3⁺ cytotoxic effectors direct and type I interferon-mediated pathogenic processes in mucosa [63,64]. Th17 effector cells are also implicated in pathogenesis and T_{regs} are well established to have protective roles. While numerical deficiency in mucosal FOXP3⁺ T_{regs} correlates with acute and chronic GVHD [65], increase in FOXP3⁺ regulatory T cells in GVHD skin biopsies is associated with lesser severity of the disease and better treatment response [66]. In murine models of bone marrow transplantation, direct T_{reg} infusion as well as agents that induce T_{regs} are shown to inhibit activation and proliferation of alloreactive donor T cells, thereby reducing GVHD [6,67–69]. However recent reports show that activated T_{reg} cells that migrate to inflamed sites in the skin tissues of cutaneous GVHD have an effector/memory cell phenotype. They are Th1-like T_{regs} expressing ICOS, CD39 and CXCR3, and also high levels of Th1 transcription factor T-bet. Although whether these T_{regs} express IFN- γ and contribute to exacerbation of the disease was not examined, it is speculated that T-bet expression in T_{regs} found in GVHD is not associated with the loss of suppression. Supporting this tenet, T-bet⁺ T_{regs} producing IFN- γ in fact have been shown to be required for regulatory T cell homeostasis and function during type 1 inflammation [70,71].

3.3. Inflammatory bowel disease (IBD)

Lack of intestinal tolerance to commensals and other innocuous antigens can lead to inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis. Both nT_{reg} cells and pT_{reg} cells are critical for commensal tolerance in the intestine [72–74]. A number of studies have shown protective roles of T_{regs} in IBD. It is also well established that Th17 like T_{reg} cells are present at elevated levels in the intestinal mucosa and in circulation of IBD patients, compared to healthy controls [10,75–77]. Although one study showed that these cells are defective in their suppressive functions [77], other studies suggest that Th17 like T_{reg} cells from CD tissues and colitic samples are immunosuppressive. These cells suppress T-cell proliferation *in vitro*, supporting the interpretation that these are not reprogrammed to lose their suppressive capacity [8,75]. Similarly, in the mouse model, although a fraction of T_{regs} produce IL-17A under Th17 conditions *in vitro*, when adoptively transferred into immunodeficient mice, they still modulate IBD and weight loss during Th17 IBD inflammation *in vivo* [78,79]. This is observed despite some of these T_{regs} may have additionally lost Foxp3 upon transfer into Rag-/- mice. Moreover, Th1 like Foxp3⁺ T_{reg} cells that produce IFN- γ have also been reported to retain their regulatory functions and inhibit colitis in mice [80]. Taken together, these data demonstrate that inflammatory conditions in IBD can induce IFN- γ or IL-17A production in a fraction of Foxp3⁺ T_{regs} *in vivo*, but do not affect their immunomodulatory functions.

3.4. Periodontitis

The progression and severity of the periodontal disease are strongly governed by an imbalance between oral commensal bacteria and host immunity. T-cells, especially CD4⁺ T helper cells, play an essential role in aberrant responses and local inflammation. Increase in Th17 cells along with the decrease in T_{regs}, i.e., Th17/T_{reg} imbalance, has been suggested recently to be critical in the development of periodontitis. Okui et al. analyzed the association between functional plasticity in T_{regs} and the pathogenesis of periodontitis [81].

Their study revealed the presence of Th17 like T_{reg} cells co-expressing Foxp3 and IL-17A in periodontitis lesions as well as in gingival tissue T-cell lines from patients. This finding indicates that T_{reg} reprogramming may be another feature of periodontitis lesions. However it remains to be seen whether these IL-17A producing T_{reg} cells contribute to inflammatory responses against periodontopathic bacteria.

3.5. Arthritis

Th17 cells are the dominant initiators of inflammation in several murine models of autoimmune arthritis. In humans, while Th17 cells play a pathological role in juvenile idiopathic arthritis [82,83] ankylosing, spondylitis and psoriatic arthritis [84], Th1/Th17 cells with intermediate phenotype are pathogenic in rheumatoid arthritis (RA) [82,85]. RA patients have been reported to exhibit a higher frequency of Th17 like T_{regs} compared to healthy subjects [86]. These T_{regs} also produce IFN- γ , IL-2, ROR- γ t and Tbet, along with IL-17A [87,88]. However, the ability of these cells to suppress proliferation, production of IFN- γ and IL-2 in effector T cells was comparable to those of conventional T_{reg} subpopulation from healthy controls, suggesting that these T_{regs} are not reprogrammed to lose their suppressive capacities.

