Original article

Frequency of alleles and haplotypes of the human leukocyte antigen system in Bauru, São Paulo, Brazil

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ABSTRACT

Background: HLA allele identification is used in bone marrow transplant programs as HLA compatibility between the donor and recipient may prevent graft rejection.

Objective: This study aimed to estimate the frequency of alleles and haplotypes of the HLA system in the region of Bauru and compare these with the frequencies found in other regions of the country.

Methods: HLA-A*, HLA-B*, and HLA-DRB1* allele frequencies and haplotypes were analyzed in a sample of 3542 volunteer donors at the National Registry of Voluntary Bone Marrow Donors (REDOME) in Bauru. HLA low resolution typing was performed using reverse line blot with the Dynal Reli™ SSO-HLA Typing Kit and automated Dynal AutoReli™48 device (Invitrogen, USA).

Results: Twenty, 36, and 13 HLA-A*, HLA-B*, and HLA-DRB1* allele groups, respectively, were identified. The most common alleles for each locus were HLA-A*02, HLA-B*35, and HLA-DRB1*07. The most frequent haplotype was A*01-B*08-DRB1*03. Allele and haplotype frequencies were compared to other regions in Brazil and the similarities and differences among populations are shown.

Conclusion: The knowledge of the immunogenic profile of a population contributes to the comprehension of the historical and anthropological aspects of different regions. Moreover, this helps to find suitable donors quickly, thereby shortening waiting lists for transplants and thus increasing survival rates among recipients.

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DOI: 10.5581/1516-8484.20140026
Introduction

The major histocompatibility complex (MHC) is a system composed by genes that encode the human leukocyte antigen (HLA) molecules. HLA genes are part of a polygenic complex located on the short arm of chromosome 6 in the 6p21.3 region. HLA genes are expressed by all nucleated cells of the human body. The function of this system is the presentation of antigenic peptides to T lymphocytes in order to trigger the proliferation and differentiation of cells capable of activating a specific immunological response.

The HLA class I molecules (HLA-A, HLA-B, and HLA-C) display endogenous antigens to CD8+ cytotoxic T lymphocytes, while the HLA class II molecules (HLA-DR, HLA-DQ and HLA-DP) present exogenous antigens to CD4+ T-helper lymphocytes.

HLA molecules differ among individuals, and these differences are associated with solid organ rejection after organ transplantation as well as with graft-versus-host disease (GVHD) after bone marrow transplantation. The identification of HLA alleles is the main tool used in bone marrow transplant programs as HLA compatibility between the donor and recipient is necessary to prevent graft rejection.

In Brazil, the National Voluntary Bone Marrow Donor Registry (REDOME) was founded in 1993 with the aim of gathering data of volunteer donors who want to donate bone marrow to patients requiring a transplant. REDOME, with about 3 million people registered, is currently considered the third largest bone marrow donor program; the United States has the world’s largest bank followed by Germany.

Similar to REDOME, the National Registry of Bone Marrow Recipients (REREME) stores information about patients waiting for bone marrow transplants. These patients are added to the registry when a matching donor is unavailable.

REDOME is important because it increases the chance of finding a matching donor for a particular recipient. In Brazil, the probability of genetic compatibility among unrelated donors is small due to miscegenation. The probability of finding a recipient compatible with an unrelated donor is about 1:100,000, and the probability of a sibling being compatible is 25%.

The genetic characterization of individuals leads to a rapid selection of unrelated donors for recipients; thus, waiting lists for transplantation and possibly graft rejection decreases, thereby improving patient survival.

In addition to the role in transplantation and susceptibility or resistance to diseases, HLA is also important for anthropological studies as the frequency of alleles varies between ethnic groups. For example, HLA-B*35 is most often found in Caucasians, whereas HLA-B*15 is common in Africans.

The population of Brazil is a good example of miscegenation. Colonization and immigration have made the population diverse; the genetic information of native Amerindians has mixed with that of Europeans and Africans brought to Brazil as slaves. Immigrants from other parts of the world, such as Germans, Arabs, Italians, Spanish and Japanese have since come to Brazil to find better living conditions; these immigrants have helped constitute the new genetic profile of Brazilians.

