Pretransplantation Cellular Alloreactivity Is Predictive of Acute Graft Rejection and 1-Year Graft Function in Kidney Transplant Recipients


ABSTRACT

Objective. To study cellular alloimmunity in kidney allograft recipients using an interferon-γ enzyme-linked immunosorbent spot assay (ELISPOT).

Material and Methods. Donor splenocyte peripheral blood mononuclear cells were obtained during kidney recovery in 53 kidney recipients including 11 with positive panel-reactive antibodies pretransplantation. For ELISPOT data analysis, the spot number, size, and intensity were calculated, reflecting the volume of cytokine secretion at the single-cell level. Results were recalculated as the ratio of the values observed for donor-stimulated to unstimulated recipient cells corrected for residual donor activity.

Results. Significantly greater pretransplantation donor-stimulated activity was observed in recipients who experienced an acute rejection episode (ARE) within 1 year (P < .05). Mean change in spot number, size, and intensity in patients without or with AREs was 0.99 vs 3.33, 1.60 vs 6.05, and 1.40 vs 6.31, respectively. The assessed parameters were prognostic of high risk of ARE: 1.5-fold increase in spot number (ARE incidence, 52% vs 9%), 2.5-fold increase in spot size (ARE incidence, 53% vs 13%), and 2.7-fold increase in spot intensity (ARE incidence, 52% vs 9%). The 3 parameters correlated with 1-year serum creatinine concentration (P < .05). In 14 recipients, AREs could have been predicted in 11 using pretransplantation ELISPOT results, and in only 2 on the basis of panel-reactive antibodies.

Conclusion. The ELISPOT-determined capacity of donor-induced reactivity observed in recipient cells obtained just before transplantation is predictive of risk of graft rejection and 1-year allograft function.

Acute rejection episodes (AREs) are a persistent complication after kidney transplantation despite progress in immunosuppression therapy and can significantly influence short- and long-term allograft function. To date, no diagnostic method has been described to reliably predict the immunologic status of transplant recipients. New predictors of posttransplantation alloreactivity are needed to individualize immunosuppression regimens. The commonly available methods focus on humoral alloimmunity (eg, panel-reactive antibodies [PRAs]) and ignore the potential contribution of cellular responses to AREs. The objective of the present study was to examine cellular alloimmunity in kidney allograft recipients using an estimate of interferon (INF)–γ production using an enzyme-linked immunosorbent spot assay (ELISPOT) after recipient lymphocyte stimulation with donor splenocytes.1,2

Materials and Methods

The study included 53 patients who had undergone kidney transplantation between 2004 and 2008. Thirty-two were male and 21...
were female patients, with mean (range) age of 45 (15–72) years. Forty-nine patients received a first graft, and 4 received a second graft. All grafts but 1 were from deceased donors (mean [SD] age, 50 [12] years). There were 3.5 (1.0) HLA mismatches. Cold ischemia time was 22.9 (5.7) hours.

Immunosuppression therapy included cyclosporine (CsA), mycophenolate mofetil (MMF), and prednisone in 19 patients; CsA, azathioprine, and prednisone in 8; tacrolimus (TAC), MMF, and prednisone in 22; TAC, sirolimus, and prednisone in 1; CsA and prednisone in 1, TAC, sirolimus, and prednisone in 1; and CsA, sirolimus, and prednisone in 1.

In 11 recipients, the most recent positive pretransplantation PRA values ranged from 4% to 40% (16% [11%]). Two patients received induction with antithymocyte globulin. Recipients were followed up for 1 year posttransplantation. Fourteen recipients (26%) experienced a biopsy-proved acute ARE in the first 12 months (mean, 1.9 months). Mean serum creatinine concentration was 1.5 mg/dL. One graft was lost during follow-up.

Peripheral blood mononuclear cells (PBMCs) in kidney allograft recipients were obtained just before the transplantation procedure, before immunosuppression therapy was initiated, and donor splenocytes were obtained during recovery. Cryopreserved cells were stored in liquid nitrogen. Before ELISPOT analysis, the cells were thawed, splenocytes were treated with mitomycin, and their viability was assessed using acridine orange and ethidium bromide staining.

