The purpose of this article is to review the literature about the Diego blood group system and to highlight anthropological studies, genetic studies, serologic characteristics of Diego antigens and antibodies, and their clinical importance in certain ethnic groups.

History and Anthropological Studies

In 1956 a low-incidence red cell antigen responsible for hemolytic disease of the newborn (HDN) was described with the aid of an antibody produced by a mother living in Caracas, Venezuela. This antigen was named Diego (Di\(^a\)) after the proposita, and the corresponding antibody was named anti-Di\(^a\).\(^1\) A study of the Diego family showed that the antigen was present in 10 of 33 members in four generations (see Fig. 1).\(^1,3\) During this study it was observed that some family members showed physical features of admixture with native Indians, many of whom are Di(a+).\(^4\) The Di\(^a\) antigen may not be completely absent in a Caucasian population free from admixture with Indians, since the second example of anti-Di\(^a\) was found in the serum of the mother of an infant with HDN from a Polish family living in New York.\(^1,2,5\) Some believe that the finding of anti-Di\(^a\) in a Polish mother and the Di\(^a\) antigen on the red cells of her husband, who is also Polish, is indicative of Mongoloid admixture, and the high incidence of the B gene in the Polish population is supportive evidence of this admixture.\(^5,4\) It was proposed that if the genetically related Di\(^b\) and anti-Di\(^b\) exist, the antibody should be sought among the homozygotes of genotype Di\(^a\)Di\(^a\) of the Caribe Indians.\(^1\)

The third example of anti-Di\(^a\) also caused HDN, this time in a family of Spanish ancestry, "Mart," living in the Dallas, Texas, area. The fourth example of anti-Di\(^a\) was described in a Puerto Rican woman, whose prematurely born twins did not develop HDN, although their red blood cells (RBCs) were direct antiglobulin test (DAT) positive. In this family there were six Di(a+) members in four generations, and there was Caribe Indian admixture. The fifth example was a naturally occurring anti-Di\(^a\) in a Caucasian woman, whose serum agglutinated RBCs from a Di(a+) blood donor. Four of her relatives of Irish origin were Di(a+). The next example of anti-Di\(^a\), in an Austrian family, caused HDN and was the first example in which alloimmunization occurred during a first pregnancy.\(^1,2,5,7\) It was concluded from these studies that Di\(^a\) is not exclusive to Mongoloids but occurs also as a low-incidence antigen in Caucasians.

Since American Indians are considered to be anthropologically related to the Mongoloid people, individuals of Chinese and Japanese descent living in Venezuela were studied. The presence of Di\(^a\) in six South American Indian populations studied, as well as in Chinese and Japanese, suggests that this gene is Mongoloid rather than American Indian. Further findings of the Di\(^a\) antigen in Chippewa Indians in Northern Minnesota and in Japanese in Winnipeg also suggested that Di\(^a\) may be an Asiatic (Mongoloid) characteristic.\(^5\) The phenotye and genotype frequencies of the Diego system in Mongoloid, Caucasian, Negroid, and hybrid Venezuelan populations are given in Table 1.\(^3,8\)

In 1956 it came as a surprise when no Di(a+) person was found among 156 Eskimos tested in the Eastern Canadian Arctic.\(^9\) Further studies on Eskimo populations showed a very low incidence of Di\(^a\).\(^10,12\) The Alaska Indians are the only American Indians that do not have at least 5 percent Di(a+) persons.\(^13\) In the study of the Canadian Indian population by Buchanan et al.,\(^13\) the Di\(^a\) antigen incidence decreased from 3 percent in northern Alberta to 0 percent in Inuvik in far northern Canada. Other studies found that the incidence of Di\(^a\) was 3–4 percent in northern Japan and 5–7 percent in southern Japan.\(^14,16\) All of these studies suggest that Di\(^a\) is an Asiatic characteristic.

Nearly 1374 Australian aborigines and 1741 New Guineans were tested for Di\(^a\) from 1967 to 1970 and
all were negative, indicating the absence or very low incidence of this antigen in these racially distinct populations. In 1986 a study by Edwards-Moulds and Alperin showed a high incidence (14.7, 8.2, and 8.9%) of Dia in three Mexican-American communities in Texas, probably reflecting their Indian ancestry. Of 4225 Mexican-Americans tested, six were negative for Dib (the antithetical high-incidence antigen), giving the Di(a+b-) phenotype an incidence of 0.14 percent. The calculated gene frequencies were 0.05–0.08 for Dia and 0.92–0.96 for Dib. In 1990 a study by Lee et al. showed that Diego typing of the Chippewa tribe in Minnesota had patterns similar to those reported in other North American Indian populations.

