T Lymphocytes: Regulatory

Zoltan Fehervari, *Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan*

Shimon Sakaguchi, *Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan*

CD4+ regulatory (Treg) T cells play a central role in the immune system by potently controlling the responses of other immunocytes. Their activity appears to be essential not only for the maintenance of immunological self-tolerance but potentially also for the control of all physiological immune responses whether against normal self-proteins, microbes or cancerous cells. A fuller understanding of these cells should allow us to effectively exploit their suppressive functions for clinical benefit.

Advanced article

Article Contents

Online posting date: 15th December 2008

Introduction

During T cell thymic development the random nature of T cell receptor (TCR) generation inevitably leads to the appearance of autoreactive clones. The vast majority of these potentially deleterious cells are however purged in the thymus by central tolerance through a process called negative selection. As efficient as negative selection is, there remains abundant evidence from both humans and animals, of autoreactive cells ''slipping through the net'' of central tolerance and into the periphery wherein they represent a potentially nocuous autoimmune hazard. The existence of such autoreactive cells can be readily demonstrated in animals by the elicitation of destructive autoimmunity following the injection of self-antigen plus a strong adjuvant. The fact that perfectly healthy individuals can harbour such harmful autoreactive cells implies the existence of effective tolerance mechanisms operating outside the thymus. Experimental evidence has indeed revealed numerous avenues by which peripheral tolerance is established and maintained, e.g. immune ignorance, peripheral deletion or anergy, or the action of regulatory T cells (Treg). Treg cells have proven to be a very dynamic area of research and recent years have provided compelling evidence for the critical roles played by this cell type in the control of perhaps all physiological immune responses. Both CD4+ and CD8+ Treg cells have been identified, and which appear to differ in their origin and mode of action. This article will focus on CD4+ Treg cells. **See also**: Autoimmune Disease; Immunological Tolerance: Mechanisms; Lymphocyte Development; Lymphocytes: Intraepithelial; Natural Antibodies

ELS subject area: Immunology

How to cite:
Fehervari, Zoltan; and, Sakaguchi, Shimon (December 2008) T Lymphocytes: Regulatory. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0004029.pub2

Treg Cells: A Definition

A consistent molecular description of Treg cells proved to be one of the early stumbling blocks in the study of these cells. Thankfully since the mid-1990s an accurate definition of Treg cells has begun to emerge; however, it should be emphasized that a completely unambiguous definition of Treg cells remains elusive. Broadly speaking, T cells with immunoregulatory properties can be divided into two types: the so-called naturally occurring regulatory cells generated in the thymus, defined hereafter as ''nTreg'', and those generated following antigenic stimulation under a variety of conditions outside the thymus, referred to as ''adaptive'' or ''induced'' regulatory T cells (iTreg) (Bluestone and Abbas, 2003). Adaptive Treg cells encompass the immunosuppressive cells referred to as ''Th3'' and ''Tr1'' cells. nTreg, iTreg and adaptive Treg cells differ fundamentally not only in the processes leading to their development but also in their mode of suppression, therefore a clear and direct lineal relationship between the two seems unlikely (**Figure 1**).

Naturally Occurring (nTreg) Cells: Function and Phenotype

Experimental evidence for nTreg cells has been suggested by animal models of autoimmune disease for many years. Perhaps the earliest clue to the existence of nTreg cells (although it was not appreciated as such at the time) was found in 1969 when autoimmune oophoritis was seen to

