Review

A paragon of self-tolerance: CD25$^+$CD4$^+$ regulatory T cells and the control of immune responses

Zoltán Fehérvári and Shimon Sakaguchi

Department of Experimental Pathology, Kyoto University, Kyoto, Japan

Correspondence: Shimon Sakaguchi (e-mail: shimon@frontier.kyoto-u.ac.jp)

Received: 23 Sep 2003 Accepted: 3 Dec 2003 Published: 19 Dec 2003

Arthritis Res Ther 2004, **6**:19-25 (DOI 10.1186/ar1037)

Abstract

The interest in naturally arising regulatory T (T_R) cells as a paradigm for maintaining immunological self-tolerance has undergone an explosive re-emergence in recent years. This renaissance was triggered by several key experimental observations and the identification of specific molecular markers that have enabled the isolation and experimental manipulation of these cells. Although their existence was once controversial, a large body of evidence now highlights the critical roles of T_R cells in maintaining immunological self-tolerance. Furthermore, abnormality of natural T_R cells can be a primary cause of autoimmune and other inflammatory diseases in humans.

Keywords: CD25$^+$CD4$^+$, Foxp3, regulatory cells, self-tolerance, suppression

Introduction

The random nature of T-cell receptor (TCR) generation inevitably leads to the appearance of deleterious autoreactive clones, but the vast majority of such cells are purged in the thymus during negative selection. However, there is abundant evidence showing that significant numbers of autoreactive cells can 'slip through the net' of central tolerance into the periphery and thereby potentially mediate autoimmunity. This phenomenon can be readily demonstrated by the experimental induction of autoimmunity when otherwise normal animals are injected with self proteins plus a strong adjuvant [1].

The fact that healthy animals harbour such destructive cells implies the existence of mechanisms operating in the periphery that are able to effectively prevent their activation. Experimental evidence has indeed revealed numerous avenues by which this can occur, among them immune ignorance, peripheral deletion/anergy, and dominant suppression (reviewed in [2]). The existence of a specific T cell subset that could dominantly suppress immune responses was first proposed by Gershon and Kondo in 1970 [3]. The concept developed from experiments suggesting that tolerance was an active cell-mediated process and could be transferred into naïve animals. Elaborate circuits involving *suppressor*, *contrasuppressor* and *veto* cells were proposed to explain the maintenance of self-tolerance; however, the inability to clone any actual suppressor cells or identify critical molecules associated with them led to the decline of such a model. Furthermore, the subsequent emergence of the Th1–Th2 paradigm seemed largely able to subsume suppression phenomena by the patterns of regulatory cytokines that these cells could secrete, and the parsimony thus offered seemed much more attractive as a theory.

In contrast, accumulated evidence from the mid-1980s has shown that depletion of a particular T cell subset from normal animals can cause autoimmune disease similar to the counterparts in humans, and that reconstitution of this subset can prevent these diseases. Subsequent detailed phenotypic characterisation of such autoimmune preventative cells now leaves no doubt of the existence of T_R cells as crucial mediators of self-tolerance in both animal models and humans.

APC = antigen-presenting cell; DC = dendritic cell; GITR = glucocorticoid-induced tumour necrosis factor family related protein; IBD = inflammatory bowel disease; IL = interleukin; IL-2R, interleukin-2 receptor; TCR = T-cell receptor; TGF = transforming growth factor; Th = T helper cell; T_R cell = regulatory T cell.

Defining a 'regulatory cell'

Broadly speaking, T cells with regulatory properties can be divided into two types: naturally occurring thymically generated regulatory cells, defined here as 'T_R cells', and those generated by antigenic stimulation under special conditions in the periphery, referred to variously as 'Th3', 'Tr1' cells or 'adaptive regulatory cells' (see, for example, [4]). This review will focus chiefly on the naturally arising suppressive T_R cells.

A discrete molecular description of T_R cells has proved to be a key issue in this field and was indeed one of the major stumbling blocks to their original exposition. Early clues hinting at the identity of regulatory cells emerged from experimental models of autoimmune disease. Many such models require the induction of lymphopoenia in genetically susceptible strains of rodents, for example 3-day-old neonatal thymectomy or adult thymectomy coupled with an immunosuppressive treatment such as cyclophosphamide [5–7]. Depending on the strain background, experimental manipulations of this kind result in a variety of autoimmune diseases such as thyroiditis, gastritis, oophoritis and orchitis. It was subsequently shown that induction of such autoimmunity could be prevented by the transfer of normal $CD4^+$ splenocytes or $CD4^+CD8^-$ thymocytes [7–10]. Collectively, such data strongly suggested that a cell population with a crucial role in maintaining self-tolerance was resident within the normal T lymphocyte pool.

