Immunohematology

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Review: the Rh blood group system: an historical calendar

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Early Studies

The question as to who discovered the Rh blood group system has been vigorously debated for many years. In 1939, Levine and Stetson correctly described the etiology of a case of HDN. They did not give a name to the causative antibody in the serum of Mary Seno; had they done so Rh might now have a different name. In 1940, Landsteiner and Wiener described antibodies produced in guinea pigs and rabbits that had been injected with RBCs from rhesus monkeys. The predominant antibody was subsequently shown apparently to have the same specificity as the causative antibody in the case of HDN described by Levine and Stetson. For the rest of their lives Levine and Wiener each claimed that he had discovered the Rh system independently of the other. Different terminologies were used to describe the first and later discovered but closely related antigens and both Levine and Wiener had supporters of their claims.

At one time it was suggested that the system had been discovered earlier. In 1933, Buchbinder published results from studies using animal sera. Rosenfield pointed out that Buchbinder was a graduate student working in Landsteiner's laboratory and suggested that some of the antibodies foreshadowed the specificities reported by Landsteiner and Wiener in 1940 and 1941. However, from his reading of the results published by Buchbinder, this author concluded that all the findings could be explained by the species differences between the animal sera and the human test RBCs.

Some early findings about Rh were difficult to explain. Fisk and Foord reported that while human cord RBCs could be divided into positive and negative using human anti-Rh, they all reacted with guinea pig anti-Rh. Murray and Clark found that human RBCs, divided into positive and negative with human anti-Rh, all adsorbed guinea pig anti-Rh. Similarly it was claimed that both Rh+ and Rh– RBCs stimulated production of anti-Rh in animals. In 1961, Levine et al. reported that human and animal anti-Rh have different specificities. The guinea pig anti-Rh was renamed anti-LW, supposedly in honor of Landsteiner and Wiener. It followed, of course, that if the antibodies raised in guinea pigs and rabbits following immunization with rhesus monkey RBCs had anti-LW specificity, then Levine and Stetson had discovered Rh in tests on the serum of Mary Seno. Wiener, of course, rejected such a claim and maintained that the sera of the immunized rabbits and guinea pigs contained both what had been called human anti-Rh and animal anti-Rh. Anti-LW closely resembles human anti-Rh because the LW antigen is more strongly expressed on Rh+ than on Rh– RBCs from human adults.

The fact that the initial experiments of Landsteiner and Wiener used RBCs from rhesus monkeys as the immunizing source led to many workers calling the subsequently discovered complex polymorphism the Rhesus blood group system. This author was as guilty as any and even published a book using that name in its title. In fact the correct name is the Rh system; the mistake of calling it the Rhesus system was never made by Race and Sanger, who did much of the early pioneer work on the polymorphism, nor by Mollison, who did much to establish the clinical significance of the system.

Up to this point the term *anti-Rh* has been used and the terms *anti-D* and *anti-Rh$_0$* have been studiously avoided. This is because two distinct terminologies for Rh were used early and it is difficult to interpret one group of workers' results in the other's terminology. As discussed in a later section, the terminology schism stemmed from different theories regarding the genetic control of Rh antigen production. Suffice it here to say that the initial studies on Rh were done with an antibody named anti-Rh$_0$ by Wiener and his colleagues and anti-D by Fisher and Race. Levine and his colleagues elected to use the name anti-D. For the rest of this review the Fisher-Race (CDE) terminology will be used (initially Wiener's Rh-Hr terms will be listed parenthetically) where names

exist. For later findings, where no CDE terms have been applied, the numerical terminology of Rosenfield et al., or the "local" names applied by the reporting authors, will be used.

Early Expansion: the First Five Antigens

It was not long after the discovery of anti-D (anti-Rh_0) that it became apparent that the situation is far more complex than a one-antigen, one-antibody system. In 1941, Wiener et al. described an antigen now known as C (rh′). It was noticed that most RBCs that carry C are D+. In the same year Levine et al. described c (hr′), an antigen found often on D+ RBCs and almost always on those that are D–. In 1943, Race et al. and Wiener and Sonn reported the discovery of an antigen E (rh″). It was seen that E was often present on D+ RBCs, particularly those that were D+, C–. E was seldom found on D– RBCs. At this point Fisher postulated that C and c have an antithetical relationship that is independent of D and E and that antithetical partners to D and E would eventually be recognized. In 1945, Mourant et al. reported discovery of e (hr″), the anticipated antithetical partner to E. No verifiable discovery of an antigen antithetical to D was ever reported and eventually the term d came to be used simply to indicate the absence of D. It was almost half a century later that biochemical and genetic evidence was produced to show that indeed most often when the D gene is absent there is no allelic gene to replace it. Indeed d and *d* truly indicate the absence of D and *D* in the overwhelming majority of instances.

Genetic Control

Fisher's concept of the genes *C, c, D, d, E,* and *e* allowed for the existence of Rh gene complexes such as *Cde, cDE, cde,* etc., that might represent three closely linked genes or three subloci within a single genetic locus. Wiener on the other hand believed that a single gene at the Rh locus controlled production of a single agglutinogen that in turn was made up of a series of blood factors such as Rh_0, rh′, and hr″. The three-gene and one-gene theories of genetic control of Rh antigen production were as widely debated as the claim as to who first discovered Rh. Ironically it was eventually shown by findings at the biochemical and molecular genetic levels that Rh antigen production is encoded by two genes. This situation was brilliantly forecast by Tippett, who based her theory on serologic findings well before molecular confirmation was available. Tippett used the MN system as a model. Just as genes at the *MN* locus encode production of one sialoglycoprotein while those at the *Ss* locus encode production of another, Tippett postulated that the *RHD* gene encodes production of one polypeptide while genes at the *CcEe* locus encode production of another. Just as in the MN system, it was supposed that crossing over, mutation, gene conversion, or a combination of these would lead to production of hybrid Rh polypeptides, the existence of which would explain the by-then well-known complexity of Rh. It has been rare in science that such a forecast has preceded confirmatory evidence yet has been so accurate.

