JCI The Journal of Clinical Investigation

Tregs and transplantation tolerance

Patrick T. Walsh, … , Devon K. Taylor, Laurence A. Turka

J Clin Invest. 2004;114(10):1398-1403. https://doi.org/10.1172/JCI23238.

Review Series

The induction and maintenance of immune tolerance to transplanted tissues constitute an active process involving multiple mechanisms that work cooperatively to prevent graft rejection. These mechanisms are similar to inherent tolerance toward self antigens and have a requirement for active immunoregulation, largely T cell mediated, that promotes specific unresponsiveness to donor alloantigens. This review outlines our current understanding of the Treg subsets that contribute to allotolerance and the mechanisms by which these cells exert their effects as well as their potential for therapy.

Tregs and transplantation tolerance

Patrick T. Walsh, Devon K. Taylor, and Laurence A. Turka

Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

The induction and maintenance of immune tolerance to transplanted tissues constitute an active process involving multiple mechanisms that work cooperatively to prevent graft rejection. These mechanisms are similar to inherent tolerance toward self antigens and have a requirement for active immunoregulation, largely T cell mediated, that promotes specific unresponsiveness to donor alloantigens. This review outlines our current understanding of the Treg subsets that contribute to allotolerance and the mechanisms by which these cells exert their effects as well as their potential for therapy.

Introduction

Peripheral tolerance to self antigens is maintained by a dynamic process involving several different mechanisms that restrict the development of a potentially destructive autoaggressive T cell response. These mechanisms include T cell depletion through activation-induced cell death, "ignorance" of self antigens (meaning the apparent absence of antigen recognition), and the induction of T cell anergy (1). While these mechanisms are clearly important in the maintenance of self tolerance, they are by themselves not sufficient, as there is also a need for active suppression of autoreactive T cells by Tregs (2). Although initial characterization of these Treg subsets defined their role in the maintenance of tolerance to self, it is now clear that such regulatory cells play an important role in suppressing immune responses directed against alloantigens expressed on transplanted organs and tissues (3).

Overview of graft rejection and tolerance

Graft rejection occurs as a consequence of polymorphisms in histocompatibility genes, primarily those located within the MHC (4). T cells respond to foreign (allogeneic) MHC molecules in the same fashion as to any foreign protein: they secrete cytokines, divide, and differentiate (5). This generates a large population of activated effector cells, primarily T cells and macrophages, which are the primary mediators of graft destruction.

Alloresponsive T cells can recognize antigens present in transplanted tissues by 1 of 2 distinct pathways. In the direct pathway, the responding T cells recognize intact allogeneic MHC molecules on the surface of donor-derived APCs, whereas in the indirect pathway, recipient APCs process donor-derived allo-MHC molecules into peptides and then present those peptides to T cells on self-MHC molecules. It is generally accepted that the direct pathway predominates in the immediate aftermath of transplantation, when graft-resident APCs (passenger leukocytes) migrate to the surrounding lymphoid tissue, where they stimulate alloresponsive T cells. As donor-derived APCs are relatively short lived, the indirect pathway of allorecognition is generally believed to predominate as the alloresponse progresses (6).

Experimental methods to induce transplantation tolerance are typically divided into 2 categories. "Central" tolerance refers (in most instances) to the use of bone marrow transplantation as a means to induce hematopoietic chimerism (7). This results in the coexistence of donor- and recipient-derived lymphoid and myeloid cells. As a result, developing T cells that are donor reactive are deleted before they can exit the thymus, in the same manner as self-reactive T cells (8). "Peripheral" tolerance refers to the use of antibodies (or occasionally pharmacologic agents) that block or modulate T cell activation or growth factor receptor pathways in mature T cells. In most instances, this has the net result of promoting apoptosis among the T cells that are responding to alloantigens (9).

An important characteristic of alloimmune responses is the high frequency of T cells that are able to recognize and respond to alloantigens (primarily the products of genes encoded within the MHC) (10). Because of this, and based on data from studies on rodent models, many investigators believe that it is necessary to achieve large-scale deletion of alloreactive T cells in order to create transplantation tolerance (9). Both central and peripheral tolerance strategies achieve this during the early "induction" phase of therapy (i.e., the first 1–2 weeks after transplantation). In the case of central tolerance, this alone appears to be sufficient, as newly developing T cells with potential anti-donor reactivity will be eliminated within the thymus following encounter with donor-derived cells (7, 8). However, in the case of peripheral tolerance strategies, a large body of data derived from experimental animals suggests that following depletion, the "maintenance" phase of tolerance requires Tregs that can act on both any remaining alloresponsive T cells and on new thymic emigrants (Figure 1).

