

# Immunohematology

---

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 21, NUMBER 1, 2005



**American Red Cross**

*Together, we can save a life*

# Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

VOLUME 21, NUMBER 1, 2005

## C O N T E N T S

1

An unusual anti-H lectin inhibited by milk from individuals with the Bombay phenotype

S.R. JOSHI, K. VASANTHA, AND J.S. ROBB

5

Gene frequencies of the HPA-1 to 6 and Gov human platelet antigens in Thai blood donors

P. KUPATAWINTU, O. NATHALANG, R. O-CHAROEN, AND P. PATMASIRIWAT

10

ABO, Rh, MNS, Duffy, Kidd, Yt, Scianna, and Colton blood group systems in indigenous Chinese

L. YAN, F. ZHU, Q. FU, AND J. HE

15

Expression of Duffy antigen receptor for chemokines during reticulocyte maturation: using a CD71 flow cytometric technique to identify reticulocytes

I.J. WOOLLEY, E.M. WOOD, R.M. SRAMKOSKI, P.A. ZIMMERMAN, J.P. MILLER, AND J.W. KAZURA

21

Rh antigens and phenotype frequencies of the Ibibio, Efik, and Ibo ethnic nationalities in Calabar, Nigeria

Z.A. JEREMIAH AND C. ODUMODY

25

*FCGR3B* polymorphism in three ethnic Chinese populations

L. YAN, F. ZHU, L. JIN, Q. LV, AND Q. FU

29

C O M M U N I C A T I O N S

Letters From the Editors

30

A N N O U N C E M E N T S

33

C L A S S I F I E D A D

34

A D V E R T I S E M E N T S

36

I N S T R U C T I O N S F O R A U T H O R S

### EDITOR-IN-CHIEF

Delores Mallory, MT(ASCP)SBB  
*Supply, North Carolina*

### TECHNICAL EDITOR

Christine Lomas-Francis, MSc  
*New York, New York*

### MANAGING EDITOR

Cynthia Flickinger, MT(ASCP)SBB  
*Philadelphia, Pennsylvania*

### SENIOR MEDICAL EDITOR

Scott Murphy, MD  
*Philadelphia, Pennsylvania*

### ASSOCIATE MEDICAL EDITORS

Geralyn Meny, MD  
*Philadelphia, Pennsylvania*

David Moolton, MD  
*Philadelphia, Pennsylvania*

S. Gerald Sandler, MD  
*Washington, District of Columbia*

Ralph Vassallo, MD  
*Philadelphia, Pennsylvania*

### EDITORIAL BOARD

Patricia Arndt, MT(ASCP)SBB  
*Los Angeles, California*

W. John Judd, FIBMS, MIBiol  
*Ann Arbor, Michigan*

Paul M. Ness, MD  
*Baltimore, Maryland*

James P. AuBuchon, MD  
*Lebanon, New Hampshire*

Christine Lomas-Francis, MSc  
*New York, New York*

Mark Popovsky, MD  
*Braintree, Massachusetts*

Geoffrey Daniels, PhD  
*Bristol, United Kingdom*

Gary Moroff, PhD  
*Rockville, Maryland*

Marion E. Reid, PhD, FIBMS  
*New York, New York*

Richard Davey, MD  
*New York, New York*

Ruth Mougey, MT(ASCP)SBB  
*Carrollton, Kentucky*

Susan Rolih, MS, MT(ASCP)SBB  
*Cincinnati, Ohio*

Sandra Ellisor, MS, MT(ASCP)SBB  
*Anaheim, California*

John J. Moulds, MT(ASCP)SBB  
*Raritan, New Jersey*

David F. Stroncek, MD  
*Bethesda, Maryland*

George Garratty, PhD, FRCPath  
*Los Angeles, California*

Marilyn K. Moulds, MT(ASCP)SBB  
*Houston, Texas*

Marilyn J. Telen, MD  
*Durham, North Carolina*

Brenda J. Grossman, MD  
*St. Louis, Missouri*

Sandra Nance, MS, MT(ASCP)SBB  
*Philadelphia, Pennsylvania*

### EDITORIAL ASSISTANTS

Linda Berenato

Judith Abrams

### COPY EDITOR

Lucy Oppenheim

### PRODUCTION ASSISTANT

Marge Manigly

### ELECTRONIC PUBLISHER

Paul Duquette

*Immunohematology* is published quarterly (March, June, September, and December)  
by the American Red Cross, National Headquarters, Washington, DC 20006.

*Immunohematology* is indexed and included in *Index Medicus* and MEDLINE on the MEDLARS system.  
The contents are also cited in the EBASE/Excerpta Medica and Elsevier BIOBASE/Current Awareness  
in Biological Sciences (CABS) databases.

The subscription price is \$30.00 (U.S.) and \$35.00 (foreign) per year.

#### **Subscriptions, Change of Address, and Extra Copies:**

Immunohematology, P.O. Box 40325

Philadelphia, PA 19106

Or call (215) 451-4902

Web site: [www.redcross.org/pubs/immuno](http://www.redcross.org/pubs/immuno)

Copyright 2005 by The American National Red Cross  
ISSN 0894-203X

# An unusual anti-H lectin inhibited by milk from individuals with the Bombay phenotype

S.R. JOSHI, K. VASANTHA, AND J.S. ROBB

There are several lectins with anti-H specificity but few of them serve as useful reagents. An anti-H lectin, extracted from the seeds of the plant *Momordica dioica Roxb. ex willd.*, was tested for its hemagglutination and inhibition properties, using standard serologic methods and panel RBCs, serum, saliva, milk, and oligosaccharides purified from milk. The extract displayed strongest agglutination with group O RBCs and was weakest with group A<sub>1</sub>B RBCs in a spectrum of O>A<sub>2</sub>>B>A<sub>2</sub>B>A<sub>1</sub>>A<sub>1</sub>B; the extract failed to react with the RBCs from 25 individuals with the Bombay (O<sub>h</sub>) phenotype and was inhibited by H secretor saliva, hence it was characterized as anti-H. However, its inhibition by milk samples from five mothers with the Bombay phenotype called into question its specificity as anti-H. The lectin reacted as strongly with group O ii (adult) RBCs as with normal OI RBCs, ruling out its specificity as anti-HI. Hemagglutination inhibition was observed with 2'-fucosyllactose (Type 2 H) and lacto-N-fucopentose-I (Type 1 H), suggesting that the binding of the lectin had preference for H structures. However, inhibition by N-acetyllactosamine, lacto-N-tetraose, and lacto-N-neotetraose suggested that the lectin also recognized unsubstituted terminal beta-linked galactose units. The hemagglutinin property in the present lectin showed an unusual anti-H specificity. The lectin was inhibited by milk from Bombay phenotype individuals and certain milk oligosaccharides not specific for the H antigen. *Immunohematology* 2005;21:1-4.

**Key Words:** lectin, Bombay (O<sub>h</sub>) phenotype, hemagglutination inhibition, *Ulex europaeus*, secretor

Anti-H of *Ulex europaeus* is a lectin widely used in identifying Bombay and para-Bombay phenotypes as well as in determining the secretor status of an individual. Anti-H-like lectins from other plants, i.e., *Cerastium tomentosum*, *Cytisus sessilifolius*, *Lotus tetragonolobus*, *Laburnum alpinum*, etc., do react to a variable extent with Bombay phenotype RBCs. However, they have anti-HI specificities and are not useful in determining secretor status.<sup>1,2</sup> We describe an anti-H lectin from the seeds of *Momordica dioica Roxb. ex willd.* The plant, popularly known in the region as *Kantola*, is a perennial dioecious climber, grown abundantly throughout India during the rainy season (July-September).<sup>3</sup> This lectin, as well as *Ulex europaeus* anti-H, can be used to confirm that RBCs are

of the O<sub>h</sub> phenotype. Although the lectin was inhibited by saliva from H secretors and not by saliva from H nonsecretors, it was inhibited by milk samples from some individuals irrespective of their secretor status. Interestingly, it was also inhibited by milk samples from Bombay phenotype individuals.

## Materials and Methods

### *Extraction of lectin*

The dry seeds were pulverized at 10% w/v in normal saline. The homogenized mixture was left at room temperature for 4 hours followed by centrifugation at 4500 rpm for 5 minutes. The opalescent extract was aspirated off the fatty layer settled at the top. The extract was treated with ether to remove lipid remnants. The pH of the extract was adjusted to 7 using 1N NaOH.

Standard serologic procedures were followed throughout<sup>4</sup> and an inhouse panel of RBCs was used to characterize the serologic specificity. Equal volumes of lectin and a 2% suspension of Group O RBCs were mixed and incubated at room temperature for 30 minutes followed by centrifugation at 1000 rpm for 1 minute and macroscopic reading of the results. Negative reactions were confirmed microscopically. Agglutination reactions were graded and scored as reported by Marsh.<sup>5</sup>

The following fluids and substances were used in the study: boiled saliva from persons of predetermined secretor status and Lewis phenotype, human milk from women who had recently delivered, and purified milk oligosaccharides.

For hemagglutination-inhibition studies, the saliva and milk samples were serially diluted in saline and 50 µl of each of the serially diluted samples was mixed with 50 µl of a 1:8 dilution of lectin. The test was incubated at room temperature for 30 minutes, then 50

$\mu$ l of the 2% RBC suspension was added. The test was further incubated at room temperature for 30 minutes and results were read after centrifugation of the test at 1000 rpm for 1 minute. The inhibition intensity was considered to be the dilutions of saliva or milk in which no hemagglutination was observed. For the study using purified milk oligosaccharides (which were kindly provided by Dr. W. Watkins), the serial dilutions (in saline) of the 0.1 M sugar solutions were mixed with an equal volume of the diluted lectin. As a procedural control, saline was substituted for the fluid or other substance and tested as described above.

To assess whether the inhibition of the anti-H by milk samples was merely due to their high osmotic molarity or pH, milk samples (frozen-stored) from two different Bombay phenotype mothers were dialyzed against 0.1 M PBS, pH 7, at 4°C for 24 hours and used for hemagglutination inhibition of the present anti-H lectin of *Momordica dioica*.

Avidity of the serologic reaction was measured as the time (expressed in seconds) taken by the lectin to form visible agglutination when added to a mixture of equal volumes of saline or citrate and RBCs on a slide.

## Results

### *Hemagglutination studies*

The raw extract showed instant gross hemolysis of the saline-suspended RBCs up to a dilution of 1:4, after which it agglutinated RBCs up to 1:64. The hemolytic property of the lectin, without affecting its agglutinating activity, was greatly reduced by the addition of human serum or bovine serum albumin to the extract. A greater strength of agglutination was observed if the RBCs were suspended in 3.8% sodium citrate solution (avidity 5 seconds, titer 64, score 45) as compared to RBCs suspended in normal saline (avidity 12 seconds, titer 32, score 30). (The results are not tabulated.) The lectin was standardized for its optimum reactivity by mixing the raw extract, 3.8% solution of sodium citrate, and 22% bovine serum albumin in a ratio of 3:2:1, respectively, and was used throughout the study.

The extract reacted with all group A, B, and O RBCs tested but failed to react with the RBCs from the 25 Bombay phenotype donors, thereby determining that it is specific for the H antigen. The reaction pattern obtained with the RBCs from various ABO phenotypes showed a minimum strength of agglutination with group A<sub>1</sub>B RBCs and a maximum strength with group O RBCs. RBCs of other ABO phenotypes reacted in

varying degrees of agglutination in the order of O>A<sub>2</sub>>B>A<sub>2</sub>B>A<sub>1</sub>>A<sub>1</sub>B. The lectin did not agglutinate O<sub>h</sub> RBCs even if they were pretreated with papain or sialidase. (The results are not tabulated.) The lectin agglutinated group O RBCs that had different Ii phenotypes, e.g., adult OI, adult Oii, and cord RBCs, in almost equal titer strength, though the score values obtained for the cord blood were slightly lower than those for the other RBCs (Table 1).

**Table 1.** Titer/score of anti-H lectin of *Momordica dioica* Roxb. ex willd. versus group O RBCs of different Ii phenotypes

Results	RBCs		
	OI (N=10)	O, ii* (N=3)	O, cord (N=3)
Titer	32 (N=8), 16 (N=2)	32, 16, 32	32, 16, 32
Score	63 (average)	54	46

\*Frozen preserved

### *Hemagglutination-inhibition studies using saliva and human milk*

Hemagglutination-inhibition studies were carried out using 30 saliva samples from different individuals with various secretor statuses and Lewis blood group phenotypes. Of these, 20 were from individuals with H substance and were of the Le(a-b+) or Le(a-b-) phenotype while the remaining 10, including five from O<sub>h</sub> phenotype individuals, were from individuals who lacked H substance and were of the Le(a+b-) phenotype. The saliva samples with H substance inhibited the agglutination of the RBCs by the lectin, whereas those that lacked the substance did not. Interestingly, 11 samples of human milk, including five samples from the Bombay phenotype mothers, exhibited hemagglutination inhibition. (A typical inhibition pattern is shown in Table 2, Nos. 12 and 13.) It is noteworthy that the lectin was inhibited by milk samples from the Bombay O<sub>h</sub> phenotype individuals, even though it did not agglutinate O<sub>h</sub> RBCs. Dialyzed milk samples showed the same hemagglutination inhibition as was seen with the nondialyzed milk samples. (The results are not tabulated.) In a separate control test, *Ulex europaeus* was not inhibited by milk samples from two of the mothers with the Bombay phenotype. (The results are not tabulated.)

### *Hemagglutination-inhibition studies using purified milk oligosaccharides*

The anti-H lectin showed maximum inhibition by 2'-fucosyllactose (sugar 5 in the list) and lacto-N-fucopentaose I (sugar 7) (Table 2). It was also inhibited

**Table 2.** Hemagglutination-inhibition titer of various milk/oligosaccharide samples obtained using lectin *Momordica dioica Roxb. ex willd.*

Sugar No.	Milk/oligosaccharide	Specificity	Inhibition titer
1	N-acetyllactosamine	-	128
2	Lacto-N-tetraose	-	128
3	Lacto-N-neotetraose	-	64
4	Lacto-difucotetraose	-	8
5	2'-fucosyllactose	H <sub>12</sub>	512
6	3-fucosyllactose	-	4
7	Lacto-N-fucopentaose-I	H <sub>11</sub>	512
8	Lacto-N-fucopentaose-II	Le <sup>a</sup>	8
9	Lacto-N-fucopentaose-III	Le <sup>x</sup>	0
10	Lacto-N-difucohexaose-I	Le <sup>b</sup>	0
11	Lacto-N-difucohexaose-II	Le <sup>a</sup>	0
12	Milk, mother, group O	-	128
13	Milk, mother, O <sub>h</sub>	-	32

by N-acetyllactosamine (sugar 1), lacto-N-tetraose (sugar 2), and lacto-N-neotetraose (sugar 3), though to a lesser degree. Lacto-difucotetraose (sugar 4), 3-fucosyllactose (sugar 6), and lacto-N-fucopentaose II (sugar 8) did show inhibition, but to a very limited extent. However, lacto-N-fucopentaose III (sugar 9) and lacto-difucohexaose I and II (sugars 10 and 11) did not inhibit the hemagglutinating property of the lectin.

## Discussion

Anti-H occurs in nature as an alloantibody in Bombay and para-Bombay (nonsecretor) individuals, as an autoantibody in persons irrespective of their ABO blood group, and as a lectin in the seeds of certain plants.<sup>6</sup> Anti-H of human origin is usually contaminated with coexisting anti-A, anti-B, or both in the serum, thereby limiting its usefulness as a monospecific reagent. The anti-H lectin prepared from *Ulex europaeus* is a useful reagent but those described from some other plants are not.<sup>6</sup>

The lectin from *Momordica dioica Roxb. ex willd.* exhibited some interesting features. The agglutinating property was obscured by a pan-hemolysin present in the raw extract that required neutralization by bovine serum albumin or human serum. The neutralized lectin agglutinated RBCs of all ABO groups except those from 25 Bombay phenotype individuals. The lectin did not react with O<sub>h</sub> RBCs even after pretreatment with enzymes, suggesting that it is highly specific for the H antigen. The agglutination pattern with various ABO blood group phenotypes further suggested its anti-H

specificity and its serologic reactivity were akin to the anti-H lectin from *Ulex europaeus*. Hemagglutination inhibition by H secretor saliva but not by nonsecretor saliva appeared to confirm H specificity. However, the lectin was totally inhibited by all samples of human milk tested, including 5 of 5 samples from Bombay phenotype individuals. Inhibitory activities retained after dialysis of the milk from Bombay phenotype individuals suggests that the inhibition was not due to the osmolarity or pH. It also suggests the presence of a high-molecular weight fucosylated glycoprotein apart from the H-specific glycoprotein that shows this hemagglutination inhibition. Bombay phenotype individuals genetically lack H. Therefore, hemagglutination inhibition of the lectin by milk from mothers with the Bombay phenotype modifies its specificity as pure anti-H. Additionally, *Ulex europaeus* was not inhibited by milk from the Bombay mothers, thus showing distinct differences from the lectin of *Momordica dioica Roxb. ex willd.* Because milk is rich in soluble I antigen,<sup>7</sup> and as the lectin was neutralized by various milk samples, its specificity was indicative of possible anti-I or anti-HI. However, both of these possibilities were ruled out because the lectin agglutinated group O ii RBCs and did not agglutinate Bombay I-positive RBCs. Inhibition of fucose-binding lectins by milk samples from mothers who are nonsecretors was also observed by N. Gilboa-Garber, Ph.D. (written communication, July 2001).

Bird and Wingham<sup>1</sup> described the lectin in Jerusalem sage (*Phlomis fruticosa*) with anti-A,B specificity wherein anti-B activity was neutralized by N-acetyl-D-galactosamine, the terminal sugar specific for blood group A antigen. They explained that the A and B antigen binding sites were situated on a single lectin molecule and both binding sites were inhibited by either of the specific sugars. It is likely that the present lectin also has dual binding sites, one for the terminal sugar specific for H antigen and the other for the terminal sugar of a different antigen, and that both binding sites were inhibited by sugars for either of the specificities.

The best inhibition by 2'-fucosyllactose (Type 2 H) and lacto-N-fucopentaose I (Type 1 H) suggests that the binding site of the lectin has preference to the H structure. However, inhibition by N-acetyllactosamine, lacto-N-tetraose, and lacto-N-neotetraose suggests that the lectin also recognizes unsubstituted terminal beta-linked galactose units. Inhibition of this lectin by milk samples from Bombay phenotype mothers may also be

attributed to such sugars present in the milk.<sup>8</sup> Interestingly, the presence of fucose on the subterminal sugar (as in lacto-difucotetraose, 3-fucosyllactose, lacto-N-fucopentaose II or III, or lacto-difucohexaose I or II) reduces the ability of the lectin to recognize the terminal galactose or the galactose when alpha-2-linked to fucose.

### Conclusion

The lectin in the *Momordica dioica* agglutinated RBCs from persons other than those of the Bombay phenotype. Its reactivity was inhibited by H secretor saliva and purified milk oligosaccharides specific for H, suggesting its specificity as anti-H. Its inhibition by milk and certain milk oligosaccharides not specific for H modified its specificity as pure anti-H. Its strong reaction with O<sub>ii</sub> RBCs and its failure to react with O<sub>n</sub>, I-positive RBCs ruled out anti-I specificity. Thus, the lectin has a unique specificity in comparison with other lectins with anti-H specificity.

### Acknowledgment

We thank Dr. W. Watkins, Imperial College, London, for generously supplying the milk oligosaccharides used in this study as well as for her valuable comments on our results. We also thank Dr. Jacques LePendu, Institute of Biology, Nantes, Cedex, France, for confirming our results in his laboratory setting.