Many More Rh Antigens

Between 1946 and the present, numerous additional Rh antigens were discovered. In some instances the serologic studies that led to recognition of a new antigen were such that the place of the antigen within the Rh system was apparent. In other instances an antigen that had been studied or described in the literature years earlier was shown by dint of family studies, by association with a highly unusual *Rh* gene complex, or by both to belong within the system. Table 1 lists the 49 Rh antigens that were included in the 2004 report of the International Society for Blood Transfusion Committee on Terminology for Red Cell Surface Antigens (reference given in table). Because this paper is a historical chronology, the antigens are listed in Table 1 in sequence of discovery. Again it should be stressed that the year of discovery may not be the year that the antigen was assigned to the Rh system. As the most extreme example, the antigen Be^a was first described by Davidsohn et al. in 1953. It was not added to the Rh system until 1975 after Race and Sanger had shown that the antigen is produced by a rare gene that also makes c and e.

Although Table 1 lists the antigens in sequence of discovery, it is more logical to consider them in groups in which some of the antigens' characteristics are the same.

C^w, C^x, E^w, and Rh26

Biochemical and molecular studies mentioned in a later section have shown that all Rh antigens are products of alternate alleles at one of the two *Rh* loci. However, such knowledge was not available when many of the antigens were first described. Accordingly some of those antigens can be considered historically in light of what was known at the time of their discovery. For example, C^w and C^x were originally

Table 1. Rh antigens listed in sequence of initial discovery*

Antigen	Year of discovery	% incidence in a Caucasian population
D	1939/1940	85
C	1941	70
c	1941	80
E	1943	30
e	1945	98
C^w	1946	1
Hr_0	1950	> 99
f	1953	64
Be^a	1953	< 1
C^x	1954	< 1
E^w	1955	< 1
V	1955	< 1
Go^a	1958	< 1
G	1958	85
Ce(rh_i)	1958	70
Hr	1960	> 99
hr^s	1960	98
VS	1960	< 1
cE	1961	30
CE	1961	< 1
C^G	1962	70
D^w	1962	< 1
Rh26	1964	80
hr^H	1964	< 1
Rh29	1967	> 99
Evans	1968	< 1
Rh32	1971	< 1
Rh33	1971	< 1
Rh35	1971	< 1
Hr^B	1972	> 99
hr^B	1972	98
Tar	1975	< 1
Rh39	1979	> 99
Rh41	1980	70
Rh42	1980	< 1
Crawford	1980	< 1
Nou	1981	> 99
Dav	1982	> 99
Riv	1983	< 1
FPTT	1988	< 1
Sec	1989	> 99
BARC	1989	< 1
JAL	1990	< 1
STEM	1993	< 1
LOCR	1994	< 1
MAR	1994	> 99
JAHK	1995	< 1
DAK	2003	< 1
CENR	2004	< 1

*From the 2004 report of the ISBT Committee on Terminology for Red Cell Surface Antigens. Vox Sang 2004;87:304-6.

thought to be the products of alleles at the *Cc* sublocus. E^w was thought to be the product of an allele at the *Ee* locus. Now, of course, it is known that C^w and C^x are carried on a polypeptide that can also carry C or c. Rh26 is included in this section because it presents, at the serologic level, as a variant form of c.

rh_i (Ce), cE, CE, f (ce), Rh41, and Rh42

At the time they were reported, these antigens were thought to be produced when certain genes at the *Cc* and *Ee* loci or subloci were in cis position (i.e., on the same chromosome). For example when *C* was at the *Cc* sublocus and *e* was at the *Ee* sublocus, Ce (rh_i) was made. Similarly when the subloci were occupied by *c* and *E,* cE was made. These explanations were sometimes strained by serologic findings. For example, while f (ce) was invariably a product of the *r* gene (i.e., *c* and *e* in cis) it was also apparently made by a *Dc–* gene complex that made no e. Now that it is known that one polypeptide is encoded by a *CcEe* gene, the production of these antigens is somewhat easier to understand.

Hr_0, Hr, Rh29, Hr^B, Rh39, Nou, Sec, Dav, and MAR

In 1951, Race, Sanger, and Selwyn described a blood sample that carried D but no representation of antigens in the Cc or Ee series. The phenotype was called D– –. Other examples of what became known as Rh-deletion phenotypes were soon found. D– – was joined by DC^w– and Dc–. In 1961, Vos et al. described a sample that lacked all Rh antigens; the term Rh_{null} was eventually applied. In studies on antibodies made by persons with Rh-deletion and Rh_{null} phenotypes and by some other persons with very rare but not fully deleted phenotypes, it became clear that there are a number of Rh antigens made by all common *Rh* genes but not by the rare genes responsible for the phenotypes described in this section. The antigens defined by these antibodies are listed in the heading of this section. It is now known that in some instances absence of the antigen is a direct consequence of absence of one of the two Rh polypeptides encoded by the *D* and *CcEe* genes. In other instances lack of the antigen represents a point mutation that leads to an amino acid change in the encoded Rh polypeptide chain. In many such instances the amino acid substitution results in the presence of an Rh antigen of low incidence.

In considering these antigens of very high incidence it will be mentioned that many of them can be targets for autoantibody production. In 1963, Weiner and Vos showed that some autoantibodies causative of warm autoimmune hemolytic anemia (WAIHA) reacted with all RBCs except those of the Rh-deletion and Rh_{null} phenotypes. Others failed to react only with Rh_{null} RBCs. Since that time thousands of similar autoantibodies have been studied. In most cases the autoantibodies are causative of WAIHA but exceptions are seen, i.e., in some hematologically normal persons and in some with drug-induced autoantibody formation.

Be^a, Go^a, D^W, Evans, Rh32, Rh33, Rh35, Tar, Crawford, Riv, FPTT, BARC, JAL, STEM, LOCR, JAHK, DAK, and CENR

The low-incidence antigens in this section heading are markers of unusual *Rh* genes at the *D* or *CcEe* loci. Some, such as Go^a and D^W, began life as "replacement" antigens when a portion of the D complex of antigens was missing. Others, such as Rh32 and Rh33, were markers of the presence of rare *Rh* genes, the relationship being painstakingly demonstrated by serologic tests. Now, of course, it is clear that a point mutation that substitutes a different from usual amino acid in either the D or the CcEe polypeptide can result in the presence of a low-incidence Rh antigen and, often, the simultaneous absence of a high-incidence antigen. Somewhat similarly, when hybrid Rh polypeptides are formed, the new sequence of amino acids at the D to CcEe or CcEe to D polypeptide joining site can result in a new antigen being present.

