

Is transplantation tolerable?

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Commentary

To test the hypothesis that chronic stimulation of T cells with a weak agonistic antigen will generate regulatory T cells and immune tolerance, a study reported in this issue employed the redesign of a minor histocompatibility antigen. Using knowledge of residues at which the antigen contacts the T cell receptor, a weak agonist was produced. Pretreatment with this altered antigen produced transplant tolerance, generation of regulatory T cells, and a loss of many antigen-reactive T cells.

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World Health Organization's plan to eradicate poliovirus from the planet will necessitate. Given some of the ongoing logistical problems with this noble effort, researchers may yet have time to uncover some of the long-standing mysteries of viral pathogenesis presented by the unique tropism and disease characteristics of poliovirus infections.

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Is transplantation tolerable?

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To test the hypothesis that chronic stimulation of T cells with a weak agonistic antigen will generate regulatory T cells and immune tolerance, a study reported in this issue (see the related article beginning on page 1754) employed the redesign of a minor histocompatibility antigen. Using knowledge of residues at which the antigen contacts the T cell receptor, a weak agonist was produced. Pretreatment with this altered antigen produced transplant tolerance, generation of regulatory T cells, and a loss of many antigen-reactive T cells.

In brackish waters once trafficked by old-school cellular immunologists, precious texts describing the wonders of somewhat

Nonstandard abbreviations used: altered peptide ligand (APL); glucocorticoid-induced TNF receptor (GITR); T cell receptor (TCR).

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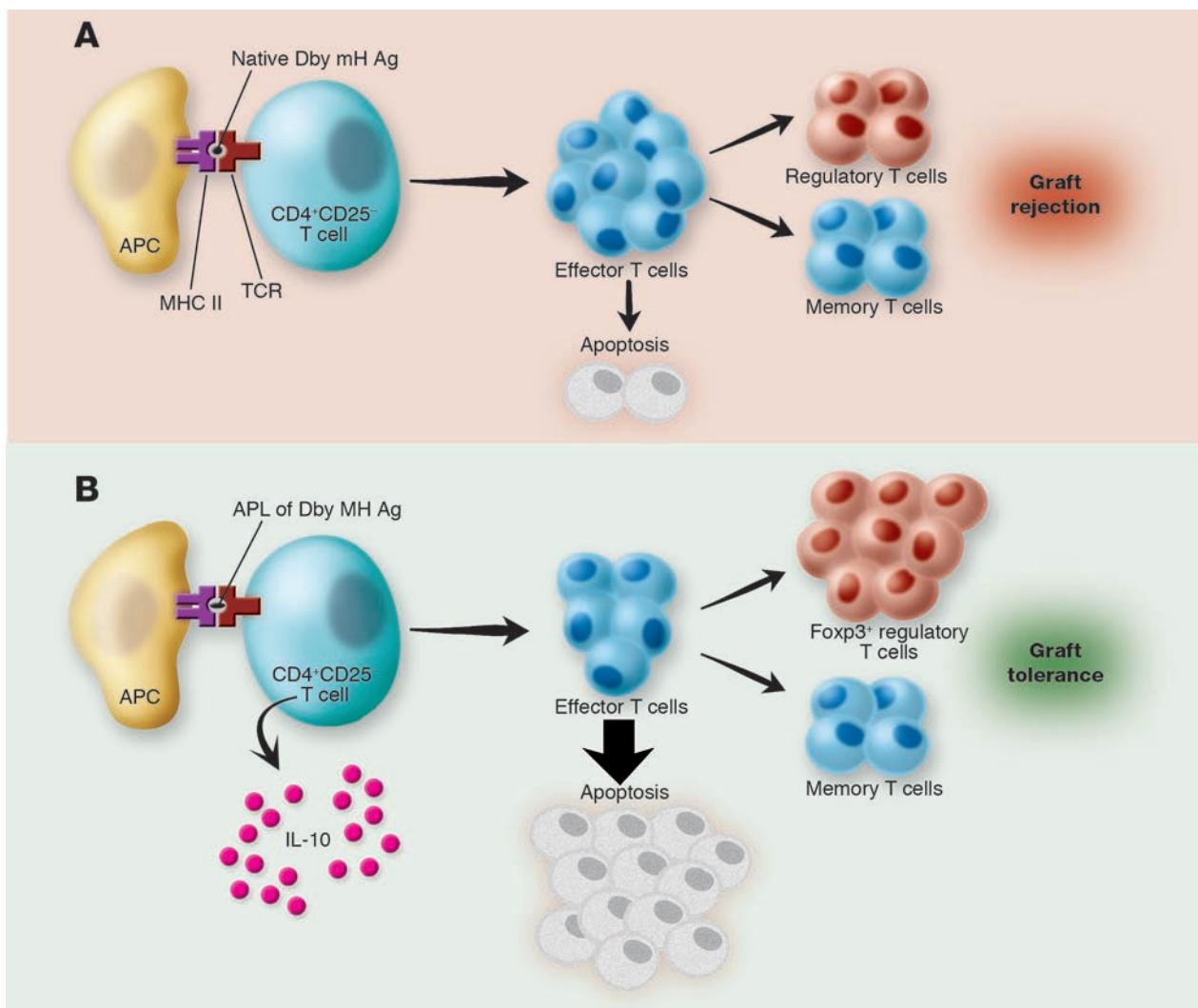
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ill-defined suppressor lymphocytes (1) were jettisoned. Later works, also discarded, described lymphocytes able to protect rather than destroy foreign tissues in adult hosts rendered tolerant to organ transplants (2-4). CD4⁺, IL-2 receptor-positive (CD25⁺) T cells capable of countering the graft-destroying properties of alloaggressive T cells were identified by Hall and his colleagues in rodent transplant models (2). Nonetheless, following the fashion of the