3.6. Multiple Sclerosis

Multiple Sclerosis (MS) is characterized by the damage in myelin sheaths surrounding axons in the brain and spinal cord. Although myelin-antigen reactive Th1 cells were previously thought to drive MS, it now appears that pathogenic Th17 cells play an important role in disease pathogenesis [89]. Murine models of experimental autoimmune encephalomyelitis (EAE), show that the factors including IL-23 [90], GMCSF [91], a high-salt diet [92] and microbiota [93], all have impact on the development and severity of Th17 mediated EAE. A higher frequency of Th1-like T_{regs} secreting IFN- γ has been reported in subjects with relapsing remitting multiple sclerosis (RRMS), compared to healthy control individuals [21]. Analysis of the Foxp3⁺ T_{reg} cell-specific demethylated region (TSDR) in isolated IFN- γ ⁺ Foxp3⁺ (Th1-like T_{regs}) and IFN- γ ⁻ Foxp3⁺ (conventional T_{regs}) in multiple sclerosis patients reveals that Th1-like T_{reg} cells possess a similar pattern of demethylation in the TSDR region to that of conventional T_{regs} from patients and healthy controls. These studies suggest that Th1-like T_{regs} may have arisen from conventional T_{reg} cells and not from effector cells expressing Foxp3 transiently. Also, while there is a direct correlation between Th1-like T_{regs} and pathology, an inverse correlation has been reported for between Th17-like T_{regs} and inflammation. Moreover, Th1 like T_{reg} cells show reduced suppressive activity *in vitro*, which can partially be reversed by IFN- γ -specific antibodies or by removal of IL-12. Consistent to this, in subjects undergoing IFN- β immunomodulatory treatment, lower frequency of Th1 like T_{reg} cells correlates with reduced inflammation. These data point to a general functional defect in the suppressive activity of Th1-like T_{regs} that might contribute to inflammation instead of regulating Th1 inflammation in MS. This is consistent to experimental autoimmune encephalomyelitis (EAE) mouse model study that demonstrated that Tbet expression in T_{regs} is not required for the resolution of central nervous system (CNS) inflammation [94]. However the functions of Th17 like T_{reg} cells, whose frequency increases in IFN- β treated patients remains to be investigated in MS patients.

3.6.1. Type 1 diabetes—Autoimmune type 1 diabetes (T1D) is a T cell-mediated autoimmune disease characterized by the destruction and dysfunction of pancreatic β cells. Numerous studies have demonstrated the beneficial role of T_{regs} in controlling T1D. In patients with type 1 diabetes, the frequency of T_{regs} is comparable to healthy controls in peripheral blood. However, these patients reveal an increase in Th1-effector-like T_{regs} enriched in CD45RO⁺ memory cells. These FOXP3⁺IFN- γ ⁺ T_{regs} are significantly increased in peripheral blood compared to the healthy controls [95]. Because Ex- T_{regs} that lose Foxp3 can be pathogenic in murine model of T1D [18], careful epigenetic analyses were performed in these human cells. These data showed that FOXP3⁺IFN- γ ⁺ cells are predominately methylated at the TSDR, in contrast to conventional n T_{regs} , suggesting a potential instability and loss of Foxp3 expression. However, consistent with n T_{reg} anergic properties, IFN- γ ⁺ T_{regs} do not produce IL-2. Most importantly, these Th1-effector-like T_{regs} retain their suppressive function, suggesting that these reprogrammed T_{regs} may not contribute to T1D pathogenesis.

3.7. Cancer

T_{reg} infiltration in tumors, and the resulting suppression of anti-tumor immunity is considered to be one of the mechanisms of immune evasion in several tumors [96,97]. Human tumor-infiltrating lymphocytes (TIL) isolated from tumor tissue samples of melanoma, ovarian, breast and colon cancers revealed that a substantial fraction of these cells can develop into FOXP3⁺ cells expressing pro-inflammatory cytokines [98]. This population of cells, arising after rounds of *in vitro* expansion of TIL, can co-produce IFN- γ and IL-17A, [99] and are shown to develop from effector Th17 cells. However, the expanded population exhibits stable expression and demethylation of FOXP3 and retain potent suppressive function *in vitro*. These data are consistent to another study, which showed a strong suppressive activity of IL-17A⁺Foxp3⁺ cells isolated from colon carcinoma [75]. This study further showed although the T_{reg} population isolated tumor tissues of melanoma, renal cell carcinoma, colon carcinoma patients can show up to 31% of IL-17A-producers *ex vivo*. However, they exhibit a strong suppressive function *in vitro*, which was comparable to T_{regs} that had little or no IL-17 producers. Taken together, these data suggest that plasticity in Th17 cells may be one of the mechanisms by which effector-like T_{regs} develop in cancer. Although the precise role of these cells in tumor progression remains to be seen, these cells may contribute to promoting active inflammation and tumor development, while still retaining their suppressive capacity on T cells.

