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Preferential Benefit of Antibody Induction Therapy in Kidney Recipients with High Pre-transplant Frequencies of Donor-reactive IFN- γ ELISPOTs

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Abstract

Background—The decision to employ induction antibody therapy in kidney transplantation is often based on perceived patient risk, as no objective biomarker has been shown to predict the effectiveness of such therapy. Because pretransplant T cell alloreactivity has been shown to increase the risk of poor posttransplant outcome, and because induction therapy is directed at alloreactive T cells, we hypothesized that antibody induction would preferentially benefit patients with high pretransplant anti-donor T cell immunity.

Methods—In a retrospective analysis of 130 patients who had enrolled in an immune monitoring study, we correlated acute rejection rates, renal allograft function, and use of antibody induction therapy with donor-reactive IFN- γ ELISPOT frequencies assessed pre- and post- kidney transplantation.

Results—Of the 32 ELISPOT (+) patients, 8 received induction therapy and had no rejection. Of the remaining 24 ELISPOT (+) patients with no induction therapy, acute rejection occurred in 11 (46%), ($p=0.02$). Twelve month glomerular filtration rate (GFR) was significantly higher in the 8 patients who received induction therapy ($p=0.0001$). Posttransplant conversion to a negative ELISPOT assay occurred in 86% of patients who received induction therapy vs. 35% of patients who did not ($p=0.02$). In the ELISPOT (–) cohort, acute rejection rates (~15%) and GFRs were similar in the 98 patients regardless of induction therapy.

Conclusions—Our results suggest that antibody induction therapy preferentially benefits kidney transplant candidates with strong pretransplant donor-reactive cellular immunity. If confirmed prospectively, pretransplant ELISPOT assessments could be used to guide decision making regarding induction therapy.

Keywords

Kidney transplantation; acute rejection; immune monitoring; T cell; alloimmunity

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Introduction

The use of induction therapy with polyclonal or monoclonal T cell antibody therapy has become routine in kidney transplantation. Antithymocyte globulins (ATG) are polyclonal T cell antibodies which induce T cell apoptosis in peripheral lymphoid tissues and also attenuate receptor signaling on circulating T cells (1). ATG induction has been shown to reduce rejection rates and improve short term outcomes in kidney transplant recipients (2,3). Anti-CD25 antibodies including basiliximab and daclizumab have also been shown to lower rejection rates in kidney recipients (4–6), and their usage has increased in kidney transplantation.

Induction therapy is not benign. ATG therapy has been linked to higher rates of cytomegalovirus (CMV) infection (2) and malignancy (7). Although anti-CD25 antibody therapy is associated with an excellent safety profile (8), some studies suggest that these agents are less effective than ATG in “high risk” patients (9). In addition to patient morbidity, the economic impact of induction antibody therapy is significant. Such therapy may not be cost effective in “low risk” patients (10).

Indeed, the decision to employ induction antibody therapy in kidney transplantation is often based on perceived risk of immune injury. Because pretransplant T cell immunity has been shown to increase the risk of poor posttransplant outcome, and because induction therapy is largely directed at alloreactive T cells, we hypothesized that antibody induction would preferentially benefit patients with high pretransplant anti-donor T cell immunity. We previously demonstrated that pretransplant cellular immunity as measured by the IFN- γ ELISPOT assay, a marker for effector/memory T cell alloreactivity, correlates with posttransplant acute rejection and renal functional impairment (11–13). In this report, we reanalyzed the data stratifying our previous cohort based on the use of induction therapy, in an effort to determine whether T cell antibody induction therapy might impact on risk of acute or chronic rejection in patients with preexisting alloimmunity. We also analyzed patients who had relatively low T cell alloreactivity as determined by a negative pretransplant ELISPOT assay, and compared outcomes with and without antibody induction.

