Published in final edited form as:

Inflamm Allergy Drug Targets. 2006 September ; 5(3): 141–148.

Natural and Induced Regulatory T Cells: Targets for Immunotherapy of Autoimmune Disease and Allergy

K.S. Nicolson and D.C. Wraith*

Department of Cellular and Molecular Medicine, University of Bristol, Bristol BS8 1TD., UK

Abstract

Recent advances in immunology have greatly increased our understanding of immunological tolerance. In particular, there has been a resurgence of interest in mechanisms of immune regulation. Immune regulation refers to the phenomenon, previously known as immune suppression, by which excessive responses to infectious agents and hypersensitivities to otherwise innocuous antigens such as self antigens and allergens are avoided. We now appreciate that various distinct cell types mediate immune suppression and that some of these may be induced by appropriate administration of antigens, synthetic peptides and drugs of various types. The induction of antigen specific immunotherapy for treatment of autoimmune and allergic diseases remains the 'holy grail' for treatment of these diseases. This goal comes ever closer as understanding of the mechanisms of immune suppression and in particular antigen specific immunotherapy increases. Here we review evidence that immune suppression is mediated by various different subsets of CD4 T cells.

Keywords

Autoimmunity; allergy; T-cell; cytokine; immune regulation; antigen; peptide

Immunological tolerance is a state of unresponsiveness that is specific for a particular antigen; it is induced by prior exposure to that antigen. Active tolerance mechanisms are required to prevent inflammatory responses to the many innocuous air-borne and food antigens that are encountered at mucosal surfaces in the lung and gut. The most important aspect of tolerance, however, is self tolerance, which prevents the body from mounting an immune attack against its own tissues. There is potential for such attack because the immune system randomly generates a vast diversity of antigen-specific receptors, some of which will be self reactive. Cells bearing these receptors therefore must be eliminated, either functionally or physically, or regulated.

Central tolerance involves selection of useful lymphocytes and deletion of those cells with potentially dangerous affinity for self-antigens. For T cells the process requires expression of self-antigens in the thymus. Genes encoding antigens normally expressed in other tissues may be transcribed in the thymus under the control of the AIRE gene [1]. AIRE regulated gene expression plays a vitally important role in self-tolerance [2].

Despite the evident efficiency of thymic selection many potentially autoreactive T cells still escape central tolerance. This reflects the fact that many antigens are either not present or are present at insufficiently high levels to induce tolerance in the thymus. Thus, for example,

peripheral blood lymphocytes from healthy individuals respond vigorously to purified myelin basic protein, a major constituent of myelin in the brain, following their culture *in vitro* [3]. So, how are these cells kept at bay in healthy individuals and why are autoimmune diseases directed to such proteins so incredibly rare?

There are five possible ways in which self-reactive lymphocytes may be prevented from responding to self antigens:

1. Self-reactive T cells in the circulation may ignore self antigens, for example when the antigens are in tissues sequestered from the circulation.
2. Their response to a self antigen may be suppressed if the antigen is present in a privileged site.
3. Self-reactive cells may be deleted at certain stages of development, or
4. Self-reactive cells may be rendered anergic and unable to respond.
5. Finally, a state of tolerance to self-antigens can also be maintained by immune regulation.

Here we discuss the function of immune regulatory T cells. These cells play a vital role in maintaining self tolerance and also hold great potential for immunotherapy of both autoimmune and allergic diseases.

NATURAL REGULATORY T CELLS

In the mid-1990s Sakaguchi and co-workers demonstrated that the inhibition of autoimmunity seen in day 3 thymectomised BALB/c mice following thymic grafting or splenocyte transfer was due to a particular type of cell. These cells were identified as a population constituting approximately 10% of normal CD4⁺ T cells that constitutively express the IL-2 receptor α chain, CD25 [4,5]. Transfer of CD4⁺ T cells depleted of CD25⁺ cells into athymic mice induced autoimmunity in a dose dependent fashion, however, transfer of purified CD4⁺CD25⁺ T cells within 10 days prevented disease [5]. CD25⁻ CD4⁺ T cells were capable of inducing autoimmunity in the absence of CD8⁺ T cells, APCs or B cells and disease could also be adoptively transferred to *nu/nu* mice, in a disease specific fashion [5]. Similar experiments were also performed in rats on the basis of CD45RB expression where reconstitution of athymic rats with CD45RB^{high} CD4⁺ T cells resulted in a severe wasting disease that was prevented by the presence of CD45RB^{lo} CD4⁺ T cells [6].

Generation of CD4⁺CD25⁺ T_{regs}

The origin of CD4⁺CD25⁺ T_{regs} has been the subject of intense research. IL-2 signalling appears to be crucial for their survival, as mice deficient for IL-2 or IL-2 receptors display a lack of CD4⁺CD25⁺ T_{regs} [7-9]. Initial evidence suggested that CD4⁺CD25⁺ T_{regs} are generated specifically in the thymus as illustrated by the observation that thymic CD4⁺ T cells can develop into T_{regs} in response to antigen [10]. The affinity of developing T cells for self-antigens presented in the thymus is also extremely important for the development of T_{regs} and cells with affinities for self-antigens just beneath the threshold for deletion during negative selection preferentially develop into T_{regs} [10].

