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HLA class I residue mismatch and renal graft outcome

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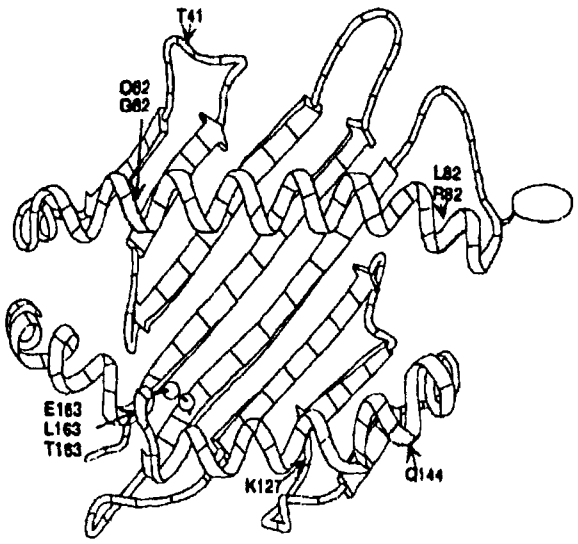
Abstract Donor-recipient HLA matching was retrospectively evaluated in 111 cadaveric renal transplants using Takemoto's ten-residue model in which HLA class I antigens are clustered by crossreactive group (CREGs) on the basis of amino acid sequence homology and the sharing of a particular public epitope. The grade and type of HLA residue mismatching were correlated to post-transplant, class I donor-specific antibody production (monitored by flow cytometry crossmatch), rejection occurrence and clinical outcome during the 1st year posttransplant. In 52 patients with 0 mismatchings (MMs) we observed a low incidence of rejection (11.1%) and antibody production (11.1%) for 0 CREG MM grade, while 1 MM was enough to increase immune response against graft (rejection 35%; antibodies 30%). Moreover, a significant correlation was observed between Q144, E163, Q62 and L82/R82 epitopes and the incidence of acute rejection and antibody production ("immunogenic" residues) in patients grouped for a single residue mismatch.

Key words HLA class I matching · Cadaveric renal transplant · Crossreactive antigens (CREG) · Flow cytometric crossmatch (FCXM) · Anti-HLA alloantibodies

Introduction

Large studies have shown a beneficial impact of HLA matching on graft survival in renal transplantation, but a complete match is only possible in a small proportion of recipients due to the extended variability of the HLA region. Recently, it was suggested that the compatibility for some public HLA epitopes, the crossreactive group (CREG) antigens, would be advantageous as private HLA matching, with the further benefit of allowing a greater number of recipients access to well-matched grafts [11–13]. CREG matching should also increase the possibility of finding donors for ethnic minority recipients as CREG frequencies between ethnic populations are more similar than allele frequencies [8]. Most HLA alleles differ one from each other only by a few amino acid residues within the peptide-binding groove which is responsible for the specific set of peptides shown to T cell receptor. They also share many epitopes: about the 90% of sera from highly sensitised patients contains anti-HLA antibodies against public antigens, shared and crossreactive epitopes, common to several HLA class I alleles. Amino acid sequence information on HLA class I has led to the identification of many polymorphic residues that correspond to certain public epitopes [1, 3, 6, 7]. For this reason, the donor-recipient compatibility might be estimated by avoiding donor mismatches only for some residues rather than of the complete HLA molecules. The ultimate goal for a successful transplant should be to distinguish between acceptable and unacceptable HLA mismatches among potential donors. Humoral response in renal transplantation can be modulated differently by HLA compatibility and it is correlated to the graft outcome, being involved in several immunological mechanisms responsible for acute and chronic rejection [2, 4, 14]. HLA class



residue	*specificity*
T41	B locus
Q62	A locus
G62	A,B loci
L82	A,B loci
R82	A,B loci
E163	A,B,C loci
L163	A,B,C loci
T163	A,B,C loci
K127	A locus
Q144	A locus

