Immunohematology

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Immunohematology

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DNA analysis for donor screening of Dombrock blood group antigens

J.R. Storry, C.M. Westhoff, D. Charles-Pierre, M. Rios, K. Hue-Roye, S. Vege, S. Nance, and M.E. Reid

Due to the scarcity of reliable antibodies, RBC typing for Do^a and Do^b is notoriously difficult. Inaccurate typing can place patients at risk for hemolytic transfusion reactions. The molecular basis of the *DOA/DOB* polymorphism is associated with three nucleotide changes: 378 C>T, 624 T>C, and 793 A>G of *DO.* While the 378 C>T and 624 T>C are silent mutations, the 793A>G polymorphism in codon 265 encodes asparagine for Do^a and aspartic acid for Do^b. We describe here the use of a PCR-RFLP assay as an alternative to traditional hemagglutination for typing donor blood for Dombrock. Primers were designed to amplify the region of *DO* containing the 793A>G polymorphism. DNA samples from blood donors were amplified and subjected to RFLP analysis. A total of 613 samples were tested for the Dombrock polymorphism (793 A>G) by PCR-RFLP. PCR-RFLP can be used to screen for Do(a–) or Do(b–) donors. This approach overcomes the scarcity of the reagents required for testing by hemagglutination. ***Immunhematology*** **2003;19:73–76.**

Key Words: Dombrock blood group system, Dombrock polymorphisms Do^a/Do^b, PCR-RFLP, typing donors

The Do^a antigen (ISBT 014001) was reported in 1965[1] and the antithetical antigen, Do^b, (ISBT 014002) was reported 8 years later.[2] While neither antigen is highly immunogenic and the corresponding allo-antibodies have not been implicated in causing hemolytic disease of the newborn,[3] they have been reported to cause transfusion reactions.[1,4–13] Anti-Do^a and anti-Do^b are usually weakly reactive, are present in sera containing other alloantibodies, and can deteriorate on storage. Furthermore, the serologic detection and identification of anti-Do^a and anti-Do^b is notoriously difficult. Thus, reagents are not available for typing donor RBCs, and selection of compatible blood is frequently based on crossmatch results. Therefore, it is possible that transfusion reactions, especially due to anti-Do^b, occur but are underreported.

Molecular techniques can overcome some of the challenges posed by conventional Dombrock phenotyping and would be valuable for determining the *DOA/DOB* status of blood donors and patients. Do^a and Do^b are encoded by the alleles *DOA* and *DOB* (chromosome 12), which are associated with three nucleotide changes, respectively, 378 C>T, 624 T>C, and 793 A>G in exon 2 of *DO* (GenBank Accession number XM_017877). The 378 C>T (126 tyrosine) and 624 T>C (208 leucine) polymorphisms are silent at the amino acid level. The 793 A>G polymorphism encodes asparagine for Do^a and aspartic acid for Do^b at amino acid residue 265.[14] Based on the reported molecular basis for the *DOA/DOB* polymorphism,[14] we developed a PCR-RFLP assay to determine the *DO* genotype of blood donors.[15] In this report, we describe another robust PCR-RFLP assay for the determination of the *DO* polymorphism, which targets the missense mutation 793 A>G, and illustrate the application of these assays for screening donor blood samples.

Materials and Methods

Blood samples

Blood samples were obtained from donors in New York and Philadelphia. New York samples were selected based on their known RBC phenotype and the requirements of patients whose serum contained multiple alloantibodies, of which one was anti-Do^a or anti-Do^b. Since the correlation between Dombrock genotype and phenotype had been determined in a previous report,[15] and due to the scarcity of anti-Do^a and anti-Do^b, antigen typing was rarely performed on the donor samples. A total of 563 donations were tested, of which 73 were duplicate samples. Duplicates were performed deliberately, using two different donations to comply with our current in-house antigen-typing criteria for labeling RBC products as "antigen-negative." In Philadelphia, 50 samples from random donors were tested.

PCR-RFLP assays

DNA was prepared from blood samples using QIAamp Blood Kit (Qiagen, Valencia, CA). The initial typing was performed using the PCR-RFLP *Eam* 1105I assay[15] to identify the polymorphism (793A>G) associated with the amino acid change (Asn265Asp). In the *Eam* 1105I assay, deliberate changes were introduced into both primers to give a distinct pattern for both alleles. Subsequently, we identified a *Bse*RI restriction site for the *DOB* allele at position 793. This permitted us to develop a new PCR-RFLP assay in which a deliberate change was introduced into one of the primers. Primers (Table 1) were designed to amplify a 368-bp product by PCR. DoF was engineered to contain an additional *Bse*RI site to serve as a positive control for the restriction enzyme digestion.

Table 1. Primers for *Bse*RI PCR-RFLP analysis

Primer name	Sequence 5' to 3'	Product size	RFLP using *Bse*RI *DOA*	*DOB*
DoF	TACCTCACCTCAGCAATCCAGCTGCTGAGGAGAGAC	368 bp	42 bp	42 bp 58 bp
DoR	TTTAGCAGCTGACAGTTATATGTGCTCAGGTTCC		326 bp	268 bp

NB: The underlined nucleotide was introduced to provide a *Bse*RI restriction site in the primer region to serve as a control for subsequent digestion.

Table 2. Donor sample Dombrock genotypes and predicted phenotypes

Genotype	Assigned phenotype	Total	Number of donations New York *Eam*1105I	New York *Bse*RI	Philadelphia *Bse*RI	Total
DOA/DOA	Do(a+b–)	81 (23)	29 [6]	52	10	91
DOA/DOB	Do(a+b+)	200 (1)	63	137	19	219
DOB/DOB	Do(a–b+)	282 (49)	104 [11]	178	21	303

NB: Numbers in parentheses ( ) represent number of duplicates tested, i.e., two different donations tested from the same donor. Numbers in brackets [ ] represent duplicates tested by each method.

PCR amplification with DoF/DoR was carried out in 50-µL volumes, each containing 100 ng of DNA, PCR buffer, 2.0 mM $MgCl_2$, 2 nmol of each dNTP, 10 pmol of each primer, 1 unit of *Taq* DNA polymerase, and sterile distilled H_2O. The PCR amplification profile involved initial denaturation for 2 minutes at 94°C, followed by 30 cycles of denaturing at 94°C for 1 minute, annealing at 60°C for 1 minute, and elongating at 72°C for 1 minute, with a final extension at 72°C for 7 minutes. PCR products were digested with *Bse*RI restriction enzyme under conditions recommended by the manufacturer (New England Biolabs, Beverly, MA). The digested products were separated on 4% MetaPhor agarose gels and visualized with ethidium bromide staining. This profile was modified in New York to include HotStarTaq (Qiagen) in thin-wall PCR tubes. The amplification profile was as follows: 95°C for 15 seconds, then 35 cycles of denaturing at 94°C for 20 seconds, annealing at 60°C for 20 seconds, elongating at 72°C for 20 seconds, with a final extension at 72°C for 7 minutes. The digested products were separated on 8% polyacrylamide gel and visualized with ethidium bromide staining.

Results

Figure 1 shows PCR-RFLP analysis after *Bse*RI restriction enzyme digestion. Examples of Do(a+b–), Do(a–b+), and Do(a+b+), phenotypes are shown on agarose (Fig. 1A) and acrylamide (Fig.1B) gels. The results of testing 563 blood donors from New York and 50 donors from the Philadelphia area are summarized in Table 2. The genotype and predicted phenotypes are shown. We were unable to correlate the observed incidence of the genes with the expected prevalence since sample selection was not random. There was complete concordance in samples for which more than one assay and/or phenotyping was performed.

Discussion

Typing RBCs for Do^a and, especially, for Do^b is notoriously difficult because the antibodies, usually present in sera containing other alloantibodies, often are weakly reactive and deteriorate on storage. Prior to the advent of molecular assays described herein, the scarcity of specific anti-Do^a and anti-Do^b made identification of appropriate antigen-negative blood a major challenge. DNA analysis for the Dombrock polymorphism provides an accurate means for typing for Do(a–) and Do(b–) donors. Previously, we relied on historical information and/or the crossmatch; however, this practice did not ensure selection of antigen-negative RBC products. We have now established a procedure that allows the results of DNA testing for predicted Do antigen status to be the test of record. The additional PCR-RFLP assay reported here reliably distinguishes *DOA* from *DOB* and is practical for typing a large number of samples from blood donors. We chose PCR-RFLP assays over allele-specific PCR because both alleles are amplified with a single set of primers and subsequent digestion with the restriction enzyme gives a distinct banding pattern for each allele.