### References

1. Bird GWG, Wingham J. Anti-H from *Cerastium tomentosum* seeds. A comparison with other seed anti-H agglutinins. *Vox Sang* 1970;19:132-9.
2. Voak D, Lodge TW. The demonstration of anti-HI-H activity in seed anti-H reagents. *Vox Sang* 1971;20:36-45.
3. Anonymous. Wealth of India: raw material volume 6. New Delhi: Council of Scientific and Industrial Research, 1962:411-2.
4. Walker RH, ed. Technical manual. 10th ed. Arlington, VA: American Association of Blood Banks, 1990.
5. Marsh WL. Scoring of hemagglutination reactions. *Transfusion* 1972;12:352-3.
6. Mollison PL, Engelfriet CP, Contreras M. Blood transfusion in clinical medicine. 10th ed. Oxford: Blackwell Science, 1997:118.
7. Marsh WL, Nichols ME, Allen FH. Inhibition of anti-I by human milk. *Vox Sang* 1970;18:149.
8. Erney R, Hilty M, Pickering L, Ruiz-Palacios G, Prieto P. Human milk oligosaccharides: a novel method provides insight into human genetics. *Adv Exp Med Biol* 2001;501:285-97.

---

*Sanmukh R. Joshi, Institute of Health Sciences, P.O. Box 3720, Ruwi Code 112, Muscat, Sultanate of Oman/Indian Red Cross Blood Center, Mumbai, India; K. Vasantha, Institute of Immunohematology (ICMR), Mumbai, India; and Janine S. Robb, Diagnostics Scotland, Edinburgh, UK.*

# Gene frequencies of the HPA-1 to 6 and Gov human platelet antigens in Thai blood donors

P. KUPATAWINTU, O. NATHALANG, R. O-CHAROEN, AND P. PATMASIRIWAT

Human platelet alloantigens (HPA) are important in neonatal alloimmune thrombocytopenia (NAIT), posttransfusion purpura (PTP), platelet transfusion refractoriness, passive alloimmune thrombocytopenia, and transplantation-associated alloimmune thrombocytopenia. Thus, HPA genotyping is essential in diagnosis and treatment. We analyzed HPA-1 to 6 and Gov alleles, using PCR with sequence specific primers (PCR-SSP) in 500 Thai blood donors who had been HLA class I antigen typed. HPA-4a was present in all samples. HPA-1b, -2b, -5b, and -6b were rare, and HPA-4b was not found. HPA-3a and -3b showed frequencies of 56.0 percent and 44.0 percent, respectively. Gov<sup>a</sup> and Gov<sup>b</sup> showed frequencies of 49.1 percent and 50.9 percent, respectively. The prevalence rates of HPA-1 to 6 gene frequencies (GFs) were consistent with those of other Asian populations rather than those of Caucasians. We also report on the GFs of Gov<sup>a</sup> and Gov<sup>b</sup>, which also are comparable to those of Asian populations. Our results could establish a useful HPA- and HLA-matched plateletpheresis donor file and provide an improvement of platelet alloantibody detection in alloimmune thrombocytopenic patients, and, therefore, a more effective platelet transfusion program. *Immunohematology* 2005;21:5-9.

**Key Words:** human platelet antigen (HPA), Gov, PCR-SSP, Thais, gene frequencies

Human platelet alloantigens (HPA) become clinically relevant if the antibody causes enhanced platelet destruction, resulting in thrombocytopenia and hemorrhagic diathesis. Five clinical entities due to platelet-specific alloantibodies can be distinguished: neonatal alloimmune thrombocytopenia (NAIT), posttransfusion purpura (PTP), platelet transfusion refractoriness, passive alloimmune thrombocytopenia, and transplantation-associated alloimmune thrombocytopenia.<sup>1</sup> Platelet transfusion refractoriness, which is defined as a low CCI after platelet transfusion, is a common problem in multitransfused patients. Immunologic refractoriness to platelet transfusion is caused by alloantibodies reacting with transfused antigens such as ABO, HLA class I, and HPA antigens. Although HLA alloimmunization is the most common, HPA antibodies are also clinically significant.<sup>1-4</sup>

In Thailand, platelet antigen typing of blood donors is not routinely performed, except for population genetic studies.<sup>5-7</sup> Usually, ABO matched and negative platelet crossmatched units are transfused to multitransfused patients with platelet refractoriness. The National Blood Centre, Thai Red Cross Society, has performed platelet antibody screening and identification in 163 thrombocytopenic patients, using the solid phase red cell adherence assay, since 1993. HLA and HPA antibodies were detected in 49.63 percent and 6.13 percent of patients, respectively. In addition, 40.0 percent of HPA antibodies could not be identified due to the limited availability of HPA-genotyped panels.<sup>8</sup> Having a panel of HLA- and HPA-typed donors would be helpful.<sup>4</sup> Different PCR techniques have been introduced, such as PCR with RFLP and PCR with sequence specific primers (PCR-SSP). The PCR-SSP technique has been shown to be a simpler and more reliable method for several HPA genotypes.<sup>9-14</sup>

This study analyzed HPA-1 to 6 and Gov alleles, using PCR-SSP in Thai blood donors who had been HLA class I antigen typed to establish HPA-genotyped panels for identification of HPA antibodies in routine testing.

## Materials and Methods

### Subjects

Five hundred healthy Thai blood donors at the National Blood Centre, Thai Red Cross Society, who had been HLA class I antigen typed, were included in this study. The study comprised 369 males and 131 females, with ages ranging from 19 to 58 years. The mean age was 36 years. EDTA blood (3 mL) was collected from each donor and informed consent was obtained from all subjects.

### DNA standards

Known *HPA-1* to *6* and *Gov* DNA samples, provided by the Australian Platelet Antibody Workshop in association with the Australian and New Zealand Society of Blood Transfusion and Central Blood Centre, Japanese Red Cross Society, were used as a standard in the PCR-SSP method.

### HPA genotyping

Genomic DNA was extracted from whole blood by the salting-out technique (Pel-Freez, Brown Deer, WI). The PCR-SSP for *HPA-1* to *6* and *Gov* systems was performed as previously described,<sup>9,15,16</sup> with some modification. To increase specificity for detection of *HPA-3b*, two bases were extended at the 5' end of the primer. The primers used in this study are listed in Table 1. Briefly, the PCR reactions were carried out in 10 µL aliquots containing 50–100 ng of genomic DNA and PCR buffer (67 mM Tris HCL pH 8.8, 16 mM ammonium sulfate, 0.01% Tween 20, 0.5 µM each of dNTP and 1.5 mM MgCl<sub>2</sub>). Each PCR reaction contained 0.1 to 0.4 µM of the control primers, 0.1 to 0.5 µM of the allele-specific primers, and 0.75 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). PCR amplifications were carried out in a GeneAmp PCR System 2700 (Applied Biosystems) or in a PTC-200 (MJ Research, Waltham, MA). The PCR program consisted of one cycle: 96°C for 1 minute; five cycles: 96°C for 25 seconds, 68°C for 45 seconds, 72°C for 30 seconds; 28 cycles: 96°C for 25 seconds, 61°C for 45 seconds, 72°C for 30 seconds; and one cycle: 72°C for 3 minutes. The amplified products were electrophoresed through 2.0% agarose gel containing 0.5 µg/mL ethidium bromide. The gel was run at 100 V for 30 minutes in 0.5X TBE and visualized under UV transilluminator. The reaction was photographed and the *HPA* alleles were assessed.

### Reproducibility of SSP typing

Ten samples were randomly repeated for *HPA-1* to *6* and *Gov* SSP-typing, to test for reproducibility.

### Statistical analysis

Genotype and gene frequencies (GF) were determined by direct counting. The validity of the Hardy-Weinberg equilibrium was tested by calculating expected numbers of subjects for each genotype using  $2 \times af[a] \times af[b] \times N$  for heterozygotes and  $af[a \text{ or } b]^2 \times N$  for homozygotes, where  $af[a]$  and  $af[b]$  are the

**Table 1.** Sequence of the primers for *HPA-1* to *6*, *Gov*, and *HGH* internal control

Primer	Sequence	Product size (bp)	Final Conc.
<i>HPA-1a</i>	5' TCA CAG CGA GGT GAG GCC A 3'	90	0.1 µM
<i>HPA-1b</i>	5' TCA CAG CGA GGT GAG GCC G 3'		
<i>HPA-1 common</i>	5' GGA GGT AGA GAG TCG CCA TAG 3'		
<i>HPA-2a</i>	5' GCC CCC AGG GCT CCT GAC 3'	258	0.1 µM
<i>HPA-2b</i>	5' GCC CCC AGG GCT CCT GAT 3'		
<i>HPA-2 common</i>	5' TCA GCA TTG TCC TGC AGC CA 3'		
<i>HPA-3a</i>	5' TGG ACT GGG GGC TGC CCA T 3'	267	0.2 µM
<i>HPA-3b</i>	5' GGT GGA CTG GGG GCT GCC CAG 3'	269	
<i>HPA-3 common</i>	5' TCC ATG TTC ACT TGA AGT GCT 3'		
<i>HPA-4a</i>	5' GCT GGC CAC CCA GAT GCG 3'	120	0.1 µM
<i>HPA-4b</i>	5' GCT GGC CAC CCA GAT GCA 3'		
<i>HPA-4 common</i>	5' CAG GGG TTT TCG AGG GCC T 3'		
<i>HPA-5a</i>	5' AGT CTA CCT GTT TAC TAT CAA AG 3'	246	0.5 µM
<i>HPA-5b</i>	5' AGT CTA CCT GTT TAC TAT CAA AA 3'		
<i>HPA-5 common</i>	5' CTC TCA TGG AAA ATG GCA GTA 3'		
<i>HPA-6a</i>	5' GAC GAG TGC AGC CCC CG 3'	238	0.2 µM
<i>HPA-6b</i>	5' GGA CGA GTG CAG CCC CCA 3'	239	
<i>HPA-6 common</i>	5' CTA TGT TTC CCA GTG GTT GCA 3'		
<i>Gov<sup>a</sup></i>	5' TTC AAA TTC TTG GTA AAT CCT GT 3'	225	0.4 µM
<i>Gov<sup>b</sup></i>	5' TTC AAA TTC TTG GTA AAT CCT GG 3'		
<i>Gov common</i>	5' ATG ACC TTA TGA TGA CCT ATT C 3'		
<i>HGH control</i>	5' GCC TTC CCA ACC ATT CCC TTA 3'	429	<i>HPA-1-4 &amp; 6</i> 0.4 µM
<i>HGH control</i>	5' TCA CGG ATT TCT GTT GTG TTT C 3'		<i>HPA-5</i> 0.1 µM <i>Gov</i> 0.2 µM

allele frequencies of the *HPA-1* to *6* and *Gov a* and *b* alleles, respectively, and N is the number of subjects typed. The differences in *HPA* genotype distribution between the two groups were tested for significance by chi-square and by Fisher's exact test.

### Results

In this study, the simultaneous determination of *HPA-1* to *6* and *Gov* genotyping by PCR-SSP resulted in PCR products of the following sizes: 90 bp for *HPA-1*, 258 bp for *HPA-2*, 267 and 269 bp for *HPA-3a* and *-3b*, 120 bp for *HPA-4*, 246 bp for *HPA-5*, 238 and 239 bp for *HPA-6a* and *-6b*, and 225 bp for *Gov*, respectively. In all reactions, the human growth hormone (*HGH*) internal control gave an expected band of 429 bp. Additional smaller, nonspecific products were seen in *HPA-3b* and *-6b*, while a larger nonspecific product was also seen in *HPA-4b*, as previously reported,<sup>4</sup> but these did not interfere with the interpretation of the *HPA* genotypes (Fig. 1).

The genotype and gene frequencies of the seven platelet antigen systems obtained from 500 Thai blood

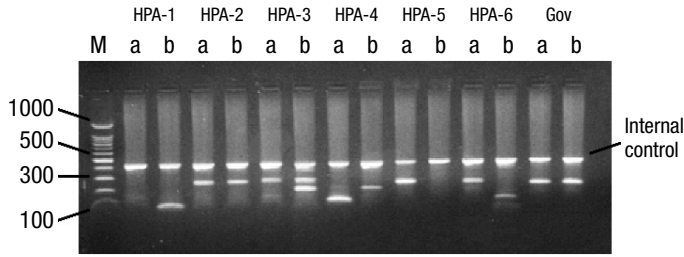


Fig. 1. A representative gel showing simultaneous *HPA-1* to *6* and *Gov* genotyping by PCR-SSP. The 429-bp amplification product of the HGH control primers is present in all lanes, which shows that amplification has occurred optimally. The genotype was deduced from the presence or absence of amplification products specific for alleles (from left to right: *HPA-1b1b*, *HPA-2a2b*, *HPA-3a3b*, *HPA-4a4a*, *HPA-5a5a*, *HPA-6a6a*, *Gov-a/b*). M: 100-bp ladder marker (Promega, Madison, WI).

Table 2. The genotype and gene frequencies of *HPA-1* to *6* and *Gov* in 500 Thai blood donors

Genotype	No.	Genotype frequencies (%)	
		Gene	(%)
<i>HPA-1a1a</i>	485	<i>HPA-1a</i>	98.5
<i>HPA-1a1b</i>	15	<i>HPA-1b</i>	1.5
<i>HPA-1b1b</i>	0		
<i>HPA-2a2a</i>	452	<i>HPA-2a</i>	95.2
<i>HPA-2a2b</i>	48	<i>HPA-2b</i>	4.8
<i>HPA-2b2b</i>	0		
<i>HPA-3a3a</i>	152	<i>HPA-3a</i>	56.0
<i>HPA-3a3b</i>	256	<i>HPA-3b</i>	44.0
<i>HPA-3b3b</i>	92		
<i>HPA-4a4a</i>	500	<i>HPA-4a</i>	100.0
<i>HPA-4a4b</i>	0	<i>HPA-4b</i>	0.0
<i>HPA-4b4b</i>	0		
<i>HPA-5a5a</i>	468	<i>HPA-5a</i>	96.8
<i>HPA-5a5b</i>	32	<i>HPA-5b</i>	3.2
<i>HPA-5b5b</i>	0		
<i>HPA-6a6a</i>	486	<i>HPA-6a</i>	98.6
<i>HPA-6a6b</i>	14	<i>HPA-6b</i>	1.4
<i>HPA-6b6b</i>	0		
<i>Gov<sup>a</sup>/Gov<sup>a</sup></i>	125	<i>Gov<sup>a</sup></i>	49.1
<i>Gov<sup>a</sup>/Gov<sup>b</sup></i>	241	<i>Gov<sup>b</sup></i>	50.9
<i>Gov<sup>b</sup>/Gov<sup>b</sup></i>	134		

donors are shown in Table 2. *HPA-4a* was present in all samples. *HPA-1b*, *-2b*, *-5b*, and *-6b* were rare and *HPA-4b* was not found. *HPA-3a* and *-3b* showed frequencies of 56.0 percent and 44.0 percent, respectively. *Gov<sup>a</sup>* and *Gov<sup>b</sup>* showed frequencies of 49.1 percent and 50.9 percent, respectively.

To test the reproducibility of the assay, ten DNA samples were randomly selected and tested for *HPA-1* to *6* and *Gov* typing. The results of the repeated assay were similar to those of the first round of testing. Moreover, this PCR-SSP HPA typing method was validated using 20 HPA reference materials typed by alternative techniques and in other laboratories. The results showed a 100 percent concordance between the two methods.

A comparison of *HPA-1* to *6* and *Gov* gene frequencies among Asian and Caucasian studies is presented in Tables 3 and 4.<sup>6,12,17-23</sup> The *HPA-1* to *6* frequencies in Thai blood donors are more similar to

those in Northeastern Thais and other Asians than to those of Caucasians. The *Gov<sup>a</sup>* and *Gov<sup>b</sup>* frequencies in our study showed results similar to those in the previous study<sup>23</sup> but were significantly different from those in the study in UK populations ( $p < 0.01$ ).<sup>24</sup>

### Discussion

The investigation of *HPA* gene and genotype frequencies is important in both population studies

Table 3. Gene frequencies of *HPA-1* to *6* in 500 Thai blood donors and different populations

HPA	Thai* (N=500)	NET† (N=300)	Taiwan <sup>17</sup> (N=300)	Hong Kong <sup>18</sup> (N=100)	Korea <sup>19</sup> (N=100)	Japan <sup>20</sup> (N=331)	Spain <sup>21</sup> (N=107)	Wales <sup>12</sup> (N=392)	Australia <sup>22</sup> (N=1,000)	Norway <sup>23</sup> (N=105)
-1a	98.5	97.2	99.7(a)	99.5	99.5	99.8(b)	74.8(c)	82.5(c)	85.8(c)	86.7(c)
-1b	1.5	2.8	0.3(a)	0.5	0.5	0.2(b)	25.2(c)	17.5(c)	14.2(c)	13.3(c)
-2a	95.2	93.8	96.0	97.5	87.0(c)	NA‡	81.8(c)	90.9(c)	92.7(b)	94.3
-2b	4.8	6.2	4.0	2.5	13.0(c)	NA	18.2(c)	9.1(c)	7.3(b)	5.7
-3a	56.0	53.3	57.5	52.5	67.0(b)	NA	68.2(b)	60.7(a)	61.9(b)	47.1(a)
-3b	44.0	46.7	42.5	47.5	33.0(b)	NA	31.8(b)	39.3(a)	38.1(b)	52.9(a)
-4a	100.0	100.0	99.8	100.0	100.0	98.9(b)	100.0	100.0	100.0	100.0
-4b	0.0	0.0	0.2	0.0	0.0	1.1(b)	0.0	0.0	0.0	0.0
-5a	96.8	96.3	98.5(a)	96.5	97.0	NA	86.1(c)	90.3(c)	90.5(c)	92.9(b)
-5b	3.2	3.7	1.5(a)	3.5	3.0	NA	13.9(c)	9.7(c)	9.5(c)	7.1(b)
-6a	98.6	98.5	96.3(b)	NA	NA	97.3	100.0	100.0(a)	NA	NA
-6b	1.4	1.5	3.7(b)	NA	NA	2.7	0.0	0.0(a)	NA	NA

\*Thai blood donors

†Northeastern Thais

‡No data available

(a)  $p < 0.05$

(b)  $p < 0.01$

(c)  $p < 0.001$

**Table 4.** Gene frequencies of *Gov* in 500 Thai blood donors and different populations

HPA	Thai* (N=500)	Norway <sup>23</sup> (N=105)	UK <sup>24</sup> (N=113)	Taiwan <sup>25</sup> (N=566)	Indonesia <sup>25</sup> (N=107)	Thai <sup>25</sup> (N=137)	Filipino <sup>25</sup> (N=100)
<i>Gov</i> <sup>a</sup>	49.1	49.5	40.0(b)	46.2	45.0	46.3	52.0
<i>Gov</i> <sup>b</sup>	50.9	50.5	60.0(b)	53.8	55.0	53.7	48.0

\*Thai blood donors (b) p < 0.01

and clinical transfusion practice, where HPA-typed platelets may be required for alloimmunized patients. Providing more data on HPA distribution among various populations enables the prediction of the risk of platelet-specific alloimmunization in different ethnic groups and improves the ability to prepare HPA-compatible platelet products.<sup>2,4</sup>

Recent studies reported gene frequencies of the *HPA-1* to *13*, *Oe*, and *Gov* alleles in Taiwanese, Indonesian, Filipino, and Thai populations, using the PCR-RFLP technique.<sup>11,25</sup> Although the results were consistent with other previous findings in Asian populations, the PCR-RFLP technique has some disadvantages, such as being time-consuming and susceptible to being misinterpreted due to incomplete enzyme digestion of the amplicon.<sup>16</sup> In this study we have established *HPA-1* to *-6* and *Gov* genotyping by PCR-SSP, using a combination of established and modified sequence-specific primers. This method is rapid, cost-effective, and suitable in large-scale platelet antigen genotyping, either for NAIT or in genetic population studies.<sup>4,6,9,10,12,14,17</sup>

Furthermore, the distribution of *HPA-1* to *6* phenotypes in Thai blood donors living in Bangkok is consistent with those found in Northeastern Thailand.<sup>6,7</sup> A previous study showed that the most common HPA antibodies in thrombocytopenic Thai patients were anti-*HPA-5b*, *-HPA-2b*, *-HPA-3a*, and unidentified antibodies. Anti-*HPA-1a*, which is the most common cause of NAIT and PTP in Caucasians, is not found in Thai populations due to the high frequency of *HPA-1a* (> 97%). Other studies showed that anti-*HPA-1b*, *-HPA-2a*, *-HPA-2b*, *-HPA-3a*, *-HPA-3b*, *-HPA-4a*, *-HPA-4b*, *-HPA-5b*, *-HPA-5a*, and *-HPA-6a* were also found in NAIT, PTP, and refractoriness to platelet transfusion therapy in Caucasians and in Japanese populations.<sup>24,26-29</sup>

The *Gov* antibodies have been reported for their clinical importance, especially in NAIT, PTP, and platelet refractoriness.<sup>24,30</sup> Although the incidence of *Gov* antibodies was lower than that of the *HPA-1* system, the incidence was equal to that of *HPA-5* system antibodies in Caucasians. The genotype frequencies of the *Gov* system in this study are comparable to those found in previous studies in Asian populations.<sup>25</sup> Thus, typing

for the *Gov* antigen system should be useful in patient diagnosis. In summary, the implementation of the HPA genotyping system using the PCR-SSP technique in both donors and patients is

beneficial in platelet transfusion therapy to provide HPA-matched platelet donors and to increase the capability for platelet alloantibody investigation.

