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T-Cell Immune Monitoring in Organ Transplantation

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Abstract

Chronic allograft injury remains the leading cause of graft loss despite improvements in immunosuppression, clinical risk stratification, and state-of-the-art antibody testing. Emerging results indicate that T-cell immune monitoring by cytokine enzyme-linked immunosorbent spot, as part of a comprehensive risk assessment platform, has the potential to guide decision making and improve outcomes after transplantation.

Keywords

Immune monitoring; T-cells; ELISPOT; IFN- γ

Chronic allograft injury (CAI) resulting in late allograft failure is a major cause of morbidity after solid organ transplantation. Studies from numerous groups indicate the pathogenesis of CAI involves genetic predisposition, nonimmune mechanisms (e.g., calcineurin toxicity, recurrent disease, and hypertension), infection, and cellular and humoral alloimmunity. The increased awareness of CAI as a clinical entity and the enlightened understanding of its intricate pathogenesis have driven the transplant community to identify the tools that can predict its development. If successful, risk assessment assays could be used to individualize therapy, for example, permitting safe calcineurin inhibitor withdrawal in those at lowest risk for immune-mediated injury or directing specific interventions to prevent incipient cell-mediated damage in high-risk transplant recipients.

Currently used risk stratification approaches in transplantation use clinical factors (donor/recipient age, race), human leukocyte antigen typing, and alloantibody screening. Although useful, these approaches are inadequate predictors of late graft failure. Multiple groups are evaluating more comprehensive strategies using genomic and proteomic testing, measurements of specific serum and urine proteins (e.g., chemokines, soluble CD30), and quantifying serum and urine RNA (e.g., perforin, granzyme, and forkhead box P3) as specific biomarkers of graft injury. The reader is referred to the recent reviews for information on these topics (1, 2).

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Based on the knowledge that alloreactive T cells are key mediators of transplant injury, significant effort has been expended in perfecting methodologies that reliably measure cellular alloimmunity and in determining the utility of these approaches as biomarkers for acute rejection, biopsy proven fibrosis, and declining allograft function.

Traditional methods of measuring T-cell alloreactivity include proliferation and cytotoxicity assays, performed either on bulk cultures (mixed lymphocyte responses) or as limiting dilution assays. Although these methods are accepted as useful research tools, their intensive labor requirements and limited reproducibility have prevented them from becoming standardized clinical tests.

The commercially available, Food and Drug Administration-approved, ImmuKnow assay (Cytex Inc., Columbia, MD), which measures mitogen-driven ATP production by CD4 T cells, was developed as a reproducible, yet nonspecific functional measure of cellular immunity. Uncontrolled studies indicate that assay results falling in the “low” range correlate with overimmunosuppression (increased risk for opportunistic infection), whereas high levels may be indicative of insufficient immunosuppression (3). Prospective controlled studies are needed to validate the utility of assay as a biomarker of immune function, and there is currently no evidence that the ImmunoKnow assay can be used to predict chronic graft injury.

Over the past decade, our research group, with collaborators, developed and tested the cytokine enzyme-linked immunosorbent spot (ELISPOT) assay as a cellular assay/biomarker for transplant outcome. The ELISPOT quantifies the frequency of antigen-reactive, cytokine-secreting lymphocytes in the peripheral blood mononuclear cells (PBMCs). We demonstrated that alloantigen-induced interferon (IFN)- γ production as determined by ELISPOT (responder cells are mixed with donor or third-party stimulators for 24 hr) quantifies the frequency of transplant-reactive, primed/memory T cells (4). We hypothesized that donor-reactive T-cell memory negatively affects transplant outcome; stronger antidonor memory responses will correlate with allograft rejection and failure.

To test this, we studied the cohorts of kidney transplant candidates on dialysis, assessing the relationship between the results of pretransplant, donor-reactive ELISPOT assays, and posttransplant outcome (5, 6). By using a cutoff of 25 IFN- γ -producing lymphocytes per 300,000 PBMCs, we found that ELISPOT(+) patients were significantly more likely to experience acute rejection compared with ELISPOT(–) patients. We also showed that the strength of the pretransplant antidonor ELISPOT results correlated inversely with calculated glomerular filtration rate at 6 and 12 months. Our findings were independently validated by other groups, including the research team led by Volk and coworkers (7) in Berlin, using similar study designs. We found that length of time on hemodialysis, an established risk factor for worse outcome, correlated with the strength of the pretransplant, antidonor ELISPOT response, and was independent of race, further supporting the concept that heightened pretransplant cellular immunity is a risk factor for poor posttransplant outcome (8). In a secondary analysis of 130 renal transplant recipients studied pretransplant, we found that induction therapy preferentially benefited those individuals with strong pretransplant, donor-reactive cellular immunity (9). These intriguing preliminary results

support the concept that ELISPOT testing could be used to guide the use of induction therapy in transplantation.

Because ELISPOT testing requires 24 hr to complete, assessing antidonor immunity by this method cannot be used for making decisions regarding deceased donor transplants. Therefore, we developed a screening strategy derived from the concept of panel reactive antibody testing. In the “panel of reactive T cell” or panel of reactive T-cell (PRT) assay, recipient PBMCs are tested in IFN- γ ELISPOTs against a panel of human leukocyte antigen-disparate stimulators (10). Our results indicate that the strength of the PRT is independent of panel reactive antibody (supported by work from the Berlin group) (10). We provided preliminary evidence that patients with weak PRT results correlate with better posttransplant outcomes compared with those with strong PRT results. We further showed that strong PRT results correlate with younger age and African American race, raising the intriguing possibility that alloreactive memory immunity could account for the observed higher incidence of rejection and poorer long-term outcomes in this latter population (11).

We and others also tested the utility of posttransplant antidonor cellular immunity by ELISPOT as a correlate of incipient injury. Our findings include (a) high mitogen-induced IFN- γ /interleukin-5 ratios at the time of allograft dysfunction predicted allograft failure within 6 months, (b) low frequency antidonor immunity correlates with stable kidney function at 6 and 12 months, and (c) high frequencies of antidonor immunity including detection of T cells reactive to indirectly presented allopeptides are correlates of poor transplant outcome (6, 12, 13). A recent study from the Berlin group confirmed and extended these observations by documenting that the strength of direct alloresponse by ELISPOT inversely correlated with graft function, and that indirect pathway reactivity correlated with the development of proteinuria (14).

The IFN- γ ELISPOT has also been used to indirectly monitor regulatory T-cell (Treg) function after kidney transplantation. If Tregs are contributing to stable kidney function, in vitro Treg depletion can uncover otherwise undetectable alloreactive IFN- γ producers (15). By using this approach, Noris et al. (16) showed that Tregs are functional in stable kidney transplant recipients on sirolimus but not cyclosporine.

Cellular immune monitoring by ELISPOT has emerged as one promising noninvasive tool for risk stratification after transplantation. Ongoing prospective validation testing in kidney and heart transplant recipients through the NIH-funded Clinical Trials in Organ Transplantation studies, among others, will determine whether ELISPOT assays in conjunction with other experimental immune monitoring approaches, and with accepted measurements of alloantibodies, will provide reliable, predictive information about incipient allograft injury. Ultimately, it is anticipated that transplant physicians will use comprehensive immune monitoring to individualize therapy for all organ transplant recipients so as to minimize toxicity, prolong graft survival, and improve patient health.

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