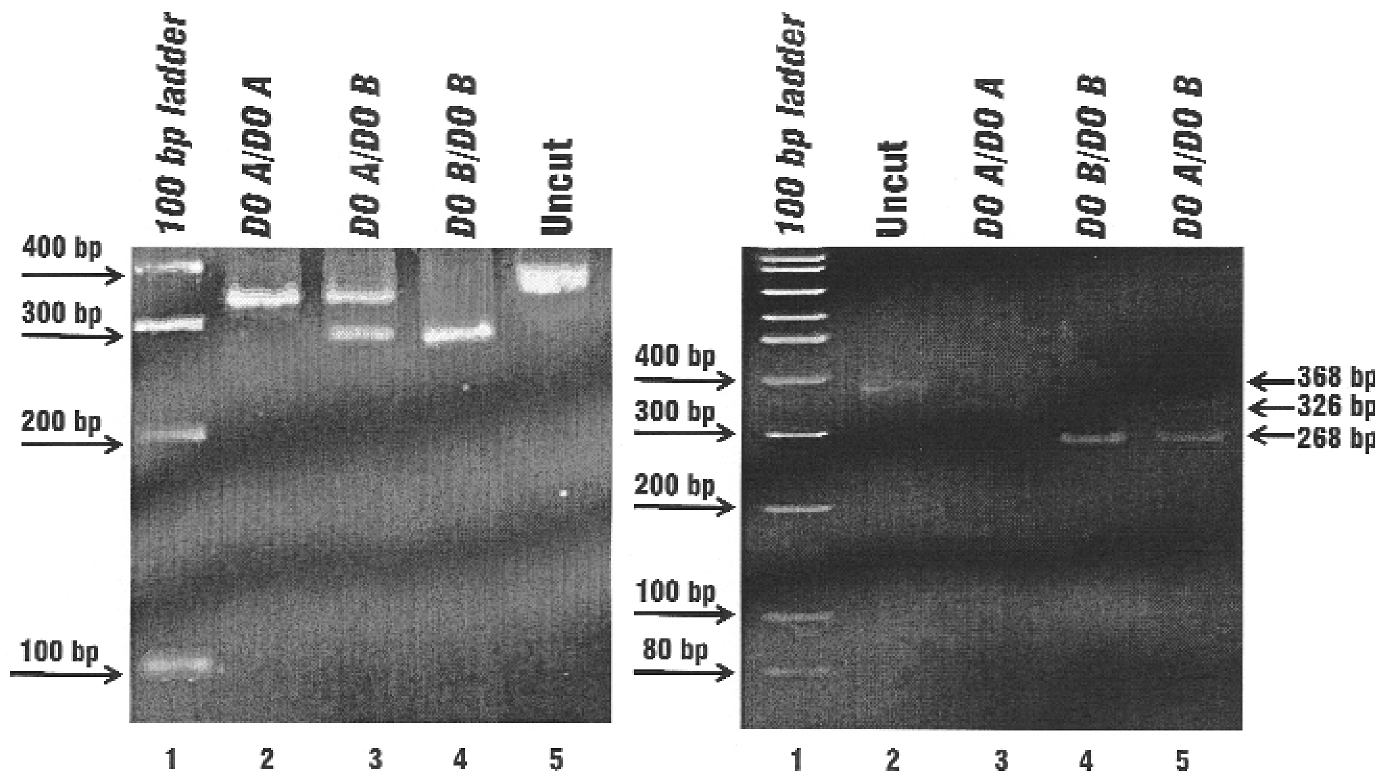

Fig. 1. Dombrock PCR-*Bse*RI RFLP: **A.** Electrophoresis on a 4% MetaPhor gel. Lane 1 = 100-bp DNA marker, lane 2 *DOA/DOA,* lane 3 *DOA/DOB,* lane 4 *DOB/DOB,* lane 5 undigested PCR product. The presence of the control *Bse*RI restriction site in the forward primer results in cleavage of 42 bp from all PCR products, resulting in the shift from 368 bp (lane 5) to 326 bp (lanes 2 and 3). The *DOB* carries an additional *Bse*RI site, resulting in fragments of 268 and 58 bp. (Note: 58- and 42-bp fragments are at too low a concentration to visualize. Results are monitored by the shift in fragment size.) **B.** Electrophoresis on an 8% polyacrylamide gel. Lane 1 = 100-bp DNA marker, lane 2 undigested PCR product, lane 3 *DOA/DOA,* lane 4 *DOB/DOB,* lane 5 *DOA/DOB.*

There are rare occasions when the genotype and phenotype may not agree.[16] For instance, in the case of null phenotypes where no antigen is expressed, a falsely positive molecular typing will result because the assay is often designed to analyze only the specific region responsible for the antigen polymorphism. The method does not detect other mutations that may result in loss of expression on the RBC surface. Importantly, however, a falsely positive result when screening blood donors means only that a rare null donor will go unrecognized and presents no danger to a patient who needs antigen-negative units. For instance, a donor who types *DOA* by the PCR-RFLP assay at nucleotide position 793 could in fact be Hy– or Gy(a–), and a donor who types as *DOB* at nucleotide 793 could in fact be Jo(a–) or Gy(a–). However, there are no known instances where molecular typing in the *DO* gene would interpret a donor as antigen negative when, in fact, he or she was antigen positive.

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Studies on the Dombrock blood group system in non-human primates

C. Mogos, A. Schawalder, G.R. Halverson, and M.E. Reid

The Dombrock blood group system consists of five distinct antigens: two antithetical antigens, Do[a] and Do[b], and three high-frequency antigens: Gy[a], Hy, and Jo[a]. Although the prevalence of Do[a] and Do[b] in different populations makes them useful as genetic markers, the scarcity of reliable antibodies to these antigens has prevented this potential from being realized. The gene (*DO;ART4*) encoding the Dombrock glycoprotein has been cloned and sequenced, and the molecular bases of the various Dombrock phenotypes have been determined. The purpose of this study was to perform DNA-based assays on the *DO* homolog in non-human primates to determine the degree of conservation in the *DO* gene. Murine MoAbs to Dombrock protein were developed by standard hybridoma technologies and used to test RBCs from non-human primates by hemagglutination. PCR-RFLP analysis for the six single-nucleotide polymorphisms (SNPs) that have been defined in human alleles were performed on DNA extracted from fresh or frozen blood samples from numerous non-human primates. Hemagglutination tests with six MoAbs to the Dombrock glycoprotein revealed distinct epitopes on RBCs from the non-human primates. The gorillas and orangutans had the same PCR-RFLP digestion pattern for the six SNPs studied as chimpanzees. Old world monkeys (macaques) were identical at nucleotides (nt) 323, 350, 624, and 793 with the chimpanzees, and at nt 898 the digestion pattern was the same as for the *HY1* allele in humans. For the new world monkeys (tamarins and squirrel monkeys) the digestion pattern was conserved for nt 793 but different for nt 624; the other SNPs could not be determined because there was no amplification. The presence of epitopes recognized by the MoAbs and PCR-RFLP results among the non-human primates shows considerable conservation of the *DO* gene. The difficulties we encountered with the amplification of DNA from the non-human primates lower in the phylogenetic tree are probably due to divergence in sequence. ***Immunohematology*** **2003;19:77–82.**

Key Words: apes, *ART 4* gene, blood group system, Dombrock blood group system, monkey, mono-ADP-riposyltransferase, non-human primates

The Dombrock blood group system comprises two antithetical antigens: Do[a] and Do[b], and three high-frequency antigens: Gregory (Gy[a]), Holley (Hy), and Joseph (Jo[a]). Based on tests with anti-Do[a] and anti-Do[b], there are three distinct phenotypes with a prevalence that differs among various ethnicities.[1-5] An absence of the high-frequency antigens Hy and Jo[a] has so far been restricted to people of African ancestry. The high-frequency antigens Gregory (Gy[a]) and Holley (Hy) are phenotypically closely related: Gy(a–) RBCs from Caucasians are Hy– and Hy– RBCs from African Americans are Gy(a+[w]).[6–8] Jo[a] has a phenotypic association with Gy[a] and Hy: Gy(a–) or Hy– RBCs are Jo(a–).[9,10] Table 1 summarizes the relationship of the Dombrock antigens, which all reside on the same glycoprotein, which is attached to the RBC via a glycosylphosphatidylinositol (GPI) linkage.[11–13]

In 1992,[14] it was shown that Do(a–b–) RBCs are also Hy–, Jo(a–), and Gy(a–)[15] and thus RBCs with the Gy(a–) phenotype are the null phenotype of the Dombrock blood group system. As a result, Gy[a], Hy, and Jo[a] antigens became part of the Dombrock blood group system.[16,17]

Antigens in the Dombrock system are resistant to papain or ficin treatment, sensitive to trypsin or DTT (200 mM) treatment, and weakened by α-chymotrypsin treatment of antigen-positive RBCs. The antigens are expressed on cord RBCs and absent from paroxysmal nocturnal hemoglobinuria (PNH) type III RBCs.[12] These characteristics can be exploited for identification of antibodies to Dombrock antigens.

