Dissecting Emerging Aspects of Regulatory Circuitry in Man and Mice: Regulatory T Cell Biology

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Abstract
Regulatory T cells (Treg), a component of adaptive immunity, are well known for their immunosuppressive roles and their ability to maintain the balance between the immunological and pathological reactions. Treg have been shown to provide protective responses and their depletion has resulted in severe pathology in some pathogen infections. The work presented here has unravelled the potential of regulatory cells in the immune system including different repertoire of Treg cell subsets, markers to distinguish them, Treg suppression mechanisms in the pathogenesis of various infections and summarize different mouse models depleting Tregs. These findings would help set up future avenues of research to elucidate a key mechanism of action of these cells and provide new therapeutic insights for pathogenesis and also for broader antibacterial/antiviral/antiproliferative immunity.

Keywords
Regulatory T Cells, Foxp3, tTreg, pTreg, IL-10, TGF-β, Helios, Neuropilin-1

1. Introduction
A hallmark of the immune system is to maintain a harmonious balance and selection among its different components. B and T cells are selected rigorously for survival during maturation phase. Among the different repertoire of cell subsets, regulatory T cells (Treg) have been established as a developmentally and func-
tionally distinct group that has been recognised as vital for keeping the immune system in check, aiding it to escape from self-targeted pathology and unhindered selection of both T and B cell populations [1] [2]. In order to maintain a delicate balance, immune system is required to respond to pathogens, nevertheless is must tolerate beneficial microbes. It is widely acknowledged that Treg play a major role in minimizing deleterious immune mediated pathology caused by harmful microbes and self-antigens and thus making a significant impact to this balance.

2. Treg

Treg are currently thoroughly investigated for their key role in the maintenance of balance between the immunological and pathological reactions over the body [3] [4]. They are crucial for the retention of self-tolerance and the control of immune responses against pathogenic organisms, tumour antigens as well as allergens [5] [6] [7] [8] [9]. Treg are developmentally and functionally different from conventional T cells. Treg constitute 5% to 10% of CD4+ T cells. In the steady state, they are generated in the thymus and can be induced from naïve CD4+ T cells.

Treg are initially characterized as expressing a CD4+CD25\textsuperscript{high} phenotype [10] [11]. However, an increasing number of markers have been identified that express constitutively on Treg. These include: cytotoxic T lymphocyte antigen 4 (CTLA-4), glucocorticoid-induced tumour necrosis factor receptor family related protein (GITR); TNFRSF18 (GITR), CD39, HLA-DR, CD45RA, OX40, CD127\textsuperscript{lo}, and CD73 [12]-[17]. In fact, none of these surface markers are expressed exclusively on Treg. Moreover, as CD25 is expressed on other activated T cells and there are some Treg in the peripheral tissues which do not express CD25 limiting the use of this marker for Treg [18] [19] [20] [21]. To date the most specific marker identified for the classification of Treg is expression of the transcription factor recognized as forkhead box P3 (Foxp3) [22], which has been exhibited to be expressed specifically in CD4+ T cells. The Foxp3 gene encodes Scurffin, which is identified as a member of forkhead-winged-helix family of transcriptional regulators and is vastly conserved in humans [23]. In mice neither activated CD4+ T cells nor differentiated Th1/Th2 cells express Foxp3 [24] [25]. Foxp3 is found to be expressed almost exclusively by CD4+CD25\textsuperscript{+} T cells in both thymus and periphery [26]. Mutation of Foxp3 causes an aggressive X-linked autoimmune disease in scurfy mice [23] and the human equivalent, immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) [27] [28]. Moreover, forced expression of Foxp3 can transform naïve T cells to Treg [24] [29]. Foxp3-sufficient or Foxp3-deficient mixed bone marrow chimeras study demonstrated that CD4+CD25\textsuperscript{+} cells only developed from Foxp3 sufficient bone marrow suggesting that Foxp3 is indispensable for the development of Treg [24] [30]. Importantly, expression of green fluorescence protein (GFP) under the control of Foxp3 gene showed that Foxp3 expression is limited.
to CD4+ T cells that exhibit suppressive potency [31]. Also, ablation of Foxp3 in Treg results loss of suppressive function and phenotype [32] [33]. Thus, Foxp3 appeared to be a lineage-specification factor of Treg and its expression is indispensable for the development and function of Treg.

