Comparative study between serological and PCR-SSP methods to determine class II HLA molecules

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ABSTRACT. The direct detection of HLA polymorphism was an extremely difficult task until a short time ago, mainly for Class II alleles. It has become easier due to Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) technique. Serological and PCR-SSP methods were compared when antigens HLA-DR and –DQ were typed in healthy donors with homozygous phenotype (blanks) and in patients who, for some reason, could not be typed by serological method. This comparative study showed that 21% of healthy donors showing blanks by serological method had their alleles defined by molecular method (PCR-SSP) and that 17% of renal patients with neither proper quantity nor quality of B lymphocytes to define their Class II HLA antigens were all defined by the PCR-SSP method. Molecular typing was adequate for patients with low B lymphocytes quantity and quality. Phenotypic homozygosis index for HLA-DR decreased, HLA-DQ1 splits alleles were better defined, and rare associations between DRB1 and DQB1 were established.

Key words: HLA, microlymphocytotoxicity, PCR-SSP.

RESUMO. Estudo comparativo entre os métodos sorológico e PCR-SSP na determinação das moléculas HLA de classe II. A detecção direta do polimorfismo HLA, que até recentemente era uma tarefa difícil, principalmente para os antígenos HLA de classe II, foi facilitada com o advento da reação de polimerização em cadeia (PCR) na definição de alelos por biologia molecular. Os métodos, sorológico e reação de amplificação em cadeia utilizando-se primer de sequência específica (PCR-SSP), foram comparados na tipificação dos antígenos/alelos HLA-DR e -DQ em doadores saudáveis com o fenótipo de homozigose (“blanks”) e em pacientes que, por alguma razão, não foram possíveis tipificá-los pelo método sorológico. Este estudo demonstrou que 21% dos doadores, apresentando “blanks” pelo método sorológico, tiveram seus alelos definidos pelo método molecular e 17% dos pacientes renais, que não apresentavam quantidade e qualidade adequada de linfócitos B para a definição de seus antígenos HLA de classe II, todos foram definidos pelo método PCR-SSP. A tipagem molecular mostrou-se adequada para tipificar pacientes com baixa quantidade e qualidade de linfócitos B circulantes, diminuindo o índice de homozigose fenotípica para HLA-DR, possibilitando melhor definição dos “splits” de HLA-DQ1 e também evidenciando o estabelecimento de associações raras entre DRB1 e DQB1.

Palavras-chave: HLA, microlinfocitotoxicidade, PCR-SSP.

Introduction

Major Histocompatibility Complex (HLA system in humans) is characterized by an extensive degree of polymorphism. During the last 40 years HLA typing to detect this polymorphism was undertaken by the serological method (Terasaki and Mc Clelland, 1964), better known as the complement-dependent cytotoxicity assay (CDC). This method is still used in some HLA laboratories especially for typifying Class I antigens (HLA-A, -B). However, the isolation of B cells is necessary to type Class II antigens (HLA-DR, -DQ). Until recently such isolation has been achieved by different methods, but typing results were not always reliable (Verduyn et al., 1993; Thorogood et al., 1993). However, the relevance of HLA-DR for solid organ transplantation and stem cells transplantation could be established even with such low reliability degree (Dupont and Hansen, 1978).

A correct assignment of HLA antigens is very important, since inadequate HLA-matching of patient-donor pairs is associated with rejection in
kidney transplants, and rejection or graft-versus-host disease in allogenic bone marrow transplants (Opelz et al., 1991; Kerman et al., 1993). Recent progress in assigning Class II HLA alleles by techniques involving DNA analysis indicates that Class II HLA typing by serology may not be sufficiently reliable (Mytilineos et al., 1990; Olerup et al., 1993).

Quality and availability of antiserum reacting with certain HLA-DR types are among the limitations in Class II HLA typing by serology. Similarly, B lymphocytes obtained by venipuncture in patients may not satisfy minimal criteria needed for successful Class II HLA typing techniques.

Molecular biological methods seemed to be a powerful tool to detect Class II HLA alleles as a substitute for serological methods. Molecular HLA typing, particularly for HLA-DR genes, has shown that there is more polymorphism than can be detected by serological typing methods (Olerup and Zetterquist, 1992; Bryan et al., 1993; Otten et al., 1995; Tiercy, 2002; Velickovic et al., 2004; Balas et al., 2004).

Serology and PCR-SSP for HLA -DR and -DQ typing have been compared in health donors, showing “blanks” in their typing results, and in renal patients who, for some reason, could not be serologically typed.

**Material and methods**

**Subjects**

Samples were obtained from 106 individuals (88 healthy donors and 18 renal patients).

**Typing HLA molecules by the serological method**

HLA serological typing method, called Complement Dependent Cell Microlymphocytotoxicity (Terasaki and McClelland, 1964), employs lymphocytes from peripheral blood since they are a good source of HLA molecules. HLA typing was performed by Special Monoclonal Typing Tray Class II, Locus: DR/DQ (SMDR72, One Lambda Inc., Canoga Park, CA).

**DNA extraction**

Genomic DNA was extracted from peripheral blood leukocytes by Qiamp spin Column (Quigen Inc. Chatsweth, CA), according to manufacturer’s recommendations. DNA concentration was adjusted to 100 ng/mL in bi-distilled water.

