Donor Antigen and Transplant Tolerance Strategies: It Takes Two to Tango!

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Over 50 years have passed since the seminal contribution by Billingham, Brent, and Medawar in the journal Nature demonstrating tolerance to skin grafts in mice after fetal/neonatal injection of bone marrow–derived donor cells (1). Although an immature immune system likely played a role in facilitating tolerance induction in these initial studies, a wealth of evidence confirms that tolerance can be acquired by a mature immune system as well, at least in experimental animals (2). The precise definition of transplant tolerance, often discussed and rarely agreed upon, can be regarded as the lack of a destructive immune response toward the graft in the absence of ongoing immunosuppressive therapy (2,3). This is manifested clinically by normal graft function in the absence of acute and chronic rejection. However, implicit in this definition is that such a state must coexist with general immune competence, including normal immune responses to pathogens and cancer risks no different than the general population.

Advances in our understanding of the induction and maintenance of tolerance have confirmed at least three major mechanisms to be active: clonal deletion, clonal anergy, and regulation/suppression (4). Most experts in the field agree that any durable tolerogenic therapy will involve manipulation of more than one mechanism, with the goal of profound reduction in clonal T cell expansion accompanied by active immune regulation (5). Multiple receptors, ligands, and signaling intermediates have been identified that are instrumental to these processes and now serve as therapeutic targets for tolerance induction strategies, including costimulatory blockade, T cell receptor targeting, and profound T cell depletion. Extensive animal and human data suggest that the administration of donor antigen concurrent with these immunomodulatory agents may be an important adjunctive therapy for the success of any clinical tolerance strategies.

The true test for the establishment of clinical tolerance in the transplant setting, the complete and successful withdrawal of immunosuppressive medications, has been achieved anecdotally and experimentally in rare renal transplant recipients (2). Although such withdrawal was not planned in most cases, a number of trials are currently underway that include withdrawal of immunosuppression as part of the protocol (2,3,6,7). These trials are not without risk; in recipients of kidney transplants, acute rejection episodes correlate strongly with the development of chronic allograft nephropathy (CAN) (8), and reduced graft function is now recognized as a risk factor for cardiovascular death (9). Clearly, identification of ideal patients before transplantation via immunologic and molecular phenotypic analyses, coupled with aggressive immune monitoring after transplantation, will be of paramount importance for the successful translation of tolerance strategies to the clinic (2,3).

The beneficial effect of blood transfusions on kidney allograft survival has been recognized for several decades (10). A prerequisite for this beneficial effect is that the blood transfusion is not leukocyte-depleted (11). Furthermore, patients who received HLA-DR matched blood transfusions showed significantly better kidney and heart transplant survival compared with patients who received HLA-DR mismatched transfusions, suggesting that MHC class II-positive leukocytes in the donor blood play a pivotal role in the blood transfusion effect (12). Enthusiasm for strategies involving the preoperative administration of donor antigen has dampened, however, in light of recent dramatic pharmacologic-related improvements in acute rejection and short-term graft survival rates. Transplant registry analysis for the period of 1988 to 1996 demonstrated an improvement in long-term graft survival in those recipients without a history of acute rejection (13), suggesting that heightened immunosuppression may be having its intended effect on long-term graft survival through reduction of acute rejection. Although the importance of the blood transfusion effect has diminished significantly since the introduction of cyclosporine, the effect still persists (14). Moreover, more recent registry analysis reveals that long-term graft survival has not improved significantly during the period of 1995 to 2000 (15), suggesting that the maximal benefit of heightened generalized immunosuppression may have been reached. These findings have sparked renewed interest in the development of protocols that provide stable, long-term graft survival independent of chronic immunosuppression.

Several recent experimental and translational studies have begun to elucidate the mechanisms behind the transfusion effect and HLA-DR matching (16–18). Preliminary findings
suggest that suppression of alloimmune responses after blood transfusion may be in part due to the induction of regulatory CD4+ T cells, which appears to be dependent on the recognition of an allopeptide shared between blood/organ donor and recipient in the context of self-HLA class II (18). Presentation in this manner induces hyporesponsiveness to the other alloantigens presented on the same antigen-presenting cell (APC) bearing the shared class II molecule, a phenomenon called linked suppression. These and similar studies should provide a better understanding of the mechanisms behind the beneficial effect, which should also help to prevent one of the most feared complications of this form of therapy, recipient sensitization.