### 2.1. Functions of Treg

Preliminary studies with Treg were based on their role in dominant tolerance and development of autoimmune disease. However, a handful of studies indicate that Treg play roles in the development of allergic diseases (reviewed in [34]), in the suppression of anti-tumour immunity [35], during pathogen infection (reviewed in [4]) and in controlling responses to commensal microbes in inflammatory diseases [36]. Treg are well known for their immunosuppressive role of varying immune cells including non-Treg CD4+ T cells [37], CD8+ T cells [38], dendritic cells (DC) [39], B cells [40], Th17 cells [41], natural killer (NK) cells [42], macrophages [43] and mast cells [44] which are activated in response to pathogen (Figure 1).

### 2.2. Different Subsets of Treg

Studies of Treg have identified several lineages of cells with different sites of induction, characterization and to a degree with various mechanisms of action. Treg are largely divided in to two major groups: thymus-derived Treg cell

![Figure 1](image)

**Figure 1.** Diverse functions of Treg. Treg control Th1 type cells which though secretion of IFNγ activate antimicrobial activity of macrophages and cytotoxic activity of CD8 T cells which lyse host cells infected with pathogen. Treg suppress Th2 type activity which through secretion of IL-4, 5 and 6 activates naïve B cells to produce antibodies as well as controlled recruitment of eosinophils. In addition, Treg alter neutrophil activity by inhibiting their recruitment on the epithelial cell membrane.
(tTreg) and peripherally derived Treg cell (pTreg) (Table 1) [45] [46] [47] [48].

2.2.1. Thymus Derived Treg (tTreg)
Treg that develop within the thymus were often referred to as natural Treg cells as opposed to peripherally derived Treg [46]. tTreg arise during the normal process of T cell development in the thymus and are imprinted with regulatory function before being released into the periphery. They help prevent autoimmunity and are resistant to thymic deletion and are non-redundantly required for the establishment of self-tolerance [49] [50]. tTreg are known to suppress effector T cell proliferation in vitro through cytokine independent fashion that is mostly cell contact dependent [51]. The proportion of tTreg appears to be small when any part of this population reacts with antigens [52]. In thymus, tTreg can develop from progenitors to tTreg through tTreg precursor cells [53] [54]. Resting tTreg express CD45RA^Foxp3^low, while activated tTreg express CD45RO^Foxp3^high.

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<th>Cell type</th>
<th>Suggested immunosuppressive mechanisms</th>
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<td>tTreg/nTreg</td>
<td>Require CD28 co-stimulation for their development in thymus</td>
<td>[58] [59] [60]</td>
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<td>IL-2 is important for thymic induction of tTreg</td>
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<td>STAT5 has been demonstrated to increase the in vivo frequency of tTreg</td>
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<td>IL-15 is essential for induction of Foxp3 expression in thymocytes</td>
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<td>c-Rel has been shown to be highly expressed by tTreg</td>
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<td>mTECs are responsible for inducing selection of thymic tTreg</td>
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<td>TGF-β and its receptor signal pathway is essential for the generation of pTreg</td>
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<td>CNS-1, a non-coding region of Foxp3 locus are a crucial regulatory element in the generation of pTreg</td>
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<td>pTreg</td>
<td>IL-2 play a vital part in the differentiation of Foxp3^+ pTreg</td>
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<td>Downstream STAT5-dependent signaling is essential for the differentiation of pTreg</td>
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<td>RA plays a significant role in enhancing the generation of Foxp3 Treg cells in the GALT</td>
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<td>Smad 2 and Smad 3 participate in the pTreg differentiation process</td>
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<td>IL-10 is the major cytokine involved in Tr1 cells differentiation pathway</td>
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<td>IL-27 are the major cytokines involved in the differentiation of Tr1 cells</td>
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<td>c-Maf which activates IL21 facilitates proliferation of Tr1</td>
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<td>IL21, an autocrine growth factor drives the proliferation of Tr1 cells</td>
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<td>ICOS stimulates the IL27-induced differentiation of Tr1</td>
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<td>AhR induced by IL27 shown to be involved in the differentiation of Tr1</td>
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<td>Th3</td>
<td>Th3 mediate their suppressive activity by the production of TGF-β</td>
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<td>Th3 exhibit a mutual relationship with Th17 cells and exert regulatory potentials</td>
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tTreg constitute about 5% - 10% of mouse peripheral CD4 cells which is corresponding to 1% - 2% of human counterpart [1].

