CD4$^+$ regulatory cells as a potential immunotherapy

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CD4$^+$ regulatory T (T$_R$) cells represent a unique lineage of thymically generated lymphocytes capable of powerfully suppressing immune responses. A large body of experimental data has now confirmed the key role played by these cells in the maintenance of self-tolerance. Increasingly, the importance of these cells is also being recognized in a host of other clinically relevant areas such as transplantation, tumour immunity, allergy and microbial immunity. Additionally, it is also possible to generate T$_R$ cells by using a variety of *ex vivo* experimental approaches. We will focus here on harnessing the suppressive abilities of both these families of regulatory cells and how this should give us access to a potent cell-based immunotherapy appropriate for clinical application.

Keywords: adaptive regulatory cell; Foxp3; immunotherapy; natural regulatory cell; self-tolerance

1. INTRODUCTION

The random nature of T cell receptor (TCR) generation and its selection on self-major histocompatability complex (MHC) inevitably leads to the appearance of deleterious autoreactive clones. The vast majority of such cells are purged in the thymus during the processes of negative selection. This process is, however, imperfect, as there is abundant experimental evidence that harmful autoreactive T cells can be activated outside of the thymus by the administration of adjuvant and self-proteins (Zamvil *et al.* 1985; Salamero *et al.* 1987; Myers *et al.* 1997). This seems also to be the case in humans, wherein it is possible to clone-out autoreactive T cells from the periphery of both normal individuals and those with manifest autoimmunity (Burns *et al.* 1983; Allegretta *et al.* 1990; Ota *et al.* 1990). Recent studies using MHC Class II tetramers have indeed unequivocally demonstrated that peripheral T cells recognizing self-antigen exist in normal humans (Danke *et al.* 2004). The fact that perfectly healthy individuals can harbour auto-aggressive cells connotes the existence of tolerance mechanisms operational in the periphery. Textbooks classically divide such mechanisms into immunological ignorance, immunological privilege, anergy/deletion and dominant tolerance, though in reality the distinctions are probably not nearly so clear-cut. In recent years, the role played by dominant tolerance mediated through the action of regulatory T (T$_R$) cells has come to pre-eminence. We herein focus on this latter mechanism of peripheral tolerance and investigate the clinical potential held by the successful therapeutic manipulation of T$_R$ cells.

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2. NATURALLY OCCURRING AND ADAPTIVE REGULATORY CELLS

Broadly speaking, CD4$^+$ T cells possessing the ability to suppress immune responses can be divided into two types: *naturally occurring* and *adaptive* regulatory cells (figure 1; Bluestone & Abbas 2003). Naturally occurring regulatory cells (defined hereafter as 'T$_R$ cells') are generated in the thymus under poorly understood conditions. Adaptive regulatory cells (also referred to variously as 'Th3' or 'Tr1') are generated outside of the thymus under specialized activation conditions. We will consider each of these populations in turn.

(a) *Naturally occurring regulatory T cells*

Experimental evidence for T$_R$ cells has been suggested by animal models of autoimmune disease for many years (Sakaguchi 2000a). One of the earliest clues to the existence of T$_R$ cells (though it was not appreciated as such at the time) was found when neonatal thymectomy of certain strains of mice was shown to elicit autoimmune oophoritis (Nishizuka & Sakakura 1969). Thymectomy of adult rats and mice followed by fractionated X-irradiation could also produce autoimmune disease (Penhale *et al.* 1973; Sakaguchi *et al.* 1994). Crucially, it was shown that the induction of such autoimmunity could be prevented by the transfer of normal CD4$^+$ splenocytes or CD4$^+$CD8$^-$ thymocytes (Sakaguchi *et al.* 1994). 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 regulatory cells appeared to migrate out from the thymus at a relatively late stage compared to conventional/autoreactive T cells (more than 3 days after birth in mice). This lag-phase enabled the experimental induction of autoimmunity by the careful timing of neonatal thymectomy
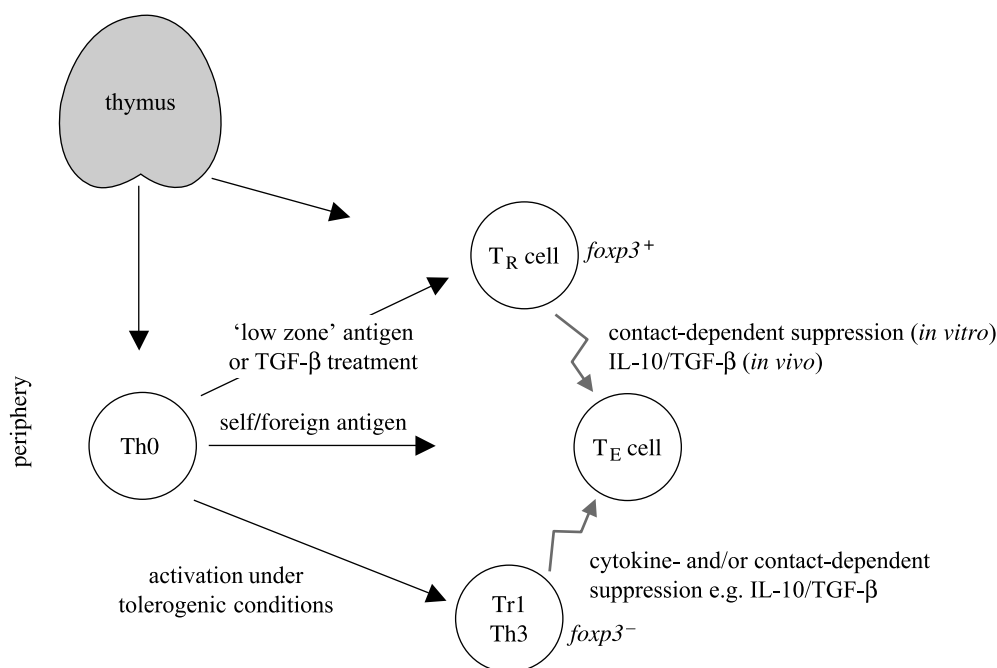
Figure 1. Relationship of thymically and extrathymically generated regulatory cells. T cells emerge from the thymus as either regulatory cells (T$_R$) or conventional naive T cells (Th0). The pathological responses of autoreactive effector cells (T$_E$) can be suppressed by the action of both thymically (T$_R$) and peripherally (Tr1/Th3) generated regulatory cells. The developmental relationship between the two classes of regulatory cells still needs to be fully clarified but some evidence suggests that Th0 are able to differentiate into T$_R$ cells in the periphery under special conditions.

or an immunosuppressive regimen which preferentially targeted the suppressive T cell population.

Attempts were subsequently made to characterize these suppressor or T$_R$ cells as they came to be known, by identifying the surface phenotype that harboured regulatory activity. Initially, CD5 was proposed as a marker for T$_R$ cells by demonstrating that otherwise normal lymphocytes depleted of CD5^{high}CD4$^+$ cells elicited autoimmunity when transferred into athymic nude mice (Sakaguchi *et al.* 1985). Unfractionated CD4$^+$ cells (which contain CD5^{high} expressers) prevented the induction of autoimmunity when co-transferred along with the CD5^{low} cells, implying that T$_R$ cells were contained specifically within the CD5^{high} compartment. Similarly, the CD45RB molecule appears to divide T cells into two distinct functional subsets: CD45RB^{high} and CD45RB^{low} cells (Powrie *et al.* 1993). The CD45RB^{high} population triggers inflammatory bowel disease (IBD) when transferred to lymphopenic mice, by eliciting an immunopathological reaction against normal gut-flora, whereas the CD45RB^{low} counterpart prevents such disease induction. To date, the most functionally useful surface marker for T$_R$ cells has proven to be their constitutive expression of the IL-2 receptor α-chain (IL-2R), CD25 (Sakaguchi *et al.* 1995). Approximately 5–10% of CD4$^+$ peripheral T cells constitutively express CD25 in normal naive mice and healthy humans (Sakaguchi *et al.* 1995; Baecher-Allan *et al.* 2001). In mice, these CD25$^+$ T 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 elicits a variety of autoimmune diseases such as thyroiditis, gastritis and insulitis, whereas co-transfer with CD25$^+$CD4$^+$ cells inhibits such disease development (see figure 2).

