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The Role of β-Pleated Sheet DRB1 Differences in Acute Rejection After Cadaveric Renal Transplant

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DONOR-recipient HLA matching influences graft survival in kidney transplantation, and it has been widely demonstrated that cadaveric graft survival decreases with increasing HLA antigen mismatches. The analysis of HLA mismatches (MMs) suggests that HLA loci do not all have the same importance in predicting graft outcome; DR compatibility, for instance, has been shown to have an important role in graft rejection, especially in the first posttransplant months. These antigens are expressed on the dendritic cells of the transplanted organ and are responsible for both early "direct" allogenic recognition of donor cells as well as "indirect" recognition of peptides from donor HLA molecules presented by the recipient's DR antigens.

The compatibility for HLA-A and -B antigens (present on all graft cells), conversely, has been shown to be important both early on in the posttransplant period and later.

The role of the DP locus is still controversial, but it would appear to be involved in retransplants in hyperimmunized subjects.[1] In contrast, it is well-known that HLA compatibility influences cellular (CTLs) and humoral (anti-HLA-non-self antibodies) alloreactivity toward the graft.[2,3]

Although matching for HLA alleles may enhance long-term graft survival,[4] the extensive heterogeneity of the HLA system[5] means that few kidneys are well-matched. Conversely, some kidney transplants from cadaveric donors have shown good long-term graft survival despite poor HLA compatibility. This apparent contradiction is probably due to the fact that most of the alleles show 95% similarity in sequence and structure, antigenic differences being determined by a relatively restricted number of amino acid residues responsible for the conformation of the peptide-binding groove. Published work has shown that some HLA antigenic subtypes can be immunogenic when confronted with certain antigens but permissive for recipients with other HLA antigens.[6] For this reason alternative approaches to HLA matching such as "epitope," cross-reactive groups (CREG), and "residue" matching were developed to discover permissive mismatches.[7–9] Sequence studies of HLA class I and class II loci have shown that different alleles have similar amino acid residues and that serologically identical antigens may have differing nucleotide and amino acid sites.

The aim of this study, on the basis of the above, was to analyze the correlation between DRB1 amino acid residue matching in cadaveric renal transplants, the occurrence of acute rejection episodes, and the posttransplant donor-specific humoral response.

MATERIALS AND METHODS

Donor-recipient renal transplant pairs were retrospectively analyzed for HLA-DRB1 amino acid residue compatibility. HLA class I and class II compatibility had been already defined using the standard complement-dependent microlymphocytotoxicity test and genomic techniques (DRB1 gene: PCR-SSO; DQA1 and DQB1 genes: PCR-SSP; DPB1 gene: Innolipa RDB). The DRB1 alleles of each recipient and donor were determined, and the correlation among DRB1 amino acid residue compatibility, acute rejection episodes, and class II humoral response in the first year after transplant was analyzed.

Patients

Fifty-one first cadaveric kidney transplants performed consecutively at the Kidney Transplant Unit of Clinical Surgery, Tor Vergata University of Rome, were enrolled in our study. All patients were transplanted on the basis of a negative pretransplant lymphocytotoxic crossmatch. Organ allocation was based on the best donor-recipient HLA matching; matching priority was HLA-DR, HLA-B, and HLA-A, respectively.

Immunosuppressive treatment consisted of a triple drug therapy (cyclosporine/prednisone/azathioprine). Acute rejection episodes were diagnosed by core biopsy, and methylprednisolone boluses were administered.