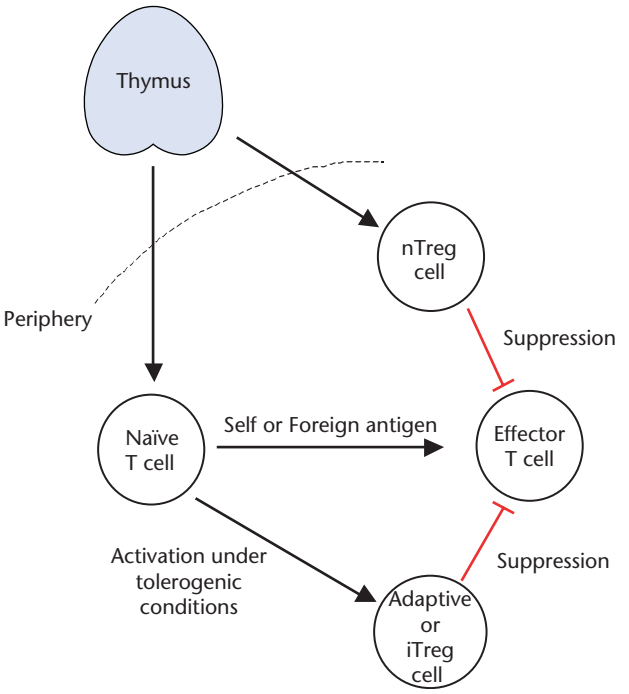

Figure 1 Development and function of regulatory T cells (Treg). The thymus is responsible for the generation and export into the periphery of both naturally occurring Treg (nTreg) cells and naïve helper T (Th0) cells. The latter can potentially differentiate into effector T cells responding physiologically against microbial pathogens or into potentially harmful autoreactive cells responding to host tissues. nTreg cells maintain self-tolerance and prevent immunopathology by controlling effector T cell responses. Additionally, adaptive induced T regulatory (iTreg) cells can develop in the periphery from naïve Th0 cells and perform similar functions to nTreg cells. iTreg and nTreg also appear to be involved in the control of antimicrobial responses and hence maintain immunohomeostasis following infection.

develop following neonatal thymectomy of mice (Nishizuka and Sakakura, 1969). These studies were followed by a number of other groups who managed to show the induction of autoimmunity in selected strains of adult rat by a combination of thymectomy and sub-lethal X-irradiation. Similarly, neonatal thymectomy coupled with cyclosporin treatment results in the spontaneous development of autoimmune diseases including gastritis, thyroiditis and oophoritis in certain strains of mice. Finally, it was shown that the induction of such autoimmunity could be prevented by the transfer of normal CD4+ splenocytes or CD4+CD8− thymocytes. Collectively, these data were strongly suggestive of the existence of a thymically produced suppressive T cell population, which was responsible for the establishment and maintenance of peripheral self-tolerance. These so-called Suppressor Cells, as they were originally known, appeared to migrate out from the thymus at a relatively late stage compared to conventional or autoreactive T cells (> 3 days after birth in mice), and it was this lag-phase which enabled the experimental induction of autoimmunity by the careful timing of neonatal thymectomy or an immunosuppressive regimen (Gershon and Kondo, 1970). **See also**: Autoimmune Disease: Aetiology and Pathogenesis; Autoimmune Disease: Pathogenesis

Subsequently, attempts were made to more specifically phenotype putative nTreg cells by isolating specific T lymphocyte fractions that harboured *in vitro* or *in vivo* regulatory activity. Initially, CD5 was proposed as a marker for nTreg cells by demonstrating that otherwise normal lymphocytes depleted of $CD5^{high}CD4+$ cells induced broad-spectrum autoimmunity when transferred into athymic nude mice (Sakaguchi *et al.*, 1985). Later studies aimed at homing-in yet further on Treg cell-specific markers have identified a number of other useful candidate molecules. For instance, CD45RB appears to divide T cells into two distinct functional subsets (Powrie *et al.*, 1993). Lymphopenic mice transferred with $CD45RB^{high}$ cells develop a lethal wasting disease characterized by severe IBD (inflammatory bowel disease) whereas unfractionated T cells or $CD45RB^{low}$ cells alone cause no disease. Importantly, co-transfer of the $CD45RB^{low}$ and $CD45RB^{high}$ populations results in protection of the mice from IBD. These pioneering studies were also the first to highlight one of the key functions of Treg cells, namely the control of immunity to gut commensals.

To date, the most useful and commonly utilized surface marker for Treg cells has proven to be their elevated constitutive expression of the IL-2 receptor α-chain, CD25 (Sakaguchi *et al.*, 1995). Approximately 5–10% of CD4+ T cells and less than 1% of CD8+ peripheral T cells constitutively express CD25 in normal naïve mice, and such cells are found in both the $CD5^{high}$ and $CD45RB^{low}$ T cell fractions. Indeed, transfer of CD25-depleted CD4+ T cells to athymic mice results in a variety of autoimmune diseases whereas co-transfer with CD25+CD4+ cells inhibits such disease development. The widespread use of CD25 has led to T cells constitutively expressing this molecule to become synonymous with Treg cells (often described as CD25+CD4+ T cells).