Interestingly, CD25 does not appear to be merely a marker for T$_R$ cells, but rather reflects an absolute dependence on IL-2 for their peripheral maintenance and function. This is dramatically demonstrated by the loss of T$_R$ cells and consequent autoimmunity which occurs if IL-2 signalling is perturbed, e.g. in the case of knock-out mice (Almeida *et al.* 2002; Malek *et al.* 2002) or antibody blockade (Setoguchi *et al.* 2005). *In vitro* T$_R$ cells are anergic to conventional TCR stimuli but this is not observed physiologically *in vivo* where they show a seemingly high rate of turnover, which again probably reflects a dependency on IL-2 (Takahashi *et al.* 1998; Almeida *et al.* 2002; Setoguchi *et al.* in press).

More recently a number of other cell surface molecules have been shown to be associated with T$_R$ cells, among them: CTLA4 (CD152), α$_E$β7-integrin (CD103), glucocorticoid induced TNF family receptor (GITR) and neuropilin-1 (a receptor more usually involved in axon guidance) (Read *et al.* 2000; Takahashi *et al.* 2000; Lehmann *et al.* 2002; McHugh *et al.* 2002; Shimizu *et al.* 2002; Bruder *et al.* 2004). It should be noted, however, that no single uniquely expressed cell surface molecule has thus far been identified for T$_R$ cells and in many cases relatively specific molecules such as GITR or CD25 are upregulated to high levels on activated non-regulatory T cells as well. This problem is made especially acute in humans who naturally show large numbers of activated CD25$^+$ T cells and, therefore, a definitive identification is usually only possible by sorting the highest CD25$^+$ expressers (Baecher-Allan *et al.* 2004). Experimental evidence now also appears to demonstrate the existence of a small T$_R$ population lacking CD25 but showing high levels of GITR expression (Lehmann *et al.* 2002; Nishimura *et al.* 2004; M. Ono, personal communication). Where these CD25$^-$CD4$^+$ T$_R$ cells
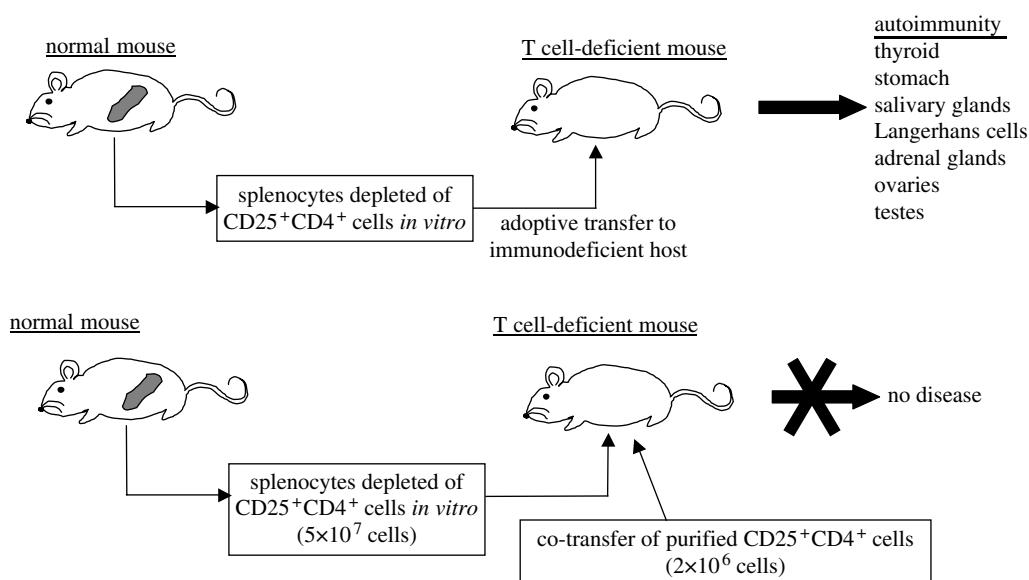
Figure 2. Demonstrating T_R cell-mediated maintenance of self-tolerance. Induction of autoimmune diseases in T cell-deficient mice by transferring $CD4^+$ splenocyte suspensions depleted of $CD25^+$ cells to T cell-deficient mice. Co-transfer of purified $CD25^+CD4^+$ T cells prevents the induction of autoimmunity. Based on Sakaguchi et al. (1995).

fit into overall T_R cell taxonomy remains unclear, but it further highlights the difficulties involved in unambiguously pinpointing natural T_R cells for clinical application.

The molecular understanding of T_R cell biology took a leap forward following the delineation of the functional and developmental role played by the transcriptional repressor *Foxp3*. The importance of *Foxp3* was first appreciated in studies using the Scurfy mouse. This mouse exhibits a fatal X-linked lymphoproliferative disease characterized by a very severe multi-organ immunopathology, allergy and IBD (Lyon et al. 1990). The Scurfy phenotype is very similar to the human immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome both in presentation and severity, and mutations in the *Foxp3* (*FOXP3* in humans) gene were found to underlie both conditions (Bennett et al. 2001; Brunkow et al. 2001; Wildin et al. 2002). The overt similarities seen between mutations in *Foxp3* and experimental depletion of $CD25^+CD4^+$ cells led to its identification as a key transcription factor involved in the development and function of T_R cells (Fontenot et al. 2003; Hori et al. 2003; Khattri et al. 2003). These mouse 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 never observed in conventional T cells following activation or differentiation to Th1 or Th2. Critically, and probably most significantly from a potential therapeutic perspective, retroviral transduction of *Foxp3* to both mouse (Hori et al. 2003) and human (Yagi et al. 2004) non-regulatory $CD4^+$ cells bestowed them with a fully functional T_R cell phenotype; i.e. they became anergic, expressed T_R 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 naturally occurring T_R 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. Essentially, a similar pattern of *FOXP3* expression is seen in human cells (Yagi et al. 2004) as in the mouse, although one report has suggested an important difference: both FOXP3 mRNA and protein appear to be induced in conventional $CD4^+$ T cells following activation (Walker et al. 2003). However, it should be noted that a second study was unable to observe this effect in human cells and reported a largely stable expression of FOXP3 (Yagi et al. 2004). By way of a possible resolution, one can speculate that the appearance of $FOXP3^+$ in the human cell cultures represents a selective expansion of a small contaminating $FOXP3^+$ population. We will return to this confusion later on.