### Acknowledgments

We thank Dr. Paul Metcalfe, National Institute for Biological Standards and Control, UK, for his helpful advice and the Australian and New Zealand Society of Blood Transfusion and Central Blood Centre, Japanese Red Cross Society, for providing known *HPA-1* to *6* and *Gov* DNA samples.

### References

1. Kroll H, Kiefel V, Santoso S. Clinical aspects and typing of platelet alloantigens. *Vox Sang* 1998; 74:345-54.
2. Kekomaki R. Use of HPA- and HLA-matched platelets in alloimmunized patients. *Vox Sang* 1998;74(Suppl 2):359-63.
3. Novotny AMJ. Prevention and management of platelet transfusion. *Vox Sang* 1999;76:1-3.
4. Verran J, Gray D, Bennett J, Lown JAG, Erber WN. *HPA-1,3,5* genotyping to establish a typed platelet donor panel. *Pathology* 2000;32:89-93.
5. O-Charoen R, Kupatawintu P, Jitjak N. Human platelet-specific alloantigen: phenotype frequency in Thai blood donors: Preliminary report. *Thai J Haematol Transfus Med* 1993;3:27-35.
6. Romphruk AV, Akahat J, Srivanichrak P, Puapairoj C, Romphruk A, Leelayuwat C. Genotyping of human platelet antigens in ethnic Northeastern Thais by the polymerase chain reaction-sequence specific primer technique. *J Med Assoc Thai* 2000;83: 1333-7.
7. Urwijitaroon Y, Barusrux S, Romphruk A, Puapairoj C. Frequency of human platelet antigens among blood donors in Northeastern Thailand. *Transfusion* 1995;35:868-70.
8. Kupatawintu P, Jitjak N, Saelee S, O-Charoen R, Nathalang O. The incidence of platelet antibody in thrombocytopenic patients. *Thai J Haematol Transfus Med* 2000;10:123-7.

9. Cavanagh G, Dunn AN, Chapman CE. HPA genotyping by PCR sequence-specific priming (PCR-SSP): a streamlined method for rapid routine investigation. *Transfus Med* 1997;7:41-5.
10. Kluter H, Fehlau K, Panzer S, Kirchner H, Bein G. Rapid typing for human antigen systems-1, -2, -3 and -5 by PCR amplification with sequence-specific primers. *Vox Sang* 1996;71:121-5.
11. Liu TC, Shih MC, Lin CL, Lin SF, Chen CM, Chang JG. Gene frequencies of the HPA-1 to HPA-8w platelet antigen alleles in Taiwanese, Indonesian, and Thai. *Ann Hematol* 2002;81:244-8.
12. Sellers J, Thompson J, Guttridge MG, Darke C. Human platelet antigens: typing by PCR using sequence-specific primers and their distribution in blood donors resident in Wales. *Eur J Immunogenet* 1999;26:393-7.
13. Skogen B, Bellissimo DB, Hessner MJ, et al. Rapid determination of platelet alloantigen genotypes by polymerase chain reaction using allele-specific primers. *Transfusion* 1994;34:955-60.
14. Tanaka S, Taniue A, Nagao N, et al. Simultaneous DNA typing of human platelet antigens 2, 3 and 4 by an allele-specific PCR method. *Vox Sang* 1995;68:225-30.
15. Hurd CM, Cavanagh G, Schuh A, Ouwehand WH, Metcalfe P. Genotyping for platelet-specific antigens: techniques for the detection of single nucleotide polymorphisms. *Vox Sang* 2002;83:1-12.
16. Schuh AC, Watkins NA, Nguyen Q, et al. A tyrosine 703 serine polymorphism of CD109 defines the Gov platelet alloantigens. *Blood* 2002;99:1692-8.
17. Lyou JY, Chen YJ, Hu HY, Lin JS, Tzeng CH. PCR with sequence-specific primer-based simultaneous genotyping of human platelet antigen-1 to -13w. *Transfusion* 2002;42:1089-95.
18. Chang YW, Mytilineos J, Opelz G, Hawkins BR. Distribution of human platelet antigens in a Chinese population. *Tissue Antigens* 1998;51:391-3.
19. Kim HO, Jin Y, Kickler TS, Blakemore K, Kwon OH, Bray PF. Gene frequencies of the five major human platelet antigens in African American, white, and Korean populations. *Transfusion* 1995;35:863-7.
20. Tanaka S, Ohnoki S, Shibata H, Okubo Y, Yamaguchi H, Shibata Y. Gene frequencies of human platelet antigens on glycoprotein IIIa in Japanese. *Transfusion* 1996;36:813-7.
21. Ferrer G, Muniz-Diaz E, Aluja MP, et al. Analysis of human platelet antigen system in a Moroccan Berber population. *Transfus Med* 2002;12:49-54.
22. Bennett JA, Palmer LJ, Musk AW, Erber WN. Gene frequencies of human platelet antigens 1-5 in indigenous Australians in Western Australia. *Transfus Med* 2002;12:199-203.
23. Randen I, Sorensen K, Killie MK, Kragh JK. Rapid and reliable genotyping of human platelet antigen (HPA)-1, -2, -3, -4 and -5 a/b and Gov a/b by melting curve analysis. *Transfusion* 2003;43:445-50.
24. Berry JE, Murphy CM, Smith GA, et al. Detection of Gov system antibodies by MAIPA reveals an immunogenicity similar to the HPA-5 alloantigens. *Br J Haematol* 2000;110:735-42.
25. Shin MC, Liu TC, Lin IL, Lin SF, Chen CM, Chang JG. Gene frequencies of the HPA-1 to HPA-13, Oe and Gov platelet antigen alleles in Taiwanese, Indonesian, Filipino and Thai populations. *Int J Mol Med* 2003;12:609-14.
26. Kickler TS, Herman JH, Furihata K, Kunicki TJ, Aster RH. Identification of Bakb, a new platelet specific antigen associated with post transfusion purpura. *Blood* 1988;71:894-8.
27. Okada N, Oda M, Sano T, Ito J, Shibata Y, Yamanaka M. Intracranial hemorrhage in utero due to fetomaternal Baka incompatibility. *Acta Haematol Jpn* 1988;51:1086-91.
28. Panzer S, Auerbach L, Cechova E, et al. Maternal alloimmunization against fetal platelet antigens: a prospective study. *Br J Haematol* 1995;90:655-60.
29. Shibata Y, Matsuda I, Miyaji T, Ichikawa Y. Yuk<sup>a</sup>, a new platelet antigen system involved in two cases of neonatal alloimmune thrombocytopenia. *Vox Sang* 1986;50:177-80.
30. Bordin JO, Kelton JG, Warner MN, et al. Maternal immunization to Gov system alloantigens on human platelets. *Transfusion* 1997;37:823-8.

---

*Pawinee Kupatawintu, BSc, National Blood Centre, Thai Red Cross Society, Henri Dunant Road, Pathumwan, Bangkok 10330, Thailand, and Department of Clinical Microscopy, Mahidol University, Bangkoknoi, Bangkok 10700, Thailand; Oytip Natbalang, PhD, Phramongkutklao College of Medicine, Rajthevi, Bangkok, Thailand; Rachanee O-Charoen, MD, National Blood Centre, Thai Red Cross Society, Bangkok, Thailand; and Pimpicha Patmasiriwat, PhD, Department of Clinical Microscopy, Mahidol University, Bangkok, Thailand.*

# ABO, Rh, MNS, Duffy, Kidd, Yt, Scianna, and Colton blood group systems in indigenous Chinese

L. YAN, F. ZHU, Q. FU, AND J. HE

The frequencies of selected alleles in the ABO, Rh, MNS, Duffy, Kidd, Yt, Scianna, and Colton blood group systems were determined among four indigenous Chinese ethnic populations: Han, Tajik, She, and Yugu. Genotypes were determined by PCR or PCR with sequence specific primers (PCR-SSP). In the Han population, the frequencies of  $A^1$ ,  $A^2$ ,  $B$ , and  $O^1$  alleles were 0.189, 0.003, 0.170, and 0.638, respectively, and the  $O^2$  allele was not identified. Among D+ Hans, the frequencies of  $C$  and  $c$  alleles were 0.67 and 0.33 and the frequencies of  $E$  and  $e$  were 0.22 and 0.78, respectively. Among D- Hans, the frequencies of  $C$  and  $c$  alleles were 0.23 and 0.77 and the frequencies of  $E$  and  $e$  were 0.04 and 0.96, respectively. The frequencies of  $M$  and  $N$  alleles were 0.478 and 0.522 among Hans and 0.655 and 0.345 among Tajiks, respectively. The frequencies of  $Fy^a$  and  $Fy^b$  alleles were 0.94 and 0.06 among Hans and 0.98 and 0.02 among Shes, respectively. The frequencies of  $Jk^a$  and  $Jk^b$  alleles were 0.49 and 0.51 among Hans and 0.56 and 0.44 among Shes, respectively. The frequency of the  $Yt^a$  allele was 1.00 among Hans. The frequencies of  $Yt^a$  and  $Yt^b$  alleles were 0.94 and 0.06 among Tajiks, respectively. The frequency of the  $ScI$  allele was 1.00 in both Han and Tajik ethnic populations. The frequency of the  $Co^a$  allele was 1.00 in Han, She, and Tajik ethnic populations. *Immunohematology* 2005;21:10–14.

**Key Words:** ABO, Rh, MNS, Duffy, Kidd, Yt, Scianna, Colton, PCR-SSP, gene frequencies

Serologic studies of blood group gene frequencies have established certain characteristics among indigenous Asian (Mongoloid) populations when compared to European (Caucasoid) populations.<sup>1</sup> In general, Asians have more  $B$ ,  $M$ , and  $Fy^a$  alleles, fewer  $S$  and  $P_i$ . Lack of D is rare in Asians; the most common Rh haplotypes are  $dCe$  and  $Dce$ . The availability of PCR and polymerase chain reaction with sequence specific primers (PCR-SSP) for detecting blood group alleles provides a means for more precise characterization of the genetic composition of different populations. We describe the results of a pilot study for determining selected alleles in the ABO, Rh, MNS, Duffy, Kidd, Yt, Scianna, and Colton blood group systems among four ethnic Chinese populations.

## Materials and Methods

### Blood samples

Peripheral blood samples were collected from healthy volunteer blood donors after obtaining informed consent. Based on information from personal interviews, subjects were categorized by ethnic group as Han (Zhejiang Province), She (Zhejiang Province), Yugu (Gansu Province), or Tajik (Xijiang Province).

### DNA extraction and genotyping

Genomic DNA was extracted from whole-blood samples, using a standard salting-out method. Blood group genotypes were determined by PCR or PCR-SSP, using previously described protocols, e.g.,  $ABO$ ,<sup>2</sup>  $RHCE$ ,<sup>3,4</sup>  $GYP A$ ,<sup>5</sup>  $FY$ ,<sup>6</sup>  $SLC14A1(JK)$ ,<sup>7</sup>  $ACHE$ ,<sup>8</sup>  $ERMAP$ ,<sup>9</sup> and  $AQP1$ .<sup>10</sup> (Table 1).

### Gene frequencies

Gene frequencies were calculated using the Hardy-Weinberg equation, as modified by Mourant.<sup>1</sup>

### Review of Chinese-language publications

We reviewed and summarized the results of other studies of ABO blood group genotypes that had been published in Chinese-language journals. The articles were selected from journals that were listed in the Qinghua computerized database for the years 1994 to 2004.

## Results

Table 2 summarizes the results of genotyping and gene frequencies, categorized by Rh, MNS, Duffy, Kidd, Yt, Scianna, and Colton blood group systems. Han samples were genotyped for selected genes in the Rh, MNS, Duffy, Kidd, Yt, Scianna, and Colton blood group systems and for  $A^1$ ,  $A^2$ ,  $B$ ,  $O^1$ , and  $O^2$ . (Table 3) We found

**Table 1.** Amplification primers

Locus		Sense (5'→3')	Antisense (5'→3')	Amplicon size(bp)	Method and reference
ABO	<i>O1</i>	tTaaGTGGAAGGATGTCCTCGTcGTA	aTatATGCAAACACAGTTAACCCAATG	137	PCR-SSP  Gassner C et al. <sup>2</sup>
	<i>non O1</i>	TaaGTGGAAGGATGTCCTCGTcGTG	aTatATGCAAACACAGTTAACCCAATG	137	
	<i>O2</i>	tCGACCCCCGAAGAAGCT	aGTGGACGTGGACATGGAGTTCC	194	
	<i>non O2</i>	CGACCCCCGAAGAAGCC	aGTGGACGTGGACATGGAGTTCC	193	
	<i>B</i>	atCGACCCCCGAAGAAGCG	aGTGGACGTGGACATGGAGTTCC	195	
	<i>non B</i>	CCGACCCCCGAAGAAGCC	aGTGGACGTGGACATGGAGTTCC	194	
	<i>A2</i>	GAGGCGGTCCGGAA gCG	ggGTGTGATTTGAGGTGGGGAC	169	
	<i>non A2</i>	gAGGCGGTCCGGAAcAG	ggGTGTGATTTGAGGTGGGGAC	170	
Rb	<i>RbC</i>	CAGGGCCACCACATTTGAA	GAACATGCCACT TCACTCCAG	357	PCR
	<i>Rbc</i>	TCGGCCAAG ATCTGACCG	TGATGACCACCT TCCCAGG	177	Avent ND <sup>3</sup>
	<i>RHE</i>	CCAAGTGTAACCTCTC	CATGCTGATCTTCT	141	PCR-SSP
	<i>Rbe</i>	CCAAGTGTAACCTCTG	CATGCTGATCTTCT	141	Faas BHW et al. <sup>4</sup>
MN	<i>M</i>	CAGCATCAAGTACCCTGGT	TGA AACTTC ATG AG CTC TAG	844	PCR-SSP
	<i>N</i>	CAGCATTAAGTACCCTGAG	TGA AACTTCATG AG CTC TAG	844	Eshleman JR et al. <sup>5</sup>
Duffy	<i>Fy<sup>a</sup></i>	CTTCCCAGATGGAGACTATGA	CATGAAGAGGACAGTGCTGCTAG	142	PCR-SSP
	<i>Fy<sup>b</sup></i>	CTTCCCAGATGGAGACTATGG	CATGAAGAGGACAGTGCTGCTAG	142	Olsson ML et al. <sup>6</sup>
Kidd	<i>Jk<sup>a</sup></i>	CCAGAGTCCAAAGTAGATGTC	CATGCTGCCATAGGATCATTCG	301	PCR-SSP
	<i>Jk<sup>b</sup></i>	CCAGAGTCCAAAGTAGATGTT	CATGCTGCCATAGGATCATTCG	301	Irshaid NM et al. <sup>7</sup>
Yt	<i>Yt<sup>a</sup></i>	CATCAACGCGGGGAGACTTCC	TAGACCCATGGTGGCTTTGCT	214	PCR-SSP
	<i>Yt<sup>b</sup></i>	CATCAACGCGGGGAGACTTCA	TAGACCCATGGTGGCTTTGCT	214	Mengli L et al. <sup>8</sup>
Sc	<i>Sc1</i>	TCACCTCCTTGGGTACCGTACC	CTCCCAGTTGGCCTTGCTC	138	PCR-SSP
	<i>Sc2</i>	TCACCTCCTTGGGTACCGTACT	CTCCCAGTTGGCCTTGCTC	138	Wagner FF et al. <sup>9</sup>
Co	<i>Co<sup>a</sup></i>	ACATCTTACGTTGTCTGGAACG	CAAGAAGAAGCTCTTCTGGAGG	143	PCR-SSP
	<i>Co<sup>b</sup></i>	ACATCTTACGTTGTCTGGAACA	CAAGAAGAAGCTCTTCTGGAGG	143	Joshi SR et al. <sup>10</sup>

**Table 2.** Genotypes and gene frequencies for Rh, MN, Duffy, Kidd, Yt, Scianna, and Colton blood group systems

Blood systems	Population	Number	Genotype			Gene frequency	
Rh	Han D+	121	<u>CC</u>	<u>Cc</u>	<u>cc</u>	<u>C</u>	<u>c</u>
	Han D-	111	53	57	11	0.67	0.33
Rh	Han D+	121	<u>EE</u>	<u>Ee</u>	<u>ee</u>	<u>E</u>	<u>e</u>
	Han D-	110	7	40	74	0.22	0.78
MN	Han	115	<u>MM</u>	<u>MN</u>	<u>NN</u>	<u>M</u>	<u>N</u>
	Tajik	100	25	60	30	0.478	0.522
Duffy	Han	102	<u>Fy<sup>a</sup>/Fy<sup>a</sup></u>	<u>Fy<sup>a</sup>/Fy<sup>b</sup></u>	<u>Fy<sup>b</sup>/Fy<sup>b</sup></u>	<u>Fy<sup>a</sup></u>	<u>Fy<sup>b</sup></u>
	She	90	91	10	1	0.94	0.06
Kidd	Han	102	<u>Jk<sup>a</sup>/Jk<sup>a</sup></u>	<u>Jk<sup>a</sup>/Jk<sup>b</sup></u>	<u>Jk<sup>b</sup>/Jk<sup>b</sup></u>	<u>Jk<sup>a</sup></u>	<u>Jk<sup>b</sup></u>
	She	90	87	3	0	0.98	0.02
Yt	Han	105	<u>Yt<sup>a</sup>/Yt<sup>a</sup></u>	<u>Yt<sup>a</sup>/Yt<sup>b</sup></u>	<u>Yt<sup>b</sup>/Yt<sup>b</sup></u>	<u>Yt<sup>a</sup></u>	<u>Yt<sup>b</sup></u>
	Tajik	100	105	0	0	1.00	0
Scianna	Han	240	<u>Sc1/Sc1</u>	<u>Sc1/Sc2</u>	<u>Sc2/Sc2</u>	<u>Sc1</u>	<u>Sc2</u>
	Tajik	100	240	0	0	1.00	0
Colton	Han	103	<u>Co<sup>a</sup>/Co<sup>a</sup></u>	<u>Co<sup>a</sup>/Co<sup>b</sup></u>	<u>Co<sup>b</sup>/Co<sup>b</sup></u>	<u>Co<sup>a</sup></u>	<u>Co<sup>b</sup></u>
	She	62	100	0	0	1.00	0
	Yugu	102	100	0	0	1.00	0

**Table 3.** ABO gene frequencies in present and previously published studies of Chinese Han populations

Han population	Number	A	B	O'	O <sup>2</sup>	Method
Zhejiang (present study)	156	0.192	0.170	0.638	0.00	PCR-SSP
Anfei <sup>11</sup>	240	0.229	0.213	0.568	nt	PCR-SSP
Wuhan <sup>12</sup>	125	0.244	0.176	0.580	nt	PCR-RFLP
Xuzhou <sup>13</sup>	104	0.249	0.245	0.504	0.00	PCR-SSP
Shanghai <sup>14</sup>	60	0.233	0.242	0.525	0.00	PCR-RFLP
ShenZhen <sup>15</sup>	260	0.232	0.185	0.583	0.00	PCR-SSP
Qinduo <sup>16</sup>	19953	0.2173	0.2438	0.5389	nt	serologic
Shandong <sup>17</sup>	14207	0.2179	0.2512	0.5309	nt	serologic

nt: not tested

no O<sup>2</sup> among the 156 Han samples tested. To compare these data with those for other populations, results for A<sup>1</sup> and A<sup>2</sup> were combined, yielding a gene frequency of 0.192 for the 156 Han samples. Tajik samples were selectively genotyped for genes in the Yt, MNS, and Scianna blood group systems. She samples were genotyped for genes in the Duffy, Kidd, and Colton blood group systems, and Yugu samples were genotyped for genes in the Colton blood group system.

