ELSEVIER

Comparison Between HLA Class I PCR-ARMS and Serologic Typing in Cadaveric Kidney Transplantation

D. Adorno, A. Canossi, F. Papola, G. Ozzella, A. Piazza, M. Di Rocco, I. Monaco, G. Liberatore, D. Maccarone, and C.U. Casciani

BIOCHEMICAL and molecular genetic techniques demonstrated that the already extensive HLA polymorphism recognized by the classic serologic approach is very understimated. Allogeneic HLA molecules are potent immunogens for both humoral and cellular responses and are responsible for organ transplantation outcome. It is well known that in kidney cadaveric donor transplants graft survival decreases with the increasing of the HLA antigens mismatches,[1] though in some cases of HLA-incompatible individuals a long-term outcome is found. Conventional typing techniques, which are usually satisfactory to determine the compatibility in related healthy donors, are less accurate when a cadaveric kidney donor has to be tested. In particular, serologic typing of cadaveric donors encounters particular problems due to a reduction of total number of lymphocyte subsets and of lymphocytes reactivity, an alteration in the relative proportions of the various types of WBCs and a decrement in lymphocyte viability. In addition, it is well known that in cadaveric donors on systemic steroid medication the serologic typing for HLA-DR antigens from peripheral blood is complicated by alterations in DR molecules that seem less expressed, suppressed, or modified.[2] Consequently, in this group of individuals the percentage of blanks results higher than that in healthy controls (30% vs 4.2%).[3]

DNA-based approaches have been established for HLA class II but not yet for routine typing of class I region, which has an extensive polymorphism, and the presence of more than one relevant exon determining the allelic variability of HLA class I antigens. Polymorphism of HLA class I genes is restricted to the hypervariable regions in exon 2 and exon 3 encoding the domains α_1, α_2, which form the peptide-binding groove; these two exons contain the great majority of class I HLA polymorphism, and it has been suggested that this high degree of variability allows the HLA class I molecule to bind and to present to T-cell, different antigenic peptides, establishing the diversity of immune responsiveness.[4]

Recently, a new molecular biology technique was perfected, the Amplification Refractory Mutation System (SSP PCR-ARMS). It allows us to discriminate HLA class I allelic variants using primers where a mismatch at the 3′ residue inhibits nonspecific amplification under appropriate conditions.[5–7]

The aim of the present study is to value the possible application of the ARMS method in clinical practice of kidney transplantation and to compare this allelic resolution with serologic techniques. For this purpose, we analyzed the incidence of HLA-A and -B serologic blanks in cadaveric donors, and we used the PCR-ARMS technique to discriminate whether they reflected a homozygous form or a mistyped allelic assignation.

MATERIALS AND METHODS

Samples

We have retrospectively investigated by the PCR-ARMS method 20 donors of 86 cadaveric donors, HLA-class I serologically typed carrying a serologic blank in HLA-A and/or -B loci.

No correlation between the presence of blanks and the time elapsed from the withdrawal to the analysis was evidenced. Organ assignment was performed on the basis of the serologically detected compatibility and of a negative crossmatch. From the 20 cadaveric donors, 25 kidney transplants were performed at the Transplant Unit of Clinica Chirurgica University of Rome "Tor Vergata".

Serological Typing

Donors were typed for HLA-A, -B, and -C antigens using cells isolated with anti-CD8 specific monoclonal antibody-coated magnetic microspheres (Dynabeads) in a standard microcytotoxicity assay (NIH).

DNA Extraction

Genomic DNA was isolated from anticoagulated (EDTA) peripheral blood. The DNA was extracted by the salting-out method, precipited with chilled absolute ethanol, and resuspended in 1:20 Tris-EDTA to a final concentration of 50 to 150 μg/mL.

From the Istituto CNR "Tipizzazione Tissutale e Problemi della Dialisi" L'Aquila, and Centro Interregionale Riferimento Trapianti Abruzzo, Molise, L'Aquila, Italy.

Supported in part by C.N.R. grant Special Project ACRO.

Address reprint requests to Domenico Adorno, Istituto CNR Tipizzazione, Tissutale E Problemi Dialisi, P/Le Collemaggio, 67100 L'Aquila, Italy.

