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Effects of Cellular Sensitization and Donor Age on Acute Rejection and Graft Function After Deceased-Donor Kidney Transplantation

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Abstract

Background—Allografts from older donors may be more immunogenic than those from younger donors. Pretransplantation cellular sensitization may interact with advanced donor age to increase the risk of immune injury after deceased-donor kidney transplantation.

Methods—The outcomes of 118 consecutive deceased-donor kidney transplant recipients with available pretransplantation donor-stimulated enzyme-linked immunosorbent spot (ELISPOT) assays for interferon gamma were analyzed retrospectively to determine the impact of cellular sensitization and other clinical variables, including donor age, on the incidence of acute rejection (AR) in the first year after deceased-donor transplantation and on estimated glomerular filtration rate 12 months after transplantation.

Results—The incidence of AR was higher in patients with positive pretransplantation ELISPOT assays versus those with negative assays (36% vs. 14%, $P=0.009$). Logistic regression indicated that the combination of donor age 50 years or older and a positive pretransplantation ELISPOT assay was more strongly associated with AR (odds ratio, 12.1; confidence interval, 1.1–133) than either variable alone. Estimated glomerular filtration 12 months after transplantation was highest in ELISPOT-negative patients receiving kidneys from donors younger than 50 years and lowest in ELISPOT-positive recipients with donors 50 years or older.

Conclusion—The combination of advanced donor age and pretransplantation cellular sensitization increases the risk of AR and poor graft function after deceased-donor kidney transplantation beyond the risk associated with each factor alone.

Keywords

ELISPOT assay for interferon gamma; Acute rejection; Expanded criteria donors; Delayed graft function; Kidney transplantation

Donor age is one of the most important factors that negatively impact the outcomes of kidney allografts from deceased donors (1, 2). However, the mechanisms accounting for this observation remain poorly understood. Senescent organs have limited ability to recover after ischemia-reperfusion (IR) injury and, as a consequence, are more likely to develop irreversible fibrosis (3, 4). There is also emerging evidence that organs from elderly donors are more immunogenic than those from younger donors (5–8).

Primed or memory antidonor T cells are commonly detected in human transplantation candidates, likely reflecting heterologous immunity that results, in part, from cross reactivity with environmental antigens (9, 10). Studies in animal models have shown that memory T cells rapidly interact with the donor endothelium of a vascularized allograft, initiating a cascade of inflammatory events that facilitate organ injury and prevent tolerance (11, 12). We and others have translated these findings to humans by demonstrating that the pretransplantation frequency of donor reactive interferon gamma (IFN γ)–producing T cells in the peripheral circulation, measured by an enzyme-linked immunosorbent spot (ELISPOT) assay, is a direct correlate of posttransplantation acute rejection (AR) and reduced glomerular filtration rate (GFR) after kidney transplantation (13–16). Pretransplantation sensitization, traditionally defined by the detection of antibodies against human leukocyte antigens (HLAs), is associated with inferior graft survival based primarily on its association with AR, itself a risk factor for premature graft failure (17, 18). In contrast to conventional crossmatches that serve as measures of pretransplantation humoral sensitization (usually against HLAs), the donor reactive IFN γ ELISPOT assay serves as a measure of pretransplantation cellular sensitization, measuring cellular responses to antigens that may or may not be HLAs.

We hypothesized that the enhanced immunogenicity of organs from older donors would facilitate immune recognition by primed donor reactive T cells. Together with the reduced ability of senescent organs to heal and, thereby, to develop fibrosis, donor reactive T cells are likely to synergize negatively with advanced donor age, increasing the risk of AR and declining kidney function after transplantation. To test this hypothesis, we analyzed outcomes in a cohort of human transplant recipients by correlating the frequencies of donor reactive IFN γ -producing peripheral blood lymphocytes with donor age, AR, and GFR. The results indicate a strong interaction between donor age and primed donor reactive T cells and suggest that patients with high pretransplantation frequencies of donor reactive IFN γ -producing peripheral blood lymphocytes are at high risk for poor outcomes if they receive grafts from older donors.