3.8. Allergy

Th1-like- T_{reg} cells were first identified in the airway hyper-reactivity (AHR) setting in mice [19]. Studies by Scott et al., showed that the IFN- γ producing Foxp3⁺ cells arise from naïve CD4⁺CD25⁻ T cells during Th1 polarized immune responses, upon stimulation with CD8 α ⁺ dendritic cells (DCs) *in vivo*. These Th1-like- T_{regs} expressing Foxp3, T-bet and inducible costimulator (ICOS) are antigen-specific. Moreover, these cells co-produce both IL-10, IFN- γ , and potently inhibit the development of allergen induced AHR, a function that can be inhibited by neutralization of IL-10, but not IFN- γ in mice. In human allergy there are no reports on Th1 like T_{regs} . However, *ex vivo* analyses of surgically removed nasal mucosa in allergic rhinitis and nasal polyposis patients show the presence of Th17-like T_{regs} cells co-

expressing FOXP3, RORC, and IL-17A. These cells are found at higher frequency in patients with polyps than in those with allergic rhinitis alone. Further studies are required to delineate whether they play pathogenic roles in human allergy. Most recently, GATA-3⁺IRF-4 + IL-4 + IL-13 + Th2 like T_{regs} have been reported in a mouse allergic model [100]. They arise due to selective augmentation of IL-4 receptor signaling in T_{reg} cells. Failure of T_{reg} cells to maintain oral tolerance in food allergy has been directly attributed to the production of Th2 cytokines and impairment of suppressive function in these cells. Such allergen-specific Th2 like T_{reg} cells are also found in the peripheral-blood of children with food allergy [100].

3.9. Infections

Although T_{regs} carry out specialized functions in various infections, their ability to produce pro-inflammatory cytokines was reported only in two infections listed below. During an acute and lethal infection by *Toxoplasma gondii*, a strong Th1 environment triggered by this microbe induces T-bet and IFN- γ expression in T_{reg} cells in mice. This is associated with IL-2 exhaustion, and decline in Foxp3⁺ T_{reg} cell numbers, coinciding with immunopathology and death in mice. These data support the idea that during an infection, Th1 environment can subvert regulatory networks and become pathogenic by promoting Th1-like T_{regs} [101]. In the context of oropharyngeal candidiasis (OPC), a Th17 infection model, Th17-like T_{regs} arise transiently in infected mice [78,102]. The increase is pronounced among T_{regs} in mouse oral lamina propria and intraepithelial cells (MOIL) isolated from tongue and palatal tissues of the infected mice [103]. Both Th17 effectors and nT_{regs} contribute to the induction of Th17-like T_{regs} *in vitro*, likely reflecting the same scenario during candidal infection *in vivo* [78]. Functionally, it is clear that the development of these cells does not contribute to pathology during acute candida infection in mice. In fact, T_{regs} as a whole, play dual but protective roles in enhancing IL-17A and decreasing TNF- α , to respectively control acute infection and reduce immunopathology in mice [79,104]. It remains to be seen how Th17-like T_{regs} are specifically involved in each of these processes during infections.

4. Markers and mechanisms of development of pro-inflammatory cytokine producing Foxp3⁺ T_{regs}

4.1. Pro-inflammatory cytokine production in natural Foxp3⁺ cells vs induced Foxp3⁺ cells

One of the key questions about pro-inflammatory cytokine Foxp3⁺ cells is whether these cells develop from conventional effector T cells expressing promiscuous Foxp3, or reprogrammed natural regulatory T cells [50,52]. Human CD4⁺ cells are known to express FOXP3 transiently upon TCR activation [105]. Unlike nT_{reg} cells, these activation induced FOXP3⁺ cells eventually lose FOXP3 expression, and do not suppress proliferation or cytokine production in effector cells. This is consistent to spontaneous up-regulation of FOXP3 in a proportion of human Th17 cells expanded from blood CD4⁺ T cells [106]. Therefore it is likely that non-suppressive Foxp3⁺ cells that are found in some diseases, and those that can be easily reprogrammed to lose Foxp3 expression, are derived from the effector cells in the periphery. However, effector like T_{regs} that retain their suppressive

properties may have arisen from nT_{regs} and pT_{regs} . Although mouse CD4 cells do not spontaneously express Foxp3, TGF- β and IL-2 together can induce Foxp3⁺ cells *in vitro*. In contrast, during Th17 inducing conditions in the presence of TGF- β and IL-6, although Foxp3 induction is substantially reduced because of the inhibitory effect of IL-6, a few cells express Foxp3. Interestingly, a small proportion of these induced Foxp3⁺ cells also co-express IL-17A [78]. Using mixed cultures where CD45.2 nT_{regs} are co-cultured with CD45.1 naïve cells under Th17 inducing conditions, it has been shown that both nT_{regs} and induced Foxp3⁺ cells are capable of producing IL-17A in a IL-6 dependent manner [78]. These data support the notion that the majority of effector-like T_{reg} cells develop from effector cells, while reprogrammed nT_{regs} may also have a contribution to this population that arises *in vivo*. Moreover, the expression of Foxp3 in these cells may also confer them with suppressive activity, as shown in some instances where effector derived Foxp3⁺ cells expressing pro-inflammatory cytokines are shown to possess potent suppressive capacity [15,99].