Recipient PBMCs were analyzed for frequency of donor-specific cells using a direct INF-γ ELISPOT procedure (Human INF-γ Development Module and ELISPOT Blue Color Module; R&D Systems, Inc, Minneapolis, Minnesota; MultiScreen HTS IP plates; Millipore Corp, Billerica, Massachusetts). Computer-assisted digital image analysis of the wells was performed using a self-developed system (SpotView; Remspec Corp, Charlton, Massachusetts). The number of spots was analyzed, as well as their size and intensity, which reflect the kinetics and intensity of INF-γ secretion at the single-cell level. Experimental results were correlated with clinical data for allograft donors and recipients.

Statistical analysis was performed using commercially available software (Statistica; StatSoft Corp, Tulsa, Oklahoma).

RESULTS
Significantly higher pretransplantation donor-stimulated cellular activity was observed in recipients who experienced at least 1 biopsy-proved ARE within 12 months posttransplantation. The ratios of mean number of donor-stimulated to unstimulated cell spots (3.33 vs 0.99; \( P < .05 \)), donor-stimulated to unstimulated spot size (6.05 vs 1.6; \( P < .05 \)), and intensity (6.31 vs 1.4; \( P < .05 \)) were also significantly greater in recipients who experienced AREs (Fig. 1).

The assessed parameters, characterizing pretransplantation cellular alloreactivity, could have been used to predict greater than 50% risk of an ARE during the first year posttransplantation: 1.5-fold increase in number of spots (ARE incidence, 52% vs 9%), 2.5-fold increase in spot size (ARE incidence, 53% vs 13%), and 2.7-fold increase in intensity (ARE incidence, 52% vs 9%) (Fig. 2). The 3 parameters were also significantly correlated with serum creatinine concentration at 1 year (\( P < .05 \)) (data not shown).

Acute rejection episodes occurred within 1 year posttransplantation in 2 PRA-positive recipients and 9 PRA-negative recipients. In 14 patients, AREs could have been predicted using the pretransplantation ELISPOT findings, 1 with both ELISPOT and PRA and 1 solely with PRA.

DISCUSSION
Currently, pretransplantation assessment of risk of an ARE posttransplantation is based on donor and recipient HLA matching, presence of serum alloantibodies (PRAs), and the final cross-match. These parameters are risk factors for posttransplantation outcome. Acute rejection episodes may influence short- and long-term allograft function and survival. Persistent cellular and humoral alloimmunity may contribute to development of chronic allograft injury. Because the rejection process is initiated by T-cell allorecognition, pretransplantation assessment of alloreactive T cells seems to be a useful adjunctive clinical tool. The ELISPOT is an impor-
tient technique for detection and quantification of antigen-specific immunologic responses at the single-cell level. Recipient PBMCs may be stimulated by stimulatory cells from the donor or from third parties or by purified protein derivative. In the present study, ELISPOT was used to analyze secretion of IFN-γ by renal transplant recipient lymphocytes after stimulation with donor splenocytes.

Pretransplantation frequency of donor-specific IFN-γ-producing lymphocytes is correlated with the risk of posttransplantation rejection episodes.1 We observed that pretransplantation cellular alloreactivity not only correlates with 1-year kidney allograft function but that the descriptors are highly predictive of a greater than 50% risk of an ARE during the first year posttransplantation. We also observed that the predictive significance of humoral evaluation of pretransplantation alloresponse is not as efficient as the T-cell–mediated reaction.

Findings of our study and previous reports indicate that pretransplantation monitoring of donor-specific T-cell-mediated reactivity may be used for treatment decision support, especially insofar as strength of immunosuppression (eg, induction therapy). It should be possible to introduce the results of this study into clinical practice to individualize immunosuppression therapy to obtain full protection of the transplanted organ with the highest possible reduction in drug dosage and, thus, drug-related adverse effects.

In conclusion, our data demonstrate that ELISPOT-determined intensity of donor-induced reactivity to recipient cells obtained just before transplantation is predictive of risk of graft rejection and 1-year allograft function.

REFERENCES


Fig. 2. Comparison of occurrence of acute rejection episodes (AREs) in patients with low or high pretransplantation cellular alloreactivity, defined by the ratios of spot numbers, mean spot size, and intensity of donor-stimulated to unstimulated cells observed with ELISPOT.