RESULTS

Clinical characteristics of the patients are displayed in Table 1. Thirty-nine of the 118 patients (33%) had a positive donor-stimulated IFN γ ELISPOT before transplantation.

Twenty-five patients (21%) experienced delayed graft function (DGF). Nine of 39 IFN γ ELISPOT-positive patients (23%) experienced DGF. Twenty-five patients (21%) had AR in the first 12 months after transplantation. All rejection episodes were clinically overt (persistent increase in serum creatinine >30% above baseline) and biopsy-proven in 22 of the 25 cases. AR occurred in 14 of 39 (36%) IFN γ ELISPOT-positive patients versus 11 of 79 (14%) IFN γ ELISPOT-negative patients ($P=0.009$). AR occurred in 10 of 25 patients (40%) with DGF versus 15 of 93 patients (16%) without DGF ($P=0.014$). The incidence of AR in patients who were IFN γ ELISPOT positive and experienced DGF was 77% versus 17% in patients who were both IFN γ ELISPOT negative and who did not experience DGF ($P<0.0001$).

In the analyses of factors associated with AR, donor age was considered both as a continuous variable or dichotomously based on thresholds of either 50 or 60 years. There were statistically insignificant trends for higher rates of AR when donors 50 years or older were compared with younger donors (27% vs. 10%, $P=0.31$) or when donors older than 60 years were compared with younger donors (31% vs. 20%, $P=0.39$). Results of the logistic regression analysis using AR as the dependent variable are displayed in Table 2. As shown in part A of Table 2, DGF and a positive pretransplantation donor-stimulated IFN γ ELISPOT test were the only significant independent correlates of AR, after adjusting for recipient age, gender, and time on dialysis, and for HLA mismatches, prior transplantation, cold ischemia time, peak panel reactive antibody (PRA), type of induction therapy, and donor age. African American ethnicity was of borderline significance. When the same logistic regression was repeated adding the interactive variables, DGF plus IFN γ ELISPOT positive and recipient age of 50 years or older plus IFN γ ELISPOT positive (part B of Table 2), the latter variables replaced both ELISPOT positivity and DGF alone and became significant correlates of AR.

We next used linear regression to analyze factors that correlated with estimated GFR 12 months after transplantation. The results, shown in part A of Table 3, indicate that DGF, donor age, and AR in the first 12 months after transplantation were significant independent correlates of GFR measured at 1 year. The number of HLA mismatches was of borderline significance. Interestingly, when AR was eliminated as an independent variable in the regression analysis (part B of Table 3), a positive pretransplantation IFN γ ELISPOT emerged as a significant correlate of GFR at 12 months, together with DGF and donor age.

The influence of an interaction between pretransplantation cellular presensitization and donor age was further examined by categorizing patients into one of four groups based on donor age less than, greater than, or equal to 50 years, and either a negative or positive pretransplantation IFN γ ELISPOT (i.e., $D<50ES-$ [n=55], $D<50ES+$ [n=31], $D \geq 50ES-$ [n=25], or $D \geq 50ES+$ [n=7]). Figure 1 depicts the incidence of AR in these subgroups of patients. By analysis of variance, the difference in the incidence of AR is highly significant ($P=0.002$), with the highest rate of AR occurring in the $D \geq 50ES+$ subgroup. Importantly, in univariate analyses, there are no significant differences in the incidence of either DGF or AR when the $D<50$ groups are compared with the $D \geq 50ES-$ group. Among the patients in the $D \geq 50ES+$ category, four of seven patients were African American, mean (SD) donor age was 58.3 (12.3) years, the mean (SD) number of HLA mismatches was 4.4 (1.2), and pretransplantation PRAs for class 1 and 2 antigens were zero with the exception of a single

patient with a peak class 1 PRA of 22%. Clinical characteristics of donor-recipient pairs for all donors 50 years or older, and categorized on the basis of IFN γ ELISPOT positivity or negativity, are shown in Table 4.