tTreg develop as a consequence of high-affinity interactions with MHC class II-peptide and their TCR repertoire is primarily self-reactive [56] [57]. They require CD28 co-stimulation for their development in thymus as mice lacking CD28 have an approximately 80% decrease in the frequency of tTreg [58] [59] [60]. IL-2, a member of γc cytokine family is important for thymic induction of tTreg as mice lacking IL-2Rβ (CD122) develop spontaneous autoimmune diseases which can be blocked by administration of donor Treg [61] [62]. Signal transducer and activator of transcription 5 (STAT5) has been demonstrated to increase the in vivo frequency of tTreg and expression of STAT5 is regulated by IL-2 [63]. IL-15 is also essential for induction of Foxp3 expression in thymocytes [64]. Transcription factor c-Rel, a member of NF-κB family has been shown to be highly expressed by tTreg. c-Rel binds to the conserved non-coding sequence 3 (CNS3) region of Foxp3 locus and in mice deficient in c-Rel, tTreg numbers are markedly reduced [65] [66]. TGF-β has been suggested to be absolutely engaged in Foxp3 induction during tTreg development. However, its role has been controversial in tTreg development [67] [68].

Medullary thymic epithelial cells (mTECs) are responsible for inducing selection of thymic tTreg, also bone marrow derived APCs can facilitate tTreg differentiation [2]. Either mTECs or bone derived APCs alone may be sufficient for the generation of tTreg numbers and both of these subsets can present self-antigens in order to induce development of tTreg [69].

2.2.2. Peripherally Derived Treg Cell

pTreg develop extra-thymically when they are exposed to certain regulatory cytokines which are released during inflammatory conditions, upon encountering cognate antigens and costimulation [70] [71]. The antigen-specific Treg repertoire is more abundant than tTreg population and in contrast to tTreg, they migrate towards the sites of inflammation [72]. pTreg play an indispensable role in establishing peripheral tolerance to commensal microbes in gut and non-pathogenic environmental antigens derived from food and they express predominantly in mucosa associated lymphoid tissues (MALT) including peyer’s patches and lamina propria of small and large intestines [73] [74]. Moreover, pTreg which present only in the placental mammals are involved in the establishment of maternal-foetal tolerance [75]. Their suppressive actions are mostly cell contact independent and depend on the availability of immunosuppressive cytokines [76] [77]. They are known to develop from CD4+Foxp3− effector T cells in the periphery and they apparently have a similar TCR repertoire to that of the effector T cells [78] [79]. In particular, pTreg can convert from CD4+Foxp3− to CD4+Foxp3, thereby expanding the range of Treg specificities to exogenous antigens [80]. Conversely, pTreg can revert to effector T cells, losing expression of Foxp3 under certain situations and thus pTreg are not irreversibly programmed [81].
TGF-β and its receptor signal pathway is essential for the generation of pTreg as diminishing TGF-β receptor signaling blocks the induction of Foxp3 expression and the resulting operational suppressive capability [82] [83] [84]. TGF-β downregulates the expression of growth factor independent 1, a transcriptional repressor that inhibits Treg differentiation [85]. TGF-β also antagonizes DNA methyltransferase 1 (Dnmt1), which inhibits the expression of Foxp3 [86]. CNS-1, a non-coding region of Foxp3 locus are a crucial regulatory element in the generation of pTreg [87]. CNS1, which possesses a TGFβ-NFAT response element are involved in pTreg differentiation in GALT [88].