DRB1 Donor-Recipient Compatibility

The HLA-DR matching in donor-recipient transplant pairs was first analyzed in terms of nucleotide similarity; nucleotide differ-

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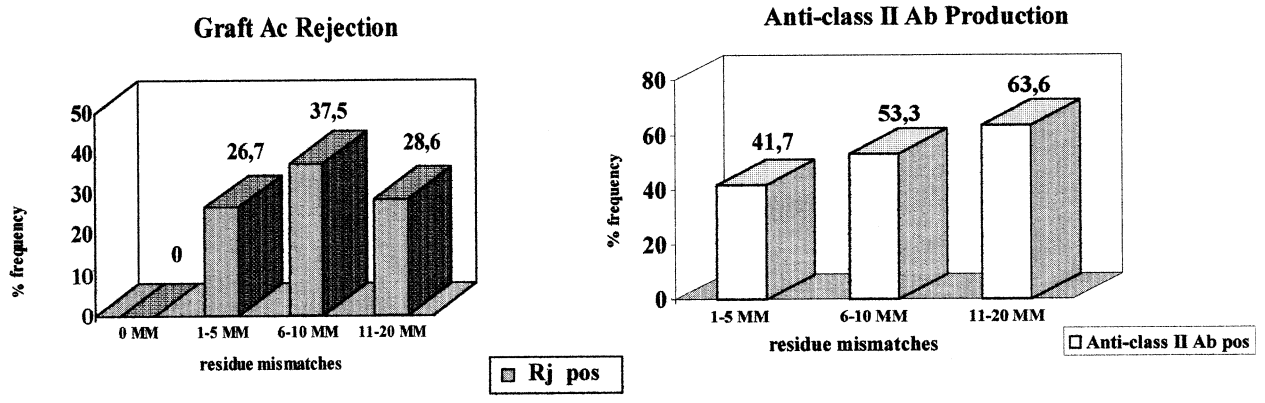

Fig 1. Frequency of kidney graft rejection and donor-specific antibody production in relation to DRB1 amino acid residue mismatches.

ences were highlighted by DNA sequencing methods. These differences were also observed once the nucleotides had been translated into amino acid residue mismatches, hence enabling us to identify differences in the three-dimensional structure of the HLA molecule. These DRB1 amino acid differences were principally located within the β-pleated sheet and α-helix of peptide-binding groove. Up to 20 residue mismatches out of the 30 possible amino acid variation sites present in DRB1 exon 2 were observed in transplant pairs.[10]

Panel and DNA Preparation

The DNA samples from EDTA-recipients' peripheral blood and donors' spleen lymphocytes were extracted by a "salting-out" procedure, as has been previously described.[11] Both the quantity and the purity of the DNA were monitored using a spectrophotometer at 260 and 280 nm.

HLA-DRB1 Sequencing Method

Higher resolution of DRB1 alleles was obtained using automated sequence-based typing (SBT). A preliminary SSP-PCR screening of the major DRB1 allele groups was carried out via 12 parallel amplifications of DRB1 allele groups (DRB1G1, DRB1G2, DRB1G3/11/6, DRB1G4, DRB1G7, DRB1G8/12, DRB1G9, DRB1G10) plus DRB3, DRB4, DRB5, and DRBALL (contamination control) (HLA-DRB Sequencing-Based Typing Kit, PE Applied Biosystems). Because PCR products have both sequencing primer sites (a −21M13 tailed 5′-primer and a custom 3′-primer), only one PCR for each amplification group was required to generate templates suitable for sequencing both strands. Sequencing of all allele groups was accomplished using one set of fluorescently labeled primers for each sequencing direction. To eliminate primer-dimer formation in the PCRs and subsequent sequencing artifacts, AmpliTaq Gold was included as a DNA polymerase in all the amplification reactions. The cycling conditions were those described by the kit protocol: 1 cycle at 95°C for 10 minutes; 36 cycles of denaturation at 96°C for 20 seconds; primer annealing at 65°C for 30 seconds; primer extension at 72°C for 30 seconds; 1 cycle at 99°C for 10 minutes.

The PCR-positive reactions were diluted 1:10 and sequenced using *forward* and *reverse* dye primers and AmpliTaq FS enzyme. Sequencing conditions consisted first of 15 cycles of denaturation at 96°C for 20 seconds, annealing at 62°C for 20 seconds, and extension at 72°C for 20 seconds, and then of another 15 cycles at 96°C for 20 seconds and at 72°C for 30 seconds. Sequencing reactions were analyzed by electrophoresis on an ABI 377 DNA sequencer in 5% Long Ranger denatured gel after ethanol/isopropanol precipitation and denaturation of samples. The sequence data obtained were processed by a specific software program. We used the new HLA MATCHMAKER program to assign IUB ambiguity codes, to detect the heterozygous positions within each electropherogram, and to assign the typing on the basis of the alignment of the processed sequence with a sequence library.