V, VS, hr^S, hr^B, and hr^H

These antigens have been separated from the others because, when discovered, each seemed to have a serologic relationship to e. As an example, in many studies VS behaved as a variant of e, while V seemed to be made by the gene complex that made VS when that gene also made c. The antigens hr^S and hr^B were initially recognized when Shapiro studied the famous Shabalala and Bastiaan samples. Each of those sera contained an antibody to a very common antigen, Hr (sometimes called Hr^S) and Hr^B, respectively, and a separable antibody, anti-hr^S and hr^B, respectively, with a specificity similar but not identical to anti-e. The antigen hr^H seemed to have some similar relationship to V and VS. Now these antigens can be regarded as variants on the CcEe polypeptide.

G and C^G

Before 1958, many workers had been puzzled by the immune response in some persons with D– RBCs. When exposed to C–, D+ or C+, D– RBCs, some of these individuals made apparent anti-CD. In 1958, Allen and Tippett showed that all genes that make C and almost all genes that make D make another antigen, which they named G. The supposed anti-CD mentioned above actually contained anti-G and, dependent on the phenotype of the immunizing RBCs, often separable anti-D, anti-C, or both as well. Allen and Tippett described the rare phenotype C–, D–, G+(r^G); later it was found that the phenotype D+, G– also exists. It is now known that a sequence of amino acids common to the D and C polypeptides represents G.

In 1962, Rosenfield et al. described anti-C^G as defining an antigen made by most genes that make C but not by those that make D without C. It has been reported that the phenotypes D–, C–, G+, C^G+ and D–, C–, G+, C^G– both exist.

Biochemical and Genetic Breakthroughs

For many years, numerous workers tried to isolate the Rh antigens or the membrane components that carry them from RBCs. Although there were a number of reports of success, none of the methods proved repeatable. When the initial breakthrough was achieved the methods devised were applied in numerous laboratories around the world. As a result, huge amounts of data were accumulated in a short time. A veritable explosion of knowledge emerged from studies in Bristol, Paris, and Baltimore. The Rh polypeptides once isolated were sequenced, the genes that encode them were recognized, and much information was obtained about the structure of the antigens. Thus many workers regard those early papers that explain the production and structure of D, C, E, etc. as the pioneer studies. In fact, all subsequent work at the biochemical and later at the molecular genetic levels is based on the initial methods devised to isolate the Rh-bearing polypeptides from RBCs. It is this author's belief that in this respect credit devolves on two independent groups: Moore and his colleagues in Scotland, who immunoprecipitated membrane components using antibodies to D, c, and E, and Gahmberg in Finland, who used a similar technique to isolate the D-bearing protein. These papers are often overlooked when reports are written describing the fine structures of, and differences between, the D-bearing and CcEe-bearing polypeptides. With regard to

giving credit where credit is due, these same workers receive too little recognition for their discovery of the Rh-related glycoprotein. Gahmberg suggested that in the membrane the nonglycosylated D-bearing polypeptide is associated with a glycoprotein. Moore and Green isolated the Rh-associated glycoproteins and showed that the one associated with the D polypeptide differs from the one associated with the CcEe polypeptide and that the Rh-associated glycoproteins also carry ABH determinants. These findings were reported at a time when isolation and sequence studies on the nonglycosylated Rh polypeptides were in vogue and were sometimes ignored or even questioned. Indeed it was not infrequently assumed that the Rh-associated glycoprotein and the nonglycosylated Rh polypeptide complex was an artificial one that formed during the isolation procedure. Such skepticism was born of the assumption that Rh could be fully explained in terms of the nonglycosylated polypeptides. Now, of course, it is known that in one form of Rh_{null} the D and CcEe polypeptide-encoding genes are normal and that the phenotype represents a defect in the independently inherited genes that encode production of the Rh-associated glycoprotein. In other words, the Rh antigen–bearing polypeptides are not expressed at the RBC membrane level unless Rh-glycoprotein–Rh-polypeptide (and possibly Ss-sialoglycoprotein) complexes form. The genes that encode production of the nonglycosylated Rh polypeptides are on chromosome 1, while those that encode production of the Rh-associated glycoproteins (that do not carry Rh antigens) are on chromosome 6. Beyond the scope of this review are findings that show that at a physiologic level tissue-located Rh-associated glycoprotein analogs are more important than the RBC Rh antigen–bearing polypeptides.

Rh Polypeptides

As mentioned several times already, two major forms of Rh polypeptide exist. One is encoded by one of the various forms of the *RHD* gene. This polypeptide carries whole or partial D, G, and, on occasion, a low-incidence antigen in lieu of a portion of D. Many of the variant polypeptides mentioned in this section contain material similar to that of a D-bearing polypeptide. The second common Rh polypeptide is encoded by one of the *RHCE* genes (*RHCe, RHCE, RHcE, RHce* are common varieties). This protein carries C or c (variant forms can carry both) and E or e and G (if C is made). Again many variant Rh polypeptides carry material similar to that encoded by *RHCE* genes. In addition to the common Rh polypeptides there are now numerous recognized variant proteins. These are encoded by genes that have arisen by gene conversion (rearrangement, crossing over) or point mutation. These variant polypeptides explain virtually all the previously inexplicable Rh serology. As mentioned in the previous section on Be^a et al., point mutation that results in insertion of a different from usual amino acid in the polypeptide chain, in a different sequence of amino acids that results when hybrid polypeptides are encoded, or both are prime sources of Rh antigens of both high and low incidence. In the white population, the D– phenotype most often represents total absence of a *D* gene. In other populations, a different explanation often obtains. Clearly it is beyond the scope of this historical chronology (and its author) to describe all the biochemical and genetic complexities in detail. Interested readers are referred to an excellent (save that it does not cite Moore et al. or Gahmberg) recent review by Westhoff (*Transfusion* 44:1663-73, 2004).

A Note about References

For the sake of clarity, detailed references to the early findings described in this review are not given. All papers mentioned are referenced in Chapter 10 of *Applied Blood Group Serology,* 4th ed., 1998. Two key references not listed there i.e., to the 2004 ISBT Committee report and the Westhoff review, are given in a previous section.

