Artigo Especial

# The history of the Diego blood group

Pedro C. Junqueira <sup>1</sup> Lilian Castilbo<sup>2</sup> Diego blood group initially, because it appeared to be rare, was considered as a family or 'private factor'. With further investigation, it was possible to trace this blood group from an individual family in Venezuela to the Indians across the continent of America and eventually to the Mongolian race in Asia. This review article follows the developments over the years and the history of the Diego blood group.

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# Diego as a "Private Factor"

In 1953, Miguel Larysse and his co-workers Túlio Arends and R. Dominguez Sisco (from the Maternidad Concepcíon, Caracas) at the Centro de Investigaciones del Banco de Sangue de Caracas studied a serum from a full term male infant who appeared at birth to be clinically and hematologically normal but at that time the billirrubin determination was not carried out. Jaundice was evident after 12 hours, it became increasingly severe and the infant expired at three days. The direct antiglobulin test carried out on the newborn's red blood cells was positive. Blood specimens of the Rh-positive mother and her Rhgroup compatible infant were sent to Philip Levine at his consultation service from Ortho Research Foundation (Raritan, New Jersey) and both samples arrived in excellent conditions in June. Although the infant's red cells were strongly "coated", no antibody was demonstrable in the maternal serum when it was tested with an extensive panel of selected cells, which, did not include the father's red blood cells. As ABO and

Rh incompatibility was excluded, the occurrence of a "low-incidence" blood factor with its corresponding antibody was suspected.

On October 26<sup>th</sup>, 1953, the father of the dead infant visited Levine in New York. At this time his red blood cells were tested against the maternal serum and a strong agglutination reaction was found. Levine and the father agreed with the name of the blood factor as Diego (Di<sup>a</sup>).

Levine also demonstrated that Di<sup>a</sup> was not identical with two other previously recognized low-incidence blood factors associated with cases of hemolytic disease of the newborn named as Mi<sup>a</sup> and Be<sup>a</sup>.

The Di<sup>a</sup> antibody was described as one of the six antibodies named as "Private" or "Family Blood Factors" (1). In this paper, they also reported that this antibody was "currently under investigation" at the Ortho Research Foundation and that it caused hemolytic disease and had negative reactions with 200 random white red blood cells tested. Table 1 shows the typing of red cell antigens in selected members of the Diego family.

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Fyª  $Jk^{\underline{a}}/Jk^{\underline{b}}$ Leª Р ABO Rh MN Ss Kk Di₫ I-1  $\circ$ Cde/cde Ν nt nt nt nt nt nt I-2 dCe/dce Α Ν nt nt nt nt nt nt II-1 Ο dcE/dce Ν nt nt nt nt nt nt II-2 Ο dcE/dce MN Kk JkªJkb SS 0 0 0 JkªJk II-3 Ο Dce/dce Μ Ss Kk 0 II-4 0 Dce/dce Ν Nt 0 nt nt nt nt nt Jk<u>ª</u>Jk III-1 Dce/dcE  $\circ$ Μ Ss Kk 0 0 ()0 III-3 dcE/dce Jkª? 0  $\circ$ MN Ss Kk + 0 0 III-5 Ο dcE/dce Μ SS Kk + Jk<sup>b</sup>Jk<sup>b</sup> 0 + + III-6 Ο DCe/DCe MN Kk Jkªjk<sup>b</sup> 0 + 0 + SS III-7 dce/dce + Jk<sup>a</sup>Jk<sup>b</sup> 0 Ο Kk 0 0 Μ Ss III-9 О Dce/dce Nt nt + nt nt nt nt nt III-10 ddE/dce Ο Μ Ss Kk 0 Jk<sup>a</sup>Jk<sup>b</sup> 0 + +  $Jk^bJk^b$ III-11 dce/dce 0 O Μ Ss Kk + + + IV-7 Jk<sup>a</sup>Jk<sup>b</sup> DCe/dcE + 0 Ο MN Ss Kk 0 + IV-8 JkªJkb Ο DCe/dcE MN Ss Kk 0 + + + IV-9 Ο DCe/dce nt nt nt nt nt nt nt + IV-10 DCe/dce 0 Ο nt nt nt nt nt nt nt