Multiple types of Tregs

Studies of Tregs in transplantation have identified multiple populations of cells with different cell-surface phenotypes and, to some extent, with different mechanisms of action (11). One population is a naturally occurring subset of $CD4^+$ T cells that arises during T cell development in the thymus and is best defined by constitutive expression the α chain of the IL-2 receptor, CD25 (2). A second population consists of induced Treg subsets that may arise during the course of a normal immune response (presumably to help terminate the response when the pathogen is eliminated and prevent secondary autoimmunity). These "induced" Tregs, while largely contained within the $CD4^+$ compartment, are distinct from their naturally occurring $CD4^+CD25^+$ counterparts. In addition, $CD8^+$ Tregs, $TCR^+CD4^-CD8^-$ T cells, and NK Tregs have also been reported to play a role in different models of transplantation tolerance (12–15).

Naturally occurring Tregs

Characterization. The study of Tregs was historically crippled by the lack of reliable cellular or molecular markers that are necessary to identify these cells. The absence of such tools led to

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **114**:1398–1403 (2004). doi:10.1172/JCI200423238.

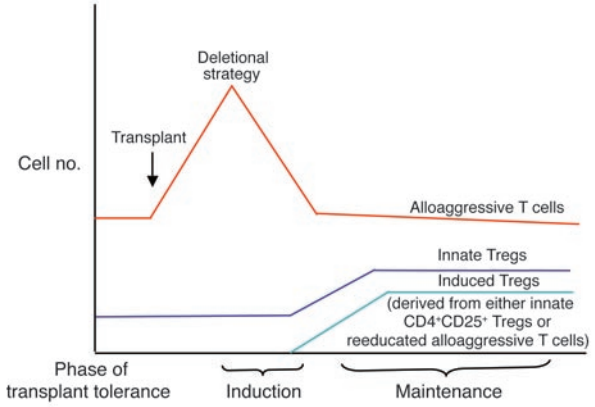

Figure 1
Altering the balance between alloaggressive and Treg subsets. Deletional strategies employed at or around the time of transplant reduce the number of potentially graft destructive T cells and facilitate the action of Treg subsets. During the maintenance phase of tolerance, these Tregs, either naturally occurring or induced, can thus act more efficiently on a greatly reduced number of effector T cells. Cell number, as denoted on the *y* axis, represents an illustration as to how the relative ratio of effector versus Treg subsets alters during the establishment of transplant tolerance and is not meant for comparison between groups.

the question of whether Tregs exist. This changed, however, with the discovery that the molecular marker CD25, previously thought to be expressed only on recently activated T cells, was also expressed on a subset of resting $CD4^+$ T cells with regulatory function (1). Naturally occurring $CD4^+CD25^+$ Tregs develop in the thymus. These Tregs constitute approximately 5–10% of mature $CD4^+CD8^-$ thymocytes and about 10% of peripheral $CD4^+$ T cells (16, 17). Other cell-surface markers, such as CD45RB, CTLA-4, glucocorticoid-induced TNF receptor family–related receptor (GITR or TNFRSF18), CD122, CD103 ($\alpha_E\beta_7$ integrin), CD134 (OX40), and CD62L (L-selectin), whose relative expression levels can be used to define and isolate $CD4^+CD25^+$ Tregs have also been identified (3). However, as with CD25, none of these molecules alone represents a definitive marker for naturally occurring Tregs, as they are also expressed on other $CD4^+$ T cell subsets, particularly activated T cells. Recently, the forkhead/winged helix transcription factor Foxp3 was shown to be uniquely expressed by naturally occurring $CD4^+CD25^+$ Tregs, and it is thought to act as a master switch controlling Treg differentiation (18, 19). However, the intracellular location of Foxp3 places an obvious limitation on its use in identifying and studying Tregs. Therefore, efforts continue to find a definitive cell-surface marker for Tregs.

Mechanism of action. Although the exact mechanism by which these cells exert their immunosuppressive effect remains elusive, $CD4^+CD25^+$ Tregs are known to suppress effector T cell proliferation in vitro through a cell contact–dependent mechanism that is largely cytokine independent (20–22). In particular, these studies implicate a role for accessory molecules such CTLA-4 and GITR expressed on the surface of Tregs (23, 24). This is in contrast to in vivo models, where blockade of both IL-10 and TGF-β has been reported to abrogate Treg-mediated unresponsiveness to alloantigens (25, 26). These apparent discrepancies could be explained by a requirement for cell contact with a third cell, such as an APC, and subsequent elaboration of cytokines that may directly suppress other cells or recruit them to become regulators (see also below).