IL-2 play a vital part in the differentiation of Foxp3+ pTreg as TGF-β fails to induce Foxp3+ pTreg from naïve CD4+CD25− cells in mice deficient in IL-2 [89] [90] [91]. Downstream STAT5-dependent IL2 signaling is also essential for the differentiation of pTreg [92] [93] [94].

Retinoic acid (RA), a vitamin A metabolite plays a significant role in enhancing the generation of Foxp3 Treg cells in the GALT [95]. However, the molecular mechanism by which RA promotes TGFβ-mediated Foxp3 induction has controversies. RA has been demonstrated to enhance Foxp3 expression by promoting the expression of Smad 3, thereby amplifying Foxp3 transcription [96] [97]. However, another study has suggested that even though RA appears to increase Smad 3, it augments Treg cell translation independently of Smad 3 [98].

Smad 2 and Smad 3 participate in the pTreg differentiation process and they are activated through TGF-β signaling pathways by inducing Foxp3 [99] [100]. Smad 2 and 3 induces the expression of TGF-β induced transcription factor, TGF-β-inducible early gene 1 product (TIEG1), which induces Foxp3 and its transcription [96]. In addition, Smad 2 and Smad 3 are also appeared to stimulate differentiation of pTreg though Foxp3-independent pathway [101].

Besides pTreg, there are other types of Treg including Tr1 cells (T regulatory type 1) and Th3 Treg [114] [115]. Tr1 population are disturbed in individuals with prolonged inflammatory conditions like colitis, arthritis and asthma. IL-10 is the major cytokine involved in Tr1 cells differentiation pathway [102]. Once mature, they exert their suppressive activity through the production of large amounts of IL-10 [46]. Usually Tr1 cells do not express Foxp3 constitutively, however, when activated, they can induce Foxp3 expression [116]. There is accumulating evidence that Tr1 cells do not require Foxp3 expression in order to exert their suppressive potency, as Tr1 cells suppress conventional T cells independent of Foxp3 expression [117] [118]. In addition, it is revealed that Tr1 cells may be differentiated from naive T cells in patients with IPEX disease [119]. An essential growth factor for the development of Tr1 is IL-15, which can mediate Tr1 cell differentiation without TCR triggering [103] [104].

IL10 and IL27 are the major cytokines involved in the differentiation of Tr1 cells [105] [106]. IL27 signaling results activation of transcription factors including c-Maf, IL21, and the costimulatory receptor ICOS [105]. c-Maf is the key
factor, which activates IL21 production. IL21, an autocrine growth factor drives the proliferation of Tr1 cells [107]. ICOS stimulates the IL27-induced differentiation of Tr1. Currently, the aryl hydrocarbon receptor (AhR), also induced by IL27, was shown to be involved in the differentiation of Tr1 [108]. c-Maf and AhR perform synergistically to facilitate proliferation of Tr1 [109]. In the case of Tr1, as IL-10 can also be produced by both Th1 (IFNγ+) and Th2 (IL-4+) effectors, definition of this subset is relatively fluid [120].

Th3 cells were first recognized due to their role in immune tolerance after oral ingestion of antigens [110]. Th3 mediate their suppressive activity by the production of TGF-β. Mice deficient in Th3 often develop spontaneous autoimmunity and Th3 exhibit a mutual relationship with Th17 cells [111] [112]. Th17 being proinflammatory are essential in autoimmune related disorders [121] [122]. No specific surface marker has been identified so far for Th3 cells, though Th3 is induced by Foxp3 expression.

Apart from Foxp3-expressing Treg there are other functional regulatory cells, which are involved in IL-35 production (Tr35) [113]. While Treg are commonly found to be CD4+, CD8+ T cells might express Foxp3 and produce the same suppressive cytokines [123].

2.2.3. Markers to Distinguish tTreg from pTreg

The tTreg vary in functionality from pTreg, however, the major problem in exploring the relative function of these two subsets is the lack of markers to distinguish the two populations.