After transplantation, recognition of alloantigen occurs via two pathways (19,20). In the direct pathway, recipient T cells recognize intact MHC molecules on the surface of donor-derived APC, mostly dendritic cells (DC). In the indirect pathway, donor MHC and minor antigens are internalized and processed by recipient APC for presentation to recipient T cells. Several lines of evidence exist to implicate indirect allore cognition in the pathogenesis of CAN (8,21). With this in mind, strategies designed specifically to target this pathway have been attempted in an effort to prevent the chronic attrition of kidney grafts (22,23). The article by Mirenda et al. (24) in this issue of JASN furthers our understanding of the mechanisms involved in the development of specific immunological hyporesponsiveness after the infusion of donor alloantigen. In this case, the authors have used donor-derived (LEW × AUG)F1 DC rendered tolerogenic by dexamethasone (Dex) therapy as the donor alloantigen, based on the premise that MHC class II matching with LEW recipients will serve to induce a tolerogenic response via the indirect pathway. These Dex-treated (LEW × AUG)F1 DC, in contrast to untreated (mature) DC, failed to stimulate a primary T cell alloresponse in vitro in the mixed lymphocyte response (MLR). Furthermore, T cells from these cultures lost the ability to respond to (LEW × AUG)F1 splenocytes but retained the ability to respond to third-party splenocytes. The authors next turned to in vivo experiments to tease out the potential mechanisms of such suppression. LEW recipients were injected intravenously with Dex-treated (LEW × AUG)F1 DC followed by a single dose of CTLA4-Ig the next day. T cells from these animals were specifically unresponsive in vitro to AUG antigen presented indirectly (i.e., LEW APC pulsed with AUG cell lysates) but not to third-party antigen. Moreover, these T cells responded to fully allogeneic AUG and third-party splenocytes but not to the semi-allogeneic (LEW × AUG)F1 splenocytes, implying at least some inhibition of direct pathway anti-AUG allospecificity. Next, a series of depletion and co-culture experiments demonstrated that the hyporesponsiveness to both pathways was dependent on a population of CD25+ cells (i.e., regulatory T cells). Finally, the investigators were able to induce operational tolerance to donor alloantigens in a kidney transplant model, as evidenced by indefinite survival of AUG kidney grafts in recipients injected with Dex-treated (LEW × AUG)F1 but not Dex-treated AUG DC. These findings confirm the role of semi-allogeneic cells in the induction of indirect pathway regulation. The authors did not test the effect of pretransplant injection with Dex-treated LEW (recipient) DC pulsed with AUG cell lysates to determine if this approach is equally effective at inducing indirect pathway regulation. However, it is likely that (LEW × AUG)F1 DC are more efficient than recipient (LEW) DC at presenting AUG antigen to LEW T cells, given the high levels of MHC-derived peptides that occupy MHC class II molecule peptide binding grooves (25), which may translate into more powerful indirect regulatory T cell induction. Interestingly, in recent reports published in JASN, regulatory T cells were found to play an important role in mediating indirect (26) but not direct (27) pathway hyporesponsiveness in renal transplant recipients (28).

In summary, the report by Mirenda et al. (24) represents a clinically applicable approach to achieving indirect pathway tolerance to alloantigens, a critical step in the effort to induce tolerance for the prevention of both acute and chronic allograft rejection. Future studies will need to define the optimal immunomodulation under which such a donor antigen challenge should occur. For instance, the present model requires the injection of CTLA4-Ig 24 h after donor antigen challenge, although the precise reason for such a requirement is not totally clear. It is possible that some attenuation of the recipient immune system is necessary to allow donor cells to persist for sufficient time to activate regulatory cells. Furthermore, the requirement for a short course of cyclosporine to achieve indefinite graft survival in this model suggests that indirect pathway regulation alone may not be sufficient to induce operational tolerance in the clinical setting. Key to the success of this and similar strategies will be the identification of optimal adjunctive immunosuppressive agents that promote rather than interfere with the induction of tolerance, and optimization of “tolerance assays” to be able to identify recipients who can be safely withdrawn from these immunosuppressive agents (2).

References