### ABO

Of the 156 Han subjects whose samples were genotyped for ABO, 4 (2.6%), 1 (0.6%), and 36 (23.1%) were A<sup>1</sup>A<sup>1</sup>, A<sup>1</sup>A<sup>2</sup>, and A<sup>1</sup>O<sup>1</sup>, respectively; 14 (8.9%) were A<sup>1</sup>B; 3 (1.9%) were BB; 33 (21.2%) were BO<sup>1</sup>; and 65(41.7%) were O<sup>1</sup>O<sup>1</sup>. The gene frequencies of A<sup>1</sup>, A<sup>2</sup>, B, and O<sup>1</sup> alleles were 0.189, 0.003, 0.170, and 0.638, respectively.

### Rb

Of the 121 D+ Hans, 53 (43.8%) genotyped as CC, 57 (47.1%) typed as Cc, and 11 (9.1%) typed as cc. Of the 111 D- Hans, 7 (6.3%) were genotyped as CC, 38 (34.2%) were Cc, and 66 (59.5%) were cc. The gene frequencies of C and c alleles among D+ Hans were 0.67 and 0.33, respectively. Among D- Hans, the frequencies of C and c alleles were 0.23 and 0.77, respectively. Of the 121 D+ Hans, 7 (5.8%) were genotyped as EE, 40 (33.0%) as Ee, and 74 (61.2%) as ee. Among the 110 D- Hans, 9 (8.2%) were genotyped as Ee and 101(91.8%) as ee. We did not detect the EE genotype in this population. The gene frequencies of E and e alleles in D+ Hans were 0.22 and 0.78; the frequencies in D- Hans were 0.04 and 0.96, respectively. The differences in RHCE allele frequencies were significant between the D+ and D- Hans.

### MNS

We typed for MN alleles, but not Ss alleles. Of the 115 Hans, 25 (21.7%) were MM, 60 (52.2%) were MN, and 30 (26.1%) were NN. The gene frequencies of M and N alleles were 0.478 and 0.522, respectively. Among 100 Tajiks, 42 (42%) were genotyped as MM, 47 (47%) as MN, and 11 (11%) as NN. The gene frequencies of M and N alleles were 0.655 and 0.345, respectively.

### Duffy

Of the 102 Hans, 91 (89.2%) were genotyped as Fy<sup>a</sup>/Fy<sup>a</sup>, 10 (9.8%) were typed as Fy<sup>a</sup>/Fy<sup>b</sup>, and 1(0.98%) was typed as Fy<sup>b</sup>/Fy<sup>b</sup>. Of the 90 Shes, 87 (96.7%) were Fy<sup>a</sup>/Fy<sup>a</sup> and 3 (3.3%) were Fy<sup>a</sup>/Fy<sup>b</sup>. No Shes typed as Fy<sup>b</sup>/Fy<sup>b</sup> and we did not find Fy/Fy in this population. Among Hans, the gene frequencies of Fy<sup>a</sup> and Fy<sup>b</sup> alleles were 0.94 and 0.06, respectively. Among Shes, the gene frequencies of Fy<sup>a</sup> and Fy<sup>b</sup> alleles were 0.98 and 0.02, respectively.

### Kidd

Of the 102 Hans, 26 (25.5%) were genotyped as Jk<sup>a</sup>/Jk<sup>a</sup>, 48 (47.0%) were Jk<sup>a</sup>/Jk<sup>b</sup>, and 28 (27.5%) were Jk<sup>b</sup>/Jk<sup>b</sup>. Of the 90 Shes, 26 (28.9%) were Jk<sup>a</sup>/Jk<sup>a</sup>; 48 (53.3%) were Jk<sup>a</sup>/Jk<sup>b</sup>, and 16 (17.8%) were Jk<sup>b</sup>/Jk<sup>b</sup>. We did not find the Jk(a-b-) phenotype in this population. Among Hans, the gene frequencies of Jk<sup>a</sup> and Jk<sup>b</sup> alleles were 0.49 and 0.51, respectively. Among Shes, the gene frequencies of Jk<sup>a</sup> and Jk<sup>b</sup> were 0.56 and 0.44, respectively.

### Yt

Among the 105 Hans, we found only Yt<sup>a</sup>/Yt<sup>a</sup> and, thus, the frequency of Yt<sup>a</sup> was 1.00. Among the 100 Tajiks, 89 (89%) were typed as Yt<sup>a</sup>/Yt<sup>a</sup>, 10 (10%) were typed as Yt<sup>a</sup>/Yt<sup>b</sup>, and 1 (1%) was typed as Yt<sup>b</sup>/Yt<sup>b</sup>. We did not find the Yt(a-b-) phenotype in either the Han or the Tajik population. Among Tajiks, the gene frequencies of Yt<sup>a</sup> and Yt<sup>b</sup> alleles were 0.94 and 0.06, respectively.

### Scianna

We typed for Sc1 and Sc2 alleles, but not for Rd and Sc3. Among 240 Hans and 100 Tajiks all were Sc1/Sc1. Therefore the frequency of Sc1 was 1.00 in both the Han and Tajik populations.

### Colton

We typed for  $Co^a$  and  $Co^b$  alleles. Among 103 Hans, 62 Shes, and 102 Yugus, we detected only  $Co^a/Co^a$ . The frequency of the  $Co^a$  allele was 1.00 in all three of these ethnic populations.

### Other studies in Chinese Han

We reviewed articles on *ABO* genotypes among Chinese populations published in Chinese-language journals (Table 3). The allele frequencies for *ABO* in the Han population were similar in all articles.

### Discussion

In our study, the most frequent *ABO* allele among Han Chinese was  $O'$  (0.638), which is similar to the frequency reported in other studies in Chinese-language publications (0.504–0.583) (Table 3) and in other Asian populations.<sup>18</sup> Among Caucasian donors in Europe, Australia, and the United States, approximately 2 to 6 percent of  $O$  alleles are  $O^2$ .  $O^2$  was not detected in the present study, which is consistent with previous findings in Asian populations.<sup>18</sup>

Two homologous genes, *RHD* and *RHCE*, encode Rh antigens. *RHD* codes for the D antigen, and *RHCE* codes for the Cc and Ee antigens. The molecular basis of the D- phenotype among Chinese may be the absence of the *RHD* gene, a partial deletion, or the absence of an intact *RHD* gene.<sup>19</sup> Therefore, PCR is not readily suited for D genotyping in this population. We used conventional serologic methods to type for the D antigen. The frequency of D+ was 99.6 percent in Hans. Only 0.4 percent of the population was D-. Using PCR, we typed the *RHCE* allele (*C,c,E,e*). *RHCE* allele frequencies are obviously different between D+ and D- Hans because of different populational haplotype frequencies. In addition, cis and trans effects of *RHCE* alleles upon D antigen expression are known, but their magnitude in this population cannot be determined from the design of the current study, which did not include antigen density studies.

We also determined selected blood group genotypes among She, Yugu, and Tajik minority populations. The Chinese population includes 56 ethnic groups; 92 percent of the population is Han and the other 8 percent represent 55 minorities. In this study, *MN* allele frequencies are significantly different between Han and Tajik populations. We did not find  $Yt^b$  in Hans, a distinct population characteristic that was reported previously by Peng et al.<sup>20</sup> We identified the  $Yt^b$  allele among Tajiks, whose  $Yt$  allele frequencies

were similar to those of Caucasians.<sup>18</sup> We did not find *Sc2* among Hans or Tajiks; nor did we find  $Co^b$  among Hans, Shes, or Yugus. These genes may be absent in Chinese populations.

The findings of this pilot study demonstrate that blood group genotypes not only distinguish Asian from European populations, but also illustrate the varied genetic compositions of different Chinese ethnic populations. Most studies of blood groups among Chinese have been conducted by serologic methods among emigrant Chinese. The results of this pilot study encourage us to pursue further studies of the genetic diversity among indigenous ethnic and minority Chinese populations.

### References

1. Mourant AE. Blood relations/blood groups and anthropology. Oxford: Oxford University Press, 1985:15:59-72.
2. Gassner C, SchmarDA A, Nussbaumer W, et al. ABO glycosyltransferase genotyping by polymerase chain sequence specific primers. *Blood* 1996;88:1852-6.
3. Avent ND. Antenatal genotyping of the blood groups of the fetus. *Vox Sang* 1998;74(suppl):2365-74.
4. Faas BHW, Simsek S, Bleeker PMM, et al. RHE/e genotyping by allele-specific primer amplification. *Blood* 1995;85:829-32.
5. Eshleman JR, Shakin-Eshleman SH, Church A, et al. DNA typing of the human MN and Ss blood group antigens in amniotic fluid and following massive transfusion. *Am J Clin Pathol* 1995;103:353-6.
6. Olsson ML, Hansson C, Avent ND, et al. A clinically applicable method for determining the three major alleles at the Duffy (FY) blood group locus using polymerase chain reaction with allele specific primers. *Transfusion* 1998;38:168-73.
7. Irshaid NM, Thuresson B, Olsson ML. Genomic typing of the Kidd blood group locus by a single tube allele specific primer PCR technique. *Br J Haematol* 1998;102:1010-4.
8. Mengli L, Dongling J. Distribution of  $Yt$  blood group in Xian Han population. *Chin J of Blood Transfus* 2002;15:320-1.
9. Wagner FF, Poole J, Flegel W. Scianna antigens including Rd are expressed by ERMAP. *Blood* 2003;101:752-7.
10. Joshi SR, Wagner FF, Vasantha K, et al. An AQP1 null allele in an Indian woman with Co(a-b-)

- phenotype and high-titer anti-Co3 associated with mild HDN. *Transfusion* 2001;41:1273-8.
11. Zhong L, Qing F, Rong L, et al. Study of identification and polymorphism distribution of human blood type ABO genotypes. *Chin J of Blood Transfus* 1998;11:178-80.
  12. Qingen Y, Chuanhong ZH. Detection of ABO genotypes by simultaneous PCR-RFLP method. *Chin J Med Genet* 1999;16:110-2.
  13. Jiongcai L, Qingbao M, Yinze ZH, et al. Genotyping of ABO blood group and its application. *Chin Immunol J* 2002;18:430-3.
  14. Zhonghui G, Dazhuang L, Ziyang Zhu, et al. Study on ABO genotype in Chinese Han population. *Journal of Clin Transfus Lab Med* 2002;14:6-8.
  15. Qiong Y, Guogang W, Yanlian L, et al. Study on genotyping of ABO blood group and its application in Chinese Han population. *Med Lab of Jiangxi* 2003;21:133-6.
  16. Yuqin W, Qi Y. Distribution of ABO and Rh group in Qingduo. *Chin J of Blood Transfus* 1997;10:150.
  17. Qun X, Zhaoyong SH, Shixun ZH, et al. Distribution of ABO, Rh, MN, P groups in Shangdong. *Chin J of Blood Transfus* 1995;8:211.
  18. Daniels G. *Human blood groups*. 2nd ed. Oxford: Blackwell Science Ltd. 2002:16-22,369-71.
  19. Ji H, Qihua F, Lei J, et al. Analysis of Del phenotype and RHD gene in RhD negative phenotype. *J of Shanghai Med Lab* 2002;17:90-2.
  20. Peng CT, Tsai CH, Lee HH, et al. Molecular analysis of Duffy, Yt, and Colton blood groups in Taiwanese, Filipinos and Thais. *Kaohsiung J Med Sci* 2000;16:63-7.
- 
- Lixing Yan, MD; Faming Zhu, MD; Qibua Fu, MD; and Ji He, BA, Institute of Transfusion Medicine, Blood Center of Zhejiang Province, Wulin Road 345, Hangzhou, Zhejiang 310006, P.R. China*

# Expression of Duffy antigen receptor for chemokines during reticulocyte maturation: using a CD71 flow cytometric technique to identify reticulocytes

I.J. WOOLLEY, E.M. WOOD, R.M. SRAMKOSKI, P.A. ZIMMERMAN, J.P. MILLER, AND J.W. KAZURA

Flow cytometric methods commonly used to identify reticulocytes are of limited usefulness in malarious areas, since RNA staining also detects plasmodia. An important antigen expressed on reticulocytes is Duffy antigen receptor for chemokines (DARC, also known as Fy), the receptor for *Plasmodium vivax*. An early marker for reticulocytes is CD71 (transferrin receptor). We have been interested in CD71 as an alternative marker for reticulocytes in the context of Fy expression. Flow cytometry was used to determine the expression of Fy on CD71-positive and -negative reticulocytes and to correlate serology and genotype. A reduction of 13 percent was seen in Fy6 expression between CD71-positive reticulocytes and RNA-positive reticulocytes. CD71 disappears early during reticulocyte maturation, while Fy6 expression is relatively preserved. CD71 is an alternative to staining for RNA for reticulocyte assays relating to Fy6 expression. *Immunohematology* 2005;21:15–20.

**Key Words:** DARC, Fy, reticulocyte, CD71, transferrin receptor, ORF, flow cytometry

With the discovery of the Duffy system, it became apparent there are two codominant alleles, *Fya* and *Fyb*, that correspond to the two codominant antigens, Fy<sup>a</sup> and Fy<sup>b</sup>, respectively.<sup>1</sup> However, in many of those of African descent, including African Americans, neither antigen is expressed.<sup>2</sup> These individuals have been described as Duffy negative, with the allelic designation *Fy*. Subsequently, it has been shown that this phenotype, which expresses neither Duffy antigen receptor for chemokines (DARC) mRNA nor protein, is caused by a nucleotide polymorphism in the GATA-1 binding site of the promoter regions: a single T to C substitution at nucleotide -46.<sup>3</sup> This change in the promoter region prevents downstream transcription of the open reading frame (ORF). It is noteworthy, though, that DARC is expressed on other tissues,

including brain, kidney, and endothelial cells of post-capillary venules, even in those with the Duffy-negative phenotype.<sup>4</sup> That is, in the Duffy-negative phenotype, Fy is silent only in the erythroid lineage.

Other Duffy-related epitopes have been discovered serologically. DARC follows the general structure of chemokine receptors, having seven transmembrane-spanning helices. Anti-Fy3 antibody attaches to the third extracellular loop in a pocket where chemokines themselves attach to their receptor.<sup>5</sup> It was initially described as a human antibody but now a mouse monoclonal is available. Anti-Fy4 and anti-Fy5 are rare and clinically unimportant human antibodies.<sup>1</sup> Anti-Fy6 is a mouse monoclonal antibody that attaches to the first extracellular domain of DARC, in an area thought to correspond to the binding site of *Plasmodium vivax* merozoites.<sup>6,7</sup>

The functional role of Fy on RBCs is not known, but it has been speculated that it acts as a “sink” for chemokines, removing them from the circulation.<sup>8</sup> The absence of Fy antigens or proteins on the RBCs of Africans, resulting in the serologic Fy(a-b-) phenotype, however, has no discernible ill effect. Similarly, no ill effect has been seen in other rare examples, of Caucasians with mutations leading to lack of functional Fy expression on all tissues.<sup>9</sup> Whether consequent elevated chemokine levels in these individuals might have a protective effect against malaria or other infectious diseases is a question for future investigation. There is certainly evidence that chemokine levels may vary according to Fy phenotype, as do outcomes in some disease models.<sup>10,11</sup>

*P. vivax* is known to preferentially invade reticulocytes. It is proposed that this is due to the presence of a second receptor on the surface of reticulocytes<sup>12</sup> that is perhaps not present on more mature RBCs. However, no second receptor has yet been identified. Recently we developed a flow cytometric assay to measure the relative amount of Fy6 on reticulocytes and demonstrated an increased expression of Fy6 on these cells compared to mature RBCs.<sup>13</sup> Expression of the Fy6 epitope was higher (49 ± 19%) on reticulocytes than on mature RBCs, regardless of donor genotype ( $p < 0.0001$ ).<sup>13</sup> We have speculated that this may be due to relative differences in size, membrane structure, RNA activity, or some combination of these.

The transferrin receptor (CD71) is a transmembrane glycoprotein involved in iron metabolism, specifically the cellular uptake of transferrin, during erythrocyte ontogeny. Its persistent presence, after release from the bone marrow, defines an early population of reticulocytes.<sup>14</sup> Therefore, the relative expression of Fy6 on CD71-positive and -negative reticulocytes may define the extent and timing of loss of Fy6 from maturing reticulocytes, all of which stain with thiazole orange (TO), the stain commonly used to identify reticulocytes in flow cytometric assays. Because TO also stains the RNA of any plasmodia present, we wished to evaluate the use of CD71 as an alternative marker for reticulocytes in this assay. Used in this way, it may be an acceptable alternative labeling method for samples from areas where malaria is endemic. The main objective of this study was to develop such a method and specifically look at the profile of DARC expression on reticulocytes less mature than those that do not express CD71, to see if that pattern was consistent with DARC's role as a "sink" for chemokines in the circulation, as theorized, or with its having an important role in RBC ontogeny in the bone marrow.

## Materials and Methods

### Samples

Samples were obtained from bag segments prepared from units of whole blood donated at the American Red Cross Blood Services, Northern Ohio Region. Racial designation was by donor self-identification.

