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Clinical Transplantation Tolerance: Many Rivers to Cross

Alan D. Salama,* Karl L. Womer,† and Mohamed H. Sayegh1‡

Modern immunosuppressive regimens for organ transplantation have resulted in excellent short-term results but less dramatic improvements in long-term outcomes. Moreover, they are associated with significant deleterious effects. One solution that should avoid the adverse drug effects and result in improved graft and patient longevity as well as positively impacting on the organ shortage is the establishment of transplantation tolerance. Ever since the original description of transplantation tolerance in rodent allografts, there have been significant efforts made to translate tolerance-promoting strategies to the clinical arena. However, >50 years later, we are still faced with significant barriers that are preventing such a goal from being widely attained. Nonetheless, pilot clinical tolerance protocols are underway in selected transplant recipients. In this review, we discuss the scientific and nonscientific issues that must be overcome for successful transplantation tolerance to become a clinical reality. The Journal of Immunology, 2007, 178: 5419–5423.

Organ transplantation is a victim of its own success. It is now considered the therapy of choice, and often the only lifesaving therapy, for end stage organ failure yet cannot satisfy the continuously increasing demand for donor organs (1). The introduction of novel immunosuppressive therapies (2) has led to significant improvement in short-term survival rates for solid organ allografts (1). Unfortunately, the use of nonspecific immunosuppression increases the rates of malignancy and opportunistic infections and, through drug-specific side effects, increases the risk of cardiovascular and metabolic diseases, all of which adversely impacts on allograft function and outcome (3). Furthermore, these agents do not prevent chronic allograft dysfunction, the leading cause of graft failure 1-year after transplantation (4). In fact, calcineurin inhibitors may themselves contribute to chronic renal dysfunction in kidney transplant recipients as well as recipients of liver, heart, and lung transplants (5). Initial optimism regarding improved long-term renal allograft survival rates based on projected half-life analysis of United States registry data (6) appears not to be borne out in reality. Indeed, the true half-lives of transplanted kidneys appear to have changed very little in recent years, even with the introduction of newer more potent immunosuppressive drugs in the past decade (7). Therefore, to improve transplantation outcomes, it is critical to continue development of novel strategies to prevent acute and chronic rejection while lessening the need for lifelong immunosuppression and promote the development of a state of donor-specific tolerance (1). So, now more than ever, the promise of a recipient who is tolerant of their allograft, can avoid the adverse effects of chronic maintenance immunosuppression, yet possesses a functioning transplanted organ that will never reject is all the more appealing. How realistic is this idea of clinical transplant tolerance and what is required to achieve it? Huge efforts continue to be made to translate the success of rodent transplantation tolerance strategies into clinical practice. However, we remain some distance from this goal, with many hurdles to overcome, both scientific and nonscientific (8).

Transplantation tolerance is a state in which there is a lack of a destructive immune response by the recipient toward the well-functioning donor organ, in the absence maintenance immunosuppression, and with a fully intact immune system. Like natural tolerance, transplantation tolerance is achieved through control of T cell reactivity by two main processes. The first, central thymic deletion, involves the elimination of cells directed against self-determinants and the positive selection of those that are not. In the context of transplantation, this requires the establishment of a chimeric immune system so that recipient T cells are educated on recipient (or donor) thymic tissue to donor Ags, which results in the deletion of potential alloreactive T cells; the second is peripheral tolerance, which uses numerous different mechanisms, including functional inactivity of T cells (anergy), suppression of T cells by other cells or factors (regulation), peripheral deletion of alloreactive T cells by apoptosis (passive or activation-induced cell death), and finally, indifference to the stimulating alloantigen (ignorance). More detailed explanation regarding these mechanisms can be found in other reviews (8, 9).