Helios, a T cell restricted member of Ikaros family transcription factor has been demonstrated to be useful for distinguishing these two Treg populations [124] [125]. Helios is preferentially expressed by tTreg, whereas Treg generated in vitro and in vivo are negative for Helios expression [124]. However, others have described that pTreg can express Helios as when T cells were stimulated with irradiated splenocytes, more than half of the pTreg expressed Helios [126]. In addition, Helios is also expressed on Th2 and T follicular helper cells and is related to the differentiation of these cells [127]. It is reported that activated Foxp3 T cells also express helios and in fact a marker for Treg activation [128]. Also expression of Helios has been shown to be associated with T cell tolerance in both thymus and periphery [129]. Thus Helios does not appear to be a marker to distinguish pTreg from tTreg.

Neuropilin-1 (Nrp1) expression, a neuronal receptor of the class 3 semaphorin subfamily and a co-receptor for vascular endothelial growth factor A, offers another biological marker to distinguish tTreg from pTreg [130] [131]. In contrast to low levels of Nrp1 expressed on pTreg, majority of tTreg express Nrp1 [130]. However, others have demonstrated that Treg generated extrathymically in the central nervous system during a spontaneous model of EAE were Nrp+ [125]. In addition, Nrp1 is not a marker for human Treg [132]. Thus Nrp1 appeared to be an imperfect marker to distinguish pTreg from tTreg.
Lap, a component of latent TGFβ and Garp (Lrrc32), a membrane anchoring molecule that binds to latent TGFβ, were found to be expressed on the surface of Treg [133]. While Lap and Garp expression selectively identifies tTreg that represent a stable subset with highly potent suppressive ability, pTreg fail to express surface Lap or Garp [134]. Recently transcription factor Krüppel-like factor 2 (KLF2) has been demonstrated for the generation of pTreg [135]. KLF2 is not required for the generation of tTreg and is only necessary for pTreg development. In addition, it has been exhibited that drugs that block KLF2 proteolysis during T cell activation augment pTreg development.

2.3. Mechanism of Suppression of Treg

To date a number of mechanisms have been associated with the suppressive action of Treg suppression. Treg execute their suppressive function rapidly when they are activated via the T cell receptor, either specifically by its natural class II ligand, or by foreign antigens that are cross-reactive to self-antigen receptors in the periphery [136].

IL-10 and TGF-β are potent immune-suppressants that facilitate Treg to inhibit Th1 inflammatory responses. IL-10 has been illustrated as the key components in the suppressive function of Treg (Figure 2) [137]. Secretion of IL-10 by Treg cells has been shown to be essential for the prevention of experimental

![Figure 2](image.png)

**Figure 2.** Potential mechanisms used by Treg cells. Illustration of various Treg cell suppressive mechanisms based on three basic modes of action: A. Inhibitory cytokines consists of TGFβ, IL-35 and IL-10. B. Modulation of DC includes mechanisms that influences DC maturation and function such as LAG3, MHC-II-mediated suppression of DC maturation, and CTLA4–CD80/CD86-mediated induction of IDO, which is an immunosuppressive molecule made by DCs. C. Cytolysis consists of granzyme A and granzyme B dependent and perforin pore dependent killing mechanisms. The concept of the figure emerged from [168].
autoimmune encephalomyelitis [138], colitis [139] [140], airway allergic response in lung [141] and skin hypersensitivity [142].

TGF-β, a well-recognized inhibitory cytokine, however, the function of TGF-β in Treg mediated suppression has some controversies (Figure 2). Mice with a T-cell specific deletion of tgfβ1 gene developed lethal immunopathology and could not inhibit inflammatory bowel disease in a transfer model [143]. TGF-β was found to be the major mechanism of suppression of prostate tumour infiltrating CD8 cells [144].

Membrane-tethered TGF-β expressed by Treg cells has also been demonstrated by Treg cell mediated suppression [145] [146]. However, another study has demonstrated conflicting findings that membrane-tethered TGF-β is dispensable for Treg function [147].