Role in transplantation. A role for naturally occurring $CD4^+CD25^+$ Tregs in the development of transplantation tolerance was first indicated by their ability to suppress graft verses host disease in murine models of allogeneic bone marrow transplantation. While transfer of allogeneic $CD4^+CD25^-$ naive or effector T cells normally leads to graft versus host disease, cotransfer of purified $CD4^+CD25^+$ Tregs along with the $CD4^+CD25^-$ T cells significantly delayed disease onset (27). Other groups have confirmed these findings, although it is not clear whether the activity of the cotransferred Tregs is amplified by or dependent upon other cell types, such as APCs or other T cells in vivo, or whether the Tregs themselves are sufficient to suppress alloresponsive T cells (28). In solid organ and tissue transplantation, cotransfer of $CD4^+CD25^+$ T cells into T cell–deficient mice along with naive $CD4^+CD25^-$ cells can block the ability of the latter cell subset to reject minor or MHC-mismatched allogeneic skin grafts (25, 29). Collectively, these studies indicate that naturally occurring Tregs can play a role in achieving transplantation tolerance.

Inducible $CD4^+$ Tregs

Characterization. The naturally occurring population of Tregs described above has inherent suppressive capabilities. However, populations of T cells whose immunosuppressive activity is induced/acquired in the periphery have also been identified. There are primarily 2 populations of these inducible Tregs important for transplantation tolerance: Th3 cells and Tr1 cells. Th3 cells were first identified because of their role, through the secretion of TGF-β, in the development of immune tolerance following the ingestion of antigens (termed oral tolerance) (30). Tr1 cells are similar to Th3 cells, but they secrete large amounts of IL-10 and were first characterized on the basis of their role in preventing autoimmune colitis (31).

There are a number of differences between naturally occurring Tregs and those induced in the periphery. First, $CD4^+CD25^+$ Tregs undergo development in the thymus, while there is no evidence to suggest thymic development of either Th3 or Tr1 cells. Instead, induced Tregs depend on peripheral factors such as the maturity or type of the stimulating APC and the availability of cytokines such as TGF-β (32). Second, in contrast to $CD4^+CD25^+$ Tregs, which exert their suppressive function through a cell contact–dependent and cytokine-independent mechanism, both Th3 and Tr1 cells appear to function independently of cell-to-cell contact and suppress immune responses through the secretion of immunosuppressive cytokines, such as IL-10 and TGF-β (33). Finally, the ability of Tr1 cells to home to anatomic sites differs from that of $CD4^+CD25^+$ T cells. Tr1 cells tend to migrate toward sites of inflammation, while naturally occurring $CD4^+CD25^+$ T cells are predominantly found in lymphoid organs (34). Agreement on this point is not universal, however, as Graca et al. recently demonstrated the existence of $CD4^+CD25^+$ Tregs within tolerated allografts (35).

At present, induced Treg subsets are largely identified on the basis of their secretion of immunosuppressive cytokines. As with $CD4^+CD25^+$ Tregs, there is no specific cell-surface marker to distinguish them from other T cell subsets. While Foxp3 is expressed by $CD4^+CD25^+$ Tregs, it is not yet clear whether it regulates the development of either Th3 or Tr1 suppressor T cell subsets (36).

However, TGF-β, a cytokine mediator of the effects of Th3 cells, has been shown recently to convert nonregulatory $CD4^+CD25^-$ T cells into regulatory $CD4^+CD25^+$ T cells, in conjunction with induction of *Foxp3* expression (37).

Role in transplantation. In the context of allograft transplantation, the induction of a regulatory T cell phenotype in otherwise alloresponsive T cells has been proposed as a major contributing factor for the maintenance of tolerance achieved through selected strategies (38). Indeed it has been reported that repetitive stimulation of naive T cells with immature allogeneic DCs results in the development of a suppressive phenotype by responding T cells (39). The maturation status and types of stimulating DCs present in the grafted tissue is undoubtedly a critical factor in determining the outcome of an alloimmune response. Phenotypically, immature DCs do not stimulate optimal effector T cell responses, due to low expression of T cell costimulatory factors and proinflammatory cytokines. In fact, such cells are often able to induce a Treg phenotype in responding T cells (5). Beyond their maturational state, however, it is also important to consider the multiplicity of existing DC subtypes, as a number of recent reports demonstrate that particular DC subsets can induce a Treg phenotype (e.g., Th3 or Tr1 cells) irrespective of their maturation state (40, 41).

While induced Tregs represent a subset distinct from their naturally occurring $CD4^+CD25^+$ counterparts, there is considerable evidence indicating that $CD4^+CD25^+$ T cells play an important role in the "development" of these cells, promoting otherwise potentially graft-destructive effector T cells to adopt a Tr1 suppressor phenotype (42, 43). At present however, the mechanism for this activity is not known and could involve either direct cell-cell interaction, involvement of a third cell (such as an APC), soluble mediators, or some combination of the three. As noted above, it has recently been demonstrated that nonregulatory T cells may also convert to a $CD4^+CD25^+$ suppressor phenotype, under the influence of TGF-β (37).