The mechanism of T_R cell suppression remains rather confusing. Abundant *in vitro* evidence has demonstrated that T_R cells require cell contact to mediate suppression, with no suggestion of any role played by soluble factors (Takahashi et al. 1998; Thornton & Shevach 1998; Sakaguchi 2004). Candidate molecules employed by T_R cells to mediate suppression have been suggested, e.g. CTLA4 and cell surface TGF-β (Nakamura et al. 2001; Fallarino et al. 2003; Munn et al. 2004; Paust et al. 2004) and the CD4 homologue LAG-3 (Huang et al. 2004); however, none of these findings appear to be definitive. For instance, in the case of CTLA4, T_R cells from $CTLA4^{-/-}$ mice appear to be just as effective as their wild-type counterparts at mediating suppression *in vitro* (Takahashi et al. 2000; Tang et al. 2004a). *In vivo*, however, there appears to be an indispensable role for soluble factors such as IL-10 and TGF-β in T_R cell mediated control of immunopathology or allograft rejection (Powrie et al. 1996; Asseman et al. 1999; Hara et al. 2001; Singh et al. 2001; Maloy et al. 2003). The importance of both these cytokines to T_R cell suppressive functions *in vivo* can be clearly demonstrated by using cells from $IL-10^{-/-}$ mice or blocking TGF-β and/or IL-10R with monoclonal antibodies

Table 1. Experimental approaches to the extrathymic generation of (adaptive) regulatory T cells.
(A large number of experimental approaches have been shown to induce regulatory cells outside of the thymus. They may take the form of *ex vivo* treatment (e.g. the addition of specific cytokines or pharmacological compounds *in vitro*) or *in vivo* induction (e.g. oral tolerance). The relationship among these various regulatory cell types is still largely unclear as is their relationship to thymically generated CD25$^+$CD4$^+$ T$_R$ cells, though the approaches (7) and (8) listed below have resulted in the production of *Foxp3$^+$* cells. The 'tailor-made' generation of such regulatory cells provides hope for a potent cell-based therapy.)

approach	references
(1) oral tolerance induction with MBP	Chen *et al.* (1994)
(2) *ex vivo* stimulation in the presence of IL-10	Groux *et al.* (1997)
(3) stimulation with immature, cytokine modified or specialized DC subsets	Jonuleit *et al.* (2000), Levings *et al.* (2004), Sato *et al.* (2003) and Wakkach *et al.* (2003)
(4) *ex vivo* treatment with the pharmacological immunosuppressants vitamin D$_3$ and dexamethasone	Barrat *et al.* (2002) and Vieira *et al.* (2004)
(5) *ex vivo* triggering of specific molecules: CD2, CD46 and Notch-1	Hoyne *et al.* (2001), Kemper *et al.* (2003), Vigouroux *et al.* (2003) and Wakkach *et al.* (2001)
(6) infection with particular bacteria or parasites	McGuirk *et al.* (2002) and Zaccone *et al.* (2003)
(7) *ex vivo* treatment with TGF-β	Chen *et al.* (2003)
(8) low-zone tolerance induction by systemic administration of minute quantities of antigenic peptide	Apostolou & Von Boehmer (2004)

(mAbs); in each case suppressive functions are abrogated.

(b) *Adaptive regulatory cells*

Abundant evidence has also demonstrated the extra-thymic generation of CD4$^+$ T cells with suppressive properties. Several quite different experimental approaches can lead to the generation of such regulatory cells (see table 1). In the original experimental demonstrations of extra-thymically generated regulatory cells, the cells were termed Tr1 or Th3 cells (Chen *et al.* 1994; Roncarolo *et al.* 2001; Weiner 2001). Th3 cells were first cloned-out from the mesenteric lymph nodes of mice orally tolerized with myelin basic protein. The majority of such cells produce TGF-β, low/absent IL-10 and varying levels of Th2 cytokines and are able to suppress the induction of experimental autoimmune encephalitis (EAE). Th3-like cells have also been shown to be important in the control of a variety of other experimental autoimmune disease models (Weiner 2001). Some clinical data has also observed a perturbation of TGF-β/Th3 function in some cases of human autoimmunity and allergy (Andersson *et al.* 2002; Perez-Machado *et al.* 2003).

Tr1 cells were first generated under conditions involving mouse T cell activation in the presence of the immunomodulating cytokine IL-10 (Groux *et al.* 1997). Such cells secrete a pattern of cytokines quite 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 anergic, functionally suppressive *in vitro* and are able to prevent the development of experimentally induced Th1 autoimmune diseases such as colitis (Groux *et al.* 1997). A variety of other *ex vivo* experimental treatments have also led to the generation of Tr1-like cells, suppressive *in vitro* or *in vivo*, chief among these being the stimulation of normally non-suppressive conventional CD4$^+$ T cells by immature or cytokine modified dendritic cells (DC) (Jonuleit *et al.* 2000; Sato *et al.* 2003; Verhasselt *et al.* 2004). A common principle which may underlie such tolerizing DC is their unique pattern of stimulatory ligands, e.g. high MHC class II coupled to low co-stimulation (CD80 and CD86). A recent paper, with potentially important therapeutic implications, apparently identified a DC subset responsible for the generation of regulatory cells *in vivo* (Wakkach *et al.* 2003). Such DCs possessed a plasmacytoid morphology and expressed the CD45RB molecule. Other molecular signals which seem to play a role in Tr1 generation (not necessarily in the presence of DC) appear to be those acting through CD2, Notch-1 and interestingly the complement receptor CD46 (Hoyne *et al.* 2001; Wakkach *et al.* 2001; Kemper *et al.* 2003; Vigouroux *et al.* 2003; Yvon *et al.* 2003). Finally, it is also possible to generate regulatory cells *ex vivo* by treating human and mouse T cells with the pharmacological immunosuppressants vitamin D$_3$ and dexamethasone (Barrat *et al.* 2002; Vieira *et al.* 2004). However, these particular cells also appear to be somewhat distinct from the previously characterized Tr1/Th3 cells in that they appear to secrete solely IL-10 as well as retain a robust proliferative capacity; such characteristics would certainly be desirable for their use in any therapeutic capacity.

(c) *What is the relationship between naturally occurring T$_R$ cells and adaptive regulatory cells such as Tr1 or Th3 cells?*

A degree of uncertainty persists as to the precise developmental relationship between thymically generated T$_R$ cells and peripherally generated adaptive regulatory cells. Although both CD4$^+$ regulatory cell types are certainly suppressive in one form or another, it is likely that T$_R$ cells and adaptive regulatory cells actually represent distinct cell types, with any similarities being a result of convergence rather than a closely shared lineage. A number of observations lead to this conclusion. Firstly, T$_R$ cells and adaptive regulatory cells appear to differ fundamentally in their *in vitro* suppressive mechanisms, with the former requiring cell contact and the latter utilizing secreted IL-10 and TGF-β either alone or in combination. Secondly, it is still unclear which of the peripherally generated regulatory cells express *Foxp3*, which is now

thought to be critical for the generation of natural T$_R$ cells. To be fair, a comprehensive analysis of *Foxp3* in all the various types of adaptive regulatory cells has not yet been made, although a couple of studies have suggested that Tr1 cells generated on immature DC (Levings *et al.* 2004) or by treatment with vitamin D$_3$–dexamethasone (Vieira *et al.* 2004) are both *Foxp3* negative. Such results would imply that they represent a differentiation state quite distinct from T$_R$ cells. One could conclude then that extra-thymically generated regulatory cells represent a heterogeneous assemblage whose primary physiological function would most likely be the homeostatic control of immune responses to exogenous antigens. Such adaptive regulatory cells would be generated concurrent/subsequent to a microbial infection with the aim of mitigating immunopathology. The corollary of this is that adaptive regulatory cells are an alternative lineage of conventional CD4$^+$ helper T cells, akin to Th1 or Th2. In contrast, thymically generated CD25$^+$CD4$^+$ T$_R$ cells, by virtue of their *Foxp3* expression, can be considered a *de facto* terminally differentiated lineage *primarily*, but not exclusively, committed to the maintenance of self-tolerance. Unfortunately this rather neat compartmentalization is challenged by recent data suggesting that T$_R$ cells, as defined by Foxp3 expression and suppressive function, can also be generated extra-thymically under particular conditions (Chen *et al.* 2003; Apostolou & Von Boehmer 2004). The addition of exogenous TGF-β to *ex vivo* cultures of conventional non-T$_R$ cells results in the generation of suppressive *Foxp3$^+$* T$_R$-like cells (Chen *et al.* 2003; our own unpublished observations). Similarly, a 'low-zone tolerance' approach involving carefully controlled dosing of minute quantities of systemic antigen generates *Foxp3$^+$* regulatory cells in otherwise T$_R$ cell-deficient and thymectomized mice (Apostolou & Von Boehmer 2004). Therefore, if one accepts *Foxp3/FOXP3* to be an unambiguous marker for T$_R$ cells then the ineluctable conclusion would be that the extra-thymic generation of 'true' T$_R$ cells can, in actual fact, occur under very particular conditions. The finding in the human system that *FOXP3$^+$* cells can be generated *in vitro* following standard TCR-mediated stimulation of conventional CD4$^+$ T cells (Walker *et al.* 2003) is similarly problematic to the formulation of a clear distinction between thymically and extrathymically generated regulatory cells. From a therapeutic perspective such epistemological questions may not even be important; what matters is the ability to controllably generate regulatory cells that are able to suppress harmful immunopathology.