time, many of us cleansed our grants and manuscripts of any mention of suppressor T cells. Following a revival of interest in suppressor, or regulatory, T cells, Chen et al. in this issue of the *JCI* (5) have now redesigned a minor histocompatibility antigen to test the hypothesis that chronic stimulation of T cells with a weak agonistic antigen will generate regulatory T cells and produce immune tolerance.

In the 1990s, an era in which the phrase "suppressor T cells" was uttered only in hushed tones, a series of brilliant experiments by Waldmann (6, 7) and his colleagues identified a crucial graft-protecting T cell-dependent network in hosts rendered tolerant to transplants by means other than creation of total and enduring deletion of antidonor clones. Tolerant host

**Figure 1**

As compared with antigenic stimulation (A), stimulation of female transgenic mice with an APL of the Dby minor histocompatibility (mH) antigen, which delivers incomplete signals to naive T cells, (a) promotes the production of $Foxp3^+$ regulatory T cells, (b) limits the development of effector T cells, and (c) magnifies the apoptotic loss of activated T cells (B), resulting in tolerance to male skin grafts. Ag, antigen.

antidonor $CD4^+$ T cells can recruit nontolerant syngeneic T cells to protect the donor graft. The detailed cellular basis of this $CD4^+$ T cell-dependent network remains somewhat elusive, although rapid progress is being made.

With the discovery that antigen-stimulated $CD4^+$ T cells can polarize into either a Th1 or a Th2 response, it became clear that graft rejection was usually the result of a Th1-type immune response. In contrast, many donor-reactive $CD4^+$ T cells in tolerant hosts manifest a Th2-type program (8, 9). The possibility that Th2-type T cells served as the cellular basis of peripheral transplant tolerance was a welcome relief to many, because, unlike the shadowy suppressor cells of old, IL-4- and IL-10-pro-

ducing Th2 cells have a defined molecular program. Unlike the situation that pertains to MHC-matched allografts, Th1-to-Th2 immune deviation does not provide a comprehensive basis for transplant tolerance in MHC-mismatched models (10). Vigorous rejection of MHC-mismatched allografts can be mounted despite marked Th1-to-Th2 immune deviation. Perhaps, Th1-to-Th2 immune deviation is necessary but not sufficient to produce tolerance in many situations.

Recovering the legacy of suppressor lymphocytes

A revival in interest in suppressor (also known as regulatory) T cells began with the discovery that $CD4^+CD25^+$ T cells pres-

ent in naive hosts can counteract autoreactive, Th1-dependent cytopathic programs (reviewed in ref. 11). Similar observations were soon made in mouse transplant models (reviewed in ref. 12). In the naive host, $CD4^+CD25^+$ regulatory T cells express cell surface glucocorticoid-induced TNF receptor (GITR) molecules (11–13). The $CD4^+CD25^+GITR^+$ phenotype does not clearly define the regulatory T cell population, since activated, nonregulatory T cells can express CD25 and GITR. Expression of $Foxp3$ provides a more precise marker for regulatory T cell development and function (14, 15). Moreover, $Foxp3$ serves as a master switch to trigger the suppressor function of regulatory T cells. Insofar as $TGF-\beta$, a cytokine with known immunosuppressive



effects, can trigger expansion of Foxp3^+ regulatory T cells (16), a link between immunosuppressive cytokines and T cell-based immunoregulation has been uncovered. While $\text{CD4}^+ \text{CD25}^+ \text{Foxp3}^+$ T cells have been clearly identified as regulatory T cells, there is no evidence that all immunoregulatory T cells express this phenotype. IL-10-producing CD4^+ Tr1 cells and other T cell subtypes have been implicated as serving an immunoregulatory function in several immune system models (17).

