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Original article

Frequency of Wrª antigen and anti-Wrª in Brazilian blood donors

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ABSTRACT

Background: Wrª is a low-incidence antigen, which is antithetical to the high prevalence red blood cell antigen, Wrᵇ. Anti-Wrª is a naturally occurring antibody that is found in approximately 1–2% of blood donors. The aim of this study was to determine the frequency of Wrª and anti-Wrª in Brazilian blood donors.

Methods: A total of 1662 Brazilian blood donors were molecularly analyzed using the SNaP-shot methodology to determine the WR*A/B alleles and to predict the frequency of the Wrª antigen. To detect the anti-Wrª, samples from 1049 blood donors were analyzed using a gel test with Wr(a+) red blood cells. The serum was treated with dithiothreitol (DTT) to determine the immunoglobulin classes. Immunoglobulin (Ig)-G isotype classification was performed in a gel test using the IgG1/IgG3 card. A monocyte monolayer assay was employed to predict the clinical significance of IgG anti-Wrª.

Results: Of the 1662 donors, only one sample had the DI*02.03 allele in heterozygous predicting the Wr(a+b+) phenotype. Anti-Wrª was detected in 34 (3.24%) samples, 64.7% in females and 35.3% in males. Regarding the immunoglobulin class, eight (23.5%) cases of anti-Wrª were classified as IgG and 26 (76.5%) as IgM. Of the eight cases of IgG anti-Wrª, four were IgG1, two were IgG3 and three anti-Wrª were not IgG3 or IgG1, and thus probably IgG2 or IgG4. The results of the monocyte monolayer assay showed that IgG anti-Wrª might be of clinical significance.

Conclusion: This study shows a very low frequency (0.06%) of the Wrª antigen in Brazilian blood donors. Additionally, it shows that the frequency of anti-Wrª in this population is higher than previously reported.

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Introduction

The Diego blood group system is carried on band 3, a multipass membrane glycoprotein, which is encoded by the *SLC4A1* gene. The Diego system is composed of 22 antigens: three pairs of antithetical antigens, Di^a and Di^b, Wr^a and Wr^b, Wu and DISK, and 16 very low frequency antigens.[1] Wr^a and Wr^b antigens are related to a SNP in exon 16 (1972G>A) that encodes a Lysine in Wr^a or a glutamic acid in Wr^b at amino acid position 658.[2]

The Wr^a antigen, first described by Holman in 1953, has an incidence of around 1 in 1000 in Caucasian populations, but it is not reported in other ethnic groups.[3] Although the Wr^a antigen has a very low incidence, anti-Wr^a is a relatively common antibody since it is often a naturally occurring antibody.[4] The described incidence of anti-Wr^a in the sera of normal donors varies in different studies; it has been estimated at 1 of 100 in healthy volunteer blood donors.[5] The immunoglobulin (Ig) class of anti-Wr^a can be IgM, IgG or IgM plus IgG. Alloanti-Wr^a is rarely involved in hemolytic transfusion reactions, however there are some cases reporting hemolytic disease of the fetus and newborn (HDFN) caused by anti-Wr^a.[1]

Antibodies against low-incidence antigens, including anti-Wr^a, are difficult to identify, because the screening and panel cells rarely express these antigens.[6,7] Hence, little is known about the frequency of anti-Wr^a in many populations. The knowledge of the molecular basis of the Diego blood group system and the development of molecular assays to identify the *DI* alleles has allowed the frequency of these alleles to be assessed in different populations. The aim of this study was to determine the frequency of the Wr^a antigen and anti-Wr^a in a Brazilian population of blood donors.

Methods

A total of 1662 blood samples were obtained from healthy volunteer Brazilian blood donors at the Associação Beneficente de Coleta de Sangue (Colsan), São Paulo, Brazil. The population studied was from Southeast of Brazil and it is composed of a highly admixed population.

Molecular analysis

DNA was extracted using the QIAmp DNA Mini Kit (Qiagen® Inc. Valencia, CA, USA) according to the manufacturer's instructions. To determine the *WR*A and *WR*B* alleles and predict the frequency of the Wr^a antigen, genotyping was performed using a previous described SNaPshot® protocol (Latini et al.[8]). Fragment analyses were performed in a 3500xL Genetic Analyzer (Applied Biosystem, Foster City, CA, USA) as shown in Figure 1.

Antibody screening

In order to investigate the occurrence of anti-Wr^a, serum samples from 1049 blood donors (638 male and 411 female donors) were initially cross-matched with a Wr(a+) red blood cell (RBC) from our collection in a gel test by an automated system (WADiana® 8XT, Grifols, Barcelona, Spain). The presence of anti-Wr^a in positive cross-matches was confirmed with two sources of Wr(a+) RBCs from commercial panels (BioRad®, Lagoa Santa, Brazil).