3. EXPERIMENTAL EVIDENCE FOR REGULATORY CELLS AS A POTENTIAL IMMUNOTHERAPY

(a) *Autoimmunity and allergy*

As described in the previous sections there is an abundance of evidence that T$_R$ cells are engaged in the maintenance of self-tolerance and both T$_R$ and adaptive T$_R$ cells can be used to control immunopathology in various animal models of experimental autoimmunity (Roncarolo *et al.* 2001; Sakaguchi

2004). Importantly, a focus on T$_R$ cell therapy is validated by a small but growing body of clinical data showing that their dysfunction may be relevant to human autoimmune disease and allergy (Akdis *et al.* 2004; Baecher-Allan & Hafler 2004; Taylor *et al.* 2004). For example, in the case of multiple sclerosis patients, there seems to be normal numbers of peripheral CD25$^+$ T$_R$ cells but their *in vitro* suppressive capacity is impaired relative to normal controls (Baecher-Allan & Hafler 2004; Viglietta *et al.* 2004). A similar impairment of T$_R$ cell function has also been reported in patients with rheumatoid arthritis (Ehrenstein *et al.* 2004).

Allergic diseases such as asthma, rhinitis and atopic dermatitis are thought to result chiefly from the differentiation and activation of Th2 cells in response to allergens. *In vitro* studies have shown that CD25$^+$CD4$^+$ cells are also able to suppress Th2 responses; therefore, moderating the reaction to allergens is potentially within the T$_R$ cell remit of control (Stassen *et al.* 2004). Suppression of allergic responses appears to occur primarily through the action of IL-10, most likely secreted by T$_R$ cells, whether natural or adaptive. IL-10 can suppress both the innate (mast cells, basophils and eosinophils) and adaptive (Th2 and B cell secretion of IgE) arms of the allergic response (Taylor *et al.* 2004). Several clinical studies have indeed found an association between diminished levels of patients' T cell IL-10 and both asthma and atopic dermatitis (Borish *et al.* 1996; Koning *et al.* 1997). More direct evidence for the role of regulatory cells in allergy is shown by a relative preponderance of allergen-specific Tr1-like cells in healthy non-atopic individuals but Th2 cells in allergic individuals (Akdis *et al.* 2004). Similarly, the *in vitro* ability of CD25$^+$CD4$^+$ cells to inhibit allergen-specific responses appears diminished in atopic individuals (Ling *et al.* 2004). Most interestingly, this relative impairment of suppression was even more pronounced in CD25$^+$CD4$^+$ cells isolated from hayfever sufferers during the grass pollen season than out of it. Whether the majority of human autoimmune and allergy cases implicate a component of T$_R$ cell dysfunction remains to be seen.

Although no clinical therapies involving T$_R$ cells have yet been attempted, some recent works using non-obese diabetic (NOD) mice (a model of autoimmune diabetes) have suggested that such an approach may well be successful (Tang *et al.* 2004b; Tarbell *et al.* 2004). The authors of these studies were able to demonstrate not only a large *in vitro* expansion of T$_R$ cells but, most excitingly, the ability to bring ongoing type 1 diabetes (T1D) under control by the transfer of T$_R$ cells combined with islet transplantation. However, in the case of one of the studies, *in vivo* suppression of diabetogenic cells was only possible by using pancreatic islet antigen-specific T$_R$ cells, presumably because only a tiny fraction of cells from a normal polyclonal T$_R$ population would actually home to the pancreas and be able to exert any meaningful suppression. A similar 'specificity' limitation was found for the suppression of T1D using *Foxp3* transduced cells (Jaeckel *et al.* 2004). This has significant implications for any clinical
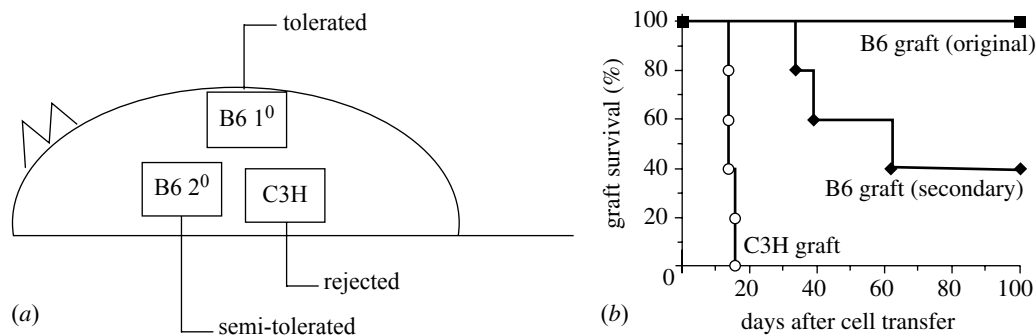
Figure 3. Population of a graft with allospecific T_R cells results in highly stable tolerance and demonstrates dominant suppression by T_R cells. BALB/c nude mice recipients received MHC Class II mismatched B6 allografts in conjunction with adoptively transferred CD25$^-$ and CD25$^+$ cells (B6 1^0). Approximately 70% of such grafts are retained more than 100 days. Mice with original surviving grafts received fresh grafts of *identical* haplotype (B6 2^0) and third party grafts (C3H) and survival examined (*a*). Animals maintained a high localized tolerance to the original graft but only partially so to the secondary graft of identical haplotype, which was rejected albeit at a slower tempo than the third party graft (*b*) (kindly reproduced with permission from Oxford University Press).

immunotherapy, since it demands the identification of islet reactive human T or T_R cell clones.

(b) Transplantation

The Holy Grail of organ transplantation would be to establish tolerance to the foreign graft as effectively as that to self-tissues, but without the need for generalized immunosuppression (Wood & Sakaguchi 2003). One way in which this ambitious goal may be realized is through the exploitation of T_R cells. BALB/c nude mice transplanted with B6 skin and subsequently reconstituted with normal BALB/c T cells show robust graft rejection, which is accelerated by the removal of CD25$^+$ cells from the reconstituting T cell population (Sakaguchi *et al.* 1995). This finding suggests that the small population (5–10%) of T_R cells resident in the BALB/c T cell transfer is able to somewhat retard the normal allogeneic response and, therefore, increasing the numbers (or function) of transferred T_R cells may engender a permanent state of allograft survival. This does indeed appear to be the case, since the transfer of highly purified CD25$^+$CD4$^+$ T_R cells to nude recipients, either prior or simultaneous to reconstitution with naive T cells, significantly prolongs graft survival (Sakaguchi *et al.* 2001). Furthermore, permanent allograft survival may be established by the transfer of large doses of T_R cells. The scarcity of T_R cells means that obtaining large numbers (as would be required for clinical applications) is highly problematic. However, it is possible to expand, *in vitro*, purified CD25$^+$ T_R cells on donor-specific splenocytes (plus IL-2 to break T_R cell *in vitro* anergy) prior to *in vivo* transfer (Sakaguchi *et al.* 2001; Trenado *et al.* 2003; Nishimura *et al.* 2004). As well as generating large numbers of T_R cells for potential *in vivo* use, such expansion has the additional advantage of selectively expanding the donor-responsive cells and, hence, improving the efficiency of suppression. Potentially T_R cells may also be expanded *in vitro* using mature DC yet still retain their suppressive functions, which may represent an appealing technique for therapy (Yamazaki *et al.* 2003; Tarbell *et al.* 2004).