Donor-Specific Class II Antibodies

Donors' lymphocytes were obtained from the spleen and stored in liquid nitrogen. Pretransplant sera were obtained from all patients and posttransplant sera were collected at regular intervals (15 days and 1, 2, 3, 4, 5, 6, 9, 12 months) and stored at −80°C until use. The sera were then analyzed by flow cytometric crossmatching (FCXM).[12] The presence of autoantibodies was determined using the same technique.

Briefly, 2.5×10^5 donor lymphocytes were incubated with 75μL of the patient's serum for 30 minutes at room temperature. Donor cells were washed twice and incubated with 50 μL of fluorescein isothiocyanate (FITC)-conjugated anti-IgG or IgM $F(ab')_2$ (Dako), at a titer of 1/20. To identify T or B lymphocytes, 5 μL of anti-CD3 and anti-CD20 monoclonal antibodies (MAbs) (Becton Dickinson), conjugated, respectively, with Peridinin Chlorophyl Protein (PerCP) and phycoerythrin (PE), were added. After incubation and washes, the samples were analyzed on a FACScan (Becton Dickinson) using FACScan Research Software (1024 channels). The presence of anti-class II antibodies was confirmed, after sera absorption for class II soluble antigens,[13] both by the appearance and an increase in antibodies binding to B lymphocytes.

Statistical Analysis

The results were expressed as means ± SD, and the differences between groups were assessed by the two-tailed Mann-Whitney U test and Fisher's chi-square test, as appropriate. The level of significance was set at $P < .05$.

RESULTS

Fifty-one first cadaveric renal transplant pairs were typed for DRB1 allele subtypes and the compatibility was as-

Table 1. Frequency of DRB1 Amino Acid Residue Mismatches in β-Sheet and α-Helix Correlated to Kidney Graft Rejection and DS Anti-Class II Antibody Production

Rejection			Antibody		
β-Sheet (codon 9–47)			β-Sheet (codon 9–47)		
	Rj Pos	Rj Neg		Ab Pos	Ab Neg
Mean ± SD	5.1 ± 4.3	3.5 ± 3.6	Mean ± SD	4.9 ± 4.0	3.7 ± 3.6
α-Helix (codon 57–86)			α-Helix (codon 57–86)		
Mean ± SD	4.4 ± 2.2	3.5 ± 2.7	Mean ± SD	4.9 ± 2.4	3.1 ± 1.9 $P = .034$

Statistical analysis: Mann-Whitney *U* test.

sessed: 6 pairs had 0 residue MMs, 15 pairs 1 to 5 MMs, 16 pairs 6 to 10 MMs, and 14 pairs 11 to 20 MMs. As for anti-class II donor-specific antibody production after the transplant, 20 patients out of 39 analyzed showed the presence of anti-HLA class II antibodies after absorption for class II soluble antigens.

Fourteen patients experienced an acute rejection episode during the first year posttransplant. In the same period, one transplanted kidney was lost because of immunological causes.

