Hemodialysis Vintage, Black Ethnicity, and Pretransplantation Antidonor Cellular Immunity in Kidney Transplant Recipients

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Prolonged exposure to dialysis before transplantation and black ethnicity are known risk factors for acute rejection and graft loss in kidney transplant recipients. Because the strength of the primed antidonor T cell repertoire before transplantation also is associated with rejection and graft dysfunction, this study sought to determine whether hemodialysis (HD) vintage and/or black ethnicity affected donor-directed T cell immunity. An enzyme-linked immunosorbent spot (ELISPOT) assay was used to measure the frequency of peripheral T cells that expressed IFN-γ in response to donor stimulator cells before transplantation in 100 kidney recipients. Acute rejection occurred in 38% of ELISPOT (+) patients *versus* 14% of ELISPOT (−) patients ($P = 0.008$). The median (HD) vintage was 46 mo (0 to 125 mo) in ELISPOT (+) patients *versus* 24 mo (0 to 276 mo) in ELISPOT (−) patients ($P = 0.009$). Black recipients had a greater median HD vintage (55 *versus* 14 mo in nonblack recipients; $P < 0.001$). Black recipients with less HD exposure had a low incidence of an ELISPOT (+) test, similar to nonblack recipients. Among variables examined, only HD vintage remained a significant positive correlate with an ELISPOT (+) result (odds ratio per year of HD 1.3; $P = 0.003$). These data suggest that the risk for developing cross-reactive antidonor T cell immunity increases with longer HD vintage, providing an explanation for the previously observed relationship between increased dialysis exposure and worse posttransplantation outcome. Longer HD vintage may also explain the increased T cell alloreactivity that previously was observed in black kidney recipients.

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Longer dialysis vintage (1–3) and black ethnicity (4) are clinical variables that are associated with inferior long-term outcomes after kidney transplantation. T cell immunity, known to play a prominent role in allograft rejection, may be linked to both of these factors. Prolonged exposure to dialysis may lead to increased exposure to environmental antigens and effector T cell formation. Increased cellular alloreactivity also has been observed in black kidney transplant recipients. Kerman *et al.* (5) reported that, compared with white patients who were awaiting transplantation, black patients exhibited higher T lymphocyte helper:suppressor ratios and panel mixed lymphocyte reactions. In addition, Poggio *et al.* (6) recently found a trend for higher frequencies of IFN-γ–producing T cells in black dialysis patients who were exposed to a panel of HLA antigens.

It is unclear whether black recipients have greater inherent T cell immunity or whether this is acquired after exposures to environmental antigens. Longer waiting times on dialysis have been noted in black transplant candidates, with less timely access to transplantation (7). Greater dialysis exposure has been linked to a greater risk for rejection relative to preemptive transplantation (2), although an underlying explanation for this risk has not been apparent.

We hypothesized that prolonged hemodialysis (HD) vintage may influence the strength of the primed cellular alloimmune repertoire and that this risk may be independent of black ethnicity. To assess T cell alloimmunity, we used a highly sensitive enzyme-linked immunosorbent spot (ELISPOT) assay that quantifies the number of IFN-γ–producing effector/memory T cells in human peripheral blood after exposure to donor cells (8). We previously demonstrated that pre- and posttransplantation cellular immunity as measured by the ELISPOT assay correlates with acute rejection (AR) and renal functional impairment in all patients and specifically in a cohort of black kidney transplant recipients (9–11). In the experience reported herein, we sought to analyze the impact of HD vintage and black ethnicity on pretransplantation donor-directed cellular immunity.

Materials and Methods

Patient Selection

Patients who received kidney transplants between January 2000 and December 2003 were enrolled under the approved guidelines of the Institutional Review Board for Human Studies at The University Hos-

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pitals 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 pretransplantation ELISPOT testing and received a kidney transplant either preemptively ($n = 8$) or after treatment with HD ($n = 92$). Recipients who were enrolled in the immune monitoring study and received multiple organ transplants ($n = 12$) or who were on peritoneal dialysis before transplantation ($n = 8$) were excluded from analysis. This cohort included a subset of black patients who were analyzed in a previous report (11).