T_R cells are also able to expand on an allograft *in vivo*, in much the same way as normal alloreactive T_E (effector) cells; however, in the case of T_R cells this expansion is non-destructive and beneficially suppressive (Nishimura *et al.* 2004). Experimental skin transplantation systems have clearly demonstrated that *in vivo* pre-expansion of regulatory cells on an allograft *prior* to exposure to alloreactive CD25$^-$ cells significantly improves graft survival, when compared to a simultaneous transfer of both CD25$^+$ T_R and CD25$^-$ cells (Nishimura *et al.* 2004). T_R cells themselves home to, and can actually be found within, the graft proper (Graca *et al.* 2002; our own unpublished observations) and once this population is established the graft is very stably tolerated, whereas a fresh graft of identical haplotype shows a lower survival (figure 3; Nishimura *et al.* 2004). Rather than being a systemic phenomenon, T_R cell-maintained tolerance instead appears to take the form of stable 'islands' of suppression. From the standpoint of immunotherapy, such properties are ideal, since it would engender a state of localized tolerance without the dangers of generalized immunosuppression. This process is summarized in figure 4.

A large number of studies have also been able to induce prolonged graft survival by the administration of mAbs to various T cell- and antigen presenting cell (APC)-associated molecules such as CD4, CD8, CD40L, CD80 and CD86 (Hara *et al.* 2001; Taylor *et al.* 2001; Graca *et al.* 2002; Nathan *et al.* 2002; Taylor *et al.* 2002; Cobbold *et al.* 2004). Many such studies employing mAbs implicate the role of regulatory cells in maintaining graft tolerance, and interestingly there is a suggestion that co-receptor blockade by non-depleting anti-CD4 mAbs in particular may act by inducing T_R cells *de novo* (Cobbold *et al.* 2004; Karim *et al.* 2004; Waldmann & Cobbold 2004). Non-depleting anti-CD4 may yet lend itself as the T_E cell-specific form of immunosuppression depicted in figure 4; however, the optimization of anti-CD4 for the clinic has still not been achieved.

Bone marrow transplantation for the treatment of haematological malignancies presents particular clinical problems. Such treatments must carefully balance the immunosuppression of potentially lethal graft versus host disease (GVHD) yet preserve a robust and beneficial lethal graft versus tumour (GVT) response. T_R cells have also been shown to be
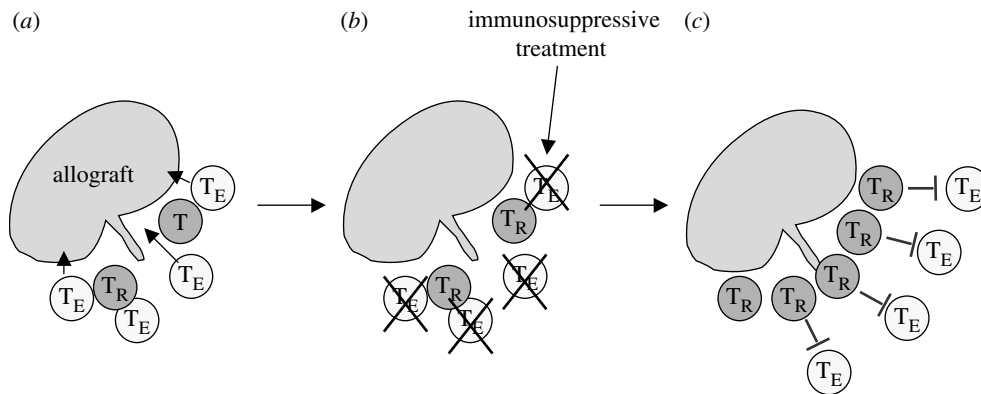
Figure 4. Blockade of effector T cells allowing the *in vivo* expansion of alloreactive/self-reactive T_R cells: a putative immunotherapy for transplantation or autoimmunity. Following transplantation both harmful effector T cells (T_E) and suppressive natural regulatory cells (T_R) are recruited to the graft. In the normal physiological state, there are too few T_R cells or their function is too weak to beneficially suppress the large precursor frequency of graft destructive T_E cells (*a*). Administration of an immunotherapeutic agent (e.g. non-depleting anti-CD4) specifically blocks T_E cells but allows the function and, more importantly, alloreactive expansion of T_R cells (*b*). The large expanded population of T_R cells then renders the graft operationally tolerant (*c*). Essentially similar principles can be applied in the case of autoimmunity.

potentially effective in such a setting. Initially, studies demonstrated that the transfer of donor-type $CD25^+CD4^+$ T_R cells with bone marrow precursor cells could markedly protect from lethal GVHD and this effect was dependent in part on IL-10 production (Hoffmann *et al.* 2002). In subsequent related studies, it was shown that T_R cells were able to not only protect from GVHD following bone marrow transplantation, but also retain strong enough immunity for an effective GVT response (Edinger *et al.* 2003; Trenado *et al.* 2003). T_R cells thus appear to offer the flexibility of function and potency required for bone marrow transplantation, by balancing the conflicting requirements of immunosuppression and immunopermissiveness.

(c) *Tumour immunity*
It is now clear that many tumour-associated antigens recognized by autologous T cells are normal self-constituents and, thus, presumably within the remit of control by self-reactive T_R cells. T_R cells may even actively be recruited by tumours as a means of immune evasion (Hontsu *et al.* 2004). The desirable elimination of tumours by cytotoxic effector T cells may thus be impeded by the action of T_R cells and evidence is accumulating that this in fact appears to be the case (Sakaguchi 2000*b*; Takahashi & Sakaguchi 2003). Experiments suggest that the simple elimination of T_R cells from splenocyte preparations by treatment with anti-CD25 mAb results in productive responses to syngeneic tumours when transferred together to lymphopenic recipients (Onizuka *et al.* 1999; Shimizu *et al.* 1999; Sutmuller *et al.* 2001). Similarly beneficial effects are seen with the *in vivo* administration of antibodies. Data appear to suggest that $CD25^+CD4^+$ T_R cells interfere with tumour-specific $CD8^+$ responses and their removal, thus enhancing anti-tumour cytolysis (Chen *et al.* 2004). Another fruitful approach may simply be to interfere with T_R cell suppression as opposed to depleting them. Signalling through GITR on T_R or activated T_E cells with either mAbs or specific ligand (GITRL) has been shown to either perturb T_R cell suppression or render T_E cells

resistant to suppression (Shimizu *et al.* 2002; Stephens *et al.* 2004). A therapy embracing both depletion and perturbation of suppression may be the most effective. In parallel to the experimental data, clinical evidence is emerging that suppressive T_R cells are present in increased numbers within tumour draining nodes or within the tumour microenvironment itself (Liyanage *et al.* 2002; Viguier *et al.* 2004). Perturbing T_R cell abrogation within the tumour may thus represent a viable treatment strategy.

