HLA Class I Donor-Specific Triplet Antibodies Detected After Renal Transplantation

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ABSTRACT

The purpose of this study was to investigate whether IgG, non–donor-specific anti–HLA class I antibodies (HLAabI) detected after renal transplantation recognize immunogenic amino acid triplets expressed on the foreign graft. In addition, we sought to evaluate the effect of these antibodies as well as other posttransplant HLAabI on graft outcome. Posttransplant sera from 264 renal recipients were tested for the presence of IgG HLAabI and HLA class II–specific alloantibodies (HLAabII) by ELISA. The HLAMatchmaker computer algorithm was used to define the HLA class I non–donor–specific antibodies, which seem to recognize immunogenic amino acid triplets. Donor-specific triplet antibodies (DSTRab) were detected in 16 of 22 (72.7%) recipients based on at least one HLA-A or -B mismatched antigen with the donor. DSTRab were found either without (n = 7) or with (n = 9) HLA donor-specific antibodies (HLA-DSA). The presence of DSTRab alone in the periphery was associated with acute rejection, whereas the presence of both DSTRab and HLA-DSA was associated with chronic rejection and graft failure.

HLAMatchmaker is a computer algorithm based on the concept that immunogenic epitopes represented by amino acid triplets on exposed parts of protein sequences of human leukocyte antigens (HLA-A, HLA-B, and HLA-C) are accessible to alloantibodies.[1] This algorithm applies the following basic ideas: Each HLA antigen represents a string of multiple epitopes defined by amino acid triplets. These epitopes are capable of causing humoral immune responses. Alloimmunized patients cannot produce antibodies directed against triplets on mismatched HLA graft antigens, if such triplets are also found in the same sequence location in any HLA molecule of the patient.[1,2] This algorithm represents a novel approach to HLA matching. Using HLAMatchmaker one can define HLA matching/mismatching by determining the number of triplets shared/not shared with recipient HLA antigens. In fact, the results of a study by Rodey et al have provided evidence that class I HLA matching at the triplet level benefits kidney transplant outcome.[3] In addition, one can utilize this algorithm to define the amino acid triplets toward which HLA-specific antibodies are directed.

According to recent publications several HLA antibody specificities detected in recipients after organ transplantation are associated with an increased incidence of rejection and graft failure.[4,5] The major purpose of this study was to investigate whether IgG non–donor-specific HLA class I alloantibodies (HLAabI) detected after renal transplantation recognize amino acid triplets expressed on the foreign graft. In addition we assessed the effect of these antibodies as well as other posttransplant HLAabI on graft outcome.

MATERIALS AND METHODS

The production of HLA-specific alloantibodies (HLAsab) posttransplantation was investigated retrospectively in 264 patients (180 men and 84 women) who received a primary renal graft. The average HLA-A,-B and -DR,-DQ mismatches were 2 and 1.2, respectively. The median follow-up period for the patients in the study was 12 months (range 1 to 48 months).

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Table 1. HLA Class I Antibodies After Renal Transplantation and Graft Outcome

Patient group	n	Good, stable graft function	Acute rejection	Chronic rejection and graft loss	Rejection and graft loss
A*	9	4	1	4	5/9 (55.5%)
B†	7	3	3	1	4/7 (57.1%)
C‡	9	6	2	1	3/9 (33.3%)
Total	25	13	6	6	12/25 (48 %)

*Patients with both DSA and donor-specific triplet antibodies (DSTRab).
†Patients with DSTRab only.
‡Patients with non–donor-specific antibodies (NDSA).

The serum samples were analyzed for the presence of IgG HLA class I– and II–specific antibodies using a commercial ELISA kit (LAT, One Lambda, Canoga Park, Calif).

All antibody-positive samples were further evaluated to determine percent PRA and antibody specificity using LAT 1288 (One Lambda), according to the manufacturer's recommendations. Antibody specificity was determined using a chi-square test and a two-by-two-tailed Fisher's Exact test with a cutoff significance level of $P < .05$. The HLAMatchmaker algorithm was used to define HLA class I non–donor-specific antibodies (NDSA), which seem to recognize amino acid triplets on donor-mismatched antigens–defined as donor-specific triplet antibodies (DSTRab). For example, antibodies detected against HLA-B7 and HLA-B42 in a recipient were characterized as NDSA; however, they also seemed to recognize the triplets b177DK and b180E expressed on HLA-B7 and HLA-B42 antigens as well as on the mismatched HLA-B60 graft antigen. These triplets were not shared between donor and recipient.