Fig. 1 Tridimensional structure of HLA class I molecule with the localisation of the ten crossreactive group (CREG) residues examined in the study

I allorecognition occurs via two distinct pathways. One is "direct" and involves the recognition of donor antigen present on the surface of the graft by activator cells of the immune system; it influences acute graft rejection mainly by cell-mediated killing. The second pathway is "indirect", through the presentation of donor's class I antigen ingested and processed by recipient's antigen-presenting cells (APC). DR antigens assembled within the APC bind these in a cleft on top of the molecule. Thus APC induce the activator cells and set-off a cascade of immune reactions and antibody production directed against the mismatched antigen. This kind of response has a decisive role in chronic rejection.

In this context donor-recipient HLA matching was retrospectively evaluated in 111 cadaveric renal transplants using S.K. Takemoto's ten-residue model. The grade and type of HLA residue mismatching (R-MM) were correlated to class I donor-specific antibody (DS-Ab) production, rejection occurrence and clinical outcome.

Patients and methods

Patients

A total of 111 patients who had undergone cadaveric kidney transplants at the Transplant Unit of Tor Vergata University in Rome were analysed. All patients were followed for at least a period of 2 years. The immunosuppressive protocol for all renal transplants consisted of a triple drug therapy (cyclosporine/prednisone/azathioprine or MMF). Rejection episodes were diagnosed by core biopsy and treated with methylprednisolone boluses. Renal function was investigated by serum creatinine monitoring.

Tissue typing and matching

HLA class I and II typing for HLA-A, -B and DR was serologically performed using a complement-dependent microlymphocytotoxicity test with immunomagnetical beads to separate T and B cells. HLA class I compatibility was evaluated either serologically or using Takemoto's ten residue–CREG model (K127, R82, G62, T41, T163, L163, E163, Q62, Q144, L82) [9]. In Takemoto's model HLA class I antigens are clustered by CREGs, according to the deduced similarities in amino acid sequence. These residues are localised on top of the alpha helices or on loops of the HLA class I molecule, oriented outwards in positions which can be recognised by anti-HLA antibodies (Fig.1). The residues were selected on the basis of their position within the HLA molecule as they interact with anti-class I antibodies and have an impact on the clinical outcome and rejection.

The donor and recipient HLA antigens, serologically assigned, were converted to the corresponding public residues, grouping HLA class I antigens on the basis of the sharing of a particular epitope. The conventional donor-recipient HLA class I compatibility was converted to ten CREGs of amino acid residues representing class I public epitopes. Private HLA class I antigens were considered mismatched when a certain antigen was present in the donor but not in the recipient.

Broad class I antigens (for example A19) were considered different if the single splits of the antigen (for example A30, A31, A32, etc.) were mismatched. HLA-DR matching was evaluated on the basis of the sharing of the "broad" HLA antigens (DR1–10 groups). A residue was counted as mismatched when it was present in the donor but not in the recipient.

Donor-specific class I antibodies

Available donor's lymphocytes were stored in liquid nitrogen until use. Pretransplant sera were obtained from all patients, and post-transplant sera were collected at regular intervals (15 days and 1, 2, 3, 4, 5, 6, 9 and 12 months) and stored at –80 °C. The sera were then analysed by flow cytometry crossmatch (FCXM). The presence of autoantibodies was determined using the same technique. Briefly, 2.5×10^5 donor lymphocytes were incubated with 75 µl patient's serum for 30 min at room temperature. Donor cells were