Table 1 - Typing of red cell antigens in selected members of the Diego Family

 $nt=not\ tested$ 

#### Diego as an "Indian Factor"

In 1955, the mother of the first documented Dia antibody carrier consulted Layrisse about a new pregnancy, which permitted further and more extensive tests in Venezuela and at Raritan, New Jersey. Layrisse and collaborators studying four generations of the original Diego family, noticed that the third and fourth generations seemed to be Caucasoid but their skins were a little darkbrown. However, the members of the second generation and the great grandmother in the first generation had a dark-brown skin and they seemed to be of Mongolian origin. Taking into account this observation, they started to observe the physical characteristics of a population from different countries with 286 individuals who had been tested with an anti-Dia serum by the indirect antiglobulin test. The frequencies of positive results found in these populations were:

Population tested	Frequencies of Di <sup>a</sup> found
Caracas	2.26%
Barcelona	3.28%
Carib Indian	35.54%
Arawaka Indian	5.26%
Curiepe mixed Negro	7.33%

From these findings Layrisse et al., 1955 (2), concluded that it was evident that the Diego Factor was not restricted to a single family, and could be placed among the relatively high incidence blood group systems in Venezuela and probably in South America, with apparently genetic, anthropologic and clinical significances. They also commented that since the Diego terminology was meaningless and this factor had probably some anthropological implications it should be changed to a more expressive one like "Indian Factor". About this comment we must remember that Chown and Lewis (Nature, 1953) said that what appears to be a rare, private

or family antigen in one population might be fairly frequent in another one, and so Layrisse was right.

In 1955, Dr Jean Dausset, a French immunologist, later awarded the Nobel Prize (1980), after working with Layrisse in Caracas, came to Rio de Janeiro to visit me because I was studying the Brazilians Indians. At the time, Dr Dausset brought me a sample of the Diego serum and this serum turned out to be very important to me. In that year we had the opportunity to test two Brazilian Indian groups with the anti-Diego serum using the indirect antiglobulin test: the Carajas living in Santa Izabel, Bananal Island, State of Mato Grosso and the Kaingaques living in a reservation near Palmas, State of Paraná. Thirteen (36%) of the red blood samples from the Carajas tested and 22 (46%) of the Kaingaques samples tested gave positive reactions with the anti-Diego serum. We had also the opportunity to test 200 red blood samples from true Black donors from the Rio de Janeiro Municipal Blood Bank that showed negative results with the anti-Diego serum. We sent our results to Layrisse, in Caracas and to Levine, in Raritan. We had our paper published in Nature, volume 17 on page 41 (3). Levine et al. published their paper in this same volume of the Nature Journal on page 40 (4).

Table 2 shows the incidence of the Di<sup>a</sup> antigen in different Indian populations.

## Diego as a "Mongolian Factor"

In 1956, in a paper published in the Nature Journal (5), Layrisse and Arends stated: "Since the Indians of the American continent are considered to be anthropological related to the Mongolian people of the old world, we decided to investigate the incidence of the Diego Factor in other available representative Asians living in Venezuela". They tested 100 unrelated males from Canton (China), living in Venezuela, and detected 5 Diego positive individuals (5% of the Chinese tested). They also tested sixty-five unrelated Japanese and found 8 Diego positive

subjects (12.5% of the Japanese tested) (6). These findings indicated that the Diego factor was not restricted to South America and suggested that this antigen was a Mongolian rather than an Indian factor.

In the same year, Lewis et al., (7) showed that the Diego antigen was found to be present in 16 of 148 unrelated Chippewa Indians from North Minnesota and in 6 of 77 unrelated Japanese from Winnipeg. This finding suggested that Diego might be an Asiatic characteristic.

In 1957, Levine and Robinson (8) said that studies carried out by Layrisse and his colleagues on the Diego blood factor in other populations including the Brazilian Indians carried out by Junqueira et al. (3), apparently suspected that the Diego factor could be Mongolian in its origin. Further, they concluded that the term Indian for the Diego blood factor was not appropriate.

Levine and Robinson, 1957 (8) also demonstrated that the Di<sup>a</sup> antigen was genetically independent from other 15 low incidence factors and from four high incidence factors previously recognized. Furthermore, Layrisse, Sanger and Race, 1959 (9) using evidence from the literature and from nine new families studied, showed that the Di<sup>a</sup> antigen had no links with most of the established blood group systems.

Many papers showing the distribution of the Di<sup>a</sup> antigen considered that it was essentially a Mongolian characteristic, absent in Whites, Blacks, Australian aborigines and other populations (10-39). Table 3 shows the incidence of the Di<sup>a</sup> antigen in the Chinese and Korean populations.

The book named "The distribution of Human Blood Groups and other polymorphisms" (Mourant et al., 1976) (40) is considered the best one to show the early worldwide race distribution of Di<sup>a</sup>.