A second study also indicated that CD4⁺CD25⁺ T_{regs} are generated intrathymically, and illustrated the alternative outcomes provided by presentation of self-antigens on different APCs. Apostolou *et al.* [11] demonstrated that presentation of self-antigens on thymic stromal cells resulted in the development of CD4⁺CD25⁺ T_{regs}, while presentation of the same antigens on haematopoietic cells resulted in the generation of CD25⁻ T cells with regulatory activity. Importantly however, the same study also revealed that CD4⁺CD25⁺

T_{regs} could actually be generated in the periphery from mature T cell populations. Naïve CD4⁺ T cells bearing transgenic TCRs specific for HA were again transferred into mice constitutively expressing HA where over the course of 2 weeks the cells expanded and contracted before stably dividing into 2 populations; CD25⁻, constituting 95% of CD4⁺ cells, and CD25⁺, representing 5% of CD4⁺ cells. However, after the prolonged exposure to antigen, both fractions of CD4⁺ T cells were anergic and suppressive of naïve T cells [11]. A further study also revealed the generation of cells with regulatory properties following prolonged exposure to a harmless antigen, although these cells were predominantly CD25⁻ and did not express significant levels of FoxP3 [12]. Together these results demonstrate that CD4⁺CD25⁺ T_{regs} are certainly generated in the thymus, but show that under non-inflammatory conditions, prolonged exposure to antigen can also result in the generation of both CD25⁺ and CD25⁻ T_{regs} in the periphery.

Phenotype of CD4⁺CD25⁺ T_{regs}

To date, CD25 has been the marker traditionally associated with regulatory function, although as described above, levels of CD45RB expression can also be used to distinguish between naïve and regulatory T cells in both rats [6] and also mice [13]. The pattern of cell surface expression of markers by CD4⁺CD25⁺ T_{regs} has now been expanded to include the observations of a higher proportion of CD62L^{lo} cells and a higher proportion of CD69⁺ cells in the regulatory T cell pool [14].

However, expression of the above markers is shared by many cells, including naïve T cells, which upregulate CD25 upon activation. Therefore, a marker unique to CD4⁺CD25⁺ T_{regs} is desirable and several candidate markers have been suggested in recent literature. The glucocorticoid receptor GPCR was suggested as a marker for CD4⁺CD25⁺ T_{regs} when it was demonstrated that CD4⁺CD25⁺ T_{regs} expressed high levels of GPCR on their cell surface, and that addition of GPCR-neutralising antibodies abrogated suppression mediated by these cells [15,16]. Depletion of GPCR-expressing cells or administration of a GPCR-neutralising antibody *in vivo* also led to autoimmune disease similar to that observed in experiments transferring CD25⁻ cells to nude mice [15]. However, it is now known that GPCR expression is also increased on normal CD4⁺ and CD8⁺ T cells following activation [17]. GPCR ligation enhances T cell activation for both CD25⁺ and CD25⁻ cells as measured by cell cycle progression, increased expression of CD69 and CD25, increased cytokine production and NFκB-mediated intracellular signalling, indicating that GPCR may instead be an effective costimulatory receptor for early T cell activation [17].

Recently, other candidate markers have been suggested for the specific identification of T_{regs} among the T cell pool. Neuropilin-1, a receptor important for axon guidance and angiogenesis as well as for T cell activation, was recently suggested as a marker specific for T_{regs} when its expression was found to be constitutive on CD4⁺CD25⁺ T cells, and decreased on CD25⁻ T cells following activation [18]. Neuropilin-1 expression was also observed to correlate with the expression of FoxP3, another candidate marker for T_{regs}.

The forkhead/winged transcription factor FoxP3 has also been identified as a marker for T cells with regulatory activity, initially for CD4⁺CD25⁺ T_{regs} in particular. FoxP3 is expressed in CD4⁺CD25⁺ cells but is absent in the majority of CD25⁻ CD4⁺ T cells [19,20]. A lack of FoxP3 *in vivo* results in lymphoproliferation and a deficiency in CD4⁺CD25⁺ T_{regs}, suggesting it is required for their development [19]. FoxP3 has been expressed in CD25⁻ T cells by transfection [21] or induced by exposure to TGFβ *in vitro* [22] and *in vivo* [23] and results in the induction of a regulatory phenotype in otherwise non-suppressive T cells. However, recent evidence suggests that in fact T cells can demonstrate strongly suppressive characteristics even in the absence of FoxP3 expression [24], indicating that a definitive marker for regulatory activity has still not been identified.

Recent literature has also raised the importance of TGF β in CD4⁺CD25⁺ T cell generation with the observation that culture of naïve peripheral CD25⁻ T cells in the presence of TGF β results in cells with high levels of FoxP3 expression, TGF β production, and suppressive characteristics both *in vitro* and *in vivo* [22,25]. Interestingly, it was found that the FoxP3 induced by TGF β down-regulated expression of Smad7, rendering CD4⁺CD25⁻ cells more susceptible to the regulatory effects of TGF β signalling, illustrating that FoxP3 expression mediates a positive autoregulatory loop for TGF β signalling by blocking Smad7 binding and allowing increased Smad 3 and 4 signalling [22]. Neutralisation of TGF β only partially reversed suppression, suggesting other factors may also be important [22].

CD4⁺CD25⁺ T_{regs} are anergic *in vitro* without the addition of exogenous IL-2 [14,26] and are suppressive both *in vitro* and *in vivo*. CD4⁺CD25⁺ regulatory T cells prevent rejection of allogeneic skin grafts in *nu/nu* mice, while mice inoculated with CD25⁻ enriched CD4⁺ T cells exert a more vigorous anti-graft response [5], indicating that CD4⁺CD25⁺ regulatory cells play a regulatory role in cellular immunity as well as in regulating autoimmune responses.

It should be noted that many of the initial studies investigating the regulatory properties of CD4⁺CD25⁺ T_{regs} were performed using reconstitution models with lymphopaenic recipients [5,13]. It has subsequently been demonstrated that under lymphopaenic conditions, transfer of CD25⁻ cells can also prevent autoimmunity providing that a larger inoculum is used to reconstitute the mice [27] suggesting that under such conditions autoimmunity results from the outgrowth of pathogenic clones. The growth of these clones may be restricted when larger cell numbers are transferred, thereby providing competition for growth factors and space [28,29]. Both CD25⁺ and CD25⁻ cells are able to prevent wasting disease in a model of cell transfer to lymphopaenic recipients [30]. While the suppressive characteristics of CD4⁺CD25⁺ T_{regs} cannot be ignored, it is important to consider the dynamics of T cell reconstitution that are occurring in lymphopaenic animals.