0041-1345/97/$17.00
PII S0041-1345(96)00620-3

Genomic Typing

PCR-SSP ARMS analysis. The HLA-A,B,C loci were studied by a sequence-specific primers (SSP) method based on the 3'-mismatch PCR (ARMS) principle (12th IHWS kit, Imperial Cancer Research Fund). Thirty-two primer mixes for split definition of HLA-A alleles (range size: 400 to 813 bp), 27 primer mixes for B alleles (range size: 180 to 784 bp), and 23 for Cw alleles (range size: 162 to 1062 bp), according to XII International Histocompatibility Workshop protocol (IHW), were used. This technique allows us to discriminate 30 HLA-A alleles, 39 HLA-B alleles, and 20 HLA-Cw alleles. Amplifications spanned from exon 2 through exon 3 including the intron of 240 bp in length; the coding primers were specific for sites in exon 2, which codify for the α1 domain, and the noncoding primers were specific for sites in exon 3, which codify for the α2 domain.

Positive internal control primers, amplifing a 256 bp fragment of exon 15 of the adenomatous polyposis coli (APC) gene for HLA-A, B loci and a 796 bp fragment from the third intron of HLA-DRB1 for HLA-C locus, were included in each reaction mix.

The PCR reactions were carried out in 13 μL volumes, according 12th International Histocompatibility Workshop conditions (99.45 ng of target DNA; 1X PCR buffer: 67 mmol/L Tris base, 16.6 mmol/L ammonium sulphate, 0.1% Tween 20; 0.25 mmol/L of each dNTP, 2 mmol/L $MgCl_2$; internal controls primers: locus A 5 μmol/L, locus B 0.8 μmol/L, and locus C 0.1 μmol/L; 0.2–1.0 μmol/L of specific primers; and 0.25 U of *Taq* polymerase).

After a single denaturation step at 96°C for 60 seconds, the amplifications were performed in a Perkin Elmer 9600 thermalcycler splitting the cycling parameters using the following conditions: 96°C for 25 seconds, 70°C for 45 seconds, 72°C for 30 seconds during five cycles; 96°C for 25 seconds, 65°C for 45 seconds, 72°C for 30 seconds in 21 cycles; 96°C for 25 seconds, 60°C for 55 seconds, 72°C for 120 seconds in four cycles. The reactions were completed with a final extension step at 72°C for 10 minutes.

A volume of 12 μL of each PCR reaction plus 1.2 μL of red cresol (1 mg/mL) was run on 2% agarose gel in 1X TAE containing a final concentration of 0.5 μg/mL ethidium bromide and analyzed on a UV transilluminator. The HLA class I typing was defined by the presence or absence of PCR products of defined size in the specific reactions, comparing the size of PCR bands with the molecular weight of the marker VIII (Boehringer Mannheim).

RESULTS

In the cadaveric donor group we found 20 cases carrying 21 serologic blanks in HLA-A and/or -B loci (24.4%); however, the incidence of 18% of HLA-A and -B undetected alleles was also assessed in a healthy bone marrow donor group (unpublished data).

The 20 cadaveric donors investigated by PCR-ARMS showed 10 HLA-A (5.8%) and 11 HLA-B (6.4%) blanks.

As far as locus A, in six cases (60.0%) we were able to define the missed antigens, while four blanks (40.0%) resulted in homozygous combinations. In particular, serologically undetected antigens were revealed as A*3101 (serologically typed as A26,blank), A*01 (A2,blank), A*11 (A1,blank), A*02 (A11,blank), A2301/24 (A9,blank) and A*24 (A2,blank). With regard to locus B, in six cases (54.5%) the second antigen serologically missed was defined, and the specificies were: B*4001 (B12,blank), B*0802 (B18,blank), B*1503-4802 (B21,blank), B*57 (B18,blank), B*41 (B*51,blank), B*5301 (B7,blank); on the other hand, in three samples (27.2%) we observed a sure homozygous situation, and in the last two cases (18.2%), B51/blank and B8/blank serologically typed, an ambiguous combination of HLA-B and -C alleles was evidenced because in our PCR-ARMS kit the presence of Cw*15 allele can mask, in some circumstances, the coexistence of the B*5901, *55, *56, *5401 specificities (Table 1).

Table 1. Serologic HLA-A,-B Blanks Retrospectively Tested by PCR-ARMS

Serologic Typing	PCR-ARMS Typing
Locus A	
2,/	A*02/24
26,/	A*26/3101
2,/	A*02/01
2,/	A*02
2,/	A*02
1,/	A*01/11
9,/	A*2301/24
11,/	A*11/0201
2,/	A*02
24,/	A*24
Locus B	
51,/	B*51,52/(5901?) Cw15
12,/	B*40/44
7,/	B*07
18,/	B*18/0802
21,/	B*4901/1503,4802
18,/	B*18/57
51,/	B*51,52
51,/	B*51,52/41
7,/	B*07/5301
8,/	B*0801/(55,56?) Cw15
14,/	B*14

Note: n = 20.

The examination of missed antigens evidenced that the most of them were the same as in healthy donors[8]: A*01, A19 group specificities and B*0802, B*1503-4802.