Manufacturing transplant tolerance

The belief that the outcome of allograft response — rejection or tolerance — following the withdrawal of immunosuppressive therapy is determined by the balance of alloaggressive to graft-protecting T cells is now emerging. In most situations, tolerance is not accompanied by permanent and complete deletion of alloaggressive donor-reactive T cells, and regulatory networks are required. Regimens that temporarily deplete cytopathic T cells aid tolerance induction (18–20) but do not replace the requirement for regulatory T cells (21).

It is in the context of favorably altering the balance of cytopathic to protective T cells that the importance of the work of Chen et al. (5), appearing in this issue of the *JCI*, must be considered. Can regulatory T cells be manufactured to aid tolerance induction? Yes, they can. How? Persistent and suboptimal stimulation of the T cell receptor (TCR) complex has been linked to the recruitment of T cells into the pool of regulatory T cells (22). To probe the implications of this hypothesis, Chen et al. rationally designed an altered peptide ligand (APL) for the antigenic epitope of the Dby peptide. The *Dby* gene, located on the Y chromosome, stimulates rejection of male skin grafts by same-strain female recipients. As anticipated, the targeted alterations in the TCR-binding epitope compromise the binding affinity with the TCR (Figure 1). Moreover, challenge of Dby-reactive TCR transgenic T cells with the APL generated “incomplete” T cell activation signals, as deduced by several assays. Interestingly, stimulation of the TCR with the APL, but not with the native epitope, triggered copious secretion of IL-10 by $\text{CD4}^+ \text{CD25}^+$ T cells. The propensity of APL-stimulated TCR transgenic T cells to robustly express IL-10 is an attribute shared with naturally occurring Tr1-type and other regulatory cells (17). Pretreatment of female mice with the APL, but not pretreatment with saline or the native Dby epitope, induced a state of prolonged

tolerance to male skin transplants even when coadministered with saturating doses of a blocking anti-IL-10 receptor mAb. IL-10 is a surrogate marker for the effector molecules driving tolerance in this model. T cells in APL-treated mice counteract the ability of untreated T cells to reject donor, male-type skin transplants.

In keeping with other models of peripheral transplant tolerance, robust Foxp3 expression in APL-treated tolerant, but not in control, transplanted tissue (skin) or spleen is manifest (5). Nonetheless, in other models of peripheral tolerance in which Foxp3^+ T cells have been found within the graft and peripheral lymphoid tissues, thymic Foxp3^+ T cells are also present. In this APL-driven model of transplant tolerance, Foxp3^+ T cells cannot be identified within the host thymus. This observation unequivocally proves that Foxp3 -expressing regulatory T cells need not be derived only from $\text{CD4}^+ \text{CD25}^+ \text{Foxp3}^+$ thymic T cells, as they can be recruited from peripheral Foxp3 -null T cells not predestined to become Foxp3^+ regulatory T cells. Finally, stimulation with a weakly agonistic APL serves not only to generate regulatory T cells; it also produced gross depletion of peripheral, but not thymic, antigen-reactive T cells (5). In keeping with the hypothesis that tolerance is efficiently promoted by generation of donor-reactive regulatory T cells plus partial depletion of donor-reactive aggressive T cells, APL treatment produced a depletion of splenic, but not thymic, Dby-reactive T cells.

Taken together, this work (5) indicates that chronic stimulation of T cells with a weak TCR agonist promotes post-thymic differentiation of potent regulatory T cells and partial but extensive post-thymic depletion of donor-reactive aggressive T cells. While there has been great emphasis on treatments that curtail the destructive properties of conventional T cells, the knowledge that T cells with graft-protective properties can be trained and propagated *in vivo* provides a new tool for attempting to tilt the allograft response toward tolerance. Insofar as the same treatment destroys antigen-reactive effector T cells, a very favorable alteration in the balance of graft-destroying to graft-protective T cells becomes manifest.

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