Mechanism of Suppression by CD4⁺CD25⁺ T_{regs}

Although CD4⁺CD25⁺ T cells require TCR stimulation to become activated, they can then suppress in an antigen-independent manner, inhibiting the growth of naïve CD4⁺ T cells in response to α CD3 coated beads [31]. The inhibitory effect of CD4⁺CD25⁺ cells appears to be mediated by the suppression of IL-2 production in naïve T cells as addition of IL-2 to co-cultures can almost completely reverse inhibition caused by the regulatory T cells [14]. However, the exact way in which this is achieved has been difficult to elucidate. Investigation of the role of cytokines in suppression by CD4⁺CD25⁺ T_{regs} has revealed their importance *in vivo*. IL-10 production by CD4⁺CD25⁺ T_{regs} is important for their suppression of a healing response to *Leishmania* [32], while production of IL-10 and TGF β is important for the reduced pathology observed following *H. hepaticus* infection in 129 mice; the addition of neutralising antibodies specific for either of these cytokines preventing CD4⁺CD25⁺ mediated protection from the pathogen [33]. IL-10 and TGF β production are also important for control of colitis [34], while systemic IL-10 production induced regulatory T cells that reduced disease in a diabetes model [35].

The role of cytokines in suppression mediated by CD4⁺CD25⁺ T_{regs} *in vitro* is less clear. In two studies published in 2001 it was observed that CD4⁺CD25⁺ T_{regs} express TGF β on their cell surface, which is necessary for their generation [36] and suppressive effects [37]. The addition of a neutralising anti-TGF β antibody prevented suppression *in vitro* in this system [37]. However, doubt was raised over this observation when it was subsequently reported that TGF β was not required for suppression by CD4⁺CD25⁺ T_{regs} [38]. A role for TGF β in suppression has recently been reiterated, with the demonstration that CD4⁺CD25⁺ cells from TGF β -deficient mice fail to protect lymphopaenic recipients from colitis in a transfer model,

despite the fact that the cells display suppressive properties *in vitro* [39]. These observations suggest that the behaviour of T_{regs} *in vivo* and *in vitro* may differ, with the importance of cytokines being more obvious *in vivo* than *in vitro*. Interestingly, while IL-10 is crucial for *in vivo* suppression it may not be required for suppression mediated by 'induced' regulatory T cells *in vitro* [40].

Factors other than cytokines may also be involved in suppression mediated by CD4⁺CD25⁺ T_{regs}. CD4⁺CD25⁺ T_{regs} express high levels of CTLA-4 [41,42] and blockade of this receptor *in vivo* abrogates suppression, implying a role for CTLA-4 at least *in vivo* [42], although CTLA-4 expression on responder cells can influence their threshold for activation, making it more difficult to activate them [43].

Cell contact may also be important for the activity of CD4⁺CD25⁺ T_{regs} as suppression is abolished in transwell culture systems where the regulatory T cells are separated from responder cells; conversely, the addition of cytokine-neutralising antibodies fails to prevent suppression in co-cultures [14].

Therapeutic Applications of CD4⁺CD25⁺ T_{regs}

The use of CD4⁺CD25⁺ T_{regs} to treat autoimmune disorders is an exciting prospect. However, it is only recently that it has been possible to generate large numbers of these cells *in vitro*, the anergic phenotype of the cells making it difficult to expand them *in vitro*. Two studies have recently reported the *in vitro* generation of large numbers of CD4⁺CD25⁺ cells with known specificities. Tarbell and colleagues utilised dendritic cells to stimulate the proliferation of CD4⁺CD25⁺ T cells isolated from transgenic mice bearing TCRs specific for a peptide mimic of a pancreatic islet β cell antigen [44]. Following culture of CD4⁺CD25⁺ cells isolated from these mice with mature BM-DCs and a stimulatory peptide, the normally anergic T_{regs} proliferated by day 3 of culture and displayed enhanced suppressive characteristics than freshly isolated cells [44]. Adoptive transfer experiments using these expanded cells revealed that 20 times less cells were required to prevent diabetes in TCR transgenic recipients compared to freshly isolated T_{regs} [44].

Similar findings were reported by Tang *et al.* using CD4⁺CD25⁺ T_{regs} expanded with α CD3, α CD28 and IL-2 [45]. Again, the T_{regs} retained their suppressive phenotype and suppressed proliferation of naïve CD4⁺ T cells both *in vitro* and *in vivo*, also preventing diabetes upon transfer of only a small number of expanded cells [44]. Together, these findings give support to the possible use of CD4⁺CD25⁺ T_{regs} in the future as a treatment strategy for various autoimmune diseases. These cells are particularly appealing in this application because of their ability to suppress proliferation to more than one antigen following TCR signalling [31] and this is evident in their suppression of a disease such as diabetes, which is likely to be directed at more than one antigen.

The prospect of utilising CD4⁺CD25⁺ T cells as a treatment strategy is further supported with the recent observations of impaired T_{reg} function in some human immune conditions. It has recently been reported that CD4⁺CD25⁺ T cell function is impaired in patients suffering with MS [46]. A similar phenomenon has also been described in allergy where suppression of proliferation and IL-5 production by CD4⁺CD25⁺ cells from atopic donors is impaired relative to non-atopic donors [47]. This lack of suppression can be reversed by treating the T_{regs} with a corticosteroid which promotes IL-10 production [48].