The 25 donor-recipient pairs showed the following distribution of HLA-A and B mismatches (MM) on the basis of serologic typing: in four pairs there were 0 MM A+B; in nine pairs 1 MM A+B; in two pairs 2 MM A+B; and three had 3 MM A+B. On the basis of PCR-ARMS typing, the MM distribution changed in: one pair with 0 MM A+B; eight pairs with 1 MM A+B; seven with 2 MM A+B; eight had 3 MM A+B; and one with 4 MM A+B.

Moreover, the degree of HLA-A,B matching in 25 ca-

Table 2. Comparative Table of Serologic and PCR-ARMS Mismatches in 25 Donor-Recipient Pairs

HLA-A,B Mismatches		Serologic Typing (No.)	PCR-ARMS Typing (No.)
0A	0B	4	1
1A	0B	5	4
0A	1B	4	4
2A	0B	5	1
1A	1B	4	6
2A	1B	0	5
1A	2B	3	3
2A	2B	0	1

daveric kidney transplant donor-recipient pairs studied according to PCR-ARMS genotyping allowed us to estimate in three recipient-donor pairs an increase in A locus mismatches. In the B locus we found an increase of mismatches in eight pairs, while in one pair an increase for both A, B loci (Table 2).

DISCUSSION

The advantages of PCR-ARMS class I typing with respect to serologic definition are that this technique may overcome the most common technical problems occurring with serologic typing, such as the requirement of viable cells and the poor quality of specimen. Moreover, serotyping is limited by the lack of availability of alloantisera and monoclonal antibodies to many specificities. Previous biochemical methods, such as one-dimensional isoelectricfocusing (1D-IEF), were able to discriminate more specificities than by serotyping, but it does not result a routine typing assay. In addition, the advent of DNA sequencing revealed the existence of new class I sequences that could not be identify by either serologic or biochemical techniques.[9,10] Only recently, as occurred for HLA class II antigens, a sufficient number of DNA sequences has been available to enable similar development of DNA-based typing for class I genes. Genomic typing has been successfully applied to the class I region, including PCR-based typing on the amplification refractory mutation system or by PCR followed by oligonucleotide probe hybridization.

PCR-ARMS seems to be a method with an useful applicability in definition of class I compatibility in cadaveric kidney transplant for its speed of execution (4 hours including DNA extraction), ease of result interpretation, and the best resolution in allele definition, even if for the present it is limited by relatively low resolution capability and by the difficulty in distinguishing some alleles and some heterozygote forms, depending on the identical reactivities of some primer mixes for certain allele combinations. It would be advisable to raise the number of mixes available to avoid these problems and to have a higher resolution, expecially for HLA-B antigens.

The better definition of HLA-A locus obtained by this molecular method to serologic typing concerned the A19 group, cases in which one allele might mask the presence of the second cross-reactive allele, such as A11 missed allele in a donor serologically typed as A1/blank or A24 missed allele in a A2/blank donor or A23,24 in a A9/blank case. Moreover, antigens that one would expect to identify easily by serology, such as A1 or A2, can be missed but detected by DNA typing, probably for a low expression of a particular antigen.

As regards HLA-B, a more polymorphic locus than HLA-A, it is very important to obtain a better definition because these antigens are mainly involved, together with HLA class II antigens, in the clinical outcome of the transplant. Therefore, the increase of HLA-B mismatches, found retrospectively in our donor-recipient pairs, underlines the importance of PCR-ARMS and suggests its possible application in routine cadaver donor typing.

REFERENCES

1. Cho YW, Cecka JM, Terasaki PI: Clinical Transplants. Los Angeles: UCLA Tissue Typing Laboratory, 1994, p 435

2. Jacobbi LM: Guidelines for specimen collection, storage, and transportation. ASHI Laboratory Manual, Third Edition, 1995

3. Papola F, Valeri M, Torlone N, et al: Transplant Proc 25:2239, 1993

4. Bjorkman PJ, Phaham P: Ann Rev Biochem 253:59, 1990

5. Krausa P, Bodmer JG, Browning MJ: Tissue Antigens 42:91, 1993

6. Saddler AM, Petronzelli F, Krausa P, et al: Tissue Antigens 44:148, 1994

7. Krausa P, Brywka M III, Savage D, et al: Tissue Antigens 45:223, 1995

8. Papola F, Ozzella G, Canossi A, et al: Hum Immunol 47:44, 1996

9. Petersdorf EW, Hansen JA: Tissue Antigens 46:73, 1995

10. Cereb N, Maye P, Lee S, et al: Tissue Antigens 45:1, 1995