In 1992 a study from Poland showed that the incidence of Dia in the southeastern region of Poland (0.91%) is greater than in the western region (0.25–0.37%). The southeastern region was invaded by Tartars during the 13th as well as the 15th to 17th centuries. Therefore, admixture with Mongoloid genes in this population cannot be excluded.

**Terminology**

A summary of the phenotypes, genotypes, antigens, and antibodies of the Diego blood group system is given in Table 2. The terminology recommended by the International Society for Blood Transfusion (ISBT) is shown in Table 3.

**Genetics**

Studies on the inheritance of Dia, conducted in the original Diego family and 40 other Indian and hybrid families, showed that the antigen is inherited as a dominant character with no linkage either to the sex chromosome or to the following red cell antigens: ABO, MNSs, Kk, Fy, Jk, Le, and P. A study

![Fig. 1. The Diego family tree carrying the Di antigen.](image-url)
Table 1. The phenotype and gene frequencies of Di\(^a\) and Di\(^b\) in Mongoloid, Caucasian, Negroid, and hybrid Venezuelan populations

<table>
<thead>
<tr>
<th>Populations</th>
<th>Number tested</th>
<th>Di((a^+))</th>
<th>Di((a^-))</th>
<th>Di(^a) (%)</th>
<th>Di(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaingangues Indians (Brazil)</td>
<td>48</td>
<td>45.83</td>
<td>54.17</td>
<td>26.44</td>
<td>73.56</td>
</tr>
<tr>
<td>Caribe Indians (Venezuela)</td>
<td>36</td>
<td>36.11</td>
<td>63.89</td>
<td>20.07</td>
<td>79.93</td>
</tr>
<tr>
<td>Guanibo Indians (Venezuela)</td>
<td>170</td>
<td>29.41</td>
<td>70.58</td>
<td>16.00</td>
<td>84.00</td>
</tr>
<tr>
<td>Parroa Indians (Venezuela)</td>
<td>76</td>
<td>14.47</td>
<td>85.52</td>
<td>7.50</td>
<td>92.50</td>
</tr>
<tr>
<td>Japanese</td>
<td>24</td>
<td>12.50</td>
<td>87.50</td>
<td>6.50</td>
<td>93.50</td>
</tr>
<tr>
<td>Chippewa Indians (Canada)</td>
<td>77</td>
<td>7.79</td>
<td>92.21</td>
<td>5.55</td>
<td>94.45</td>
</tr>
<tr>
<td>Japanese</td>
<td>148</td>
<td>10.81</td>
<td>89.19</td>
<td>5.56</td>
<td>94.44</td>
</tr>
<tr>
<td>Negroid (Curiepe, Venezuela)</td>
<td>150</td>
<td>7.33</td>
<td>92.67</td>
<td>3.74</td>
<td>96.26</td>
</tr>
<tr>
<td>Ciudad Bolivar Indians</td>
<td>100</td>
<td>7.00</td>
<td>93.00</td>
<td>3.57</td>
<td>96.43</td>
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<tr>
<td>Arawaco Indians (Venezuela)</td>
<td>152</td>
<td>5.26</td>
<td>94.73</td>
<td>2.80</td>
<td>97.20</td>
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<tr>
<td>Chinese (Canton)</td>
<td>100</td>
<td>5.00</td>
<td>95.00</td>
<td>2.54</td>
<td>97.46</td>
</tr>
<tr>
<td>Negroid (Yaracuy, Venezuela)</td>
<td>119</td>
<td>5.36</td>
<td>96.64</td>
<td>2.70</td>
<td>98.30</td>
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<tr>
<td>Barcelona</td>
<td>61</td>
<td>3.28</td>
<td>96.72</td>
<td>1.65</td>
<td>98.35</td>
</tr>
<tr>
<td>Caracas Indians</td>
<td>500</td>
<td>2.00</td>
<td>98.00</td>
<td>1.01</td>
<td>99.99</td>
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<tr>
<td>Pregonero Indians</td>
<td>148</td>
<td>0.67</td>
<td>99.33</td>
<td>0.34</td>
<td>99.66</td>
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<tr>
<td>Poles (1992)</td>
<td>9661</td>
<td>0.46</td>
<td>99.54</td>
<td>0.71</td>
<td>99.29</td>
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<td>Poles (1957)</td>
<td>200</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
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<tr>
<td>Americans (U.S.)</td>
<td>1000</td>
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<td>100.00</td>
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<tr>
<td>Italians</td>
<td>400</td>
<td>0.00</td>
<td>100.00</td>
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<td>100.00</td>
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<tr>
<td>Spaniards</td>
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<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Dutch</td>
<td>200</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Russians</td>
<td>200</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
<tr>
<td>Hungarian</td>
<td>200</td>
<td>0.00</td>
<td>100.00</td>
<td>0.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Note: Further population studies can be found in: Mourant AE, Kopec AC, Domaniewska-Sobczak K. The distribution of the human blood groups. 2nd ed. London: Oxford University Press, 1976.

