Published in final edited form as:
Curr Opin Organ Transplant. 2008 August ; 13(4): 419–424. doi:10.1097/MOT.0b013e3283071463.

T cell Immune Monitoring in Organ Transplantation

Rajani Dinavahi, MD and
Instructor, Medicine/Nephrology, Mount Sinai School of Medicine, Division of Nephrology, Recanati/Miller Transplantation Institute, One Gustave Levy Place, Box 1243, NY, NY 10029

Peter S. Heeger, MD
Professor of Medicine, Mount Sinai School of Medicine, Division of Nephrology, Recanati/Miller Transplantation Institute, Immunology Institute, One Gustave Levy Place, Box 1243, NY, NY 10029

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 ELISPOT, as part of a comprehensive risk assessment platform, has the potential to guide decision-making and improve outcomes following transplantation.

Keywords

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

Chronic allograft injury (CAI) resulting in late allograft failure is a major cause of morbidity following solid organ transplantation. Studies from numerous groups indicate the pathogenesis of CAI involves genetic predisposition, non-immune mechanisms (e.g., calcineurin toxicity, recurrent disease, 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 tools that can predict its development. If successful, risk assessment assays could be used to individualize therapy, e.g., 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 employed risk stratification approaches in transplantation employ clinical factors (donor/recipient age, race), HLA typing and alloantibody screening. While 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, Foxp3) as specific biomarkers of graft injury. The reader is referred to recent reviews for information on these topics (1,2).

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

Address all correspondence to: Peter S. Heeger, MD, Professor of Medicine, Mount Sinai School of Medicine, One Gustave Levy Place, Annenberg Building Box 1243, NY, NY 10029, Tel: 212-241-6324, peter.heeger@mssm.edu.

Conflict of Interest Statement: The authors have no conflicts of interest to report.

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. While 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, FDA approved, ImmunoKnow® assay, 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 over-immunosuppression (increased risk for opportunistic infection) while high levels may be indicative of insufficient immunosuppression (3). Prospective controlled studies are needed to validate the assay’s utility 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 peripheral blood mononuclear cells (PBMCs). We demonstrated that alloantigen-induced IFNγ production as determined by ELISPOT (responder cells are mixed with donor or third party stimulators for 24h) quantifies the frequency of transplant-reactive, primed/memory T cells (4). We hypothesized that donor-reactive T cell memory negatively affects transplant outcome; stronger anti-donor memory responses will correlate with allograft rejection and failure.

To test this, we studied cohorts of kidney transplant candidates on dialysis, assessing the relationship between the results of pretransplant, donor-reactive ELISPOT assays and posttransplant outcome (5,6). Using a cutoff of 25 IFNγ-producing lymphocytes per 300,000 PBMCs, we found that ELISPOT(+) patients were significantly more likely to experience acute rejection compared to ELISPOT(−) patients. We also showed that the strength of the pretransplant anti-donor ELISPOT results correlated inversely with calculated GFR at 6 and 12 months. Our findings were independently validated by other groups, including the research team led by Volk and colleagues in Berlin, using similar study designs (7). We found that length of time on hemodialysis, an established risk factor for worse outcome, correlated with the strength of the pretransplant, anti-donor ELISPOT response, 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 use of induction therapy in transplantation.

Because ELISPOT testing requires 24 h to complete, assessing anti-donor immunity by this method cannot be used for making decisions regarding deceased donor transplants. We therefore developed a screening strategy derived from the concept of panel of reactive antibody (PRA) testing. In the “panel of reactive T cell” or PRT assay, recipient PBMCs are tested in IFNγ ELISPOTs against a panel of HLA-disparate stimulators (10). Our results indicate that the strength of the PRT is independent of PRA (supported by work from the Berlin group). (10). We provided preliminary evidence that patients with weak PRT results correlate with better posttransplant outcomes compared to 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 anti-donor cellular immunity by ELISPOT as a correlate of incipient injury. Our findings include a) high mitogen-induced IFNγ/IL-5 ratios at the time of allograft dysfunction predicted allograft failure within six months, b) low frequency anti-donor immunity correlates with stable kidney function at 6 and 12 mo, and c) high frequencies of anti-donor 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 IFNγ ELISPOT has also been used to indirectly monitor regulatory T cell (Treg) function after kidney transplantation. If Treg are contributing to stable kidney function, in vitro Treg depletion can uncover otherwise undetectable alloreactive IFNγ producers (15). Using this approach Noris et al showed that Treg are functional in stable kidney transplant recipients on sirolimus but not cyclosporine (16).

