

ORIGINAL ARTICLE

ANALYSIS OF HLA CLASS I ALLOANTIBODIES IN THE SERA OF SENSITIZED PATIENTS ON HEMODIALYSIS

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Background – The specificity of HLA class I (HLA-A and B) alloantibodies was studied in 30 serum samples from antibody-positive, potential kidney transplant recipients who had percent panel reactive antibody values (%PRAs) of more than 5%.

Methods – Antibody detection was performed using the microlymphocytotoxicity technique. In this study, the specificity of antibodies was categorized as either private epitopes or cross-reactive group (CREG) epitope clusters. A *p* value of less than 0.05 and χ^2 values greater than 3.841 indicated a significant association between a known antigen and an unknown serum sample.

Results – No specific antibody was defined in seven (23.33%) serum samples with %PRA values of less than 20%. At 88% to 100% %PRA values (7 patients, 23.33%), most of the serum reactions were positive because the patients had developed multiple antibodies against a large array of HLA antigens. Identifiable antibodies were found in 16 (53.3%) serum samples with %PRA values between 20% and 87%. Anti-CREG antibodies with or without antiprivate antibodies were identified in nine of the 16 samples (59.25%), whereas only six (37.5%) of these 16 samples contained an apparent antiprivate antibody without evidence of anti-CREG antibodies and one (6.25%) contained two different private antibodies.

Conclusion – Antibody reactivity against CREG clusters was more common among patients with definable antibodies. Knowing the specificity of HLA antibodies in patients' sera, helps to define a suitable kidney with negative cross-matching for sensitized patients, among previously HLA-typed donor banks. The records of these banks are maintained in computer programs at the Isfahan Transplantation Laboratory.

Keywords • alloantibodies • HLA antigens • kidney transplantation

Introduction

Highly sensitized renal dialysis patients presents present an enigma to most transplant programs. Not only it is difficult to find a suitable cross-matched negative donor, but it is also apparent that a kidney transplant is generally less successful than unsensitized patients.¹ The number of patients who are on the waiting list for renal transplantation is a universal problem.²

Many patients who become sensitized have HLA-specific antibodies due to previous graft failures, blood transfusions, and pregnancies.³

Humoral sensitization and antibody definition are determined by testing patient sera using lymphocytotoxicity assays against at least 60 selected lymphocyte panels from donors with known HLA-A, B type. The percentage of lymphocytes killed by the sera is referred to as %PRA (percent panel reactive antibody).

These assays are designed primarily to detect antibodies that are specific for the products of the HLA-A and B loci. Patients with %PRA activity of more than 50% are considered to be highly sensitized.² The higher the %PRA value, the more difficult it is to find a cross-matched negative donor.

The chance of a successful transplant is improved by defining the specificity of HLA antibodies and selecting a suitable kidney without the HLA antigens, corresponding to the antibodies

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in the patients' sera.¹

In this study, which was performed on 30 serum samples from sensitized patients awaiting kidney transplantation, in Aliasghar Hospital, Isfahan, the epitope specificity of HLA antibodies was defined and it was observed that the majority of antibodies have specificity for definable clusters of public epitopes.

Materials and Methods

Patients and serum samples

A total of 122 potential renal recipients were admitted for HLA antibody screening to the Immunogenetic Laboratory of Aliasghar Hospital in Isfahan, during a period from 1996 to 1998. Among these patients a total of 30 individuals who had unknown HLA antibodies in their sera with persistent %PRA values greater than 5% were selected and included in this study for antibody specificity analysis.

Serum samples (1 mL) were collected from these subjects and stored at -20°C, until the beginning of serum analysis experiments.

Panel cells

Lymphocyte panels used for screening were obtained locally from 81 HLA-typed donors including medical students and kidney donors who were referred to laboratory on different days. Each time, 3 to 5 different HLA-typed lymphocytes were chosen and each of them was added to a separate Terasaki plate containing 30 different serum samples derived from the patients prepared previously.

Donor lymphocytes were prepared from 5 mL of peripheral blood according to the Boyum method⁴ and using Ficoll hypaque (Axis Shield, Oslo, Norway) as a separating agent.

The lymphocytes were used on the same day. Donor types included most of the local common original HLA class I antigens and some of the splits: A1, A2, A3, A9, A23, A24, A25, A26, A11, A29, A30, A31, B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, B22, B27, B35, B37, B40, B44, B60.