Figure 2 displays the 1-year estimated GFRs for each group. By analysis of variance, the differences among the four patient groups is significantly different ($P=0.003$). Mean GFR was highest in the $D<50ES-$ group and lowest in the $D \geq 50ES+$ group (mean [SD], 57 [21] vs. 30 [11] mL/min; $P=0.029$). There were no significant differences in GFRs between the $D<50$ groups and the $D \geq 50ES-$ group.

DISCUSSION

Results of this study confirm previous observations suggesting that pretransplantation cellular sensitization, assessed by the donor-stimulated IFN γ ELISPOT assay, is associated with an increased risk of AR in the first year after kidney transplantation (13, 15, 16). More importantly, our results suggest for the first time that the combination of cellular presensitization and advanced donor age may be particularly detrimental, markedly increasing the risk of AR and poor renal function, especially in the presence of DGF.

Based on our data, we can speculate on the mechanisms that might account for these interactions. It is generally accepted that nonspecific injuries induce a proinflammatory milieu that can activate both innate and adaptive immune responses. The aging organ's compromised capacity to repair injury may accelerate these immune responses. As a consequence of the inability to repair injury, older parenchymal cells express more major histocompatibility complex molecules (8), a phenomenon that is aggravated further by IR injury. Older grafts contain more apoptotic cells that create milieu for local inflammation. Normally, phagocytosis and clearance of apoptotic cells by dendritic cells inhibit the production of proinflammatory cytokines (19). The reduced phagocytic function of older dendritic cells thus can lead to higher concentrations of these cytokines that augment innate and adaptive immune responses. In vitro studies suggest that older dendritic cells exhibit enhanced antigen-presenting capacity (20, 21). Furthermore, IR injury may enhance the immunogenicity of dendritic cells via toll-like receptor 4 and nuclear factor- κ B activation (22). In this milieu of heightened immune readiness, primed donor-reactive T cells can quickly be engaged by both direct and indirect antigen recognition pathways, thus converting the nonspecific injury of IR to immune-mediated injury that is ultimately expressed as AR.

Expanded criteria donors (ECDs) are now used routinely at most transplantation centers, based on the belief that the patient survival benefit associated with kidney transplantation using such donors outweighs the increased risk of graft failure (23). Among the clinical variables included in the current definition of ECDs, age is by far the variable contributing most potently to the relative risk of graft failure. Using donor age older than 50 years as surrogate for ECD status, our study results suggest that the use of an ECD kidney in a recipient with a positive pretransplantation donor-stimulated IFN γ ELISPOT may be unwise. However, our results indicate that the use of ECD kidneys in recipients who lack cellular presensitization may yield outcomes that are not appreciably different from those of

recipients receiving standard-criteria donor kidneys. In addition, the results suggest that transplantation of a patient with a positive pretransplantation donor-stimulated IFN γ ELISPOT may be associated with a prohibitively high risk of AR when the likelihood of DGF is great, especially when the donor is old.

In a previous study, we suggested that a positive pretransplantation donor-stimulated IFN γ ELISPOT assay was associated with a reduced GFR after 12 months, independent of clinically overt AR (14). The results of the current study differ slightly because a positive ELISPOT correlated with a reduced 1-year GFR only when AR was eliminated as an independent variable. Keeping in mind that we included only clinically overt episodes of AR in the analysis, we interpret this finding to suggest that, beyond the detrimental effects of overt rejection, a positive pretransplantation IFN γ ELISPOT may be associated with subclinical immune injury that adversely affects graft function over time. Use of interactive variables in the multivariate regression analyses indicate that the combinations of older age and a positive pretransplantation IFN γ ELISPOT and the combination of DGF and a positive pretransplantation IFN γ ELISPOT are more potent correlates of AR than any of the individual component variables. It is also noteworthy that this study confirms that the negative associations of a positive pretransplantation IFN γ ELISPOT on AR and GFR are independent of conventional humoral sensitization, suggesting that the two techniques may be complementary in pretransplantation immune risk assessment.

Although our results provide further validity for use of the IFN γ ELISPOT assay as a pretransplantation immune risk assessment tool, measurement of a donor-specific IFN γ ELISPOT assay to make decisions about accepting a deceased-donor kidney may not be practical given the time required for the performance of the assay. Routine measurement of pretransplantation IFN γ ELISPOTs against a panel of donors (24) may be more practical, but further studies are needed to determine whether such panels provide the same information as the donor-specific assay.

