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-mismatched blood transfusions showed significantly better kidney and heart transplant survival compared with patients who received HLA-DR-matched 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....

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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 recog-
nition 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 alloan-
tigens 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
allorecognition 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 mecha-
nisms 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). Further-
more, T cells from these cultures lost the ability to respond to
(LEW × AUG)F1 splenocytes but retained the ability to re-
spond 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 allospecific-
ity. 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 opera-
tional 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 con-
firm the role of semi-allogeneic cells in the induction of indi-
rect 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 regula-
tory 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 immu-
nomodulation 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 opti-
mization of “tolerance assays” to be able to identify recipients
who can be safely withdrawn from these immunosuppressive
agents (2).

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