According to the demographic census carried out by the Brazilian Institute for Geography and Statistics (IBGE), the population of the city of Bauru in 2010 was estimated to be 343,937, the majority of city residents were White (70.66%), followed by Mulatto (22.69%), Black (4.95%), Asian (1.57%), and Amerindian (0.13%). Until 1850, the region was inhabited solely by Amerindians, but pioneers from Minas Gerais and São Paulo explored this land but did not adopt the slavery system which was prevalent in other regions of the state until 1868.

The distribution of HLA variants has been analyzed in different Brazilian regions and throughout the world. Monte et al. established the frequencies of HLA alleles in Teresina, Piauí and concluded that multiracial people, the dominant ethnic group in this region, have predominately Caucasian and African genes with a small proportion of Amerindian genes.

Bortolotto et al. compared the HLA alleles of ethnic groups from different regions of Brazil and noticed some similarities and differences among them. The HLA-A*02 allele was common in populations from Rio Grande do Sul and Paraná, regions mostly populated by Caucasians. The frequency of this allele was also high in mulattos from Piauí; however, the HLA-B*15 allele, common in Black people, was found in Caucasians, Blacks, and Mulattos.

Middleton et al. and Williams et al. analyzed the distribution of the HLA-A and HLA-B alleles, respectively, in diverse populations including Brazilians. Both studies showed common alleles among the studied populations, thus indicating the extent of miscegenation possibly due to migration.

The characterization and determination of population allele frequencies is important because this can identify whether these predominant alleles exist in a specific region, if they are shared with other populations and if they are related to disease susceptibility or protection.

Through HLA class I (HLA-A and -B) and class II (HLA-DRB1) allele typing from the registry of volunteers (REDOME) of Bauru, São Paulo, we aimed to estimate the frequencies of HLA alleles and haplotypes prevalent in the region, and to compare the frequencies of these alleles to those of other regions of Brazil.

Methods

Population

Among donors listed in the REDOME from 2008 to 2012, 3542 volunteer bone marrow donors were selected and evaluated at the Immunogenetics Laboratory of the Instituto Lauro de Souza Lima in Bauru. Volunteers were evaluated according to gender, age, marital status, ethnic group and HLA type.

Results from published studies on the populations of Ribeirão Preto (n = 184), São Paulo (n = 239), Paraná...
(n = 2775),20 Rio Grande do Sul (n = 5000),11 Minas Gerais (n = 1000),20 Pernambuco (n = 101),20 and Piauí (n = 97)12 were used to compare HLA frequencies.

The study was approved by the Ethics Committee of the Instituto Lauro de Souza Lima, Bauru.

**Extraction of DNA and HLA typing**

DNA was isolated by the salting-out method using venous blood kept in ethylenediaminetetraacetic acid (EDTA) anticoagulant.21

The typing of HLA class I alleles (loci A* and B*) and class II alleles (locus DRB1*) was performed by the reverse line blot technique at a low resolution (Dynal Reli™ SSO-HLA Typing Kit and Dynal AutoReli™48, Invitrogen, USA).

The DNA samples were amplified using specific biotinylated primers to each HLA region (0.5 µM) using 20 mM of Tris-HCl solution (with 30% glycerol and 100 mM KCl), dNTPs (400 µM of dATP, dCTP, and dGTP; 800 µM of dUTP), Taq polymerase (100 μ/mL), and sodium azide (0.05%). Samples underwent 35 cycles at 95°C, 60ºC, and 72°C denaturation, annealing and extension temperatures, respectively. After amplification by polymerase chain reaction (PCR), the amplicons were chemically denatured and added to a nylon membrane containing sequence-specific oligonucleotide probes. The amplicons marked with biotin hybridized with the corresponding probes with the complementary target sequence and were observed using a colorimetric reaction; conjugated streptavidin, hydrogen peroxide and tetramethylbenzidine (TMB) substrate were added. Perfect matching program (PMP) software was used for interpretation.

**Statistical analysis**

Allele frequencies were obtained by direct count and the haplotype construction was performed using a probabilistic computational model. The distribution of gene frequencies in the population was checked using the Hardy-Weinberg equilibrium, and the analysis of haplotypes was performed using the Arlequin software version 3.1.22 Statistical differences between populations were determined by the chi-square test with the SISA online software.23 Significance was set at a p-value of ≤ 0.05.