We found a significant decrease in rejection rates in ELISPOT (+) patients who received induction, a clinical outcome associated with an attenuation of IFN- γ production posttransplant. Conversely, ELISPOT (–) patients had comparable outcomes with and without induction therapy. The findings suggest that pretransplant measurements of anti-donor immunity may be useful for individualizing the use of induction therapy.

Methods

Patients

One hundred thirty patients who received kidney transplants between January 2000 and December 2003 were enrolled in an immune monitoring study under the approved guidelines of the Institutional Review Board for Human Studies at The University Hospitals of Cleveland. Patients provided informed consent before transplantation and were selected for ELISPOT immune monitoring on the basis of the availability of donor stimulator cells

that consisted of donor splenocytes in deceased-donor (DD) transplants or of donor peripheral blood mononuclear cells in living-donor (LD) transplants. All patients had pre-transplant ELISPOT testing and received a solitary kidney (n=119) or a combined kidney-pancreas (KP) (n=11) transplant. An ELISPOT assay was performed using recipient cells that were collected before immunosuppression and before kidney implantation. T cell-depleted donor cells were used as stimulators in the assay. Choice and adjustment of immunosuppression were not influenced by the result of the ELISPOT assay, which was not prospectively available to the transplant clinicians.

Demographic variables were collected on patients at the time of enrollment and included donor age, donor source (living vs. deceased), HLA mismatch, panel reactive antibody (PRA), KP transplant, recipient ethnicity (black vs. non-black), induction therapy, maintenance immunosuppression (sirolimus vs. other), delayed graft function (DGF) and acute rejection. Black recipients more often received tacrolimus/sirolimus therapy in the absence of induction therapy, as per the protocol at the time of enrollment. Renal biopsies were performed on patients with suspected rejection, and biopsy-proven acute rejection was defined by a Banff IA or higher score (Banff '97). DGF was defined as the need for dialysis during the first week after transplantation. There was one graft loss during the first year in a patient who had positive testing by ELISPOT and had biopsy-proven AR within 1 month of transplantation. This patient was included in the analysis. Estimated glomerular filtration rate (GFR) was calculated using the simplified MDRD equation (14).

HLA Typing and Alloantibody Determinations

Antigens that were encoded by HLA class I loci (A and B) were identified by the basic microlymphocytotoxicity assay using local antisera. Class II alleles were determined by sequence-specific priming and PCR. Pretransplantation panel reactive antibody (PRA) was determined by flow cytometry using Flow PRA beads (One Lambda, Canoga Park, CA) according to the manufacturer's recommendations. PRA > 60% was used to classify patients as highly sensitized.

ELISPOT assays

IFN- γ ELISPOT assays were performed as described previously in detail (11). The resulting spots were counted with a Series 1 Immunospot computer-assisted ELISPOT image analyzer (Cellular Technology, Cleveland, OH). Results were depicted as the mean number of IFN- γ spots per 300,000 recipient peripheral blood lymphocytes based on duplicate or triplicate measurements in a given assay. Based on previous analyses, a positive test was predefined as a >25 spots per 300,000 peripheral blood lymphocytes. Control wells that assessed cytokine production by stimulators alone were included in all assays (<20 spots per 300,000), and detected spots in these control wells were subtracted from the total number of spots in wells in which responders and stimulators were mixed.

Statistical Analyses

Values are shown as mean $\pm$ SD, median (range), or percentage. Baseline demographic data between patient groups was analyzed using *t* test for continuous variables and Pearson χ^2 test for dichotomous variables. A student's *t* test was used to compare rejection rates

between ELISPOT groups and between groups who received induction vs. no induction, to compare eGFR between groups, and to compare rates of negative post-transplant ELISPOT testing. We performed a backward stepwise regression analysis when analyzing correlates with eGFR in the ELISPOT (+) and (–) cohorts. Two-sided $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using SPSS version 11.5 (SPSS, Chicago, IL).

