

# Regulatory T cells

**EDITOR'S COMMENT:** One of the most important papers of 2004 was that from David Hafler's group (Viglietta 2004 J.Exp.Med. 199: 971) showing that the basic immunological defect in multiple sclerosis is impaired function of a new type of cell: the "regulatory T cell". Post-doc scientist, Vicki Robertson, summarises what is known about this novel class of immune cells.

In the late 1960s and 1970s it was proposed that T cells could act as suppressor cells to suppress the immune response by producing soluble factors.<sup>1</sup> The existence of a dedicated population of 'suppressor' T cells was the subject of significant controversy among immunologists for many years, and by the 1980s the mechanisms behind T cell suppression had failed to be characterised by both molecular and biochemical studies. The discovery of T helper 1 (Th1) and Th2 cells led immunologists to believe that suppression was achieved through counter-regulatory cytokines and not by a distinct subset of T cells; thus the field of suppressor T cells was widely discredited. In 1993, Green and Webb even went as far as describing suppressor cells as "the nearest thing we have to a dirty word in cellular immunology".<sup>2</sup>

Over 30 years ago a series of experiments completed by Nishizuka and Sakakura<sup>3</sup> corroborated the existence of regulatory cells (Tregs) as we know them today. These experiments demonstrated that mice thymectomised between day two and four of age developed organ specific autoimmune disease which could be prevented by 'adding back' syngeneic T cells obtained from adult thymus and spleen.<sup>3</sup> However, until recently it has been difficult to distinguish Tregs from effector cells due to the lack of a robust expression marker.<sup>4</sup> Current interest in Tregs was revived in 1995 by Sakaguchi et al<sup>5</sup> who characterised the cells responsible for the prevention of organ specific autoimmunity. Sakaguchi et al showed that these regulatory cells are CD4+ T cells which express the CD25 antigen (interleukin-2 receptor  $\alpha$ -chain), the transcription factor forkhead box P3 (FoxP3), and protect against autoimmunity in murine models of gastritis and thyroiditis.<sup>5</sup>

Now Tregs are regarded as separate lineages of cells with specific differentiation patterns and distinct functions, which regulate peripheral self-tolerance by suppressing the activity and expansion of autoreactive T cells. Several different subsets of Tregs have been characterised and described in the literature but they can generally be divided into two groups: Naturally occurring/innate Tregs (expressing CD4+CD25+ and FoxP3) and adaptive/inducible Tregs (Th3 and Tr1 cells) (Figure 1).

## Naturally occurring Tregs

Naturally occurring Tregs are the most widely studied population of regulatory cells and constitute 8-12% of the CD4+ T cell pool in mice and 2-5% of CD4+ T cells isolated from human peripheral blood. Naturally occurring Tregs express high levels of the CD25 antigen, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), glucocorticoid-inducible tumour necrosis factor receptor (GITR) and FoxP3.<sup>6</sup> Whilst FoxP3 is currently considered the best marker for Treg identification, low expression of FoxP3 has been detected in CD4+CD25- populations and in CD8+ T cells. 'Knock in' experiments in mice have shown that FoxP3 is highly specific for Tregs and it was thought that low levels of FoxP3 detected in CD4+CD25- was likely to be due to contaminating FoxP3+ Tregs in these cell populations.<sup>7</sup> However, there have been recent data to suggest that FoxP3 is expressed transiently by activated non-suppressive CD4+CD25- T cells in humans<sup>8</sup> and there have been many reports of CD4+CD25+ Tregs being induced in the periphery in response to antigen from naive CD4+CD25- cells. To date no distinguishing features, either phenotypic or functional, have been found between these cells or naturally occurring Tregs.<sup>9</sup>

Clonal deletion of self-reactive T cells in the thymus is a major mechanism of immunologic self-tolerance, although it is uncertain how much of T cell self-reactivity can be removed by thymic negative selection, especially T cells which have T cell receptors (TCRs) for self-antigens, expressed outside the thymus.<sup>6</sup> Tregs are generated in the thymus during negative selection<sup>10</sup> and have TCRs specific for self-antigens with an intermediate affinity so that they are not deleted. Shevach et al