INDUCED REGULATORY T CELLS

Tr1 Cells

In 1997 Groux and co-workers reported the generation a new subset of regulatory T cells, called T regulatory cells 1, or Tr1, generated from both OVA-specific TCR transgenic mice and human peripheral mononuclear cells by repeated stimulation in the presence of IL-10 [49]. The resulting T cell clones had a characteristic cytokine profile, secreting high levels of IL-10 and moderate amounts of TGF β , IFN γ and IL-5, low IL-2 and no IL-4 [49]. Tr1 clones inhibited the growth of naïve T cells upon stimulation with antigen although this was abrogated by the addition of neutralising anti-TGF β and IL-10 antibodies [49]. Tr1 cells have also been shown to be suppressive *in vivo*, preventing irritable bowel disease in susceptible transgenic mice. However, suppression was only observed if the mice were fed the antigen used to generate the Tr1 cells indicating that these cells mediate their suppressive effect in an antigen-dependent manner [49].

Tr1 cells have been isolated from the peripheral blood of SCID patients, following allogeneic stem cell transplantation suggesting they are a population occurring naturally *in vivo* [49]. The fact that they can be generated from naïve peripheral T cells suggests that they represent a distinct population of T_{regs} to CD4⁺CD25⁺ cells [50].

Recently, the generation of regulatory cells with a Tr1-like phenotype has been described following stimulation of T cells with antigen presented by immature dendritic cells, both *in vivo* and *in vitro* [51]. Tr1 cells generated in this way demonstrate a highly stable phenotype, even upon restimulation with mature DCs and antigen [51], making them attractive for therapeutic applications. Tr1 cells have also been described in a human system following stimulation of CD4⁺ T cells *via* CD3 and the complement receptor CD46 in the presence of IL-2 [52]. Again, the resulting cells were characterised by high levels of IL-10 production and suppression of naïve cell proliferation [52]. A further population of IL-10-producing cells has been described following culture in the presence of vitamin D3 and dexamethasone [53]. The development of methods for the reliable generation of homogenous populations of T cells with known antigen-specificities and regulatory properties may be useful for therapeutic applications.

Interactions between populations of regulatory T cells may be important for the control of autoimmune conditions. CD4⁺CD25⁺ T_{regs} can induce the generation of Tr1-like cells from the CD25⁻ population following co-culture *in vitro* [54]. In a mouse model of inflammatory bowel disease, endogenous CD4⁺CD45RB^{lo} or CD4⁺CD25⁺ T_{regs} administered after disease induction were important for the maintenance of tolerance through subsequent induction of Tr1 cells even though they were unable to alone prevent disease [55].

Th3 Cells

In 1994 Chen and co-workers described the identification of a group of T cell clones that secreted TGF β and IL-10 in culture following a protocol of feeding large doses of whole MBP protein to SJL mice. When lymphocytes from the fed mice were cultured *in vitro* with the same antigen, it was observed that the cells secreted TGF β preferentially as well as Th2 like cytokines [56]. TGF β was found to be secreted by CD4⁺ and CD8⁺ T cells, while IL-4 and IL-10 were found to be secreted only by CD4⁺ T cells [56]. The fact that TGF β secretion was regulated separately from that of IL-4 and IL-10 suggested that these cells were not normal Th2 cells.

Th3 cells were also observed to inhibit the effect of other T cells *in vivo*, reducing the severity of PLP-induced EAE in SJL mice, although the proliferation of PLP-specific cells

was only reduced if MBP was added to cultures, suggesting that these cells also act in an antigen-specific manner [56].

Another group of regulatory T cells similar to Th3 cells was described in 1996 when isolation of T cell clones from infiltrating T cells in pancreatic islet cells revealed that some of the clones were strongly suppressive when stimulated *in vitro* with irradiated splenocytes from NOD mice [57]. It was subsequently revealed that the observed suppression was due to the secretion of TGF β into culture supernatant [57]. In a manner similar to that described in Th3 cells, the suppressive effect of the NOD-derived cells could be abrogated by addition of neutralising anti-TGF β antibodies to cultures, and the cells could prevent disease in an *in vivo* model if transferred to NOD recipients [57].

The importance of TGF β in the generation of Th3 cells was demonstrated by Zheng *et al.* who showed the conversion of conventional CD4⁺ T cells to Th3 cells by stimulating the cells in the presence of TGF β [58]. Again these cells displayed suppressive characteristics, preventing IgG production by B cells in a TGF β -dependent mechanism [58]. These cells were derived from the CD25⁻ T cell compartment, although they upregulated CD25 expression upon treatment with TGF β [58]. Again, these results imply that the peripheral pool of T_{regs} may contain more than one population of cells, with different cell types specialised for different functions; Th3 cells in particular seem important for tolerance on mucosal surfaces.

PI-T_{Reg} and Th10 Cells

Tolerance induction has also been reported following administration of the peptide Ac1-9[4K] derived from the protein MBP [59,60]. Substitution of the lysine residue present in the wildtype sequence at position 4, with alanine or tyrosine (Ac1-9[4A] and Ac1-9[4Y] respectively) results in peptides with increasing affinity for MHC class II [61]. A single intraperitoneal injection of either of the higher affinity peptides prior to EAE induction in (PL/J $\times$ B10.PL)F1 mice resulted in reduced disease severity, which was accompanied by reduced T cell responsiveness *in vitro* [62]. Intranasal administration was investigated as an alternative route of administration and it was found that a single administration of any of the peptides before EAE induction protected from disease in the same mice, while only the highest affinity peptide (Ac1-9[4Y]) was protective if administered after disease induction [59,60].