RESULTS

HLAabI were detected among 25 of 264 (9.5%) cases, either alone (n = 15) or with HLA class II–specific alloantibodies (HLAabII; n = 10). Moreover, only HLAabII were detected in 19 cases. Three of the 25 recipients with HLAabI had zero HLA-A,-B mismatch with their respective donors (HLA-identical siblings). Therefore, these three cases were excluded from the total number of patients with HLAabI when deriving the percent detection of HLA donor-specific antibodies (DSA) and DSTRab.

DSA were detected in 9 of 22 (40.9%) of the recipients with HLAabI and at least one HLA-A or -B mismatched antigen with the donor. The DSA were always detected with NDSA, which were actually DSTRab (group A, n = 9) (Table 1). The DTRSab in the patients of group A were directed against an antigen sharing a triplet with the donor-mismatched HLA class I antigen, against which a DSA was detected in the patient.

NDSA alone were detected in 16 of 25 (60%) recipients with HLAabI. In 7 of 16 cases, the detected antibodies were in fact DSTRab (group B), whereas, in the other 9 cases, the antibodies did not seem to recognize donor-specific triplets on mismatched antigens (group C) (Table 1).

DSTRab were detected in 16 of 22 (72.7%) recipients with HLAabI and at least one HLA-A or -B mismatched antigen with the donor. When both DSTRab and DSA were considered, the detected antibodies seemed to recognize HLA class I donor-mismatched antigens in 16 of 22 (72.7%) cases compared with 9 of 22 (41%) when only DSA were considered.

The incidence of rejection and graft failure among recipients with both DSA and DSTRab (group A) versus DSTRab alone (group B) was 55.5% and 57.1%, respectively (Table 1). The presence of DSTRab alone (Group B) in the periphery was associated with an acute rejection episode (AR) in three of seven recipients. The presence of both DSA and DSTRab (group A) was associated with CR and graft failure in four of nine cases (Table 1).

DISCUSSION

Most (72.7%) IgG non–donor-specific HLA class I antibodies detected after renal transplant seem to recognize amino acid triplets that are expressed on the graft alloantigens. Oher investigators have reported the detection of such antibodies as well as DSA in patients who have lost a renal graft and are now candidates for retransplantation.[6] The actual percentage of patients with detectable HLAabI recognizing mismatched alloantigens in the present study was much higher when both DSTRab and DSA and not only DSA were considered (72.7% versus 41% respectively).

The presence of DSTRab alone seems to result in the same incidence of rejection and graft failure as the presence of both DSTRab plus DSA. In this study the presence of DSTRab alone in the periphery was associated with acute rejection, whereas the presence of both DSTRab and DSA was associated with chronic rejection and graft failure: In three of six cases of AR, DSTRab alone were present in the periphery, whereas, in four of six cases of CR and graft failure, both DSTRab and DSA were present (Table 1).

The problem in understanding humoral responses in clinical renal transplantation is that one often observes HLA antibodies directed toward non-donor alloantigens. Several explanations have been proposed: for example, the transplanted organ may provoke inflammation, triggering previously undetected memory responses. This explanation may account for cases in women (pregnancies) or unreported blood transfusions. Alternatively, consistent with the concept of HLAMatchmaker, several epitopes (triplets) can be the target for a humoral immune response. One must take into account that antibodies have different binding sites for an antigen, which may increase or decrease binding efficiency. Antibodies recognizing private epitopes (triplets) will remain in the graft and thus cannot be seen in the

periphery of the patient. In contrast, antibodies toward triplets shared between donor HLA antigens and other non-donor HLA antigens may be found in the patient's serum if they show lower avidity.

In conclusion, HLA class I DSTRab seem to be involved in rejection and graft outcome, but more studies are required to clarify their exact role in the humoral alloimmune response and whether their presence is due to mismatched graft antigens or to other sensitization events not related to transplantation.

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