More recently a number of other cell surface molecules have been shown to be associated with nTreg cells, among them: CTLA4 (CD152), CD103 ($\alpha_E\beta7$-integrin), GITR (glucocorticoid-induced TNF family receptor), and Neuropilin-1 (a receptor more usually involved in axon guidance) and LAP (latency associated peptide) (**Table 1**). It is important to note that no single uniquely expressed Treg cell surface molecule has so far been identified, and in many cases relatively nTreg cell-specific molecules such as GITR or CD25 are upregulated to high levels on activated non-regulatory T cells as well. In short, the nTreg cell surface phenotype is very similar to an activated conventional T cell, making the unambiguous discrimination of the two cell types troublesome. However, some recent data have provided encouraging results which suggest that $Foxp3^+$ nTreg cells can be distinguished from activated T cells on the basis of IL-7 receptor (IL-7R or CD127) expression. IL-7 acts as an important growth factor for effector T cells but not nTreg cells, therefore IL-7R is down-regulated on the surface of the latter. This difference in expression should provide a useful tool for the specific isolation of

Table 1 Comparison of thymically and peripherally produced regulatory cells (Treg). Treg can be divided into two broad types depending principally on the means of their generation: those produced in the thymus (commonly referred to as Foxp3$^+$ Treg cells or nTreg) and those produced extrathymically or *ex vivo* under specialized conditions i.e. iTreg (e.g. Tr1, Th3)

	Naturally occurring Treg cells (nTreg cells)	Induced regulatory Treg cells (iTreg cells)
Phenotype	CD25, CTLA4, GITR, IL-7R^{low} Anergic *Foxp3*$^+$	CD25, CTLA4 Usually anergic Foxp3 generally absent
Developmental signal(s)	B7-CD28 interactions CD40-CD40L interactions IL-2 Retinoic acid Endogenous TCR-β chain ligation	Cytokines (TGF-β, IL-4, IL-10) Sub-optimal T cell activation (e.g. stimulation with immature DC) Vitamin D_3 and Dexamethasone Oral tolerance induction protocols
Suppression mechanism		
In vitro	Contact dependent and/or short-range mediators (CTLA4, LAG3, surface TGF-β, IL-35, Granzyme B, adenosine)	IL-10 and/or TGF-β
In vivo	Contact dependent IL-10 and/or TGF-β	IL-10 and/or TGF-β

nTreg cells from an activated but otherwise nonregulatory T cell population.

The migration of effector T cell subsets to different tissues is controlled by the selective expression of adhesion molecules and chemokine receptors. Naïve T cells recirculate throughout the systemic immune system but upon activation in specific lymphoid compartments these cells acquire and exhibit distinct homing tropisms. For example, the integrin $\alpha_4\beta_7$ and the chemokine receptor CCR9 mediate the homing of T cells to the GALT (gut-associated lymphoid tissue) and mesenteric lymph nodes. In a similar manner, skin-homing populations are characterized by the expression of both E- and P-selectin and CCR4. nTreg cells in both humans and mice exhibit a similar heterogeneity with both naïve and effector-type populations and are characterized the same set of homing receptors. Indeed, the vast majority of nTreg cells in peripheral blood express not only CCR4 but also CLA (cutaneous lymphoid antigen). These same nTreg can also be found abundantly in the skin and may thus play an important role in immune surveillance and tolerance of this tissue compartment. Therefore similar mechanisms to those of effector T cells govern nTreg migration and organ-selective homing.

A final property of nTreg (and indeed most iTreg) is a profound state of *in vitro* anergy i.e. an unwillingness to respond to normal levels of TCR stimulation. This anergy can be overcome by the addition of exogenous IL-2 or high levels of costimulation. The physiological relevance of this *in vitro* anergy is unclear since nTreg are known to proliferate readily *in vivo* and indeed exhibit a naturally high basal rate of turnover. Given this contradictory behaviour it is likely that Treg cell anergy is actually an *in vitro* artefact reflecting simply an absence of some signal normally required for proliferation. Therefore Treg cell anergy seems to be less of a conceptual problem than a practical one needing to be overcome if these cells are to be expanded effectively *in vitro*.

Treg Cell Development and the Transcription Factor Foxp3

Several lines of evidence suggest that nTreg cells are produced by a normal thymus. First, CD25+CD4+ thymocytes show a very similar phenotype and function to their peripheral counterparts, expressing both the characteristic surface markers as well as being suppressive *in vitro*. Second, thymic CD25+CD4+ cells express the classic constitutive pattern of nTreg cell-associated surface markers. Third, depletion of CD25+CD4+ cells from thymocytes followed by their adoptive transfer to immunodeficient hosts results in autoimmunity in a manner identical to that of the transfer of splenic CD25−CD4+ cells. Data suggest that nTreg cells arise from relatively highly self-reactive thymocytes, though why they fail to be deleted by negative selection is as yet unclear (Jordan *et al.*, 2001). Several accessory or costimulatory signals, such as those through CD28-B7 or CD40-CD40L interactions, are also thought to play an important role in nTreg cell development. The elevated expression of CD25 by nTreg indicates the crucial role played by IL-2 in nTreg cell development since knockout mice, both for CD25 or IL-2 itself, show not only a drastic reduction in nTreg cell numbers but severe

manifestation of autoimmunity. Importantly, this suggests that the CD25 molecule is not merely a handy marker for nTreg cells but an essential component for their development and function. Collectively, the data demonstrate that thymic and peripheral nTreg cells share a lineage continuity and the thymus is actively engaged in the production and export of immunoregulatory cells.