The antibodies in the Dombrock blood group system represent a challenge for even the most

Table 1. The Dombrock blood group system

RBC	Reactivity with anti-						
Phenotype	Do[a]	Do[b]	Gy[a]	Hy	Jo[a]	Whites	Blacks
Do(a+b–)	+	0	+	+	+	18	11
Do(a+b+)	+	+	+	+	+	49	44
Do(a–b+)	0	+	+	+	+	33	45
Gy(a–)	0	0	0	0	0	Rare	1 proband[40]
Hy–	0	wk	wk	0	0*	Not found	Rare
Jo(a–)	wk	0/wk	+	wk	0	Not found	Rare

*Some Hy– samples may express Jo[a] very weakly.[41]

Table 2. **A.** Differences between expressed human *DO* alleles

	nt (aa)	nt (aa)	nt	nt	nt (aa)	nt (aa)
Allele	323 (108)	350 (117)	378	624	793 (265)	898 (300)
DOA	G (Gly)	C (Thr)	C	T	A (Asn)	C (Leu)
DOB	G (Gly)	C (Thr)	T	C	G (Asp)	C (Leu)
HY1	T (Val)	C (Thr)	C	C	G (Asp)	G (Val)
HY2	T (Val)	C (Thr)	C	C	G (Asp)	C (Leu)
JO	G (Gly)	T (Ile)	T	T	A (Asn)	C (Leu)

B. Differences between non-human primate *DO* alleles; nucleotides presumed from RFLP patterns

	nt 323	nt 350	nt 378	nt 624	nt 793	nt 898
Chimp	G	C	T	C	A	C
Gorilla	G	C	T	C	A	C
Orangutan	G	C	T	C	A	C
Macaque	G	C	ND	C	A	G
Tamarin	NA	NA	NA	T	A	ND
Squirrel	NA	NA	NA	novel RFLP pattern	A	ND

nt = nucleotide aa = amino acid NA = no amplification ND = not done

experienced serologist. The antibodies are usually IgG and react optimally by column agglutination technology or by the IAT using papain- or ficin-treated antigen-positive RBCs. They are weakly reactive, are usually present in sera containing other alloantibodies, and often deteriorate in vitro and in vivo. Moreover, anti-Hy and anti-Jo^a are particularly difficult to differentiate from each other. The molecular basis of the Hy and Jo^a antigens has provided valuable insight in understanding the serologic difficulties, and this has been reviewed by Reid.[18]

Antibodies to Dombrock antigens have caused transfusion reactions and a positive DAT on newborn RBCs, but not HDN.[19–29] It is possible that transfusion reactions are underreported because the classic signs are not always manifested: the DAT is often negative, antibody may not be recovered in an eluate, and the antibody titer may not increase.

The *DO* gene consists of three exons distributed over 14 kbp of DNA and is identical to the *ART4* gene[30,31] (GenBank accession #XM017877). The messenger RNA, which consists of 1.1 kbp, is predicted to encode a protein of 314 amino acids that has both a signal sequence and a GPI-anchor motif. The *DOA* and *DOB* alleles differ in three nucleotide positions. Two are silent mutations (378C>T; 126Tyr and 624T>C; 208Leu) and one is a missense mutation (793A>G; Asn265Asp), which encodes Do^a and Do^b (Table 2A).[30] These three mutations each can be readily differentiated by PCR-RFLP.

The nucleotide change associated with Hy+/Hy– is 323 G>T, which is predicted to encode Gly108Val. Interestingly, the change associated with the absence of the Hy antigen is on an allele carrying 378C (*DOA*), 624C (*DOB*), and 793G (*DOB*). Its association with 793G, 265Asp, explains why RBCs with the Hy– phenotype are invariably Do(a–b+). There are two forms of the *HY*– allele, one with 898G encoding 300Val and the other with 898C encoding 300Leu (which is present on Hy+ wild type). Nucleotide 350C>T (Thr117Ile) is associated with the absence of the Jo^a antigen and is on an allele carrying 378T (*DOB*), 624T (*DOA*), and 793A (*DOA*). The genotype of people whose RBCs have the Jo(a–) phenotype can be *JO/JO* or *HY/JO* (Table 2A).

To date, four molecular bases of silent *DO* genes have been described. Two of them, a mutation in the donor splice site[32] and a mutation in the acceptor splice site,[33] lead to outsplicing of exon 2. A third proband has a deletion of eight nucleotides within exon 2 that leads to a frameshift and a premature stop codon,[34] and the fourth mechanism is a non-sense mutation in a novel allele (350C; 378T [*DOB*]; 624T [*DOA*]; 793A [*DOA*]) that has been named *GY5*.[32]

The chimpanzee *DO* gene is identical to human *DO* at nt positions 323, 350, and 898 but has a novel combination of three SNPs that distinguish *DOA* and *DOB* (378T [*DOB*], 624C [*DOB*], and 793A [*DOA*])[35] (GenBank accession #AF373016, AF373017, and AF374727) (Table 2B). The sequence of the *DO* homolog in mouse has also been determined.[36]

In the study presented here, we provide results of testing RBCs from non-human primates with monoclonal antibodies to the Dombrock glycoprotein and of PCR-RFLP analyses performed on genomic DNA from greater apes, old world monkeys, and new world monkeys for the six *DO* SNPs that define the human Dombrock phenotypes.

Material and Methods

Preparation of monoclonal antibodies

MoAbs were produced using standard hybridoma methods. Briefly, Balb-C mice were immunized intramuscularly with cDNA from the *DO* gene and then by intraperitoneal injection of transfected 293T human kidney cells expressing either the Do^a or the Do^b antigen. After three immunizations, the mice were bled and the serum was tested by hemagglutination to determine the serologic response. Mice with a sufficient response were boosted with an additional

immunization of transfected 293T cells, and 2 days later spleen cells were harvested for fusion with mouse myeloma X63 Ag8.653 cells. Fusions were done with PEG4000 and selection for hybrids was accomplished using DMEM with hypoxanthine-aminopterin-thymidine (HAT) added (Sigma Chemicals, St. Louis, MO). The medium was supplemented 7 days later with ClonaCell HY Medium E (StemCell Technologies, Vancouver, BC), with hypoxanthine-thymidine (HT) added. Screening for antibody-producing hybrids was done 7 to 10 days later by hemagglutination in V-well trays with Dombrock-positive RBCs. Cloning of positive hybrids was done by limiting dilution over three passes, and the final hybridoma was overgrown for maximum antibody production.

PCR amplification

Genomic DNA was extracted from fresh or frozen blood samples from at least two animals from each species, using the QIAamp Blood Kit (Qiagen, Valencia, CA). The samples were from the Catarrhini suborder: the great apes (gorillas, orangutans), old world monkeys (macaques), and new world monkeys (tamarins, squirrel monkeys) (LEMSIP, NY). The quality and quantity of the DNA was determined by testing 5 μL of each sample in parallel with a K562 genomic DNA control (Invitrogen/Life Technologies, Grand Island, NY) on ethidium bromide–impregnated 0.8% agarose gels (Bio-Rad Laboratories, Hercules, CA) in Tris-Borate EDTA buffer (TBE), visualized and documented with the FluorChem Imaging System version 3.1 (Alpha Innotech Corporation, San Leandro, CA).

Approximately 200–500 ng of genomic DNA from each sample was amplified by PCR using three sets of primers flanking the region of interest (Table 3) in a 50-μL reaction volume containing 50 pmol of each primer, 2 nmol of each dNTP, 2.5 mM $MgCl_2$, 1.0 U HotStarTaq (Qiagen) and PCR Reaction Buffer. Genomic template DNA was added to the PCR mix and amplification was performed in a Perkin Elmer Gene Amp PCR System 2400 Thermocycler (Perkin Elmer, Norwalk, CT) using the cycling profile 95°C for 15 minutes, followed by 35 cycles of 94°C for 20 seconds, 20 seconds at the temperature appropriate for the primer set (Table 3), 72°C for 20 seconds, followed by 72°C for 10 minutes. PCR products were analyzed on ethidium bromide–impregnated agarose gels (Bio-Rad Laboratories) in parallel with a DNA ladder (Invitrogen). Gels were visualized and documented with the FluorChem Imaging System version 3.1 (Alpha Innotech Corporation).

RFLP analysis

PCR products were incubated with the appropriate restriction enzyme (Table 3) for 2 to 16 hours, using conditions recommended by the manufacturer. The digests were run on 8% non-denaturing polyacrylamide gels for 2 hours at 200 V in TBE in parallel with the uncut PCR product and a Gene Ruler 50-bp DNA ladder (Fermentas Inc., Hanover, MD). Gels were stained with ethidium bromide and visualized and documented with the FluorChem Imaging System version 3.1 (Alpha Innotech Corporation).

Table 3. Primers and restriction enzymes used for Dombrock PCR-RFLP analysis.

Primer Pair	Primer Sequences (5'→3'), annealing temperature and expected product size	Restriction enzyme (nt #)	RFLP band pattern (bp) cut	uncut
DoX2F	tcagtaccaaggctgtagca	*Bsa*JI (nt 323)	120, 92, 8 (wild-type)	212, 8 (*HY*)
Do378R	agtaaagtcagaatgaacattgctgcacaat (annealing temperature 58°C) Expected product 220 bp	*Xcm*I (nt 350) *Dra*III (nt378) In exon 2	167, 53 (wild type) 170, 27, 23 (*DOA*)	220 (*JO*) 170, 50 (*DOB*)
DoABF	cactttaatgcctacacagggaccaccagtcga	*Mnl*I (nt 624)	83, 55, 8 (*DOB*)	83, 63 (*DOA*)
DoMnlR	agggagaagtactgtacaggtgcacccaggca (annealing temperature 62°C) Expected product 146 bp	In exon 2		
DoF	cactttaatgcctacacagggaccaccagtcga	*BseR*I (nt 793)	268, 58, 42 (*DOB*)	326, 42 (*DOA*)
DoR	tatgtgctcaggttcccagttgacctcaacgacaac (annealing temperature 62°C) Expected product 368 bp	In exon 2		
DoEx3F	tcaatggatagatgaggtag	*Bsm*AI (nt 898)	170, 121 (*HY1*)	291 (wild-type and *HY2*)
DoEx3R	tggtttcagcagaagtatga (annealing temperature 55°C) Expected product 291 bp	In exon 3		

The nucleotides are numbered as in Gubin et al.,[30] i.e., nucleotide 1 is the A of the first AUG initiation codon, and amino acid 1 is methionine.