### Methods

RBC phenotyping for Fy<sup>a</sup> and Fy<sup>b</sup> was performed using standard blood bank reagents and methods. DNA

was isolated from samples using the QIA blood kit (Qiagen, Santa Clarita, CA). Genotyping of the FY promoter and open reading frame polymorphisms was performed by PCR-RFLP strategies.<sup>13</sup>

Flow cytometry studies were done using an anti-Fy6 antibody (NYBC-BG6), kindly provided by John Barnwell, Centers for Disease Control and Prevention, Atlanta, Georgia. This antibody binds to the extracellular portion of the Fy glycoprotein involved in interaction of the reticulocyte surface with the *P. vivax* merozoite ligand.<sup>7</sup> This antibody was conjugated directly to a phycoerythrin (PE) label (ProZyme, San Leandro, CA) according to the manufacturer's instructions. Prior experiments were done to determine the optimum concentration and incubation time for labeling erythrocytes. A 5 µL aliquot of blood was washed three times in 50 µL of 2% BSA/PBS with 0.1% sodium azide. The RBCs were then incubated with 50 µL of a 1:50 dilution of PE-labeled anti-Fy6 for 15 minutes at 37°C. Cells were again washed twice with 2% BSA/PBS with 0.1% sodium azide. A second mouse antibody against human CD71 (Caltag, Burlingame, CA) labeled with TC (Tricolor) was added at a dilution of 1:50 and incubated for 15 minutes. The RBCs were then washed twice in PBS and resuspended in TO solution (Becton Dickinson, San Jose, CA) for 30 minutes to stain reticulocytes, according to the manufacturer's specifications. Flow cytometry readings were performed using a Coulter Elite instrument (Coulter Corp., Miami, FL) equipped with a 488-nm air-cooled argon ion laser at 15 mW. TO, PE, and TC fluorescence was collected with band pass filters at 525 nm, 575 nm, and 675 nm, respectively. Compensation was applied at the level of the hardware after the appropriate initial experiments, i.e., after controls were run, negative fluorescent signals were adjusted until orthogonal. Forward scatter, side scatter, and fluorescence data were analyzed. A count of 5000 TO-positive cells was taken per sample. This was approximately 1 to 2 percent of the total number of cells assayed for each individual. Experiments were standardized using Quantum Simply Cellular Beads (Flow Cytometry Standards Corp., San Juan, PR) and Immunobrite beads (Coulter) according to the manufacturers' instructions. Analysis was undertaken using Win MD 2.8 software (Dr. Joe Trotter, BD Biosciences, accessed at <http://facs.scripps.edu/software.html>), using the quadrant tool to gate upon the populations of interest. Care was taken in the use of the quadrant tool to reduce background.

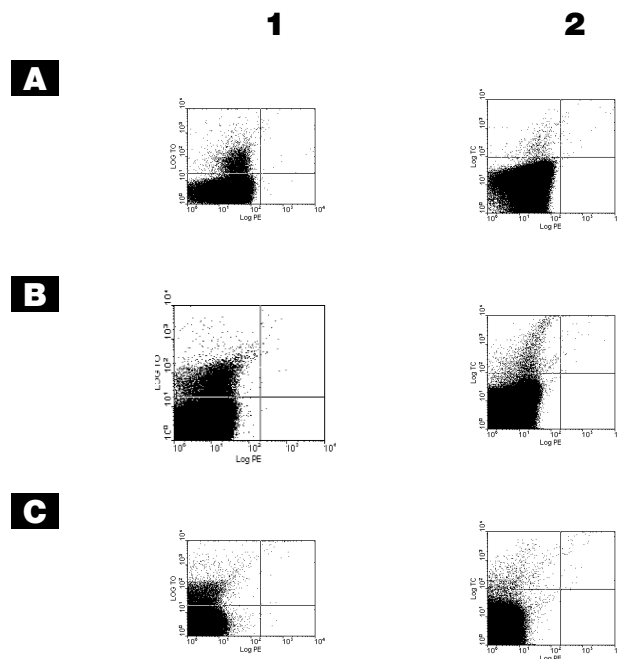
Correlation coefficients for mean fluorescence were calculated using Pearson's bivariate analysis, and statistical analysis was undertaken with the SPSS 10 software package (SPSS Inc., Chicago, IL).

## Results

Genotyping was performed on samples from 33 donors. Samples were classified according to promoter and ORF polymorphisms. Twenty were homozygous for the wild-type promoter, six were heterozygous, and seven were homozygous for the promoter polymorphism. In the group homozygous for the wild-type promoter, ten were *FY\*A/FY\*A* for the ORF, one was *FY\*A/FY\*B*, and nine were *FY\*B/FY\*B*. All but three of these donors identified themselves as white; the others self-identified as African American. Among the six persons who were heterozygous for the promoter polymorphism, two were *FY\*A/FY\*B* for the ORF and four were *FY\*B/FY\*B*. Five of these donors were self-identified as African American and one as white. As expected, all seven who were homozygous for the promoter polymorphism were *FY\*B/FY\*B*. These individuals were all self-identified as African American. Serologic findings were consistent with genotyping in these donors.

Characteristic flow cytometric dot plots obtained for RBCs from individuals with each of the three different promoter genotypes are shown in Figure 1. The X axis demonstrates the amount of PE fluorescence (Fy6) and the Y axis the reticulocyte markers as measured by TO fluorescence (RNA) or TC fluorescence (CD71). Graphs were prepared with Win 2.8 MD software. Donor A is homozygous for the wild-type promoter, donor B is heterozygous, and donor C is homozygous for the GATA-1 promoter polymorphism (i.e., having no Fy expression on RBCs). In the first column, TO expression is a marker for reticulocytes. In the second column, TC is used as the marker for the presence of CD71. PE fluorescence represents Fy6 expression. A vertical line has been drawn to delineate the main population of cells. Values from the upper left quadrant of each panel therefore represent reticulocytes, and values from the lower left quadrant represent mature RBCs.

The flow cytometry and genotyping results for all 33 subjects are summarized in Table 1 (mature RBCs) and Table 2 (reticulocytes). They show a high degree of correlation between the two methods of measuring Fy expression. Table 3 shows the percentage of each population labeled with TC (CD71-positive) and TO



**Fig. 1.** Representative flow cytometric curves for RBCs from donors with and without Fy expression on RBCs. The panels in row (A) represent a serologically Duffy-positive individual who is homozygous for the wild-type promoter. The panels in row (B) represent a donor heterozygous for that polymorphism; those in row (C) show a serologically negative individual who is homozygous for the promoter polymorphism. Panels in the first column show PE fluorescence, which represents the Fy6 epitope versus TO. Panels in the second column show PE versus TC, which represents CD71, or the transferrin receptor, for the same individuals. The mean fluorescent intensity (MFI) of the left upper quadrant has been used as representative of double-labeled cells to reduce background. These MFI values for the top panel are 32.7 and 30.7 (TO/PE and TC/PE respectively), for the middle 10.0 and 14.0, and for the bottom 1.9 and 3.6, respectively.

(RNA-positive). The mean reduction in Fy expression between early, CD71-positive reticulocytes and the population of reticulocytes as a whole was only 13 percent. Therefore, there is a strong degree of correlation between the results of the two flow cytometric assays for Fy6 expression on reticulocytes.

## Discussion

The key finding from this study was that DARC expression is relatively preserved during reticulocyte maturation. Comparing immature reticulocytes and the total reticulocyte population (at least four-fifths of which are CD71 negative), Fy6 expression is relatively stable in the life of the reticulocyte, falling by a mean of 13 percent as CD71 is lost. This is in keeping with previously published data showing the antigen appears late in the bone marrow during ontogeny of RBCs.<sup>15</sup>

**Table 1.** Level of Fy6 expression on mature RBCs according to Fy promoter and ORF genotype as measured by MFI

Promoter genotype	ORF genotype	Number	Mature RBCs	Mature RBCs	Correlation coefficient (Pearson's bivariate)
			RNA (TO) negative MFI ± SD	CD71 (TC) negative MFI ± SD	
Homozygous wild-type promoter	<i>FY*A/FY*A</i>	10	14.55 ± 4.95	14.66 ± 4.99	1.00
	<i>FY*A/FY*B</i>	1	6.2	6.2	
	<i>FY*B/FY*B</i>	9	10.38 ± 2.57	10.44 ± 2.58	
	All	20	12.26 ± 4.56	12.34 ± 4.60	
Heterozygous wild-type promoter	<i>FY*A/FY*A</i>	0	-	-	1.00
	<i>FY*A/FY*B</i>	4	5.63 ± 0.98	5.73 ± 0.98	
	<i>FY*B/FY*B</i>	2	3.75 ± 0.35	3.75 ± 0.35	
	All	6	5.12 ± 1.20	5.07 ± 1.28	
Total Fy positive		26	10.5 ± 5.08	10.66 ± 5.11	1.00
Homozygous promoter polymorphism (Fy negative)	<i>FY*B/FY*B</i>	7	1.57 ± 0.10	1.57 ± 0.10	1.00

**Table 2.** Level of Fy6 expression on reticulocytes according to Fy promoter and ORF genotype as measured by MFI

Promoter genotype	ORF genotype	Number	Reticulocytes	Reticulocytes	Correlation coefficient TO+ versus TC+ populations
			RNA (TO) positive MFI ± SD	CD71 (TC) positive MFI ± SD	
Homozygous wild-type promoter	<i>FY*A/FY*A</i>	10	24.25 ± 8.30	24.37 ± 11.82	0.771
	<i>FY*A/FY*B</i>	1	9.9	15.2	
	<i>FY*B/FY*B</i>	9	19.42 ± 3.33	24.25 ± 8.30	
	All	20	21.36 ± 7.10	23.91 ± 9.13	
Heterozygous wild-type promoter	<i>FY*A/FY*A</i>	0	-	-	0.469
	<i>FY*A/FY*B</i>	4	10.63 ± 1.04	13.53 ± 2.35	
	<i>FY*B/FY*B</i>	2	7.9 ± 0.00	12.35 ± 0.49	
	All	6	9.72 ± 1.62	13.13 ± 1.93	
Total Fy positive		26	18.67 ± 7.99	21.42 ± 9.25	0.831
Homozygous promoter polymorphism (Fy negative)	<i>FY*B/FY*B</i>	7	2.04 ± 0.26	4.2 ± 1.2	

**Table 3.** Percentage of reticulocytes (TO+) staining positive for CD71 (TC+) by genotype

Promoter genotype	ORF genotype	Number	Race		Percentage of reticulocytes TO+ also CD71 (TC) +	Serology
			White	African American		
Homozygous wild-type promoter	<i>FY*A/FY*A</i>	10	10	0	16 ± 7	10 Fy(a+b-)
	<i>FY*A/FY*B</i>	1	1	0	19	1 Fy(a+b+)
	<i>FY*B/FY*B</i>	9	6	3	13 ± 3	9 Fy(a-b+)
	All	20	17	3	15 ± 5	
Heterozygous wild-type promoter	<i>FY*A/FY*A</i>	0	0	0	-	
	<i>FY*A/FY*B</i>	4	1	3	15 ± 6	4 Fy(a+b-)
	<i>FY*B/FY*B</i>	2	0	2	23 ± 10	2 Fy(a-b+)
	All	6	1	5	18 ± 8	
Total Fy positive		26			16 ± 7	
Homozygous promoter polymorphism (Fy negative)	<i>FY*B/FY*B</i>	7		7	18 ± 8	7 Fy(a-b-)

Note: No significant differences are noted.

Assuming a constant rate of reticulocyte release into the circulation and subsequent maturation to RBCs, these results also demonstrate the relatively early disappearance of CD71 from reticulocytes. The loss of CD71 is consistent with previous studies. Although the mean percentage of cells expressing CD71 (15% ± 5%) in the present study is lower than has been reported in

some previous studies using similar methods on homozygous wild-type promoter populations,<sup>14</sup> it is consistent with the findings of others. For instance, in a flow cytometry-based study that did not examine Fy expression, Dertinger et al.<sup>16</sup> found it was the youngest 10 to 20 percent of reticulocytes that label with anti-CD71. Thus very large numbers of cells must be

analyzed to obtain a sufficiently large sample of CD71-bearing cells for statistical analysis of their antigen expression.

The persistence of relatively constant quantities of Fy on the RBC surface supports the prevailing theory that DARC's major role is as a "sink" for circulating chemokines. On the other hand, a large difference between the two sets of reticulocytes may have implied a role for Fy6 in RBC development.

Our findings that, first, there is a gene dosage effect associated with the promoter polymorphism and, second, the Fy<sup>b</sup> polymorphism in the ORF is independently associated with lower Fy expression, confirmed results previously reported.<sup>13</sup> An incidental finding is the apparent higher expression of DARC on the cells of persons with the Fy(a+b-) phenotype as compared to the Fy(a-b+) phenotype, a finding consistent with previous studies using the same antibody.<sup>13</sup> Whether this is due to a true difference in Fy expression or a difference in antibody affinity is unclear.

This study demonstrates the ease of using three-color flow cytometry to examine RBC antigen expression during maturation. Similar techniques may be used to define subpopulations of maturing reticulocytes in individual patients and to distinguish between mixtures of cell populations, for example, identifying donor or patient reticulocytes in the posttransfusion setting.<sup>17</sup> This technique may be useful when the putative reticulocyte-specific receptor for *P. vivax* malaria is identified and to measure the decline of other RBC surface markers in the circulation. Clearly this technique is unlikely to be of use in African populations who have no Fy expression on RBCs. However, the global burden of malaria includes 70 to 80 million cases annually, about 10 to 20 percent of which occur in Africa. The rest occur predominantly in South America, the Southwest Pacific, and Asia,<sup>18</sup> so there are large populations potentially suitable for study of this technique. In addition, it seems unlikely that in vitro, and perhaps in vivo, RBC exposure to malaria alters DARC antigen expression on RBCs.<sup>19</sup> Consequently, we would not likely see artifactual changes postvenipuncture secondary to malaria. However, it cannot be ruled out, since these studies were performed on healthy blood donors, in whom other factors, such as anemia, malaria, or hemoglobinopathies, might not be significantly different. As DARC is only expressed on erythrocytes, there should be no confounding effect due to the

relatively small number of WBCs in the circulation that express CD71 and contain RNA. In addition, of course, the absolute number of WBCs is much smaller than the number of RBCs.

Finally, this study evaluated the utility of CD71 as a reticulocyte marker alternative to RNA labeled by TO. Comparable Fy6 expression on reticulocytes is obtained using staining for either CD71 or RNA, which allows comparison of data from different populations, including those exposed to malaria. The role of transferrin receptor in malaria pathogenesis, if any, is unknown. Studies of soluble levels of transferrin receptor in malaria have led to contradictory results, with levels said to be both lower and higher than expected,<sup>20,21</sup> possibly dependent upon disease severity. The techniques reported here may be a useful adjunct to exploring reticulocyte biology, in the future, with special relevance to malaria.

### Acknowledgments

We thank Ms. Claire McGrath, MT(ASCP)SBB, for assistance with provision of samples; Dr. Margaret Hellard for statistical assistance; and Dr. Rosemary Sparrow for helpful advice and review of the manuscript.

### References

1. Wasniowska K, Hadley TJ. The Duffy blood group antigen: an update. *Transfus Med Rev* 1994;8:281-8.
2. Sanger R, Race RR, Jack J. The Duffy blood groups of New York Negroes: the phenotype Fy (a-b-). *Br J Haematol* 1955;1:370-4.
3. Tournamille C, Colin Y, Cartron JP, Le Van Kim C. Disruption of a GATA motif in the *Duffy* gene promoter abolishes erythroid gene expression in Duffy-negative individuals. *Nat Genet* 1995;10:224-8.
4. Hadley TJ, Peiper SC. From malaria to chemokine receptor: the emerging physiologic role of the Duffy blood group antigen. *Blood* 1997;89:3077-91.
5. Tournamille C, Le Van Kim C, Gane P, et al., Close association of the first and fourth extracellular domains of the Duffy antigen/receptor for chemokines by a disulphide bond is required for ligand binding. *J Biol Chem* 1997;272:16274-80.

6. Nichols ME, Rubenstein P, Barnwell J, et al. A new human Duffy blood group specificity defined by a murine monoclonal antibody. Immunogenetics and association with susceptibility to *Plasmodium vivax*. *J Exp Med* 1987;166:776-85.
7. Wasniowska K, Blanchard D, Janvier D, et al. Identification of the Fy6 epitope recognized by two monoclonal antibodies in the n-terminal extracellular portion of the Duffy antigen receptor for chemokines. *Mol Immunol* 1996;33:917-23.
8. Darbonne WC, Rice GC, Mohler MA, et al. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. *J Clin Invest* 1991;88:1362-9.
9. Mallinson G, Soo KS, Scall TJ, Pisaka M, Anstee DJ. Mutations in the erythrocyte chemokine receptor (Duffy) gene: the molecular basis of the Fya/Fyb antigens and identification of a deletion in the Duffy gene of an apparently healthy individual with the Fy(a-b-) phenotype. *Br J Haematol* 1995;90:823-9.
10. Akalin E, Neylan JF. The influence of Duffy blood group on renal allograft outcome in African Americans. *Transplantation* 2003;75:1496-500.
11. Velzing-Aarts FV, Muskiet FA, van der Dijs FP, Duits AJ. High serum interleukin-8 levels in afro-caribbean women with pre-eclampsia. Relations with tumor necrosis factor-alpha, Duffy negative phenotype and von Willebrand factor. *Am J Reprod Immunol* 2002;48:319-22.
12. Galinski MR, Medina CC, Ingrovallo P, Barnwell JW. A reticulocyte-binding protein complex of *Plasmodium vivax* merozoites. *Cell* 1992;69:1213-26.
13. Woolley IJ, Hotmire KA, Sramkoski RM, Zimmerman PA, Kazura JW. Differential expression of the Duffy antigen receptor for chemokines according to RBC age and FY genotype. *Transfusion* 2000;40:949-53.
14. Serke S, Huhn D. Identification of CD71 (transferrin receptor) expressing erythrocytes by multiparameter-flow-cytometry (MP-FCM): correlation to the quantitation of reticulocytes as determined by conventional microscopy and by MP-FCM using a RNA-staining dye. *Br J Haematol* 1992;81:432-9.
15. Southcott MJ, Tanner MJ, Anstee DJ. The expression of human blood group antigens during erythropoiesis in a cell culture system. *Blood* 1999;93:4425-35.
16. Dertinger SD, Torous DK, Hall NE, et al. Enumeration of micronucleated CD71-positive reticulocytes with a single laser flow cytometer. *Mutat Res* 2002;515:3-14.
17. Perry ES, Moore RH, Berger TA, et al. In vitro and in vivo persistence of reticulocytes from donor red cells. *Transfusion* 1996;36:318-21.
18. Mendis K, Sina BJ, Marchesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg* 2001;64(1-2 Suppl):97-106.
19. Chambers DR, Procter J, Muratova O, et al. In vitro RBC exposure to *Plasmodium falciparum* has no effect on RBC antigen expression. *Transfus Med* 2002;12:213-9.
20. Williams TN, Maitland K, Rees DC, et al. Reduced soluble transferrin receptor concentrations in acute malaria in Vanuatu. *Am J Trop Med Hyg* 1999;60:875-8.
21. Mockenhaupt FP, May J, Stark K, et al. Serum transferrin receptor levels are increased in asymptomatic and mild *Plasmodium falciparum*-infection. *Haematologica* 1999;84:869-73.