Maintenance immunosuppression consisted of calcineurin inhibitor therapy in all patients (91 with tacrolimus, nine with cyclosporine). Sirolimus was used adjunctively in 62 patients, whereas mycophenolate mofetil therapy was given in the remaining 38. At the time of enrollment, our protocol for black patients consisted of tacrolimus/sirolimus combination, which was used in 93% of black patients. Alternatively, 79% of nonblack patients received tacrolimus/mycophenolate mofetil. All patients were initiated on corticosteroids; 25 withdrew from steroids within 6 mo after transplantation. Induction therapy was given in select cases, primarily in patients with equivocally positive cross-matches as determined by flow cytometry. Antithymocyte globulin or basiliximab was used as an induction agent. 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 was 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. HD vintage was recorded in months and represented total cumulative months on HD in patients who received nonprimary kidney transplants ($n = 7$). Renal biopsies were performed on patients with suspected AR, and biopsy-proven AR was defined by a Banff IA or higher score (Banff '97). Delayed graft function (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 mo of transplantation. This patient was included in the analysis.

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. We defined patients as highly sensitized when their final pretransplantation PRA was >60%.

ELISPOT Assays

IFN-γ ELISPOT assays were performed as described previously in detail (9). 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 ± 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 Mann-Whitney U test was used to compare HD vintage between groups and to compare the total number of IFN-γ spots between groups, whereas χ^2 testing was used to compare ELISPOT status between groups. Spearman correlations were used to determine variables that were associated with HD vintage. Multivariable logistic regression analysis was used to determine factors that were associated with AR and factors that were associated with an ELISPOT (+) test. Variables with $P \leq 0.10$ in univariable analyses were included in multivariable models. In addition, the variable "black ethnicity" was forced into the regression analysis for AR. Two-sided $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using SPSS version 11.5 (SPSS, Chicago, IL).

Results

Baseline demographic and transplant data are shown in Table 1. Patients were divided fairly evenly between black and nonblack patients, with 53 black recipients in the cohort of 100 patients. The majority of transplants were performed using DD, and 81% of patients received no induction therapy. Sixteen percent were highly sensitized, and 7% were recipients of a previous kidney transplant.

We first looked at factors that were associated with biopsy-proven AR diagnosed in the first 12 mo after transplantation. The overall incidence of AR was 21%. Confirming known risk factors, univariable analysis revealed associations between AR and both HLA mismatch (odds ratio [OR] per HLA mismatch 1.4; $P = 0.04$) and DGF (OR 3.8; $P = 0.012$). In addition, there was a trend for more AR in DD transplant recipients (OR 2.5; $P = 0.09$). There was no correlation with age, gender, previous transplant, high PRA, induction therapy, or type of maintenance immunosuppression. Black recipients had a 25% incidence of AR compared with 17% in nonblack recipients, a nonsignificant trend but consistent with previous reports (4,12). In addition, patients with AR had a median HD vintage of 47 (8 to 120) *versus* 33 mo (0 to 276 mo) in patients without AR ($P = 0.06$).

Table 1. Baseline characteristics ($n = 100$)[a]

Characteristic	Value
Age (yr)	48 ± 13
Male (%)	63
Black ethnicity (%)	53
DD transplant (%)	60
Cold time (h)	13 ± 11
Previous transplant (%)	7
HLA mismatch (mean ± SD)	3.8 ± 1.6
PRA > 60 (%)	16
Induction therapy (%)	19

[a]DD, deceased donor; PRA, panel reactive antibody.

