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HLA-DMB in Amerindians: Specific linkage of DMB*01:03:01/DRB1 alleles

Antonio Arnaiz-Villena [a,*], Jose Palacio-Grüber [a], Ester Muñiz [a], Diego Rey [a], Maria Jose Recio [b], Cristina Campos [a], Narcisa Martinez-Quiles [b], Jose Manuel Martin-Villa [b], Jorge Martinez-Laso [c]

[a] Department of Immunology, University Complutense, School of Medicine, The Madrid Regional Blood Center, Madrid, Spain
[b] Department of Immunology, University Complutense, School of Medicine, Madrid, Spain
[c] Immunogenetics and Molecular Diagnosis Unit, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain

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ABSTRACT

Background: HLA-DMB proteins are important for intracellular microbial metabolism in order other major histocompatibility complex (MHC) molecules present peptides to lymphocytes. In addition, HLA-DMB alleles have been found linked to diseases in some ethnic groups and HLA-DMB molecules may be important to explain HLA disease association.
Objective: To detect HLA-DMB alleles profile in Amerindians for the first time and compare them to other populations. This will establish the bases to study HLA-DMB linkage to disease in Amerindians.
Method: A group of 168 voluntary Amerindians have been typed for HLA-DMB alleles. They have been characterized both, by genetic and genealogical bases. Cloning and automated HLA-DMB DNA (exons 2, 3 and 4) sequencing have been performed for allele assignation.
Results: HLA-DMB*01:01:01 and HLA-DMB*01:03:01 show the highest frequencies. These have been compared to other World wide populations. HLA-DMB*01:03:01 is tightly associated to certain specific HLA-DRB1 alleles in Amerindians.
Conclusion: The specific Amerindian HLA-DMB allele frequencies and their linkage disequilibrium with other MHC alleles may be crucial to determine HLA-DMB World wide variation, evolution and specific linkage to disease in Amerindians and other populations.

1. Introduction

Class II major histocompatibility complex (MHC) molecules are glycoproteins that play an essential role in the immune system. Five families of class II MHC genes have been described in humans (HLA-DR, -DQ, -DP, -DM, and -DO [1]). Each one of them are constituted by a pair of genes named A and B (i.e.: MHC-DMB) that encode α and β chains, respectively. HLA-DR, -DQ and -DP are cell-surface glycoproteins with a high degree of polymorphism and present antigenic peptides to CD4 T lymphocytes. HLA-DM proteins are almost absent from the surface of antigen presenting cells and they accumulate in certain intracellular compartments, including the MIIC (MHC class II compartment) [2,3]; this is mostly driven by a tyrosine-based signal that is placed in the cytoplasmic tail of the DMβ chain and corresponds to residue 230 at exon 5 [4], this signal is defined as ITIM (Immunoreceptor tyrosine-based inhibitory motif). MHC-DMB molecules that present and do not bear these motifs are found both in humans [5] and primates [6]. Since these ITIM motifs may be important for stopping (presence) or continuing (lack) MHC-DR molecules antigenic peptides loading, functionality of MHC-DMB directed Class II presentation may be variable according to differences in alleles. It might influence still unknown Class II presentation metabolism details, necessary to start new responses by T-cells and also influence HLA pathologies associated to diseases [5,6] and HLA transplant and pharmacogenomics physiopathology [5,6]

The role of MHC-DM molecules [2,4,7] has been described as a peptide exchange factor required to help the removal of endogenous invariant chain (class II-associated invariant chain peptides or CLIP) from antigen presenting class II (HLA-DR, -DQ, and -DP) αβ-CLIP complexes in the MIIC compartment, facilitating the generation of class II complexes that can accommodate antigenic peptides. These chains will later be exported to the cytoplasmatic

* Corresponding author at: Departamento de Inmunología, Facultad de Medicina, Universidad Complutense, Pabellón 5, planta 4. Avda. Complutense s/n, 28040 Madrid, Spain.
E-mail address: arnaizantonio@gmail.com (A. Arnaiz-Villena).

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membrane [8–10]; this process is also modulated by HLA-DO molecules that inhibit HLA-DM-mediated catalysis of MHC-II peptide exchange. In addition, it has been put forward that MHC-DM may play a role in the release of peptides other than CLIP, having a potential function as a peptide editor that selects a certain high-stability subpopulation of peptides for presentation at the cell surface [11]. Following this postulated function, MHC-DM molecules would not have to bind the peptide itself in order to promote peptide exchange on class II molecules. Rather, they would act as chaperones interacting with the class II αβ heterodimers promoting the release of low-stability peptides, thereafter stabilizing empty class II heterodimers in the acidic pH of the MIIC and facilitating a subsequent loading until a high-stability peptide occupies the groove [11]. These findings led to different authors to consider that editing by HLA-DM molecules is a key aspect for T cell epitope selection [12]. P1 pocket of the peptide binding groove has been recently described as a crucial factor for resistance to HLA-DM editing since peptides that retain this pocket occupancy are resistant to HLA-DM [13,14]. However, MHC Class II conformational changes could be considered as a key feature determining HLA-DM susceptibility to removal [12]. These characteristics could explain the limited polymorphism of the HLA-DM molecule, in contrast to classical HLA class II molecules [7,15].