Results

Tests with monoclonal antibodies

Six MoAbs were cloned; they agglutinated all human RBCs strongly except those with the Gy(a–) phenotype: MIMA-52, MIMA-53, MIMA-55, MIMA-64, MIMA-73, and MIMA-98. All MoAbs recognized trypsin- and pronase-sensitive and papain-resistant epitopes on human RBCs. MIMA-52 and MIMA-98 reacted weakly with DTT-treated (200 mM) antigen-positive RBCs. MIMA-52 did not react with neuraminidase-treated RBCs. MIMA-55, MIMA-64, and MIMA-98 reacted weakly with α-chymotrypsin–treated RBCs. MIMA-52 and MIMA-53 strongly agglutinated RBCs from great apes but not from lesser apes, old world monkeys, new world monkeys, prosimians, rabbits, dogs, sheep, or mice.[37] The other MoAbs reacted with different patterns with RBCs from non-human primates (Table 4).

Table 4. Results of hemagglutination with RBCs from non-human primates

RBCs	MIMA-52 MIMA-53	MIMA-55	MIMA-64	MIMA-73 MIMA-98
Chimpanzee (n = 2)	4+	2+	2+	3+
Gorilla (n = 1)	4+	0	0	0
Orangutan (n = 2)	4+	2+	3+	3+
Gibbon (n = 1)	0	1+	1+	1+
Baboon (n = 2)	0	0	1+ and 0	2+
Macaque (n = 3)	0	0	0	1+
Squirrel monkey (n = 1)	0	0	0	0
Lemur (n = 1)	0	0	0	0
Sheep (n = 1)	0	0	0	0
Dog (n = 1)	0	0	0	0
Rabbit (n = 1)	0	0	0	0
Mouse (n = 1)	0	0	0	0

PCR-RFLP analysis

The interpretation of results obtained by PCR-RFLP analyses is shown in Table 2B. The RFLP patterns obtained for exon 2 at nt 323 (*HY*) and at nt 350 (*JO*) were the same in chimpanzees (n = 3), gorillas (n = 2), orangutans (n = 2), macaques (n = 3), and humans. At nt 624 and nt 793, macaques gave the same RFLP patterns as the great apes; at nt 898, the pattern was the same as that of the human *HY1* allele. The gorillas and orangutans had the same RFLP band pattern at nt 898 as chimpanzees and humans. No PCR amplification was obtained with DNA from new world monkeys (two tamarins and two squirrel monkeys) when using primers located at the 5' end of exon 2. The PCR-RFLP band pattern for nt 793 analysis was the same as for chimpanzees and nt 624 in the tamarin gave the same pattern PCR-RFLP band as human *DOA*.

Discussion

The hemagglutination tests with MoAb anti-Dombrock and PCR-RFLP assays performed with human primers allowed us to study Dombrock homologs in greater apes, old world monkeys, and new world monkeys. Our results imply that there is a high degree of conservation in the nucleotide sequence among the higher species of primates. The non-amplification of the 5' portion of exon 2 in new world monkeys suggests that the sequence was different enough that the human *DO* sequence-specific primers did not bind. The gorillas and orangutans had the same RFLP pattern as chimpanzees, i.e., human *DOB* at nts 378 and 624 and *DOA* at nt 793. The old world monkeys had a RFLP pattern at nt 898 that was the same as the human *HY1* allele.[35] In new world monkeys, only two RFLP analyses were possible; the pattern at nt 793 was the same as for chimpanzees, while at nt 624 the pattern was the same as for human *DOA*. Reactivity of MoAb anti-Dombrock with RBCs from non-human primates shows that the MoAb epitopes are conserved with the higher order primates.

Our findings imply that the SNP associated with *DOA* at nt 793 is the primordial gene and that the rearrangements at nts 323, 350, 378, and 624 are recent changes in the phylogenetic tree. Due to the small number of each species tested, it is not possible to determine whether the *DOA/DOB* polymorphism exists in non-human primates. The high degree of homology in *DO* suggests that the protein it encodes has an important role. The human *DOB* allele encodes an Arg-Gly-Asp (263-RGD-265) motif. RGD motifs within adhesive ligands are commonly involved in cell-to-cell interactions involving integrin binding.[38] However, because the *DOA* allele and the non-human primate *DO* homologs disrupt the aspartic acid moiety, which in other proteins has been correlated with overall antigenicity of RGD-containing proteins, it is unlikely that the RGD motif in the *DOB* allele is a critical one.[30,35,39] There is no known pathology associated with the Do^a form of the Dombrock glycoprotein where aspartic acid (D) is replaced by asparagine (N) (RGD→RGN) nor, indeed, with an absence of the entire glycoprotein (Gy[a–]). The presence of RGN in non-human homologs[35] strongly suggests that this, and not RGD, is the primordial sequence. Expression of Dombrock and other ectoenzymes (Kell and Yt) on RBCs may provide a readily transportable steady-state level of these enzymes for tissues in the vascular space.

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Murine monoclonal antibodies can be used to type RBCs with a positive DAT

G.R. Halverson, P. Howard, H. Malyska, E. Tossas, and M.E. Reid

RBCs with a positive DAT due to IgG coating require the use of directly agglutinating reagents or treatment with chemicals to remove sufficient IgG to permit typing of the RBCs with antisera that require use of the IAT. In this study we demonstrate that murine IgG MoAbs to human RBC antigens can be used as an alternative if the anti-mouse IgG is neutralized or affinity purified to prevent cross-reaction with cell-bound IgG. We performed DATs on RBC samples coated with IgG in vivo and in vitro, comparing two anti-human IgG reagents (Organon Teknika, Durham, NC, and Ortho-Clinical Diagnostics, Raritan, NJ) with two affinity-purified anti-mouse IgG reagents (The Binding Site, San Diego, CA, and Sigma, St. Louis, MO), and one non-purified anti-mouse IgG reagent. The affinity-purified anti-mouse IgG reagents were nonreactive with the four in vitro sensitized RBC samples and were nonreactive with 8 of 11 in vivo sensitized RBC samples. Non-purified anti-mouse IgG and both anti-human IgG reagents reacted with every sample. Use of murine MoAbs to antigen type RBCs coated with human IgG is reliable only when the anti-mouse IgG reagents have been affinity purified or neutralized to prevent cross-reactivity. Our results also show the importance of including a saline/RBC control as well as an anti-mouse IgG/RBC control. Murine MoAbs are valuable reagents and we have applied them successfully in typing patients' RBCs that have a positive DAT. ***Immunohematology* 2003;19:83–85.**

Key Words: murine monoclonal antibodies, anti-mouse IgG, DAT

RBCs that are coated with human IgG are DAT-positive and therefore cannot be antigen typed using normal human reagent antibodies that react by the IAT. Various alternative methods have been described to overcome this problem.[1] These include the use of saline-reactive antibodies (which are rare), removal of IgG from the RBC surface by various elution methods (acid, chloroquine, heat) before testing with reagents requiring the IAT, adsorption/elution of alloantibodies, and DNA testing.[2] It is also possible to use murine IgG MoAbs as alternative reagents for typing DAT-positive RBCs, but it is critical to use anti-mouse IgG reagents that have been properly purified to prevent cross-reactivity with human IgG, which causes falsely positive reactions.

Materials and Methods

RBCs coated with human IgG in vivo were obtained from patients with warm autoantibodies. In vitro sensitized RBCs were prepared by incubating 500 μL of washed, packed, antigen-positive RBCs with 500 μL of commercial polyclonal antisera for 30 minutes at 37°C. Anti-human IgG reagents were purchased from Ortho-Clinical Diagnostics (Raritan, NJ) and Organon Teknika (Durham, NC). Anti-mouse IgG affinity-purified reagents were purchased from The Binding Site (San Diego, CA) and Sigma Chemical Co. (St. Louis, MO). To determine the optimal dilution of the anti-mouse IgG, the reagents were diluted in 6% BSA/PBS at pH 7.4 and tested in dilution studies with four murine MoAbs: MIMA-9 (anti-Kp^b), MIMA-19 (anti-Fy^a), MIMA-23 (anti-K), and MIMA-27 (anti-Kp^a).[3–5] Both anti-mouse IgG reagents reacted at an optimal dilution of 1 in 50. Rabbit anti-mouse IgG was produced by immunizing rabbits with mouse IgG at Cocalico Biologics, Inc., Reamstown, Pennsylvania. The rabbit serum was then diluted 1 in 50 in PBS for testing. To prevent cross-reactivity with human IgG, this reagent was neutralized with antibody-free human serum. Antiglobulin tests using anti-human IgG and anti-mouse IgG commercial reagents were performed by standard methods in test tubes. RBCs were washed × 4 in PBS immediately prior to testing with the panel of antiglobulin reagents.

Results

Of 11 RBC samples sensitized with IgG in vivo, nine were strongly reactive (4+) and two were weakly reactive (1+ and 2+) in the DAT with anti-human IgG reagents. With both affinity-purified anti-mouse IgG reagents, three samples that were weakly reactive (1+) by IAT were also agglutinated in the PBS control; the other eight samples were nonreactive. In contrast, the

non-neutralized anti-mouse IgG agglutinated all RBC samples except for one with a weakly positive (1+) DAT. There was variation in reaction strength, but in general the strongest reactivity was obtained with the RBCs with the strongest DAT (Table 1).