**Amplification reaction of genomic DNA**

Amplification was performed by Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) (Newton et al., 1989) employing Micro SSP™ DNA (One Lambda, Inc. Canoga Park, CA, USA). Assay was performed for DNA typing of alleles HLA DRB1*, 3*, 4*, 5* and DQB1*. Pre-optimized primers for the amplification of Class II HLA genes in the reaction were distributed in wells of PCR plate for later addition of DNA samples, Taq polymerase (Invitrogen, Life technologies) and dNTP-buffer mix. After amplification, electrophoresis in agar gel 2.5% was undertaken, at 150 volts, for approximately 5 minutes. A pair of specific primers amplifying a limited region of β-human globulin gene, present in all DNA samples, was used as a positive control of reaction.

**Stataistical analysis**

Statistical analysis was performed using simple kappa coefficient (Landis and Koch, 1977).

**Results**

Serological and molecular PCR-SSP methods were compared for HLA typing in 106 individuals (88 health donors and 18 renal patients). Figure 1 demonstrates that 62% of health donors had concordant results with HLA-DR typing. However, a difference in HLA-DR typing was found in 21% of health donors, whereas in 17% of renal patients (17%) the HLA-DR typing was possible only by PCR-SSP (kappa coefficient = 0.60).

**Table 1.** Discrepancies in HLA-DR typing results between serology and Polymerase Chain Reaction-Sequence Specific Primer methods in 106 individuals.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Serology</th>
<th>PCR-SSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>DR17.X</td>
<td>DRB1*03,*13</td>
</tr>
<tr>
<td>4</td>
<td>DR4,4</td>
<td>DRB1*04,*13</td>
</tr>
<tr>
<td>3</td>
<td>DR11,1</td>
<td>DRB1*11,*13</td>
</tr>
<tr>
<td>1</td>
<td>DR7,1</td>
<td>DRB1*07,*13</td>
</tr>
<tr>
<td>1</td>
<td>DR1,1</td>
<td>DRB1*01,*04</td>
</tr>
<tr>
<td>1</td>
<td>DR11,1</td>
<td>DRB1*08,*11</td>
</tr>
<tr>
<td>1</td>
<td>DR1,1</td>
<td>DRB1*01,*11</td>
</tr>
<tr>
<td>1</td>
<td>DR15,5</td>
<td>DRB1*11,*13</td>
</tr>
</tbody>
</table>
Results showed that among 88 health donors, 21 (23.8%), HLA-DQ1 splits (HLA-DQ5 and HLA-DQ6) were better defined by PCR-SSP methodology (Table 2).

Table 2. Discrepancies in HLA-DQ typing results between serology and Polymerase Chain Reaction-Sequence Specific Primer.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Serology</th>
<th>PCR-SSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DQ1, X</td>
<td>DQ3, 5</td>
</tr>
<tr>
<td>2</td>
<td>DQ1, X</td>
<td>DQ3, 5</td>
</tr>
<tr>
<td>3</td>
<td>DQ1, X</td>
<td>DQ3, 5</td>
</tr>
<tr>
<td>4</td>
<td>DQ1, X</td>
<td>DQ3, 5</td>
</tr>
<tr>
<td>1</td>
<td>DQ1, 7</td>
<td>DQ3, 5</td>
</tr>
<tr>
<td>2</td>
<td>DQ1, 2</td>
<td>DQ3, 5</td>
</tr>
<tr>
<td>3</td>
<td>DQ1, 3</td>
<td>DQ3, 5</td>
</tr>
<tr>
<td>4</td>
<td>DQ1, 3</td>
<td>DQ3, 5</td>
</tr>
</tbody>
</table>

Discussion

Since HLA-DR and DQ typing results obtained by serologic and molecular PCR-SSP methods were compared, we may conclude that PCR-SSP method was more accurate and reliable than serology for DRB1 and DQ typing. Our research showed that 38% of individuals had their typing HLA improved when PCR-SSP method was used. About 21% of the above percentages were healthy donors that had missed alleles and 17% were renal patients that could not be typed by serology due to low viability and small quantity of cells. In the case of HLA-DQ typing, 23.8% of healthy donors could have their HLA-DQ1 better defined and consequently the matching process in the bone marrow receptors could be improved also.

A reliable HLA typing method to select suitable kidney and bone marrow donors is obviously very important. Missed alleles and incorrect assignments by serology may result in incorrect donor selection (Opelz et al., 1991; Wu et al., 2002; Noreen et al., 2001). Patients undergoing haemodialysis for a long period of time could have their B-cell viability decreased and the separation of these cells from blood are affected, making the output small (Mytilineos et al., 1990; Usberti et al., 2002). In current study 17% of renal patients were injured due to delay or inconclusive HLA-DR typing. Consequently they could not be in the waiting list to apply for a cadaver donor. No such problem exists in the case of DNA typing since DNA samples may be easily obtained from fresh blood buffy-coat. HLA-DR typing by the PCR-SSP method may be generated from all patients regardless the quantity or quality of B-cells in their circulation system. In addition, when the number of circulating nucleated cells is too low to get enough DNA, it may be extracted from any nucleated cell source (Otten et al., 1995; Hayney et al., 1996).

Conclusion

The results showed that when the serological and molecular methods are compared for HLA typing, moderate agreement was found (kappa coefficient = 0.60). Nevertheless, molecular method proved to be useful in determining HLA-DR molecules, mainly for patients in which certain problems in serological tests may be expected. PCR-SSP method may detect HLA Class II alleles that could be easily lost or incorrectly determined by the serological technique.

References


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