(d) *Microbial immunity*
Although the main function of T_R cells is thought to be the maintenance of self-tolerance, it is clear that they can also control excessive immune responses to pathogens or inappropriate responses to commensal/mutualistic microbes. As described above, it is well known that severe combined immunodeficiency (SCID) mice transferred with $CD25^-CD45RB^{hi}CD4^+$ cells develop IBD, whereas similarly treated mice lacking intestinal microbes under germ-free conditions show no such immunopathology (Singh *et al.* 2001). The sensitivity of the gut in general to perturbation of T_R cell number or function intimates the critical involvement that these cells have in the control of immune responses to non-self but otherwise harmless symbiotes. The fundamental nature of this microbial interaction is further suggested by the expression on T_R cells of the TLR4 pattern recognition receptor (Caramalho *et al.* 2003). It now seems that inflammatory signals, whether host (e.g. IL-6) or pathogen (e.g. lipopolysaccharide, LPS) derived either directly or indirectly, disable T_R cell suppressive function (Pasare & Medzhitov 2003; Yamazaki *et al.* 2003; Fehervari & Sakaguchi 2004). This suggests a model whereby immune responses are freed-up from T_R cell suppression only in the presence of microbial 'danger signals'. In fact, it is becoming increasingly clear that naive T cell responses can only be *initiated* by DC matured with 'danger signals' such as LPS and that this control is maintained, at least in part, through the action of regulatory cells (Pasare & Medzhitov 2004; Sporri & Reis e Sousa 2005).

T$_R$ cells also have a role to play in infectious diseases by blunting overactive and damaging immune responses to pathogens. Evidence for this comes from the rapid and fatal pulmonary inflammation seen following CD25$^+$CD4$^+$ T$_R$ cell depletion during chronic infection with *Pneumocystis carinii* (Hori *et al.* 2002). In this model, immunopathology was mediated by CD25$^-$CD4$^+$ effector T cells and their damage could be prevented by the presence of CD25$^+$CD4$^+$ T$_R$ cells. It may well be that the necessity to attenuate harmful immunopathology was a key selective pressure leading to the evolution of the T$_R$ subset. Another study has examined the role of T$_R$ cells during chronic infection with the protozoan parasite *Leishmania major* (Belkaid *et al.* 2002). This model suggested that the long-term persistence of *L. major* is maintained through the action of T$_R$ cells and the removal of CD25$^+$CD4$^+$ cells resulted in a sterilizing response, i.e. the complete elimination of the parasite. Interestingly, eradication of the parasite in this way prevented immunity to subsequent re-infection and so demonstrated the importance of T$_R$ cells for the support of long-term immunological memory. The perseverance of immunological memory has been long-suspected to be mediated by the presence of a small 'antigen depot' and the action of T$_R$ cells may thus provide a hitherto unappreciated mechanism by which this can be achieved. This observation with *L. major* has obvious implications for a vaccine development strategy, i.e. using an adjuvant formulation that enables a reasonable level of T$_R$ cell function would encourage the generation of memory. These findings may also throw light on the puzzling nature of the so-called *Koch's Phenomenon*, i.e. a tuberculous individual is often highly resistant to a new infection yet unable to overcome the original infection. This could be seen as akin to the dual transplant tolerance effect depicted in figure 3, with the original infection being protected by an established T$_R$ population, while fresh infections are subject to the full force of the immune response (Sakaguchi 2003).

Multicellular parasites such as helminths and nematodes provide a particularly compelling area in which to study regulatory cells. Owing to their size such organisms are unable to pursue a classic pathogen evasive strategy of sequestering themselves in host cells, yet they are still able to reside in immunologically exposed environments of their host often for many years. To achieve such a feat they appear to possess multiple and potent mechanisms of immune subversion (Maizels *et al.* 2004). There is much evidence that helminths and nematodes trigger a switch from an immunopathological Th1 to a less harmful Th2 response; however, this cannot explain all the data and increasingly it appears that regulatory cell function is being co-opted by such parasites (Yazdanbakhsh *et al.* 2002). Basic evidence for the involvement of regulatory cells comes from the observations of elevated lymphocyte IL-10 and TGF-β production following nematode infection (Mahanty *et al.* 1996). A further example is seen in the infection of mice with the helminth *Schistosoma mansoni*. In this case, T$_R$ cell-derived IL-10 appears critical for the prevention of immunopathology following such infection and IL-10-producing CD25$^+$ cells can be recovered from parasite-induced granulomas

(Hesse *et al.* 2004). Several reports have also shown that infection with *S. mansoni*, along with other microbes, can prevent a host of experimental autoimmune diseases such as T1D, EAE and collagen induced arthritis (CIA) (Cooke *et al.* 2004). Interestingly, the T1D-protective effect of *S. mansoni* is also observed with adult parasite and egg-derived antigens (Cooke *et al.* 1999; Zaccone *et al.* 2003). Importantly, exposure to such antigens may result in the *in vivo* generation of regulatory cells, since parasite antigen-treated mice were unable to cause diabetes in NOD-SCID recipients following adoptive transfer. More direct evidence for T$_R$ cell involvement has been shown in a mouse model of experimental filariasis, where increased expression of *Foxp3* and T$_R$ cell surface markers are observed in draining lymph nodes following nematode infection (Maizels *et al.* 2004). Increasingly, such parasite data are painting a more complex picture of immune evasion, i.e. it is not just a product of simple Th1–Th2 switching but rather the recruitment of T$_R$ cell functions. As with many such host–parasite interactions, it is often difficult to disentangle which party benefits from a particular immune response; i.e. is dampening down immunity beneficial to the host through the prevention of immunopathology or is the parasite 'co-opting' the T$_R$ cells to ensure its survival/transmission? One thing is clear though: the efficient ability of parasites to engage T$_R$ cell functions may be exploited for immunotherapeutic benefit. This has, in fact, already been achieved, with one study demonstrating encouraging results in the treatment of Crohn's disease and IBD following ingestion of Whipworm (*Trichuris suis*) ova (Summers *et al.* 2003, 2005). In this particular case, the nematode parasite is by-and-large non-pathological, but hopefully more advanced therapies should be able to utilize totally harmless parasite-derived antigens with similar efficacy.

