Immune Monitoring in Kidney Transplant Recipients Revisited

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The discovery and clinical implementation of several new immunosuppressant agents along with a variety of advances in clinical care have made a significant impact on the outcome of organ transplantation. Acute rejection rates for renal allograft recipients have fallen precipitously over the last decade, and recently reported data demonstrate prolongation of the half-lives of transplanted kidneys to >11 yr (1). Despite these accomplishments, the transplant community remains appropriately focused on devising strategies to further improve the care of their patients. Basic science experiments have brought attention to the primary role of T and B cell immunity as mediators of the graft rejection process (2), and it has thus become desirable to determine whether functional correlates of immune reactivity can provide clinically useful information regarding graft outcome.

One important clinical issue derives from the fact that presently employed pharmaceutical agents are dosed either empirically or on the basis of classic pharmacokinetic algorithms. Use of immunosuppressants in this manner is often accompanied by significant comorbidities related to either excessive immunosuppression (i.e., infection) or to "side" actions of the drug (i.e., hyperlipidemia, osteopenia, and diabetes mellitus). As a result, it is desirable to use the minimum effective dose of each medication. Transplant physicians would ideally prefer to taper or even eliminate the use of some medications to prevent such long-term morbidities. The risk of empiric minimization of immunosuppressive drugs is the development of acute rejection and potentially graft loss in a significant number of patients. Nonetheless, it is notable from several trials that such rejection episodes occur in a minority of the individuals who undergo drug withdrawal posttransplantation (3,4). Many patients can tolerate drug withdrawal, but they cannot be prospectively identified by conventional criteria. If a functional measure of the donor-reactive immune response could predict which patients are likely to tolerate drug withdrawal and which

patients are not, it would be possible to tailor therapy as required for each individual patient.

Acute rejection remains a problem in kidney transplantation, despite the lower incidence that has been achieved through the use of some of the newer immunosuppressants. High-dose corticosteroid or antilymphocyte antibody therapy is often required to reverse acute rejection. Although generally effective, these treatments are associated with significant acute and chronic side effects. A small number of acute rejection episodes are resistant to any therapy and result in graft failure. Ideally, preemptive treatment of an expanding alloimmune response before clinical recognition of organ pathology (i.e., elevation in serum creatinine), if it could be detected, would be desirable. Such early intervention may be more effective than therapy initiated after organ damage has already occurred and could theoretically limit morbidity associated with the treatment itself.

A related issue of significance is that chronic allograft dysfunction or "chronic rejection" remains the major cause of late graft loss, and there is no effective therapy available to treat this condition after it has become established. Studies in animal models and in humans reveal that chronic allograft dysfunction is the end result of a complex pathophysiologic process that involves both T and B cell immune mechanisms along with nonimmune factors such as hypertension, graft ischemia, side effects of medication, and a number of other factors (2). Although there are some clinical correlates associated with increased risk of the development of chronic allograft dysfunction (such as the previous development of an acute rejection episode), there are no reliable methods yet available that are capable of predicting which patients will develop this syndrome. Again, if such a predictor were available, it would provide a clinical tool for assessment of risk and for tailored preemptive therapy in high-risk individuals.

It is now well established that alloreactive T cells can recognize transplant antigens through two distinct pathways (2). Recipient T lymphocytes can recognize donor MHC, peptide complexes directly found on donor cells (direct pathway), and can recognize donor-derived peptides (principally derived from donor HLA molecules) processed and presented by recipient antigen presenting cells (APCs) through the indirect pathway. As donor passenger APCs exit the transplanted organ, they are replaced over time by recipient APCs, resulting in a situation in which indirect allorecognition could theoretically

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dominate the chronic alloimmune repertoire. On the basis of this foundation, it has been hypothesized by many in the transplant immunology community that T cells responding through the indirect pathway may be the principal immune mediators of chronic allograft dysfunction. One problem with testing this hypothesis in humans has been that the frequencies of cells responding to such peptides are often below the detection limit of many available assays.