Results

We recorded data on 130 consecutive kidney and KP recipients who enrolled in the ELISPOT immune monitoring study between January 2000 and December 2003. All patients were treated with calcineurin inhibitors, with 119 (92%) on tacrolimus and the remaining 11 (8%) on cyclosporine. Adjunctive sirolimus therapy was used in 71 (55%) patients, MMF in 52 (40%), and a few patients were treated with enteric coated mycophenolic sodium (n=3), FTY720 (n=3) or azathioprine (n=1). All patients were treated initially with corticosteroids, and 31 patients participating in clinical trials were tapered off of steroid therapy within the first year posttransplant. Induction therapy was used in 32 (25%), with basiliximab in 24 and antithymocyte globulin (ATG) in 8. Patients who received induction therapy included the KP recipients (n=11), patients who participated in multicenter protocols (n=9), patients with a positive B cell flow crossmatch (n=4) or patients who received induction due to surgeon preference (n=8).

Thirty-two (25%) patients were ELISPOT (+) based on the pretransplant assay. Comparative data between ELISPOT (+) and ELISPOT (–) patients are shown in Table 1. As previously described (12), acute rejection rates were higher in ELISPOT (+) patients, with rates of 34% (11/32) in ELISPOT (+) vs. 13% (13/98) in ELISPOT (–) patients ($p=0.007$), (Table 1). There were 8 ELISPOT (+) patients who received induction therapy with basiliximab (n=5) or ATG (n=3). Demographic characteristics of ELISPOT (+) patients with induction therapy vs. none are shown in Table 2a. Of the eight positive patients who received induction therapy, none experienced a rejection episode during 12 months posttransplant. Conversely, rejection occurred in 46% of the remaining 24 ELISPOT (+) patients with no induction therapy ($p=0.02$). Of these ELISPOT (+) patients with no induction therapy, rejection rates were similar between recipients of living donor and deceased donor kidneys (50% vs. 45%, $p=ns$).

In the ELISPOT (–) cohort, the incidence of acute rejection was relatively low overall, with no significant difference in rejection rates between patients who received induction (n=24) vs. no induction (n=74), (Table 2b). Seventy-four patients who were ELISPOT (–) received no induction therapy. This cohort included 42 (57%) recipients of deceased donor kidneys, and 37 (50%) black recipients. Acute rejection rates were just 12% in the first year and were similar in black and non-black recipients. Additionally, 17 (23%) of these patients withdrew successfully from steroid therapy within six months of transplant, with no rejection episodes after steroid withdrawal. Conversely, of the five ELISPOT (+) patients with no induction therapy and steroid withdrawal, two developed acute rejection at four and six months after steroid elimination, respectively.

Renal function was measured by calculating the MDRD GFR at one year post-transplant. One ELISPOT (+) patient had early rejection and graft loss and was not included in this analysis. GFR was 65 ± 22 ml/min/1.73m² in ELISPOT (+) patients who received induction, vs. 38 ± 14 ml/min/1.73m² in ELISPOT (+) patients without induction therapy ($p=0.001$), (Figure 1a). This difference increased further after assigning a GFR of zero to the patient with a failed transplant ($p<0.001$). Calculated GFR remained superior in the induction group after controlling for donor age, donor source, recipient ethnicity, KP transplant, maintenance immunosuppression, DGF, and rejection (slope=14.5, $p=0.02$). Conversely, in the ELISPOT (–) cohort, GFR was similar between patients who received and did not receive induction (54 ± 22 and 53 ± 17 ml/min/1.73m² respectively, $p=ns$), (Figure 1b). In this cohort, younger donor age, KP transplant, and absence of acute rejection all correlated with higher twelve-month GFR. However, there was no association between the use of induction therapy and renal function (data not shown).