Potential benefits of transplantation tolerance

The hope is that successful transplantation tolerance will promote better outcomes, in terms of the duration of graft survival,
but also with regards to the overall health of the recipient. Ideally, tolerance would be associated with no acute graft rejection, but this should also translate into a lack of chronic rejection and late graft loss. However, this first goal remains somewhat of an uncertainty because in some animal models long-term graft survival and transplant tolerance do not always prevent chronic rejection (10, 11). Clearly, not all late graft loss is immunologically based, and thus, transplant tolerance may not impact the alloantigen-independent factors contributing to graft attrition (1, 4). Furthermore, cases of human tolerance, which have been described, have also been revised when subsequent rejection and graft loss were found to have occurred (12). There is no doubt that a true tolerant state, in which the recipient does not mount a harmful immune response toward their graft, will avoid the complications of maintenance immunosuppressive drug therapy, which is known to contribute toward the morbidity and mortality of transplant recipients (3). However, this does not take into account any possible deleterious effects, which a novel tolerance induction regimen may have on the recipient. Undoubtedly, the avoidance of maintenance immunotherapy will minimize the issue of noncompliance, which accounts for a consistent proportion of late graft failures (13), and should also be associated with an improved quality of life for the recipient. While it should be remembered that even with a robust tolerance protocol, patient compliance to monitor any potential alteration in the immunological state will also be essential. Finally, by improving long-term transplant outcome, tolerance should mitigate the problem of organ shortage as more organs would be available to primary transplant recipients. Moreover, it should also have a major economic impact by lessening the cost of chronic maintenance immunosuppression and overall medical care related to the mortality and morbidity of transplant recipients.

A number of tolerance trials are currently being conducted under the auspices of the immune tolerance network (www.immunetolerance.org), which is also collating data on renal transplant recipients who have become tolerant of their grafts, as demonstrated by stable graft function, while not taking any maintenance immunosuppressants (14). Such cases are rare and have been described in patients receiving total lymphoid irradiation (15), in recipients of kidney transplants after bone marrow transplantation using the same donor (16), in patients with multiple myeloma who received simultaneous bone marrow and kidney transplant (17), and in a subgroup of noncompliant patients who stopped their immunosuppression (18). In addition, it occurs in an unpredictable manner in ~20% of liver transplant recipients in whom immunosuppression may be successfully (intentionally) withdrawn (19). However, the long-term outcomes of these cohorts are still not known, and identifying the patients in whom this strategy is likely to succeed remains difficult. The hope is that from these subjects an immunological “tolerance signature” will be discovered that should enable the development of reproducible nontoxic strategies that will promote tolerance in all patients. Devising such protocols for tolerance induction in transplantation will also obviously impact on the development of treatments for autoimmune and possibly allergic diseases.

### Challenge with moving to the clinic

One of, if not, the most significant hurdles in developing clinical tolerance strategies is the lack of reproducible assays to detect tolerance (8) (Table I). Although numerous assays exist that provide some form of readout regarding donor-specific alloimmune responses, none has been shown reliably in rigorous clinical studies to provide a positive tolerance signature (20). In part, this reflects our current ignorance regarding the complex immunological interactions and their chronology, which are required for tolerance to be established. Without such a signature, moving into the clinical arena will be fraught with difficulty, as knowing when we have achieved tolerance and when it may be perturbed will be difficult to ascertain early enough to allow withdrawal of immunosuppression or pre-emptive therapy if tolerance is to be lost. These concerns are important because acquired immunological tolerance may be less robust than natural tolerance and thus will require more vigilant and long-term monitoring.

There remains some debate about whether tolerance induction will require a completely novel strategy or whether a drug minimization approach would ultimately allow complete withdrawal of immunosuppressive drugs and culminate in the induction of tolerance. The introduction of newer induction protocols has allowed for minimal maintenance regimens (e.g., alemtuzumab induction with tacrolimus monotherapy maintenance) (21). However, attempts to minimize therapy further have resulted in episodes of graft rejection (22), often with more uncommon effector mechanisms such as predominant monocyte-induced rejection (23), which may not have been diagnosed using conventional (lymphocyte-biased) alloimmune assays. Moreover, following such minimization protocols, it is apparent that the recipients are not truly tolerant, with evidence

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<thead>
<tr>
<th>Scientific</th>
<th>Nonscientific</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Strategy: central or peripheral depletion in association with drug minimisation or novel adjunctive therapies.</td>
<td>2. Ethical issues with complete immunosuppression withdrawal and risk of graft loss.</td>
</tr>
<tr>
<td>3. Confirmation in large animal models and the reproducibility in patients.</td>
<td>3. Optimising the tolerogenic regimens in association with industry.</td>
</tr>
<tr>
<td>4. Defining the mechanisms through which innate immunity, immune memory, and infections prevent or break tolerance induction.</td>
<td>4. Financing the trials.</td>
</tr>
<tr>
<td>5. Tolerance trial end points.</td>
<td></td>
</tr>
</tbody>
</table>
of acute and chronic rejection developing in a significant number of cases. The long-term consequences following such rejection remains to be seen (22, 23). Although such “prope” tolerance may be an improvement in terms of overall immunosuppressive burden (24), it is not the robust state persistently sought by transplantation biologists. Longer-term follow-up is required to appreciate fully whether it offers significant additional benefits (other than financial) over standard transplantation therapies.