---

*Ian J. Woolley, MBBS, FRACP, Department of Infectious Diseases & Clinical Epidemiology, Monash Medical Centre, 246 Clayton Rd, Clayton, Victoria 3168, Australia; Erica M. Wood, MBBS, FRACP, FRCPA, Australian Red Cross Blood Service, Southbank, Victoria, Australia; R. Michael Sramkoski, BS, MT(ASCP)H, and Peter A. Zimmerman, PhD, Cancer Center, The Center for Global Health and Diseases, Case Western Reserve University; John P. Miller, MD, PhD, American Red Cross North Central Region, St. Paul, Minnesota; James W. Kazura, MD, The Center for Global Health and Diseases, Case Western Reserve University, Cleveland, Ohio.*

# Rh antigens and phenotype frequencies of the Ibibio, Efik, and Ibo ethnic nationalities in Calabar, Nigeria

Z.A. JEREMIAH AND C. ODUMODY

This report forms part of the study on the Rh phenotypes within the various ethnic nationalities in the south-south region of Nigeria. The aim is to demonstrate the Rh polymorphisms among the people of African descent. The frequencies of Rh blood group antigens and phenotypes of the Ibibio, Efik, and Ibo ethnic nationalities in Calabar municipality, Nigeria, were determined using standard serologic techniques. Of the 720 Calabar individuals tested, the frequencies of the Rh antigens within the nationalities were c (100%), e (96.38%), D (96.38%), E (15.22%), and C (3.62%) for the Ibibios; c (100%), e (95.60%), D (96.70%), E (21.98%), and C (0%) for the Efiks; and c (100%), e (94.29%), D (91.43%), E (28.57%), and C (2.86%) for the Ibos. The overall frequencies of the Rh antigens in these 720 individuals were c (100%), e (95.56%), D (94.44%), E (18.89%), and C (2.78%). Forty (5.56%) were found to be D-, while all were found to possess the c antigen. The most frequently occurring Rh phenotype was Dccee, with a frequency of 73.61 percent. The alternative allele, C, did not appear in homozygous form (CC) in the population tested. This study further demonstrates the variability of Rh blood group phenotypes in Nigeria and Africa. *Immunohematology* 2005;21:21-24.

**Key Words:** Rh antigens, Rh phenotypes, Ibibio, Efik, Ibo, Nigeria

A total of 29 blood group systems have been recognized by the ISBT Working Party on Terminology for Red Cell Surface Antigens.<sup>1</sup> Rh remains the most complex and polymorphic of the blood group systems.<sup>2,3</sup> Antibodies developed against these antigens have been known to cause HDN.<sup>4-6</sup> In addition, the distribution and frequencies of these antigens have been known to vary between racial groups.<sup>2,3,7</sup>

Nigeria is a multiethnic country whose peoples include the Ijaws, Ogonis, Ikwerres, Ibibios, Ekpeyes, Annangs, Itsekiris, Binis, Urhobos, Isokos, Yorubas, Hausa/Fulani, Ibos, Tivs, Jukuns, and Efiks.<sup>8</sup> Among these ethnic nationalities, the Rh blood group phenotypes of the Yorubas (Western Nigeria), Hausa/Fulani (Northern Nigeria), and Ibos (Eastern Nigeria) have

been reported.<sup>9-11</sup> In a recent study conducted in Port Harcourt (South-South Nigeria), the Rh phenotypes of the four main ethnic groups, Ijaws, Ikwerre, Ekpeye, and Ogoni, were reported.<sup>12</sup> There are no published data on the Rh phenotypes among other ethnic nationalities in the south-south region of Nigeria.

In this report, the Ibibio, Efik, and Ibo ethnic nationalities in Calabar, Nigeria, were randomly selected for study and their Rh phenotypes determined. It is hoped that the data generated from this study will provide information on the Rh antigens and phenotype frequencies of the ethnic nationalities of the people of South-South Nigeria. Also the study seeks to demonstrate the variability and polymorphism of the blood group antigens among the ethnic groups in Nigeria.

## Materials and Methods

### Subjects

A total of 720 persons of both sexes and of various ages and ethnic backgrounds randomly selected from patients and donors at the University of Calabar Teaching Hospital (UCTH); the staff of UCTH; students at the School of Medical Laboratory Sciences, UCTH, Calabar; and students of Holy Child Secondary School; all in the Calabar metropolis, were recruited into the study. The study population consisted of three ethnic nationalities: Ibibio (276), Efik (182), and Ibo (70). The remaining 192 were people of other groups in Nigeria.

### Serology

Venous blood (2 mL) was collected from each of the 720 persons into EDTA tubes. RBCs were phenotyped for D, C, E, c, and e antigens according to standard

serologic protocols (tube method)<sup>13</sup> with an IgM anti-D MoAb (IG 1017) and IgM MoAbs specific for C (AC 2207), c (RH 1268), E (ME 093), and e (AE) 3080 (BIOTEC, Ipswich, Suffolk, UK). Albumin 30% (RE 2728) and an anti-CDE MoAb (CDE 490) were also used. Six tubes were arranged in a row and labeled anti-D, -C, -E, -c, -e, and autocontrol. Two drops of antisera and one drop of a 5% washed RBC suspension were added to each tube. 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. All of the negative results were confirmed microscopically. Anti-CDE, albumin, and antihuman globulin (AHG) tests were used as controls and for weak D determination. To determine the presence of weak D antigen, one drop of anti-CDE reagent and one drop of the RBC suspension were added to a labeled test tube and incubated at 37°C for 30 minutes. Agglutination was read macroscopically and confirmed microscopically. In the final stage, bovine albumin was added to the test and further incubated at 37°C for 30 minutes. At the end of the incubation, the test was washed three times with 0.9% physiological saline. The RBC button was resuspended and one drop of AHG reagent was added. Agglutination at this stage confirmed the presence of weak D antigen.

**Statistical method**

We calculated allele frequencies under the standard assumption of a Hardy-Weinberg equilibrium, using the counting method of Cepellini et al.<sup>14</sup> Frequencies of the Rh antigens were obtained using chi-square analysis. Results were expressed as a percentage.

**Results**

Seven hundred and twenty individuals of both sexes and of various ages residing in the Calabar municipality in South-East Nigeria were randomly screened for the presence of Rh antigens.

Table 1 shows the frequency (%) of the five Rh antigens in the population. The c antigen was found to have the highest frequency (100%), followed by e and D antigens (95.56% and 94.44%, respectively). The E and C antigens were found to have the lowest frequency in the population (18.89% and 2.78%, respectively). The Rh phenotype frequencies in the population are as shown in Table 2. Seven phenotypes were found to occur in the population, the most common being Dccee (73.61%). Ccee was the most

**Table 1.** Rh antigen frequency of the 720 Calabar participants

Rh antigen	Number positive (%)	Number negative (%)
D	680 (94.44)	40 (5.56)
C	20 (2.78)	700 (97.22)
E	136 (18.89)	584 (81.11)
c	720 (100)	0
e	688 (95.56)	32 (4.44)

**Table 2.** Probable Rh phenotypes and most probable genotypes of the 720 Calabar participants

Probable Rh phenotype	Number positive (%)	Most probable genotype*
Dccee	530 (73.61)	(R <sup>o</sup> R <sup>o</sup> )
DccEe	100 (13.89)	(R <sup>o</sup> R <sup>2</sup> )
ccee	38 (5.27)	(rr)
DccEE	32 (4.44)	(R <sup>2</sup> R <sup>2</sup> )
DCcee	41 (1.94)	(R <sup>o</sup> R <sup>1</sup> )
DCcEe	4 (0.56)	(R <sup>o</sup> R <sup>2</sup> )
Ccee	2 (0.28)	(rr <sup>1</sup> )

\*Obtained from Calculator for Rh genotype determination, Ortho-Clinical diagnostics, Raritan, New Jersey

rare phenotype in the population (0.28%). Of the 40 subjects that were D-, 38 were ccee, while only two were Ccee.

Table 3 shows the occurrence of the Rh antigens and the probable genotypes in the populations studied. It is noteworthy that apparent homozygosity for *cc*, *ee*, and *EE* genes is found in 97.22 percent, 81.12 percent, and 4.44 percent of the population, respectively. Apparent homozygosity of *C* was not found among the samples tested. Weak D antigen was not encountered in this study. Variant e+ phenotypes such as hr<sup>s</sup>- and hr<sup>B</sup>- phenotypes and the r<sup>s</sup> phenotype with altered C expression could not be tested for.

Table 4 shows the distribution of the Rh antigens in the three main groups in Calabar. The c antigen was present in every individual irrespective of his or her ethnic nationality. The e antigen has a very high

**Table 3.** RH genes and their occurrence in the 720 Calabar participants

Probable gene combination	Number (%)
<i>cc</i>	700 (97.22)
<i>Cc</i>	20 (2.78)
<i>CC</i>	0 (0)
<i>ee</i>	584 (81.12)
<i>Ee</i>	104 (14.44)
<i>EE</i>	32 (4.44)
<i>DD</i> } <i>Dd</i> * }	680 (94.44)
<i>dd</i> *	40 (5.56)

\*d denotes absence of functional RHD gene.

**Table 4.** Distribution of the Rh antigens within the three main ethnic groups tested in Calabar

Rh antigen	Ethnic nationalities*		
	Ibibio	Efik	Ibo
c	276 (100)	182 (100)	70 (100)
e	266 (96.38)	174 (95.60)	66 (94.29)
D	266 (96.38)	176 (96.70)	64 (91.43)
E	42 (15.22)	40 (21.98)	20 (28.57)
C	10 (3.62)	0 (0)	2 (2.86)

\*Only the frequencies within the three main ethnic nationalities were compared. Minor groups were not included.

frequency within each of the three groups. The highest frequency (96.38%) was recorded for the Ibibios, while the lowest (94.29%) was recorded for the Ibos. None of the Efik subjects had the C gene and very low frequencies of 2.86% and 3.62% were recorded for the Ibo and Ibibio subjects, respectively.

## Discussion

This report forms part of the study on the Rh phenotypes within the various ethnic nationalities in Nigeria. The aim of the study was to demonstrate the ethnic variations of the Rh antigens and how polymorphic the Rh blood group can be in Nigeria. The results obtained in this study were dependent on the genetic constitution of the individuals tested and not on sex, age, or disease conditions. All the subjects resided in Calabar, which is the administrative head of the then South-East Nigeria (now split into Cross Rivers State and Akwa Ibom States) with Ibibios, Efiks, and Ibos as the main ethnic groups.

In this study, the c antigen was detected in all subjects (100%) and the results show that 97.22 percent of the 720 persons tested were homozygous for c. There were no c- individuals recorded, so the possibility of HDN due to anti-c in this population is minimal when viewed against the report of Frazer and Tovey,<sup>15</sup> where 12 percent of the cases of HDN in Southwest England were due to anti-c. We had expected anti-C to occur more frequently in this population: only 2.78 percent were found to possess the C antigen in single dose. It is interesting to observe in this study that none of the 182 Efiks enlisted in this study possessed the C antigen. This may be of great importance in anthropological studies of the Efik ethnic nationality. The frequency of the c antigen in this study is quite similar to the 99.8 percent frequency obtained recently in Port Harcourt.<sup>12</sup> There is, however, a marked difference in the occurrence of the

C antigen, i.e., 17.7 percent was recorded in the Port Harcourt population.<sup>12</sup> Previous studies in Nigeria showed a frequency of 26.9 percent of the C antigen among the Yorubas<sup>10</sup> and 43.7 percent among the Ibos.<sup>11</sup> These values, when compared with the 2.78 percent frequency of C antigen in the Calabar population, demonstrate a wide variation in the frequency distribution of Rh antigens within various ethnic groups in Nigeria. In addition, Worlledge et al.<sup>10</sup> reported that 40 (25%) Rh D- Ibo donors at UCH Ibadan possessed the C antigen, whereas in Calabar only 2.86 percent of 70 random Ibo subjects possessed the C antigen. Various reports seem to support that c is a high-frequency antigen in Nigeria (96.4% in Yoruba, 99.8% in Port Harcourt, 92.7% in Ibo, and 100% in the Calabar population).<sup>10-12</sup>

There was a marked difference in the frequency of the E antigen among the three groups. The highest frequency (28.57%) was among the Ibos, with 21.98 percent and 15.22 percent among Efiks and Ibibios, respectively, while 19.8 percent and 20.5 percent were previously documented for the Yoruba and Port Harcourt populations, respectively.<sup>10-12</sup> With the low frequency of the E antigen in Calabar, the possibility of having individuals with naturally occurring anti-E should be considered, because it could affect a major crossmatch if the donor RBCs encountered are positive for the E antigen.

Of the 40 (5.56%) D- individuals found in this study, 38 were of the phenotype ccee, while only two were Ccee. None of the D- individuals were E+, suggesting that E is usually present on the RBCs of D+ individuals. The frequency of the D antigen in this study was 94.44 percent, very close to the Port Harcourt value of 95 percent. From calculations using the Hardy-Weinberg formula, it was found that 61.8 percent of the D+ individuals were probably homozygous for the D gene and will therefore pass on a D gene to each of their offspring. Rh D- wives of such men have a greater chance of having children with HDN due to anti-D. This clearly contradicts the statement of Dacie and Lewis<sup>16</sup> that the chances of being homozygous for D in Nigeria are so high that every Rh-positive person must be assumed to be homozygous for the D gene, because only 61.8 percent of all D+ individuals in Calabar were calculated to be homozygous for the D gene.

The Dccee phenotype was found to be the most common in Calabar, with a frequency of 73.61 percent.

Results obtained from this study further demonstrate the great variability of Rh antigens within

the various ethnic groups in Nigeria. Differences in Ibibio, Efik, and Ibo Rh phenotype frequencies are further proof of Rh polymorphism in blacks. These results are of both clinical and anthropological interest.

## References

1. Daniels GL, Cartron JP, Fletcher A., et al. International Society of Blood Transfusion Working Party on Terminology for red cell surface antigens. *Vox Sang* 2003;84:244-7.
2. Race RR, Sanger R. Blood groups in man. 6th ed. Oxford: Blackwell Scientific Publications, 1975:179-260.
3. Rupee C, Myers J, Gindy L. Blood groups, In: Petz LD, Swisher SN, Kleinan S, Spencer RK, Strauss RG, eds. Clinical practice of transfusion medicine. 3rd ed. New York: Churchill Livingstone, 1996:71-151.
4. Knowles S, Poole G. Human blood group systems. In: Murphy MF, Pamphilon DH, eds. Practical transfusion medicine. 1st ed. London: Blackwell Scientific Publications, 2002;24-31.
5. Bowman JM. Maternal alloimmunization and fetal hemolytic disease. In: Reace EA, Hobbins JC, Mahoney MJ, Petrie RH, eds. Medicine of the fetus and mother. Philadelphia: JB Lippincott, 1992:1152-4.
6. Jovanovic-Srzentic S, Djokic M, Tijanic N, et al. Antibodies detected in samples from 21,730 pregnant women. *Immunohematology* 2003;19: 89-91.
7. Issitt PD. Applied blood group serology. 3rd ed. Miami: Montgomery Scientific Publications, 1985:105-10.
8. Niger Delta Ethnic Nationalities. Conference on sustainable development and conflict resolution in the Niger Delta. Feb. 4-6, 1999.
9. Onwukeme KE. Blood group distribution in blood donors in a Nigerian population. *Niger J Physiol Sci* 1990;6:67-70.
10. Worlledge S, Ogiemudia SE, Thomas CO, Ikoku BN Luzzatto. Blood group antigens and antibodies in Nigeria. *Ann Trop Med Parasitol* 1974;68:249-64.
11. Ukaejiofor EO, Okonkwo WE, Tagbo EN, Emeribe AO. ABO and Rhesus in a Nigerian population In: Blood transfusion in the tropics. 1st ed. Nigeria: Salem Media, 1996:38-9.
12. Jeremiah ZA, Buseri FI. Rh antigen and phenotype frequencies and probable genotypes for the four main ethnic groups in Port Harcourt, Nigeria. *Immunohematology* 2003;19:86-88.
13. Judd JW. Methods in immunohematology. 2nd ed. Durham: Montgomery Scientific Publication, 1998:2-3.
14. Cepellini R, Siniscalco M, Smith CAB. The estimation of gene frequencies in a random mating population. *Am Hum Genet* 1955;22: 97-114.
15. Frazer ID, Tovey GH. Haemolytic disease of the newborn due to antibodies other than D. *Br Med J* 1981;283:514-5.
16. Dacie JV, Lewis SM. Practical Haematology. 5th ed. London: Churchill Livingstone, 1982:515-36.

---

*Z. Awortu Jeremiah, MSc, AMLSCN Lecturer/Career Medical Scientist (Haematology/Immunohaematology), College of Health Science and Technology, Kilometer 6 (Mile 4), Rumueme, Port Harcourt, Nigeria, and Ministry of Health, Rivers State; and Chris Odumody, AMLSCN, Medical Laboratory Scientist, Rehoboth Specialist Hospital, Port Harcourt, Nigeria.*

**REMEMBER: THE PASSWORD IS "2000" For [www.redcross.org/pubs/immuno](http://www.redcross.org/pubs/immuno)**

**Now, as a subscriber, you can enter the password, 2000, and access the back issues.** That means cover to cover! You will receive every article in total, every letter to the editor, every review, every ad, every notice, and every literature review! All of the other services will continue to be available on the Web site, including sending a letter to the editor, subscribing with a credit card on the secure order site, performing a literature search, reviewing instructions for authors, and linking to other important sites. Log on now to see this great service!

# *FCGR3B* polymorphism in three ethnic Chinese populations

L. YAN, F. ZHU, L. JIN, Q. LV, AND Q. FU

Fc $\gamma$ RIIIb receptor is expressed primarily on neutrophils as three polymorphic antigens (HNA-1a, HNA-1b, and HNA-1c) that are encoded by alleles *FCGR3B\*1*, *FCGR3B\*2*, and *FCGR3B\*3*, respectively. These antigens play an important role in immune neutropenia; their absence predisposes individuals who lack them to life-threatening infections. This study investigated the *FCGR3B* gene frequencies in three ethnic Chinese populations: Han, She, and Tajik. *FCGR3B\*1*, *FCGR3B\*2*, and *FCGR3B\*3* were genotyped by PCR using sequence specific primers (PCR-SSP). The results showed the gene frequencies were 0.55 for *FCGR3B\*1* and 0.45 for *FCGR3B\*2* in 177 Han individuals, 0.69 for *FCGR3B\*1* and 0.31 for *FCGR3B\*2* in 87 She individuals, and 0.35 for *FCGR3B\*1* and 0.65 for *FCGR3B\*2* in 99 Tajik individuals, respectively. The *FCGR3B<sub>null</sub>* genotype was not found, but the *FCGR3B\*3* allele was identified in only three individuals in the Tajik population. DNA clone and sequencing confirmed that these individuals had the C $\Rightarrow$ A mutation at position 266 on exon 3. This study found that the gene frequencies in Han and She ethnic groups were similar to those previously reported in the Asian population, but the *FCGR3B* allele frequencies in the Tajik population were more similar to that of Caucasians. *Immunohematology* 2005;21:25–28.