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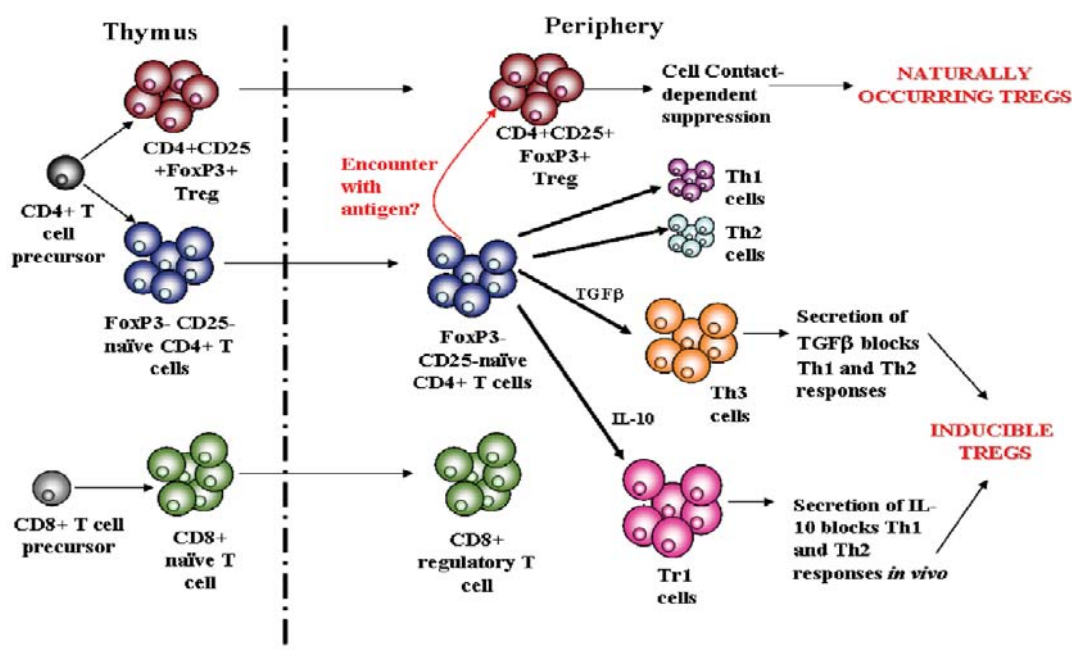


Figure 1: The development of regulatory T cells. Naturally occurring Tregs are produced in the thymus during negative selection, express CD25 and FoxP3 and mediate suppression via cell contact-dependent mechanism. CD4+CD25-FoxP3- can differentiate into Tregs in the periphery that are phenotypically and functionally identical to naturally occurring Tregs, possibly through encountering an antigen. CD4+ naive T cells can differentiate into both Th1 and Th2 cells, however if stimulated with high levels of either TGFβ or IL-10 they differentiate into Th3 or Tr1 cells respectively which exert suppression via the secretion of cytokines. CD8+ T cells can also differentiate to have a regulatory function.

have proposed that Tregs go through a further process, which they term altered negative selection. This results in their TCR signal transduction process being permanently altered so that Tregs leave the thymus in a partially incapacitated state, and are precommitted to function as regulatory cells when they encounter their target antigen in the periphery.<sup>6</sup> The survival of Tregs in the periphery is dependent on them encountering their target antigen and on the presence of interleukin-2 (IL-2); IL-2 knock-out mice do not have Tregs.<sup>11</sup> In humans, only Tregs expressing high levels of CD25 are considered to be regulatory as intermediate levels of the CD25 antigen are expressed by activated T effector cells;<sup>5</sup> however, the functional role of CD25 in T cell mediated immunoregulation is, as yet, unknown.

Naturally occurring Tregs are anergic in vitro when stimulated via their TCR or with IL-2 alone; however, stimulated with a combination of anti-CD3 antibodies and IL-2, or IL-15 they proliferate vigorously, but this abolishes their suppressive properties.<sup>12,13</sup> In vivo Tregs are capable of clonal expansion and proliferate in an MHC class II-dependent manner in response to an antigen and lymphopaenia whilst retaining their suppressor function.<sup>14,15</sup> Naturally occurring Tregs mediate their suppression in a cell-contact dependent, cytokine-independent manner,<sup>6</sup> and suppression can be abrogated in vitro by the addition of high concentrations of IL-2 to the culture. Suppression is accompanied by cell cycle arrest and is mediated via the inhibition of IL-2 production by effector cells at the mRNA level, although the mechanisms behind this process have yet to be defined.<sup>6</sup> Suppression mediated by naturally occurring Tregs is, however, insensitive to IL-10 and TGF- $\beta$  blockade, although there have been some recent reports to suggest that TGF- $\beta$  may be required to sustain FoxP3 expression in human Tregs.<sup>16</sup> Cross-linking of CTLA-4 to CD80 and CD86 on the surface of activated T effector cells and antigen presenting cells (APCs), which results in Tregs secreting TGF- $\beta$ , is thought to be one mechanism by which Tregs exert suppression.<sup>17</sup> Blocking CTLA-4 with antibodies abrogates Treg-mediated suppression.<sup>6,18</sup> In addition to suppression, activated human Tregs can directly kill activated CD4+ and CD8+ T cells, dendritic cells and activated B cells in a perforin-dependent but Fas/FasL-independent manner.<sup>19,20</sup>

### Adaptive Tregs

Adaptive Tregs are generated in the periphery, and require IL-2 for their survival and are thought to suppress immune responses by releasing anti-inflammatory cytokines such as IL-10 and TGF $\beta$ . The two most commonly studied adaptive Tregs are Type 1 regulatory T cells (Tr1) and Th3 cells.