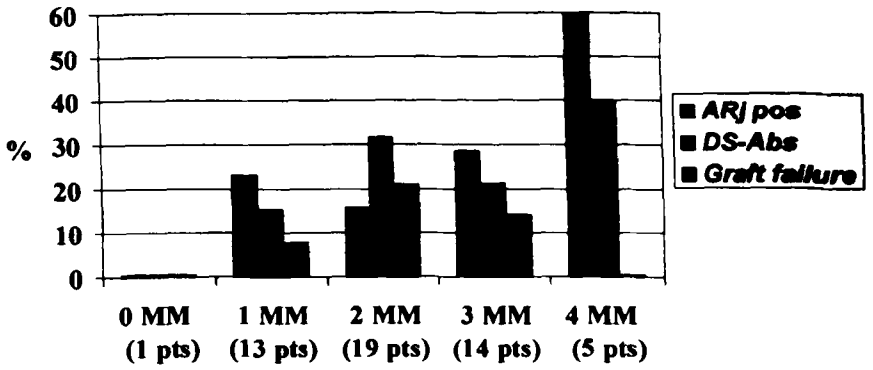


Fig. 2 Clinical outcome and antibody occurrence in 0 DR mismatching (*MM*) patients (*pts*) in relation to AB MM grade. *ARj* Acute rejection, *DS-Abs* donor-specific antibodies

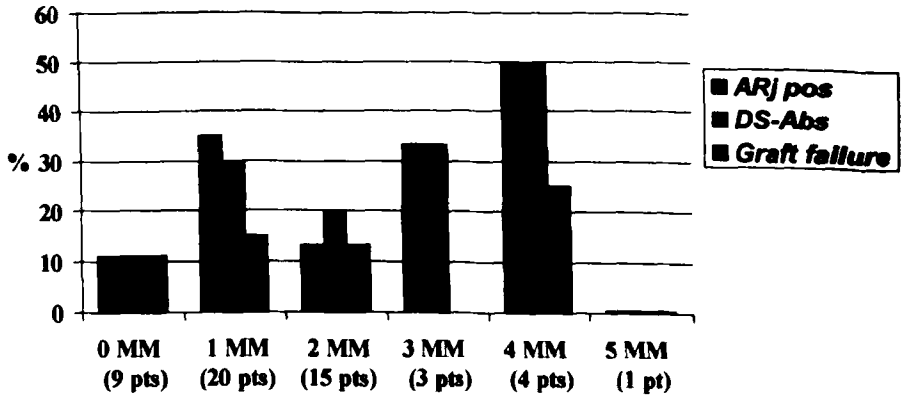


Fig. 3 Clinical outcome and antibody occurrence in 0 DR MM patients in relation to CREG MM grade

washed twice and incubated with 50 µl fluorescein isothiocyanate (FITC) conjugated anti-IgG or IgM F(ab')2 (Dakopatts, Denmark). To identify T or B lymphocytes, 5 µl anti-CD3 and anti-CD20 monoclonal antibodies (Becton Dickinson, Calif., USA), conjugated respectively with peridinin chlorophyll protein and phycoerythrin (PE), were added. After incubation and washes, the samples were analysed on a FACScan (Becton Dickinson) using the Cell Quest Software (1024 channels).

Statistical analysis

The results were expressed as means ± standard deviation and the differences between groups were assessed by the Mann-Whitney *U*-test and Fisher's Chi-squared test. The level of significance was set at $P < 0.05$.

Results

Acute rejection was observed in 31 (27.9%) of 111 transplanted patients, 25 within the first 6 months post-transplantation and the others after 6 months. As far as the posttransplant DS-Ab production is concerned, 24 patients were FCXM positive during our observation period and 7 out of these suffered graft lost. Correlating HLA R-MMs with acute rejection occurrence and DS-Ab production, we observed that the antibody appearance increased progressively among the groups with different class I R-MM grades, 0 MMs 3/20 patients (15%), 1 MM 7/39 (17.9%), 2 MMs 7/32 (21.9%), 3 MMs 3/11 (42.9%), 4 MMs 3/7 (42.9%) and 5 MMs 1/2 (50%), while rejection increased strongly going from 0 MMs to 1 MM group (20 vs 35.9%) after which an irregular trend was observed (2 MMs 28.1%, 3 MMs 9.1%, 4 MMs 28.6%, 5 MMs 50%). As for class I serological MMs, rejection and antibody appearance increased greatly going from 0 MMs to 1 MM (0 vs 39.1%, 0 vs 21.7%), with no wide changes for the most incompatible groups. From our group of patients, we selected 52 cases with 0 MMs DR, in order to eliminate the influence of DR incompatibility on alloreactivity against graft. In these patients we observed a low incidence of rejection (11.1%) and antibody production (11.1%) for 0 CREG MM grade but 1 MM was enough to have an increased immunological response against the graft (rejection 35%; antibodies 30%). The trend of rejection onset and antibody appearance was similar considering both AB MMs and CREG MMs. (Figs. 2, 3). The important effect of CREG R-MM on the humoral response was confirmed by the major incidence, statistically significant, of CREG R-MMs in antibody-positive versus antibody-negative patients (antibody-positive patients: CREG MM mean ± SD 1.9583 ± 1.3666 vs antibody-negative patients: 1.4597 ± 1.1186; $P = 0.0343$).