Table 1. DAT results: RBC samples sensitized with IgG in vivo

Sample	Saline	H # 1	H # 2	M # 1	M # 2	M # 3
1	0	4+	4+	0	0	4+
2	0	2+	2+	0	0	1+
3	0	4+	4+	0	0	3+
4	0	1+	1+	0	0	0
5	0	4+	4+	0	0	0
6	1+	4+	4+	1+	1+	3+
7	1+	4+	4+	0	0	3+
8	0	4+	4+	0	0	4+
9	1+	4+	4+	1+	1+	2+
10	0	4+	4+	0	0	4+
11	1+	4+	4+	1+	1+	2+

H # 1 = Organon Teknika anti-human IgG
H # 2 = Ortho-Clinical Diagnostics anti-human IgG
M # 1 = The Binding Site affinity purified anti-mouse IgG
M # 2 = Sigma affinity purified anti-mouse IgG
M # 3 = MTS anti-mouse IgG (not neutralized)

To determine whether specificity of the immunoglobulin coating the RBCs was a factor, we sensitized antigen-positive RBCs in vitro with human polyclonal anti-K, anti-k, anti-s, and anti-Jk[a]. As shown in Table 2, the results were similar to those obtained with the RBCs that had been sensitized in vivo.

To eliminate unwanted cross-reactivity, 1 part human serum to 3 parts of rabbit serum containing anti-mouse IgG were mixed. This was incubated for at least 1 hour at room temperature before use. Whereas the "raw" reagent strongly agglutinated Coombs Control Cells (Ortho-Clinical Diagnostics), the neutralized reagent did not. All RBC samples sensitized, whether in vivo or in vitro with human IgG, were not agglutinated by this neutralized reagent. The ability of this neutralized anti-mouse IgG to strongly agglutinate RBCs coated with monoclonal murine IgG antibodies was not affected by the addition of human serum.

Table 2. DAT results: RBC samples sensitized with IgG in vitro

Sample	Saline	H # 1	H # 2	M # 1	M # 2	M # 3
Anti-Jk[a]	0	3+	4+	0	0	3+
Anti-s	0	4+	4+	0	0	3+
Anti-K	1+	4+	4+	0	0	3+
Anti-k	0	4+	4+	0	0	3+

H # 1 = Organon Teknika anti-human IgG
H # 2 = Ortho-Clinical Diagnostics anti-human IgG
M # 1 = The Binding Site affinity purified anti-mouse IgG
M # 2 = Sigma affinity purified anti-mouse IgG
M # 3 = MTS anti-mouse IgG (not neutralized)

Conclusion

Our results show that while affinity-purified anti-mouse IgG reagents do not cross-react with human IgG, reagents that are not purified or neutralized may cross-react. These results demonstrate the importance of using anti-mouse IgG reagents that have been affinity-purified or neutralized to prevent cross-reactivity. The need for use of proper controls to validate testing is also evident. When used with appropriate anti-mouse IgG reagents, murine MoAbs are valuable tools for performing RBC phenotyping in the presence of a positive DAT. They are used extensively in our laboratory to resolve antigen typing problems of this kind.

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Rh antigen and phenotype frequencies and probable genotypes for the four main ethnic groups in Port Harcourt, Nigeria

Z.A. Jeremiah and F.I. Buseri

Rh is the most complex and polymorphic of the RBC group systems and is of major importance in transfusion medicine. Data are not available on the frequency of Rh antigens D, C, E, c, and e in Port Harcourt, Nigeria. Two mL of venous blood was collected into an EDTA tube from each of 400 persons of mixed ethnic groups recruited for the study. The study population comprised 167 Ijaws (41.8%), 141 Ikwerres (35.2%), 50 Ekpeyes (12.5%), and 42 Ogonis (10.5%). The RBCs were phenotyped for D, C, E, c, and e antigens according to standard serologic methods. The most frequently occurring antigen was found to be c (99.8%), followed by e (98.7%), then D (95.0%), E (20.5%), and finally C (17.7%). The antigens occurred independently of the ethnic groups ($p > 0.05$) except the antithetical antigens Ee, which were found to be statistically significant in the Ijaw ethnic group when subjected to Pearson chi-square test ($\chi^2 = 9.890$, $p < 0.02$). One (0.2%) of the study population was found to be c– while 20 (5.0%) were D–. ***Immunohematology*** **2003;19:86–88.**

Key Words: Rh antigens, Rh phenotypes, ethnic groups in Nigeria

Since the discovery of the Rh system in 1940, more than 40 antigens have been discovered, making it the most complex RBC antigen system.[1,2] In addition, the frequency of Rh antigens has been found to vary between racial groups.[1–3] Information relating to the frequencies of Rh phenotypes in Blacks has been determined by testing African Americans or small groups of Africans with little regard for ethnic variation. It is hoped that the data obtained from this study of the major ethnic groups in Port Harcourt will provide needed information about the ethnic groups in Nigeria.

Materials and Methods

Subjects

A total of 400 persons of both sexes and of various ages and ethnic backgrounds attending Rivers State College of Health Science and Technology in the heart of the Port Harcourt metropolis were recruited into the study. The study population consisted of four major ethnic groups, namely, Ijaw (167), Ikwerre (141), Ekpeye (50), and Ogoni (42). The health conditions of the subjects were not considered critical for this study.

Serology

Two mL of venous blood was collected from each subject into EDTA tubes. RBCs were phenotyped for D, C, E, c, and e antigens according to standard serologic protocols (tube method)[4] with an IgM anti-D MoAb (IG 1016) and IgM MoAbs recognizing C (AC 2206), c (RH 1267), E (ME 092), and e (AE 3079) (Biotec, London). An anti-human globulin (AHG) reagent, AHG (RE 2470), albumin 30% (RE 2727), and an anti-CDE MoAb (CDE 490) were also used. The titers of the five Rh antisera were determined by serial dilution; the titers ranged from

Table 1. Observed frequencies of the Rh D, C, c, E, and e antigens for the 400 persons within the four ethnic groups

Antigens	Number positive (% positive)	Number negative (% negative)
D	380 (95.0)	20 (5.0)
C	71 (17.7)	329 (82.3)
c	399 (99.8)	1 (0.2)
E	82 (20.5)	318 (79.5)
e	395 (98.7)	5 (1.3)

Table 2. Frequency distribution of Rh antigens for the 400 persons within the four ethnic groups

Ethnic Groups (Number Tested)	D Percent Pos	D Percent Neg	C Percent Pos	C Percent Neg	c Percent Pos	c Percent Neg	E Percent Pos	E Percent Neg	e Percent Pos	e Percent Neg
IJAW(167)	40.0	1.8	8.0	33.8	41.8	0	9.8	32.0	41.5	0.3
IKWERRE(141)	32.8	2.5	4.8	30.5	35.3	0	6.0	29.3	34.8	0.5
EKPEYE(50)	11.8	0.8	3.3	9.3	12.3	0.3	1.7	10.8	12.3	0.3
OGONI(42)	0.5	0	1.7	8.8	10.5	0	3.0	7.5	10.3	0.3
Pearson chi-square (χ^2) value (p > 0.05)	3.845		4.358		7.018		4.856		1.269	
Degrees of freedom	3		3		3		3		3	
	ns*		ns		ns		ns		ns	

* Not significant

Table 3. Frequency of the antithetical antigens within the four ethnic groups

	Cc Freq (%)†	CC Freq (%)	cc Freq (%)	Ee Freq (%)	EE Freq (%)	ee Freq (%)	DD/Dd Freq (%)	dd* Freq (%)
IJAW	32 (19.2)	0	135 (80.8)	38 (22.8)‡	1(0.6)	128 (76.6)	158 (95.8)	7 (4.2)
IKWERRE	19 (13.5)	0	122 (86.5)	22 (15.6)	2 (1.4)	117 (83.0)	138 (93.2)	10 (6.8)
EKPEYE	12 (24.6)	1 (2.0)	37 (74.0)	6 (12.0)	1 (2.0)	43 (86.0)	46 (92.0)	4 (8.0)
OGONI	7 (16.7)	0	35 (83.3)	11 (26.2)	1 (2.4)	30 (71.4)	40 (100)	0

* d represents the absence of the D antigen

† Frequency (% of total)

‡ Pearson chi-square (χ^2) value = 9.890; p < 0.02

16 to 64. All the reagents were manufactured in October 2000 and had an expiration date of October 2002.

Six tubes were set in a row for each sample and labeled anti-D, anti-E, anti-e, anti-c, anti-C, and auto control. Two drops of antisera were added to each tube and a drop of 5% washed RBC suspension was added to the tubes. This was followed by incubation at 37°C for 30 minutes. At the end of 30 minutes, the tubes were centrifuged and the agglutination was read macroscopically after resuspending the RBCs. All negative results were confirmed microscopically. Anti-CDE, albumin, and AHG tests were used as controls and for weak D confirmation.

Statistical methods

Results were analyzed using the computer statistical analytical system (SAS). Pearson chi-square (χ^2) test was used for comparison of the frequency distribution of the antigens in the various ethnic groups. Values less than 0.05 ($p < 0.05$) were considered to be statistically significant.

Genotypes

Probable genotypes were determined by using a calculator for determination of Rhesus genotypes supplied by Ortho-Clinical Diagnostics, Raritan, New Jersey.

Results

Of the 400 samples tested, 95 percent were D+, 17.7 percent were C+, 99.8 percent were c+, 20.5 percent were E+, and 98.7 percent were e+ (Table 1). The frequency distribution of the Rh antigens within the various ethnic groups is shown in Table 2. The pattern of distribution did not show any statistical significance when subjected to Pearson chi-square analysis ($p > 0.05$). As shown in Table 3, the frequency of the antithetical antigens Ee was significant in the Ijaw group ($p < 0.02$). Based on the observed frequencies of the Rh antigens (see Table 1), Table 4 lists the nine most probable Rh phenotypes in the four ethnic groups. Also cited are common Rh genotypes for the listed phenotypes.