Interestingly, TGF-β has been found in tolerated grafts, which suggests that induced Tregs may develop and exert their influence directly at the site of the graft (26). Karim et al. have also shown that $CD4^+CD25^+$ Tregs can develop from $CD25^-$ precursors in thymectomized mice (44) and that these Tregs can suppress skin allograft rejection. These data suggest that inducible Treg subsets can prolong allograft survival without newly formed innate Tregs entering the periphery. Although appropriate strategies were employed to deplete innate $CD4^+CD25^+$ Tregs, one cannot completely exclude the possibility that residual nondepleted cells contributed to tolerance. A role for $CD4^+CD25^+$ T cells in the induction of a regulatory phenotype in otherwise nonsuppressive T cells provides an attractive hypothesis bringing together the observations of numerous groups concerning the respective roles of both innate and induced Treg subsets in promoting transplantation tolerance (34, 45). This suggests a model in which the 2 subsets act in a cooperative fashion to suppress potentially inflammatory immune responses directed toward transplanted tissues. The ability of these cells to induce regulatory function in other populations would also explain a paradox that has been raised regarding the potency of $CD4^+CD25^+$ Tregs. In vitro, meaningful suppression of activated T cells by $CD4^+CD25^+$ Tregs generally requires at least a 1:3 ratio of Tregs to effectors; lower ratios yield little suppression (46). However, the frequency of $CD4^+CD25^+$ Tregs in vivo is only approximately 10% that of $CD4^+$ T cells, and approximately 3% of all T cells (16). Thus, some combination of selective homing and/or induction of suppressive function in other cells must be occurring in vivo.

Other Treg types

As mentioned above, Treg subsets have also been described outside the $CD4^+$ compartment. $CD8^+$ Tregs generated through oral exposure to alloantigen have been observed in tolerated allografts. The precise mechanism of regulation by this subset is unclear but may be associated with increased IL-4 production (13). Similarly, Seino et al. demonstrated a requirement for NK T cells in acquiring long-term cardiac allograft acceptance after costimulatory blockade (15). Additionally, there is evidence for a $TCR^+CD4^-CD8^-$ Treg subset that can mediate acceptance of skin allografts, possibly by inducing the deletion of alloreactive $CD8^+$ T cells (14). The full significance of these Treg subsets outside the $CD4^+$ compartment with regard to transplantation tolerance, however, remains unknown given the still-early stages of investigation on these cell types.

Indirect allorecognition, linked suppression, and infectious tolerance

A great deal of work on the role of Tregs in transplantation tolerance has addressed their mode of allorecognition. As discussed above, it is believed that indirect allorecognition predominates with increasing duration of engraftment. This finding, coupled with observations from a number of groups that there is a requirement for the continuous presence of antigen in order to maintain transplant tolerance (47, 48), suggests that it is the indirect pathway that is the predominant mode of allorecognition by Tregs (49). Yamada et al. explored the relative contribution of the direct and indirect pathways of antigen presentation to transplant tolerance by using either donor mice deficient in MHC class II molecules (in which recognition is indirect only) or recipients that lack MHC class II molecules in the periphery but possess $CD4^+$ T cells as a consequence of transgenic expression of MHC II on thymic epithelium (in which recognition is direct only). Using agents that block T cell costimulatory pathways, the authors found that tolerance could be easily achieved in the absence of direct recognition but that recipients lacking indirect allorecognition pathways were notably difficult to tolerize (50).

If Tregs recognize alloantigens via indirect allorecognition, one might ask how cells that recognize only a small subset of graft-derived antigens can block the response to all graft-expressed antigens. Indeed, even before the clear identification of $CD4^+CD25^+$ Tregs, these questions were already being investigated. For example, more than a decade ago, transplantation tolerance in a rat model was achieved through the oral administration of multiple MHC-derived peptide alloantigens (51). Later studies implicated a role for immunoregulatory T cells in contributing to tolerance in this model (52). Niimi and colleagues further extended these observations with the finding that allograft tolerance could be achieved through oral administration of a single alloantigen present in the graft (53). The tolerizing potential of a single alloantigen, which can subsequently dominantly confer nonresponsiveness against all other antigens present within the graft, has been termed "linked suppression" and is dependent on the action of Tregs (38). Linked suppression occurs when a potentially alloreactive T cell comes under the tolerizing influence of a Treg, such as a $CD4^+CD25^+$ Treg, as both cells recognize their respective alloantigens presented by the same APC (33, 54, 55). In effect this leads to a "reeducation"