Immunoglobulin classes

To determine the Ig classes (IgG or IgM), the serum was treated with dithiothreitol (DTT, Sigma-Aldrich, Brazil). The IgG isotype classification was performed in a gel test using the IgG1/IgG3 card (BioRad®, Lagoa Santa, Brazil).

Monocyte monolayer assay

To predict the clinical significance of anti-Wr^a, the monocyte monolayer assay (MMA) was performed as previously described[9] in two samples with anti-Wr^a classified as IgG1 and one sample classified as IgG3. Using an optical microscopy, 600
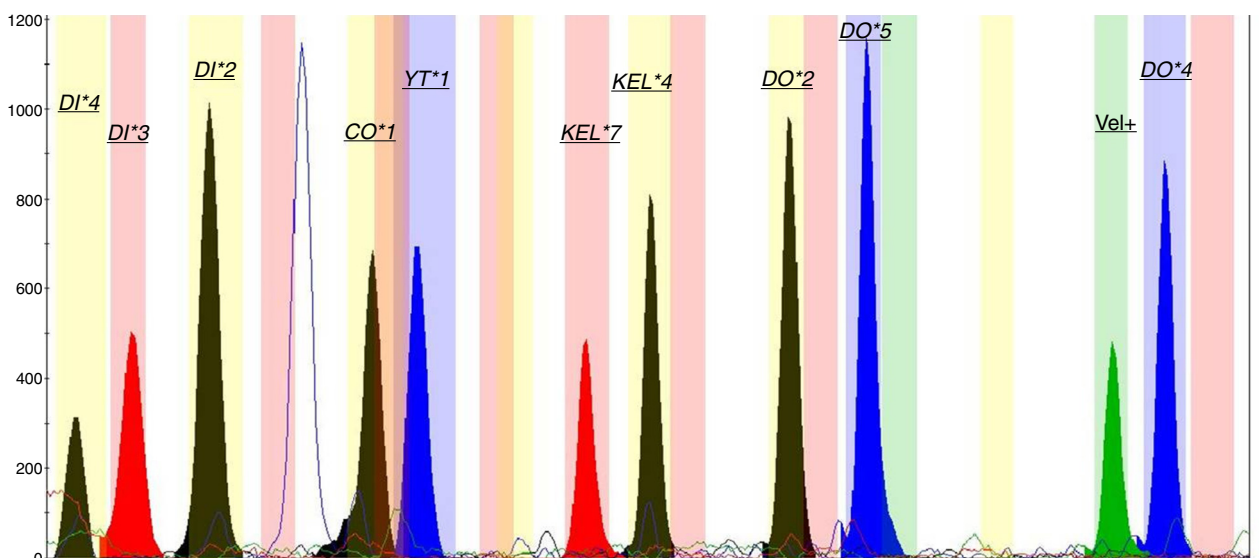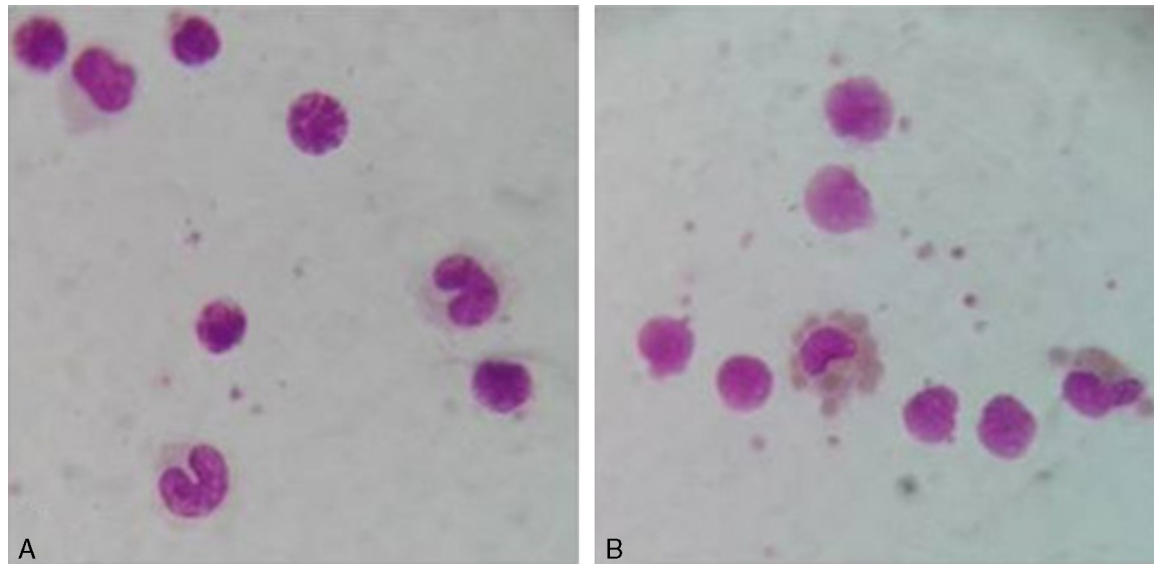

Figure 1 – GeneMapper electropherogram of representative SNaPshot fragment in the analysis of the Wr(a+) donor.

