HLA-DR AND HLA-DQ TYPING: A COMPARATIVE STUDY USING SEROLOGY AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) ANALYSIS

TIPIFICAÇÃO DAS ESPECIFICIDADES HLA-DR E HLA-DQ: ESTUDO COMPARATIVO UTILIZANDO SOROLOGIA, E AVALIAÇÃO DO POLIMORFISMO DO DNA GENÔMICO POR INTERMÉDIO DO TAMANHO DOS FRAGMENTOS GERADOS POR CLIVAGEM COM ENZIMAS DE RESTRIÇÃO

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ABSTRACT: Serology has been used for HLA typing for many decades; however, serological typing of histocompatibility class II molecules depends on the adequate expression of these molecules on the surface of B lymphocytes, the availability of viable cells and a complete set of antisera. HLA typing at the genomic level has supplanted these pitfalls. The utilization of restriction fragment length polymorphism (RFLP) was the first approach to the HLA typing at molecular level. Although serology and RFLP methods define HLA specificities at low resolution level, RFLP has been considered to be better than serology. In this study, we performed HLA class II (HLA-DR and DQ) typing comparing these two methods.

UNITERMS: HLA Antigens. Polymorphism, Restriction Fragment Length.

1. INTRODUCTION

The identification of class II HLA-DR and HLA-DQ molecules on the surface of lymphomononuclear cells has been traditionally carried out by a complement-dependent microcytotoxicity assay using a panel of HLA alloantisera (serology assay). Since HLA class II molecules have a restricted cellular distribution in the peripheral blood B lymphocytes, and due to the unavailability of specific antisera, serology has been gradually replaced by methods with better resolution. Besides serology, HLA class II molecules have also be identified on cell membranes by mixed lymphocyte culture assays (cellular assays), using a panel of cells with previously known phenotypes. However, the assignment of HLA class II specificities using cellular methods has been difficult to compare with those defined by serology. The last decade has been characterized by a vast array of technological changes in terms of HLA typing. Molecular typing methods became available, in particular those using sequence-specific oligonucleotide primers/probes (SSP/SSOP) hybridized to polymerase chain reaction (PCR)-amplified genomic DNA [(1/4)]. These methods are able to type HLA class II alleles at low, medium and high resolution levels and have replaced serology and cellular methods.

Allelic polymorphism arising from nucleotide sequence variation following the digestion of genomic DNA with specific restriction endonucleases, i. e., the restriction fragment length polymorphism (RFLP) analysis was one of the very first molecular methods used for HLA class II typing[5]. Although with low resolution, RFLP typing method is considered to be better than serology [6]. In this study, we assessed HLA class II typing using serology and RFLP analysis, comparing both methods of HLA typing.

2. MATERIAL AND METHODS

2.1. Study population

A total of 115 individuals were studied. Of these, 23 were patients presenting with rheumatic chorea, and 92 were healthy individuals.

2.2. Serology typing

HLA-DR or HLA-DQ typing was carried out by a standard complement-dependent microlymphocytotoxicity assay[7]. Peripheral blood lymphocytes were enriched for B lymphocytes by passage through nylon wool columns. A panel of commercially available HLA antisera (Pel Freez, Gen Track-USA, and Biotest-Germany) which recognized 12 HLA-DR (HLA-DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR10, DR52 and DR53) and 3 HLA-DQ (DQ1, DQ2 and DQ3) antigens was used

2.3. Restriction fragment length polymorphism (RFLP) typing

RFLP typing was carried out as previously described[8]. Shortly, genomic DNA was obtained from peripheral blood cells after digestion with proteinase K, followed by phenol-chlorophorm extraction and ethanol precipitation. Genomic DNA was digested with the specific restriction endonuclease *Taq I* (BRL, USA). Endonucleolytic fragments were submitted to electrophoresis using 1% agarose gels. After electrophoresis, the gels containing fragments of duplex DNA were submitted to *in situ* denaturation and neutralization, and then, the gels were dried onto Whatman 3MM paper. Single-stranded DNA immobilized on dried gel was hybridized with radiolabeled cDNA probes specific for HLA-DRB or DQB genes. Radiolabeled DNA fragments were revealed through exposition to XAR-5 (Kodak, Japan) films. Variation in nucleotide sequence between alleles of HLA-DRB or HLA-DQB genes were reflected by allele-specific hybridization signal patterns. Table I shows schematically the HLA-DRB and DQB RFLP band pattern. A typical hybridization pattern of HLA-DRB and HLA-DQB genes is shown in Figure 1.

2.4. Analysis of data

HLA-DR and HLA-DQ group of alleles detected by serology or RFLP analysis were very similar, i. e., HLA-DR1, DR2, DR3, DR4, DR5, DR6, DR7, DR8, DR9, DR10, DQ1, DQ2 and DQ3. Results of serology and RFLP analyses were compared in terms of concordant or discordant HLA typing. Inconclusive HLA assignment, missed antigen/allele and technical failure were also evaluated.