A key finding in understanding the development of nTreg was the identification of the transcriptional repressor, Foxp3. The important role played by Foxp3 first came to light through studies of the Scurfy mutant mouse. This mouse exhibits a fatal X-linked lymphoproliferative disease characterized by a severe multi-organ immunopathology, allergy and IBD. The Scurfy phenotype is very similar to the human syndrome IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) both in presentation and severity, and mutations in the *Foxp3* (*FOXP3* in humans) gene, were found to underlie both conditions. The overt similarities seen between mutations in *Foxp3* and *FOXP3* and depletion of CD25 + CD4 + cells, prompted several groups to investigate the relationship of this gene to nTreg cell development and function (Fontenot *et al*., 2003; Hori *et al*., 2003; Khattri *et al*., 2003). Studies demonstrated *Foxp3* mRNA and protein to be expressed specifically in CD25 + CD4 + T cells, and in contrast to the cell surface markers used to date, was not observed in conventional T cells following standard *in vitro* activation or differentiation. Critically, retroviral transduction of *Foxp3* to nonregulatory CD4 + cells, bestowed upon them a fully functional nTreg cell phenotype; i.e. they became anergic, expressed nTreg cell surface markers and could mediate suppression both *in vitro* and *in vivo*. Collectively, the data from mice show *Foxp3* to be not only a seemingly unambiguous marker for nTreg cells, whose genetically programmed expression is sufficient to drive their development and function, but also demonstrates them to be a genuinely distinct lineage rather than simply another activation state of conventional T cells. The signals both upstream and downstream of Foxp3 await detailed characterization. **See also**: Transcription Factors; Transcription Factors and Human Disorders

Although typically thought to be generated via thymic development, later evidence has complicated the picture somewhat by demonstrating that Treg cells, as defined by Foxp3 expression and suppressive function, can in some instances also be generated extrathymically. These cells are commonly termed ($Foxp3^+$) iTreg. Differentiaion signals for iTreg typically involve either the exogenous addition of TGF-β (transforming growth factor β) to *in vitro* cultures of conventional naïve T cells, or *in vivo* through the addition of carefully controlled minute doses of systemic antigen in otherwise nTreg cell-deficient mice. IL-2 is known to be critical not only for Treg survival but also the extrathymic generation of $Foxp3^+$ iTreg cells and its addition to *in vitro* cultures can significantly increase the efficiency of TGF-β mediated induction. Another important signal recently identified to be involved in the generation of $Foxp3^+$ iTreg cells is retinoic acid (Schambach *et al*., 2007). This common metabolite signals through the nuclear retinoic acid receptor and synergizes with TGF-β to drive naïve helper differentiation down a Treg cell pathway and away from inflammatory Th17 cells. Retinoic acid is known to be important in $Foxp3^+$ iTreg development in the GALT where it might supplant the developmental function of the IL-2. Finally, nTreg themselves also seem to be able to trigger Foxp3 expression and conversion to a suppressive phenotype of otherwise normal T cells. The mechanism of this process is unclear as is its *in vivo* significance but one can readily see how it could provide a mode of 'infectious tolerance' and dissemination of suppression. **See also**: Mucosal Surfaces: Immunological Protection; Nuclear Receptor Genes

In summary, the acquisition of Foxp3 expression is not an event unique to the thymus but can also occur in the peripheral immune system, typically under conditions of sub-optimal T cell stimulation. However, the extrathymic acquisition of Foxp3 (e.g. through the action of TGF-β alone) does not seem to result in stable expression indicating that other accessory signals, perhaps such as retinoic acid, are also required for full differentiation into *de facto* nTreg cells. The precise physiological significance of the extrathymic generation of $Foxp3^+$ iTreg cells remains to be determined.