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Reactivity of FDA-approved anti-D reagents with partial D red blood cells

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Individuals whose RBCs are characterized as having a partial D phenotype may make anti-D if exposed to normal D+ RBCs; thus it is desirable that they be typed as D– should they require blood transfusion or Rh immune globulin (RhIG) prophylaxis. Further, use of different anti-D reagents by blood centers and transfusion services can account for FDA-reportable errors. For this study, anti-D reagents for use in tube tests were obtained from three U.S. manufacturers. They included three examples of IgM monoclonal anti-D blended with monoclonal IgG anti-D, one IgM monoclonal anti-D blended with polyclonal IgG anti-D, and two reagents formulated with human anti-D in a high-protein diluent. One anti-D formulated for use by gel column technology was also tested. Direct agglutination tests by tube or gel were strongly positive (scores 9–12), with partial D RBCs of types DII, DIIIa, DIIIb, and DIVa. No reagent anti-D caused direct agglutination of DVI type 1, DVI type 2, or DFR phenotype RBCs. One tube anti-D reagent formulated with an IgM monoclonal anti-D plus a polyclonal IgG anti-D failed to cause direct agglutination of DVa, DBT, and R_0^{Har} RBCs, while DVa RBCs reacted weakly with two high-protein reagents formulated with human IgG anti-D. In contrast, the anti-D used by gel column technology was strongly reactive (score 11) with DVa, DBT, and R_0^{Har} RBCs. The single monoclonal IgM–polyclonal IgG blended anti-D and the two high-protein reagents were also the only reagents that failed to react with R_0^{Har} RBCs by the IAT. Elimination of the test for weak D on all patient samples, using currently available FDA-licensed reagents, will ensure that partial D category VI (DVI) patients will type as D– for the purpose of RhIG prophylaxis and blood transfusion. However, RBCs of other partial D phenotypes will be classified as D+ in direct agglutination tests with some, if not all, currently available reagents. Testing donors for weak expression of D continues to be required, albeit that Rh alloimmunization by RBCs with a weak or partial D phenotype is uncommon. Further, because of differences in performance characteristics among FDA-approved reagents, conflicts between donor center D typing and transfusion service confirmatory test results are inevitable. ***Immunohematology* 2005;21:146–8.**

Key Words: anti-D reagents, partial D red blood cells, gel column technology

Flegel and Wagner[1] have shown that weak D phenotypes arise from missense mutations to regions of the *RHD* gene that encode the transmembrane portion of the D protein. Consequently, less than the expected amount of normal D protein is inserted into the RBC membrane. Individuals whose RBCs are characterized as having a partial D phenotype usually do not make anti-D if exposed to D+ RBCs and most examples should type as D+ in direct agglutination tests with currently available reagents formulated in part with high-affinity IgM monoclonal anti-D.

In contrast, partial D phenotypes are the result of hybrid genes or missense mutations to regions of *RHD* that encode portions of D protein external to the RBC membrane. Individuals with a partial D phenotype or with RBCs expressing a partial D phenotype are at risk of making anti-D if exposed to D+ RBCs. Therefore, it is desirable that they be typed as D– if they are candidates for transfusion or RhIG prophylaxis. Further, partial D phenotypes can account for FDA-reportable errors when blood centers and hospitals use different reagents that give dissimilar reactions with partial D phenotype RBCs.

To determine the performance characteristics of anti-D reagents with respect to partial D phenotype cells, we tested currently available FDA-approved reagents. Our findings, which have implications for both transfusion service and blood donor testing laboratories, are presented in this report.

Materials and Methods

The partial D RBCs used in this investigation were from our frozen RBC collections. Many had been obtained through the SCARF International Exchange Program.

Gel column technology using ABO and Rh typing cards (preloaded with anti-D) was from Ortho-Clinical Diagnostics, Raritan, New Jersey. Anti-D reagents for tube testing included a monoclonal IgM anti-D blended with polyclonal IgG anti-D (BioClone, Ortho) and three monoclonal IgM anti-Ds blended with monoclonal IgG anti-D (ImmucorGamma, Norcross, GA). The specific

Table 1. Formulation of currently available FDA-approved anti-D reagents

Reagent Type	Source	IgM anti-D	IgG anti-D
Tube	Ortho BioClone	MAD2	Polyclonal
Tube	Gamma-clone	GAMA401	F8D8
Tube	Immucor Series 4	MS201	MS26
Tube	Immucor Series 5	Th28	MS26
Gel	Ortho (ID-MTS)	MS201	NA*

* Not applicable

monoclonal antibodies used in the formulation of these reagents are shown in Table 1. In addition, we tested two anti-D reagents formulated in a high-protein diluent with human IgG anti-D as well as their matched inert control reagents (ImmucorGamma). All reagents were used in accordance with the manufacturers' product circulars. Reactions were graded and scored as described by Marsh.[2]

Results

Direct Agglutination Tests

The results of direct agglutination tests with anti-D reagents and partial D RBCs are shown in Table 2. RBCs of partial D categories II, IIIa, IIIb, and IVa were strongly reactive (scores 9–12) with the five tube reagents and with the anti-D used by gel column technology. RBCs of categories DVI type 1 (BARC– [RH:–52]), DVI type 2 (BARC+ [RH:52]), and DFR (FPTT+ [RH:50])[3] did not react in direct tests with tube and with reagents used by the gel column technology. The tube tests were also examined for direct agglutination after incubation, as permitted by the manufacturer; again no agglutination was observed with the DVI and DFR samples.

Table 2. Results of direct agglutination tests with IgM/IgG blended anti-D reagents and partial D RBCs*†

	Monoclonal Anti-D					Polyclonal Anti-D	
Partial D RBCs	GAM Tube	IMM-4 Tube	IMM-5 Tube	ORT Tube	ORT Gel	GAM Tube	IMM Tube
DII	10	12	11	11	12	9	10
DIIIa	10	12	12	12	12	11	10
DIIIb	9	9	8	8	11	8	9
DIVa	9	12	10	12	12	10	10
DVa	6	9	8	0(0)	11	5	5
DVI.1	0(0)	0(0)	0(0)	0(0)	0	0	0
DVI.2	0(0)	0(0)	0(0)	0(0)	0	0	0
DFR	0(0)	0(0)	0(0)	0(0)	0	0	0
DBT	10	11	10	0(0)	11	0	0
R_0^{Har}-1	6(8)	0(6)	0(0)	0(0)	12	0	0
R_0^{Har}-2	6(6)	6(10)	6(10)	0(0)	12	0	0
R_0^{Har}-3	3(4)	0(3)	0(3)	0(0)	9	0	0

*Agglutination score of direct tests with anti-D.

†Number in parentheses denotes agglutination score following incubation, as permitted by the manufacturer.