Discussion

In this study, the observed frequency of Rh antigens and phenotypes was determined in the Port Harcourt metropolis. The result of this study depended on the genetic constitution of the subjects and not on sex, age, or disease conditions. The persons studied were residents of Port Harcourt and were students of a multiethnic tertiary institution.

Table 4. Number and frequency of the most probable Rh phenotypes for the 400 persons from the four ethnic groups

Phenotypes	Frequency (%)	Probable genotype*
Dce/Dce	243 (60.8)	R^0R^0
DcE/Dce	70 (17.5)	R^2R^0
DCe/Dce	58 (14.5)	R^1R^0
dce/dce	12 (3.0)	rr
DCe/DcE	7 (1.8)	R^1R^2
DcE/dCE	4 (1.0)	R^2r^y
dCe/dce	4 (1.0)	$r'r$
DCe/dCe	1 (0.2)	R^1r'
DCe/dCE	1 (0.2)	R^1r^y

* Taken from an Ortho-Clinical Diagnostics calculator

In England, about 85 percent, 70 percent, 30 percent, 80 percent, and 98 percent of the population are positive for the D, C, E, c, e antigens, respectively.[5] These figures show marked differences from those obtained in Port Harcourt. Similar differences were also found between the Port Harcourt and German and Japanese populations.[6,7]

The D– phenotype is found in approximately 15 percent of Caucasians, and much earlier studies done in Africa suggested that frequencies of 20 to 30 percent existed in Middle and West African countries.[8] Among the subjects in Port Harcourt, only 5 percent were D–, and this did not differ significantly between the four ethnic groups. These data are similar to a recent report of blood donors in Mali, where only 7 percent were D–.[9] Interestingly, no D– persons were found among the Ogoni ethnic group, which could be due to a small sample size. Of the 5 percent D– donors (n = 20) in Port Harcourt, 12 were of the *dce/dce* genotype while eight were of the *dCe/dce* genotype.

Except for C and E, results from our study were similar to those obtained from the Eastern part of Nigeria.[10] The following frequencies were observed in that study: c (92.7%), e (94.7%), C (43.7%), D (96.7%), and E (44.4%). In Port Harcourt the Dce/Dce phenotype was found to be very frequent (60.8%), and of the 400 persons tested, only one was c– (0.2%). In comparison, the most common Rh phenotype in England was DCe/dce (31.7%).[5] Thus, as opposed to a 1981 study in Southwest England[11] that found anti-c to be a common cause of HDN, we would expect anti-C to occur more frequently in our obstetric population.

This paper illustrates the great variability of blood group phenotypes within the ethnic groups of Africa. Studies performed in South Africa cannot be applied to populations residing in North Africa. The Duffy blood group is a prime example, as the Fy(a–b–) phenotype reached frequencies of almost 100 percent but only in the areas highly endemic for *Plasmodium vivax,* i.e., West Africa. The differences between the Ijaws and the other three ethnic groups tested from Nigeria further demonstrate just how polymorphic blood groups can be in Africans and those of African decent.

Acknowledgment

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Antibodies detected in samples from 21,730 pregnant women

S. Jovanovic-Srzentic, M. Djokic, N. Tijanic, R. Djordjevic, N. Rizvan, D. Plecas, and D. Filimonovic

Although anti-D is still the main cause of HDN, many other antibodies have been implicated. From September 1995 to April 2000, screening for RBC antibodies was performed on samples from 21,730 pregnant women regardless of RhD type. Standard tube and gel methods were used. Anti-D was identified in 254 samples; other antibody specificities were detected in 376 samples, for a total of 630 antibodies. For this study, 522 antibodies were considered clinically significant. The incidence of potentially clinically significant antibodies was 2.4 percent. The majority belonged to the Rh system, followed by anti-M, -Fy^a, -S, -Jk^a, and -Jk^b. Among antibodies of no clinical significance, the most frequent were anti-H, -Le^a, and -P_1. ***Immunohematology*** **2003;19:89–92.**

Key Words: non-anti-D antibodies, fetomaternal hemorrhage, screening, third trimester, pregnancy

Prenatal immunohematologic care of pregnant women requires the investigation of unexpected RBC antibodies in their sera during pregnancy. When RBC antibody screening is positive, it is necessary to determine specificity of the antibody, its clinical importance, and the ability to cross the placenta and cause HDN.

Anti-D is still the main cause of HDN, despite the use of systematic RhD immunoprophylaxis since 1968 in many developed countries. The incidence of anti-D decreased from 17 percent to 1.5 percent after the administration of postpartum prophylaxis, and from 1.5 percent to 0.6 percent after antepartum Rh immunoprophylaxis.[1] In Yugoslavia, the incidence of alloimmunization to D is still high despite postpartum RhD immunoprophylaxis. According to several authors, the incidence of anti-D in our country, where RhD immunoprophylaxis is administered only postpartum, is between 1.28 percent and 1.68 percent.[2,3]

According to published data, the incidence of clinically significant antibodies during pregnancy in Croatia is approximately 1 percent (64.8% anti-D), 0.58 percent in Tyrol (54% anti-D), 0.82 percent in Salzburg (48% anti-D), and 0.24 percent in Sweden (32% anti-D).[4–6]

The aim of our study was to determine the current incidence of non-anti-D antibodies in both D– and D+ pregnant women in our country.

Materials and Methods

From September 1995 to April 2000, RBC antibody screening was performed on blood samples from 21,730 D+ and D– pregnant women at the Laboratory for Antenatal Screening of the National Blood Transfusion Institute (NBTI). Transfusion and previous pregnancy histories were unobtainable in most cases. RBC antibody screening was done at room temperature, using enzyme-treated RBCs (papain, Merck), and by the IAT using a tube method. Enzyme-treated and non-treated group O reagent RBCs were prepared in our Institute according to standard procedures. Suspect and positive results of antibody screening were checked by the gel method (DiaMed ID, Cressier sur Morat, Switzerland), with commercially prepared reagent RBCs from the same manufacturer. Antibody identification was performed by either the tube or the gel method. Commercially prepared RBC panels were provided by the NBTI and by DiaMed. Polyspecific and monospecific anti-human globulin (AHG) test reagents were products commercially prepared in our Institute and by DiaMed. Antibody titration was done, whenever possible, using a manual method by IAT using monospecific anti-IgG.[7–10]

Results

The incidence of anti-D found in the 21,730 samples in our study was 1.16 percent, which is similar to the incidence of anti-D found in a previous investigation (1.36%).[2] Anti-D was demonstrated in 254 (40.0%) of the 630 antibody-positive samples (Table 1), however, 54 were attributed to "passive" anti-D postpartum prophylaxis.

Most of the clinically significant non-anti-D antibodies belonged to the Rh system (77%). The most common antibody in this group was anti-C (23%), which was, with one exception, always identified with anti-D (Table 1).

There were 67 anti-E (11%) in the sera of both D+ and D– pregnant women. There were 17 anti-c (2.7%) and 2 anti-C^w (0.3%); 12 of 17 anti-c and the majority of anti-C were confirmed using enzyme-treated RBCs. The remaining five anti-c antibodies were identified by IAT, with antibody titers between 8 and 16. All 67 anti-E and the two anti-C^w were identified only using enzyme-treated RBCs (Table 1).

Table 1. Specificity and percentage of 485 clinically significant Rh antibodies detected in 630 positive blood samples from pregnant women

Antibody	Number detected	% detected
D	254	40.0
C	145	23.0
E	67	11.0
c	17	2.7
C^w	2	0.3
Total	485	77.0

Anti-M was identified in 23 samples (3.7%). All were identified at room temperature. Three were also present in the IAT using anti-IgG AHG.

Anti-Fy^a was identified in six cases (0.9%) in our study; the antibodies of lowest incidence were four anti-S (0.6%), three anti-Jk^a (0.5%), and one anti-Jk^b (0.2%) (Table 2). All of these antibodies were detected by IAT.

Table 2. Specificity and percentage of 37 clinically significant non-Rh antibodies detected in 630 positive blood samples from pregnant women

Antibody	Number detected	% detected
M	23	3.7
Fy^a	6	0.9
S	4	0.6
Jk^a	3	0.5
Jk^b	1	0.2
Total	37	5.9

Among the antibodies without clinical significance, the most frequent were 49 anti-H (7.8%), which were identified using enzyme-treated RBCs, mostly at room temperature. Anti-Le^a was present in 30 samples (4.7%), anti-Le^b in 17 samples (2.7%), and anti-Le^a+Le^b in 3 samples (0.5%). In most of the investigated cases, Lewis antibodies were demonstrated using enzyme-treated RBCs and, less commonly, by IAT. They usually caused hemolysis using enzyme-treated RBCs.

Table 3. Specificity and percentage of 108 non-clinically significant antibodies detected in 630 positive blood samples from pregnant women

Antibody	Number detected	% detected
H	49	7.8
Le^a	30	4.7
Le^b	17	2.7
P_1	9	1.4
Le^a and Le^b	3	0.5
Total	108	17.1

Nine examples of anti-P_1 (1.4%) were detected at room temperature and using enzyme-treated RBCs (Table 3).

Due to the number of samples (21,730) sent for testing from various sources over a period of 5 years, it was not feasible in most cases to obtain individual pregnancy and transfusion histories.