Table 2. The Diego blood group system

<table>
<thead>
<tr>
<th>Classification</th>
<th>Symbols</th>
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<tbody>
<tr>
<td>Genes</td>
<td>Di(^a) and Di(^b)</td>
</tr>
<tr>
<td>Genotypes</td>
<td>Di(^a)/Di(^b), Di(^a)/Di(^b), Di(^a)/Di(^b)</td>
</tr>
<tr>
<td>Phenotypes</td>
<td>Di(a+b-), Di(a+b+), Di(a-b+), Di(a-b-)</td>
</tr>
<tr>
<td>Antibodies</td>
<td>anti-Di(^a) and anti-Di(^b)</td>
</tr>
</tbody>
</table>

*Not found yet

Table 3. ISBT terminology for the Diego blood group system*

<table>
<thead>
<tr>
<th>Symbol</th>
<th>DI</th>
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<tbody>
<tr>
<td>System number</td>
<td>010</td>
</tr>
<tr>
<td>Antigen Di(^a) number</td>
<td>010.001</td>
</tr>
<tr>
<td>Antigen Di(^b) number</td>
<td>010.002</td>
</tr>
</tbody>
</table>


ABH secretor (Sese) genes, allowing the conclusion that Diego and ABH secretor genes segregate independently. 30

Serologic Characteristics

Di\(^a\) antigen

Di\(^a\) is serologically independent of the rare human blood antigens Mi\(^a\), Ven, Ca, Be\(^a\), Wi\(^a\), By\(^a\), Rm, Gr, C\(^a\),
C, E, He, and V, and there is no serologic or genetic relationship to the common blood antigens H, Vel, P, PP1, MN, Rh, Lewis, K, Duffy, or Kidd.1,2,21,22,23 Trypsin, bromelain, and papain treatment of Di(a+) RBCs has no apparent effect on agglutinability by anti-Dia.3,21,31 The Dia antigen is well developed at birth, as evidenced by the fact that Di(a+) RBCs from a newborn react as strongly as adult Di(a+) RBCs.13,21 In vitro, Dia antigen is well preserved in Alsever's solution at 4°C for at least 4 weeks, and on RBCs frozen by Chaplin's method for at least 1 year.21

**Anti-Dia**

Anti-Dia is usually an immune IgG antibody of subclasses IgG1 and IgG3,31 detectable in the indirect antiglobulin test (IAT) with either polyspecific or anti-IgG anti-human globulin (AHG).1-3,32 Naturally occurring anti-Dia has been found in a white Australian woman of Irish origin.6 Anti-Dia is active over a wide pH range (5.0 to 8.0)5,21 and has no hemolytic activity in vitro.5 Except for one complement-fixing example described in 1990,20,33 anti-Dia normally does not bind complement.31

**Macrophage assay**

Di(a+b-) RBCs were coated with anti-Dia and incubated with human macrophages in the macrophage binding assay.33 Of 400 macrophages, 108 contained phagocytosed RBCs for a rate of 27 percent, implying that anti-Dia is of clinical importance.

**Clinical significance of anti-Dia**

In any population with a high incidence of incompatible matings for Di, this antigen will play a role in causing HDN. Anti-Dia has caused severe HDN.1,3,7,32,34 However, in 40 other studies in which the father was Di(a+) and the mother was Di(a-) and had more than one Di(a+) child, the mothers were not allosensitized to Di.3,21 A case report of an immediate hemolytic transfusion reaction (HTR) apparently caused by anti-Dia was reported by Hinckley and Heustis in 1979.32 Anti-Dia was incriminated as the probable cause of the HTR because its titer increased significantly in the post-transfusion period.