**Key Words:** *FCGR3B*, gene frequency, Chinese population, PCR-SSP, Fc receptor

Fc $\gamma$ RIII is a low-affinity Fc gamma receptor on leukocytes, existing as two isoforms, Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIb.<sup>1</sup> Fc $\gamma$ RIIIa is expressed on natural killer cells, monocytes, and macrophages. Fc $\gamma$ RIIIb is expressed primarily on neutrophils and as three polymorphic antigens (human neutrophil antigen, HNA-1a [formerly NA1]), HNA-1b (formerly NA2), and HNA-1c (formerly SH, NA3), which are encoded by alleles *FCGR3B\*1*, *FCGR3B\*2*, and *FCGR3B\*3*, respectively.<sup>2</sup> *FCGR3B\*1* and *FCGR3B\*2* differ at five nucleotide (nt) positions (141G $\Rightarrow$ C, 147C $\Rightarrow$ T, 227A $\Rightarrow$ G, 277G $\Rightarrow$ A, and 349G $\Rightarrow$ A) that encode four amino acid changes.<sup>1,2</sup> *FCGR3B\*3* is identical to *FCGR3B\*2* except for a single base change (266C $\Rightarrow$ A) that encodes amino acid Ala78Asp.<sup>3</sup>

Polymorphisms of neutrophil antigens are important clinically because of their role in antibody-mediated immune neutropenia. In addition, alleles of *FCGR3B* distinguish certain racial and ethnic populations. In French, German, American Caucasian, and African Black populations, *FCGR3B\*2* is the most

prevalent *FCGR3B* allele,<sup>4,5,6</sup> whereas in certain Chinese and Japanese populations, *FCGR3B\*1* is more prevalent.<sup>7,8</sup> *FCGR3B\*3* occurs in 0.05 percent of Caucasians,<sup>3</sup> but has not been reported in Asians.<sup>7,8</sup> Rarely, individuals lack Fc $\gamma$ RIIIb neutrophil antigens (NA<sub>null</sub>), predisposing them to life-threatening infections. Also, several low-prevalence *FCGR3B* variant alleles that carry a single-base substitution of one of the five polymorphic sites on *FCGR3B* have been identified in certain Caucasian and Black populations.<sup>5,9</sup>

Frequencies of *FCGR3B* genes and of seven *FCGR3* variants have been described in Chinese individuals from Zhejiang Province.<sup>8</sup> China's population is large and genetically heterogeneous. Most indigenous Chinese are ethnically Han (92%). The remaining 8 percent of indigenous Chinese represent 55 ethnic minorities. Ethnic She (630,000 individuals) reside in Fujian, Zhejiang, and Jiangxi Provinces. Ethnic Tajik (33,000 individuals) reside in Xinjiang Province. We describe the frequencies of *FCGR3B* alleles and the results of screening for Fc $\gamma$ RIII variants among Han, She, and Tajik populations.

## Materials and Methods

### Subjects

Peripheral blood samples were collected from volunteer blood donors after obtaining informed consent. We interviewed subjects to categorize them as Han (Zhejiang Province), She (Zhejiang Province), or Tajik (Xinjiang Province).

### DNA extraction and genotyping *FCGR3B*

We extracted genomic DNA from whole blood, using a QIAamp Blood Kit (Qiagen GmbH, Hilden, Germany). Allele-specific DNA amplification was performed according to the protocol of Bux et al.<sup>3,10</sup> Primer sequences are listed in Table 1.<sup>3</sup> Genotypes were assigned by the presence or absence

**Table 1.** Primers for PCR-SSP and DNA sequencing

Name	Primer sequence
FCGR3B*1 sense	5'cag tgg ttt cac aat gtg aa3'
FCGR3B*1 antisense	5'atg gac ttc tag ctg cac 3'
FCGR3B*2 sense	5'caa tgg tac agc gtg ctt 3'
FCGR3B*2 antisense	5'atg gac ttc tag ctg cac 3'
FCGR3B*3 sense	5'aag atc tcc caa agg ctg tg 3'
FCGR3B*3 antisense	5'tct gtc gtt gac tgt gtc at 3'
Exon 3 forward	5'tgagctcattctggcttga3'
Exon 3 reverse	5'tcaggacccttggttccac3'

of an electrophoretic band in 2% agarose gel stained with ethidium bromide and visualized by ultraviolet light (GeneGenius, Syngene, UK).

### Sequencing FCGR3A and FCGR3B

We performed DNA sequencing on samples from 12 individuals from the Tajik population with three different FCGR3B forms. DNA fragments encompassing the full exon 3 coding region were amplified using primers designed by our laboratory (Table 1), which can amplify both FCGR3A and FCGR3B genes. The PCR reaction mixture contained 50 to 100 ng genomic DNA, 2.5 µL of dNTP (200 µmol/L each), 2.5 µL 10X PCR buffer (100 mmol/L Tris-HCl, pH 8.3, and 500 mmol/L KCl), 2.0 mmol/L MgCl<sub>2</sub>, 0.5 µmol/L of forward and reverse primers, and 1.0 U Taq DNA polymerase (Roche company) in a final volume of 25 µL. PCR amplification was performed with initial denaturing at 95°C for 5 minutes followed by 30 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C, plus a final extension at 72°C for 10 minutes. DNA amplified fragments were cloned using TOPO cloning sequencing kits (Invitrogen Co., Carlsbad, CA) according to manufacturer's instructions. Positive clones according to blue and/or white selection were cultured in LB medium. Plasmid DNA was extracted by using a plasmid DNA purification kit (Shanghai Bocai, China). DNA sequences were determined by amplified primers, using cycle sequencing kits (BigDye Terminator, Applied Biosystems, Foster City, CA) and a Genetic Analyzer (ABI Prism 377, Applied Biosystems), according to the manufacturer's instructions.

### Calculation of allele frequencies and statistical analysis

Allele frequencies were calculated using the formula of Steffensen et al.<sup>6</sup> Briefly, FCGR3B\*1 and FCGR3B\*2 were treated as two existing alleles at the

same locus. Based on this assumption, the frequencies were calculated independently for FCGR3B\*3 by counting the number of each allele and calculating the percentage. FCGR3B\*3 frequency was calculated as one-half of the percentage of allele-positive individuals. We used chi-squared analyses to test for Hardy-Weinberg equilibrium for FCGR3B genes. A  $p < 0.05$  was considered to be statistically significant.

## Results

### Allele-specific DNA amplification

Among the 172 Hans, 87 (50.6%) genotyped as FCGR3B\*1+,\*2+,\*3-; 34 (19.8%) genotyped as FCGR3B\*1-,\*2+,\*3-; and 51 (29.6%) genotyped as FCGR3B\*1+,\*2-,\*3- (Table 2). Allele frequencies were 0.55 for FCGR3B\*1, 0.45 for FCGR3B\*2, and 0.00 for FCGR3B\*3 (Table 3). Among the 87 Shes, 36 (41.4%) individuals genotyped as FCGR3B\*1+,\*2+,\*3-; 9 (10.3%) genotyped as FCGR3B\*1-,\*2+,\*3-; and 42 (48.3%) genotyped as FCGR3B\*1+,\*2-,\*3-. Allele frequencies were 0.69 for FCGR3B\*1, 0.31 for FCGR3B\*2, and 0.00 for FCGR3B\*3. Among the 99 Tajiks, 48 (48.5%) genotyped as FCGR3B\*1+,\*2+,\*3-; 38 (38.4%) genotyped as FCGR3B\*1-,\*2+,\*3-; 10 (10.1%) genotyped as FCGR3B\*1+,\*2-,\*3-; 1 (1%) individual genotyped as FCGR3B\*1+,\*2+,\*3+; and 2 (2%) individuals genotyped as FCGR3B\*1-,\*2+,\*3+.

**Table 2.** Genotype frequencies observed in the Han, She, and Tajik populations

Genotype	Han		She		Tajik	
	n	%	n	%	n	%
FCGR3B*1+,*2+,*3-	87	50.6	36	41.4	48	48.5
FCGR3B*1-,*2+,*3-	34	19.8	9	10.3	38	38.4
FCGR3B*1+,*2-,*3-	51	29.6	42	48.3	10	10.1
FCGR3B*1-,*2-,*3-	0	0.0	0	0.0	0	0.0
FCGR3B*1+,*2+,*3+	0	0.0	0	0.0	1	1.0
FCGR3B*1-,*2+,*3+	0	0.0	0	0.0	2	2.0
FCGR3B*1+,*2-,*3+	0	0.0	0	0.0	0	0.0
FCGR3B*1-,*2-,*3+	0	0.0	0	0.0	0	0.0

**Table 3.** FCGR3B allele frequencies in different populations

Population	n	FCGR3B*1	FCGR3B*2	FCGR3B*3
Han(Zhejiang)	172	0.55	0.45	0.00
She minority	87	0.69	0.31	0.00
Tajik minority	99	0.348	0.652	0.015
Japanese <sup>7</sup>	400	0.622	0.378	0.00
Danish <sup>6</sup>	200	0.365	0.635	0.030
Ugandan <sup>5</sup>	43	0.395	0.558	0.174
Northern German <sup>5</sup>	260	0.373	0.627	0.025

Allele frequencies were 0.348 for *FCGR3B\*1*, 0.652 for *FCGR3B\*2*, and 0.015 for *FCGR3B\*3*. Differences between *FCGR3B\*1*, *FCGR3B\*2*, and *FCGR3B\*3* frequencies among Hans, Shes, and Tajiks were significant ( $p < 0.001$ ). We did not find the *FCGR3B\*1-,\*2-,\*3-* (*FCGR3B<sub>null</sub>*) genotype among the 358 samples tested. Three Tajik individuals expressed the *FCGR3B\*3* allele. Table 3 also lists previously published allele frequencies in certain other populations.

*FCGR3* sequencing

DNA cloning and sequencing confirmed the results of PCR-SSP analysis by showing that three *FCGR3B\*3* positive samples had a C⇒A mutation at position 266 on exon 3. We sequenced 76 clones of exon 3 of *FCGR3* from 12 Tajik subjects (Table 4). We identified three individuals (7 clones) as having *FCGR3B\*2* variants (141C, 147T, 227G, 266C, 277G, 349A), two individuals (2 clones) as having *FCGR3A* variants (141G, 147C, 227G, 266C, 277A, 349A), and one individual (1 clone) as having *FCGR3B\*2* variant (141G, 147T, 227G, 266C, 277A, 349A). Each variant demonstrated either a single base substitution of one of the six polymorphic sites of the *FCGR3* gene or a new combination of nucleotides at the corresponding position.

**Discussion**

Our results confirm that *FCGR3B\*1* is more prevalent than *FCGR3B\*2* among Chinese Hans and Shes, as reported previously.<sup>7,8</sup> We also found that *FCGR3B* allele frequencies in the Tajik population were very similar to those of Caucasians.<sup>5,6</sup> We identified three Tajik individuals with the *FCGR3B\*3* allele, which is the first time this allele has been identified in indigenous Asians. We did not find *FCGR3B\*3* in our Han or She populations, which was consistent with previous observations in other Asian populations. We also did not find the *FCGR3B<sub>null</sub>* genotype in any of the three Chinese populations tested, but larger scale testing is required to ascertain that it is totally absent in the Chinese population.<sup>8</sup>

Several *FCGR3B* variants have been described in African Blacks and American Caucasians.<sup>9</sup> Our finding of three new *FCGR3B* variants demonstrates that such variant genes also exist in Chinese populations. It is not known whether these variants reflect somatic

**Table 4.** *FCGR3* variants at nucleotide positions within exon 3 from 12 Tajik subjects

<i>FCGR3</i> form	Number of individuals	Number of clones	Nucleotide positions					
			141	147	227	266	277	349
<i>FCGR3A</i>	12	35	G	C	G	C	G	A
Variant 2	2	2	G	C	G	C	A	A
<i>FCGR3B*1</i>	7	10	G	C	A	C	G	G
<i>FCGR3B*2</i>	9	15	C	T	G	C	A	A
Variant 1	3	7	C	T	G	C	G	A
Variant 3	1	1	G	T	G	C	A	A
<i>FCGR3B*3</i>	3	6	C	T	G	A	A	A

mutations or multiple gene loci. Also unknown is what impact mutations resulting in variant genes have on the function of *FCGR3B* gene-encoded neutrophil antigens or their corresponding Fc gamma receptors.

**References**

1. Qiu WQ, de Bruin D, Brownstein BH, et al. Organization of the human and mouse low-affinity Fc gamma R genes: duplication and recombination. *Science* 1990;248:732-5.
2. Bux J. Nomenclature of granulocyte alloantigens. *Transfusion* 1999;39:662-3.
3. Bux J, Stein EL, Bierling P, et al. Characterization of a new alloantigen (SH) on the human neutrophil Fc gamma receptor IIIb. *Blood* 1997;89(3): 1027-34.
4. Kissel K, Hofmann C, Gittinger FS, et al. HNA-1a, HNA-1b, HNA-1c (NA1, NA2, SH) frequencies in African and American Blacks and in Chinese. *Tissue Antigens* 2000;56:143-8.
5. Flesh BK, Doose S, Siebert R, et al. FCGR3 variants and expression of human neutrophil antigen-1a, -1b, -1c in the population of northern Germany and Uganda. *Transfusion* 2002;42:469-75.
6. Steffensen R, Gulen T, Varming K, et al. FcγRIIIB polymorphism: evidence that NA1/NA2 and SH are located in two closely linked loci and that the SH allele is linked to the NA1 allele in the Danish population. *Transfusion* 1999;39:593-8.
7. Fujiwara K, Watanabe Y, Mitsunaga S, et al. Determination of granulocyte specific antigens on neutrophil Fcγreceptor IIIb by PCR preferential homoduplex formation assay, and gene frequencies in Japanese population. *Vox Sang* 1999;77:218.
8. Yin T, Jie J, Lixing Y, et al. *FCGR3B* gene frequencies and *FCGR3* variants in a Chinese population from Zhejiang Province. *Ann Hematol* 2003;82:574-8.

9. Matsuo K, Procter J, Stroncek D. Variants in genes encoding neutrophil antigens NA1 and NA2. *Transfusion* 2000;40:645-53.
10. Bux J, Stein EL, Santoso S, et al. NA gene frequencies in the German population, determined by polymerase chain reaction with sequence-specific primers. *Transfusion* 1995;35: 54-7.

---

*Lixing Yan, MD; Faming Zhu, MD; Lei Jin, BA; Qinfeng Lv, BA; and Qibua Fu, PhD, Institute of Blood Transfusion Medicine, Blood Center of Zhejiang Province, Hangzhou, Zhejiang, 310006 P.R. China.*

**IMPORTANT NOTICE ABOUT MANUSCRIPTS  
FOR  
IMMUNOHEMATOLOGY**

Please **e-mail** all manuscripts for consideration to Marge Manigly at [mmanigly@usa.redcross.org](mailto:mmanigly@usa.redcross.org)

**Free Classified Ads and Announcements**

*Immunohematology* will publish classified ads and announcements (SBB schools, meetings, symposia, etc.) **without charge**. Deadlines for receipt of these items are as follows:

**Deadlines**

- 1st week in January for the March issue
- 1st week in April for the June issue
- 1st week in July for the September issue
- 1st week in October for the December issue

E-mail or fax these items to Cindy Flickinger, Managing Editor, at (215) 451-2538 or [flickingerc@usa.redcross.org](mailto:flickingerc@usa.redcross.org).

## COMMUNICATIONS

### Letters From the Editors

#### **Moving into 2005**

The first issue of 2005 has an international aspect, with articles discussing blood groups and leukocyte polymorphism in China, Rh antigens in Nigeria, platelet antigens in Thailand, a lectin from a plant native to India, and a collaborative effort by researchers from Australia and the United States to determine Duffy antigen expression on reticulocytes. We know you will read these articles with interest.

The next issue will feature two review articles. Dr. Douglas Lublin will discuss the clinical aspects of Cromer/DAF and Dr. Anne Eder will provide an update on paroxysmal cold hemoglobinuria (PCH). To complement the review article by Dr. Lublin, the issue will include two recent molecular studies of unusual phenotypes within the Cromer blood group system.

We appreciate your support of the journal and welcome your comments. We look forward to continuing to provide you with the informative serologic and educational articles that have been the hallmark of *Immunohematology* for the past 20 years.

#### **Many thanks!**

Dr. S. Gerald Sandler will be making the transition from his position as medical editor of *Immunohematology* to a role on the Editorial Board. He has served as medical editor, first unofficially and later officially, since the founding of the journal. His contributions to the journal have been immense, as both editor and contributor. Dr. Sandler was presented with a plaque at the Editors' Breakfast at the AABB conference in Baltimore last October, to honor his many years of support for *Immunohematology* and his work to make it possible for the journal to be cited in *Index Medicus*.

The editors and staff of *Immunohematology* must say farewell to Ms. Linda Berenato, who has served admirably as the editorial assistant of the journal for the past 7 years. We all think we are the backbone of the journal, but Linda is the one who made sure that the right words were always capitalized, italicized, bolded, and indented. In short, she knew the style of the journal as well as anyone. This took a very large load off our shoulders when we edited the manuscripts. She is moving on to an ink-free and *Immunohematology*-free position. We wish her a world of happiness in her new endeavor. We will miss her more than we can say.

We extend a heartfelt welcome to Ms. Judy Abrams as she assumes the role of editorial assistant.

#### ***Immunohematology* cited in *Index Medicus***

We know that both the authors and the readers of *Immunohematology* are as excited as we are that the journal has been accepted for citation in *Index Medicus*. In addition, the past 8 years of articles have been placed into *Index Medicus* and MEDLINE. Articles from these issues can be retrieved by searching PubMed®. Citations from the indexed articles, the indexing terms, and the abstracts will now be available to authors and readers worldwide. This is a great honor for our journal, and we once again thank Dr. S. Gerald Sandler for his valiant—and, ultimately, successful—efforts to reach this goal.

Delores Mallory  
Editor-in-Chief

Cindy Flickinger  
Managing Editor

## ANNOUNCEMENTS

**Monoclonal antibodies available at no cost.** The Laboratory of Immunochemistry at the New York Blood Center has developed a wide range of monoclonal antibodies (both murine and humanized) that are useful for screening for antigen-negative donors and for typing patients' RBCs with a positive DAT. Monoclonal antibodies available include anti-M, -Fy<sup>a</sup>, -Fy<sup>b</sup>, -K, -k, -Kp<sup>a</sup>, -Js<sup>b</sup>, -Do<sup>b</sup>, -Wr<sup>b</sup>, and -Rh17. For a complete list of available monoclonal antibodies, please see our Web site at <http://www.nybloodcenter.org/framesets/FS-4C7.htm>. Most of those antibodies are murine IgG and, thus, require the use of anti-mouse IgG for detection, i.e, anti-K, -k, and -Kp<sup>a</sup>. Some are directly agglutinating (anti-M, -Wr<sup>b</sup>, and -Rh17), and a few have been humanized into the IgM isoform and are directly agglutinating (anti-Js<sup>b</sup> and -Fy<sup>a</sup>). The monoclonal antibodies are available at no charge to anyone who requests them. **Contact:** Marion Reid (mreid@nybloodcenter.org) or Gregory Halverson (ghalverson@nybloodcenter.org), New York Blood Center, 310 East 67th Street, New York, NY 10021.

**Notice to Readers:** *Immunohematology, Journal of Blood Group Serology and Education*, is printed on acid-free paper.

**IMMUNOHEMATOLOGY IS ON THE WEB!**

**[www.redcross.org/pubs/immuno](http://www.redcross.org/pubs/immuno)**

**Password "2000"**

For more information or to send an e-mail message "To the editor"

**[immuno@usa.redcross.org](mailto:immuno@usa.redcross.org)**

### **Attention: State Blood Bank Meeting Organizers**

If you are planning a state meeting and would like copies of *Immunohematology* for distribution, please **contact** Cindy Flickinger, Managing Editor, 4 months in advance, by fax or e-mail at (215) 451-2538 or [flickingerc@usa.redcross.org](mailto:flickingerc@usa.redcross.org).

## ANNOUNCEMENTS CONT'D

### **Masters (MSc) in Transfusion and Transplantation Sciences**

**At**

### **The University of Bristol, England**

Applications are invited from medical or science graduates for the Master of Science (MSc) degree in Transfusion and Transplantation Sciences at the University of Bristol. The course starts in October 2005 and will last for 1 year. A part-time option lasting 2 or 3 years is also available. There may also be opportunities to continue studies for PhD or MD following MSc. The syllabus is organized jointly by The Bristol Institute for Transfusion Sciences and the University of Bristol, Department of Pathology and Microbiology. It includes:

- Scientific principles of transfusion and transplantation
- Clinical applications of these principles
- Practical techniques in transfusion and transplantation
- Principles of study design and biostatistics
- An original research project

Applications can also be made for Diploma in Transfusion and Transplantation Science or a Certificate in Transfusion and Transplantation Science.

The course is accredited by the Institute of Biomedical Sciences.