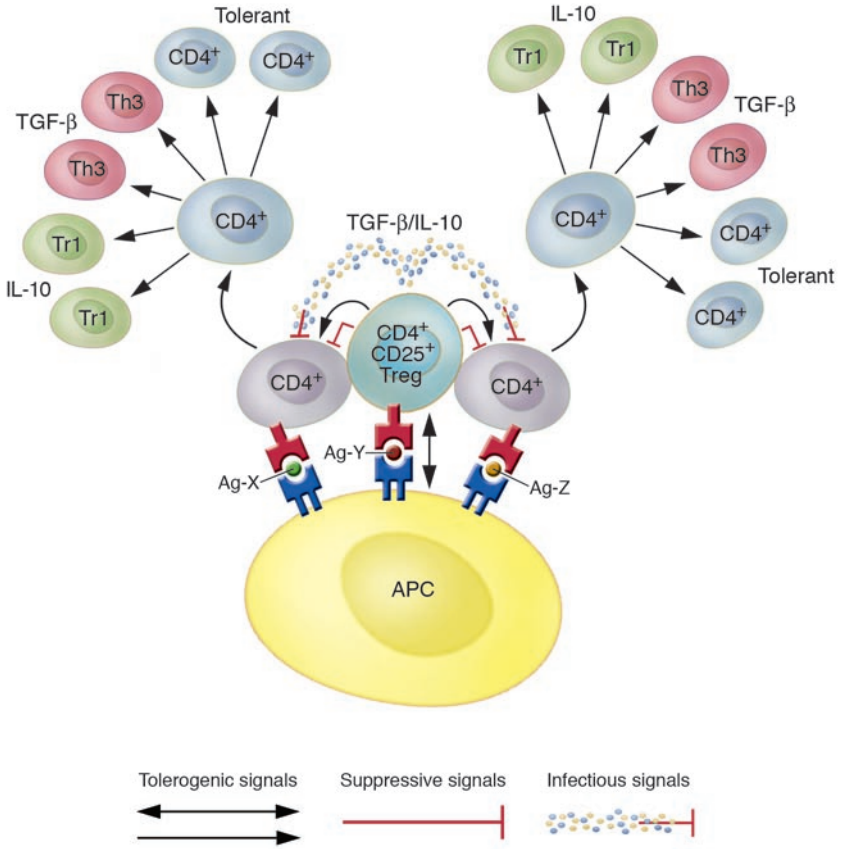

Figure 2
Infectious tolerance and linked suppression induced by $CD4^+CD25^+$ Tregs. $CD4^+CD25^+$ Treg cells can suppress alloreactive $CD4^+$ T cells either directly via cell contact or secretion of IL-10 and TGF-β or alternatively by influencing the stimulating APC. Linked suppression arises when tolerance generated against a specific antigen (Ag-Y) leads to tolerance against unrelated or third-party antigens (Ag-X and Ag-Z), providing that these unrelated antigens are expressed on the surface of the same APC. The secretion of IL-10 and TGF-β by Tregs has been implicated in this process, which is thought to reinforce the infectious nature of transplant tolerance.

of the potentially destructive alloresponsive T cell with the induction of a Treg phenotype (Figure 2).

Importantly, the reeducated alloaggressive cell, which becomes instead a "secondary" regulatory cell, can in turn induce other naive $CD4^+$ T cells to adopt a regulatory phenotype, thus propagating the tolerant state. Such mechanisms are thought to explain some seminal observations with regard to suppressive T cells and transplantation tolerance made almost 30 years ago (56). However, it was not until recently that the term "infectious tolerance" was coined, by Waldmann and colleagues, to describe the transferable nature of allograft tolerance from one recipient to another (reviewed in ref. 38). Tolerance achieved after a short course of nondepleting CD4 and CD8 antibodies prior to transplantation of minor histocompatibility–mismatched skin grafts was associated with the development of a regulatory $CD4^+$ T cell phenotype within the recipient (57). Transfer of $CD4^+$ T cells from these tolerized mice could prevent graft rejection in naive recipients. Most strikingly, the regulatory cells from the tolerized mice induced new Tregs in the naive-transplanted recipients, and these secondary Tregs could do the same if transferred to "tertiary" recipients, which illustrates the infectious nature of the process. Later studies by a number of groups confirmed these observations using more stringent models of transplant tolerance, such as cardiac allograft across major histocompatibility barriers, leading to a general acceptance of the existence of this phenomenon (47, 58). The generation of these Treg subsets has also been implicated in tolerance achieved through numerous strategies including costimulatory blockade and treatment with cyclosporine (59–61). The precise mechanisms by which infectious tolerance is mediated by $CD4^+$ suppressor T cells remain elusive, and it is not possible to assign functions to specific Treg subsets, although evidence indicates that immunosuppressive cytokines such as IL-10 and TGF-β play an important role (62).