Tolerance induction following intranasal peptide administration can be a powerful way to control responses to more than one self-peptide as the repeated administration of a single peptide can affect responses to other peptides in a mechanism termed bystander suppression. Previous work, using H-2^{u^{xs}} mice, demonstrated that administration of the MBP peptide Ac1-9 was suppressive of responses mediated against Ac1-9, 89-101 and whole MBP protein, demonstrating a linked suppression [63]. By contrast, the administration of PLP 139-151 was suppressive of responses both to itself and also to the MBP epitopes Ac1-9 and 89-101, and could suppress EAE induced by MBP peptides, demonstrating bystander suppression that was not mediated by a switch to a Th2 phenotype [63]. Such inhibition of multiple epitopes could be useful in a therapeutic setting in order to control multiple specificities of self-reactive T cells responding to more than one self-antigen.

Further experimentation using peptide antigen administration has since been carried out using the MBP-specific TCR transgenic mouse strain Tg4 [64]. Initial work demonstrated that a single intranasal administration of Ac1-9[4Y] resulted in transient cell death of the peripheral T cell repertoire [65]. However, it was observed that repeated administration of the peptide resulted in complete protection from EAE induction [65]. The number of peptide administrations required to induce tolerance was found to correlate with the number of

precursor T cells, so that transgenic animals with a high precursor frequency required more peptide administrations for complete tolerance induction than nontransgenic littermates [65]. Following repeated antigen administration cell death was observed in central and peripheral lymphoid organs but did not account for the tolerance observed. As shown previously by Sundstedt *et al.* [66], a single administration resulted in strong proliferative responses and production of Th1 and Th2 cytokines. However, after repeated inhalation of peptide there was almost no detectable proliferation after *in vitro* restimulation [65]. As there was no change in the absolute numbers of CD4⁺ T cells in the mice this could not have been due to deletion of a subset of cells and instead must have been due to active suppression of their proliferation. Cytokines may have been involved in this process since IL-10 levels increased with multiple administrations of peptide. As seen previously, administration of neutralising anti-IL-10 antibodies abrogated the suppressive effect [49,56,65,66].

The mechanisms involved in the induction of tolerance have since been investigated further and it is now known that a population of CD4⁺ T cells with regulatory characteristics are generated following repeated peptide inhalation. These cells have since been termed PI-T_{Reg} cells. PI-T_{Reg} cells are anergic upon peptide stimulation *in vivo* and they fail to make IL-2 in response to peptide stimulation, instead secreting high levels of IL-10 [40]. PI-T_{Reg} cells are predominantly CD25⁻ and CTLA-4⁺; the proportion of CTLA-4⁺ cells in tolerant mice is 10 times higher than in naïve animals. PI-T_{Reg} cells suppress the proliferation of naïve CD4⁺ T cells when co-cultured *in vitro* by an as yet undetermined mechanism; neutralisation of IL-10 or TGFβ does not abrogate suppression, although cell-cell contact is required for suppression to occur [40]. It is possible that suppression *in vitro* is linked to IL-2 as the addition of IL-2 to co-cultures reverses suppression.

PI-T_{Reg} cells are also suppressive *in vivo*, preventing induction of EAE in treated mice [65]. Interestingly however, the protection mediated *in vivo* is dependent on IL-10; injection of IL-10-neutralising antibody abrogates suppression [40]. PI-T_{Reg} mediated tolerance can also be transferred from one mouse to another; the transfer of 5×10⁷ tolerant splenocytes to a naïve recipient results in both reduced proliferation among naïve cells and serum IL-2 production upon antigenic challenge, again in an IL-10-dependent mechanism [40].

A similar population of T_{regs} termed Th10 cells have also been described following repeated administration of superantigen when it was observed that repeated injection of staphylococcal enterotoxin A (SEA) resulted in the inhibition of proliferation of naïve cells and an increase in IL-10 production [66]. A single injection of SEA elicited significant amounts of IFNγ, IL-2 and TNFα and only marginal amounts of IL-4 and IL-10 in the serum [66]. However, after a third injection there was a downregulation in IL-2, TNFα, IFNγ and IL-4 whereas IL-10 levels were significantly upregulated. The upregulation of IL-10 coincided with reduced responsiveness to SEA. Administration of neutralising anti-IL-10 antibody restored serum levels of TNFα and IFNγ, and also to some degree, the IL-4, indicating the importance of IL-10 in the inhibitory effect of the cells. Elimination of subsets of T cells revealed that CD4⁺ T cells were crucial for the IL-10 production [66].

It is clear that the differentiation of cells secreting predominantly IL-10 can be encouraged *in vitro* by culture in the presence of IL-10 or drugs such as vitamin D3 and dexamethasone; as reviewed elsewhere by O'Garra and colleagues [67]. It is important to note, however, that work from various laboratories has now emphasised ways in which these cells may be induced *in vivo* [40,65,66,68]. It appears likely that these cells can differentiate from naïve precursor cells since they may differentiate in the absence of natural T_{reg} cells and do not necessarily express FoxP3 [24]. In certain situations, however, it has been possible to induce the differentiation of FoxP3 expressing T_{reg} cells from naïve precursors [69]. Either way, the fact that T_{reg} cells may be induced to differentiate from naïve precursors *in vivo* opens up an

important avenue for immune modulation and the development of antigen specific therapies for hypersensitivity disorders.

CONCLUSIONS

Work from various laboratories has shown that it is possible to induce regulatory cells that do not require the presence of CD25⁺ cells in order to mediate their tolerogenic properties. In combination with the observation that expression of FoxP3 was not necessarily detected in these cells, it suggests that induced T_{Reg} cells do in fact represent a population of T_{regs} distinct in origin from conventional CD4⁺CD25⁺ T_{regs} (see Fig. 1). This finding is echoed in the current literature where it is now obvious that there are indeed several different populations of T_{regs}, allowing classification beyond simply the point of endogenous versus induced populations.