The requirement to use larger experimental animals such as primates to confirm findings in rodent models before initiating human tolerance trials has been applied frequently. However, these models do not always accurately predict outcomes in humans (23). This sobering reality has been demonstrated most recently in the first human trial of an agonist CD28 mAb in the U.K., which resulted in a life-threatening cytokine storm following administration to normal healthy volunteers, of a much lower dose of drug that was shown to be safe in nonhuman primates (25, 26). Significant immunological differences between humans (as well as nonhuman primates) and rodents, with respect to the proportion of memory and immunologically naive T cells (27), may explain why strategies used in rodents have not been successfully translated to nonhuman primates (28). For example, differences in requirements for costimulatory blockade between immunologically naive and experienced cells mean that tolerance strategies based on costimulatory blockade are very successful in rodents (immunologically naive) and less efficacious in animals with a higher proportion of memory cells (29) such as primates or humans. Moreover, induction therapy using depleting strategies appears to promote homeostatic proliferation and an expansion in the memory T cell compartment (30) while effectively depleting regulatory cell populations (31). Such strategies effectively attenuate tolerance induction through costimulatory blockade, even in rodents (30, 31). In addition, human studies using depleting agents such as alemtuzumab or antithymocyte globulin demonstrate that the postdepletion residual cells appear to be uniquely memory T cells, which are subsequently capable of promoting rejection (23, 32). Moreover, these cells appear to be selectively inhibited by particular immunomodulatory mechanisms, including regulatory T cells (30, 31, 33), but not others (34). In addition, other less common effectors of rejection may appear following depletion, such as monocyte-rich cellular infiltrates in alemtuzumab-treated patients using tacrolimus monotherapy (Fig. 1) and predominant humoral rejection in those treated with alemtuzumab and rapamycin (35). These findings suggest that other additional mechanisms to peripheral depletion may be required for successful tolerance induction in nonhuman primate and human transplant recipients. Alternative strategies that appear to promote both deletion and regulation have proven to be highly efficacious in promoting tolerance in stringent small animal models of transplantation but require testing in larger animals to confirm their potential (36–38).

Strategies to induce central (thymic) deletional tolerance through induction of mixed allogeneic chimerism have been translated to the clinic with some success, albeit in selected patient populations, such as those with hematological malignancies and concurrent renal failure (39). Recent long-term (>7 years) follow-up of a cohort of six patients treated with bone marrow and kidney grafts from HLA identical donors has been published. Although chimerism was not sustained in 67% of the patients, three had complete remissions from their myeloma, whereas kidney graft rejection only occurred in one recipient. Three of the six had immunosuppression successfully withdrawn within the first 3 mo, whereas graft vs host disease occurred in three and required further immunotherapy. These results demonstrate that such an approach is feasible, but, for more general applicability, in a recipient population that does not have hematological malignancy, greater refinement is required. These data also raise a significant issue, which is the

![FIGURE 1. Renal biopsy sections from a transplant patient treated with alemtuzumab and tacrolimus monotherapy who developed rejection within the first postoperative year. a, H&E stain demonstrating interstitial inflammation and hemorrhage. b, Immunohistochemistry demonstrating relatively sparse staining of CD3+ T cells. c, Immunohistochemistry demonstrating abundant CD68+ monocyte/macrophage infiltration within the same area of the biopsy.](http://www.jimmunol.org)
stability of the tolerant state. Although tolerance is inducible under certain circumstances, for it to be a therapeutic option for a wider patient population, it has to be robust and permanent. In considering this issue, it is important to realize that, while laboratory animals may not be exposed to many infectious agents, patients are confronted by a multitude. Heterologous immunity, the production of memory T cells generated to infectious agents, allows for potent alloimmune responses and the prevention of tolerance induction through cross-reactive T cell responses or bystander effects (40, 41). In part, the difficulties in translating the success of rodent transplant tolerance to larger animals and patients are due to the difference in the memory T cell compartment between immunologically naive rodents and Ag-experienced primates and humans (42). Recent data demonstrate that infectious agents, signaling through TLRs and recruitment of the MyD88 adaptor, can prevent tolerance induction and promote graft rejection (43–45). Moreover, it appears that TLR signaling directly to CD4+ T cells enhances activation, proliferation, and survival (46). Viral infections can also abrogate deletion of alloreactive CD8+ cells through TLR signaling (45), which is also associated with a defective recruitment of regulatory T cells into the grafts (44). These data may also explain some of the differential susceptibility of particular organs to tolerance induction, with those organs in which there is a greater microbial exposure being the hardest in which to induce tolerance (44). This intrinsic susceptibility or resistance to tolerance induction will undoubtedly impact on which grafts are considered most suitable for the first translational trials. Identical tolerance-inducing protocols appear to have differential effects depending on the organ transplanted, suggesting that specific protocol modifications would have to be made for certain grafts (47). Moreover, the organ/ceIl susceptibility to tolerance may be balanced by a susceptibility to develop recurrent disease (48), more likely if the organs are initially lost through an autoimmune process (such as the pancreas in type 1 diabetes) than a nonimmune mechanism.