Potential for utilizing Tregs in transplantation

One can consider 2 separate issues regarding $CD4^+CD25^+$ Tregs in transplantation: What is their role in models of tolerance? and What is their potential as a therapeutic tool? At present, there are almost no data in humans, although the issue has been examined extensively in animal models. Many studies establish the ability of Tregs to prevent graft rejection and facilitate tolerance in manipulated situations, such as using immune reconstituted immunodeficient hosts (reviewed in ref. 6). Because of confounding effects of homeostatic proliferation in T cell–deficient animals (63), this does not provide definitive proof of their role in tolerance in a normal host. However, such a role is strongly suggested by other studies, such as those showing Tregs in tolerated grafts (35). Moreover, both sets of studies clearly show the potential to use Tregs as a deliberate therapeutic tool. This might be achieved either by ex vivo expansion and activation of Tregs followed by infusion or by manipulating the immune response in vivo in a manner that promotes Treg development (64). Recent advances regarding the role of TGF-β, IL-10, and Foxp3 in the development of subsets of Tregs may provide a means to achieve these goals.

Concluding remarks

The large number of alloreactive cells present even in naive hosts is believed to underlie the requirement for deletion of these cells in the induction of tolerance across MHC-mismatched

barriers by strategies such as costimulatory blockade that target mature peripheral T cells (65, 66). From these investigations, we and others theorized that such a reduction in the uniquely high numbers of potentially graft destructive T cells would facilitate immunoregulation by Treg subsets, thus promoting and maintaining a tolerant state (9). Subsequently, this hypothesis has been strengthened by other groups who have demonstrated roles for both naturally occurring and induced Treg subsets in the development of allotolerance achieved through costimulatory blockade (27, 54). As a result, it is being increasingly proposed that clinical transplantation tolerance protocols that target alloreactive T lymphocytes for deletion need to specifically spare Tregs (1). Along those lines, Strom and colleagues recently demonstrated that selective lysis of nonregulatory $CD25^+$ alloreactive T cells and persistence of Treg cells could be achieved through administration of an agonistic IL-2–Fc receptor fusion protein. Indeed, administration of this fusion protein in combination with selective blockade of IL-15 signaling, which is important for effector T cell proliferation and memory generation, resulted in graft acceptance in a very stringent transplantation model (67). Taken together, these reports strongly support the idea that deletion and regulation play complementary roles in the development of transplantation tolerance. This has raised the potential for ex vivo expansion/promotion of regulatory cells, which could then be used as adoptive immunotherapy in transplantation. Determining whether such approaches are feasible will undoubtedly be the focus of study for many years to come. It may be premature to predict whether or not they will succeed, but it is not premature to state that Tregs in transplantation have finally "arrived."

Address correspondence to: Laurence A. Turka, University of Pennsylvania, 700 CRB, 415 Curie Boulevard, Philadelphia, Pennsylvania 19104-6144, USA. Phone: (215) 898-1018; Fax: (215) 573-2880; E-mail: turka@mail.med.upenn.edu.