Attempts were then made to phenotype putative T_R cells more specifically by isolating the T lymphocyte fraction that harboured regulatory activity. Sakaguchi and colleagues managed to first identify the CD5 molecule as a marker for T_R cells by demonstrating that otherwise normal lymphocytes depleted of $CD5^{high}CD4^+$ cells induced broad-spectrum autoimmunity when transferred into athymic nude mice [11]. Unfractionated $CD4^+$ cells (which contain $CD5^{high}$-expressing cells) prevented the induction of autoimmunity when transferred together with the $CD5^{low}$ cells, implying that the T_R cells were contained specifically within the $CD5^{high}$ compartment. Subsequent experiments aimed at homing in yet further on T_R cell-specific markers have identified a number of other potential candidate molecules. For instance, CD45RB seems to divide T cells into two distinct functional subsets [12]. Lymphopoenic mice transferred with $CD45RB^{high}$ cells develop a lethal wasting disease characterised by severe inflammatory bowel disease (IBD), 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 colitis.

More recently, the most useful surface marker for T_R cells has proved to be the interleukin-2 (IL-2) receptor α-chain, CD25 [13]. About 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 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 transfer with $CD25^+CD4^+$ cells inhibits such disease development. Moreover, $CD25^+CD4^+$ cells in normal naïve mice exhibit clear immunosuppressive properties *in vitro* and *in vivo* [13,14]. It now seems that the naturally occurring $CD25^+CD4^+$ population could account for the regulatory effect of $CD5^{high}$ and $CD45RB^{low}$ $CD4^+$ T cells.

A comprehensive characterisation of the surface profile of T_R cells has revealed them to be quite distinct from conventional naïve effector T cells. Aside from the constitutive expression of CD25, T_R cells show elevated levels of adhesion molecules such as CD11a (LFA-1), CD44, CD54 (ICAM-1), CD103 ($\alpha_E\beta_7$ integrin) in the absence of any apparent exogenous antigenic stimulation [14,15]. Naturally occurring $CD25^+CD4^+$ cells additionally express CD152 (CTLA-4), a molecule classically only expressed after T cell activation [16–18]. There is some evidence to suggest that T_R cells might also exhibit a characteristic chemokine receptor profile, with mouse $CD25^+CD4^+$ cells expressing elevated levels of CCR5 and their human counterparts expressing CCR4 and CCR8 [19,20]. Such a distinctive pattern of chemokine receptors suggests that T_R cells might be rapidly recruited to sites of inflammation and thereby efficiently control immune responses. Most recently, several groups have demonstrated that glucocorticoid-induced tumour necrosis factor family related protein (GITR) is predominantly expressed at both the RNA and protein levels by $CD25^+CD4^+$ cells [15,21,22]. Administration of the anti-GITR monoclonal antibody, DTA-1, *in vivo* elicits autoimmune disease, suggesting that this molecule has an important functional role in maintaining T_R cell suppression [22].

The surface marker profile of T_R cells is thus quite different from that of naïve T cells. However, it should be noted that most, if not all, of their apparently characteristic molecules are upregulated during conventional T cell activation. This similarity to otherwise normal but primed T cells is potentially problematic when trying to identify or isolate true T_R cells and precludes the use of CD25 alone (or any other surface molecule yet found) as an infallible marker. This caveat aside, several important distinctions still remain between the surface phenotype of T_R and primed T cells, but they are more relative than absolute. For example, although both primed T cells and T_R cells express CD25, the latter does so to a higher level and more stably. Indeed, when stimulation of normal T cells ceases, CD25 expression is lost, whereas T_R cells revert to their original constitutive expression level [23]. In addition, $CD25^+$ cells generated from originally $CD25^-CD4^+$ cells show no suppressive ability either *in vitro* or *in vivo* [23]. As a component of the high-affinity IL-2 receptor, CD25 itself is

essential for the survival of T_R cells, and the cells are exquisitely sensitive to an absence of signalling through this receptor [24]. Clear evidence for this can be seen by the almost total absence of $CD25^+CD4^+$ cells in IL-2-deficient mice. In conclusion, the similarities between T_R cells and primed T cells are therefore probably only a reflection of a shared activation state.

As noted above, the search for a definitive T_R cell marker has been fraught with complications and an occasional lack of certitude regarding their undeniable existence as a functionally distinct population rather than simply another activation state of conventional T cells. However, some very recent data have gone some way to demonstrating conclusively that T_R cells are a genuine T cell lineage, in the process identifying a seemingly unambiguous marker [25–27]. Studies with the Scurfy (*sf*) mutant mouse model provided the required breakthrough. The Scurfy mouse exhibits a fatal X-linked lymphoproliferative disease that is mediated by highly activated $CD4^+$ T cells and is akin to the phenotype of both CTLA-4 and transforming growth factor (TGF)-β knockout mice [28–32]. Subsequent work mapped the *sf* mutation to a novel forkhead/winged-helix family transcriptional repressor termed *Foxp3*, which encodes the protein scurfin [33]. A mutation in the human orthologue, *FOXP3*, has also been identified as the underlying cause of the aggressive autoimmune syndrome IPEX (for Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked syndrome) [33–35].