4. A PUTATIVE THERAPEUTIC SCHEME INVOLVING THE USE OF REGULATORY CELLS

Naturally occurring T$_R$ cells exhibit several properties that make them potentially useful for therapeutic applications. Firstly, they have a diverse repertoire reactive not only with self- but also with non-self antigens. Secondly, they exhibit a functionally stable phenotype, which appears terminally differentiated for the control of immune responses. Finally, T$_R$ cells can be expanded both *in vitro* and *in vivo* yet retain a suppressive phenotype. Such properties could be applied to a number of clinically important areas. Figure 5 summarizes a potential therapeutic scheme involving T$_R$ cells as applied to the control of transplant rejection. Once a donor is identified the organ is harvested and a source of donor antigen taken, e.g. biopsy material or peripheral blood mononuclear cells (PBMCs), to be used for later expansion of donor-specific regulatory cells. Such donor-specific antigen can be banked if necessary. In the case of a non-solid organ transplant such as bone marrow, recipient T$_R$ cells could be pre-mixed with the graft prior to surgery (*a*), such an enforced localization may reduce the number of T$_R$ cells ultimately required for tolerance induction by encouraging *in situ* regulatory cell expansion. Following grafting, the recipient is put
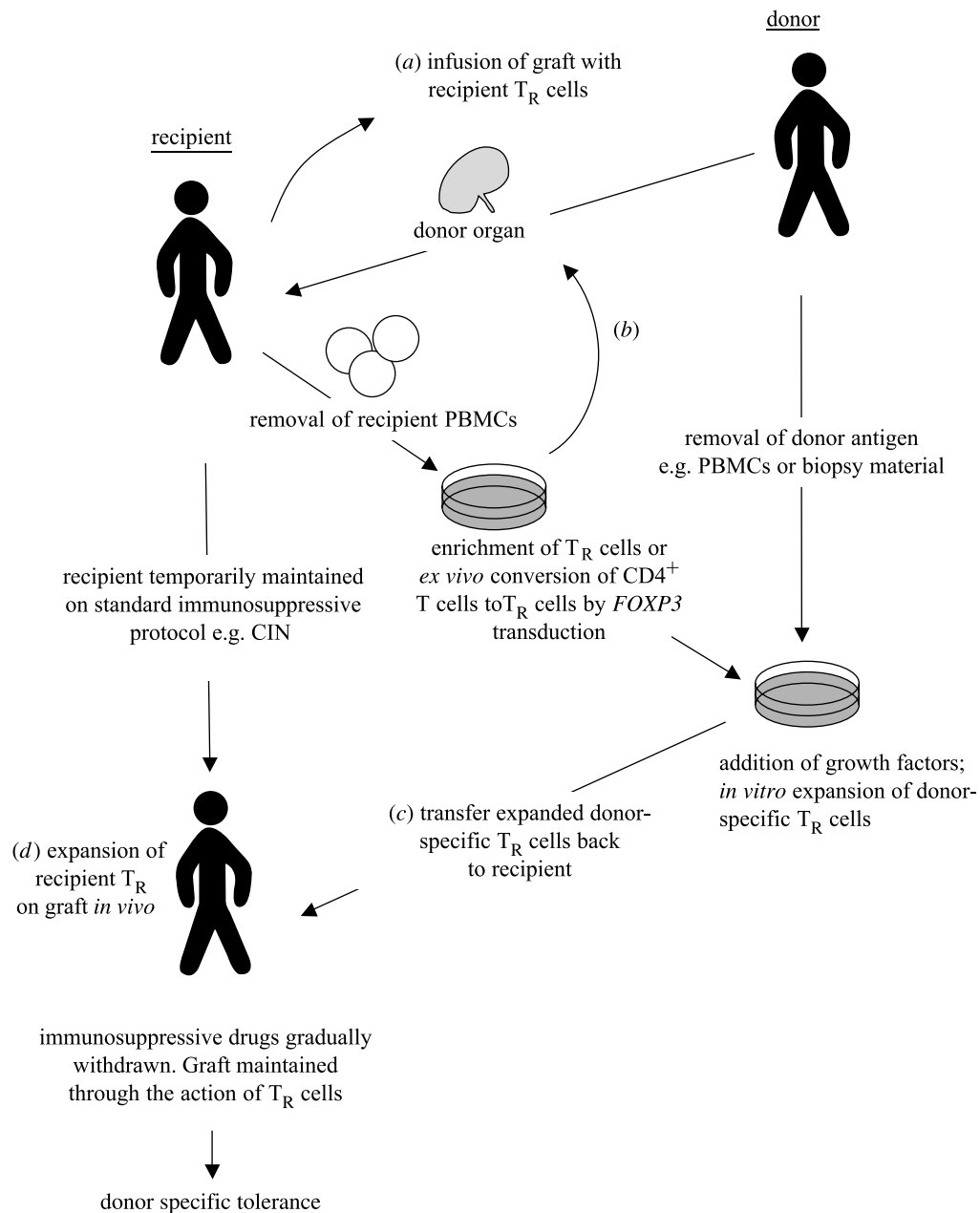
Figure 5. A putative therapeutic scheme using regulatory cells applied to organ transplantation. Both T$_R$ and adaptive regulatory cells could potentially be used to establish allograft tolerance in the absence of standard immunosuppression. Intervention could be made at several stages. (*a*) In the case of bone marrow grafting, fresh recipient T$_R$ cells or (*b*) *ex vivo*-expanded T$_R$ cells could be suffused with the graft. (*c*) Donor-specific T$_R$ or adaptive regulatory cells could be generated and expanded *ex vivo* on a source of donor antigen (e.g. PBMCs or biopsy material). (*d*) Recipient T$_R$ cells could expand naturally on the graft *in vivo* following transplantation. Meanwhile host effector cell responses would be held in check by immunosuppressive treatment allowing expansion of T$_R$ cells to effective levels, before drug treatment is phased-out. CIN, Calcineurin inhibitors.

onto a standard immunosuppressive regimen. Two routes of therapy could then potentially be taken. In the first, the recipient undergoes leukapheresis followed by enrichment of T$_R$ cells or conversion of conventional T cells to regulatory cells *ex vivo*, either by gene transduction of *FOXP3* or the generation of Tr1-like cells using a pharmacological approach (*b*). These newly generated regulatory cells are then sorted and expanded on the previously frozen donor-derived antigenic source. To support expansion, growth factors such as IL-2 may need to be added at this stage. The expanded and more importantly donor-specific, regulatory cells are then re-sorted and transferred back to the patient (*c*). Once an effective population of

regulatory cells has been established in the recipient, pharmacological immunosuppression is gradually withdrawn and the graft is maintained long-term by dominant tolerance alone. If one views an organ targeted by autoimmunity as an allograft, then a broadly similar scheme could also be used to alleviate autoimmunity.

As outlined here, such a therapeutic approach seems rather straightforward, however applying it to a real-life situation would present formidable technical challenges. Basically, these could be summarized as problems of *number* and *specificity*, i.e. obtaining enough regulatory cells which are reactive with the allograft or, in the case of autoimmunity, self-antigen.

Table 2. Potential clinical applications of CD25$^+$CD4$^+$ T$_R$ cells.
(Depending on the target condition, clinical benefit may be accrued by either enhancing or attenuating/depleting T$_R$ cell number or function. While none of these approaches has yet been approved for clinical trials, abundant experimental data have demonstrated the potential of a regulatory cell based therapy.)

	target condition	potential therapeutic approach
increase of T$_R$ cell number or function	transplantation	*ex vivo* gene transduction of *Foxp3* to conventional T cells
	autoimmune disease	*ex vivo* generation of regulatory cells from conventional T cells using cytokines, pharmacological agents or modified DC
	allergy	expansion of host T$_R$ cells *ex vivo* using transplantation antigens or known autoantigens plus growth factors such as IL-2
reduction of T$_R$ cell number or perturbation of function *in vivo*	cancer	numerical reduction or transient elimination of T$_R$ cells and/or perturbation of suppressive function *in vivo* (anti-CD25, anti-CTLA4)
	infectious disease	rendering effector cells resistant to suppression (signalling through GITR with anti-GITR or GITRL)

These problems could be ameliorated to some extent by the use of living related donors which is becoming an increasingly attractive transplant modality (Prasad *et al.* 2002; Takada & Tanaka 2004). The luxury of foreknowing the donor haplotype and time of grafting would give ample opportunity for pre-expanding donor-reactive recipient T$_R$ cells. The most desirable scheme would involve the use of an immunosuppressive agent that selectively targeted effector cells, so staving-off rejection, yet allow the expansion of beneficial graft-reactive T$_R$ cells (figure 5*d* and as depicted in figure 4). Again, once T$_R$ cell mediated suppression had reached a critical threshold of efficacy, immunosuppression could be withdrawn. Immunosuppressive drugs routinely used in transplantation, such as cyclosporin and basiliximab (a depleting anti-IL2R mAb), are probably wholly inappropriate at engendering such a situation, since they would likely impair T$_R$ cell function (Sakaguchi & Sakaguchi 1989; Kovarik *et al.* 1999). However, numerous animal models have demonstrated the ability of anti-CD4 and to a lesser extent, anti-CD40L mAb to facilitate the *in vivo* expansion and/or function of T$_R$ cells or adaptive regulatory cells (Nathan *et al.* 2002; Cobbold *et al.* 2004; Waldmann & Cobbold 2004).