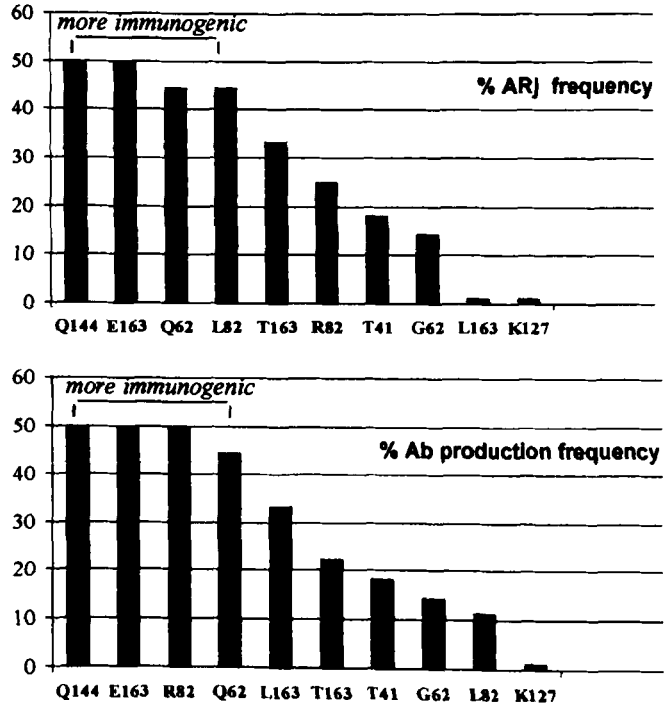


Fig. 4 Incidence of acute rejection (*ARj*) and donor-specific anti-class I antibody (*Ab*) production in patients grouped for mismatch in single CREG residues

Analysing single CREG residue frequencies, a fairly homogeneous distribution was evidenced in our patient population (frequency range 11.5–21.2%). Moreover, a significant correlation between Q144, E163, Q62 and L82/R82 residues, the incidence of acute rejection and antibody production was evidenced in patients grouped for mismatch in the single CREG residues (Fig. 4).

In order to better characterise the different degree of response triggered by the "more immunogenic" residues or by the others, we compared the two groups for acute rejection occurrence, mean serum creatinine levels and DS-Ab production. Our analysis highlighted a significantly higher incidence of rejection ($P = 0.006$), higher levels of 2-year post-transplant serum creatinine and a greater production of anti-HLA antibodies ($P = 0.05$) in the group of patients presenting mismatch for the Q144, E163, Q62 and L82/R82 residues.

When analysing locus specificity of the single immunogenic residues, the presence of two different locus A-exclusive residues was evidenced. Since among the residues considered in our study only three were specific for locus A, we investigated the different incidence of the mentioned graft-status parameters in the two more immunogenic residues versus the other one. Patients with mismatched Q62 and/or Q144, but without K127, showed a worse renal function, as indicated by higher serum creatinine levels, a significantly higher incidence of rejection ($P = 0.04$) and humoral response ($P = 0.04$), although the CREG or AB MMs mean was lower than the K127 residue (Table 1).