Cellular immune monitoring by ELISPOT has emerged as one promising noninvasive tool for risk stratification following 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.

Abbreviations

CAI
: Chronic Allograft Injury

ELISPOT
: Enzyme-linked Immunosorbent spot

GFR
: glomerular filtration rate

HLA
: Human Leukocyte Antigen

IFNγ
: Interferon gamma

PBMC
: Peripheral Blood Mononuclear Cell

PRA
: Panel Reactive Antibody

PRT
: Panel of reactive T-cell

Treg

Regulatory T cells

References

1. Anglicheau D, Suthanthiran M. Noninvasive prediction of organ graft rejection and outcome using gene expression patterns. Transplantation 2008;86 (2):192. [PubMed: 18645476]
2. Dinavahi R, Heeger PS. T-cell immune monitoring in organ transplantation. Curr Opin Organ Transplant 2008;13 (4):419. [PubMed: 18685339]
3. Israeli M, Yussim A, Mor E, Sredni B, Klein T. Preceeding the rejection: in search for a comprehensive post-transplant immune monitoring platform. Transpl Immunol 2007;18 (1):7. [PubMed: 17584596]
4. Heeger PS, Greenspan NS, Kuhlenschmidt S, et al. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. J Immunol 1999;163 (4):2267. [PubMed: 10438971]
5. Augustine JJ, Siu DS, Clemente MJ, Schulak JA, Heeger PS, Hricik DE. Pre-transplant IFN-gamma ELISPOTs are associated with post-transplant renal function in African American renal transplant recipients. Am J Transplant 2005;5 (8):1971. [PubMed: 15996247]
6. Hricik DE, Rodriguez V, Riley J, et al. Enzyme linked immunosorbent spot (ELISPOT) assay for interferon-gamma independently predicts renal function in kidney transplant recipients. Am J Transplant 2003;3 (7):878. [PubMed: 12814480]
7. Nickel P, Presber F, Bold G, et al. Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. Transplantation 2004;78 (11):1640. [PubMed: 15591953]
8. Augustine JJ, Poggio ED, Clemente M, et al. Hemodialysis vintage, black ethnicity, and pretransplantation antidonor cellular immunity in kidney transplant recipients. J Am Soc Nephrol 2007;18 (5):1602. [PubMed: 17389735]
9. Augustine JJ, Poggio ED, Heeger PS, Hricik DE. Preferential benefit of antibody induction therapy in kidney recipients with high pretransplant frequencies of donor-reactive interferon-gamma enzyme-linked immunosorbent spots. Transplantation 2008;86 (4):529. [PubMed: 18724221]
10. Poggio ED, Clemente M, Hricik DE, Heeger PS. Panel of reactive T cells as a measurement of primed cellular alloimmunity in kidney transplant candidates. J Am Soc Nephrol 2006;17 (2):564. [PubMed: 16382020]
11. Poggio ED, Augustine JJ, Clemente M, et al. Pretransplant cellular alloimmunity as assessed by a panel of reactive T cells assay correlates with acute renal graft rejection. Transplantation 2007;83 (7):847. [PubMed: 17460554]
12. Tary-Lehmann M, Hricik DE, Justice AC, Potter NS, Heeger PS. Enzyme-linked immunosorbent assay spot detection of interferon-gamma and interleukin 5-producing cells as a predictive marker for renal allograft failure. Transplantation 1998;66 (2):219. [PubMed: 9701268]
13. Poggio ED, Clemente M, Riley J, et al. Alloreactivity in renal transplant recipients with and without chronic allograft nephropathy. J Am Soc Nephrol 2004;15 (7):1952. [PubMed: 15213286]
14. Bestard O, Nickel P, Cruzado JM, et al. Circulating alloreactive T cells correlate with graft function in longstanding renal transplant recipients. J Am Soc Nephrol 2008;19 (7):1419. [PubMed: 18417724]
15. Salama AD, Najafian N, Clarkson MR, Harmon WE, Sayegh MH. Regulatory CD25+ T cells in human kidney transplant recipients. J Am Soc Nephrol 2003;14 (6):1643. [PubMed: 12761267]
16. Noris M, Casiraghi F, Todeschini M, et al. Regulatory T cells and T cell depletion: role of immunosuppressive drugs. J Am Soc Nephrol 2007;18 (3):1007. [PubMed: 17287424]