4.2. Markers of effector-like T_{regs}

The most important markers of effector-like T_{regs} are the relevant lineage specific transcription factors: T-bet for Th1 like effector T_{regs} , GATA-3 for Th2 like T_{regs} , and ROR- γ t for Th17 like T_{regs} . Although they express key transcription factors of effector cells, it is important to remember that not all the T-bet, GATA3, and ROR- γ t-expressing Foxp3⁺ cells produce cytokines. Besides these intracellular markers, there are attempts to further classify these cells based on the cell surface markers. Recently, human T_{regs} are classified into three populations based on their stability, FOXP3, CD25 and CD45RA expression, namely CD4⁺CD25⁺CD45RA⁺ (stable resting T_{regs}), CD4⁺CD25⁺⁺⁺Foxp3^{high} CD45RA⁻ (activated T_{regs}), and CD4⁺CD25⁺Foxp3^{low}CD45RA⁻ (unstable T_{regs}) [107]. It has been shown that the cells with pro-inflammatory cytokine producing ability are enriched among unstable CD4⁺CD25⁺Foxp3^{low}CD45RA⁻ T_{reg} cells. Specifically for IL-17A⁺Foxp3⁺ cells, CCR6, CD49d, HLA-DR^{neg} and IL1R- β have also been described as markers [8,26,108,109]. Most recent studies further defined CD161, a natural killer cell marker, as a reliable marker for the Th17-like T_{regs} [11,24,86,87,108,110]. Taken together, effector like T_{regs} expressing proinflammatory cytokines can be detected by the expression of CD4⁺CD25^{hi} CD127^{lo} CD45RA⁻ HLA-DR^{neg} CD161⁺Foxp3^{low} markers, along with the specific lineage transcription factors and chemokine receptors. Although the markers for Th1 like T_{regs} are not well established, they may also express the above markers, and can be distinguished from Th17 like T_{regs} by the expression of T-bet. Not surprisingly, there are variations in the expression of some of these markers, which can be explained by variations in the milieu of different inflammatory conditions.

4.3. Mechanisms of induction of proinflammatory cytokine production in T_{regs}

4.3.1. Proinflammatory cytokines—The development of pro-inflammatory cytokine producing T_{regs} is strongly determined by the cytokine microenvironment. Foxp3⁺ T_{regs} have similar requirements like naïve cells to differentiate into respective lineages. For example, they require the cytokine IL-12 to develop into Th1-like T_{regs} . IL-6, IL-23 and IL-1- β promote the differentiation of T_{regs} into Th17-like T_{regs} [26,78,86,111–113]. Mechanistically, the conversion of T_{regs} into Th17-like T_{regs} relies on IL-6 and IL-1 β

dependent epigenetic modifications [8,26,109]. When this phenomenon was first reported in mice, IL-6 alone was shown to be able to induce Th17 producing cells in Foxp3⁺ T_{regs} in the presence of TGF- β [114]. Thus, as in Th17 cells, IL-6 dependent STAT3 activation, along with ROR- γ t and ROR- α are required for IL-17A expression in T_{reg} cells [26,112,115]. Most recent report also suggests that IL-6 promotes PIM1 kinase expression in *in vitro* expanded human T_{regs}. This kinase, by specifically phosphorylating FOXP3 at Ser(422), negatively regulates FOXP3 chromatin binding activity, likely reprogramming the cells [116]. While knockdown of PIM1 promoted FOXP3-induced suppressor functions, the role of PIM1 in the development of effector like T_{regs} remains to be studied. Because IL-2 and IL-15 are known suppressors of IL-17A production [117,118], and T_{regs} express higher levels of the receptors for these cytokines, it is probable that these cytokines control the differentiation of Th17-like T_{regs}. It remains to be seen whether these cytokines can be employed for therapeutic intervention to control IL-17A expression in T_{regs}. Indoleamine 2,3-dioxygenase (IDO), as well as Eos (Ikzf4), an obligate co-repressor for Foxp3, are also implicated in controlling the development of Th17 like T_{regs} *in vitro* and *in vivo* [119–121]. Interestingly, in most situations, only a small population of T_{regs} becomes effector like and most T_{regs} do not. It is not clear whether it is due to very low frequencies of Ag-specific T_{regs} that respond in the tissue during inflammation. Suppressor of cytokine signaling 1 (SOCS1) plays an important role in T_{reg} cell integrity and function, by protecting the cells from excessive inflammatory cytokines [16]. Although the underlying mechanisms of why only a small fraction of T_{regs} become effector like T_{regs} are unknown, SOCS1 may prevent most of the cells from becoming pro-inflammatory cytokine producers, while a few cells are relieved from these signals to become effector like T_{regs}.