In 1967, thirteen years after the detection of the anti-Di<sup>a</sup>, Thompson, Childers and Hatcher identified the anti-Di<sup>b</sup> (41). As the phenotype Di(a–b–) has not been reported yet, we may assume that only two alleles (Di<sup>a</sup> and Di<sup>b</sup>) control the Diego blood group system.

Both antibodies, anti-Di<sup>a</sup> and anti-Di<sup>b</sup> are

**Table 2** – Incidence of the  $Di^a$  antigen in different indian populations

Population	Indians	Number tested	Number Di(a+)	Percent Di(a+)	Reference
		icsica	DI(a+)	DI(a+)	
	Carib				
	(Cachama)	121	43	35.6	Layrisse et al,1955
Venezuela	Carib				
	(Aribi)	49	7	14.3	
	Arawako				
	(Guajiros)	152	8	5.3	
Brazil	Carajas				
	(São Domingos)	36	13	36.1	Junqueira et al., 1956
	Kaingangues				
	(São Domingos)	48	22	45.8	
North Americans	Chippewa	148	16	10.8	Lewis et al., 1956
Mexico		152	31	20.4	Salazar et al., 1959
USA	Penobscots	244	20	8.0	Allen et al., 1960
	Xavantes	78	24	30.4	Neal et al., 1964
Brazil	(São Domingos)				
	Xavantes	289	78	27.0	Gershowitz et al., 1967
	(São Marcos)				
	Xavantes	171	62	36.3	
	(Simoes Lopes)				
Guatemala	Mayan Indians	255	57	22.3	Cann et al., 1968
Mexican Americans		1685	172	10.2	Edwards-Moulds et al., 1986
USA	Chippewa	119	9	7.0	Lee et al., 1990
Venezuela	Caracas	65	8	12.3	Layrisse et al., 1956
Canada	Winnepeg	77	6	7.8	Lewis et al., 1956
	Tokio	88	2	2.3	Furuhata et al., 1957
	Kumamoto	153	12	7.8	Ueno et al., 1957
Japan	Jumma	500	16	3.2	Masakis et al., 1959
	Tokio	146	6	4.1	Yokoyama et al., 1960
	Kumamoto	227	17	7.5	
	Hiroshima	309	25	8.1	Tsuchiya et al., 1964
Brazil	Rio de Janeiro	207	13	6.7	Cerqueira et al., 1968

responsible for the hemolytic disease of the newborn and for hemolytic transfusion reactions (42). Due to the composition of our population, and supported by studies showing that 3.6% of the multi-transfused patients in Brazil have anti-Di<sup>a</sup> (43) the Brazilian red cell panels used for

antibody screening ought to include a Di(a+).

In 1975, Race and Sanger (42) said in the last edition (6<sup>th</sup>) of their book: "The Venezuelan discovery of Di<sup>a</sup> will make an outstanding contribution to the anthropology of the Mongolian world".

Population	Chinese	Number	Number	Percent	Reference
		tested	Di(a+)	Di(a+)	
Venezuela	Canton	100	5	5.0	Layrisse et al., 1956
Taiwan	Fukein and Canton	1000	32	3.2	Lin-Chu et al., 1988
Korea	Seoul	227	17	6.1	Won et al., 1960

**Table 4** – Antigen assigned to Diego blood group system by the ISBT Nomenclature (Daniels et al., 2001)

System	Number	Symbol	Amino Acid Substitution
010DI	0100001 DI 1	Di <u>a</u>	Leu 854
010DI	0100002 DI 2	Di <sup>b</sup>	Pro 854
010DI	0100003 DI 3	Wr <u>a</u>	Lys 658
010 DI	010004 DI 4	Wr <sup>b</sup>	Glu658
010DI	0100005 DI 5	$Wd^{\underline{a}}$	Val 557 Met
010DI	0100006 DI 6	Rbª	Pro 548 Leu
010DI	0100007 DI 7	WARR	Thr 552 lle
010DI	0100008 DI 8	ELO	Arg 432 Trp
010DI	0100009 DI 9	Wu	Gly 565 Ala
010DI	0100010 DI 10	Bpª	Asm 569 Lys
010DI	0100011 DI 11	Mo <sup>a</sup>	Arg 656 His
010DI	0100012 DI 12	Hg <u>a</u>	Arg 656 Cys
010DI	0100013 DI 13	Ug <u>a</u>	Tyr 555 His
010DI	0100014 DI 14	Sw <sup><u>a</u></sup>	Arg 646 Gln
010DI	0100015 DI 15	BOW	Pro 561 Ser
010DI	0100016 DI 16	NFLD	Glu 429 Asp
			Pro 561 Ala
010DI	0100017 DI 17	Jn <sup>a</sup>	Pro 566 Ser
010DI	0100018 DI 18	KREP	Pro 566 Ala
010DI	0100019 DI 19	Tra	Lys551Asn
010 DI	0100020 DI 20	Fra	Glu480Lys
010DI	0100021 DI 21	SW1	Arg646Trp