Peripherally Generated Treg: Adaptive Treg (Tr1 and Th3 Cells)

Abundant evidence has demonstrated the extrathymic generation of T cells with suppressive properties and an anergic phenotype. Several quite different experimental approaches can lead to the generation of such Treg cells so it would be erroneous to consider them as a single discrete T cell lineage. In the original demonstration of extrathymically generated regulatory cells, the cells were termed Tr1 or Th3 cells (Roncarolo, 2001; Weiner, 2001). These suppressive cells are now collectively termed adaptive Treg cells. Typically Tr1 cells are generated under conditions involving T cell activation in the presence of immunomodulating cytokines or repetitive stimulation with nonprofessional antigen-presenting cells. Tr1 cells were initially generated by chronic stimulation of normal nonregulatory T cells in the presence of IL-10. Such cells secrete a pattern of cytokines distinct from that of the more usual Th1/Th2 profile, and are characterized by high levels of IL-10 and generally low levels of TGF-β and IL-5. Moreover, Tr1 cells are functionally suppressive *in vivo* and able to prevent the development of Th1 autoimmune diseases such as colitis. Th3 cells, however, were cloned-out from the mesenteric lymph nodes of mice orally tolerized with myelin basic protein. The majority of such cells produces TGF-β and varying levels of Th2 cytokines and suppresses the induction of experimental autoimmune encephalitis. *In vitro* treatment of human and mouse T cells with the immunosuppressants vitamin D_3 plus dexamethasone has also

resulted in the generation of Treg cells but with properties somewhat distinct from those reported for Tr1 or Th3 cells. In almost all cases these peripherally generated Treg fail to express Foxp3 and are therefore developmentally distinct from the nTreg generated within the thymus. It would therefore be probably more accurate to describe these Treg as another T cell activation stage rather than a truly distinct lineage.

DC also have a role to play in the extrathymic development of Treg cells. Stimulation with both immature DC (i.e. low levels of costimulatory molecules) and DC modified by pre-treatment with IL-10 or TGF-β have been shown to result in the induction of anergic cells with suppressive capabilities *in vitro* and *in vivo* (Rutella and Lemoli, 2004). Current models of DC-based tolerance state that T cell antigen recognition on immature DC results in tolerization whereas mature DC elicit effector responses (Steinman *et al*., 2003). A system structured in this way would be effective at maintaining self-tolerance in the physiological steady-state, i.e. in the absence of inflammatory 'danger signals' yet support productive immune responses following DC maturation triggered by the presence of microbes.

Extrathymically generated regulatory cells or Treg cells thus represent a heterogeneous assemblage with no direct ontogenic relationship to nTreg cells. The only really clear point of convergence between the two broad families of regulatory cells is that they share a suppressive capability. Why should the immune system accommodate these different types of Treg cell? One possibility is that adaptive Treg and iTreg cells represent a specialized activation state of conventional CD4+ cells 'designed' to control immunopathology during microbial responses whereas nTreg cells are a terminally differentiated *de facto* lineage committed to the maintenance of self-tolerance. Evidence for this comes from studies showing that nTreg cell suppression is broken by IL-6, a cytokine often present during microbial infection. In contrast, iTreg cells are resistant to the effects of IL-6 owing to their down-regulation of the IL-6 receptor, and can thus maintain their suppressive functions. These findings might offer some useful clues to a specific role for iTregs *in vivo*.

Treg Cell Mode of Action

The cardinal feature of both thymically and peripherally produced Treg cells is their ability to suppress immune responses. At least *in vitro*, nTreg cells and iTreg cells seem to differ fundamentally in their mechanism of suppression, with the former requiring cell contact and the latter using relatively far-reaching soluble inhibitory factors such as IL-10 and TGF-β. A variety of experimental models have, however, shown that both families of Treg rely to various extents on TGF-β and/or IL-10 to exert suppressive effects *in vivo*. **See also**: Immunoregulation; Transforming Growth Factor Beta: Role in Cell Growth and Differentiation