4.3.2. TLR signaling—Another important signal that has been implicated in pro-inflammatory cytokine production in T_{regs} emanate from microbial stimuli that activate Toll like receptors (TLRs) [78,122]. TLRs are not only expressed by the innate immune cells and antigen presenting cells (APC) [123,124], they are also expressed in CD4 T cells, including Foxp3⁺ T_{regs} [123,125–131]. It is now clear that majority of the tissue T_{regs} express high levels of TLR-2, and thus have an ability to respond to TLR-2 ligands directly [78]. TLR-2 signaling activation in the presence of APC *in vitro*, reverses T_{reg}'s suppressive functions [112,122,129,132–137], because of the pro-inflammatory cytokines produced by the APC responding to TLR-2 ligands [138]. Also, loss of Foxp3 is a prominent feature [139] in a heterogenous mixture of stable and unstable T_{regs} that were isolated only on the basis of CD25 marker [128,140]. However, when highly purified CD4⁺CD25^{high}Foxp3^{GFP} T_{regs} isolated from Foxp3^{GFP} mice are stimulated with TLR-2 ligands and IL-2 in the absence of APC, they show moderate proliferation without losing Foxp3 expression [31,78,141,142]. Most recent reports support the idea that T_{regs} proliferate in response to TLR-2 ligands, but they retain their regulatory activity under TLR activation conditions [8,78,139,140,143,144]. The latter tenet is more consistent with the view that TLR-2 ligands promote tolerance by inducing peripheral T_{regs} (pT_{regs}), and do not reverse suppression [145]. Thus while TLR-9 signaling from commensal bacteria may negatively control the development of T_{regs} [146], TLR-2 signaling may promote the homeostasis of T_{regs} under steady-state conditions [78]. This is in line with the association of TLR-2 polymorphisms with changes in neonatal T_{reg} numbers as well as allergies and atopic dermatitis [147]. However, under inflammatory Th17

conditions, when nT_{regs} are stimulated with TLR-2 ligands in the presence of APC, IL-6, IL-23 and IL-1 β , a minor fraction (<20%) of nT_{regs} lose Foxp3. Interestingly, among nT_{regs} that do not lose Foxp3, TLR-2 ligands also induce proliferation and IL-17A production [12,78]. Importantly, when mice are infected with a TLR-2 activating fungus, *C. albicans*, Foxp3⁺ cells expand and produce IL-17A *in vivo* [12,78]. Myd88 [148] [129,138] and TLR-2 are required, both in T_{regs} and APC for Foxp3⁺ cells to produce IL-17A [78]. TLR-2 dependent increase in Foxp3⁺IL-17A⁺ROR- γ t⁺ cells is an additive effect of direct proliferation of T_{regs} , as well as IL-6 mediated induction of IL-17A. These findings are consistent with the role of TLR-2 signaling in promoting IL-17A in effector CD4 cells [149]. As *C. albicans* also activates dectin signaling, which has been implicated in IL-17A induction in Foxp3⁺ cells [115,150], it is possible that dectin1 is also involved in inducing Th17-like T_{reg} cells during OPC. Moreover, the role of IL-1 β dependent Myd88 signaling in T_{regs} cannot be excluded in the induction of Th17-like T_{reg} cells. Thus excess of TLR-2 signaling from commensal bacteria, infections and endogenous ligands may all contribute to the development of Foxp3⁺ IL-17A⁺ cells in various inflammatory disease conditions. In this context, Staphylococcal enterotoxin B is also implicated in the differentiation of T_{regs} into RORC(+) T_{regs} that may be involved in the pathogenesis of eosinophilic nasal polyposis [151].

5. Effector transcription factors contribute to effector cytokine production in T_{regs}

The development of naive CD4 T cells into T helper cell lineages such as Th1, Th2, T_{reg} and Th17 cells rely on lineage specific transcription such as T-bet, GATA-3, Foxp3 and ROR- γ t respectively. Each Th lineage develops as a result of specific response to unique set of cytokines that instruct a specific differentiation program, and in turn exhibits unique profile of cytokines [152–154]. Along with lineage specific master transcription factors, these lineages are also associated with other factors including signal transducer and activator of transcription (STAT) factors. While STAT1 and STAT4 are critical for Th1 regulation, STAT6 directs Th2 cells. While Th17-cell differentiation is dependent on a balance between signaling through STAT3 and interferon regulatory factor 4 (IRF-4) [155], T_{reg} differentiation is dependent on STAT-5 and SMAD factors. Emerging concepts of Th lineage commitment suggest that Th-cell fate is not as irreversible as previously thought, and that lineage reprogramming can occur through the inducible expression of key transcription factors in all the Th subsets [152]. Similarly, the expression of effector Th lineage transcription factors exemplifies the plasticity in T_{regs} , and is a prerequisite for their effector characteristics.

Besides the classical Th subsets are the T follicular helper (Tfh) cells, which are required for survival, differentiation and class-switching of B cells in the germinal centers. Tfh cells are vital for the generation and maintenance of germinal center reactions and the long-lived humoral immunity. B-cell lymphoma (Bcl)-6, CXCR5, PD-1, SAP (SH2D1A), IL-4, IL-21, and ICOS, and the absence of Blimp-1 (prdm1) are the characteristic markers of Tfh cells [156–158]. Similar to Tfh cells, CD4⁺CD25⁺ Foxp3⁺CD69⁻ T_{reg} cells are able to downregulate CCR7 and upregulate Bcl-6, CXCR5, programmed cell death 1(PD)-1 and

ICOS. Functionally, these T_{reg} cells migrate into the B-cell zone and suppress germinal center reactions [159–161]. Although a fraction of Tfh like T_{reg} cells lose Foxp3 expression and produce IL-17A in gut Peyer's patches, they are incapable of producing the characteristic Tfh cytokines, namely, the IL-4 and IL-21 [17,162–164].