Limitations of this study include the retrospective nature of the analysis and the relatively small number of patients studied. Indeed, only seven patients in the study fit the criteria for donor age older than 50 years and a positive pretransplantation IFN γ ELISPOT. However, analysis of this small group of patients confirms that the risk of these two variables is independent of conventional humoral sensitization. In addition, immunosuppression was heterogeneous, making it difficult to dissect out any independent effects of immunosuppressive drugs on the endpoints studied. Finally, our population of patients includes a large number of African Americans, and results of the study may not apply to populations with less ethnic heterogeneity. Clearly, further prospective studies with larger numbers of patients receiving relatively homogeneous immunosuppression are needed to validate our observations. Also needed are further studies to define the role of presensitized T lymphocytes in linking innate and adaptive mechanisms of immune injury early after the transplantation of an allograft. However, we believe that our preliminary results suggest that cellular presensitization measured by a positive donor-stimulated IFN γ ELISPOT test may be particularly disadvantageous in patients receiving kidneys from elderly donors, especially in the presence of DGF.

MATERIALS AND METHODS

Patients

We retrospectively analyzed the outcomes of 118 patients who received deceased-donor kidney transplants between 2001 and 2010 at either the University Hospitals Case Medical Center or the Cleveland Clinic, who were alive at 1 year to allow for the measurement of estimated GFR at 12 months and for whom pretransplantation donor-stimulated IFN γ ELISPOTs were available based on an institutional review board–approved protocol. Selection of patients for the study was strictly limited to the availability of cells from either the spleen or lymph nodes of the deceased donor. We also excluded patients with a positive flow cytometry–based crossmatch at the time of transplantation. Maintenance immunosuppression was heterogeneous during the 9-year study period. Fifty-seven of the 118 patients (48%) received induction antibody therapy (29 patients with basiliximab and 28 with rabbit antithymocyte globulin). The major endpoints were the cumulative incidence of AR and estimated GFR at 1 year after transplantation. DGF was defined as the need for dialysis during the first week after transplantation. GFR was estimated using the abbreviated Modification of Diet in Renal Disease formula (25). PRAs were measured using flow cytometry.

ELISPOTS

Donor-stimulated IFN γ ELISPOTs were measured using previously described techniques (26, 27). Donor cells were derived from a T-cell depleted pool cells obtained from the spleen or lymph nodes. In some cases, an expanded clone of B cells derived from the donor pool was used to provide stimulators, using the technique described by Zand et al. (28). A positive ELISPOT was defined as 25 or more interferon-producing cells per 300,000 cells plated in the ELISPOT well.

Statistical Analyses

One-way analysis of variance was used to compare the incidence of AR and the mean GFRs among subgroups of patients. Univariate analyses for dichotomous independent variables were performed with a chi-square analysis using Fisher exact test. An independent *t* test was used for assessing the effects of single variables on estimated GFR. Stepwise backward logistic regression was used to analyze the independent correlations of multiple clinical variables with AR. Linear regression was used to analyze the independent correlations of clinical variables with estimated GFR at 12 months. Donor age was analyzed both as a continuous variable and, in some analyses, as a dichotomous variable. For the latter, cutoff values of 50 and 60 years were based on age cutoffs used for conventional definitions of expanded criteria donors. $P < 0.05$ was considered to represent statistical significance for the univariate analyses. For logistic and linear regression analyses, independent variables yielding $P < 0.10$ were considered to be of borderline significance.

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FIGURE 1.

Incidence of acute rejection in patients categorized according to donor age and the presence or absence of a positive pretransplantation IFN γ assay. *D*<50, donor younger than 50 years; *D* $\geq$ 50, donor 50 years or older; ES-, IFN γ ELISPOT negative; ES+, IFN γ ELISPOT positive. By analysis of variance, *P*=0.002; **P*=0.036 comparing the incidence of acute rejection in the *D*>50ES+ group to the incidence in all of the other groups combined.

FIGURE 2.