The overt immunological similarities seen with genetic defects of *Foxp3* and the experimental depletion of $CD25^+CD4^+$ T_R cells led several groups to investigate the potential role of *Foxp3* in the development and function of T_R cells. Three independent groups were able to demonstrate that *Foxp3* mRNA [25–27] and the encoded protein [27] were specifically expressed only in naturally arising $CD25^+CD4^+$ T_R cells and, critically, were never observed in normal T cells even after they had been activated and acquired the expression of CD25/GITR. However, a very low level of *Foxp3* expression was observed in $CD25^-CD4^+$ T cells; this appeared to be attributable to a small population of $CD25^-CD45RB^{low}GITR^{high}$ T_R cells ([26], M Ono, manuscript in preparation). In addition, T_R cells were unable to develop in the absence of *Foxp3*, as demonstrated by the use of *sf* mice or by the targeted deletion of *Foxp3* [25,27]. Finally, and most convincingly, retroviral transduction of *Foxp3* into conventional $CD25^-$ *Foxp3*$^-$ T cells converted them into phenotypical and functional T_R cells capable of effectively suppressing both *in vitro* and *in vivo* [26,27]. Thus *Foxp3* seems to be a 'master gene' controlling the normal development and/or function of naturally occurring T_R cells.

As yet there are very few data detailing the role and expression patterns of *FOXP3* in human cells. Some of the early indications, both published and unpublished, have shown *FOXP3* expression in human $CD25^+CD4^+$ T cells ([36], H Yagi and S Sakaguchi, unpublished results); however, it already seems that there are some discrepancies with the murine data. For example, there seem to be considerable differences in *FOXP3* expression between individuals and, more significantly, *FOXP3* might be inducible in human $CD25^-CD4^+$ cells (which start off apparently *FOXP3*$^-$) after anti-CD3/anti-CD28 stimulation [36]. It remains to be determined whether this simply represents an expansion to detectability of the tiny *Foxp3*$^+$$GITR^+CD25^-CD4^+$ population described above ([26], M Ono, manuscript in preparation) or is a genuine property of human T cells radically different from that of mice.

The suppressive properties of T_R cells can be modelled *in vitro* by mixing titrated numbers of highly purified $CD25^+CD4^+$ cells and $CD25^-CD4^+$ (or $CD25^-CD8^+$) responder cells plus a TCR stimulus such as anti-CD3, ConA or antigen-presenting cells (APCs) plus antigenic peptide. Under such conditions, the $CD25^+$ population suppresses both the proliferation and IL-2 production of the $CD25^-$ cells in a dose-dependent manner [37,38]. The T_R cells require TCR stimulation to exert any suppressive effects, but once this condition has been satisfied the ensuing suppression is non-specific for antigen [37,38]. Suppression is therefore an active process and can be directed against bystander cells.

Curiously, the $CD25^+CD4^+$ T_R cells themselves are anergic *in vitro*; that is, they do not proliferate or produce IL-2 in response to conventional T cell stimuli. However, this anergy can be broken by a sufficiently potent stimulus such as the addition of exogenous IL-2 or anti-CD28, or the use of mature dendritic cells as APCs [37,39]. Interestingly, anergy seems to be the default state for T_R cells, because they revert to it once IL-2 is withdrawn [37,38]. However, the anergy *in vitro* is not reflected *in vivo*, wherein T_R cells seem to have a highly active rate of turnover [24]. An anergic state also seems to be closely related to T_R cells' suppressive ability because if it is broken there is a concomitant loss of regulatory activity both *in vitro* and *in vivo* [37]. Table 1 summarises what is currently known about the T_R cell phenotype.

Development and origin

$CD25^+CD4^+$ T_R cells are produced by the normal thymus as fully functioning suppressive cells, and such thymocytes exhibit apparently all the properties of their matured peripheral counterparts [14]. Itoh and colleagues showed that the adoptive transfer of CD25-depleted thymocytes to syngeneic nude mice recipients led to a similar spectrum of autoimmune disease to that with $CD25^-CD4^+$ peripheral cells [14]. $CD25^+CD4^+$ thymocytes are also anergic and suppressive *in vitro* and exhibit a classic T_R cell surface phenotype, for example elevated levels of activa-

Table 1

Comparison of the phenotype of conventional naïve CD4+ T cells and CD4+ regulatory cells (T_R)