**Table 2 - Frequencies of the HLA-A, B and DRB1 alleles in the Bauru population.**

| HLA-A | AF | HLA-B | AF | HLA-DRB1 | AF |
|-------|----|-------|----|----------|----|
| A*01  | 0.088 | B*07  | 0.061 | DRB1*01  | 0.100 |
| A*02  | 0.263 | B*08  | 0.050 | DRB1*03  | 0.092 |
| A*03  | 0.094 | B*13  | 0.020 | DRB1*04  | 0.092 |
| A*11  | 0.055 | B*14  | 0.051 | DRB1*07  | 0.149 |
| A*23  | 0.045 | B*15  | 0.078 | DRB1*08  | 0.047 |
| A*24  | 0.098 | B*18  | 0.051 | DRB1*09  | 0.015 |
| A*25  | 0.012 | B*27  | 0.024 | DRB1*10  | 0.018 |
| A*26  | 0.034 | B*35  | 0.120 | DRB1*11  | 0.129 |
| A*29  | 0.042 | B*37  | 0.011 | DRB1*12  | 0.017 |
| A*30  | 0.058 | B*38  | 0.021 | DRB1*13  | 0.129 |
| A*31  | 0.042 | B*39  | 0.032 | DRB1*14  | 0.043 |
| A*32  | 0.034 | B*40  | 0.047 | DRB1*15  | 0.111 |
| A*33  | 0.029 | B*41  | 0.013 | DRB1*16  | 0.041 |
| A*34  | 0.006 | B*42  | 0.012 |       |    |
| A*36  | 0.004 | B*44  | 0.106 |       |    |
| A*66  | 0.007 | B*45  | 0.014 |       |    |
| A*68  | 0.055 | B*46  | 0.0004 |      |    |
| A*69  | 0.001 | B*47  | 0.002 |       |    |
| A*74  | 0.008 | B*48  | 0.005 |       |    |
| A*80  | 0.001 | B*49  | 0.028 | B*50  | 0.027 |
|       |       |       |    | B*51  | 0.083 |
|       |       |       |    | B*52  | 0.019 |
|       |       |       |    | B*53  | 0.021 |
|       |       |       |    | B*54  | 0.001 |
|       |       |       |    | B*55  | 0.009 |
|       |       |       |    | B*56  | 0.003 |
|       |       |       |    | B*57  | 0.029 |
|       |       |       |    | B*58  | 0.024 |
|       |       |       |    | B*59  | 0.001 |
|       |       |       |    | B*67  | 0.001 |
|       |       |       |    | B*71  | 0.001 |
|       |       |       |    | B*73  | 0.001 |
|       |       |       |    | B*78  | 0.001 |
|       |       |       |    | B*81  | 0.002 |
|       |       |       |    | B*82  | 0.001 |

AF: allele frequency.
Discussion

The present study evaluated the frequency expression of HLA, class I (HLA-A* and B*) and class II (HLA-DRB1*) alleles and haplotypes of bone marrow donors registered in the REDOME in Bauru.

We found that the donors were predominantly White, female and tended to be young adults. Our results agree with the demographics reported for this city as presented by the IBGE census\(^ {15} \) and by a study of the northern region of Paraná State by Bardi et al.\(^ {24} \)

The most frequent alleles observed in Bauru were HLA-A*02, HLA-B*35, and HLA-DRB1*07. Similar to this study, the most common allele found in all Brazilian populations (Ribeirão Preto,\(^ {20} \) São Paulo,\(^ {20} \) Paraná,\(^ {20} \) Rio Grande do Sul,\(^ {11} \) Minas Gerais,\(^ {20} \) Pernambuco\(^ {20} \) and Piauí\(^ {12} \)) was HLA-A*02.

The frequency of the HLA-B and HLA-DRB1 alleles differed between certain regions. For HLA-B, the most frequent allele was HLA-B*44 in Ribeirão Preto;\(^ {20} \) and São Paulo;\(^ {20} \) and this was the second most common allele in the current study. In Pernambuco,\(^ {20} \) the HLA-B*42 allele was the most common, however this allele was rare in our population. Moreover, the HLA-B*07 allele was the most common in Piauí\(^ {12} \) and the fifth most common in our study.