#### Tr1 cells

Tr1 cells resemble naturally occurring Tregs in many ways and are essential for the maintenance of peripheral tolerance. The role of Tr1 cells is in the regulation of immune responses

in transplantation, allergy, and autoimmunity. Tr1 cells are inducible from naïve cells both in vivo and in vitro, and exposure to high levels of IL-10 is required for their generation.<sup>21</sup> Whilst having a low proliferative capacity, Tr1 cells can be expanded in the presence of IL-2 and IL-15 without the need for TCR activation.<sup>22</sup> Antigen specific Tr1 cells do need to be activated via their TCR as well as by IL-10, but once activated they can mediate bystander suppression against other antigens, probably regulated by the local production of IL-10 and TGF- $\beta$ .<sup>23</sup> IL-10 downregulates the expression of costimulatory molecules and pro-inflammatory cytokines produced by APCs and directly inhibits IL-2 and TNF- $\alpha$  production by CD4+ T cells,<sup>24</sup> whilst TGF- $\beta$  downregulates the function of APCs and inhibits cytokine production and proliferation of T cells.<sup>25,26</sup> Once fully differentiated Tr1 cells produce large amounts IL-10 themselves as well as TGF- $\beta$  and IL-5. Tr1 cells can be easily distinguished from Th1 and Th2 cells as they produce interferon- $\gamma$  (IFN- $\gamma$ ) at a level of at least one log lower than Th1 cells,<sup>21</sup> low amounts of IL-2, and IL-4 is undetectable.<sup>21</sup>

Tr1 cells are largely found in the gut where their primary role is thought to be the induction of tolerance to the large number of antigens that pass through an animal's intestine as part of their diet. Induction of Tr1 cells in the gut is as a result of priming by specialised APCs such as dendritic cells. Mice deficient in IL-10 suffer from inflammatory bowel disease, however adding back IL-10 into IL-10-deficient mice transiently cures this inflammation.<sup>27</sup> Unlike naturally occurring Tregs, Tr1 cells do not express high levels of CD25 or FoxP3 but do express high levels of the IL-15 receptor- $\alpha$  chain. Tr1 cells mediate their suppression through a cell contact-independent mechanism by the secretion of IL-10 and TGF- $\beta$  to suppress both memory and naïve T cell responses, both in vitro and in vivo.<sup>21,28</sup> However, Tr1 cells can take on a cytotoxic function when induced by anti-CD3/CD46 resulting in apoptosis in target cells by the production of perforin and granzyme B in a CD18 dependent manner.<sup>19</sup> Tr1 cells have also been shown to suppress B cell immunoglobulin production and can modulate the antigen-presenting capacity of monocytes and dendritic cells.<sup>29</sup>

#### Th3 regulatory cells

Th3 regulatory cells were first discovered during experiments to investigate oral tolerance.<sup>30</sup> These cells are dependent on the costimulation of the TCR and TGF- $\beta$  signalling for their development, and once differentiated secrete TGF- $\beta$ , IL-4, IL-10 and provide help for IgA production.<sup>31</sup> Th3 cells are generated from gut associated lymphoid tissue in the presence of high levels of TGF- $\beta$ , primarily after the ingestion of a foreign antigen. It has also been proposed that Th3 development can be induced by the presence of IL-10 with the simultaneous inhibition of IL-12 to downregulate the development and maturation of Th1 cells which can inhibit Th3 expansion.<sup>31</sup> Th3 cells express CTLA-4 on their cell surface and it is the stim-

ulation of CTLA-4, which results in TGF- $\beta$  secretion. Upon stimulation by TGF- $\beta$ , Th3 cells also up regulate CD25 and FoxP3; however, unlike naturally occurring Tregs, the main mechanism of Th3 cell-mediated suppression is the production of TGF- $\beta$  which suppresses the proliferation of both Th1 and Th2 cell subsets. Oral administration of myelin basic protein (MBP) in SJL/J mice has been shown to induce tolerance and Th3 cells taken from MBP tolerised mice inhibit the proliferation and cytokine secretion of MBP-specific Th1 cells and suppress the development of experimental autoimmune encephalitis (EAE); the rodent model of multiple sclerosis (MS).<sup>31</sup> Suppression of EAE by Th3 cells is abrogated by injection of anti-TGF- $\beta$  antibodies.<sup>30</sup> Unfortunately, a trial of oral bovine myelin in the 1990s in humans with multiple sclerosis was negative; thankfully, the myelin was not sourced from British beef.