Conventional naïve helper T cell	Natural regulatory T cell (T_R)
Foxp3$^-$	*Foxp3*$^+$
CD5^{low}, CD11a^{low}, CD25^{low}, CD38^{low}, CD44^{low}, CD45RB^{high}, CD54^{low}, CD103ε^{low}, GITR^{low}	CD5^{high}, CD11a^{high}, CD25^{high}, CD38^{high}, CD44^{high}, CD45RB^{low}, CD54^{high}, CD103^{high}, GITR^{high}
About 90–95% of splenic CD4$^+$ T cells	About 5–10% of splenic CD4$^+$ T cells
Responsive to conventional T cell stimuli	Anergic to conventional T cell stimuli
Non-suppressive	Suppressive

Many of the distinctions are not absolute; for instance, activated non-regulatory effector T cells express cell surface markers with a pattern similar to that of T_R cells, so such discrimination is possible only with constitutive expression. Currently, expression of *Foxp3* seems to be the most accurate marker for T_R cells because this does not vary with the activation state.

tion markers such as CTLA-4 and GITR; importantly they are also *Foxp3*$^+$ [14,22,26].

T_R cells can develop in TCR transgenic mice specific for an exogenous peptide; however, those cells that do develop show a strong bias for expressing an endogenous TCR-α chain paired with the transgenic β-chain, in contrast to CD25$^-$CD4$^+$ cells, which predominantly expressed only the whole transgenic TCR [14,40]. When these mice were bred onto a RAG-2$^{-/-}$ or TCR$\alpha^{-/-}$ background (both of which lack endogenous α-chain gene rearrangements), CD25$^+$CD4$^+$ cells were eliminated, suggesting that signalling through TCRs expressing the endogenous TCRα-chains was necessary for their development [14,40].

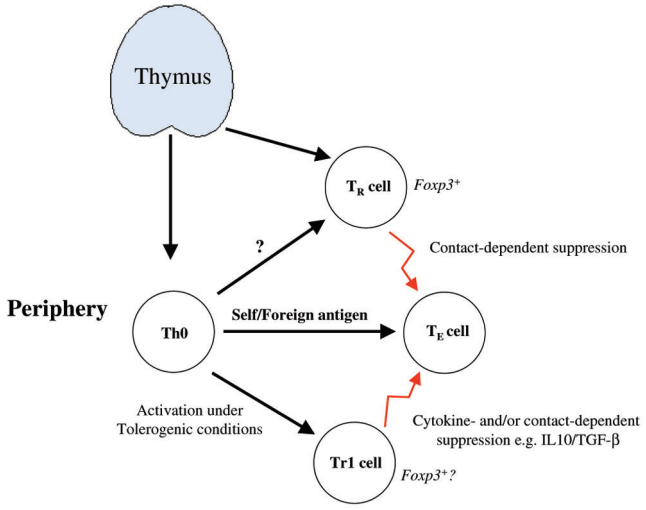
Furthermore, studies with a doubly transgenic mouse have also demonstrated that the CD25$^+$CD4$^+$ T_R cells show a high self-reactivity and differentiate on thymic epithelial cells [41,42]. Thus, the central generation of CD25$^+$CD4$^+$ T_R cells is dependent on relatively high-avidity TCR interactions with self-peptide/MHC complexes within the thymic stroma. However, it is still not clear why the relatively self-reactive T_R cell precursors escape thymic negative selection and instead begin a developmental programme involving *Foxp3*. Although apparently not required for the activation of suppressive functions, the classic co-stimulatory molecule CD28 seems to be important in the thymic production of T_R cells and/or their peripheral maintenance, as demonstrated by markedly reduced T_R cell numbers in CD28$^{-/-}$ animals [18]. A similar decrease in the T_R cell population could also be observed by blockading CD28–B7 interactions with CTLA-4-immunoglobulin fusion protein [18]. Finally, CD40–CD40L interactions also seem to be important in the development of T_R cells, as shown by their marked decrease in CD40$^{-/-}$ mice [43].

The extra-thymic generation of T_R cells from conventional CD25$^-$CD4$^+$ cells is still an open question. It is clear that T cells with regulatory properties and an anergic phenotype (such as the aforementioned Tr1 cells) can be generated in the periphery, but whether these are identical to naturally occurring T_R cells remains to be established. Several approaches have led to the peripheral generation of regulatory cells. For instance, activation of conventional T cells in the presence of TGF-β/IL-10 or with the immunomodulatory agent 1-α-25-dihydroxyvitamin D_3 produces a suppressive T cell [44,45]. Also of potential interest is the induction of regulatory cells by immature or 'tolerogenic' dendritic cells (DCs) [46,47]. Additionally, in some now classic studies, Qin and colleagues were able to generate regulatory cells by the administration of non-depleting anti-CD4 monoclonal antibodies *in vivo* to thymectomised mice (reviewed in [48]). A final confirmation of whether such peripherally generated regulatory cells are contiguous with naturally occurring T_R cells or are simply another T cell activation state will have to await the assessment of *Foxp3* expression. A summary of T_R cell developmental steps is shown in Fig. 1.