Another cytokine, IL-35, a member of IL-12 family has also been attributed to the in vitro and in vivo suppressive potency of Treg [148]. IL-35 consists of Epstein-Barr virus induced gene 3 (Ebi3) and IL-12α/p35, both are highly expressed by Treg but not by naïve or activated T cells [113] [149]. Treg cells from Ebi3 and IL-12α-deficient mice have a reduced suppressive capability in vitro and fail to cure inflammatory bowel disease in vivo [148].

There is evidence that Treg can indirectly suppress other cells through modulation of DC function (Figure 2). Treg cells suppress the capacity of DCs to activate T cells by down-regulating costimulatory molecules CD80 and CD86 [150] [151]. A handful of molecules expressed at high levels in Treg have been identified which facilitate the blockade of DC maturation and DC mediated activation of effector cells. Among them the most well-known is CTLA4. CTLA4 a member of CD28/B7 family and CTLA4 mediated immunosuppression has been demonstrated in several settings including autoimmune diseases and different tumour types [152] [153]. Mice with a Treg cell specific deletion of CTLA-4 develop systemic autoimmunity and CTLA4-deficient mice inhibit upregulation of CD80 and CD86 even in the presence of strong DC maturation stimuli [152] [154] [155] [156]. Moreover, anti-CTLA4 inhibits the ability of Treg cells to suppress colitis [154].

Treg cells can induce the expression of indoleamine 2, 3-dioxygenase (IDO) in DCs in a CTLA4-dependent manner [157]. IDO is a potent immunosuppressive enzyme which induces catabolism of tryptophan in to pro-apoptotic metabolites, resulting in the suppression of effector cells [158]. Ligation of CTLA-4 to CD80 and CD86 induce DCs by activating transcription factor Foxo3, which down-regulate production of IL-6 and TNFα by DCs [159].

Other molecules expressed by Treg that can affect the function of DCs are: Lymphocyte activation gene-3 (LAG-3) [160] [161], immunoreceptor tyrosine-based activation motif (ITAM) [162] and Fibrinogen-like protein 2 (FGL2) [163]. Treg cells from LAG-3 deficient mice had a decreased suppressive activity and anti-LAG-3 blocked suppressive activity of Treg cells both in vitro and in vivo [160] [164].
Treg cells can suppress DC maturation by inhibiting down-regulation of MARCH1 and upregulation of CD83 [165]. MARCH1, a membrane bound E3 ubiquitin ligase, was found to degrade CD86 and MHC-II on DC by directing them to the late endosomal or lysosomal compartment [166]. CD83 is accompanied by DC maturation and it inhibits the action of MARCH1 [167]. Treg use IL-10 to influence MARCH1-CD83 pathway.

There is growing evidence that Programmed death 1 (PD-1) pathway plays a role in Treg mediated suppression [169] [170]. PD-1 is expressed predominantly on exhausted CD8 cells and PD-L1, the ligand for PD-1s expressed on Treg and tumour cells [171]. In a BL/6 model, Treg from tumour draining lymph node suppress via PD-1/PD-L1 pathway. In samples of T cells taken from melanoma patients, PD-1 blockade was found to enhance effector T cell proliferation and inhibit the suppressive function of PD-L1 expressing Treg [172]. Treg have also been shown to mediate their suppressive activity through Fas-FasL interaction resulting in apoptosis of B cells [173].

Treg are able to mediate immune suppression by triggering direct cytolysis of target cells [174] [175] [176]. Granzyme and perforin pathway are two suggested mechanisms for Treg mediated immune suppression (Figure 2). Treg cells from mice deficient in granzyme B had a reduced suppressive activity [177]. Also granzyme B deficient mice were able to clear tumour more effectively than wild type mice since Treg cells inhibit anti-tumour immunity in a granzyme B and perforin dependent manner [178].

Another mechanism of suppression is the induction of metabolic disruption in target cells. Treg can be multiplied significantly by TCR stimulation in presence of high concentrations of IL-2 [179] [180]. As IL-2 is secreted by Th1 type cells, and elevated level of IL-2 acts as a negative-feedback mechanism thereby halts the effector T cell activity by the expanding Treg [181].