Discussion and Comment

Anti-D can cause both a moderate and a severe form of HDN. The incidence of anti-D alloimmunization in D– women without the administration of prophylactic anti-D during pregnancy is usually noted at the end of a second pregnancy, with an incidence of 8 to 10 percent, and after the fourth or fifth pregnancy, at 50 percent.[1,4,11]

The most frequent and potentially significant non-anti-D antibody in our study was anti-C. The incidence among both D+ and D– pregnant women was 23 percent. Bowell found an incidence of 14 percent in D+ pregnant women.[18] HDN caused by anti-C is usually mild, as the C antigen has weak immunogenicity.[1,11–13]

Anti-E was found in 11 percent of our cases. Anti-E can be a naturally occurring IgM antibody, as it was in most of our cases. IgG anti-E can be found in the sera of pregnant women with a history of previous transfusions and pregnancies. This immune form of anti-E is able to cause mild or moderate HDN.[1,4,11,14]

The incidence of anti-c in our investigation was 2.7 percent, which is similar to Bowell's results for pregnant women.[15] In most women, alloimmunization to the c antigen is found after multiple pregnancies, transfusion of c+ RBCs, or both. Mild and moderate cases of HDN usually appear when the titer of anti-c is higher than 8 by IAT.[4,15]

Anti-C^w in our study had an incidence of 0.3 percent. This antibody can sometimes cause mild HDN.[1,4]

Anti-M had an incidence of 3.7 percent in our study, which correlates with results in similar investigations. These antibodies are usually naturally occurring IgM + IgG antibodies. A clinically significant IgG anti-M has been reported as a cause of hydrops fetalis.[16] The incidence of clinically significant IgG anti-M is 0.1 percent.[16,17]

Anti-S seldom causes HDN.[1,4] In our study, the incidence of this antibody was 0.6 percent.

According to published data, anti-Fy^a can be found in 33 percent of Fy(a–) persons transfused with Fy(a+) RBCs. Anti-Fy^a rarely causes HDN, but some of the described cases were fatal.[1] There were six examples of anti-Fy^a in our study, with an incidence of 0.9 percent.

Anti-Jk^a and -Jk^b (0.5% and 0.2%, respectively), complement binding antibodies, were rarely found in our investigation. Published data show that they seldom cause HDN, regardless of antibody titer.[1] So far there is no explanation for that, although complement is not fully developed during fetal life nor right after delivery.[4]

Anti-K was not seen in our investigation. The incidence of the K antigen in Caucasians is 9 percent and, after the D antigen, the K antigen is the most immunogenic. HDN caused by anti-K can be severe.[1] There is evidence that anti-K can recognize K antigens expressed at an early stage of erythroid development in the fetal liver and can cause anemia by suppressing erythropoiesis.[1,18,19]

Anti-H, -Le^a, -Le^b, and -P_1 have little or no clinical significance even though they can often be found in the sera of pregnant women. It is well known that expression of Lewis antigens is reduced during pregnancy. The explanation is in the slight decrease of Le glycolipid in the plasma during pregnancy and in the increased ratio of lipoprotein to RBC mass that occurs in pregnant women. There is not an exact explanation for the higher appearance of anti-H and -P_1 during pregnancy. The incidence of these antibodies in our study was 7.7 percent and 1.4 percent, respectively.

Guidelines for prenatal immunohematology in most countries suggest testing for unexpected antibodies at the initial visit, regardless of the woman's D type. Third trimester testing is recommended for D– women and for both D– and D+ women when there is a history of previously detected clinically significant antibodies, blood transfusions, or complicated deliveries.[20]

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BOOK REVIEWS

Information Technology in Transfusion Medicine. Suzanne H. Butch, MA, MT(ASCP)SBB, and Marcus B. Simpson, MD, Editors. Bethesda, MD: American Association of Blood Banks (AABB) Press, 2002. 288 pp. List price $105; member price $75. ISBN: 1-56395-163-0. Stock number: 022035. To order: call (866) 222-2498.

The AABB Press has published another winner—a highly readable paperback on information technology (IT) for blood bankers in hospital transfusion services or blood centers. Editors Suzanne H. Butch, MA, MT(ASCP)SBB, and Marcus B. Simpson, MD, recruited eight authors, whose chapters span the field from the fundamentals of bar code science to the selection, implementation, and validation of computerized blood bank information systems.

Three chapters were particularly useful for this reviewer. First, a 37-page chapter entitled "Bar Code Data Collection in the Blood Bank" by Bruce R. Way, MBA, Director of Marketing, Computype, Inc., provides a comprehensive description of bar codes and scanners, supported by 10 tables and 25 illustrations. The focus is blood bank application, which is illustrated by two subtitles, "Codabar: The Bar Code Standard in Blood Banks" and "ISBT 128—The New Bar Code Standard in Blood Banks." Second, Alan Kusinitz, Managing Partner, SoftwareCPR, wrote a highly resourceful chapter: "Electronic Records and Electronic Signatures." Beginning with a detailed outline of the *Code of Federal Regulations,* 21 CFR 11 part 11—Electronic Records: Electronic Signatures, Mr. Kusinitz outlines FDA's requirements in meticulous detail. He explains that the intent of the regulation is to ensure that electronic records and signatures have the integrity of traditional paper records. Although the FDA regulation is only two and one-half pages long, Mr. Kusinitz's 40-page chapter dissects the regulation and provides expert guidance and practical tips for meeting FDA's requirements. Third, Dr. Marcus B. Simpson's chapter, "Internet Web-Based Applications in Blood Banking and Transfusion Medicine," is a unique resource for accessing the ever-increasing volume of blood bank information available on the Internet. One table lists the URLs (uniform resource locators/"Web addresses") for pertinent search engines, including Yahoo Blood Transfusion. Another table lists URLs for 15 online journals of special interest to blood bankers. Other tables include the URLs for pertinent professional organizations and transfusion medicine educational resources.

Additional chapters providing valuable practical guidance include "To Build or Buy: Selection of Blood Establishment Computer Software," "Validating the System," and "Electronic Data Interchange." The chapter on "Privacy and the Health Insurance Portability and Accountability Act" was written prior to the April 14, 2003, compliance date and, therefore, adds little to the abundant official and interpretive material that has become available since implementation. In summary, whether you are an IT novice or an expert, this concise volume has much to offer as a resource for managing practical issues in a transfusion service or blood center.

S. Gerald Sandler, MD
Professor of Medicine and Pathology
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Attention: State Blood Bank Meeting Organizers

If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please **contact** Mary McGinniss, Managing Editor, 4 months in advance, by phone or fax at (301) 299-7443.

BOOK REVIEWS

Current Perspectives in Cellular Therapy 2000. Zbigniew M. Szczepiorkowski, MD, PhD, and Edward L. Snyder, MD, FACP, eds. Bethesda, MD: American Association of Blood Banks (AABB) Press, 2001. 55 pages. Softcover. List price: $15.00; member price: $12.00. Stock #013150. To order: call (866) 222-2498 or fax (301) 951-7150.

This slim paperback publication consists of a collection of reviews that cover a breadth of topics within the field of cellular therapy. Although the intended audience is not stated, it could include physicians as well as technologists or apheresis personnel. The table of contents is separated into four main categories: novel cell sources for cellular therapy; laboratory aspects of cellular therapy; emerging clinical applications of cellular therapy; and regulatory aspects of cellular therapy. Within a category, each topic follows the same format, with brief coverage of the background, current status, and future directions of the topic. The brevity of the publication will lend itself well to revision as new developments within the field emerge.

The first section, on novel cell sources, is a fine "jumping off" point for an introduction to cellular therapy. Cord blood and mesenchymal stem cells are discussed. The mysteries yet to be solved regarding stem cell plasticity, and the therapies which may follow from such understanding, are outlined. In addition, the section briefly touches upon ethical and political issues that have emerged to impact the available cell sources. Because of the import of these decisions upon progress (or lack thereof) within this field, this topic could easily be expanded to become its own category for discussion in the future.

The second and longest section, on laboratory aspects, is also the most technical. The topics covered give the reader a solid introduction to the guidelines on and issues involved with collecting, storing, and shipping hematopoietic progenitor cells. Controlled rate freezing compared to passive freezing using less dimethyl sulfoxide is discussed. Also touched upon are the challenges of developing new products with acceptable quality parameters and adequate functional tests. Techniques on CD34-positive selection and tumor purging, as well as new technologies in cellular engineering, are listed in an unbiased fashion. The final section, on HLA typing, is a brief summary of three DNA typing methods and their typical usefulness in the HLA lab. Though the chapter does not flow directly from the antecedent topics, it is a useful synopsis of the latest technology used in the HLA typing laboratory.

The section on emerging clinical applications is interesting and informative. Topics such as donor leukocyte infusion and graft-versus-host disease will prove useful to those of us who counsel potential bone marrow and peripheral blood stem cell donors. Other topics, such as adoptive cellular therapy for cancer, speak to the incredible potential that underlies the need for continued research in the field of cellular therapy. The observation that advanced melanoma will regress in a small percentage of cases with the use of T-cell antitumor activity is a tantalizing finding, most deserving of ongoing research efforts to further define the mechanisms behind the observation. The closing discussion on ex-vivo expansion of hematopoietic stem cells is well-written and educates the reader as to why pursuing this endeavor may also prove quite fruitful.

The final section on regulations is very brief, but alludes to enough references and regulatory documents that the interested reader would know how to proceed if more detail was desired.