**Di antigen**

Di antigen is the high-incidence antigen antithetical to Di.22 Di is not inactivated by proteolytic enzymes or by 2-mercaptoethanol.32 Di is well developed at birth.18,35 Di(a-b+) RBCs have about 15,400 Di antigen sites per cell as measured by quantitative immunoferritin microscopy.36 Di has been demonstrated on phagocytic leukocytes, but not on platelets or lymphocytes.37

**Anti-Dib**

The first two examples of anti-Dib were described by Thompson et al.35 in 1967 in Mexican-Indian women. The sera of both women reacted with the RBCs from approximately 200-300 donor units in the IAT. Both women delivered healthy newborns without clinical evidence of HDN. Anti-Dib usually reacts in the IAT, and the reactions are not enhanced by the addition of complement.5,35 Anti-Dib is not denatured by 2-mercaptoethanol,39 and may agglutinate Di(a-b+) RBCs more strongly than Di(a+b+) RBCs.35

**Clinical significance of anti-Dib**

In 1971 two more examples of anti-Dib were described. One caused mild HDN in the child of a Mexican woman.40 Another example was detected in a Japanese woman whose second child had died of kernicterus.38 Her third child, who became jaundiced within 24 hours after birth, was treated by exchange transfusion and survived. In the Japanese population, the frequency of Di(b-) is calculated to be 0.07 percent.38 In 1978 Orlina and Unger described a case of moderately severe HDN due to anti-Dib, in which two exchange transfusions with incompatible blood were performed. Another example of anti-Dib causing HDN was described in a Japanese woman with a history of blood transfusion.42

Anti-Dib may be more benign than anti-Dia as a cause of HDN. Many studies have revealed that newborns of Di(b-) women with anti-Dib exhibit either mild or subclinical HDN.35-39,40 There have been studies, however, in which anti-Dib has been implicated as the cause of moderately severe HDN, requiring exchange transfusion.38,41,42 In the case report by Orlina and Unger, the affected newborn was treated with phototherapy as well as two exchange transfusions with incompatible blood. The newborn eventually made a complete recovery. Anti-Dib has also been implicated as a cause of a delayed HTR in one study.35

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M. ZAFAR AND M.E. REID

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Association of Di\(\text{a}\) With Band 3

It has recently been shown that the Di\(\text{a}\) antigen is associated with Band 3 Memphis\(^4\). Band 3 Memphis is a consequence of a point mutation of AAG to GAG (Lys\(\rightarrow\)Glu) in codon 56 of cDNA for Band 3 and thus is present on the cytoplasmic domain of Band 3. Two variants of Band 3 Memphis have been described (variants 1 and 2) that are distinguished by differences in their ability to bind stilbene-disulphonate. Di\(\text{b}\) is associated with the variant that most readily binds stilbene-disulphonate, i.e., Band 3 Memphis, variant 2.

It is interesting to note that antigens that were depressed on RBCs from a Mexican donor\(^4\) included Di\(\text{b}\) and many blood group antigens now known to be associated with Band 3.

Conclusion

It can be concluded that the presence of Di\(\text{a}\) is practically confined to people of Mongoloid extraction. Anti-Di\(\text{a}\) causes severe HDN and has been incriminated as a probable cause of HTR among recipients of certain racial groups. In any population in which Di\(\text{a}\) has a relatively high incidence, Di(a+) RBCs should be included in the RBCs used for antibody screening. In an ABO-incompatible mating with HDN, it is difficult to exclude the possibility that RBC destruction is caused by an antibody for a low-incidence antigen, rather than by a high-titered anti-A or anti-B. In such cases, the father's RBCs should be tested against the mother's serum. Further, a female patient with childbearing capability should not be transfused with blood from her husband or her children.

Anti-Di\(\text{b}\) seems more benign as compared to anti-Di\(\text{a}\) in causing HDN, but it has caused moderately severe HDN. This specificity should be considered when investigating fetal hemolysis caused by an antibody reacting with an unknown high-frequency antigen in persons of Mongoloid ancestry.

Acknowledgments

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References


37. Kuriyan MA, Øyen RE, Marsh WL. Demonstration of Diego (Diβ) and Scianna (Scβ) antigens on phagocytic leukocytes of the blood. Transfusion 1978;18:361-4.


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