The suppressive ability of Treg cells can be modelled *in vitro* using the so-called Treg assay. In this technique titrated numbers of highly purified CD25+CD4+ cells (i.e. Treg) are mixed with CD25−CD4+ T cell (i.e. naïve) responders plus a TCR stimulus. Under such conditions, the CD25+CD4+ population suppresses both the proliferation and IL-2 production of the CD25−CD4+ cells in a dose-dependent manner (Takahashi *et al*., 1998). Treg cells require TCR stimulation plus a small amount of IL-2 to actually elicit their suppressive functions, although they are themselves anergic (i.e. unresponsive as measured by proliferation or IL-2 production) to conventional *in vitro* T cell activation stimuli. Once nTreg cells have actually been activated they are able to suppress in an antigen nonspecific manner and can control bystander responses to irrelevant antigens. Certain signals or agents are able to abrogate Treg cell suppression *in vitro* or *in vivo*. In the case of adaptive Treg and some iTreg, blockade of secreted suppressive factors such as TGF-β or IL-10 can prevent suppression. However, nTreg suppression *in vitro* can be circumvented by the supply of strong costimulatory or accessory signals, e.g. anti-CD28, mature DC or exogenous IL-2. Similarly, if DCs are present, the supply of TLR (toll-like receptor) ligands such as LPS (lipopolysaccharide) will circumvent Treg cell suppression. These effects seem to be mediated, at least in part, by LPS-triggered secretion of IL-6 from DCs which then blocks nTreg cell suppressive function (see aforementioned). Although one study has hinted at a direct effect of LPS on Treg cells other studies have been unable to replicate this observation and it therefore remains controversial.

Cell-contact-dependent Suppression by nTreg Cells

The precise basis of cell-contact-dependent suppression mediated by nTreg is a subject of considerable confusion and many possibilities have been proposed (**Figure 2**). At this stage it should be emphasized that no single definitive mechanism of suppression has yet been identified despite numerous *in vitro*, *in vivo* and gene expression profiling studies.

Most simplistically, nTreg cells may compete with responder cells for stimulatory ligands, be they cell surface (e.g. costimulatory molecules) or soluble growth factors (e.g. IL-2). The high level of adhesion molecule (e.g. LFA-1) and CD25 expression would make nTregs proficient competitors for space on the surface of DCs as well as an effective 'IL-2 sink' together capable of depriving effector T cells of growth signals. Some data, however, support a less passive mode of suppression involving the expression of relatively nTreg cell-specific molecules such as suppressive surface bound TGF-β. Another obvious candidate molecule is CTLA4 (CD152), which is constitutively expressed by Treg cells and binds B7.1 and B7.2 (CD80 and CD86, respectively) with high affinity. Indeed, CTLA4

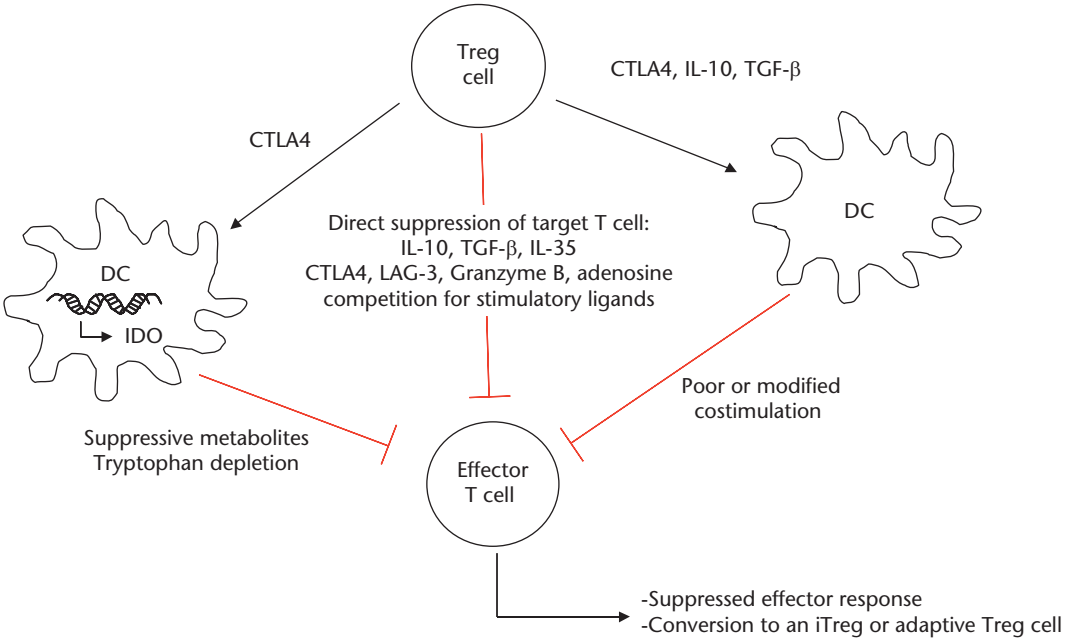

Figure 2 Potential suppression mechanisms of Treg cells. A number of potential mutually nonexclusive mechanisms can be employed by the various types of Treg cells (nTreg, iTreg and adaptive Treg cells). These can take the form of contact-dependent mechanisms or through the secretion of suppressive cytokines. Additionally, Treg cells can modify the function of dendritic cells either by rendering them inefficient APC or turning them into actively suppressive cells by inducing their expression of IDO.

