Anti-Idiotypic Antibodies Specific for HLA in Heart and Kidney Allograft Recipients

Abstract
Chronic rejection is the major threat to both heart and renal allograft survival. We have explored the possibility that some patients with anti-donor HLA antibodies (Ab1) develop specific anti-idiotypic antibodies (Ab2) which suppress the production of Ab1, and subsequently, the progression of chronic rejection. Analysis of Ab2 in sera obtained from Ab1 producers showed that 22% of heart and 18% of kidney recipients produced Ab2. The 4- and 5-year actuarial graft survivals in Ab2 producers were 100% and 83%, respectively, compared to 57% in patients who formed Ab1 but not Ab2 (p < 0.004). Patients carrying the DR2 alleles, DRB1*1501, *1502 or *1601 were at a lower risk of producing anti-donor HLA antibodies.

Chronic rejection, causing the persistent attrition of functioning allografts, represents the major threat to long-term survival of organ transplants [1-5]. Although the pathophysiologic mechanisms leading to late graft failures is ill understood, it has long been suspected that antibodies reacting with allograft antigens are involved [5].

Supporting this view is the consistent finding of immunoglobulin and complement deposition in affected vessel walls of the graft [5-8] and the presence of anti-donor HLA antibodies in recipient sera [9, 10]. The production of anti-HLA antibodies implies the activation of T helper cells which recognize peptides derived from the processing and presentation of allogeneic major histocompatibility complex (MHC) molecules by host antigen-presenting cells (APCs) [11, 12]. Such a process of indirect 'allo-recognition' may in turn depend on the ability of host MHC class II molecules to bind allopeptides [11-15]. A corollary of this postulate is that an Ir gene effect may be evidenced in transplantation by the association of responder/nonresponder status with certain MHC class II genotypes. Evidence for positive or negative associations between the outcome of the graft and recipient MHC phenotype has already been described [16-22], yet it has been difficult to confirm mostly because of ambiguities in serologic typing of human HLA class II anti-
gens. Such Ir gene effects may be obscured, however, by the development in the host of immunoregulatory mechanisms suppressing rejection, such as anti-idiotypic anti-HLA antibodies, for example. In an attempt to explore these possibilities, we have analyzed the HLA-DR genotype of heart and kidney allograft recipients, and evaluated their sera for anti-HLA (Ab1) and anti-anti-HLA (Ab2) antibodies. The data presented in this paper substantiate the existence of an Ir gene effect, as well as the regulatory role of Ab2 which may suppress the progression of chronic organ allograft rejection.

Materials and Methods

Patient Population

Selection of patients for these studies was limited to recipients who had been monitored for anti-HLA antibodies at least four times during the first year following transplantation and had available cryopreserved lymphocytes from the corresponding cadaver donor. A total of 111 heart and 59 kidney allograft recipients fulfilled these criteria. All patients were recipients of primary cadaver grafts and received Cyclosporine-based immunosuppressive therapy.

Statistical Analysis

Actuarial graft survival rates were computed by the Kaplan-Meier estimate, and the log rank test was used for statistical comparison of survival curves (BMDP I-L, BMDP Statistical Software). The χ² test was used to determine significant associations between DRB1 alleles and anti-HLA antibody production (SAS/STAT software). The duration of transplant survival was computed from the date of transplantation to the date of recipient death or retransplantation in the case of cardiac allograft recipients and to the date of return to dialysis for renal graft recipients. Since unsuccessful immunosuppression may result in rejection, malignancies or infections, the assumption was made that immunological problems were the major underlying cause of transplant failure.

The hypothesis that anti-HLA antibody producers (i.e. responders) differ with respect to their HLA-DR genotype from patients who do not form antibodies (i.e. nonresponders) was tested by constructing 2 × 2 contingency tables for each of the 37 DRB1 genes encountered in the patient population. p values were corrected to control for type I error introduced by the performance of multiple comparisons: the raw p value was multiplied by the number of comparisons performed, which is 37 for DRB1 analysis [23].

HLA-A,B,C and DR Typing

Recipients and donors were typed for HLA-A,B,C and DR antigens by conventional serology, as previously described [24]. The HLA-DRB1 genotype of 161 recipients was determined by polymerase chain reaction amplification of the first domain of the DRB1* gene and hybridization with sequence specific oligonucleotide probes, as previously described [25, 26]. Molecular typing was performed for the following DRB1* alleles: 0101, 0102, 0103, 1501, 1502, 1601, 1602, 0301, 0302, 0401, 0402, 0403, 0404, 0405, 0406, 0407, 0408, 0409, 0410, 0411, 1101, 1102, 1103, 1104, 1201, 1202, 1301, 1302, 1303, 1304, 1305, 1401, 1402, 1403, 1404, 1405, 07, 0801, 0802, 0803, 0804, 09 and 1001.

Evaluation of Anti-HLA Antibodies

Sera obtained at the time of transplantation and at 3-month intervals following transplantation were depleted of soluble HLA class I and class II antigens [9, 10, 27] and then screened for anti-HLA-A,B,C, DR and DQ antibodies on an HLA reference panel of 70 cells [24]. Assignment of anti-HLA antibody specificity was based on the tail analysis program (UCLA Software) indicating a combined correlation coefficient ≥ 0.7 between serum reactivity and the presence of certain HLA antigens on target lymphocytes. All sera were tested for reactivity with donor T and B cells and for autoreactivity with recipient lymphocytes. To exclude the possibility that lymphocytotoxicity of the patients' sera was caused by antilymphocytic antibodies used for treatment, such as monoclonal antibody (mAb) OKT3 or ATG, sera were tested by immunocytofluorometry for mouse and horse antibodies using FITC-conjugated goat-anti-mouse Ig (Tago, Burlingame, Calif., USA) and FITC-conjugated goat-anti-horse Ig (Chemicon, Temecula, Calif.). For this, 5 × 10⁵ lymphocytes were incubated with 50 μl of patient serum for 30 min at 4°C. After 30 min of incubation, the cells were washed 3 times in PBS containing 2.5% fetal calf serum and 0.05% sodium azide. Cells were then stained with 50 μl of the appropriate secondary antibody. After 30 min of incubation at 4°C, cells were washed and analyzed on a FACSTAR cytofluorograph (Becton-Dickinson, Mountain View, Calif.). Sera containing xenogeneic antibodies were excluded from the analysis.