Analysis of HLA residue matching in donor-recipient pairs showed that there were no rejection episodes in the 0 MM residue patients, whereas the incidence of rejection increased in the presence of residue mismatches, although rejection episodes in the 1 to 5 MM (26.7%), 6 to 10 MM (37.5%) groups, and the 11 to 20 DRB1 residue mismatch group (28.6%) were not significantly different one from the other. A linear correlation existed between posttransplant anti-class II antibody production and the number of DRB1 residue mismatches (1 to 5 MM 41.7%, 6 to 10 MM 53.3%, 11 to 20 MM 63.6%) (Fig 1). Analysis of the mismatched DRB1 amino acid residues in the β-pleated sheet (codon 9–47) and the α-helix (codon 57–86) of the DRB1 peptide-binding groove revealed that the frequency of β-sheet residue mismatches was higher in the rejection-positive group than in the rejection-negative group (5.1% ± 4.3 vs 3.5% ± 3.6), whereas residue differences within the α-helix showed less variation (4.4% ± 2.2 vs 3.5% ± 2.7). A significant increase in the mismatched DRB1 residues localized in the α-helix was observed in transplanted patients with anti-class II specific antibody production (4.9% ± 2.4 vs 3.1 ± 1.9, $P = .034$) (Table 1); this increase, however, was only slight for mismatched residues in the β-pleated sheet.

More than one amino acid residue mismatch (maximum 20 differences for each transplant pair) was observed out of the 30 polymorphic residues in the DRB1 exon 2 in the 51 kidney transplants included in this study, apart from 6 DRB1 completely matched pairs.

We studied in detail the most frequent DRB1 amino acid residue mismatches associated with rejection and humoral response. The frequency of the mismatches localized in codon 9 (35.7% Rj-positive vs 13.9% Rj-negative) and codon 28 (35.7% Rj-positive vs 16.7% Rj-negative) in the β-pleated sheet, as well as in codon 57 (57.1% Rj-positive vs 25% Rj-negative, $P = .04$) in the α-helix was higher in patients who experienced rejection episodes within the first posttransplant year. Mismatches in codon 58 of the α-helix were more frequently associated, on the other hand, with anti-class II antibody production (30% vs 15.8%) (Table 2).

DISCUSSION

In accordance with the published data,[7,14] our results confirmed that acute rejection episodes after cadaveric kidney transplantation are influenced by the number of mismatched DRB1 residues. It was particularly interesting to note that DRB1 residue compatibility was always correlated to the absence of rejection episodes, whereas the presence of amino acid differences, either in moderate or high numbers, was associated with a similar frequency of rejection. This pattern could be due to the possible role played by differences in particular regions of the HLA cleft or by single amino acid residue mismatches in alloreactivity onset following organ transplantation.

In fact, our data suggest that rejection occurrence is correlated to mismatched DRB1 amino acid residues in the β-pleated sheet rather than in the α-helix. These findings lead us to hypothesize that the amino acid differences in the DRB1 molecule have an important role in triggering posttransplant alloreactivity. A higher frequency of differences in residue 9 and 28 in the β-sheet and in residue 57 in the α-helix in the rejection-positive group underlines the importance of these amino acid positions in immunological response. In addition, from the recent light shed on the three-dimensional conformation of HLA-DR molecule it can be assumed that these amino acid residues are part of the peptide-binding pockets (codon 9 and 57 in peptide

Table 2. Mismatched DRB1 Amino Acid Residues More Frequently Associated With Rejection Episodes and Antibody Production

Rejection				Antibody		
β-Sheet				α-Helix		
	Rj Pos	Rj Neg			Ab Pos	Ab Neg
Residue 9				Residue 58		
	37.5%	13.9%			30.0%	15.8%
Residue 28						
	35.7%	16.7%				
α-Helix						
Residue 57						
	57.1%	25.0%	$P = .04$			

Statistical analysis: Fisher's chi-square test.

position (P) 9; codon 28 in P4, P5, P6, P7), and so the peptide presentation pattern could influence alloreactivity phenomena implicated in rejection.[15–17]

Unlike rejection, donor-specific anti-class II antibody production seems to be significantly influenced by differences in the α-helix and particularly by codon 58 rather than by differences in the β-sheet. This amino acid residue has an effect on peptide binding and T-cell response.[15]

Finally, identification of the residues that appear to have the most important role in alloreactivity onset will allow investigators to define the existence of "permissive" or "immunogenic" allele combinations. These combinations could then form the basis of new criteria for organ allocation, increasing not only the chances of transplant but also of finding more suitable donor-recipient combinations, thus reducing the risk of rejection.

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