Partial D RBCs of types DVa, DBT, and R_0^{Har} did not react with or were variably agglutinated by these reagents. Of note is the failure of the Ortho tube reagent to react with these RBCs in direct agglutination tests while the same RBCs reacted strongly (score 11) with the anti-D used by gel column technology marketed by the same manufacturer. In contrast, Gamma and Immucor monoclonal reagents reacted with DVa and DBT RBCs in direct tests and they gave weak and variable reactions with R_0^{Har} RBCs; the direct reactions with R_0^{Har} RBCs were only slightly enhanced after incubation.

Indirect Antiglobulin Test (IAT)

Partial D RBCs that reacted weakly or not at all with one or more tube anti-D reagents in direct agglutination tests were further tested against these reagents by the IAT (test for weak D). The results are summarized in Table 3. Gamma and Immucor reagents formulated with monoclonal anti-D reacted with DVa, DVI, DFR, and R_0^{Har} RBCs; DBT RBCs were not tested by IAT since these RBCs were strongly reactive in direct agglutination tests. In contrast, reagents formulated with human anti-D reacted with DVa, DVI, DFR, and DBT, but not with R_0^{Har} RBCs.

Table 3. Results of IATs with tube anti-D reagents and those partial D RBCs that did not react in direct agglutination tests*

	Monoclonal Anti-D		Polyclonal anti-D†		
Partial D RBCs	GAM	IMM-4/5	ORT	GAM	IMM
DVa	10	12	11	8	8
DVI.1	12	11	8	9	8
DVI.2	11	10	8	9	9
DFR	12	12	11	8	9
DBT	/‡	/	8	9	9
R_0^{Har}-1	9	9	0	0	0
R_0^{Har}-2	10	10	0	0	0
R_0^{Har}-3	8	8	0	0	0

*Agglutination score of IATs with anti-D.

†ORT is reagent formulated with IgM monoclonal anti-D and human IgG anti-D in a low-protein diluent; GAM and IMM are both high-protein reagents formulated from human IgG anti-D.

‡Not tested; RBCs directly agglutinated.

Discussion

To paraphrase Flegel and Wagner,[1] it is desirable that partial D RBCs, especially those of the most commonly encountered form (DVI), be typed as D–

when the sample is from a patient who is pregnant or requires transfusion. Such patients are at risk of alloimmunization to D and should be transfused with D– RBCs; if pregnant, they should receive appropriate RhIG therapy. DVI RBCs can be appropriately typed as D– by direct agglutination tests with any of the currently available FDA-approved monoclonal anti-D reagents. However, RBCs of some partial D phenotypes (DII, DIIIa, DIIIb, and DIVa) typed as D+ in direct tests with all anti-D reagents tested and yet other phenotypes reacted variably.

The test for weak D (formerly D^u) need not be performed, nor is it required in a hospital setting except for testing apparent (by direct agglutination tests) D– newborns of D– women.[4] Some consideration should also be given to testing new patients with more than one anti-D reagent, the second test performed after reidentification of the sample to prevent erroneous D typings due to sample misidentification within the laboratory. The use of two different anti-D reagents, based on data we have presented, will facilitate recognition of potential R_0^{Har} samples. Conflicts between the two anti-D results, or between current and historical records, may also be an indication of partial D and the patient should be managed accordingly.

The overall reactivity of Ortho's tube reagent was most consistent with that of the two high-protein reagents formulated with human IgG anti-D. All three reagents failed to react with R_0^{Har} in both direct and indirect tests, in contrast to the Gamma and Immucor monoclonal reagents. However, it should be noted that the reactivity of these reagents by IAT is due to carry-over agglutination from direct tests (T. Frame, FIBMS, personal communication, 2004).

Depending upon the reagents and method used, conflicts between donor center D typing and transfusion service confirmatory test results can be expected, especially when testing DVa, DBT, and R_0^{Har} RBCs. Since these conflicts may be considered FDA-reportable errors, managers of donor testing facilities may want to give consideration to reagent selection and their methods for testing for weak D. Reliable detection of some partial D phenotypes appears to require a tube test for weak D; we have no data on the reactivity of tube anti-D reagents with partial D cells when tested on automated platforms.

It should be noted that in most cases only a single example of each partial D category was tested. That there is undoubtedly heterogeneity among RBCs of a particular category is evident from the variability of reactions noted with three different examples of R_0^{Har} RBCs. R_0^{Har}-1 was confirmed as such by both molecular and serologic analysis; it tested positive for Rh33 and Rh50. All three examples reacted with an eclectic human anti-D known to react with R_0^{Har} RBCs.

In conclusion, tests for weak D need not be performed on apparent D– pregnant women or potential transfusion recipients.[4] Rather, such individuals can be considered D– for the purpose of RhIG prophylaxis and blood transfusion.[5] While such a testing strategy will appropriately type DVI RBCs (the most common form of partial D) as D–, RBCs of other partial D phenotypes will be classified as D+ in direct agglutination tests with some, if not all, currently available reagents. Testing donor blood for weak expression of D continues to be required.[4] Due to differences in performance characteristics among FDA-approved reagents, as revealed in this investigation, conflicts between donor center D typing and transfusion service confirmatory test results are inevitable.

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Case report: immune anti-D stimulated by transfusion of fresh frozen plasma

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FFP has occasionally been reported to generate an immune response to RBC antigens (e.g., anti-D and anti-Fy^a). The Council of Europe requires that each unit of FFP have less than 6×10^9/L RBCs. However, there is considerable variation internationally in the method of production and the level and assessment of RBC contamination of FFP. This study reports the case of a 63-year-old group B, D– man who received multiple transfusions of D– blood products over a 4-month period. Seven months later the patient's antibody screen remained negative and he was transfused with seven units of D– RBCs and six units of FFP, four of which were D+. Two months later anti-D, -E, and -K were detected in his plasma. Although the anti-E and anti-K could have resulted from transfusion of antigen-positive RBCs, the anti-D could have resulted only from transfusion of the D+ FFP. The D status of FFP is currently not considered when selecting products for transfusion even though the D antigen is highly immunogenic and the level of RBC contamination of FFP is not always known. This case highlights that transfusion of FFP is a stimulus for RBC antibodies and that when a patient has had a recent transfusion of FFP, consideration should be given to obtaining a sample for pretransfusion testing within 3 days before a scheduled RBC transfusion. In addition, the D status of FFP should be considered before administering FFP to premenopausal D– women. ***Immunohematology* 2005;21:149–51.**

Key Words: red blood cell, antibody, anti-D, contamination, fresh frozen plasma, FFP

On rare occasions FFP can generate an immune response to RBC antigens. The literature includes reports of anti-D,[1–3] -Fy^a,[3] -E,[4] and -Jk^a [4] being identified or increasing in titer after transfusion of as few as two units of FFP. The Council of Europe (CE) set guidelines in 2004[5] for maximum levels of RBC contamination in FFP in an attempt to circumvent immunization by RBC antigens. However, there is considerable variation internationally in the method of production and the level and assessment of RBC contamination of FFP.[6] We describe a patient who developed anti-D after the transfusion of D+ FFP.