On the other hand, thirteen HLA-DMB alleles that encoded for seven proteins have been identified [15]. Changes in the genetic polymorphism of HLA-DM molecule may lead to changes in its molecular structure and function, thus possibly affecting antigen presentation of classical MHC class II molecules. Therefore, certain HLA-DM alleles are regarded as candidates to disease-susceptibility genes, in pathologies such as rheumatoid arthritis [16–18], psoriasis [19], systemic lupus erythematosus [20] or type I diabetes [21].

On the other hand, a massive migration to Spain has occurred from South America in the last 10 years, particularly from rural Andean populations (and also from Santo Domingo, Hispaniola Island). In Madrid region, about 10% of the population was Amerindian by 2011 [22,23]. Classical HLA genes in Amerindians are very different from those of the rest of the World, including Spaniards [24,25]

Greenberg first postulated the triple migration theory for explaining the peopling of the Americas across the Bering Straight from Siberia [26]: Amerindians (most North and South American Indians; 12,000 years BP), Na-Dene (Athabaskans, Navajo, Apache; 8000 years BP) and Eskimo-Aleuts (6000 years BP). Other authors have postulated different numbers of migration waves based on HLA, Y, mtDNA and other genetic markers, and also a Trans-Pacific and Trans-Atlantic people input to America has been put forward [25,27–38].

However, Amerindian populations have never been typed for HLA-DMB allele polymorphism. In the present work, we address the question of obtaining HLA-DMB allele frequencies in Amerindians in order to compare them with the distribution of this polymorphism in other World populations and to establish epidemiology Preventive Medicine programmes of diseases linked to HLA-DM and find out possible new variants, since Amerindian shows different and particular HLA allele sets which are different to the rest of the World [24,25,39].

2. Material and methods

2.1. Population sample

168 healthy unrelated Amerindian were HLA-DMB typed. Samples were collected from volunteer Amerindian blood donors at The Madrid Regional Blood Center. A written consent to participate in the present study was signed by each individual blood donor. All were born in an isolated rural or tribal community, and their ancestors were traced there at least for two generations in the same place of origin (Andean or other Iberian-American country; for more origin details see Ref. [23]). Seven other populations were also included in the analyses for comparison of HLA-DMB allele frequencies, and belong to different ethnic groups (Europeans and Asians) (see Results).

2.2. DNA extraction and HLA typing

Genomic DNA from peripheral blood mononuclear cells was extracted with the Nucleic Acid Isolation System QuickGene-810 (Fujifilm, Japan) by using reagents and protocols provided by the manufacturer (QuickGene Whole Blood Extraction Kit S, Fujifilm, Japan). DNA amplification of exons 2 and 3 of HLA-DMB was carried out separately by PCR for sequence-based HLA-DMB typing method using specific forward primers (exon 2 DMB2AMPA: 5′-TATTGCCAGGCCAGTAGAAG-3′, and exon 3 PL DMBEx3: 5′-TGCAAGTAGCCAAAACCACTCC-3′) and specific reverse primers (exon 2 DMB2AMPB: 5′-ACTCACGTCAGCCACCTTGT-3, and exon 3 PR DMBEx3: 5′-CCAGTCCCGAAGGATGGGCT-3′) according to previously described protocols [40,41]. The PCR conditions consisted of 35 cycles of 94 °C, 30 s; 59 °C, 30 s; and 72 °C, 35 s for exon 2 and 35 cycles of 94 °C, 30 s; 54.5 °C, 30 s; and 72 °C, 35 s for exon 3 in a programmable heat block (Mastercycler epgradient S, Eppendorf, Germany). The PCR products obtained were electrophoresed in a 2% agarose, detected by staining with ethidium bromide and confirmed with a molecular weight marker (Roche Diagnostics GmbH, Mannheim, Germany). Amplified products were sequenced in both directions using the ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Sequence of both strands was determined using Chromas 2.31 software and HLA-DMB polymorphism was identified by aligning the sequences with identified alleles [15] using Mega 5 software [42]. If any doubt arose standard DNA cloning was carried out [40]. HLA class II (DRB1 and DQB1) genotyping was performed as referenced in [23].

2.3. Statistical analyses

Both HLA-DMB allele frequencies and HLA class II haplotypes frequencies (DRB1-DMB, DQB1-DMB and DRB1-DQB1-DMB) in Amerindians were calculated with Arlequin 3.0 computer program [43]. This program was also used to test the Hardy–Weinberg equilibrium and linkage disequilibrium (LD) [44,45]. Allele frequencies in different populations were compared by a Chi-squared test. Statistical significance was defined as $p < 0.05$. These analyses were performed using the SPSS/PC statistical program (version 17.0 for Windows; SPSS, Inc., Chicago, IL, USA). World wide HLA allele frequencies were in part obtained of www.allelefrequencies.net.

3. Results

3.1. Distribution of HLA-DMB allele frequencies in Amerindian population and comparison with other World populations: novel findings

Amerindian population was found to be in Hardy–Weinberg equilibrium with respect to HLA-DMB ($P < 0.05$). Allele frequencies are shown in Table 1. In this study, a total of five HLA-DMB alleles were detected. The two HLA-DMB alleles with the highest frequencies were HLA-DMB*01:01:01 and HLA-DMB*01:03:01, while the rest of alleles, HLA-DMB*01:02, HLA-DMB*01:04 and HLA-DMB*01:07, showed frequencies lower than 3%, and were detected only in eight, four and eight individuals, respectively. Amerindian