Estimated glomerular filtration (mean [SD]) 1 year after transplantation in patients categorized according to donor age and the presence or absence of a positive pretransplantation IFN γ ELISPOT assay. *D*<50, donor younger than 50 years; *D* ≥50, donor 50 years or older; ES-, IFN γ ELISPOT negative; ES+, IFN γ ELISPOT positive. **P*=0.007 versus patients from the other groups.

TABLE 1

Clinical characteristics of the patients (n=118)

Variable	Mean (SD) or Percent	Range
Recipient age, y	47.5 (11.8)	22–75
Recipient gender, male	63%	
Recipient ethnicity, African American	52%	
Time on dialysis, mo	48.6 (30.7)	0–136
HLA mismatches (A, B, DR)	3.95 (1.9)	0–6
Prior transplantation	8%	
Induction antibody therapy	48%	
Cold ischemia time, hr	18.6 (6.5)	1–36
Cold ischemia time, >24 hr	20%	
Positive pretransplantation IFN γ ELISPOT	33%	
Peak panel reactive antibodies		0–99
PRA, >80%	5%	
PRA, >60%	13%	
Delayed graft function	21%	
Glomerular filtration rate at 1 y, mL/min	50.8 (21.4)	13.7–105.3
Incidence of acute rejection within 1 y	21%	
Biopsy-proven rejection	19%	
Donor age, y	40.8 (15.4)	1–66
Donor age, 50	35%	
Donor age, 60	11%	

HLA, human leukocyte antigen; PRA, panel reactive antibody.

TABLE 2

Significant correlates of acute rejection using logistic regression

Variable	Odds Ratio	95% Confidence Limits	P
A. Without Interactive Variables			
Positive pretransplantation IFN γ ELISPOT	3.601	(1.37–9.46)	0.009
Delayed graft function	3.47	(1.22–9.84)	0.019
African American ethnicity	1.91	(0.95–4.08)	0.069
B. Using the Interactive Variables Delayed Graft Function Plus Positive IFNγELISPOT and Recipient Age of 50 y Plus Positive IFNγELISPOT			
Delayed graft function plus positive IFN γ ELISPOT	14.4	(2.6–79.0)	0.002
Donor age 50 y plus positive IFN γ ELISPOT	12.1	(1.1–133)	0.041

IFN γ ELISPOT, interferon-gamma enzyme-linked immunosorbent spot.

TABLE 3

Significant correlates of glomerular filtration rate at 1 year using linear regression

Variable	Standardized Beta Coefficient	<i>P</i>
A. Including Acute Rejection as an Independent Variable		
Donor age	−0.383	<0.001
Acute rejection	−0.300	<0.001
Delayed graft function	−0.204	0.014
HLA mismatches	0.137	0.079
B. Excluding Acute Rejection as an Independent Variable		
Donor age	−0.485	<0.001
Delayed graft function	−0.238	0.005
Positive pretransplantation IFN γ ELISPOT	−0.180	0.028

HLA, human leukocyte antigen; IFN γ ELISPOT, interferon-gamma enzyme-linked immunosorbent spot.

TABLE 4

Clinical characteristics of donor-recipient pairs in which the donor was older than 50 years, based on the pretransplantation IFN γ ELISPOT results

Variable	IFN γ ELISPOT+ (n=7)	IFN γ ELISPOT- (n=25)	P
Donor age, mean (SD), y	58 (12.3)	57 (4.6)	NS
Number of HLA mismatches	4.4 (1.2)	4.2 (1.6)	NS
African American recipients, %	57	56	NS
Peak PRA class 1, mean (SD), %	11 (21)	16 (34)	NS
Peak PRA class 2, mean (SD), %	0	8.7 (2.5)	NS
AR in the first 12 mo, %	71	21	0.025
eGFR at 12 mo, mean (SD), mL/min	30.3 (10.3)	41.5 (21)	0.022

AR, acute rejection; eGFR, estimated glomerular filtration rate; HLA, human leukocyte antigen; IFN γ ELISPOT, interferon-gamma enzyme-linked immunosorbent spot; NS, not significant; PRA, panel reactive antibody.