If induction therapy functions in part by inhibiting the high frequency of IFN- γ producing memory/effector T cells, then posttransplant anti donor immunity would be diminished in treated patients. Of the 32 ELISPOT (+) patients, 24 had follow-up post-transplant ELISPOT testing at varying time points within the first 6 months post-transplant, ranging from one to five assays per patient. Posttransplant ELISPOT data were available in seven of the eight patients who received induction therapy. Of these seven patients, six had testing all within the negative range (median of 2 assays per patient), and the seventh patient had one positive test in the first month followed by two negative assays. Thus 6/7 (86%) converted to a persistently negative ELISPOT test early post-transplant (Figure 2a).

Seventeen ELISPOT (+) patients who received no induction therapy had posttransplant ELISPOT monitoring (median of 3 assays per patient). Only 6/17 (35%) had persistently negative ELISPOT testing posttransplant ($p=0.02$ vs. ELISPOT (+) patients with induction therapy), (Figure 2b). Of the six who did convert to ELISPOT (–) posttransplant, two had received ATG therapy for acute rejection prior to posttransplant testing. Ten patients with posttransplant monitoring had no induction therapy and no acute rejection episodes. Even in the absence of acute rejection, the majority of these patients (6/10) remained ELISPOT (+) after transplantation.

Discussion

In this retrospective analysis we sought to determine whether preexisting cellular alloimmunity could function as a surrogate marker for responsiveness to antibody induction therapy. While there are a number of limitations of the study (see below), our data are consistent with the interpretation that pretransplant ELISPOT positivity can provide this information. None of the patients with potent anti-donor T cell alloimmunity pretransplant who received induction therapy went on to develop acute rejection, while the rejection rate was 46% in the ELISPOT (+) patients who did not receive induction. Moreover, induction therapy was an independent correlate of superior renal function at twelve months in ELISPOT (+) patients.

In contrast, patients lacking donor reactive cellular immunity at baseline did not appear to benefit from induction therapy in prevention of rejection or improvement in allograft function. The twelve month rejection rate was low at 12% in 74 ELISPOT (–) patients who received no antibody induction, similar to the rate in ELISPOT (–) patients who received induction therapy. The absence of memory/effector T cell response to donor cells pretransplant may identify a cohort for whom less aggressive therapy is conducive with good outcomes. Lack of induction therapy in such patients may be associated with less patient morbidity and lower healthcare cost.

Our data, based on the pretransplant IFN- γ ELISPOT, also show that this assay may provide a surrogate marker for responsiveness to induction therapy after transplantation. The utility of the assay as a useful marker is bolstered by the finding that treatment led to a reduction in posttransplant anti donor immunity when compared to untreated patients. Importantly, our data do not imply that the efficacy of induction therapy is due to its effects on pretransplant T cell memory, a hypothesis that will require further study.

Induction antibody therapy may not actually eliminate effector/memory cells from circulation. Pearl et al. demonstrated that after depleting therapy with ATG, cells with an effector memory phenotype (CD4+CD45RA–) predominated despite the significant T cell depletion overall (15). However, ATG has been shown to modulate surface receptors on T cells through antibody binding (1,16) and to prevent “bystander activation” of naïve T cells (15). Such effects could lead to a diminished cytokine response as measured by the ELISPOT assay. The combination of ATG induction and calcineurin inhibitor maintenance therapy may be an effective means of preventing rejection despite the presence of preexisting T cell alloimmunity (17).

In this experience, induction therapy with the anti-CD25 antibody basiliximab also led to a reduction in anti donor immunity after transplantation. Anti-CD25 antibody therapy diminished IFN- γ expression in circulation relative to placebo in a small series of heart transplant recipients (18). A recent report also suggested a benefit of IL-2 blockade in inhibiting memory T cell activation (19). In that study, IL-2 levels peaked at 18–24 hours after antigen-specific T cell engagement in vitro, leading to cellular activation and an increase in IFN- γ . The addition of an anti-CD25 antibody efficiently blocked IFN- γ production by CD8 cells.