Treg has been found to exert metabolic disruption in target cells by their expression of ectoenzymes CD39 and CD73. Treg from CD39 deficient mice have reduced suppressive functions both in vitro and in vivo [182]. CD39 and CD73 convert proinflammatory nucleotides to anti-inflammatory adenosine and these ectoenzymes catalyse the generation of perinuclear adenosine from extracellular ATP or ADP [183]. Adenosine generated in this process then suppresses effector T cells via binding to adenosine A2A receptor.

IL-17 and IFN-γ has been shown to modulate the suppressive capacity of Treg on Th2 immune responses [184] [185].

IRF4 a transcription factor responsible for the differentiation of Th2 cells has been shown to play a role in the in Treg mediated suppression of Th2 responses. Mice wherein irf4 is depleted in Treg developed a lymphoproliferative disease that occurs due to a selective dysregulation of Th2 cells [186]. T-bet, a transcription factor is indispensable for Th1 cell differentiation. It has been demonstrated that Treg mediated suppression of Th1 is regulated by Treg expression of T-bet [187]. Treg mediated suppression of Th17 responses is regulated by Treg expres-
sion of STAT3. Selective ablation of STAT3 in Treg cells results selective uncontrolled Th17 dependent pathology of intestinal mucosa [41]. Other researchers have described a subset of Foxp3+Treg that express the Tfh cell master transcriptional regulator, Bcl-6/Blimp-1 and accumulation at sites of B cell germinal centre responses where they function to control these reactions [188] [189] [190] [191].

2.4. Depletion of Treg Using Mouse Model

A number of different methods to deplete Treg depletion have been used to study their function and variable degrees of depletion have been seen. The two commonly used methods are: antibody depletion method and transgenic mouse model depletion. With the recognition of the IL-2 receptor CD25 on Treg, antibodies specific to CD25 have been developed. About 65% - 70% of Treg populations are depleted following introduction of anti-CD25 antibody clone i.e., PC-61 [192]. Also, a population of CD25- Foxp3+ Treg cells cannot be depleted using PC61. Importantly, expression of CD25 is induced on activated conventional CD4+T cells [18] [19] [20] [21].

To deal with the issue of in vivo function of Treg in immunopathology, mice with fully depleted Treg have been engineered. These mice permit selective ablation of Foxp3+ Treg while without impairing CD25+ effector T cells. The genetic introduction of human diphtheria toxin receptor (DTR) targeted to a specific murine cell type has become a prevailing tool to selectively ablate Treg upon DT injection [193]. Rudensky and colleagues generated a knock-in mice called Foxp3DTR mice in which human DTR is introduced in to the 3’ untranslated region of Foxp3 which allow specific depletion of Treg by injecting DT. This model showed more than 97% depletion of Foxp3+ cells after DT administration [194]. However, these mice subjected to DT succumb to catastrophic autoimmune disease within 2 - 3 weeks, as a result of massive expansion of diverse array of immune cells.

Suffner et al generated a novel set of BAC (Bacterial Artificial Chromosome) transgenic mouse, called Foxp3.LuciDTR mouse, in which Treg express luciferase and the human DTR [195]. They developed several founder lines with different degrees of Treg depletion such as Foxp3.LuciDTR-3 and Foxp3.LuciDTR-4 and Foxp3.LuciDTR-5. Mice from lines Foxp3.LuciDTR-3, Foxp3.LuciDTR-4 and Foxp3.LuciDTR-5 exhibited ~75%, 90% and >95% depletion of Tregs, respectively, while Foxp3.LuciDTR-5 mice did suffer from wasting disease due to autoimmunity. Lahl and Sparwasser [193] produced another BAC technology based transgenic mouse model known as DEpletion of REGulatory T cell (DEREG) mice, which express a DTR fused with GFP protein under the control of an additional Foxp3 promoter, allowing efficient depletion of Foxp3+ Treg by DT injection. Depletion in DEREG mice led to ablation of Foxp3+ Treg to 95% - 98% [196].