Preparation of microplates

The 30 previously frozen serum samples were thawed and 1 µL of each sample was dispensed into a marked well, in each of the 81 Terasaki microplates, and stored at -20°C until the beginning of the experiments.

Lymphocytotoxicity assay

HLA-A or B typing on panel cells was performed by the microlymphocytotoxicity method developed by the National Institutes of Health (NIH).⁵ The NIH technique was also used for antibody screening of patients' sera against 81 panel cells from HLA-typed individuals.

Analysis of anti-HLA specificity

Reactivity of each serum sample against the 81 local cell panels was evaluated visually for testing the specificity according to Rodey and Fuller.⁶ HLA antibody identification in some of the patients' sera was difficult because of the heterogeneity of HLA antibodies, and the presence of mixtures of different antibodies in the same sera. In addition, some HLA class I antigens have similarities in their structure, and this makes cross-reactive groups (CREGs) difficult to identify. The HLA class I antigens are divided into eight major CREGs as determined in Table 1.^{7,8}

Defined antibody reactivities of patients' sera were assigned to one of the two categories according to apparent epitope specificity: a) antibodies directed against private specificities were defined by reactivity against a single HLA specificity and the absence of reactivity with any other member of the same CREG (e.g., a serum "anti-A2" reacting with HLA-A2-positive cells, but not with HLA-A28, or A9 cells, was assigned to this group) and b) antibodies directed against public specificities were defined by reactivity with more than one member of a CREG (e.g., a serum "anti-A2, 28" reacting with HLA-A2 and-A28-positive cells, was assigned to this group).

Table 1. Definition of major histocompatibility HLA class I CREGs (public epitope clusters).⁸

Public epitope clusters	Associated private epitopes
A1 CREG (1C)	A1, 3, 9, 10, 11, 28, 19
A2 CREG (2C)	A2, 9, 28
B5 CREG (5C)	B5, 15, 17, 18, 35, 70
B7 CREG (7C)	B7, 13, 27, 22, 40, 41, 42, 47, 48
B8 CREG (8C)	B8, 18, 16, 15
B12 CREG (12C)	B12, 21, 13, 40, 41

Statistical analysis

Patients' serum samples were analyzed for the presence of HLA antibodies separately.

Antibody specificity assignment was based on a 2×2 table analysis using χ^2 statistics to determine significant correlations ($\chi^2 > 3.84$) between serum reactivity patterns and the presence of specific markers in the lymphocyte panel.³

The closeness of agreement between a serum sample and a recognized antigen was also measured by using a correlation coefficient (R value). R values greater than 0.6 ($p < 0.05$) indicated a good correlation.³ All the antibodies listed in Table 3 had χ^2 values greater than 3.841 and p values less than 0.05.

Results

Collectively, 32 recognizable HLA antibodies were defined in our patients. Results of the serologic studies are summarized in Table 2.

HLA antibody specificity and definition of the CREGs that each antibody belongs to, are summarized in Table 3.

Discussion

Patients awaiting kidney transplantation may produce anti-HLA class I antibodies in their sera due to multiple transfusions.³

These sensitized patients tend to accumulate on renal transplantation waiting lists, because the HLA antibodies in their sera, lead to positive cross-matches with many potential organ donors.⁷

Determining HLA phenotype and also the specificity of HLA-A and B antibodies in the patients' sera help to find a negative cross-matched, and acceptable mismatch kidney donor, against which the patients do not form alloantibodies. According to the results of this cross sectional study that was performed on 30 sensitized patients with %PRA of more than 5%, seven serum samples (23.33%) had %PRA values from 5% to 20%.

%PRA values were 20% to 87% in 16 serum samples (53.33%), and 88% or more in seven (23.33%) samples.

In the lower ranges of %PRA (less than 20%), the observed success rate was low for defining antibody specificity, due to negative or false-positive reactions. HLA specificity analysis rapidly improved with increasing %PRA values.

Table 2. Summary of serum analysis.