3. RESULTS

A typical RFLP analysis for HLA-DRB and HLA-DQB alleles is shown in Figure 1. RFLP and serological typing were concordant in 77% of the analyses for HLA-DRB alleles, and in 46% for HLA-DQB alleles. Compared to RFLP, serological typing missed 13% of the HLA-DR alleles, and 38.6% of the HLA-DQB alleles. Discrepant analyses were seen in 9.4% of HLA-DR typings, and in 17% of the HLA-DQ typings. Both RFLP and serology failed to

Table I - *Taq I* bands detected with HLA-DRβ and HLA-DQβ cDNA probe

HLA-DR	HLA-DRβ	HLA-DQβ
HLA-DR1	7,9	2
HLA-DR2	2,4, 16 (common) or 1, 14, 15 (rare)	6 (common) or 2 (rare)
HLA-DR3	2,5, 10 or 11 or 3, 5, 10 or 11	3,7
HLA-DR4	1,7, 8,12	8 (common) or 3(rare)
HLA-DR5	2,6, 10 or 11	3
HLA-DR6	2,5, 10 or 11	6
HLA-DR7	1, 7, 11, 12 or 1, 5, 11, 13	1 or 8, or 1, 7
HLA-DR8	4	8 (common) or 3 (rare)
HLA-DR9	1, 7, 11, 12 or 1, 5, 11, 13	5, 7 or 3 (rare)
HLA-DR10	17, 9	3

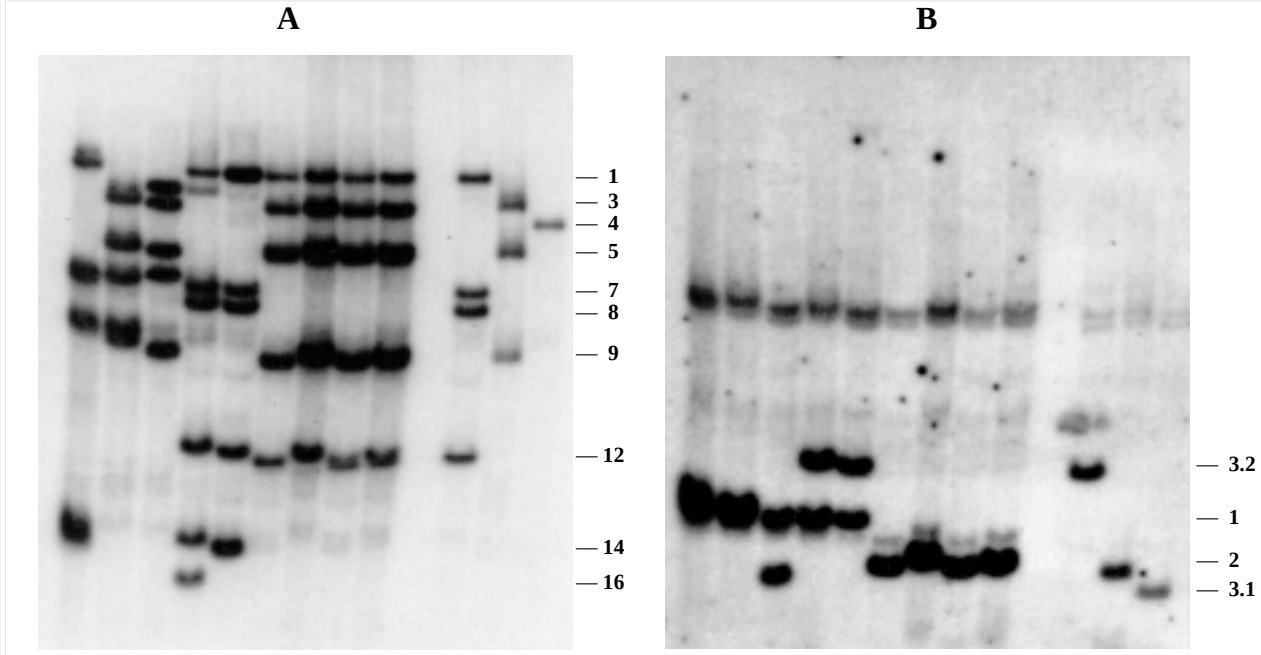

Figure 1: Illustration of 2 Restriction fragment length polymorphism analyses. Panel **A** shows a HLA-DRB typing. *The number of the bands is shown on the right side.* Lanes 1 to 9 (from the left) represents patients and healthy individuals of this study, and lanes 1 to 3 (from left to right) represents homozygous control individuals. Control #1 shows bands 1, 7, 8 and 9 compatible with HLA-DR4, control #2 shows bands 3, 5 and 10 compatible with HLA-DR3/6, and control #3 shows only band 4 compatible with HLA-DR8.(See Table 1 for band patterns). Panel **B** shows HLA-DQ typing. *The allele assignment is shown on the right side*. Lanes 1 to 9 (from the left) represents patients and healthy individuals of this study, and lanes 1 to 3 (from left to right) represents homozygous control individuals. Control #1 represents a HLA-DQ3.2 individual (HLA-DQ8 by serology and HLA-DQB1*0302 by the new nomenclature), control #2 represents a HLA-DQ2 individual (HLA-DQ2 by serology and DQB1*02 new nomenclature), and control #3 represents a HLA-DQ3.1 individual (HLA-DQ7 by serology and HLA-DQB1*0301 new nomenclature).

type HLA-DR and DQ alleles in 5.9% of the cases. Some of the ambiguities for discriminating alleles were due to different bands which were not currently identified. Major ambiguities seen for both serology and RFLP analysis was related to the distinction between HLA-DR3 and HLA-DR6 alleles.

