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HLA-*A*, -*B*, and -*DRB1* allelic and haplotypic diversity in a sample of bone marrow volunteer donors from Rio Grande do Sul State, Brazil

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ABSTRACT

The HLA *A, B*, and *DRB1* allele, phenotype, and haplotype frequencies were studied in a sample of 5,000 volunteer bone marrow donors registered at the Brazilian Volunteer Bone Marrow Donor Registry. The participants live in the state of Rio Grande do Sul and were classified according to ethnic group (4,428 Caucasians, 324 mestizos [mixed race], and 248 blacks). Typing was performed using the polymerase chain reaction sequence-specific oligonucleotide method combined with Luminex technology. Twenty-one *HLA-A*, 33 *HLA-B*, and 13 *HLA-DRB1* allele groups were identified. The most frequent allele groups for each locus were A*02, B*35, and DRB1*13. The most frequent haplotypes were A*01 B*08 DRB1*03 in Caucasians and mestizos and A*02 B*15 and DRB1*04 in blacks. The allele frequencies were compared with samples from different Brazilian regions. In most comparisons no significant differences were found. The most significant differences were observed in the comparison of the groups of our sample, indicating that human leukocyte antigen (HLA) is a good marker to distinguish among people from different ethnic groups. The data provide insight on the knowledge of HLA diversity in the population of Rio Grande do Sul and in the search for a better match for transplant.

1. Introduction

Human leukocyte antigen (HLA) typing is an important immunologic tool, as well as a valuable asset in the investigation of risk factors for diseases and in organ transplant programs. These genes are located in the major histocompatibility complex region and encode cell surface molecules, which play an important role in the presentation of antigens and the recognition of specific "nonself" and "self" antigens [1].

The transplant of bone marrow and solid organs is an alternative therapy to some diseases and its success depends mostly on the HLA compatibility between a donor and a recipient. Despite the advancements in immunosuppressive therapies and pretransplant investigation using more sensitive methods for the detection of anti-HLA antibodies, allele typing and understanding of the allelic diversity are still important [2]. This system is characterized by great polymorphism, which is an obstacle in the search for a compatible donor. Some theories were developed to explain this feature. One model suggests that this variability is maintained by positive selection of these molecules, which has been occurring since the early stages of human evolution by means of local antigen stimuli to which the populations have been exposed [3,4]. The HLA system is also characterized by extensive linkage disequilibrium, which has an important clinical application. Patients with haplotypes in linkage disequilibrium are more likely to find a compatible donor [5]. Therefore, the investigation of such alleles and haplotypes provides insight on the study of genetic composition and the origin of different populations. Brazil has an extremely diversified population, and the frequencies of alleles may vary according to the predominant ethnic group in the studied region. Previous studies were conducted on HLA frequency in Brazilian regions such as the states of Paraná [6–8], São Paulo [9–12], Rio de Janeiro [13], Minas Gerais [14], Goiás [15], Piauí [16], and Pernambuco [17]. Some of the data available in the literature were obtained by serologic typing methods. Studies on samples from individuals of the 5 continents have revealed differences between the identified HLA alleles and their consequent increase in mixed-race populations [18,19]. A comparative study of 5 different Brazilian samples, including an analysis of a group of individuals from Rio Grande do Sul composed of Caucasians and blacks, has demonstrated that comparison of these populations reveals more similarities than differences [15]. When that study was published, the World Health Organization Nomenclature Committee for factors of the HLA system had 97 specificities serologically defined. In 2010, more than 4,000 *HLA-A*, *HLA-B*, and *DRB1* alleles had been identified in the world population [20]. With the advancements in typing tech-

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niques, the introduction of molecular biology methods, and the increased number of new alleles identified, such information requires updating.

Rio Grande do Sul is the southernmost state in Brazil, with an area of approximately 282,000 km^2 and a population estimated at more than 10 million. The capital, Porto Alegre, has 1,409.939 inhabitants. This Brazilian region is characterized by a strong influence of European settlers. The population of Rio Grande do Sul is classified as 81.4% Caucasian, 5.0% black, 13.3% mulatto, and 0.3% Oriental or Amerindian [21]. Information on the diversity of HLA alleles enables us to estimate the probability of a patient on the transplant waiting list finding a donor with better immunologic compatibility and contributes to studies on population genetics and disease-related studies. Knowledge of the phenotypic frequencies of these alleles is essential to determine HLA antibody panel reactivity. This test is performed in the pretransplant investigation, where the anti-HLA antibodies present in the recipient's serum are defined. In past years, advances in these tests have made it possible to detect antibodies using solid-phase tests, which do not necessarily contemplate an HLA distribution that is representative of the assessed population [22], because they mostly include the global frequency of HLA alleles. Knowledge of the data on phenotypic frequency makes it possible to determine the real reactivity represented by a percentage value based on the recipient's population group.

The purpose of this study was to determine the phenotype, allele, and haplotype frequencies of *HLA-A*, *-B*, and *-DRB1* in a sample of volunteer bone marrow donors from Rio Grande do Sul State and to compare the allele frequencies of the ethnic groups within our sample as well as to published data related to different Brazilian populations.