The article by Najafian *et al.* (5) in this issue demonstrates that transplant physicians are one step closer to having clinically useful tools for immune monitoring of allograft recipients. The study makes use of a cytokine ELISPOT (enzyme linked immunosorbent spot) assay, a derivative of the ELISA that detects cytokine-secreting T cells at single-cell resolution (6). Importantly, the ELISPOT detects peripheral blood T cells that have been sensitized to antigens *in vivo*, thus providing a direct *ex vivo* reflection of the *in vivo* immune response (6). Najafian *et al.* used this approach to evaluate the frequency and cytokine profile of donor-reactive cellular immunity in the peripheral blood of a small cohort of kidney transplant recipients. The investigators showed that T cells primed through the indirect pathway to donor HLA DR-derived peptides are readily detectable in some immunosuppressed recipients of HLA DR–mismatched allografts but not in recipients of HLA DR–matched allografts. More importantly, the authors demonstrated that the indirectly primed, DR-peptide–specific T cells were more prevalent in patients with a history of an acute rejection episode compared with patients who did not experience acute rejection. The correlation between the development of an acute rejection episode and the detection of an expanded population of donor-peptide–reactive T cells is anticipated on the basis of studies in animals but has not been documented in humans. The results are important because they demonstrate feasibility for rapidly detecting and characterizing donor-reactive cellular immunity posttransplantation by using a peripheral blood sample and because the results nicely confirm previous studies by this group and others that indirect reactivity is associated with poor outcome posttransplantation.

The findings from this small, cross-sectional study do not of course establish a cause and effect relationship between acute or chronic rejection and indirect T cell alloreactivity. It would also be premature, on the basis of the data, to state that the detection of peripheral blood T cells sensitized to indirectly presented allopeptides is predictive of a poor outcome. Long-term, prospective studies involving serial analyses of peripheral immune responses specific for directly and indirectly presented alloantigens in large numbers of patients need to be performed to determine the clinical utility of this approach for predicting acute rejection or chronic graft dysfunction.

There are notably a number of other monitoring assays presently being evaluated by multiple investigators in an effort to address similar questions. Proliferation to donor cells in one-way mixed lymphocyte responses and detection of allo-reactive helper or cytotoxic T cells by limiting dilution analysis have been used by clinical and research laboratories for many years. However, the labor-intensiveness and time-consuming nature of these assays, along with inconsistent correlations

with transplant outcomes, have prevented their broad acceptance as reliable immune monitoring tools. More recent studies by Suthanthiran *et al.* (7) have shown that quantitative reverse transcription–PCR analysis of message for T cell–derived inflammatory mediators (*i.e.,* granzyme B, perforin, transforming growth factor–β) from allograft biopsies, peripheral blood cells, or urine cells obtained from transplant recipients can provide supplemental diagnostic information regarding the presence or absence of acute or chronic rejection. Preliminary work by other groups also suggests that intracellular cytokine staining with detection by flow cytometry or delayed type hypersensitivity can be used to evaluate alloantigen-sensitized T cells and that flow cytometry can be effectively used to detect alloantibodies that have developed posttransplantation. Intriguing data by Nickerson *et al.* (8) further suggest that specific urine spectrographic patterns of samples taken from transplant recipients act as strong correlates of rejection. For each of these approaches, however, prospective trials are required to determine whether any of these promising readouts, alone or in combination, will provide the required information with which to make clinical therapeutic decisions.

As transplantation medicine moves into the 21st century, it is possible to envision an era in which serial immunologic monitoring analyses will rationally guide the day-to-day care of transplant recipients. Drug doses could be individually optimized, preemptive therapies could be instituted for incipient acute rejection episodes, and early treatment for prevention of chronic allograft dysfunction could be started in high-risk individuals, all on the basis of the results of simple yet reliable studies of alloreactive immunity performed with blood and/or urine samples. The implications of such an approach, including the cost savings and avoidance of side effects related to use of the minimum amount of immunosuppressive therapy while maximizing benefit to the allograft recipient are enormous. It is hoped that over the next several years cooperative, prospective, multicenter trials will be designed to address these important questions and that the findings from such studies will ultimately result in improved patient care.

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