Mechanisms of suppression

The suppression mechanism of activation-induced regulatory cells such as Tr1 cells is based primarily on the secretion of anti-inflammatory cytokines such as IL-10 and TGF-β (reviewed in [49]). The situation with naturally occurring T_R cells is not nearly so clear-cut and despite intense interest remains strangely inconclusive. Potential T_R cell suppression mechanisms can basically be divided into those mediated by secreted factors and those requiring intimate cell–cell contact. Most of the experiments *in vivo* examining T_R cell suppression have been based on the murine IBD model described above and have, as with Tr1 cells, flagged the importance of IL-10 and TGF-β. By blocking IL-10 signalling *in vivo* with monoclonal antibodies against the IL-10 receptor, Asseman and colleagues were able to abrogate the normal IBD-preventative action of CD45RB^{low} T cells [50]. The same group was also able to show that CD45RB^{low} T cells from IL-10-deficient mice were unable to prevent colitis and, moreover, were even colitogenic themselves [50]. The importance of IL-10 in

Figure 1



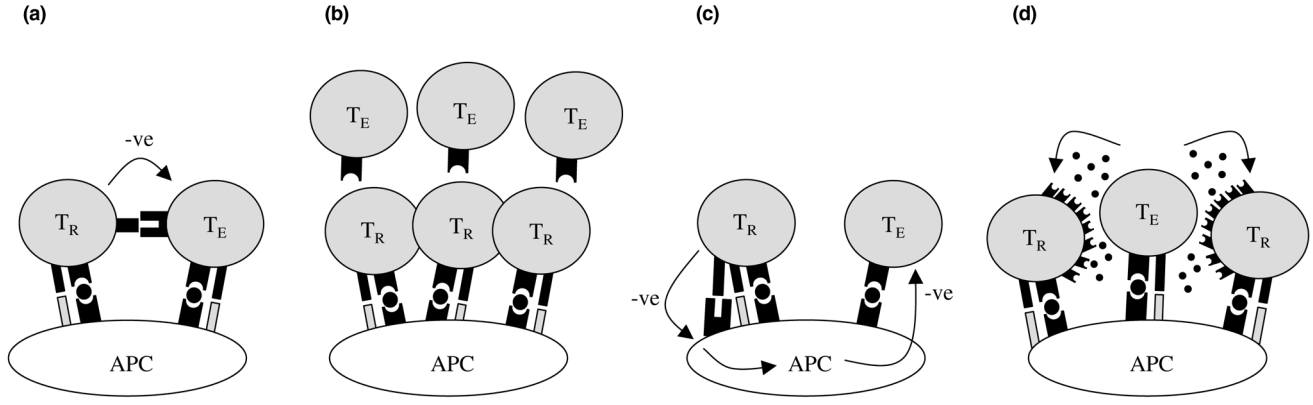
A putative scheme for the development of regulatory T (T_R) cells. T_R and naïve conventional helper T cells (Th0) develop within a normal thymus through the processes of positive and negative selection. Precursor T cells of relatively high avidity trigger a T_R cell developmental programme involving the activation of *Foxp3*, whereas T cell receptors of intermediate avidity yield Th0 cells. Additionally, regulatory cells can be peripherally generated (for example, Tr1 cells) when activated under tolerogenic conditions (for example, with immature dendritic cells). As yet it is unclear whether *de facto Foxp3*+ T_R cells can be generated in the periphery or whether the Tr1 cells produced from conventional Th0 cells are equivalent to naturally present T_R cells. IL, interleukin; T_E cell, effector T cell; TGF, transforming growth factor.

the control of IBD is also implied by the observation that IL-10$^{-/-}$ mice spontaneously develop IBD even though these mice are not lymphopoenic [51].

Similarly, several groups have shown that a monoclonal-antibody-mediated blockade of TGF-β abrogates T_R cell suppressive functions both *in vivo* and *in vitro* [52,53]. Interestingly, TGF-β does not necessarily have to act as a soluble factor but can be expressed exclusively on the surface of CD25+CD4+ cells after stimulation through the TCR and might therefore mediate its effects in a membrane-proximal manner [53]. The level at which these anti-inflammatory cytokines operate to maintain tolerance is also uncertain, but it might be through the inhibition of APCs or pathogenic T cells, by maintenance of the T_R cell population and/or by enhancement of their function (reviewed in [54]).