blockade without cross-linking (e.g. using Fab fragments) is able to prevent *in vitro* nTreg suppression. CTLA4 expressed on nTreg could not only compete with responder T cells seeking activatory costimulation, but there is now evidence that CTLA4 ligation of B7 on a particular class of DC elicits the induction of the immunosuppressive enzyme IDO (indoleamine dioxgenase). In addition to ligand competition, nTreg cell CTLA4 has also been shown to downregulate costimulatory molecule expression on the surface of antigen-presenting cells. Though still controversial, recent data have even demonstrated that CTLA4 ligation of B7 *directly* on responder T cells (a so-called outside-in signal) is also suppressive in an as yet ill-defined manner. Although CTLA4 certainly has some role to play, a cautionary note is still probably merited, since nTreg cells from CTLA4$^{-/-}$ mice appear to suppress comparably to their wild-type counterparts. Furthermore, high levels of CTLA4 expression following activation or genetic manipulation do not render conventional T cells suppressive. In such cases the absence of CTLA4 might be compensated for or synergize with other suppressive molecules expressed by nTreg cells such as surface bound TGF-β. **See also**: Immunological Accessory Molecules; Placental Immune Defences – Protection Against Rejection and Infection; Reproductive Immunology

Another candidate suppressive mechanism is via the CD4-related molecule LAG-3. This molecule is expressed on the surface of both nTreg and iTreg following activation, especially in the presence of effector cells. The level of LAG-3 expression correlates with suppressive function; knockout cells show sub-optimal effectiveness whereas ectopic expression bestows suppressive function on otherwise nonsuppressive Foxp3^{-} T cells. How LAG-3 actually mediates suppression is unclear though presumably it would be related to its major histocompatability class II binding ability. IL-35 is another molecule potentially involved in suppression. This cytokine is over-represented in CD25+CD4+ T cells and appears to be a downstream target of *Foxp3*, it furthermore appears to mediate at least some of the suppressive functions of nTreg cells. This interesting pathway awaits further characterization. A final mechanism of suppression worth discussing is cAMP (cyclic adenosine monophosphate). Elevation of cAMP has long been known to arrest T cell proliferation and differentiation, in part by activation of the transcriptional repressor inducible cAMP early repressor (ICER) which blocks IL-2 production. Evidence suggests that nTreg might deliver cAMP directly to target cells through gap junctions (still controversial) or by the local generation of adenosine. Indeed, nTreg expression of the ectonucleotidases CD39 and CD73 has been shown to generate adenosine and is at least in part responsible for the suppressive action of nTreg cells. Generally the suppressive mechanisms of Treg cells described above do not result in the death of the target cell but rather anergy or deviation of the response away from an inflammatory one. However, Granzyme B has also been reported as a mechanism of nTreg suppression and this appears to be mediated at least

partly by triggering apoptosis in the target cell. On surveying the diversity of suppression mechanisms above, one is ineluctably drawn to the conclusion that several, mutually nonexclusive, mechanisms exist and which one predominates will depend greatly on the context and milieu, e.g. target cell, inflammatory vs. noninflammatory environment, etc.

Human Regulatory Cells and Clinical Perspectives

The existence of Treg cells in rodents and the vital role they play is beyond reproach, and it now seems that an almost directly equivalent population also exists in humans. Human Treg cells share a similar molecular and functional phenotype to their mouse counterparts; being anergic, Foxp3 positive, having constitutive expression of CD25 and demonstrating contact-dependent suppression *in vitro* (Baecher-Allen and Hafler, 2004). IPEX, described above, perhaps provides the most compelling piece of evidence for the importance of Treg cells to human health. Studies with human Treg cells provide a particular challenge both in the isolation of the cells themselves but also in the kind of experiments that can be performed. For instance especial care needs to be taken when isolating human Treg cells since only the highest CD25-expressing T cells are $Foxp3^+$ nTreg cells, with the low expressors simply representing activated conventional T cells. Another complication when investigating human Treg cells is the more promiscuous expression of *FOXP3* which is upregulated following activation of normal T cells. Human Treg cells also appear to suppress with generally similar mechanisms to those found in the mouse, with the possible additional use of perforin by human Treg cells. Therefore human Treg cells might directly kill their target cells.