Table 1 Locus A-specific residues Q62, Q144 ("immunogenic") vs K127: donor-specific anti-HLA class I antibody (*Ab*) production and clinical outcome in kidney transplantation. (*ARj* Acute rejection, *CREG* crossreactive group, *MMs* mismatchings)

	Residues		Significance
	Q62, Q144	K127	
Class I Ab-positive patients	43.8%	0%	$P = 0.04$
ARj-positive patients	43.8%	0%	$P = 0.04$
Creatinine at 3 months	2.2 ± 1.1	1.5 ± 0.6	
Creatinine at 6 months	2.1 ± 1.0	1.7 ± 0.6	
Creatinine at 1 year	2.1 ± 1.0	1.6 ± 0.6	
Creatinine at 2 years	2.2 ± 1.3	1.5 ± 0.7	
CREG MMs	2.1 ± 1.1	3.0 ± 1.1	
A, B MMs	2.4 ± 0.8	2.7 ± 0.9	

Discussion

Our data demonstrated the useful application of CREG matching in renal transplantation and in particular of the ten-residue model in terms of humoral immune triggering and acute rejection occurrence. In fact, the trend of rejection onset and anti-HLA antibody production was similar when considering serological or R-MM class I incompatibilities in 0 MM DR patients. An important finding was that the identity for class I serological antigens was always associated with the absence of acute rejection and humoral response while the same condition, in terms of CREG MMs, involved a certain incidence, even if of a smaller degree compared to other CREG mismatched antigens (11.1%), of alloreactivity towards the graft. The presence of an immune response also within the same CREG antigens, as reported also by other authors [5, 7], suggests the importance of other immunogenic private epitopes in addition to the public ones. Some public and possibly non-immunogenic epitopes presented to recipient's T cells could produce tolerance rather than alloreactivity. Consequently, a matching strategy based on the identification of the acceptable mismatches to the recipient's own CREG together with an epitope analysis of the anti-HLA antibodies with intra-CREG specificities could be essential to replace the conventional HLA matching.

As for the immunological impact of the ten CREG residues analysed, we evidenced the presence of some more "immunogenic" amino acid positions (Q144, E163, Q62, R82/Bw4, L82/Bw6). From the correlation between grade and type of CREG mismatch and anti-HLA class I antibody production or rejection occurrence, it is possible hypothesise that it is not so much de-

termining the number of CREG MMs between recipient and donor but especially the type, and maybe also the charge of the amino acid residue, which is recognised as foreign on the donor's cells. Among the ten CREG residues analysed, we evidenced the remarkable influence of the Q144, E163 and Q62 residues on both donor-specific humoral response after transplantation and acute rejection episodes. The identification of these more alloreactive epitopes confirmed the important role of the positions 62, 65 and 163 as prominent T cell or antibody epitopes, as postulated by Bjorkman [1], because of their only upwardly oriented residues with amino acid substitutions in the alpha helix, and then demonstrated to have a positive influence on graft outcome in transplants matched for these positions, as deduced by Takemoto in the three-residue model [12].

In detail, two residues specific for locus A antigens (Q62 and Q144) are more alloreactive than the other locus A specific K127 residue, never associated with acute rejection or posttransplant antibody production in our patient population. We could hypothesise the stronger impact of some HLA-A allele combinations than others on alloreactivity and subsequently the clinical outcome of transplant. The identification of the residues with the major role in alloreactivity might represent the basis of a new organ allocation criterion that gives priority to the more widely distributed and potentially more immunogenic public epitopes rather than on the whole number of private epitopes characteristic of allelic variants. Complete immunological and clinical understanding of these class I epitopes reputed to be more "immunogenic", together with the well-established notion of the donor HLA class I peptides best fitting the recipient's DR peptide motifs [10] will be determinant in suggesting new therapeutic patterns to induce tolerance, diagnosing rejection episodes more accurately and improving the number of well-matched grafts thus resulting in a drastic reduction of graft losses due to immunologic complications.

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