5.1. Th effector lineage specific transcription factors

In many of the above mentioned diseases, IL-17A⁺Foxp3⁺ Th17 like T_{regs} also co-express ROR- γ t, suggesting that ROR- γ t is involved in IL-17A production in Foxp3⁺ cells. Most recent data reveal also the requirement of RUNX transcription factors for the induction of a Th17-like phenotype in T_{regs} [165,166]. Th17-like T_{regs} represent either early stages of cells undergoing Th17 and T_{reg} differentiation, or plastic T_{regs} losing their activity during Th17 inflammation. As Th17 cells and T_{regs} have evolved greater developmental plasticity than Th1 and Th2 subsets, it is believed that these subsets can interconvert. This is because of the involvement of TGF β , a cytokine that is common to both Th17 and T_{reg} cell differentiation [167–169]. While TGF- β receptor I signaling is required for both Foxp3 and IL-17A induction, the differential up-regulation of Foxp3 and IL-17A is dependent on its differential ability to activate mothers against decapentaplegic homologue (SMAD)4. Also, TGF β induces Foxp3 in collaboration with IL-2 dependent STAT5 activation, whereas it induces ROR- γ t in collaboration with IL-6 dependent STAT3 activation. As a result, ROR- γ t and Foxp3 are often co-expressed at early stages of T cell differentiation, and these two transcription factors antagonize each other partly through protein–protein interaction in steady state conditions [170,171]. In the absence of IL-6 in the milieu, Foxp3 inhibits Th17 differentiation by antagonizing the function of the transcription factors ROR- γ t and ROR- α . However, IL-6 overcomes this suppressive effect of Foxp3 and promotes Th17-like T_{regs} in a ROR- γ t and STAT3 dependent manner [8,115]. These data suggest that during immune imbalance caused by inflammatory conditions, despite Foxp3 expression, the antagonistic effects of the protein may be lost. Studies in human T_{regs} have shown that, compared to IL-17A^{neg} T_{regs} and conventional T cells, IL-17A⁺ T_{regs} express higher levels of ROR- γ t after co-culture with conventional T cells and APCs, suggesting a complete absence of Foxp3 mediated antagonism [113]. It is clear that IL-6 mediated STAT3 activation is also critical in this phenomenon, as T_{reg} cells from patients with STAT3 mutations are unable to produce IL-17A [86]. Interestingly, STAT-3 signaling is not only essential for the development of Th17-like T_{regs} , it has also been shown to be required in T_{regs} to suppress Th17 immunopathology in mice [172,173]. However, it is unknown whether Th17 like T_{regs} with active STAT-3 signaling, are programmed to engage in distinct effector-specific suppression of Th17 immunopathology in an inflammatory environment.

In the context of Th1-like T_{regs} , T-bet has been implicated in CXCR3 expression and IFN- γ production [70,71]. To delineate whether T-bet in T_{regs} causes IFN- γ dependent Th1 inflammation instead of suppressing it, mice with T_{reg} -specific T-bet ablation were made. These mice do not develop autoimmune diseases at steady state suggesting that T-bet is dispensable for T_{regs} to suppress auto-reactive Th1 cells at steady state [174]. However, T-bet-deficient T_{regs} do not thrive in a Th1 environment and these cells are unable to suppress *Scurfy* effector Th1 cells in a transfer model. It appears that the actions of T-bet expressing T_{regs} at steady state are different from those under inflammatory conditions [54,175].

However, the role of antigen-specific T-bet-expressing T_{regs} and the function of T-bet and IFN- γ in these cells during Th1 immune responses are unknown and require future investigation.

Similarly, Th2 like T_{reg} cells express GATA3, the master transcription factors of Th2 cells [166,174,176,177,178]. Genome-wide pattern of GATA3 binding in various T cells has been profiled through chromatin immune-precipitation followed by high throughput sequencing (ChIP-Seq) analysis. Although GATA3 binds to the CGRE site in the *Il13* distal promoter in regulatory T cells as in Th2 cells, GATA3-expressing T_{regs} do not produce IL-13. It is possible that Foxp3 suppresses the function of GATA3 to induce the expression of Th2 cytokines. In accordance to this tenet, a modest reduction of Foxp3 expression in T_{regs} results in the production of Th2 cytokines in these cells [179]. GATA3 also regulates many Th2-specific genes including *Il1rl1*, which encodes the IL-33 receptor T1/ST2, and chemokine receptor gene *Ccr8* in T_{regs} [180]. Although T_{reg}-specific deletion of GATA3 in mice result in spontaneous autoimmunity in one study [177], three other studies report that mice with T_{reg}-specific GATA3 deletion do not develop any disease up to 6 months of age [166,174,176]. Therefore, GATA3 expression in T_{regs} may not be required to for these T_{regs} to control auto-reactive Th2 cells at steady state. It seems that the suppressive effect of Foxp3 on GATA3 is strong enough to restrain the production of Th2 cytokines in T_{regs}. However, whether GATA3 can overcome the restraining effect of Foxp3 and contribute to production of these cytokines in T_{regs} during a strong Th2 inflammation remains to be seen.