The frequency of the HLA-DRB1*07 allele was the same for our region as well as for Ribeirão Preto;\(^ {20} \) however, the HLA-DRB1*13 allele was the most common in São Paulo;\(^ {20} \) Rio
### Table 4 - Statically significant differences of the HLA-B alleles in the Bauru population compared with the populations of Ribeirão Preto, São Paulo, Paraná, Rio Grande do Sul, Minas Gerais and Piauí.

| Locus | Bauru (n = 3542) | Ribeirão Preto (n = 184) | São Paulo (n = 239) | Paraná (n = 2775) | Rio Grande do Sul (n = 5000) | Minas Gerais (n = 1000) | Pernambuco (n = 101) | Piauí (n = 97) |
|-------|----------------|--------------------------|-------------------|------------------|--------------------------|--------------------------|------------------|-----------------|
|       | AF             | p-value                  | AF                | p-value          | AF                       | p-value                  | AF               | p-value         |
| B*07  | 0.0561         | 0.058                    | 0.073             | 0.069            | 0.133                    | 0.066                    | 0.099            | 0.0423          |
| B*08  | 0.0503         | 0.049                    | 0.036             | 0.055            | 0.118                    | 0.0135                   | 0.047            | 0.015           |
| B*13  | 0.0200         | 0.016                    | 0.010             | 0.022            | 0.031                    | 0.0293                   | 0.016            | 0.020           |
|       |                |                          |                   |                  |                          |                          |                  |                 |
| B*14  | 0.0511         | 0.077                    | 0.0479            | 0.067            | 0.036                    | 0.0001                   | 0.103            | 0.060           |
| B*15  | 0.0787         | 0.060                    | 0.102             | 0.080            | 0.163                    | 0.077                    | 0.124            | 0.0283          |
| B*35  | 0.1200         | 0.126                    | 0.089             | 0.0372           | 0.113                    | 0.236                    | 0.115            | 0.074           |
| B*38  | 0.0211         | 0.016                    | 0.030             | 0.028            | 0.0142                   | 0.048                    | 0.015            | 0.005           |
| B*40  | 0.0477         | 0.047                    | 0.040             | 0.048            | 0.098                    | 0.037                    | 0.037            | 0.044           |
| B*41  | 0.0133         | 0.016                    | 0.020             | 0.008            | 0.036                    | 0.021                    | 0.018            | 0.015           |
| B*42  | 0.0120         | 0.025                    | 0.005             | 0.004            | 0.0001                   | 0.011                    | 0.0001           | 0.198           |
| B*45  | 0.0144         | 0.011                    | 0.020             | 0.010            | 0.0150                   | 0.023                    | 0.034            | 0.0001          |
| B*47  | 0.0002         | 0.000                    | 0.002             | 0.000            | 0.0001                   | 0.0002                   | 0.001            | 0.015           |
| B*48  | 0.0088         | 0.005                    | 0.005             | 0.005            | 0.001                    | 0.003                    | 0.003            | 0.0001          |
| B*49  | 0.0281         | 0.027                    | 0.020             | 0.021            | 0.0069                   | 0.054                    | 0.031            | 0.040           |
| B*53  | 0.0211         | 0.027                    | 0.040             | 0.0124           | 0.012                    | 0.0001                   | 0.030            | 0.039           |
| B*54  | 0.0012         | -                        | 0.005             | 0.000            | 0.0317                   | -                       | -                |                 |
| B*55  | 0.0090         | 0.008                    | 0.010             | 0.021            | 0.013                    | 0.005                    | 0.005            | 0.031           |
| B*57  | 0.0297         | 0.025                    | 0.062             | 0.0001           | 0.031                    | 0.058                    | 0.037            | 0.040           |
| B*58  | 0.0244         | 0.036                    | 0.005             | 0.0669           | 0.015                    | 0.0002                   | 0.040            | 0.025           |
| B*73  | 0.0001         | -                        | 0.000             | 0.001            | 0.003                    | 0.0463                   | -                | -               |
| B*81  | 0.0013         | 0.011                    | 0.0362            | 0.000            | 0.0192                   | 0.0001                   | 0.0001           | 0.005           |
| B*82  | 0.0013         | -                        | 0.000             | 0.000            | 0.001                    | 0.001                   | -                |                 |

HLA: human leukocyte antigen; AF: allele frequency; -: untyped alleles.