Elucidation of the mechanism of T_R cell suppression is complicated by the fact that most evidence *in vitro* shifts the emphasis of suppression to mechanisms solely based on cell contact. First, anti-IL-10 or anti-TGF-β monoclonal antibody fails to perturb the suppressive activity of CD25+CD4+ cells *in vitro* [54], although the use of soluble IL-10R seems to have a partial effect [55]. A study showing the successful neutralisation of suppression with anti-TGF-β monoclonal antibodies at the same time also demonstrated the TGF-β to be bound to the cell surface [53]. Second, culture supernatants from activated CD25+CD4+ cells show no inherent suppressive ability, nor is any suppression observed across a semi-permeable membrane [37,38]. Taken together, the data *in vitro* thus seem to obviate the role of not merely IL-10/TGF-β but also soluble factors in general, suggesting that T_R cell suppression is dependent on close cell–cell contact, although it is still impossible to discount completely the possibility that suppression is mediated in an extreme paracrine fashion. The membrane events that occur during cell contact-dependent suppression are entirely unclear, but presum-

Figure 2



Possible mechanisms of regulatory T (T_R) cell suppression. These mechanisms are not necessarily mutually exclusive, and potentially two or more might act in concert. **(a)** Antigen-presenting cell (APC)-activated T_R cells transduce an unidentified *active* negative signal to nearby effector T (T_E) cells located on the same APC or an adjacent one. **(b)** T_R cells outcompete T_E cells for stimulatory ligands on the APC surface by virtue of their high expression of adhesion molecules. **(c)** T_R cells modulate the behaviour of the APC so that they become ineffective or suppressive stimulators of T_E cells. **(d)** CD25 expression by the T_R cells acts as an interleukin-2 sink and hinders the autocrine/paracrine stimulation of T_E cells.

ably an as yet uncharacterised inhibitory molecule is expressed on the surface of activated T_R cells (see Fig. 2).

Another mechanism of suppression mediated by cell contact could proceed via simple competition for APCs and specific major histocompatibility complex-peptide antigenic complexes. The high level of adhesion molecules and chemokine receptors present on the surface of T_R cells would make them particularly well suited to homing to, and stably interacting with, APCs, thereby physically excluding normal $CD25^-CD4^+$ effector cells. Furthermore, constitutive expression of the high-affinity IL-2R would make T_R cells into an effective sink for IL-2, depriving potential autoreactive cells of this essential growth factor. A final, conceptually attractive model of suppression would be T_R cell-mediated inhibition or alteration of APC function. Supporting this model is the observation that $CD25^+CD4^+$ cells could alter the antigen-presenting function of DC by downregulating their expression levels of CD80/CD86 [56] or, as has recently been demonstrated, by triggering the immunosuppressive catabolism of tryptophan by DC [57]. Although APC perturbation might well occur *in vivo*, it is not essential because T_R cells are able to suppress effectively even in the absence of any APCs [58].

Conclusion

Solid evidence now strongly supports the existence of the once controversial T_R cells as key controllers of self-tolerance. Although limitations of space have forced this review to focus primarily on the role of T_R cells and autoimmunity, there are ample data to suggest that this lineage might be crucial wherever immune reactions need to be regulated or tuned. For instance, T_R cells might limit anti-tumour or microbial immune responses. A strategic manipulation of T_R cells might thus be used either to enhance or to dampen immune responses as required. The identification of molecular markers, in particular *Foxp3*, has permitted the accurate isolation and study of these cells in ways not previously possible and will, it is hoped, facilitate therapeutic intervention with this potentially powerful immunological ally.

Competing interests

None declared.

Acknowledgements

We thank our colleagues at Kyoto University for stimulating discussion and for permission to cite prepublication work. ZF is supported by a research fellowship from the Japan Society for the Promotion of Sciences.