Figure 2 – Monocyte monolayer assay slides (1000×). (A) Negative control and (B) Monocyte monolayer assay with anti-Wrᵃ.

monocytes were counted to determine the percentage of reactive monocytes (RBC adhered and phagocytized). MMA results <4% were considered negative while results ≥4% were considered positive.

Results

Wrᵃ antigen

Of the 1662 genotyped blood samples, only one sample presented the *WR*A* allele in heterozygous. It was genotyped as *WR*A/WR*B* predicting the Wr(a+b+) phenotype.

Anti-Wrᵃ

Anti-Wrᵃ was detected in 34 samples from 1049 screened blood donors representing a frequency of 3.24%. Regarding the Ig classes, 8/34 (23.5%) were IgG and 26/34 (76.5%) were IgM. Of the eight IgG anti-Wrᵃ, four were classified as IgG1 and one was isotyped as IgG3. Three samples were not classified as IgG1 or IgG3; these are probably IgG2 or IgG4, Ig classes that are not involved in severe transfusion reactions. As shown in Table 1, a higher frequency of anti-Wrᵃ was observed in female donors ($p = 0.0036$, Fisher's exact test).

Table 1 – Frequency of anti-Wrᵃ and immunoglobulin (Ig) class.

Ig class	Isotype	Donor		Total
		Female	Male	
IgG	IgG1	3	1	4
	IgG3	0	1	1
	IgG2 or IgG4	3	0	3
IgM	–	16	10	26
Total		22	12	34

MMA results (Figure 2) show that Wrᵃ antibodies classified as IgG can potentially be clinically significant, as IgG1 antibodies presented 7–7.5% of reactive monocytes and 12.7% of IgG3 had reactive monocytes.

Discussion

This study shows novel information regarding the presence of anti-Wrᵃ in a Brazilian population of blood donors. Although the frequency of the Wrᵃ antigen (1:1662) is lower than that previously reported in Europeans (1:1000),[3] the occurrence of anti-Wrᵃ was higher (1:31) when compared to other studies where it ranged from 1 in 80 to 1 in 200. The frequency of anti-Wrᵃ found in this study is similar to that found in Spain (1:37), however the presence of the antigen in Spanish population is around 2-times (1:785)[6] the frequency found in Brazilians.

The mechanisms involved in anti-Wrᵃ production are still unclear. Some authors believe that, besides the alloimmunization in response to antigen exposure, certain proteins that can cross-react with the Wrᵃ antigen are formed when the immune system becomes more active.[7] Situations described to be involved in anti-Wrᵃ alloimmunization are also related to immune system activation, including pregnancy, autoimmune hemolytic anemia and patients with other RBC antibodies.[7] Therefore, our hypothesis is that the difference in anti-Wrᵃ distribution between genders could be associated to pregnancy, as anti-Wrᵃ was found in 5.2% of women and 1.8% of men.

The nature of alloimmunization might determine the antibody behavior. Our results comprising Ig class showed that IgM anti-Wrᵃ was the predominant class, corroborating with the hypothesis of it being a naturally occurring antibody. On the other hand, four IgG1 and one IgG3 anti-Wrᵃ with possible clinical significance were identified. Even though anti-Wrᵃ is described to rarely cause HDFN or hemolytic transfusion reactions, probably due to the fact that anti-Wra are usually nonimmune antibodies[10] 1.4% of anti-Wrᵃ found in this study

can be of clinical significance. Due to the low incidence of the Wra antigen and the low risk of hemolytic transfusion reaction, the use of screening panels containing Wr(a+) RBCs is not required. Thereby Wr^a incompatible transfusion can occur, but few cases of hemolytic transfusion reaction were described, been estimated to be 1 in 500,000.[11]

In summary, the Wr^a antigen has a very low frequency in Brazilian blood donors and anti-Wr^a has a higher frequency than reported in other populations. Considering the low frequency of the antigen and the few cases of mild HDFN related to anti-Wr^a, clinical impact is discussable as well the requirement of RBC reagent to identify them.

Conflicts of interest

The authors declare no conflicts of interest.

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