4. DISCUSSION

HLA-DR antigen assignment by serology presents several technical problems including: **a)** lack of specific typing antisera panel, **b)** crossreactivity between HLA-DR antigens, **c)** unavailability of HLA-DR molecule expression due to underlying diseases such as chronic myelocytic leukemia, influence of drugs which interfere with the expression of the molecule on lymphocyte membrane such as chloramphenicol, **d)** unavailability of viable B lymphocytes or inadequate number of these cells. Molecular methods do not depend on viable B cells; however, RFLP typing method has some drawbacks, it is time-consuming, it requires at least one week for each HLA-DR or DQ typing, and involves the use of radioactive reagents [2, 6].

Although serology and RFLP assays are low resolution methods for HLA typing, RFLP analysis, as observed in this study, was more accurate than serology. The ambiguity between HLA-DR3 and DR6 specificities was not resolved by any of the 2 methods employed in this study. The discrimination between these allele was possible only after using other molecular methods such as sequence specific oligonucleotide probe (SSOP) or sequence specific primer (SSP) analysis or by sequence based typing (SBT). The HLA typing using these methods showed that most of the HLA-DR3/DR6 antigen/alleles, as typed by serology or RFLP, were in fact HLA-DRB1*13 or *14, i.e., splits of the HLA-DR6 group (results not shown). The comparison of HLA-DR typing using serology and

RFLP analysis conducted by other investigators on a large group of individuals showed results similar to ours, i. e., RFLP discriminated more alleles than serology [6]. In addition, other studies encompassing large number of individuals, comparing HLA typing by serology or other molecular methods such as SSP or SSOP have also reported better results with molecular methods [4, 9, 10]. Nowadays, serological HLA class II typing has been almost completely replaced by molecular methods.

The correct assignment of HLA alleles is very important for adequate selection of donors for solid organ or hematopoietic stem cell transplantation, for disease association and evolution studies. In a large retrospective study encompassing 3455 cadaver kidney transplants, Opelz et al (1993) compared the effect of HLA-DR matching by serology and DNA-RFLP analysis. The HLA-DR matching using DNA-RFLP analysis improved significantly the one-year graft survival rate [11], emphasizing the relevance of correct HLA class II assignment for graft survival.

Among the molecular methods currently used, the SSP, SSOP and SBT certainly are the most employed. Molecular methods have many advantages compared to serology. The probe and primer reagents used to identify HLA-DR and DQ alleles function uniformly in a highly reproducible way. Probes and primers can be produced in unlimited amounts[4]. The resolution power of these methods is almost unlimited, new alleles can be assigned, and ambiguities can be easily discriminated. The most recent list regarding HLA class II alleles encompasses 221 HLA-DRB1 alleles, 39 HLA-DQB1, 19 HLA-DQA1, 15 HLA-DPA1 and 84 HLA-DPB1 alleles[12]. Although high resolution methods are currently in use, recently developed methods using DNA-arrays have further improved HLA typing in terms of resolution level and amount of tests performed at each time.

DONADI EA; PAULA-SANTOS CM & NEPOM GT. Tipificação das especificidades HLA-DR e HLA-DQ: estudo comparativo utilizando sorologia e avaliação do polimorfismo do DNA genômico por intermédio do tamanho dos fragmentos gerados por clivagem com enzimas de restrição. **Medicina, Ribeirão Preto, 33:** 27-31, jan./mar. 2000.

RESUMO: Embora as tipificações sorológicas dependam da expressão adequada de moléculas HLA de classe II, nas superfícies celulares, da viabilidade celular e da presença de um painel adequado de anti-soros, esse método tem sido utilizado há muitos anos e as tipificações por biologia molecular têm suplantado os problemas. A avaliação do polimorfismo dos genes HLA por intermédio da variação do tamanho dos fragmentos gerados pós digestão com enzimas de restrição (RFLP) foi o primeiro método molecular a ser utilizado para esse fim. A sorologia e o método utilizando RFLP definem os alelos HLA sem muita resolutividade, no entanto, o método utilizando RFLP tem sido considerado melhor do que a sorologia. Assim, neste estudo, fizemos análise das tipificações dos antígenos/alelos HLA de classe II (HLA-DR e HL-DQ), comparando os dois métodos.

UNITERMOS: Antígenos HLA. Polimorfismo de Fragmento de Restrição.

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