References

1. Cohen IR: **Regulation of autoimmune disease physiological and therapeutic.** *Immunol Rev* 1986, **94:**5-21.
2. Arnold B: **Levels of peripheral T cell tolerance.** *Transpl Immunol* 2002, **10:**109-114.
3. Gershon RK, Kondo K: **Cell interactions in the induction of tolerance: the role of thymic lymphocytes.** *Immunology* 1970, **18:**723-737.
4. Bluestone JA, Abbas AK: **Natural versus adaptive regulatory T cells.** *Nat Rev Immunol* 2003, **3:**253-257.
5. Nishizuka Y, Sakakura T: **Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice.** *Science* 1969, **166:**753-755.
6. Sakaguchi S, Sakaguchi N: **Organ-specific autoimmune disease induced in mice by elimination of T cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease.** *J Immunol* 1989, **142:**471-480.
7. Sakaguchi N, Miyai K, Sakaguchi S: **Ionizing radiation and autoimmunity. Induction of autoimmune disease in mice by high dose fractionated total lymphoid irradiation and its prevention by inoculating normal T cells.** *J Immunol* 1994, **152:**2586-2595.
8. Seddon B, Mason D: **Regulatory T cells in the control of autoimmunity: the essential role of transforming growth factor beta and interleukin 4 in the prevention of autoimmune thyroiditis in rats by peripheral $CD4^+CD45RC^-$ cells and $CD4^+CD8^-$ thymocytes.** *J Exp Med* 1999, **189:**279-288.
9. Sakaguchi S, Takahashi T, Nishizuka Y: **Study on cellular events in post-thymectomy autoimmune oophoritis in mice. II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis.** *J Exp Med* 1982, **156:**1577-1586.
10. Kojima A, Tanaka-Kojima Y, Sakakura T, Nishizuka Y: **Prevention of postthymectomy autoimmune thyroiditis in mice.** *Lab Invest* 1976, **34:**601-605.
11. Sakaguchi S, Fukuma K, Kuribayashi K, Masuda T: **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.** *J Exp Med* 1985, **161:**72-87.
12. Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL: **Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice.** *Int Immunol* 1993, **5:**1461-1471.
13. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M: **Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases.** *J Immunol* 1995, **155:**1151-1164.
14. Itoh M, Takahashi T, Sakaguchi N, Kuniyasu Y, Shimizu J, Otsuka F, Sakaguchi S: **Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance.** *J Immunol* 1999, **162:**5317-5326.
15. McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC: **$CD4^+CD25^+$ immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor.** *Immunity* 2002, **16:**311-323.
16. Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S: **Immunologic self-tolerance maintained by $CD25^+CD4^+$ regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4.** *J Exp Med* 2000, **192:**303-310.
17. Read S, Malmstrom V, Powrie F: **Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of $CD25^+CD4^+$ regulatory cells that control intestinal inflammation.** *J Exp Med* 2000, **192:**295-302.
18. Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA: **B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes.** *Immunity* 2000, **12:**431-440.
19. Bystry RS, Aluvihare V, Welch KA, Kallikourdis M, Betz AG: **B cells and professional APCs recruit regulatory T cells via CCL4.** *Nat Immunol* 2001, **2:**1126-1132.
20. Iellem A, Mariani M, Lang R, Recalde H, Panina-Bordignon P, Sinigaglia F, D'Ambrosio D: **Unique chemotactic response profile and specific expression of chemokine receptors CCR4 and CCR8 by $CD4^+CD25^+$ regulatory T cells.** *J Exp Med* 2001, **194:**847-853.
21. Gavin MA, Clarke SR, Negrou E, Gallegos A, Rudensky A: **Homeostasis and anergy of $CD4^+CD25^+$ suppressor T cells *in vivo*.** *Nat Immunol* 2002, **3:**33-41.
22. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S: **Stimulation of $CD25^+CD4^+$ regulatory T cells through GITR breaks immunological self-tolerance.** *Nat Immunol* 2002, **3:**135-142.