Data on the clinical relevance of Treg cells are steadily accumulating. For instance, nTreg cells isolated from patients with MS (multiple sclerosis) or rheumatoid arthritis are significantly less suppressive than those of normal control individuals. Therefore defective nTreg function or reduced frequency may well play an important role in a number of human autoimmune diseases. In the case of cancer, Treg cells likely have a negative influence since they can hamper normally desirable anti-tumor immune responses. Some evidence already suggests that cancer patients show increased numbers of active Treg cells specific for tumour proteins both in their blood and within the tumour mass itself. This suggests that tumours co-opt the functions of Treg cells during the process of immunoediting and indeed numerous animal studies have demonstrated the efficacy of depleting Treg cells to unmask otherwise suppressed anti-tumour immunity. Indeed, this phenomenon might underlie the beneficial effects of anti-CTLA4 monoclonal antibodies in cancer. **See also**: Tumour Immunology

Establishing the roles played by Treg cells in humans is critical for any approach involving their therapeutic manipulation. In this regard, IPEX, despite its severity, offers a measure of hope. Since hemizygous mothers carry only one copy of the mutant gene, half of their Treg cells are nonfunctional or absent yet such individuals are completely normal, suggesting that such harmful immunopathology can be dominantly controlled even by significantly reduced numbers of Treg cells. Major practical and theoretical hurdles remain before Treg cells can be successfully exploited in the clinic, but should we succeed the benefits would be enormous.

References

Bluestone JA and Abbas AK (2003) Natural versus adaptive regulatory cells. *Nature Reviews in Immunology* **3**: 253–257.

Baecher-Allan C, Viglietta V and Hafler DA (2004) Human CD4 + CD25 + regulatory T cells. *Seminars in Immunology* **10**: 89–98.

Fontenot JD, Gavin MA and Rudensky AY (2003) Foxp3 programs the development and function of CD4 + CD25 + regulatory T cells. *Nature Immunology* **4**(4): 330–336.

Gershon RK and Kondo K (1970) Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology* **18**: 723–737.

Hori S, Nomura T and Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**: 1057–1061.

Jordan MS, Boesteanu A, Reed AJ *et al*. (2001) Thymic selection of CD4 + CD25 + regulatory T cells induced by an agonist self-peptide. *Nature Immunology* **2**: 301–306.

Khattri R, Cox T, Yasayko SA and Ramsdell F (2003) An essential role for Scurfin in CD4 + CD25 + T regulatory cells. *Nature Immunology* **4**(4): 337–342.

Nishizuka Y and Sakakura T (1969) Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. *Science* **166**(906): 753–755.

Powrie F, Leach MW, Mauze S, Caddle LB and Coffman RL (1993) Phenotypically distinct subsets of CD4 + T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *International Immunology* **5**: 1461–1471.

Roncarolo M-G, Bacchetta R, Bordignon C, Narula S and Levings MK (2001) Type 1 regulatory cells. *Immunological Reviews* **182**: 68–79.

Rutella S and Lemoli R (2004) Regulatory T cells and tolerogenic dendritic cells: from basic biology to clinical applications. *Immunology Letters* **94**: 11–26.

Sakaguchi S, Fukuma K, Kuribayashi K and Masuda T (1985) Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance: deficit of a T cell subset as a possible cause of autoimmune disease. *Journal of Experimental Medicine* **161**: 72–87.

Sakaguchi S, Sakaguchi N, Asano M, Itoh M and Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causesvarious autoimmune diseases. *Journal of Immunology* **155**: 1151–1164.

Schambach F, Schupp M, Lazar M and Reiner S (2007) Activation of retinoic acid receptor α favours regulatory cell induction at the expense of IL-17-secreting T helper cell differentiation. *European Journal of Immunology* **37**: 2396–2399.

Steinman RM, Hawiger D and Nussenzweig MC (2003) Tolerogenic dendritic cells. *Annual Reviews in Immunology* **21**: 685–711.

Takahashi T, Kuniyasu Y, Toda M *et al*. (1998) Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *International Immunology* **10**: 1969–1980.

Weiner H (2001) Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunological Reviews* **182**: 207–214.

Further Reading

Baecher-Allan C and Hafler DA (2004) Suppressor T cells in human diseases. *Journal of Experimental Medicine* **200**: 273–276.

Hong J and Chess L (2004) An integrated view of suppressor T cell subsets in immunoregulation. *Journal of Clinical Investigation* **114**: 1198–1208.

Murphy K, Travers P and Walport M (2007) *Janeway's Immunobiology*, 7th edn. New York: Garland Publishing.

Sakaguchi S (2004) Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annual Review of Immunology* **22**: 531–562.

Shevach E (2007) From vanilla to 28 flavors: multiple varieties of T regulatory cells. *Immunity* **25**: 195–201.