Case Report

A 63-year-old man was diagnosed in May 2003 with locally extensive adenocarcinoma of the esophagus. Laser therapy and single-fraction radiotherapy were applied to the esophagus for control of dysphagia and bleeding. Cytotoxic chemotherapy with fluorouracil plus epirubicin and cisplatin from June to December 2003 resulted in significant reduction in the size of the tumor. The patient's warfarin therapy, administered after aortic valve replacement in 2002, was maintained when possible. The patient's RBCs typed as group B, D– and from March to August 2003 he was transfused with a total of 21 units of group B, D– or group O, D– RBCs for anemia. No atypical RBC antibodies were detected during this time. On February 12, 2004, the patient presented to the emergency department with acute gastrointestinal hemorrhage. The patient's Hb was 6.0 g/dL and the International Normalized Ratio was 8.9. No atypical RBC antibodies were detected in the patient's plasma and he was transfused over a 15-hour period with seven units of D– RBCs and six units of FFP, four of which were D+ (Table 1). Endoscopy and computerized tomography scan showed regrowth and progression of the tumor, with extensive upper mediastinal lymphadenopathy and a persisting pulmonary deposit. No further blood product support was required during this admission and the patient was discharged for palliative care. When the patient's sample was tested 2 months later, on April 8, the antibody screen was positive. Anti-D, -E, and -K were identified, with reaction strengths of 4+, 3+, and 2+, respectively, and remained detectable on subsequent testing two months later.

Materials and Methods

Antibody identification was performed by LISS-IAT gel cards (DiaMed AG, Switzerland) using commercial reagent RBCs (Commonwealth Serum Laboratories, Melbourne, Australia) diluted to 1 percent in Diamed-ID Diluent 2. Reactions were graded from 0 (no agglutination) to 4+ (maximum agglutination).

Table 1. Blood products transfused on 2/12/2004*

	Product	ABO/Rh	Antigen typing	
			E	K
1	FILRBC	O Neg	NT	Neg
2	FILRBC	O Neg	NT	Pos
3	FILRBC	O Neg	Neg	Neg
4	FILRBC	B Neg	Neg	Neg
5	FILRBC	B Neg	NT	NT
6	FILRBC	B Neg	Neg	Neg
7	FILRBC	B Neg	Neg	Neg
8	FFP	B Neg	Neg	Neg
9	FFP	B Neg	Neg	Neg
10	FFP	B Pos	Pos	Neg
11	FFP	B Pos	Neg	Neg
12	FFP	B Pos	NT	Neg
13	FFP	B Pos	Neg	Neg

* FILRBC = leukodepleted RBCs, NT = not tested

Transfusion history of the patient was obtained from the laboratory information systems at two health care institutions. All other Western Australian transfusion services confirmed the absence of any transfusion history.

Discussion

This report describes the development of anti-D in a multiply transfused D– patient after stimulation by residual RBC antigens in D+ FFP. Anti-K and anti-E also developed, most likely due to the transfusion of antigen-positive RBCs (Table 1). One of the transfused units of RBCs was K+ and, although the E phenotype of some of the transfused units was unknown and the incidence of the E antigen in D– Australian donors is only 4 percent,[7] the possibility that anti-E resulted from transfusion of E+ RBCs cannot be excluded.

The D antigen is highly immunogenic: historic experiments demonstrated primary immunization after a cumulative dose of only 0.03 mL or 0.24×10^9 RBCs.[3] For this dose, injections of 0.01 mL of whole blood (approximately 0.005 mL RBCs) were given at 2-week intervals. A total of six injections was administered.

The minimum volume of a single dose of RBCs required to stimulate primary antibody sensitization is unproved and will vary among subjects. Similarly the number and volume of RBCs required to stimulate an anamnestic response are uncertain, though they will be considerably less. In an attempt to circumvent immunization by RBC antigens, CE guidelines require that each unit of FFP contain less than 6×10^9/L RBCs.[5] Compliance would allow up to 1.5×10^9 RBCs in a 250-mL unit of FFP, although there are often less than 0.18×10^9 RBCs.[8,9] RBC stroma, as found in FFP, is less immunogenic than intact RBCs.[3] However, this and previously reported cases[1,2] implicating FFP indicate that there is sufficient antigenicity to stimulate a response after frozen storage and subsequent thawing and infusion. As few as two units of FFP have been shown to stimulate a secondary anti-D response.[1,2] It is not known whether the anti-D produced in this case was a primary or secondary response. Although no D+ RBCs had been transfused since 2002, the possibility of prior sensitization to D antigen cannot be completely discounted.

Current transfusion guidelines[10,11] do not require the D status of FFP to be considered before transfusion and CE Guidelines[5] do not require FFP products to be labeled with the Rh type. The FFP transfused in this case was prepared from whole blood. In some countries FFP may be subjected to pathogen reduction with methylene blue (MB) or solvent detergent (SD). RBC stroma is present in untreated FFP and in MBFFP, but not in SDFFP, because it is removed by the solvent.[12] This case report highlights that the D status of FFP is relevant in transfusion practice. Specifically, a patient who has had a recent transfusion of FFP may be sensitized to RBC antigens, indicating the need for an antibody screen within three days before RBC transfusion. In addition, transfusion of D+ FFP to D– premenopausal women may have significant implications, and it has been reported that D– girls and premenopausal women requiring FFP transfusion should receive D– FFP only if SDFFP is not available.[12]

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Incidence of weak D in blood donors typed as D positive by the Olympus PK 7200