As in our previous work, patients with positive primed antidonor cellular immunity before transplantation had a higher incidence of posttransplantation AR. The 12-mo incidence of AR was 38% in ELISPOT (+) patients *versus* 14% in ELISPOT (−) patients ($P = 0.008$; Figure 1). We used a multivariable logistic model for AR controlling for HLA, DGF, donor source (LD *versus* DD), black ethnicity, and HD vintage (Table 2). In this model, ELISPOT (+) status remained an independent correlate of AR (OR 4.6; $P = 0.009$). Addition of use of induction therapy and pretransplantation PRA resulted in a persistent association of ELISPOT (+) status with AR (OR 4.1; $P = 0.02$). In these models, the trend that was seen in univariate analyses for HD vintage in association with AR dropped out. This suggested a potential interaction between ELISPOT status and exposure to HD.

We therefore next examined the relationship between IFN-γ ELISPOT results and HD vintage. Median HD vintage for the entire cohort was 36 mo (0 to 276 mo). HD vintage was significantly greater in ELISPOT (+) patients with a median of 46 (0 to 125) *versus* 24 mo (0 to 276 mo) in ELISPOT (−) patients ($P = 0.009$). Eight patients received a preemptive transplant, and of these, one (13%) was ELISPOT (+). In patients who were on HD <36 mo ($n = 48$), 15% were ELISPOT (+). Conversely, in patients who were on HD ≥36 mo ($n = 52$), 42% were ELISPOT (+) ($P = 0.002$; Figure 2).

We next examined the relationship among pretransplantation antidonor T cell immunity, HD vintage, and ethnicity (black *versus* nonblack). Median HD time for nonblack patients was 14 mo (0 to 276 mo), with one outlier in this group. Alternatively, median time for black patients was 55 mo (6 to 136 mo) ($P < 0.001$). There were no preemptive transplants in the black cohort, and even among black recipients of LD kidneys, the median HD vintage was 20 mo (6 to 84 mo).

Total median number of ELISPOTs and the percentage of ELISPOT (+) patients relative to the median HD vintage of 36 mo were examined for nonblack and black recipients (Figure 3). In the nonblack cohort, patients who were on HD ≥36 mo had a 47% incidence of ELISPOT (+) testing, *versus* 17% in those who were on HD <36 mo ($P = 0.03$). Similarly, in the black cohort, those who were on HD ≥36 mo had a 40% incidence of ELISPOT (+) testing *versus* 11% in those who were on HD <36 mo ($P = 0.03$). Therefore, HD vintage, rather than black ethnicity, was the primary risk factor for heightened cellular alloimmunity measured at the time of transplantation. Rates of AR trended in a similar manner when examined comparing ethnic groups and dividing by the median dialysis exposure. AR occurred in four (15%) of 27 of non-black recipients with a vintage <36 mo *versus* four (24%) of 17 with a vintage ≥36 mo (NS). Black recipients with an HD vintage <36 mo had AR occur in one (6%) of 18 patients, *versus* 12 (34%) of 35 patients who were on HD ≥36 mo ($P = 0.02$).

Finally, we examined the relationship between ELISPOT status and other clinical variables and confirmed that post-ELISPOT immunosuppressive therapy was similar between groups (Table 3). Among variables examined, only HD exposure had a positive correlation with ELISPOT result. There was a trend for more DD recipients in the ELISPOT (+) group, which disappeared after controlling for HD vintage (data not shown). There was no correlation between HLA matching and ELISPOT result. Interest-

Table 2. Logistic regression analysis for 12-mo incidence of AR

Variable	OR	95% CI	*P*
HLA mismatch	1.48	0.96 to 2.29	0.08
DGF	3.25	0.95 to 11.11	0.06
DD transplant	1.30	0.32 to 5.25	NS
Black ethnicity	1.00	0.29 to 3.44	NS
HD vintage (per yr)	1.00	0.81 to 1.23	NS
ELISPOT (+)	4.60	1.46 to 14.47	0.009

[a]AR, acute rejection; CI, confidence interval; DGF, delayed graft function; ELISPOT, enzyme-linked immunosorbent spot; HD, hemodialysis; OR, odds ratio.