Although T-bet- and GATA3-expressing T_{regs} can be found at steady state, these cells do not represent stable T_{reg} subsets in mice [174]. The expression of T-bet and/or GATA3 in T_{regs} is highly dynamic and is often associated with TCR activation of the T_{regs}. When T_{regs} are stimulated by TCR and/or IL-2 stimulation, they can either up-regulate T-bet or GATA3, each contributing to the suppression of ROR- γ t expression and stabilization of Foxp3 [176]. T-bet-expressing T_{regs} are enriched among CD44^{hi} population, and express higher levels of T_{reg}-specific genes such as GITR and CTLA-4 than T-bet-non-expressing T_{regs} [71]. Interestingly, although specific deletion of either *Thx21* or *Gata3* genes alone in T_{regs} does not alter the functions and phenotype of T_{regs}, combined deletion of both the genes in these cells results in the development of autoimmune-like diseases even at steady state. T-bet-GATA3 double-deficient T_{regs} lose their suppressive functions. Strikingly, this is accompanied by the up-regulation of ROR- γ t expression and down-regulation of Foxp3 expression. Without T-bet and GATA3, Foxp3 expression cannot be maintained in T_{regs}, possibly because of the up-regulation of antagonistic protein, ROR- γ t. Most importantly, T-bet-GATA3 double-deficient T_{regs} also produce IL-17A, suggesting that T_{reg} cells may utilize several cross-regulatory mechanisms that are adopted by T effector cells.

Taken together, a balance between the expression and cross-regulation of hallmark transcription factors T-bet, GATA3 and ROR- γ t in T_{regs} may determine their suppressive functions and pro-inflammatory cytokine production [174]. It is similar to T helper cells whose lineage transcription factors can cross-suppress other lineages during differentiation [181–187]. Similarly, under steady state conditions, a delicate balance between the transcription factors would normally stabilize Foxp3 and suppress the function of effector transcription factors in T_{regs}. However, an inflammatory situation may disrupt this balance

and cause dys-regulation in T_{regs}. Thus inflammation driven activation of one or more effector transcription factors in T_{regs} contributes to the reprogramming T_{regs} into effector-like T_{regs} *in vivo*.

5.2. IRF4

In effector T cells, IRF4 plays a critical role during the differentiation of Th2, Th17 and T follicular helper (Tfh) cells [188]. Although T_{regs} acquire effector Tfh cell characteristics to regulate Tfh and germinal center responses, it is dependent on Bcl-6 and CXCR5 expression but not IRF4 [159,160]. In T_{regs}, IRF4 in collaboration with Foxp3, regulates a subset of T_{reg}-specific genes. It is well known that, to gain full suppressive activity, T_{regs} need to be activated through their TCR [189]. Together with IL-2-mediated STAT5 activation, TCR stimulation induces the activation of several constitutively expressed transcription factors such as NFATs, NF- κ b and AP-1 family members, along with the expression of many transcription factors including Egr1, Egr2 and NFATc1 and IRF4 in T_{regs} [190]. Though all of these transcription factors may contribute to T_{reg} suppressive functions, it is believed that IRF4 is critical for maintenance of T_{reg} functionality. Consistent to this idea, acute ablation of TCR in mature T_{regs}, accompanied by the down-regulation of IRF4 in activated T_{regs}, also induces autoimmune diseases [191,192]. T_{reg} specific deletion of *Irf4* results in severe Th2-related autoimmunity [193]. Since IRF4 is expressed in all activated T_{regs} [35] and IRF4 is involved in optimal T_{reg} activation, it is speculated that the suppression of auto-reactive Th2 cells may require higher T_{reg} suppressive activity than auto-reactive Th1 or Th17 cells. Thus, transcriptional regulatory network and IRF4 activity fueled by continuous TCR stimulation in T_{regs} is essential for maintaining immune tolerance. Whether IRF4 contributes to pro-inflammatory cytokine production in T_{regs} during immune dys-regulation has not been explored.