### CD8+ Tregs

Recently, a CD8+ T cell population with suppressive properties has been identified. It is thought that these cells can have a suppressive effect on both activated CD4+ cells and B cells through an interaction that is dependent on target cells expressing the HLA class Ib Molecule, Qa-1. The human homologue of Qa-1 is HLA-E and it is proposed that Qa-1 restricted cells expand as part of the primary immune response to inhibit the expansion of autoreactive CD4+ cells and consequent autoimmunity.<sup>32</sup> Experiments in animals have suggested an important role for suppressor CD8+ cells in the protection against disease recurrence and exacerbation in MS.<sup>32</sup> Tr1-like CD8+ cells have also been described. The induction of these cells is IL-10-dependent and once differentiated these CD8+ T cells secrete high levels of IL-10, are anergic and have a suppressive function.<sup>33,34</sup>

### Tregs and Autoimmune Disease

A breakdown in the homeostasis of the immune system is a hallmark feature of autoimmune disease. There has been wide interest in the role of Tregs in various autoimmune diseases. Clinically, loss of function mutations in the FoxP3 gene is strongly linked to immune dysregulation.<sup>35</sup> In mice this results in multiple organ autoimmunity and uncontrolled lymphoproliferation,<sup>12</sup> whilst in humans it triggers a syndrome of lymphoproliferation and myeloproliferation, autoimmunity and allergic dysregulation.<sup>36</sup>

In autoimmune diseases such as systemic lupus erythematosus (SLE), juvenile idiopathic arthritis (JIA), and Kawasaki disease, naturally occurring Treg numbers have been reported to be lower than in healthy individuals, and these lower Treg numbers correlate with either a higher disease activity or a poorer prognosis. It is hypothesised that a reduced number of Tregs results in a shift in balance between Tregs and pre-inflammatory T cells and so there is a breakdown in tolerance. No difference in Treg numbers has been observed in other autoimmune diseases such as spondyloarthritis, myasthenia gravis,

immune-mediated Type 1 arthritis or MS. However, Tregs have been shown to have impaired function, in *in vitro* assays, in myasthenia gravis<sup>37</sup> and in MS<sup>38</sup> compared with healthy controls, and there is evidence to suggest that Tregs from MS patients have reduced FoxP3 expression which correlates to reduced suppressive function.<sup>39</sup>

Th3 regulatory cells are also clearly important in protecting against autoimmunity. It has been shown that abrogation of TGF- $\beta$  differentiation leads to autoimmune disease<sup>40</sup> and that TGF- $\beta$  plays a part in natural resistance to EAE in rats.<sup>41</sup> Thus, Th3 cells are an important regulatory cell type through which TGF- $\beta$  exerts suppression of immune responses to self.

Tr1 cells were first implicated in the modulation of T cell mediated disease when it was observed that patients with severe combined immunodeficiency (SCID) could be transplanted with HLA-mismatched allogeneic stem cells and did not develop GVHD. These patients had high levels of IL-10 in their plasma and had high numbers of donor derived T cells, which were producing the IL-10. High numbers of these donor derived cells correlated to a low incidence of GVHD after allogeneic HSC transplantation in cancer patients.<sup>42</sup>

While the immunosuppressive function of regulatory T cells prevents the development of autoimmune disease, it is not desirable during immune responses to infectious microorganisms. Current hypotheses suggest that upon encounter with infectious microorganisms the activity of regulatory T cells may be downregulated, either directly or indirectly, by other cells to facilitate elimination of the infection. Experimental evidence from mouse models suggests that some pathogens may have evolved to manipulate regulatory T cells to immunosuppress the host and so potentiate their own survival. For example, regulatory T cell activity has been reported to increase in several infectious contexts, such as retroviral infections and various parasitic infections including *Leishmania* and malaria.<sup>43,44</sup>

Tregs are also pose a problem in the context of tumour immunity. CD8+ cells have shown to be potent mediators of anti-tumour immunity, however they require CD4+ T cell help for CD8+ T cell activation, function and survival, but Tregs suppress the anti-tumour properties of CD8+ cells by invoking immunological tolerance to self antigens.<sup>45</sup>

**Summary**

Tregs are essential components of the immune system and self-tolerance. A dysregulated Treg response can lead to a severe or even fatal immunopathology. It is clear that all types of Tregs are inter-dependent on one another to achieve regulation, maintain self-tolerance, and prevent autoimmunity. It is likely that naturally occurring Tregs are recruited at the beginning of an immune response to control the degree of response, but that adaptive Tregs, which are induced after repeated antigen stimulation, act later to diminish the immune response and re-establish homeostasis. To date, all the evidence sug-

gests that Tregs have the potential to be used therapeutically as targets to enhance tumour immunotherapy or to manage autoimmune disease and aid transplantation tolerance.<sup>15</sup>

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