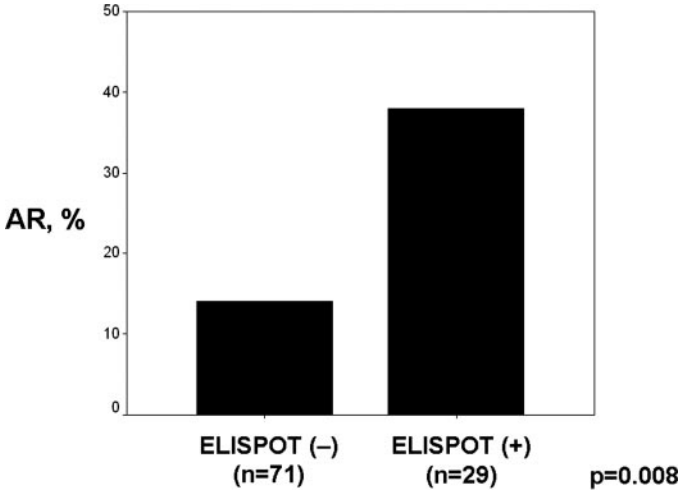

Figure 1. Twelve-month incidence of acute rejection (AR) in enzyme-linked immunosorbent spot–negative [ELISPOT (−)] patients ($n = 71$) *versus* ELISPOT (+) patients ($n = 29$).

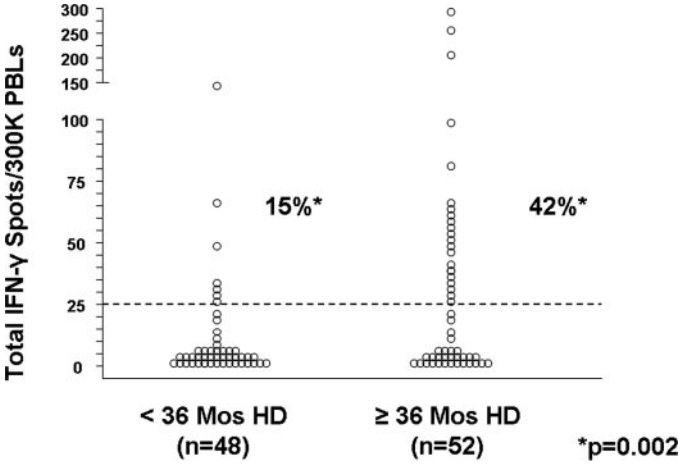

Figure 2. Total number of IFN-γ ELISPOT in patients who were on hemodialysis (HD) <36 mo (left) and ≥36 mo (right). The dashed line represents the cutoff for an ELISPOT (+) test (≥25 spots). Percentages represent the percent ELISPOT (+) from each group.

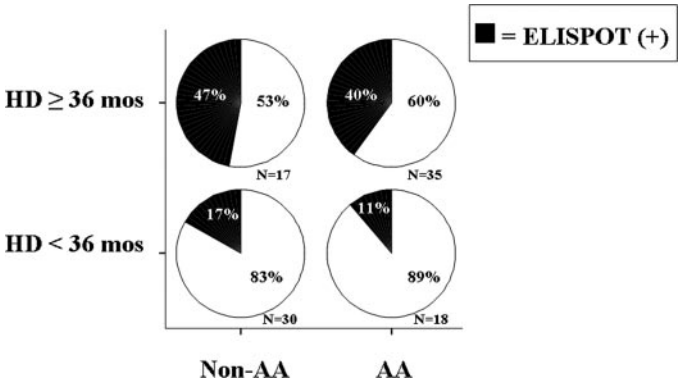

Figure 3. The percentage of patients with an ELISPOT (+) test divided by nonblack or black ethnicity (*x* axis) and by HD vintage <36 or ≥36 mo (*y* axis).

ingly, we noted a *negative* correlation between antibody sensitization and cellular alloimmunity and found that only one of 16 highly sensitized patients (PRA >60%) was also ELISPOT (+). The association between an ELISPOT (+) result and HD vintage remained significant after controlling for antibody sensitivity (OR per additional year on HD 1.3; $P = 0.003$).