23. Kuniyasu Y, Takahashi T, Itoh M, Shimizu J, Toda G, Sakaguchi S: **Naturally anergic and suppressive CD25$^+$CD4$^+$ T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation.** *Int Immunol* 2000, **12:**1145-1155.
24. Almeida AR, Legrand N, Papiernik M, Freitas AA: **Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers.** *J Immunol* 2002, **169:**4850-4860.
25. Khattri R, Cox T, Yasayko SA, Ramsdell F: **An essential role for Scurfin in CD4$^+$CD25$^+$ T regulatory cells.** *Nat Immunol* 2003, **4:** 337-342.
26. Hori S, Nomura T, Sakaguchi S: **Control of regulatory T cell development by the transcription factor Foxp3.** *Science* 2003, **299:**1057-1061.
27. Fontenot JD, Gavin MA, Rudensky AY: **Foxp3 programs the development and function of CD4$^+$CD25$^+$ regulatory T cells.** *Nat Immunol* 2003, **4:**330-336.
28. Lyon MF, Peters J, Glenister PH, Ball S, Wright E: **The scurfy mouse mutant has previously unrecognized hematological abnormalities and resembles Wiskott–Aldrich syndrome.** *Proc Natl Acad Sci USA* 1990, **87:**2433-2437.
29. Godfrey VL, Wilkinson JE, Rinchik EM, Russell LB: **Fatal lymphoreticular disease in the scurfy (sf) mouse requires T cells that mature in a sf thymic environment: potential model for thymic education.** *Proc Natl Acad Sci USA* 1991, **88:**5528-5532.
30. Blair PJ, Bultman SJ, Haas JC, Rouse BT, Wilkinson JE, Godfrey VL: **CD4+CD8$^-$ T cells are the effector cells in disease pathogenesis in the scurfy (sf) mouse.** *J Immunol* 1994, **153:**3764-3774.
31. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH: **Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4.** *Immunity* 1995, **3:** 541-547.
32. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T: **Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease.** *Nature* 1992, **359:**693-699.
33. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F: **Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse.** *Nat Genet* 2001, **27:**68-73.
34. Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF, Ochs HD: **The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3.** *Nat Genet* 2001, **27:**20-21.
35. Wildin RS, Smyk-Pearson S, Filipovich AH: **Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome.** *J Med Genet* 2002, **39:** 537-545.
36. Walker MR, Kasprowicz DJ, Gersuk VH, Bènard A, Van Landeghen M, Buckner JH, Ziegler SF Jr: **Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25$^-$ T cells.** *J Clin Invest* 2003, **112:**1437-1443.
37. Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S: **Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state.** *Int Immunol* 1998, **10:**1969-1980.
38. Thornton AM, Shevach EM: **CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production.** *J Exp Med* 1998, **188:**287-296.
39. Yamazaki S, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K, Steinman RM: **Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells.** *J Exp Med* 2003, **198:**235-247.
40. Suto A, Nakajima H, Ikeda K, Kubo S, Nakayama T, Taniguchi M, Saito Y, Iwamoto I: **CD4$^+$CD25$^+$ T-cell development is regulated by at least 2 distinct mechanisms.** *Blood* 2002, **99:**555-560.
41. Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Holenbeck AE, Lerman MA, Naji A, Caton AJ: **Thymic selection of CD4$^+$CD25$^+$ regulatory T cells induced by an agonist self-peptide.** *Nat Immunol* 2001, **2:**301-306.
42. Bensinger SJ, Bandeira A, Jordan MS, Caton AJ, Laufer TM: **Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4$^+$25$^+$ immunoregulatory T cells.** *J Exp Med* 2001, **194:**427-438.
43. Kumanogoh A, Wang X, Lee I, Watanabe C, Kamanaka M, Shi W, Yoshida K, Sato T, Habu S, Itoh M, Sakaguchi N, Sakaguchi S, Kikutani H: **Increased T cell autoreactivity in the absence of CD40–CD40 ligand interactions: a role of CD40 in regulatory T cell development.** *J Immunol* 2001, **166:**353-360.
44. Gregori S, Casorati M, Amuchastegui S, Smiroldo S, Davalli AM, Adorini L: **Regulatory T cells induced by 1 alpha,25-dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance.** *J Immunol* 2001, **167:**1945-1953.
45. Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA: **A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood.** *J Immunol* 2001, **166:**7282-7289.
46. Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH: **Induction of interleukin 10-producing, nonproliferating CD4$^+$ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells.** *J Exp Med* 2000, **192:**1213-1222.
47. Sato K, Yamashita N, Baba M, Matsuyama T: **Regulatory dendritic cells protect mice from murine acute graft-versus-host disease and leukemia relapse.** *Immunity* 2003, **18:**367-379.
48. Cobbold S, Waldmann H: **Infectious tolerance.** *Curr Opin Immunol* 1998, **10:**518-524.
49. Groux H: **Type 1 T-regulatory cells: their role in the control of immune responses.** *Transplantation* 2003, **75:**8S-12S.
50. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F: **An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation.** *J Exp Med* 1999, **190:** 995-1004.
51. Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, Thompson-Snipes L, Leach MW, Rennick D: **Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4$^+$ TH1-like responses.** *J Clin Invest* 1996, **98:**1010-1020.
52. Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL: **A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells.** *J Exp Med* 1996, **183:**2669-2674.
53. Nakamura K, Kitani A, Strober W: **Cell contact-dependent immunosuppression by CD4$^+$CD25$^+$ regulatory T cells is mediated by cell surface-bound transforming growth factor beta.** *J Exp Med* 2001, **194:**629-644.
54. Takahashi T, Sakaguchi S: **The role of regulatory T cells in controlling immunologic self-tolerance.** *Int Rev Cytol* 2003, **225:**1-32.
55. Zhang X, Izikson L, Liu L, Weiner HL: **Activation of CD25$^+$CD4$^+$ regulatory T cells by oral antigen administration.** *J Immunol* 2001, **167:**4245-4253.
56. Cederbom L, Hall H, Ivars F: **CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells.** *Eur J Immunol* 2000, **30:**1538-1543.
57. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P: **Modulation of tryptophan catabolism by regulatory T cells.** *Nat Immunol* 2003, **4:**1206-1212.
58. Piccirillo CA, Shevach EM: **Cutting edge: control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells.** *J Immunol* 2001, **167:**1137-1140.

Correspondence

Shimon Sakaguchi, Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan. Tel: +81 75 751 3888; fax: +81 75 751 3820; e-mail: shimon@frontier.kyoto-u.ac.jp