Conclusions drawn from this study are limited by its retrospective nature, lack of randomization, and heterogeneous patient population. Patients were treated with variable maintenance immunosuppressive therapy and had other differences which may have influenced outcomes. The decision to give induction therapy to some patients may have been determined by perceived patient risk. Nonetheless, our results support the need for future prospective trials aimed at directing the use of induction therapy based on biomarkers including pretransplant IFN- γ ELISPOT testing. An ongoing NIH sponsored multicenter observational trial using ELISPOT and other immune markers may help validate these findings.

In conclusion, the use of T cell antibody induction therapy with either polyclonal antibody or antibody to CD25 correlated with decreased acute rejection and improved renal allograft function only in patients with heightened pretransplant donor reactive cellular immunity. Sensitive immune monitoring tests such as the donor specific IFN- γ ELISPOT assay may permit individualized use of induction antibody therapy in patients who will most benefit from the treatment, coincidently limiting associated morbidity and expense.

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Figure 1.

a: Estimated GFR at twelve months postransplant in ELISPOT (+) recipients who had no induction therapy (left, n=24)) versus induction therapy (right, n=8).

b: Estimated GFR at twelve months postransplant in ELISPOT (-) recipients who had no induction therapy (left, n=74)) versus induction therapy (right, n=24).

Figure 2.

a: Total IFN- γ ELISPOTs in seven patients measured pretransplantation (left) and posttransplantation after receiving induction therapy (median 2 posttransplant assays per patient).

b: Total IFN- γ ELISPOTs in seventeen patients measured pretransplantation (left) and posttransplantation in the absence of induction therapy (median 3 posttransplant assays per patient).

Table 1

Demographic and outcomes data in ELISPOT (+) vs. ELISPOT (–) patients

Variable	ELISPOT (+) (n=32)	ELISPOT (–) (n=98)	p Value
Age	49 ± 12	46 ± 13	ns
Female (%)	38	40	ns
Black (%)	59	43	ns (p=0.1)
Dialysis months (median)	46 (0–125)	24 (0–276)	0.03
Deceased donor (%)	69	59	ns
Donor age	40 ± 15	41 ± 13	ns
Kidney pancreas (%)	3	11	ns
PRA > 60 (%)	3	15	0.07
HLA mismatch	3.8 ± 1.4	3.6 ± 1.8	ns
Induction therapy (%)	25	24	ns
Sirolimus therapy (%)	53	55	ns
Prednisone withdrawal (%)	22	24	ns
Delayed graft function (%)	19	14	ns
Acute rejection (%)	34	13	0.007

Table 2**a: ELISPOT (+) Recipients Stratified by Induction Therapy vs. No Induction**

Variable	Induction (n=8)	No Induction (n=24)	p Value
Age	49 ± 16	49 ± 11	ns
Deceased donor (%)	25	83	0.001
Donor age	37±17	41±15	ns
Black recipient (%)	38	67	ns (0.15)
Kidney pancreas (%)	13	0	ns
HLA mismatch	3.6±1.5	3.9±1.3	ns
PRA > 60 (%)	0	4	ns
Sirolimus (%)	12.5	67	0.007
Prednisone withdrawal (%)	25	21	ns
Delayed graft function (%)	0	25	ns
Acute rejection %	0	46	0.02

b: ELISPOT (–) Recipients Stratified by Induction Therapy vs. No Induction

Variable	Induction (n=24)	No Induction (n=74)	p Value
Age	45 ± 11	47 ± 13	ns
Deceased donor (%)	67	57	ns
Donor age	34 ± 11	43 ± 13	0.002
Black recipient (%)	21	50	0.01
Kidney pancreas (%)	42	0	<0.001
HLA mismatch	4.1 ± 2.1	3.4 ± 1.7	ns
PRA > 60 (%)	21	14	ns
Sirolimus (%)	54	55	ns
Prednisone withdrawal (%)	29	23	ns
Delayed graft function (%)	8	16	ns
Acute rejection (%)	17	12	ns