3. Concluding Remarks

There are complex dynamic suppression processes controlling other cells by
Treg and that involve various inhibitory cytokines, DC function and cytolysis. These mechanisms are different depending upon Treg cell subtypes and the nature of the pathogens. Also they can act together or independently, according to the requirements of the immune system and homeostasis maintenance, or during the progression of various pathological processes. This gives two layers of reinforcement of Treg function: the suppressive effects of Treg and protective potential of Treg against different pathogens and they also alter the functional Treg pool in response to tissue and inflammatory cues. Together, these data suggest Treg mediated suppression might be a valuable component in different pathogens, which might provide novel therapeutic approaches for vaccination against them. Further investigations are required on Treg in different type of infection models in order to extend the current understanding on the salient features of these cells, their either beneficial or detrimental role during infection and their mechanisms participating to the immunity against various pathogens.

Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

References

ripheral Blood. The Journal of Immunology, 167, 1245-1253. https://doi.org/10.4049/jimmunol.167.3.1245


[86] Li, C., Ebert, P.J. and Li, Q.J. (2013) T Cell Receptor (TCR) and Transforming Growth Factor Beta (TGF-Beta) Signaling Converge on DNA (Cytosine-5)-Methyltransferase to Control Forkhead Box Protein 3 (Foxp3) Locus Methylation and Inducible Regulatory T Cell Differentiation. *The Journal of Biological Chemistry*, 288, 19127-19139. [https://doi.org/10.1074/jbc.M113.453357](https://doi.org/10.1074/jbc.M113.453357)


of FOXP3 Mutations in Patients with IPEX Syndrome. European Journal of Immunology, 41, 1120-1131. https://doi.org/10.1002/eji.201040909


European Journal of Immunology, 39, 1466-1471.
https://doi.org/10.1002/eji.200839040

https://doi.org/10.3389/fimmu.2013.00253


https://doi.org/10.1073/pnas.1323493111

https://doi.org/10.1084/jem.20052056

https://doi.org/10.1016/j.coi.2009.09.011


https://doi.org/10.1084/jem.190.7.995

https://doi.org/10.1016/S0896-8411(03)00045-3

https://doi.org/10.4049/jimmunol.178.3.1433

https://doi.org/10.1016/j.immuni.2008.02.017


Abbreviations

**CTLA**-**4**, Cytotoxic T lymphocyte antigen 4  
**DEREG**, Depletion of regulatory T cells  
**DTR**, Diphtheria toxin receptor  
**DT**, Diphtheria toxin  
**EAE**, Experimental autoimmune encephalomyelitis  
**Ebi3**, Epstein-Barr virus induced gene 3  
**Foxp3**, Forkhead box P3 protein  
**Foxo**, Forkhead box O transcription factor  
**ICOS**, Inducible T cell costimulatory  
**IDO**, Indoleamine 2, 3-dioxygenase  
**IPEX**, Immune dysregulation, polyendocrinopathy, enteropathy X-linked  
**IRF**, Interferon regulatory factor  
**ITAM**, Immunoreceptor tyrosine-based activation motif  
**KLF2**, Krüppel-like factor 2  
**LAG-3**, Lymphocyte activation gene 3  
**LAP**, Latency associated peptide  
**MARCH1**, Membrane-associated E3 ubiquitin ligase RING-CH1  
**mTECs**, Medullary thymic epithelial cells  
**NFAT**, Nuclear factor of activated T cells  
**Nrp1**, Neuropilin-1  
**nTreg cells**, Naturally occurring regulatory T cells  
**pTreg**, Peripherally derived Treg cell  
**PD-1**, Programmed death 1  
**PD-L**, Programmed death 1 ligand  
**RA**, Retinoic acid  
**STAT**, Signal transducer and activator of transcription  
**T-box**, T-box expressed in T cells  
**TCR**, T cell receptor  
**Tfh cell**, T follicular helper cell  
**TGFβ**, Transforming growth factor β  
**TIEG1**, TGF-β-inducible early gene 1 product  
**TNF**, Tumour necrosis factor  
**TNFRSF18**, Tumour necrosis factor receptor superfamily 18  
**Tr1**, T regulatory type 1  
**Treg**, CD4+Foxp3+ Regulatory T cell  
**tTreg**, Thymus derived Treg cell