1. Lechler, R.I., Garden, O.A., and Turka, L.A. 2003. The complementary roles of deletion and regulation in transplantation tolerance. *Nat. Rev. Immunol.* **3**:147–158.
2. Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., and Toda, M. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* **155**:1151–1164.
3. Wood, K.J., and Sakaguchi, S. 2003. Regulatory T cells in transplantation tolerance. *Nat. Rev. Immunol.* **3**:199–210.
4. Rogers, N.J., and Lechler, R.I. 2001. Allorecognition. *Am. J. Transplant.* **1**:97–102.
5. Walsh, P.T., Strom, T.B., and Turka, L.A. 2004. Routes to transplant tolerance versus rejection; the role of cytokines. *Immunity.* **20**:121–131.
6. Chiffoleau, E., Walsh, P.T., and Turka, L. 2003. Apoptosis and transplantation tolerance. *Immunol. Rev.* **193**:124–145.
7. Nikolic, B., and Sykes, M. 1997. Mixed hematopoietic chimerism and transplantation tolerance. *Immunol. Res.* **16**:217–228.
8. Sykes, M. 2001. Mixed chimerism and transplant tolerance. *Immunity.* **14**:417–424.
9. Li, X.C., Strom, T.B., Turka, L.A., and Wells, A.D. 2001. T cell death and transplantation tolerance. *Immunity.* **14**:407–416.
10. Suchin, E.J., et al. 2001. Quantifying the frequency of alloreactive T cells in vivo: new answers to an old question. *J. Immunol.* **166**:973–981.
11. Jonuleit, H., and Schmitt, E. 2003. The regulatory T cell family: distinct subsets and their interrelations. *J. Immunol.* **171**:6323–6327.
12. Gilliet, M., and Liu, Y.J. 2002. Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J. Exp. Med.* **195**:695–704.
13. Zhou, J., Carr, R.I., Liwski, R.S., Stadnyk, A.W., and Lee, T.D. 2001. Oral exposure to alloantigen generates intragraft CD8+ regulatory cells. *J. Immunol.* **167**:107–113.
14. Zhang, Z.X., Yang, L., Young, K.J., DuTemple, B., and Zhang, L. 2000. Identification of a previously unknown antigen-specific regulatory T cell and its mechanism of suppression. *Nat. Med.* **6**:782–789.
15. Seino, K.I., et al. 2001. Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. *Proc. Natl. Acad. Sci. U. S. A.* **98**:2577–2581.
16. Itoh, M., et al. 1999. 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.* **162**:5317–5326.
17. Jordan, M.S., et al. 2001. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol.* **2**:301–306.
18. Hori, S., Nomura, T., and Sakaguchi, S. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* **299**:1057–1061.
19. Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* **4**:330–336.
20. Takahashi, T., et al. 1998. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* **10**:1969–1980.
21. Suri-Payer, E., and Cantor, H. 2001. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by CD4(+)CD25(+) T cells. *J. Autoimmun.* **16**:115–123.
22. Piccirillo, C.A., et al. 2002. CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J. Exp. Med.* **196**:237–246.
23. Takahashi, T., et al. 2000. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* **192**:303–310.
24. Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y., and Sakaguchi, S. 2002. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. *Nat. Immunol.* **3**:135–142.
25. Hara, M., et al. 2001. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. *J. Immunol.* **166**:3789–3796.
26. Josien, R., et al. 1998. A critical role for transforming growth factor-β in donor transfusion-induced allograft tolerance. *J. Clin. Invest.* **102**:1920–1926.
27. Taylor, P.A., Noelle, R.J., and Blazar, B.R. 2001. CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J. Exp. Med.* **193**:1311–1318.
28. Hoffmann, P., Ermann, J., Edinger, M., Fathman, C.G., and Strober, S. 2002. Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. *J. Exp. Med.* **196**:389–399.
29. Graca, L., et al. 2002. Both CD4(+)CD25(+) and CD4(+)CD25(-) regulatory cells mediate dominant transplantation tolerance. *J. Immunol.* **168**:5558–5565.
30. Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., and Weiner, H.L. 1994. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science.* **265**:1237–1240.
31. Groux, H., et al. 1997. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature.* **389**:737–742.
32. Steinman, R.M., Hawiger, D., and Nussenzweig, M.C. 2003. Tolerogenic dendritic cells. *Annu. Rev. Immunol.* **21**:685–711.
33. Stassen, M., Schmitt, E., and Jonuleit, H. 2004. Human CD(4+)CD(25+) regulatory T cells and infectious tolerance. *Transplantation.* **77**(1 Suppl.):S23–S25.
34. Cottrez, F., and Groux, H. 2004. Specialization in tolerance: innate CD(4+)CD(25+) versus acquired TR1 and TH3 regulatory T cells. *Transplantation.* **77**(1 Suppl.):S12–S15.
35. Graca, L., Cobbold, S.P., and Waldmann, H. 2002. Identification of regulatory T cells in tolerated allografts. *J. Exp. Med.* **195**:1641–1646.
36. Sakaguchi, S. 2003. The origin of *FOXP3*-expressing $CD4^+$ regulatory T cells: thymus or periphery. *J. Clin. Invest.* **112**:1310–1312. doi:10.1172/JCI200320274.
37. Chen, W., et al. 2003. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* **198**:1875–1886.
38. Waldmann, H., and Cobbold, S. 2001. Regulating the immune response to transplants: a role for CD4+ regulatory cells? *Immunity.* **14**:399–406.
39. Jonuleit, H., Schmitt, E., Schuler, G., Knop, J., and Enk, A.H. 2000. Induction of interleukin 10-producing, nonproliferating CD4(+) T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med.* **192**:1213–1222.
40. Wakkach, A., et al. 2003. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity.* **18**:605–617.
41. Lavelle, E.C., et al. 2003. Cholera toxin promotes the induction of regulatory T cells specific for bystander antigens by modulating dendritic cell activation. *J. Immunol.* **171**:2384–2392.
42. Jonuleit, H., et al. 2002. Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. *J. Exp. Med.* **196**:255–260.
43. Dieckmann, D., Bruett, C.H., Ploettner, H., Lutz, M.B., and Schuler, G. 2002. Human CD4(+)CD25(+) regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells [corrected]. *J. Exp. Med.* **196**:247–253.
44. Karim, M., Kingsley, C.I., Bushell, A.R., Sawitzki, B.S., and Wood, K.J. 2004. Alloantigen-induced CD25+CD4+ regulatory T cells can develop in vivo