There are a number of other important issues that the transplant community will face in taking such translational work forward, which must be agreed upon for there to be a meaningful chance of success (Table I). Clinical issues, such as which end points are to be measured as well as how and when to define trial success, must be clarified. It is obvious that, while we rely on short-term outcomes, such as 1-year graft survival, the incidence of infectious or malignant complications over 2 or 3 years when comparing newer drug protocols, along with other outcomes, must be considered in a tolerance trial. There must be an agreement on what stage it would be acceptable to move from a pilot study to a larger trial, given that it is not desirable to subject patients to challenging, intensive protocols if long-term success is not likely. In addition, current, immediate success in organ transplantation with drug-minimizing regimens means there may be resistance on behalf of both patients and physicians to enroll in such an experimental trial, and if enrolled, whether they remain agreeable to completely withdraw immunosuppression while patients and grafts are doing well on minimal drug therapy.

No less important and possibly as difficult to solve are some of the nonscientific hurdles that exist (Table I). These include ethical issues regarding which patient population to recruit for tolerance trials and how best to counsel them regarding the risks and benefits. The age of the patients recruited (adults vs children), the type of graft (from deceased or living donors), and ultimately which organ are all important considerations. The consequences of a graft failing may be less with renal transplants compared with hearts, lungs, or livers, whereas sensitization from a failed graft may be more costly in pediatric recipients compared with adults. On the other hand, pediatric recipients would benefit the most because they are potentially exposed to immuno-suppression for longer. Similarly, losing a living donor organ is considered greater harm than loss of a deceased donor organ. Should high-risk (such as previously sensitized patients) or low-risk renal transplant recipients be chosen first? The former have the most to lose if the experimental procedure fails because they may have to wait even longer for a retransplant but perhaps the most to gain. In addition, such trials will have to be conducted in partnership with industry. Understanding which therapeutic combination is most tolerogenic will require cooperation and support from the pharmaceutical and biotechnology companies, which may be less interested in funding such studies, as true tolerance should ultimately negate the need for maintenance immunosuppression. In that regard, support from governmental agencies and private foundations becomes critical if clinical tolerance strategies are to expand in the future.

Conclusions
Pilot clinical transplantation tolerance protocols are just beginning to be tested and will likely succeed in some patients and fail in others. Lessons learned from failures are as important as those learned from successes. The first and most critical step is devising methods to measure and monitor the tolerant state. Therefore, investment in investigation of potential biomarkers of tolerance is a high priority. From the currently available data, it seems that peripheral deletional strategies will not induce tolerance but can reduce the immunosuppressive burden. Modification of deletion strategies by promoting active regulation may provide a solution. However, as with all novel tolerance strategies, we will be comforted to know that such a new regimen is capable of inducing robust tolerance in larger animals, when appropriate, before subjecting patients to such a protocol, necessitating support of nonhuman primate research. Strategies to induce central tolerance are hopeful but, as they are used currently, have significant limitations that would preclude their more widespread use. Their refinement in stringent rodent models and nonhuman primates is required to make them suitable for wider clinical applicability. Understanding the mechanisms of how innate immune activation, memory T cells, and other cell populations (B cells and monocytes) resist tolerance, while developing novel strategies to overcome such resistance, are critical for induction of reproducible and durable tolerance in humans. Finally, realizing the importance of nonscientific hurdles and developing means to address them are as important as overcoming the scientific challenges to achieve tolerance in the clinic. There are indeed many rivers to cross to achieve clinical transplantation tolerance, but we are continuously building new bridges that will ultimately allow us to reach that elusive goal.

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References