Overall, *Current Perspectives in Cellular Therapy 2000* is a useful reference for readers who might not otherwise have time to keep up with the latest developments in this field. In the preface, Drs. Szczepiorkowski and Snyder state that the topics chosen are "intended to provide an overview and to serve as an introduction to the burgeoning and rapidly changing field" of cellular therapy. With that as the intended purpose, the book clearly succeeds. The novice will be able to come away feeling more articulate about, and more intrigued by, cellular engineering as it interfaces with transfusion, transplantation, and internal medicine. We await the results of the clinical trials that were ongoing in this first edition, in hopes that the next edition is in press.

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COMMUNICATIONS

Letter to the Editors

Irregular RBC antibodies in the sera of Brazilian pregnant women

Irregular RBC antibodies found in the sera of pregnant women have been studied mainly in developed countries, e.g., Sweden (0.57%)[1] and the Netherlands (2.7%).[2] Other surveys have been carried out in developing countries such as Mexico (10.6%).[3]

In this study, 2338 Brazilian pregnant women from Leonor M. Barros Maternity Hospital in São Paulo were screened by IAT using the DiaMed gel system. In this survey, 239 pregnant women (10.2%) were found to have irregular antibodies identified by the same method used for antibody screening, a figure similar to that observed in Mexico in 1991.[3]

Antibodies identified among the 239 women included 37 anti-D (15.5%), 22 anti-C+D (9.2%), 3 anti-D+E (1.3%), 1 anti-D+Le^a (0.4%), 4 anti-C^w (1.7%), 4 anti-c (0.4%), 11 anti-E (4.6%), 3 anti-e (1.3%), 1 anti-C (0.4%), 1 anti-C+Le^a (0.4%), 2 anti-K (0.8%), 6 anti-M (2.5%), 1 anti-N (0.4%), 1 anti-Jk^a (0.4%), 59 anti-Le^a (24.7%), 11 anti-Le^a+Le^b (4.6%), and 72 cold antibodies (30.1%), specificity not identified with the panel used. Note: almost 60 percent of detected antibodies were clinically insignficant Lewis and cold antibodies.

In order to avoid the most common antibody in pregnant women (anti-D), RhIg is widely administered soon after birth; this has successfully decreased alloimmunization to D in countries such as the United States, England, and Sweden. However, a decrease in the incidence of anti-D has not been observed in developing countries, probably due to deficient preventive care for pregnant women.

In this survey, a high frequency (26.4%) of anti-D was detected alone or together with other irregular antibodies. This finding requires special attention from Brazilian public health authorities in order to achieve better general care for pregnant women by supplying RhIg to all Brazilian maternity hospitals.

In addition, a screening technique that avoids detection of Lewis and cold antibodies, in this case 142 examples, would lessen cost and workload and would significantly reduce the frequency of irregular antibodies detected in the sera of pregnant women.

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Letter From the Editors

Ortho Dedication

Thirteen is not unlucky for *Immunohematology.* That is the number of years that Ortho-Clinical Diagnostics has supported the publication of the September issue—the "Ortho" issue—of the journal; and that represents 13 of the 19 years that *Immunohematology* has been published. In addition, Ortho-Clinical Diagnostics distributes copies of each of the four issues of the journal published each year to members of their Bankers Club. Thirteen years of receiving the journal! Wonderful!

That represents a major contribution to the support of publication of this journal and demonstrates once again the commitment that Ortho-Clinical Diagnostics has to promoting education for its customers in the field of immunohematology.

If you appreciate this generous gift as much as we do, please tell your Ortho-Clinical Diagnostics representative how much you enjoy the journal and appreciate their support of it.

Delores Mallory
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Mary McGinniss
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IN MEMORIAM

Dr. Bertil Cedegren
1925 – 2003

Dr. Bertil Cedegren began his career in bacteriology and serology and started his medical career in 1954. He studied electron microscopy in the United States for 2 years and then began his clinical work at Karolinska Hospital in Karolinska, Sweden. He then went to the Department of Blood Group Serology at the National Laboratory for Forensic Chemistry. In 1965, he went to Umea, Sweden, to the Department of Clinical Chemistry and, in 1968, he became Senior Consultant in Transfusion Medicine and Head of the Blood Bank.

Dr. Cedegren was known around the world for his search for rare blood types among blood donors, patients, and their families in Sweden. He and his staff found an amazing number of rare donors in Sweden. He was always eager to share a fresh or frozen unit of pp, Vel–, Ge–, or other RBCs with any patient in need and he felt strongly that all blood banks should do the same.

I had the honor and pleasure of meeting Dr. Cedegren at an ISBT meeting a number of years ago and we discussed the problem of exchanging and finding rare blood. From his idea came the ISBT Working Party on Rare Donors, which is still an active working party today.

Dr. Cedegren was a kind, dedicated physician and immunohematologist with a great concern for both patient and donor, particularly when there was a need for rare blood.

Delores Mallory
Editor-in-Chief

ANNOUNCEMENTS

Monoclonal antibodies available. The New York Blood Center has developed murine monoclonal antibodies that are useful for donor screening and for typing red cells with a positive DAT. Anti-Rh17 is a direct agglutinating monoclonal antibody. Anti-Fy^a, anti-K, anti-Js^b, and anti-Kp^a are indirect agglutinating antibodies that require anti-mouse IgG for detection. These antibodies are available in limited quantities at no charge to anyone who requests them. **Contact:** Marion Reid, New York Blood Center, 310 E. 67th Street, New York, NY 10021; e-mail: mreid@nybc.org

Annual Symposium. The National Institutes of Health, Department of Transfusion Medicine, will hold their annual symposium, **Immunohematology and Blood Transfusion,** on October 2 and October 3, 2003. The symposium will be co-hosted by the Greater Chesapeake and Potomac Region of the American Red Cross and is free of charge. Advance registration is encouraged. For more information and registration, **Contact:** Karen Byrne, NIH/CC/DTM, Bldg. 10/Rm. 1C711, 10 Center Drive, MSC 1184, Bethesda, MD 20892; e-mail: kbyrne@mail.cc.nih.gov; or visit our Web site: www.cc.nih.gov/dtm.

Workshop on Blood Group Genotyping. The ISBT/ICSH Expert Panel in Molecular Biology has recommended that a workshop be held on blood group genotyping by molecular techniques. The results would culminate in a report at the ISBT Congress in 2004 in Edinburgh. It was decided that only laboratories that provide a reference service in blood group genotyping would be included in the workshop. One of the aims of the workshop would be to establish an external quality assurance plan. If you have any suggestions as to how the workshop should be organized, we would be grateful for your opinions. If you are interested in taking part in such a workshop, please **contact** Geoff Daniels (geoff.daniels@ nbs.nhs.uk). Offer presented by Geoff Daniels, Martin L. Olsson, and Ellen van der Schoot.

HEMATOLOGÍA HABANA′ 2005 – First Announcement. The 5th National Congress and the 7th Latin American Meeting in Hematology, Immunology, and Transfusion Medicine will present a scientific program at the International Conference Center, Havana, Cuba, May 16–20, 2005. A preliminary program lists malignant hemopathies, disorders of RBC membranes, immuno-therapy, histocompatibility, immunohematology, hemolytic disease of the newborn, and blood components as some of the topics. For more information **contact:** Prof. José M. Ballester, President, Organizing Committee, Hematology Habana′ 2005, Apartado 8070, Ciudad de la Habana, CP 10800, Cuba.

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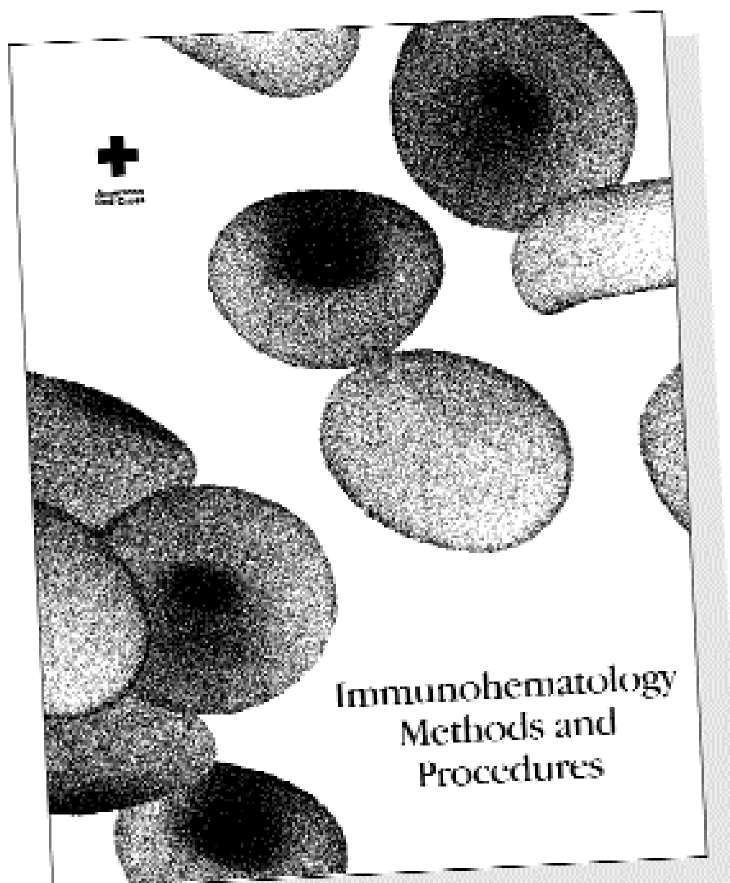

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