Condition	Number (%)
Sera tested	122 (100)
PRA = 0%	92 (75.5)
PRA > 5%	30 (24.5)
Sera with undefined antibodies	
PRA < 20%	7 (23.33)
Sera with multispecific antibodies	7 (23.33)
PRA > 88%	
Sera with defined antibodies	16 (53.3)
Anti-CREG \pm antiprivate	9 (56.25)
Only one antiprivate antibody	6 (37.5)
Two antiprivate	1 (6.25)
Total no. of antibodies detected	32 (100)
Anticross reactive \pm antiprivate	26 (81.2)
Only one antiprivate	6 (18.7)

PRA = panel reactive antibody.

The highest success rate for defining HLA antibodies was in %PRA ranges of 20% to 87%. In this range, six single, private HLA antibodies were defined. The most frequent antibody specificities were anti-A2 and anti-A9.

At 88% to 100% %PRA values, no specificities could be determined in seven (23.33%) serum samples. Almost all of the reactions in this range were positive, because the patients had developed multiple specific antibodies against a large array of HLA antigens due to multiple transfusions or hyperresponsiveness of the immune system. No auto-HLA antibodies were identified in this analysis.

These studies confirm and extend further support to previous observations⁸⁻¹⁰ that the predominant HLA class I alloantibodies produced by alloimmunized patients are directed against cross reactive epitopes. These data show that the detailed specificity analysis of highly reactive sera (%PRA > 90%) do not help to define the antibody specificity.

The observation that alloimmunized individuals predominantly develop antibodies to clusters of public epitopes has important implications for developing algorithms to improve donor-recipient matching in organ transplantation.

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Table 3. The HLA phenotype, % panel reactive antibody (PRA), and HLA antibody specificity for each of the patients.

Patient no.	HLA phenotype	%PRA	Antibody specificity detected in sera	CREG
1	A2/B5 / DR12, 52 / DQ1,3	40	A10, A11	1 C
2	A11 / B15, 35 / CW4 / DR2, 52, 53 / DQ1	45	A2, A28	2 C
3	A28 / B14, 21 / DR7, 53 / DQ2	20	Not defined (ND)	—
4	A10, 31 / B35 / DR5, 14, 52, 53 / DQ1	35	B7, B27	7C
5	A- / B14, 17 / CW3 / DR1,52 / DQ1	66	B5	Private
6	A1,3 / B15, 35 / DR1, 5, 52, 53 / DQ1,3	90	Multispecific (MS)	Many
7	A- / B14 / DR4, 7, 52 / DQ1,3	90	MS	Many
8	A1, 29 / B7, 8 / DR1, 3,52 / DQ1, 2	100	MS	Many
9	A3, 11 / B35 / DR5, 52 / DQ1, DQ3	40	B7	Private
10	A1 / B35, 63 / DR6, 8, 52, 53 DQ1	25	A9	Private
11	A28 / B52 / DR2, 3, 53 / DQ1, 2	25	A9	Private
12	A1, 30 / B21 / DR5, 52	20	ND	—
13	—	95	MS	Many
14	A9, 28 / B21, 35 / CW3 / DR4, 11, 52, 53 / D	45	B14	Private
15	—	30	A2, A9	2C
16	A9 / B5 / DR2, 11, 52 / DQ1	30	A2	Private
17	—	30	A2, 28 / B16	2C + private
18	A2, 29 / B35, 60 / DR2, 11, 52 / DQ1, 3	15	ND	—
19	A1, 2 / B5 / DR2, 52 / DQ1	15	ND	—
20	A1, 9 / B13 / DR7, 52, 53 / DQ2	18	ND	—
21	A28 / B5, 14 / DR1, 11, 52 / DQ1, 3	17	ND	—
22	A11 / B5, B7 / DR7, 2, 53 / DQ1	15	ND	—
23	A3 / B35 / DR1, 52 / DQ1	35	A9, A28	2C
24	A24, 29 / B17, 35 / CW4	35	A2 / B7, 27, 13, 22, 40	Private + 7C
25	A10, 33 / B8, 35 / DR1, 5, 52	88	A2, A10	1C
26	A3 / B5, 22 / CW1 / DR10, 11, 52 / DQ1,3	80	A2, A10	Two privates
27	A11 / B22 / CW4 / DR4, 53 / DQ3	25	B5, B35	5 C
28	A1, 29 / B7, 8 / DR1, 5, 52 / DQ2	100	MS	Many
29	A1, A3 / B22 / CW1 / DR3, 4, 52, 53 / DQ2, 3	100	MS	Many
30	A11 / B12, 22 / CW1	90	MS	Many

CREG = cross-reactive group.

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