Discussion

Our data suggest that longer HD vintage augments the pretransplantation frequency of activated alloreactive T cells and provide an explanation for the association between dialysis vintage and renal allograft survival. Greater dialysis exposure, known to have a negative impact on patient survival after kidney transplantation (13,14), also correlates with AR and death-censored graft survival (1–3). It has been argued that dialysis may increase the risk for AR by correcting immune dysfunction related to the predialysis uremic state (2). However, dialysis also may be associated with greater exposure to infectious and environmental antigens over time, and such immunologic stimuli may lead to the generation of activated or memory T cells that are capable of cross-reacting with alloantigens after transplantation (15,16).

Because previous reports have suggested greater T cell alloresponses in black kidney transplant recipients, we sought to determine the impact of black ethnicity on IFN-γ expression relative to HD vintage. When we stratified black and nonblack patients, we found a significant increase in HD vintage among black patients but low levels of IFN-γ expression in black patients who spent <3 yr on HD. These data suggest that dialysis-related environmental stimuli, rather than inherent variability of the T cell response, may contribute to immunologic differences between ethnic groups.

One recent analysis of black European kidney transplant recipients found no increased rate of rejection compared with a white cohort (17). The black European patients who were analyzed seemed to have greater resources than many black transplant candidates (18), and dialysis vintage was not different between ethnic groups in this study (E. Thervet, Hospital Necker, Paris, France; personal communication, April 10, 2006). In the United States, it is likely that multiple variables influence inferior outcomes in black transplant recipients, including problems with posttransplantation access to care and prescription coverage (19). However, increased HD vintage seems to convey a direct immunologic risk before transplantation and may further disadvantage black recipients.

The ELISPOT test of IFN-γ expression reflects donor-reactive effector/memory T cell activity and likely represents cross-reactivity after previous antigenic stimulation (20). A recent analysis using a panel of stimulator cells demonstrated increased IFN-γ frequencies against an increasing variety of HLA proteins with increased dialysis vintage (15), thereby demonstrating cross-reactivity against multiple foreign antigens. Such a screening test using a so-called "panel-reactive memory T cell" analysis may provide a determination of overall T cell immunization. Alternatively, the donor-reactive ELISPOT test performed in this study may be analogous to the final antibody cross-match by assessing primed T cell reactivity specific to donor cells. These tests may enhance our pretransplantation assessment of immunologic risk, because donor reactive IFN-γ expression and its association with AR were independent of both PRA and HLA matching.

One limitation of this analysis is that we did not track potential influences of T cell stimulation in these patients before transplantation. Potential sources of immunostimulation on HD include the dialysis membrane, synthetic vascular access, exposure to blood

Table 3. ELISPOT result and clinical variables[a]

Variable	ELISPOT (−) ($n = 71$)	ELISPOT (+) ($n = 29$)	*P*
Recipient age (yr)	47 ± 14	50 ± 12	NS
Male (%)	61	69	NS
Black ethnicity (%)	52	55	NS
DD transplant (%)	56	69	NS (0.25)
Previous transplant (%)	6	10	NS
HLA mismatch	3.8 ± 1.7	3.7 ± 1.3	NS
Highly sensitized (%)	21	3	0.03
HD vintage (mo)	35 ± 31	53 ± 32	0.009
Induction therapy (%)	18	21	NS
ATG induction (%)	8	10	NS
MMF therapy (%)	37	41	NS

[a]ATG, antithymocyte globulin; MMF, mycophenolate mofetil.

products, and recurrent infections (21). Prospective measurements of IFN-γ frequencies using a panel-reactive memory T cell assay in an HD population would be ideal to track the influence of such factors over time.

Conclusion

These data demonstrate for the first time that a measure of cellular alloreactivity correlates positively with both longer HD vintage and immunologic injury after kidney transplantation. This experience offers a novel explanation for the inferior immunologic outcomes that previously were observed in patients with longer dialysis vintage. Further efforts to shorten waiting time on dialysis before transplantation, particularly in black recipients, may lead to improved outcomes and perhaps less disparate outcomes between ethnic groups.

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Disclosures

None.

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