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The incidence of weak D has been reported to be between 0.23 and 0.5 percent in Europe and 3.0 percent in the United States. All studies were performed before the introduction of monoclonal anti-D reagents. Using current commercial reagents, this study evaluated D+ samples for the presence of weak D. D+ donors, typed by the Olympus PK 7200, using diluted monoclonal blend anti-D and diluted polyclonal anti-D, were selected by sampling batches of 100 to 200 samples from the previous day's collection. Anti-D reagents used on the Olympus PK 7200 are required to detect RBCs with the weak D phenotype which do not agglutinate at immediate spin (IS) when tested with polyclonal anti-D by manual tube methods. More than 95 percent of donors tested were Caucasian. Using tube tests with two different monoclonal blend anti-D reagents and one polyclonal anti-D typing reagent, the presence or absence of the D antigen was evaluated after the IS reading. Donors found negative or weakly positive (< 2+) at IS were further typed for weak D by the IAT. The weak D samples were *RHD* genotyped by allele-specific PCR. Of 1005 donors tested, 4 (0.4%) were classified as weak D by one or more anti-D reagents. Polyclonal anti-D reagent demonstrated weaker reactions when compared with the monoclonal blends. All weak D samples were found positive for exon 4, intron 4, and exon 10, a finding consistent with most D+ samples. The incidence of weak D found in this study is not significantly different from that found in earlier studies using polyclonal anti-D reagents. ***Immunohematology* 2005;21:152–4.**

Key Words: incidence of weak D, anti-D typing reagents

The D antigen is carried on an integral protein inserted into the RBC membrane. It is proposed that point mutations that cause amino acid changes in the intracellular or transmembrane portions of the protein result in less protein being inserted into the membrane. This leads to a quantitative difference in reactivity with anti-D reagents when testing D+ and weak D RBCs. Depending on the anti-D reagent, RBCs with a weak D phenotype may not react or may react weakly (< 2+) in direct agglutination tests, but are reactive by the IAT.

Point mutations in extracellular loop regions or genetic mutations, such as recombination or frameshift mutations leading to absence of portions of the Rh protein, can result in partial D antigens. Some anti-D reagents can react with partial D RBCs by direct agglutination while others require IATs.

The incidence of serologically defined weak D has been reported to be between 0.23 and 0.5 percent in Europe and 3.0 percent in the United States. In 1974, Garretta reported an incidence of 0.56 percent with 203,240 samples typed with a Groupamatic using polyclonal anti-D.[1] In 1989, Contreras reported 49 out of 16,484 donors (0.3%) typed as weak D by the Kontron Groupamatic G2000.[2] Of these 49 donors, 27 were initially typed as weak D by the Kontron. In addition, 14 of 87 Groupamatic-typed D–, C+, E– donors and 8 of 90 D–, C–, E+ Groupamatic-typed donors were confirmed weak D. All 49 donors were confirmed weak D by a manual IAT.

An incidence of 0.23 percent (32 of 13,500) weak D donors was found in the Netherlands when testing with various anti-D reagents, including four polyclonal anti-D with enhancers, four monoclonal anti-D available in the Netherlands, two modified IgG anti-D, and bromelin anti-D.[3] In this study, weak D was identified as samples typing D– with at least two of these anti-D reagents.

In the United States, Stroup Walters reported an incidence of 3.0 percent in typing 23,000 donors in 1988 with at least two reagents.[4] This study was performed prior to the introduction of monoclonal anti-D reagents in the United States.

Materials and Methods

Sample selection

D+ blood donors, typed by the Olympus PK 7200 (Olympus America, Inc., Melville, NY) using diluted monoclonal blend anti-D and diluted polyclonal anti-D, were selected by sampling batches of 100 to 200

samples from the previous day's collection. A total of 1005 D+ donor samples were tested. All samples were collected in EDTA-containing tubes (Becton Dickinson and Co., Franklin Lakes, NJ). More than 95 percent of samples were from Caucasian donors.

Anti-D reagents used on the PK 7200 included anti-D monoclonal blend Gamma-clone (ImmucorGamma, Houston,TX) diluted 1 to12 with physiologic saline and polyclonal anti-D (ImmucorGamma) diluted 1 to 8 with 6% dextran (Mol.wt. 68800). The optimal working dilution was determined for each lot of anti-D and Rh control reagent. Following the Olympus PK 7200 *Operator's Manual,* a series of dilutions were made for each reagent and tested with 16 D+, 50 D–, and 8 weak D RBC samples. All donor RBCs were treated with 0.8% bromelin. All batches typed on the PK 7200 included D–, D+, and weak D controls.

Testing for D antigen

D antigen testing by conventional tube method was performed using the following anti-D reagents: polyclonal anti-D (ImmucorGamma, Houston, TX), anti-D monoclonal blend, Gamma-clone (ImmucorGamma, Houston, TX), and anti-D monoclonal blend, Series 4 (ImmucorGamma, Norcross, GA). RBCs found to be D– or weakly D+ (< 2+) were further tested for weak D by the IAT. A positive result in the IAT defined weak D in this study.

RHD *genotyping*

All weak D samples were *RHD* genotyped by allele-specific PCR. DNA was prepared from blood samples using one of the following methods: QIAamp Blood Kit (Qiagen, Venlo, the Netherlands), PUREGENE (Gentra Systems, Minneapolis, MN), or MagNA Pure LC DNA Isolation Kit I (Roche, Indianapolis, IN). *RHD* genotyping was performed in two multiplex PCR reactions. The first reaction detected intron 4 and exon 10.[5,6] The second reaction detected exon 4, including the position of the 37-bp insert found in the *RHD* pseudogene.[7] PCR products were analyzed by agarose gel electrophoresis in the presence of ethidium bromide followed by documentation by UV illumination.

Results

Four donors (0.4%) were classified as weak D by one or more anti-D reagents (Table 1). Polyclonal anti-D reagent demonstrated weaker reactions when compared with the monoclonal blends. Gamma-clone monoclonal blend anti-D showed the strongest reactions on direct agglutination.

Table 1. Results of donor weak D RBCs with anti-D reagents

	Polyclonal Anti-D		Monoclonal blend Gamma-clone		Monoclonal blend Immucor (Series 4)	
Sample	IS	IAT	IS	IAT	IS	IAT
1	+/–*	2+	w+†	2+	w+	2+
2	0	2+	1+	3+	w+	3+
3	0	w+	w+	2+	0	2+
4	+/–	3+	1+	3+	+/–	3+

*+/– = weak granularity in the RBC suspension. A few macroscopic agglutinates in a turbid red background.

†w+ = tiny agglutinates in a turbid red background (free RBCs)

All weak D RBC samples were found positive for exon 4, intron 4, and exon 10, a finding consistent with most D+ RBC samples.