5. MAJOR OBSTACLES TO THE USE OF REGULATORY CELLS AS AN IMMUNOTHERAPY
As detailed earlier, a large body of data from murine studies have demonstrated the potent ability of both natural and adaptive regulatory cells to control immune responses under a wide range of clinically important conditions (table 2). Although, of course, this should be seen as very encouraging, it is now almost axiomatic that many efficacious therapies in the mouse are very difficult to translate into humans or at least if there is a beneficial effect then the results are more subdued. So in order to avoid regulatory cells from becoming a failed 'next big thing', a number of significant hurdles need to be overcome (see table 3).

(a) *Ex vivo generation of T$_R$ cells*
The identification of *FOXP3* as a critical gene for T$_R$ cell development and function was a major advance, since it opened the possibility of *in vitro* T$_R$ cell generation with the obvious clinical benefits that this implies. However,

Table 3. Major hurdles to the development of regulatory T cells as an immunotherapy.
(A number of theoretical and experimental hurdles need to be overcome before the clinical potential of T$_R$ cells can be fully realized.)

identification of unambiguous surface markers for the isolation of human T$_R$ cells
clear delineation of the molecular mechanism of T$_R$ cell mediated suppression
characterization of the signals both downstream and upstream of *Foxp3*
identification of factors capable of viably expanding T$_R$ cells *ex vivo*
resolving the accuracy to which *FOXP3* can be used as a marker in human cells

a sober analysis of the experimental details of the mouse work should temper our excitement somewhat. For instance, a simple linear extrapolation of cell numbers used to control immunopathology in the mouse colitis model or skin transplant rejection would require the generation of hundreds of millions of T$_R$ cells for a single dose of systemic therapy in a human patient. Relative to the spleen, human peripheral blood yields only small numbers of transduction targets and, furthermore, even if the requisite number of T$_R$ cells could be generated then sort times alone would be prohibitive. Clearly, such an approach is impractical and alternatives need to be found, whether theoretical or technical. High-volume magnetic bead-based sorting could be much more practical and would only require the generation of a cell-surface reporter for isolation of T$_R$ cell transductants. It is also likely that such exorbitant numbers of T$_R$ cells are not required if the antigen is known, such as in the case of transplantation antigens, where there is a high precursor frequency of responders and less-so if there is a defined autoantigen, e.g. myelin basic protein in multiple sclerosis or glutamic dehydrogenase in T1D. A T$_R$ cell therapy designed to control autoimmunity may therefore require the identification of real human autoantigens and the cloning of specific cells. A more targeted, rather than systemic, administration of the T$_R$ cells may also enable the use of significantly fewer cell numbers. Another technical hurdle concerns the vectors used to introduce *Foxp3*. All the *Foxp3* gene transduction studies in the mouse have been performed using retroviral vectors, which would be an undesirable

approach in humans, since it could risk both the transformation of the target cells as well as resulting in sensitization of the host immune system to viral antigens. Ideally, efficient non-viral transfection would need to be used in the clinical setting.

(b) *Potential activation of autoimmunity by perturbation of T_R cell function*

Blocking T_R cell function or their numerical reduction/elimination, e.g. in the case of cancer therapy, raises a different problem, namely the potential induction of autoimmunity. At one level it could be argued that a transient autoimmunity would be an acceptable price to pay to be rid of an otherwise intractable cancer, but clinically this is clearly far from ideal. Thankfully, though there may be no need for a systemic reduction of T_R cell numbers to elicit effective cancer reactivity, rather only within the tumour mass itself. Similarly, an incomplete reduction of T_R cell number/function may be enough to elicit potent tumour immunity yet preserve self-tolerance. Such risks need to be carefully assessed prior to abrogation of T_R cell function in the clinical setting.

(c) *In vivo expansion/enhancement of T_R cells*

A T_R cell-based therapy, while being 'intelligent' in that it is highly targeted as well as flexible, would also be incredibly laborious and expensive to perform. Therefore, from a large-scale therapeutic perspective, the identification of compounds which could specifically target T_R cells *in vivo* by either intensifying/stymieing their function or increasing/decreasing their numbers would be the most desirable approach. This would also make it amenable to the kind of high throughput technologies at which the pharmaceutical and biotech industry excel. Attaining such a goal would require a considerable extension of our knowledge of fundamental T_R cell biology. Specifically it would be critical to understand the signalling events both upstream and downstream of *FOXP3*. How is the *FOXP3* gene switched on? How does it exert its effects, is it through direct action or via an intermediary? Finally, the discrepancy between mouse and human as regards the induction of FOXP3 following conventional TCR triggering needs to be resolved (Walker *et al.* 2003; Yagi *et al.* 2004). If this were to be substantiated, it would alter our understanding of how regulation is generated and maintained in humans, as well as have important implications for the usefulness of FOXP3 as a T_R cell specific marker and the validity of mice as a model of human T_R cells.

(d) *Identification and perturbation of T_R cell suppressive mechanisms*

The inhibition of T_R cell-mediated suppression should also be a major therapeutic target. Ideally, this would lead on from the identification of a T_R cell-associated molecule specifically involved in suppression, should one actually exist. As outlined in previous sections here, this is still a controversial area. Alternatively, rendering effector cells resistant to suppression could also be a feasible approach. Clearly, identifying target molecules on either effector or T_R cells and unambiguously delineating the mechanism of suppression would be absolutely fundamental to the success of any such therapy.

(e) *Identification of highly specific human T_R cell surface marker(s)*

Finally, there is a lacuna regarding a highly specific surface marker to identify T_R cells. Although several candidate molecules such as CD25 or GITR have been identified, none of them are able to definitively discriminate between activated T cells and T_R cells. This is an especially acute problem in humans who naturally have large numbers of activated T cells and where T_R cell isolation can be performed definitively only by flow sorting the very highest $CD25^+$ expressers. The ability to confidently identify and isolate T_R cells from patients would be fundamental to their clinical exploitation; therefore, the discovery of appropriate cell surface markers should remain a priority.

6. CONCLUSIONS

Abundant evidence now strongly supports the existence of T_R cells as key controllers of self-tolerance. It now seems that their suppressive role can be expanded to many areas of immunology, in fact potentially to any scenario, where the suppression and/or tuning of an immune response is required. A strategic manipulation of regulatory cells, whether naturally occurring or adaptive, to dampen or enhance their functions as appropriate promises great clinical benefit. Already manipulation of $CD25^+CD4^+$ T_R cells in various animal models has provided encouraging results for the enhancement of tumour immunity, maintenance of allograft tolerance or the control of autoimmunity. Increasingly, studies are also demonstrating the significant roles $CD25^+CD4^+$ T_R cells can play in human pathologies as varied as autoimmunity, HIV infection and allergy. Recent advances in our understanding of $CD25^+CD4^+$ T_R cell development and important functional markers such as the association with *Foxp3/FOXP3*, has permitted the accurate isolation and manipulation of these cells in mice and importantly their human counterparts. Understanding the events both upstream and downstream of *Foxp3/FOXP3* may enable us to 'tailor-make' large numbers of $CD25^+CD4^+$ T_R cells for the specific suppression of immune responses or antagonizing them, where a boost of immunity is required. It is the strong belief of many researchers in this area that T_R cells will, in a not-too-distant futurity, become a potent cell based therapy.

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