from CD25-CD4+ precursors in a thymus-independent process. *J. Immunol.* **172**:923–928.
45. Waldmann, H., et al. 2004. Regulatory T cells and organ transplantation. *Semin. Immunol.* **16**:119–126.
46. Kuniyasu, Y., et al. 2000. Naturally anergic and suppressive CD25(+)CD4(+) T cells as a functionally and phenotypically distinct immunoregulatory T cell subpopulation. *Int. Immunol.* **12**:1145–1155.
47. Chen, Z.K., Cobbold, S.P., Waldmann, H., and Metcalfe, S. 1996. Amplification of natural regulatory immune mechanisms for transplantation tolerance. *Transplantation.* **62**:1200–1206.
48. Onodera, K., Volk, H.D., Ritter, T., and Kupiec-Weglinski, J.W. 1998. Thymus requirement and antigen dependency in the "infectious" tolerance pathway in transplant recipients. *J. Immunol.* **160**:5765–5772.
49. Scully, R., Qin, S., Cobbold, S., and Waldmann, H. 1994. Mechanisms in CD4 antibody-mediated transplantation tolerance: kinetics of induction, antigen dependency and role of regulatory T cells. *Eur. J. Immunol.* **24**:2383–2392.
50. Yamada, A., et al. 2001. Recipient MHC class II expression is required to achieve long-term survival of murine cardiac allografts after costimulatory blockade. *J. Immunol.* **167**:5522–5526.
51. Sayegh, M.H., Khoury, S.J., Hancock, W.W., Weiner, H.L., and Carpenter, C.B. 1992. Induction of immunity and oral tolerance with polymorphic class II major histocompatibility complex allopeptides in the rat. *Proc. Natl. Acad. Sci. U. S. A.* **89**:7762–7766.
52. Hancock, W.W., Sayegh, M.H., Kwok, C.A., Weiner, H.L., and Carpenter, C.B. 1993. Oral, but not intravenous, alloantigen prevents accelerated allograft rejection by selective intragraft Th2 cell activation. *Transplantation.* **55**:1112–1118.
53. Niimi, M., Shirasugi, N., Ikeda, Y., and Wood, K.J. 2001. Oral antigen induces allograft survival by linked suppression via the indirect pathway. *Transplant Proc.* **33**:81.
54. Honey, K., Cobbold, S.P., and Waldmann, H. 1999. CD40 ligand blockade induces CD4+ T cell tolerance and linked suppression. *J. Immunol.* **163**:4805–4810.
55. Davies, J.D., Leong, L.Y., Mellor, A., Cobbold, S.P., and Waldmann, H. 1996. T cell suppression in transplantation tolerance through linked recognition. *J. Immunol.* **156**:3602–3607.
56. Kilshaw, P.J., Brent, L., and Pinto, M. 1975. Suppressor T cells in mice made unresponsive to skin allografts. *Nature.* **255**:489–491.
57. Qin, S., et al. 1993. "Infectious" transplantation tolerance. *Science.* **259**:974–977.
58. Yin, D., and Fathman, C.G. 1995. CD4-positive suppressor cells block allotransplant rejection. *J. Immunol.* **154**:6339–6345.
59. Larsen, C.P., et al. 1996. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature.* **381**:434–438.
60. Graca, L., Honey, K., Adams, E., Cobbold, S.P., and Waldmann, H. 2000. Cutting edge: anti-CD154 therapeutic antibodies induce infectious transplantation tolerance. *J. Immunol.* **165**:4783–4786.
61. Hall, B.M., Jelbart, M.E., Gurley, K.E., and Dorsch, S.E. 1985. Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. Mediation of specific suppression by T helper/inducer cells. *J. Exp. Med.* **162**:1683–1694.
62. Cobbold, S., and Waldmann, H. 1998. Infectious tolerance. *Curr. Opin. Immunol.* **10**:518–524.
63. Goldrath, A.W., Bogatzki, L.Y., and Bevan, M.J. 2000. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *J. Exp. Med.* **192**:557–564.
64. Waldmann, H., and Cobbold, S. 2004. Exploiting tolerance processes in transplantation. *Science.* **305**:209–212.
65. Wells, A.D., et al. 1999. Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. *Nat. Med.* **5**:1303–1307.
66. Li, Y., et al. 1999. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nat. Med.* **5**:1298–1302.
67. Zheng, X.X., et al. 2003. Favorably tipping the balance between cytopathic and regulatory T cells to create transplantation tolerance. *Immunity.* **19**:503–514.