Statistical differences set for p-values ≤ 0.05.

### Table 5 - Statically significant differences of the HLA-DR alleles in the Bauru population compared with the populations of Ribeirão Preto, São Paulo, Paraná, Rio Grande do Sul, Minas Gerais and Piauí.

| Locus | Bauru (n = 3542) | Ribeirão Preto (n = 184) | São Paulo (n = 239) | Paraná (n = 2775) | Rio Grande do Sul (n = 5000) | Minas Gerais (n = 1000) | Pernambuco (n = 101) | Piauí (n = 97) |
|-------|----------------|--------------------------|-------------------|------------------|--------------------------|--------------------------|------------------|-----------------|
|       | AF             | p-value                  | AF                | p-value          | AF                       | p-value                  | AF               | p-value         |
| DRB1*04 | 0.0922         | 0.113                    | 0.115             | 0.122            | 0.0001                   | 0.233                    | 0.0001           | 0.115           |
| DRB1*07 | 0.1497         | 0.137                    | 0.115             | 0.199            | 0.0001                   | 0.246                    | 0.0001           | 0.142           |
| DRB1*08 | 0.0477         | 0.060                    | 0.052             | 0.048            | 0.119                    | 0.0004                   | 0.048            | 0.186           |
| DRB1*09 | 0.0155         | 0.038                    | 0.0028            | 0.028            | 0.011                    | 0.0216                   | 0.031            | 0.018           |
| DRB1*10 | 0.0182         | 0.025                    | 0.015             | 0.011            | 0.0008                   | 0.030                    | 0.023            | 0.031           |
| DRB1*11 | 0.1290         | 0.129                    | 0.125             | 0.130            | 0.220                    | 0.0001                   | 0.108            | 0.0121          |
| DRB1*13 | 0.1297         | 0.137                    | 0.147             | 0.117            | 0.0387                   | 0.256                    | 0.157            | 0.0016          |
| DRB1*14 | 0.0432         | 0.022                    | 0.038             | 0.038            | 0.082                    | 0.031                    | 0.0132           | 0.093           |
| DRB1*15 | 0.1111         | 0.110                    | 0.094             | 0.090            | 0.0001                   | 0.173                    | 0.0001           | 0.108           |
| DRB1*16 | 0.0411         | 0.016                    | 0.0220            | 0.032            | 0.033                    | 0.0108                   | 0.070            | 0.0217          |

HLA: human leukocyte antigen; AF: allele frequency.

Statistical differences set for p-values ≤ 0.05.
Table 6 - Comparison of the most common haplotypes in the population of Bauru with the populations of Paraná and Rio Grande do Sul.

| Haplotype       | Bauru | Paraná | p-value<sup>a</sup> | Rio Grande do Sul | p-value<sup>a</sup> |
|-----------------|-------|--------|---------------------|-------------------|---------------------|
| A*01 B*08 DRB1*03 | 0.019 | 0.028  | 0.0003              |                   |                     |
| A*03 B*07 DRB1*15 | 0.006 | 0.013  | 0.0001              |                   |                     |
| A*02 B*44 DRB1*01 | 0.004 | 0.008  | 0.0022              |                   |                     |
| A*02 B*15 DRB1*04 | 0.002 | 0.007  | 0.0001              |                   |                     |
| A*02 B*44 DRB1*07 | 0.004 | 0.007  | 0.0248              |                   |                     |
| A*02 B*44 DRB1*07 | 0.011 | 0.007  | 0.0353              |                   |                     |

<sup>a</sup> p-value ≤ 0.05.

Conclusion

The HLA compatibility between donors and recipients is essential for a successful bone marrow transplantation. Therefore, the estimation of the immunogenic profile in our region and other regions in Brazil can assist in targeting, developing, and maintaining the REDOME database. The identification of the frequencies of HLA alleles within a nation is important because it eases the burden of searching compatible donors, decreases the wait for transplants and therefore increases the chances of survival of the recipients. In addition, knowledge of HLA frequencies can improve our understanding of the historical and anthropological composition of populations, that may also allow for areas of interest in each region to be identified.

Conflicts of interest

The authors declare no conflicts of interest.

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