2. Subjects and methods

2.1. Sample

The current study included a sample of 5,000 volunteer bone marrow donors registered at the Transplant Immunology Laboratory at Santa Casa Hospital in Porto Alegre from January 2006 to October 2008. Sixty-nine percent of the donors were women, and donors' ages varied between 18 and 54 years. Most individuals in the sample live in different municipalities of the mesoregion of the Porto Alegre metropolitan area (http://www.ibge.gov.br/). The sample was divided into subsamples according to the type of ethnicity as informed by the donor. Therefore, 3 different groups were generated: Caucasians (n = 4,428), blacks (n = 248), and mestizos (individuals of European, African, and Amerindian origin, n = 324). All donors signed an informed consent. The samples used for comparisons between different Brazilian states and their characteristics were as follows: Paraná (n = 3,500, Caucasians, blacks, Orientals, and mestizos) [8], São Paulo (n = 103, Caucasians) [11], Minas Gerais (n = 95, Caucasians) [14], Piauí (n = 97, mestizos) [16], and Pernambuco (n = 101, Caucasians and non-Caucasians) [17]. These studies were selected because they are the most recent publications on the Brazilian population that facilitate comparison because they all use molecular typing methods. The study was approved by the University Research Ethics Committee.

2.2. DNA extraction and typing

DNA was extracted using a QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Typing was performed using the Luminex multianalyte system with the LABType SSO commercial kit (One Lambda, Inc., Canoga Park, CA). In this polymerase chain reaction biotinylated primers are used. For the HLA class I amplification (locus A and B) 2 primers are used, 1 for exon 2 and the other for exon 3. For the class II amplification (locus DRB1) a single primer is used for exon 2. The amplified biotinylated product is subjected to a hybridization process with specific sequences of given HLA alleles. These sequences are conjugated to the surface of microspheres (beads) coded by colors that are part of the Luminex system. After the hybridization stage, a streptavidin–phycoerythrin conjugate solution is added, which binds itself to the biotinylated product. The reaction is read using advanced flow cytometry (LABScan 100). Analyses of the allele typings were performed using HLA Fusion v 1.2.1. software (One Lambda, Inc.). Although this methodology allows the identification of some alleles, in most cases only the allelic groups were identified. Therefore, only the referred information was used in our analysis.

2.3. Statistical analysis

The allele and haplotype frequencies and verification of Hardy–Weinberg equilibrium were calculated using Arlequin v 3.11 software [23]. The expectation–maximization algorithm was used to determine haplotype frequencies, as described by Excoffier and Slatkin [24]. This method allows the estimate of random haplotype frequencies based on the allelic frequencies of the sample. Hardy–Weinberg equilibrium was verified using the method of Guo and Thompson [25]. The phenotypic frequencies were calculated by direct count with the aid of SPSS 17.0 software (Chicago, IL). The significance of the differences between the allelic frequencies in our sample and in samples of other Brazilian populations was calculated by χ^2 test with Yates' correction using PEPI 4.0 software [26]. Allele frequencies >0.05 were used as a cutoff to compare frequencies among populations. Bonferroni's correction was used with an initial significance level of $p < 0.05$.

3. Results and discussion

The *HLA-B* and *-DBR1* loci were in Hardy–Weinberg equilibrium ($p > 0.05$). In the B locus the observed heterozygosity was 92.8% and the expected heterozygosity was 93.2%. For the DRB1 locus it was 88.7 and 89.6%, respectively. The values observed for the A locus were 86.3 and 87.4%. However, a significant difference was observed for the A locus between the observed and expected heterozygosity (p = 0.003). This difference can perhaps be explained by the size of the sample because it was not observed in the separate analysis of the ethnic groups. In the analysis of the total sample, 21 *HLA-A,* 33 *HLA-B*, and 13 *HLA-DRB1* allelic groups were identified. Table 1 presents the allele and phenotype frequencies in the total sample for the 3 studied loci. The *HLA-A*02* allele was the most frequent during the entire analysis. In locus A the *HLA-A*03*, *-A*24*, and *-A*01* alleles presented more than 10% of the frequency. In locus B the most common allelic groups were *HLA-B*35*, *-B*44*, *-B*51*, and *-B*15*, and in locus *DRB1* the *HLA-DRB1*13*, *-DRB1*07*, *-DRB1*04*, and *-DRB1*11* alleles were the most common. The data indicate a more representative contribution of alleles of European origin [27–29]. By contrast, the data also indicate the occurrence of alleles of African influence, such as *HLA-B*15*.

Table 2 demonstrates the comparison of these frequencies between the ethnic groups studied in our population. Bonferroni's correction reduced the significance from $p < 0.05$ to $p < 0.0024$ for locus A, $p < 0.0015$ for locus B, and $p < 0.0038$ for locus DRB1.

The frequency of the *HLA-A*02* allele was greater in Caucasians (28.2%). Nevertheless, no significant differences were observed in the comparison of the frequency of this allele among Caucasians, mestizos, and blacks. Some alleles of locus A have exhibited different frequencies in the comparison between ethnic groups. Among them, the *HLA-A*23* and *-A*30* alleles presented 8.0 and 9.6% of frequency in blacks compared with Caucasians (3.7 and 3.1%) and mestizos (4.6 and 5.0%). However, a significant difference was observed only in the comparison between Caucasians and blacks for these alleles after Bonferroni's correction. There was a difference in the *HLA-A*01* allelic group in the comparison between blacks and