Further information can be obtained from the Web site:

<http://www.blood.co.uk/ibgrl/MSc/MScHome.htm>

For further details and application forms please contact:

**Dr. Patricia Denning-Kendall**

**University of Bristol**

**Paul O'Gorman Lifeline Centre, Department of Pathology and Microbiology, Southmead Hospital**

**Westbury-on-Trym, Bristol**

**BS10 5NB, England**

**Fax +44 1179 595 342, Telephone +44 1779 595 455, e-mail: [p.a.denning-kendall@bristol.ac.uk](mailto:p.a.denning-kendall@bristol.ac.uk).**

## SPECIAL ANNOUNCEMENTS

### Spring Meetings!

#### **March 22–23      Kentucky Association of Blood Banks (KABB)**

2005 Annual Kentucky Association of Blood Banks (KABB) Meeting will be held March 22 and 23, 2005, in Louisville, Kentucky. The meeting is held concurrently with the Kentucky Society for Clinical Laboratory Sciences. For more information or registration information, **contact:** Karla Smith (859) 576-8424, email: info@kabb.org or <http://www.kabb.org>.

#### **April 7–8            MidAtlantic Association of Blood Banks (MAABB)**

MAABB invites you to join us in Colonial Williamsburg on April 7 and 8, 2005, for our Annual Meeting. Visit our Web site at <http://www.maabb.org> to view the program and download a registration form. AABB new assessor training is scheduled for Saturday, April 9. For more information, **contact:** Judy Sullivan at <http://www.jsullivan@aabb.org>.

#### **April 22–24        AABB Immunohematology Reference Laboratory (IRL) Conference 2005**

The AABB Immunohematology Reference Laboratory Conference 2005 will be held April 22 through 24, 2005, at the Hilton New Orleans Riverside Hotel in New Orleans, Louisiana. Through lectures, case studies, and audience participation, the conference will address both technical and administrative issues vital to the operation of a state-of-the-art IRL. For more information or to register, visit <http://www.aabb.org> AABB Spring Conference 2005 or **contact:** the AABB Meetings and Programs Department at (301) 215-6480 or [meeting@aabb.org](mailto:meeting@aabb.org).

#### **May 16–20         HEMATOLOGÍA HABANA' 2005**

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, immunotherapy, histocompatibility, immunohematology, hemolytic disease of the newborn, regenerative medicine, 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, e-mail: [ihidir@hemato.sld.cu](mailto:ihidir@hemato.sld.cu); Web site: <http://www.loseventos.cu/hematologia2005>.

#### **June 9–10           Heart of America Association of Blood Banks (HAABB)**

The Heart of America Association of Blood Banks (HAABB) 2005 Spring Meeting will be held June 9 and 10, 2005 at the Embassy Suites in Kansas City, Missouri. For more information, visit the Web site at <http://www.HAABB.org> or **contact:** Jennifer Jung at (314) 494-0937 or [jungje@usa.redcross.org](mailto:jungje@usa.redcross.org).

#### **June 21–22         Florida Association of Blood Banks (FABB)**

The 59th annual Florida Association of Blood Banks (FABB) conference will be held on June 21 and 22, 2005, at the Renaissance Vinoy Resort and Golf Club in St. Petersburg, Florida. The meeting's theme is *Look Ahead Through Education*. There will be two days of expert speakers, addressing topics such as medical and technical issues, donor recruitment, marketing and networking. We are hoping to have a Fun Run along the bay for attendees. There will be many exhibitors with the latest in equipment, automation, and other blood bank necessities. For more information, visit the FABB Web site at <http://www.floridaabb.com>.

## CLASSIFIED AD

**Wake Forest University Baptist Medical Center** in Winston-Salem, North Carolina, is accepting applications for an Assistant Manager position. Supervisor experience required. SBB preferred. Experience with FDA and AABB regulations desired. To apply, send a resume **directly to:** rjoseph@wfubmc.edu.

**Phone, Fax, and Internet Information:** If you have any questions concerning *Immunohematology*, *Journal of Blood Group Serology and Education*, or the *Immunohematology Methods and Procedures* manual, **contact** us by e-mail at immuno@usa.redcross.org. For information concerning the National Reference Laboratory for Blood Group Serology, including the American Rare Donor Program, please contact Sandra Nance, by phone at (215) 451-4362, by fax at (215) 451-2538, or by e-mail at snance@usa.redcross.org

**Attention SBB and BB Students:** You are eligible for a **free** 1-year subscription to *Immunohematology*. Ask your education supervisor to submit the name and complete address for each student and the inclusive dates of the training period to *Immunohematology*, P.O. Box 40325, Philadelphia, PA 19106.

**Manuscripts:** The editorial staff of *Immunohematology* welcomes manuscripts pertaining to blood group serology and education for consideration for publication. We are especially interested in case reports, papers on platelet and white cell serology, scientific articles covering original investigations, and papers on new methods for use in the blood bank. **Deadlines** for receipt of manuscripts for consideration for the March, June, September, and December issues are the first weeks in November, February, May, and August, respectively. For instructions for scientific articles, case reports, and review articles, see "Instructions for Authors" in every issue of *Immunohematology* or on the Web. **Include fax and phone numbers and e-mail address with your manuscript.**

## ADVERTISEMENT S

### **NATIONAL REFERENCE LABORATORY FOR BLOOD GROUP SEROLOGY**

#### **Immunohematology Reference Laboratory**

AABB, ARC, New York State, and CLIA licensed

(215) 451-4901—24-hr. phone number

(215) 451-2538—Fax

#### **American Rare Donor Program**

(215) 451-4900—24-hr. phone number

(215) 451-2538—Fax

ardp@usa.redcross.org

#### ***Immunohematology***

(215) 451-4902—Phone, business hours

(215) 451-2538—Fax

immuno@usa.redcross.org

#### **Quality Control of Cryoprecipitated-AHF**

(215) 451-4903—Phone, business hours

(215) 451-2538—Fax

#### **Granulocyte Antibody Detection and Typing**

- Specializing in granulocyte antibody detection and granulocyte antigen typing
- Patients with granulocytopenia can be classified through the following tests for proper therapy and monitoring:

—Granulocyte agglutination (GA)

—Granulocyte immunofluorescence (GIF)

—Monoclonal Antibody Immobilization of Granulocyte Antigens (MAIGA)

For information regarding services, call Gail Eiber at: (651) 291-6797, e-mail: eiber@usa.redcross.org,

or write to:

#### **Neutrophil Serology Reference Laboratory**

**American Red Cross**

**St. Paul Regional Blood Services**

**100 South Robert Street**

**St. Paul, MN 55107**

CLIA LICENSED

### **National Platelet Serology Reference Laboratory**

Diagnostic testing for:

- Neonatal alloimmune thrombocytopenia (NAIT)
- Posttransfusion purpura (PTP)
- Refractoriness to platelet transfusion
- Heparin-induced thrombocytopenia (HIT)
- Alloimmune idiopathic thrombocytopenia purpura (AITP)

Medical consultation available

Test methods:

- GTI systems tests
  - detection of glycoprotein-specific platelet antibodies
  - detection of heparin-induced antibodies (PF4 ELISA)
- Platelet suspension immunofluorescence test (PSIFT)
- Solid phase red cell adherence (SPRCA) assay
- Monoclonal antibody immobilization of platelet antigens (MAIPA)
- Molecular analysis for HPA-1a/1b

For information, e-mail: immuno@usa.redcross.org

or call:

Maryann Keashen-Schnell

(215) 451-4041 office

(215) 451-4205 laboratory

Sandra Nance

(215) 451-4362

Scott Murphy, MD

(215) 451-4877

#### **American Red Cross Blood Services**

**Musser Blood Center**

**700 Spring Garden Street**

**Philadelphia, PA 19123-3594**

CLIA LICENSED

## ADVERTISEMENTS CONT'D

### **IgA/Anti-IgA Testing**

IgA and anti-IgA testing is available to do the following:

- Monitor known IgA-deficient patients
- Investigate anaphylactic reactions
- Confirm IgA-deficient donors

Our ELISA assay for IgA detects antigen to 0.05 mg/dL.

For information on charges and sample requirements, call (215) 451-4909, e-mail: flickingerc@usa.redcross.org, or write to:

**American Red Cross Blood Services  
Musser Blood Center  
700 Spring Garden Street  
Philadelphia, PA 19123-3594  
ATTN: Cindy Flickinger**

CLIA LICENSED

### **Reference and Consultation Services**

Antibody identification and problem resolution

HLA-A, B, C, and DR typing

HLA-disease association typing

Paternity testing/DNA

For information regarding our services, contact Mehdizadeh Kashi at (503) 280-0210, or write to:

**Pacific Northwest Regional Blood Services  
ATTENTION: Tissue Typing Laboratory  
American Red Cross  
3131 North Vancouver  
Portland, OR 97227**

CLIA LICENSED, ASHI ACCREDITED

### **National Neutrophil Serology Reference Laboratory**

Our laboratory specializes in granulocyte antibody detection and granulocyte antigen typing.

Indications for granulocyte serology testing include:

- Alloimmune neonatal neutropenia (ANN)
- Autoimmune neutropenia (AIN)
- Transfusion related acute lung injury (TRALI)

Methodologies employed:

- Granulocyte agglutination (GA)
- Granulocyte immunofluorescence by flow cytometry (GIF)
- Monoclonal antibody immobilization of neutrophil antigens (MAINA)

TRALI investigations also include:

- HLA (PRA) Class I and Class II antibody detection

For further information contact:

Neutrophil Serology Laboratory  
(651) 291-6797

Randy Schuller  
(651) 291-6758  
schullerr@usa.redcross.org

**American Red Cross Blood Services  
Neutrophil Serology Laboratory  
100 South Robert Street  
St. Paul, MN 55107**

CLIA LICENSED

**Notice to Readers:** All articles published, including communications and book reviews, reflect the opinions of the authors and do not necessarily reflect the official policy of the American Red Cross.

# Immunohematology

JOURNAL OF BLOOD GROUP SEROLOGY AND EDUCATION

## Instructions for Authors

### SCIENTIFIC ARTICLES, REVIEWS, AND CASE REPORTS

Before submitting a manuscript, consult current issues of *Immunohematology* for style. Type the manuscript on white bond paper (8.5" × 11") and double-space throughout. Number the pages consecutively in the upper right-hand corner, beginning with the title page. Each component of the manuscript must start on a new page in the following order:

1. Title page
2. Abstract
3. Text
4. Acknowledgments
5. References
6. Author information
7. Tables—see 7 under Preparation
8. Figures—see 8 under Preparation

### Preparation of manuscripts

1. Title page
  - A. Full title of manuscript with only first letter of first word capitalized (bold title)
  - B. Initials and last name of each author (no degrees; all CAPS), e.g., M.T. JONES and J.H. BROWN
  - C. Running title of ≤ 40 characters, including spaces
  - D. 3 to 10 key words
2. Abstract
  - A. One paragraph, no longer than 300 words
  - B. Purpose, methods, findings, and conclusions of study
3. Key words—list under abstract
4. Text (serial pages)

Most manuscripts can usually, but not necessarily, be divided into sections (as described below). Results of surveys and review papers are examples that may need individualized sections.

  - A. Introduction

Purpose and rationale for study, including pertinent background references.
  - B. Case Report (if study calls for one)

Clinical and/or hematologic data and background serology.
  - C. Materials and Methods

Selection and number of subjects, samples, items, etc. studied and description of appropriate controls, procedures, methods, equipment, reagents, etc. Equipment and reagents should be identified in parentheses by model or lot and manufacturer's name, city, and state. Do not use patients' names or hospital numbers.
  - D. Results

Presentation of concise and sequential results, referring to pertinent tables and/or figures, if applicable.
  - E. Discussion

Implications and limitations of the study, links to other studies; if appropriate, link conclusions to purpose of study as stated in introduction.

### 5. Acknowledgments

Acknowledge those who have made substantial contributions to the study, including secretarial assistance; list any grants.

### 6. References

- A. In text, use superscript, arabic numbers.
- B. Number references consecutively in the order they occur in the text.
- C. Use inclusive pages of cited references, e.g., 1431-7.
- D. Refer to current issues of *Immunohematology* for style.

### 7. Tables

- A. Head each with a brief title, capitalize first letter of first word (e.g., Table 1. Results of ...), and use no punctuation at the end of the title.
- B. Use short headings for each column needed and capitalize first letter of first word. Omit vertical lines.
- C. Place explanations in footnotes (sequence: \*, †, ‡, §, ¶, \*\*, ††).

### 8. Figures

- A. Figures can be submitted either by e-mail or as photographs (5" × 7" glossy).
- B. Place caption for a figure on a separate page (e.g., Fig. 1. Results of ...), ending with a period. If figure is submitted as a glossy, place first author's name and figure number on back of each glossy submitted.
- C. When plotting points on a figure, use the following symbols if possible: ○ ● △ ▲ □ ■.

### 9. Author information

- A. List first name, middle initial, last name, highest academic degree, position held, institution and department, and **complete** address (including zip code) for **all** authors. List country when applicable.

### SCIENTIFIC ARTICLES AND CASE REPORTS SUBMITTED AS LETTERS TO THE EDITOR

#### Preparation

1. Heading—To the Editor:
2. Under heading—title with first letter capitalized.
3. Text—write in letter format (paragraphs).
4. Author(s)—type flush right; for first author: name, degree, institution, address (including city, state, ZIP code, and country); for other authors: name, degree, institution, city, and state.
5. References—limited to ten.
6. One table and/or figure allowed.

Send all manuscripts by e-mail to:  
Marge Manigly at [mmanigly@usa.redcross.org](mailto:mmanigly@usa.redcross.org)

## Becoming a Specialist in Blood Banking (SBB)

### What is a certified Specialist in Blood Banking (SBB)?

- Someone with educational and work experience qualifications who successfully passes the American Society for Clinical Pathology (ASCP) board of registry (BOR) examination for the Specialist in Blood Banking.
- This person will have advanced knowledge, skills, and abilities in the field of transfusion medicine and blood banking.

### Individuals who have an SBB certification serve in many areas of transfusion medicine:

- Serve as regulatory, technical, procedural, and research advisors
- Perform and direct administrative functions
- Develop, validate, implement, and perform laboratory procedures
- Analyze quality issues, preparing and implementing corrective actions to prevent and document issues
- Design and present educational programs
- Provide technical and scientific training in blood transfusion medicine
- Conduct research in transfusion medicine

### Who are SBBs?

Supervisors of Transfusion Services	Managers of Blood Centers	LIS Coordinators Educators
Supervisors of Reference Laboratories	Research Scientists	Consumer Safety Officers
Quality Assurance Officers	Technical Representatives	Reference Lab Specialist

### Why be an SBB?

Professional growth	Job placement	Job satisfaction	Career advancement
---------------------	---------------	------------------	--------------------

### How does one become an SBB?

- Attend a CAAHEP-accredited Specialist in Blood Bank Technology Program **OR**
- Sit for the examination based on criteria established by ASCP for education and experience

**Fact #1:** In recent years, the average SBB exam pass rate is only 38%.

**Fact #2:** In recent years, greater than 73% of people who graduate from CAAHEP-accredited programs pass the SBB exam.

### Conclusion:

The **BEST** route for obtaining an SBB certification is to attend a CAAHEP-accredited Specialist in Blood Bank Technology Program

### Contact the following programs for more information:

PROGRAM	CONTACT NAME	CONTACT INFORMATION
Walter Reed Army Medical Center	William Turcan	202-782-6210; William.Turcan@NA.AMEDD.ARMY.MIL
Transfusion Medicine Center at Florida Blood Services	Marjorie Doty	727-568-5433 x 1514; mdoty@fbsblood.org
Univ. of Illinois at Chicago	Veronica Lewis	312-996-6721; veronica@uic.edu
Medical Center of Louisiana	Karen Kirkley	504-903-2466; kkirk1@lsuhsc.edu
NIH Clinical Center Department of Transfusion Medicine	Karen Byrne	301-496-8335; Kbyrne@mail.cc.nih.gov
Johns Hopkins Hospital	Christine Beritela	410-955-6580; cberite1@jhmi.edu
ARC-Central OH Region, OSU Medical Center	Joanne Kosanke	614-253-2740 x 2270; kosankej@usa.redcross.org
Hoxworth Blood Center/Univ. of Cincinnati Medical Center	Catherine Beiting	513-558-1275; Catherine.Beiting@uc.edu
Gulf Coast School of Blood Bank Technology	Clare Wong	713-791-6201; cwong@giveblood.org
Univ. of Texas SW Medical Center	Barbara Laird-Fryer	214-648-1785; Barbara.Fryer@UTSouthwestern.edu
Univ. of Texas Medical Branch at Galveston	Janet Vincent	409-772-4866; jvincent@utmb.edu
Univ. of Texas Health Science Center at San Antonio	Bonnie Fodermaier Linda Smith	SBB Program: 210-358-2807, bfodermaier@university-health-sys.com MS Program: 210-567-8869; smithla@uthscsa.edu
Blood Center of Southeastern Wisconsin	Lynne LeMense	414-937-6403; Irlemense@bcsew.edu

*Additional information can be found by visiting the following Web sites: [www.ascp.org](http://www.ascp.org), [www.caahep.org](http://www.caahep.org), and [www.aabb.org](http://www.aabb.org)*

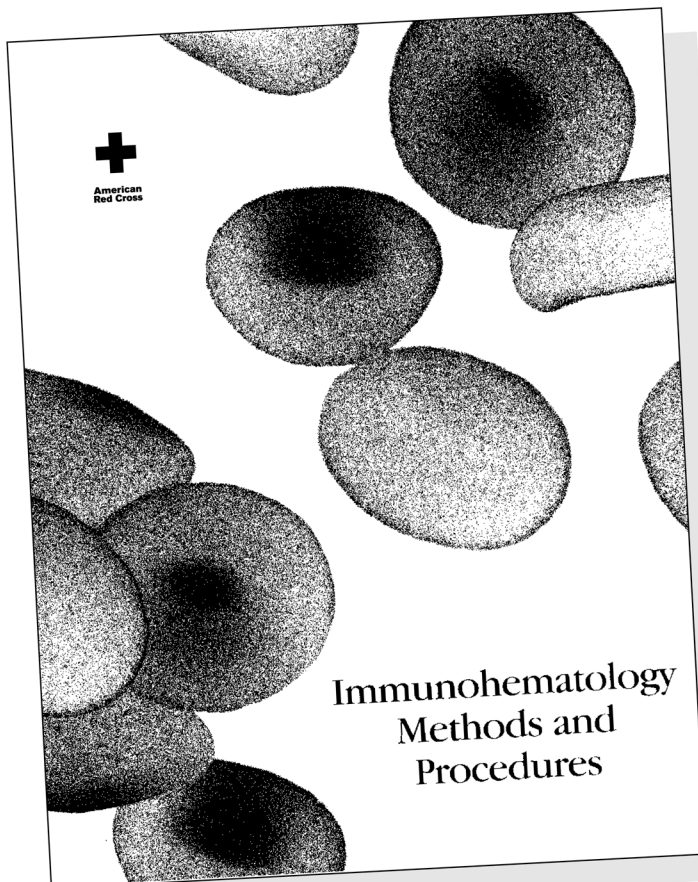
**From the publishers of *Immunoematology***

**A**

---

***Comprehensive  
Laboratory  
Manual***

---



Featuring—

- Over 100 methods—  
just about every method used in a reference lab.
- Eleven chapters discussing problems faced by blood group serologists and the procedures and methods that can be used to solve them.
- An extra set of the methods to use at the bench, printed on durable waterproof paper.
- See business reply order card enclosed in this issue or order on the Web at [redcross.org/immunoematology](http://redcross.org/immunoematology)