6. Do pro-inflammatory cytokine producing Foxp3+ T_{regs} retain suppressive capacities?

Induction of Foxp3⁺ effector-like T_{reg} cells under strong pro-inflammatory conditions is either associated with or without the loss of Foxp3 [51,139,194]. In instances where they lose Foxp3 expression, they either fail to control inflammation or even exacerbate pathogenic immune responses [18,194,195]. This raises an interesting possibility that T_{reg} functions are not limited solely to suppression but also to other effector functions that may be largely dependent on cytokine milieu or innate immune responses [79,196,197]. Although a small fraction of T_{regs} themselves loses Foxp3 in Th17 inflammatory conditions *in vitro*, the destabilization is only a transient phenomenon. Adoptive transfer of such T_{regs}, a proportion of which includes the destabilized cells, exhibits delayed but strong potential to suppress Th17 inflammation chronic Th17 IBD model [79]. The transient loss of Foxp3 and/or suppressive functions in T_{regs} is attributed to the inflammatory cytokines in the milieu [138], as they lose their suppressive capacity only upon strong activation in the presence of IL-1 β and IL-6 [8,79]. Withdrawal of these inflammatory cytokines restores the suppressive functions in T_{regs}. This mechanism of transient silencing of T_{regs}, along with the strong Th effector responses might allow subset-specific protective immune responses against microbes to proceed normally in cytokine-rich environments, such as during acute infections

[196,198,199]. Although a small proportion of Foxp3⁺ IL-17A⁺ cells develop in response to *C. albicans* in the oral cavity, the mice clear the infection and immunopathology normally in a Th17 and T_{reg} dependent manner [79,102,200]. Although the fungal clearance and immunopathology required the presence of T_{regs} as a whole, the role of Foxp3⁺ IL-17A⁺ cells in mediating the protection is not known. Although these Foxp3⁺ IL-17A⁺ cells do not cause immunopathology during acute infection, whether these cells contribute to protective functions of T_{regs} remains to be investigated. As the expression of lineage specific transcription factors are required in T_{regs} to control the respective lineages [201], it can be cautiously speculated that RORγt⁺ IL-17A⁺ Foxp3⁺ cells are the Th17-specific regulatory cells that may control infection associated Th17 immunopathology. These data support the possibility that effector like Foxp3⁺ cells found in tissues can retain the suppressive capacities. Thus the accumulation of these pro-inflammatory T_{reg} cells in the inflammatory tissues could be a result of auto-antigen mediated proliferation and activation of T_{regs}. These effector/memory T_{regs} may be actively recruited to the tissue for controlling the immunopathology. This idea is consistent to the data that showed thymus derived regulatory T cells become activated, proliferate and differentiate into memory T_{regs} and serve as more potent suppressors mediating resolution of organ-specific autoimmunity in mice [202]. As pro-inflammatory cytokine producing T_{regs} are known to be enriched among T_{regs} with memory characteristics, it is tempting to speculate that these cells may express the respective inflammatory cytokines as well to establish “regulatory memory”. At least in regards to IBD, RA and multiple sclerosis, the pro-inflammatory cytokine producing T_{reg} cells with memory characteristics are functionally as suppressive as conventional T_{reg} cells. However, one cannot rule out that their increased persistence during chronic inflammation such as psoriasis may contribute to worsening of the immunopathology. The differences in the cytokines, metabolites and commensal diversity in the milieu are likely critical in determining whether T_{regs} may adopt suppressive or non-suppressive modes in different diseases.

7. Concluding remarks and perspective

Pro-inflammatory cytokine producing Foxp3⁺ T_{reg} cells expressing lineage-specific effector transcription factors are extensively documented in various inflammatory diseases. A growing number of recent studies from mouse models reveal that pro-inflammatory cytokines and TLR signaling promote the induction of these cells. The expression of effector transcription factors in T_{regs} does not necessarily imply that they lose their suppressive capacities. In fact T_{regs} require lineage specific effector transcription factors along with Foxp3 to suppress respective lineages and the resulting inflammation [71,173,193]. It appears that T_{regs} may adopt the transcriptional program of lineage-specific effector cells to selectively suppress cells of that same lineage. However, a considerable overlap between the transcriptional program, with a concomitant loss of FOXP3 expression presents the potential for conversion of T_{regs} into effector cells and T_{reg} lineage instability [18]. This is seen in the context of a lethal infection by *Toxoplasma gondii* where T-bet and IFN-γ expressing Foxp3⁺ cells have been associated with immunopathogenesis. Similarly, Th17 like T_{regs} are associated with dys-regulation in psoriasis patients. However in other diseases such as IBD, MS, RA, allergy, candidal infection and cancer, effector like T_{regs} retain their suppressive

properties. These T_{reg} s, whether suppressive or non-suppressive, may be functionally specialized to their local environment that determines their phenotype, suppressive function and pro- and anti-inflammatory cytokine production [1,15]. Therefore the functions of the effector-like $Foxp3^+$ cells is strongly dependent on environmental cues and local pro-inflammatory cytokines, metabolites, and possibly whether they originated from nT_{reg} s, pT_{reg} s or effector cells. Improved understanding of how T_{reg} subsets respond to ranging environmental cues that direct the heterogeneity within these subsets is key to direct efforts to employ these cells for clinical intervention.

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Fig. 1. Origin and functions of pro-inflammatory cytokine producing T_{regs} in various diseases.