It seems likely that there may be good reason for the presence of so many distinct populations of T_{regs} as individual populations may fulfil separate roles in the control of autoimmunity. Although CD4⁺CD25⁺ T_{regs} are clearly important for the prevention of autoimmune responses, their importance in homeostatic regulation is also becoming increasingly apparent and these cells may have developed specifically to fulfil this function. CD4⁺CD25⁺ T cells have high affinity for self-peptides, only slightly under the threshold for deletion in the thymus [10]. As a result, these cells are highly sensitive to MHC and can respond to MHC bearing almost any antigen, enabling suppression to a range of antigen specificities. The initial observations that autoimmune responses were induced specifically by CD25⁻ populations with self-reactive repertoires may have been confused by the absence of CD25⁺ cells in both transferred populations and recipient animals; while CD25⁺ T cells undoubtedly prevented disease when added to the cells transferred to lymphopaenic recipients, it is now likely that the mechanism by which this occurred was by controlling the homeostatic proliferation of CD25⁻ cells rather than inhibiting specific self-reactive T cell populations [28]. In a lymphopaenic animal, there is a large cell void to be filled and as such selects for populations with the most efficient reconstitution ability rather than particular antigen specificities. In fact, since these early experiments, it has now been demonstrated that CD25⁻ cells can also be protective from autoimmune disease using an EAE model in TCR transgenic mice [70].

Alyanakian and colleagues further demonstrated the distinct niches occupied by populations of T_{regs} in a NOD.SCID model; while CD25⁺ cells were essential for the control of gastritis, it was CD45RB^{lo} cells that were important for control of colitis. CD62L⁺ T cells were important for controlling diabetes while CD25⁺ T cells were protective to a lesser extent [71]. Differences within CD4⁺CD25⁺ populations have also been described on the basis of integrin expression. Stassen and colleagues demonstrated that $\alpha_4\beta_7$ ⁺ human CD4⁺CD25⁺ T_{Reg} cells induced a Tr1-like phenotype upon suppression, characterised by IL-10 production, while $\alpha_4\beta_1$ ⁺ cells induced a Th3 response characterised by TGF β production, indicating distinct subsets of T_{regs} fulfilling separate roles in suppression [72].

Induced populations of T_{regs} appear to be more specific in their suppression. Thus suppression is often only observed in response to the same antigen used for their induction [49,73]. Specific recognition of the antigen used in their induction may be important for their subsequent suppression, as many populations of induced T_{regs} appear to mediate suppression through cytokine production and therefore may require T cell signalling. Many populations of induced T_{regs} secrete cytokines such as TGF β and IL-10 that are inhibitory for proliferation by responding T cells. As shown previously, PI-T_{Reg} cells mediate their suppression in a mechanism that is at least partly dependent on IL-10 production, with partial reversion of their suppressive effects when IL-10 signalling is blocked [40]. A similar

pattern is also demonstrated for other populations of induced T_{regs} , with neutralisation of IL-10 detrimental to the suppression mediated by these cells [11,53,66]. This would seem to indicate that the main way in which induced T_{regs} exert their suppression is by the secretion of inhibitory cytokines, while the mechanisms utilised by $CD4^+CD25^+$ T_{regs} may be more varied. Together, this data would support the finding that there are many populations of T_{regs} with distinct roles, and this would not be unexpected in the immune system; the development of different populations each designed to control a specific facet of autoimmune disease or immune pathology in general would be beneficial to the immune system, allowing individual antigen responses to be controlled independently. Much as there are several populations of helper T cells in the immune system, it seems logical that there should be several populations of regulatory T cells.

Induction of selective, autoantigen-specific tolerance is the “holy grail” for the treatment and prevention of autoimmune diseases. We believe that this may now be achieved through the induction of regulatory T cells. At this time there is evidence that the induction of such cells accounts for the success of specific immunotherapy in the treatment of allergy; as reviewed recently elsewhere [74]. There is now increasing evidence that regulatory T cells provide protection in experimental models of autoimmunity [75-77] and preliminary evidence suggests that such cells may be induced in human autoimmune conditions [78]. A number of groups are extending this approach to other autoimmune conditions and we await the results of these trials with interest.

Acknowledgments

Work in the author's laboratory is supported by grants from the Wellcome Trust and the Multiple Sclerosis Society of Great Britain and Northern Ireland. KSN acknowledges support from the RJ Daniels family trust.

REFERENCES

1. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D. Science. 2002; 298:1395. [PubMed: 12376594]
2. Su MA, Anderson MS. Curr. Opin. Immunol. 2004; 16:746. [PubMed: 15511668]
3. Ponsford M, Mazza G, Coad J, Campbell MJ, Zajicek J, Wraith DC. Clin. Exp. Immunol. 2001; 124:315. [PubMed: 11422210]
4. Asano M, Toda M, Sakaguchi N, Sakaguchi S. J. Exp. Med. 1996; 184:387. [PubMed: 8760792]
5. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. J. Immunol. 1995; 155:1151. [PubMed: 7636184]
6. Powrie F, Mason D. J. Exp. Med. 1990; 172:1701. [PubMed: 2258700]
7. Almeida AR, Legrand N, Papiernik M, Freitas AA. J. Immunol. 2002; 169:4850. [PubMed: 12391195]
8. Malek TR, Yu A, Vincek V, Scibelli P, Kong L. Immunity. 2002; 17:167. [PubMed: 12196288]
9. Papiernik M, de Moraes ML, Pontoux C, Vasseur F, Penit C. Int. Immunol. 1998; 10:371. [PubMed: 9620592]
10. Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Hohenbeck AE, Lerman MA, Naji A, Caton AJ. Nat. Immunol. 2001; 2:301. [PubMed: 11276200]
11. Apostolou I, Sarukhan A, Klein L, von Boehmer H. Nat. Immunol. 2002; 3:756. [PubMed: 12089509]
12. Chen TC, Cobbold SP, Fairchild PJ, Waldmann H. J. Immunol. 2004; 172:5900. [PubMed: 15128770]
13. Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. J. Exp. Med. 1994; 179:589. [PubMed: 7905019]
14. Thornton AM, Shevach EM. J. Exp. Med. 1998; 188:287. [PubMed: 9670041]

15. McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC. *Immunity*. 2002; 16:311. [PubMed: 11869690]
16. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. *Nat. Immunol.* 2002; 3:135. [PubMed: 11812990]
17. Kanamaru F, Youngnak P, Hashiguchi M, Nishioka T, Takahashi T, Sakaguchi S, Ishikawa I, Azuma M. *J. Immunol.* 2004; 172:7306. [PubMed: 15187106]
18. Bruder D, Probst-Keppler M, Westendorf AM, Geffers R, Beissert S, Loser K, von Boehmer H, Buer J, Hansen W. *Eur. J. Immunol.* 2004; 34:623. [PubMed: 14991591]
19. Fontenot JD, Gavin M, Rudensky A. *Nat. Immunol.* 2003; 4:330. [PubMed: 12612578]
20. Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. *Immunity*. 2005; 22:329. [PubMed: 15780990]
21. Hori S, Nomura T, Sakaguchi S. *Science*. 2003; 299:1057. [PubMed: 12522256]
22. Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF. *J. Immunol.* 2004; 172:5149. [PubMed: 15100250]
23. Peng Y, Laouar Y, Li MO, Green EA, Flavell RA. *Proc. Natl. Acad. Sci. U S A.* 2004; 101:4572. [PubMed: 15070759]
24. Vieira PL, Christensen JR, Minaae S, O'Neill EJ, Barrat FJ, Boonstra A, Barthlott T, Stockinger B, Wraith DC, O'Garra A. *J. Immunol.* 2004; 172:5986. [PubMed: 15128781]
25. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. *J. Exp. Med.* 2003; 198:1875. [PubMed: 14676299]
26. Levings MK, Sangregorio R, Roncarolo MG. *J. Exp. Med.* 2001; 193:1295. [PubMed: 11390436]
27. Barthlott T, Kassiotis G, Stockinger B. *J. Exp. Med.* 2003; 197:451. [PubMed: 12591903]
28. Stockinger B, Barthlott T, Kassiotis G. *Immunology*. 2004; 111:241. [PubMed: 15009422]
29. Barthlott T, Moncrieffe H, Veldhoen M, Atkins CJ, Christensen J, O'Garra A, Stockinger B. *Int. Immunol.* 2005; 17:279. [PubMed: 15684039]
30. Annacker O, Pimenta-Araujo R, Burlen-Defranoux O, Barbosa TC, Cumano A, Bandeira A. *J. Immunol.* 2001; 166:3008. [PubMed: 11207250]
31. Thornton AM, Shevach EM. *J. Immunol.* 2000; 164:183. [PubMed: 10605010]
32. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. *Nature*. 2002; 420:502. [PubMed: 12466842]
33. Maloy KJ, Salaun L, Cahill R, Dougan G, Saunders NJ, Powrie F. *J. Exp. Med.* 2003; 197:111. [PubMed: 12515818]
34. Liu H, Hu B, Xu D, Liew FY. *J. Immunol.* 2003; 171:5012. [PubMed: 14607897]
35. Goudy KS, Burkhardt BR, Wasserfall C, Song S, Campbell-Thompson ML, Brusko T, Powers MA, Clare-Salzler MJ, Sobel ES, Ellis TM, Flotte TR, Atkinson MA. *J. Immunol.* 2003; 171:2270. [PubMed: 12928371]
36. Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. *J. Immunol.* 2001; 166:7282. [PubMed: 11390478]
37. Nakamura K, Kitani A, Strober W. *J. Exp. Med.* 2001; 194:629. [PubMed: 11535631]
38. Piccirillo CA, Letterio JJ, Thornton AM, McHugh RS, Mamura M, Mizuhara H, Shevach EM. *J. Exp. Med.* 2002; 196:237. [PubMed: 12119348]
39. Nakamura K, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W. *J. Immunol.* 2004; 172:834. [PubMed: 14707053]
40. Sundstedt A, O'Neill EJ, Nicolson KS, Wraith DC. *J. Immunol.* 2003; 170:1240–8. [PubMed: 12538682]
41. Read S, Malmstrom V, Powrie F. *J. Exp. Med.* 2000; 192:295–302. [PubMed: 10899916]
42. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S. *J. Exp. Med.* 2000; 192:303. [PubMed: 10899917]
43. Eggena MP, Walker LS, Nagabhushanam V, Barron L, Chodos A, Abbas AK. *J. Exp. Med.* 2004; 199:1725. [PubMed: 15210748]
44. Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. *J. Exp. Med.* 2004; 199:1467. [PubMed: 15184500]

45. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, Masteller EL, McDevitt H, Bonyhadi M, Bluestone JA. *J. Exp. Med.* 2004; 199:1455. [PubMed: 15184499]
46. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. *J. Exp. Med.* 2004; 199:971. [PubMed: 15067033]
47. Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, Carr VA, Robinson DS. *Lancet.* 2004; 363:608. [PubMed: 14987885]
48. Dao Nguyen X, Robinson DS. *J. Allergy Clin. Immunol.* 2004; 114:296. [PubMed: 15316506]
49. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries J, Roncarolo MG. *Nature.* 1997; 389:737–742. [PubMed: 9338786]
50. Roncarolo M-G, Levings MK, Traversari C. *J. Exp. Med.* 2001; 193:5.
51. Wakkach A, Fournier N, Brun V, Breitmayer JP, Cottrez F, Groux H. *Immunity.* 2003; 18:605. [PubMed: 12753738]
52. Kemper C, Chan AC, Green JM, Brett KA, Murphy KM, Atkinson JP. *Nature.* 2003; 421:388. [PubMed: 12540904]
53. Barrat FJ, Cua DJ, Boonstra A, Richards DF, Crain C, Savelkoul HF, de Waal-Malefyt R, Coffman RL, Hawrylowicz CM, O'Garra A. *J. Exp. Med.* 2002; 195:603. [PubMed: 11877483]
54. Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. *J. Exp. Med.* 2002; 196:247. [PubMed: 12119349]
55. Foussat A, Cottrez F, Brun V, Fournier N, Breitmayer JP, Groux H. *J. Immunol.* 2003; 171:5018. [PubMed: 14607898]
56. Chen Y, Kuchroo VK, Inobe J-I, Hafler DA, Weiner HL. *Science.* 1994; 265:1237. [PubMed: 7520605]
57. Han HS, Jun HS, Utsugi T, Yoon JW. *J. Autoimmun.* 1996; 9:331. [PubMed: 8816968]
58. Zheng SG, Gray JD, Ohtsuka K, Yamagiwa S, Horwitz DA. *J. Immunol.* 2002; 169:4183. [PubMed: 12370347]
59. Metzler B, Wraith DC. *Int. Immunol.* 1993; 5:1159. [PubMed: 7694644]
60. Metzler B, Wraith DC. *Annals of the New York Academy of Sciences.* 1996; 778:228. [PubMed: 8610976]
61. Fairchild PJ, Wildgoose R, Atherton E, Webb S, Wraith DC. *Intern. Immunol.* 1993; 5:1151.
62. Liu GY, Wraith DC. *Int. Imm.* 1995; 7:1255.
63. Anderton SM, Wraith DC. *Eur. J. Immunol.* 1998; 28:1251. [PubMed: 9565365]
64. Liu GY, Fairchild PJ, Smith RM, Prowle JR, Kioussis D, Wraith DC. *Immunity.* 1995; 3:407. [PubMed: 7584132]
65. Burkhart C, Liu GY, Anderton SM, Metzler B, Wraith DC. 1999 *International Immunology.* 1995; 11:1625. [PubMed: 10508180]
66. Sundstedt A, Hoiden I, Rosendahl A, Kalland T, Van Rooijen N, Dohlsten M. *J. Immunol.* 1997; 158:180. [PubMed: 8977189]
67. O'Garra A, Vieira PL, Vieira P, Goldfeld AE. *J. Clin. Invest.* 2004; 114:1372. [PubMed: 15545984]
68. Buer J, Lanoue A, Franzke A, Garcia C, vonBoehmer H, Sarukhan A. *J. Exp. Med.* 1998; 187:177. [PubMed: 9432975]
69. Apostolou I, Von Boehmer H. *J. Exp. Med.* 2004; 199:1401. [PubMed: 15148338]
70. Olivares-Villagomez D, Wensky AK, Wang Y, Lafaille JJ. *J. Immunol.* 2000; 164:5499. [PubMed: 10799918]
71. Alyanakian MA, You S, Damotte D, Gouarin C, Esling A, Garcia C, Havouis S, Chatenoud L, Bach JF. *Proc. Natl. Acad. Sci. USA.* 2003; 100:15806. [PubMed: 14673094]
72. Stassen M, Fondel S, Bopp T, Richter C, Muller C, Kubach J, Becker C, Knop J, Enk AH, Schmitt S, Schmitt E, Jonuleit H. *Eur J Immunol.* 2004; 34:1303–11. [PubMed: 15114663]
73. Bynoe MS, Evans JT, Viret C, Janeway CA Jr. *Immunity.* 2003; 19:317–28. [PubMed: 14499108]
74. Larche M, Wraith DC. *Nat. Med.* 2005; 11:S69–76. [PubMed: 15812493]
75. Wraith, DC. *New Generation Vaccines.* Myron, M.; Levine, JBK.; Rappuoli, Rino; Liu, Margaret; Good, Michael, editors. Marcel Dekker Inc.; New York: 2004.

76. Anderton SM. Immunology. 2001; 104:367. [PubMed: 11899421]
77. Wraith DC, Nicolson KS, Whitley NT. Curr. Opin. Immunol. 2004; 16:695. [PubMed: 15511660]
78. Prakken BJ, Samodal R, Le TD, Giannoni F, Yung GP, Scavulli J, Amox D, Roord S, de Kleer I, Bonnin D, Lanza P, Berry C, Massa M, Billetta R, Albani S. Proc. Natl. Acad. Sci. USA. 2004; 101:4228. [PubMed: 15024101]

Fig. (1).

CD4⁺ regulatory T cells. The thymus generates both CD25⁻ and CD25⁺ Treg cells. T cells respond to antigen presented by mature dendritic cells by differentiating into effector T cells (Teff), secreting cytokines and providing help for B cells and cytotoxic T cells. Antigen presented by immature dendritic cells drives the differentiation of CD25⁻ cells, such as IL-10⁺ Treg cells, that suppress effector cell generation. Thymus-derived CD25⁺ Treg cells block expansion of the effector cell population. CD25⁺FoxP3⁺ Treg cells can also be generated from CD25⁻ precursors in peripheral lymphoid tissues following repeated encounter of antigen presented by immature DC. Both these and other regulatory populations depend on the production of cytokines, such as IL-10 and TGF- β , for suppression *in vivo*, although the requirement for cytokines depends on the nature of the effector T cell response.