## The Diego blood group system

The Diego blood group system is a rapidly expanding system and today it consists of two pairs of antithetical antigens (Di<sup>a</sup> and Di<sup>b</sup>, and Wr<sup>a</sup> and Wr<sup>b</sup>) and 17 low incidence antigens (44) (Table 4).

Spring et al., in 1992 (45) recognized an association between band 3 (anion exchange 1 - AE1), the most abundant integral protein of the red blood cell (RBC) membrane, and the Diego blood group system. They found by SDS-PAGE that Di(a+) red cells always have band 3 variant Memphis, although not all band 3

Memphis red cells are Di(a+). This observation led to the investigation of band 3 from red cells of known Diego blood group in order to ascertain whether the expression of the Di<sup>a</sup> antigen is linked to band 3 Memphis, and to define the molecular basis of this variant.

In 1993, the Diego blood group locus was assigned to chromosome 17 by Zelinski et al., (46) and in 1994, Bruce et al. (47) showed by H DIDS (4,4' –diisothiocyanato-2,2' –dihydrostibene disulfonate) binding studies on samples of known Diego phenotypes that the expression of the Di<sup>a</sup> antigen is associated with an increased susceptibility of band 3 to labeling

by H DIDS. This provided evidence for a link between the expression of the Di<sup>a</sup> antigen and the presence of band 3 variant Memphis (45).

DNA sequence analysis (47) showed that the  $Dt^a/Dt^b$  polymorphism results from a point mutation at nucleotide 2561 (C>T) resulting in a single amino acid substitution in position 854, with a proline corresponding to the Di<sup>b</sup> antigen and leucine to the Di<sup>a</sup> antigen. Molecular analysis of band 3 from individuals with red cells expressing the Di<sup>a</sup> antigen showed the simultaneous occurrence of the mutations 2561T (854Leu) and 166G (56Glu) responsible for the Band 3 variant Memphis (47).

Carries of band 3-Memphis are asymptomatic and show no morphologic abnormalities of their erythrocytes (48). Studies to determine the frequencies of band 3-Memphis in some populations have been performed, proving that band 3-Memphis is not a rare polymorphism and that the gene frequency of band 3-Memphis varies among different populations, with a high frequency among Indians and the Japanese (48-51).

Bruce et al. (1995) also reported that the  $Wr^a/Wr^b$  polymorphism results from a glutamic acid in position 658 of band 3 corresponding to  $Wr^b$  and a lysine in the same position, corresponding to  $Wr^a$  (52).

Evidence suggests that the Wr<sup>b</sup> antigen is also associated with the glycophorin A (GPA) because Wr<sup>b</sup> antigen requires both band 3 and GPA for it's expression in the red cell membrane (52-54).

The recognition that band 3 carries antigens of the Diego blood group system and the elucidation of the  $Di^a/Di^b$  and  $Wr^a/Wr^b$  polymorphisms have led several investigators to elucidate the molecular basis of the other low incidence Diego antigens and to create a more accurate structural model of band 3 (55).

New studies with band 3 and Diego are being developing and new findings are emerging. We have found in our population by molecular studies a high frequency of 166G mutations (Memphis) and the possibility that the Di<sup>a</sup> antigen can not be associated with the band 3 variant Memphis (56).

In the 1980s and 1990s, serological, biochemical and molecular studies have given a new face to Immuno-hematology. Most of the papers published are cooperative works and are improving the understanding of the Diego system. It is important that these studies should continue in different populations to expand the knowledge of the structure and function of band 3.

# A história do sistema de grupos sangüíneos Diego

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#### Resumo

O sistema de grupos sangüíneos Diego, devido à sua raridade, era considerado um fator privado familiar. Investigações posteriores, de estudos familiares na Venezuela e em índios do continente americano e mongóis na Ásia, evidenciaram a sua existência.

Neste relato apresentamos o desenvolvimento do conhecimento e da sua história. Rev.bras.hematol.hemoter.,2002,**24**(1):15-23

**Palavras-chave**: Diego, grupos sangüíneos, bistória

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