Discussion

When testing 1005 D+ donors, as defined by testing with the Olympus PK 7200, we found the incidence of weak D to be 0.4 percent. This is not significantly different from the results of earlier studies using polyclonal reagents, where the incidence of weak D was reported to be between 0.23 and 3.0 percent. Monoclonal reagents showed similar results to those seen with polyclonal reagents when using less than 2+ agglutination as the defining limit to determine the presence or absence of D antigen by direct agglutination.

It is possible that the incidence of weak D is higher than that found in our study because only donors who typed D+ on the Olympus PK 7200 were studied. Donors with very weakly expressed D antigens not detected by the Olympus methodology may have been missed. Gassner and colleagues performed molecular analysis on 1700 serologically typed D–, C+ or E+ RBC samples from Central European blood donors. Eighty-nine had various forms of *RHD* alleles when screened for *RHD*-specific DNA sequences.[8] Five of these donors would have presumably been detected by serologic weak D testing. Wagner et al.[9] investigated 1068 donors who serologically typed D–. Of these donors, 48 carried the *RHD* gene and all were C or E antigen positive. In addition, 7374 RBC samples determined to be D–, C–, E–, c+, e+ were tested and 2 *RHD* positive donors were found. Five donors in all were subsequently typed as weak D.[9] The frequency of weak D in individuals typing as D– is low, on the basis of these two studies.

In our study all weak D samples were genotyped to confirm the presence of the *RHD* gene and to look for unusual PCR results, in an attempt to determine whether the weak D status was due to quantitative or qualitative differences. All four weak D samples tested as having "normal" *RHD* genes. It is unlikely that these weak D samples are any of the common partial D types. DVI, the most common partial D among Caucasians, reacts only in the IAT using a test tube method, while R_0^{Har} reacts with Gamma-clone anti-D upon immediate spin (IS) and does not react with Ortho BioClone anti-D reagent. Partial D categories, including DII, DIII, DIV, and DVa, are detected by monoclonal anti-D reagents upon IS.[10] The donors in our study all reacted weakly by direct agglutination with both examples of monoclonal anti-D used but reactivity was significantly enhanced in the IAT used to detect weak D. In addition, our PCR method would have detected the presence of DVI and R_0^{Har}, as DVI would be negative for exon 4 and intron 4 and R_0^{Har} would have tested as D–. As most weak D phenotypes are due to point mutations[11] that would not be detected by our PCR assay, our results are consistent with those samples having either a *RHD* allele encoding a weak D phenotype, or having *RHC* in trans to D, causing weakened D antigen expression.

While only serologically determined D+ donors were tested in this study and the number tested was relatively small, the incidence of 0.4 percent compares to that previously found by serologic methods. Further molecular studies on the U.S. donor population, like those reported by Gassner and Wagner, may provide further information on the incidence and significance of weak D antigen.

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Review: the Rh blood group D antigen . . . dominant, diverse, and difficult

C.M. WESTHOFF

Our understanding of the Rh blood group system has been greatly advanced since the genes were cloned in the late 1990s. We have witnessed the explosion of information about the Rh blood group antigens with the development of PCR and the rapid elucidation of the genetic basis for the antigens and phenotypes. This new genetic information explains some of the longstanding questions about the Rh system, especially the D antigen, leading to reemergence of discussions about appropriate D testing approaches.

Serologic Foundation of Rh

Recent genetic information has confirmed many of the predictions of the serologists whose primary, and often only, tools were the antibodies made by immunized individuals. Exploiting adsorption and elution approaches, along with selected RBC testing strategies, they uncovered many of the details concerning the specificity and complexity of the Rh blood group system. For example, the prediction that Rh antigens are encoded by two genes, not one or three, was adeptly forecast by Patricia Tippett based solely on serologic observations.[1] Many investigators contributed to knowledge of the variability of the D antigen[2–6] and variants of the e antigen were elucidated by Issitt[7] and others.[6,8,9] That work is the foundation of our understanding today, as the new genetic information builds upon the serologic footing. Serologic reactivity is the basis for blood groups important in blood transfusion practice because serology defines an antigen. That fact will not change as we use different testing methods in the future, including those that are DNA based. The suggestion that agglutination of RBCs is still the mainstay of blood bank technology because the field is reticent to change and stuck in the early 1900s is shortsighted; without a serologic relationship, a variation in a blood group gene at the DNA level is just another single nucleotide polymorphism (SNP) that occurs once in every 100 to 300 bp in the human genome.[10] These polymorphisms are of only academic interest until associated with a phenotype and found to be relevant to transfusion medicine, by stimulation of an antibody, or to RBC function, as when they result in a null phenotype.

Rh Background

Two genes *(RHD, RHCE)* located on chromosome 1p34–p36[11] encode the Rh proteins designated RhD and RhCE; one carries the D antigen and the other carries CE antigens in various combinations (ce, Ce, cE, or CE).[12–15] The genes are 97 percent identical and each has 10 exons, but they encode proteins that differ by 32 to 35 of 416 amino acids (shown as circles on the RhD protein in Figure 1). Both proteins are predicted to cross the membrane 12 times. On the RhCE protein, the E and e antigens differ by one amino acid, Pro226Ala, located on the fourth extracellular loop (Fig. 1). However, the requirement for e antigen expression is more complex than the 226Ala polymorphism and expression can be altered by changes in other regions of the protein. These changes are often encountered in people of African or mixed ancestry[6,16] and are responsible for weak or altered e expression. C and c antigens differ by four amino acids but only the amino acid change at position Ser103Pro is predicted to be extracellular (Fig. 1). Additionally, three of the polymorphic amino acids in RhC are identical to RhD and explain the expression of G antigen on both proteins.

Why is the Rh System So Complex?

The majority of blood group systems are encoded by single gene loci. In contrast, the Rh system is encoded by two genes that have many identical regions

and the fact that they are in very close proximity on the same chromosome affords the opportunity for numerous exchange events between them. This results in new hybrid proteins that carry portions of RhD and portions of RhCE or vice versa. Most of these exchanges occur by a process called gene conversion. In this process, one member acts as donor template during replication of the other but, unlike in homologous recombination, the donor template remains unaltered. The donated region can span several base pairs, single exons, or even multiple exons. These exchanges between *RHD* and *RHCE* generate new polymorphic proteins and these hybrid proteins are responsible for the myriad of antigens observed in the Rh blood group system (reviewed in Westhoff[17] and Reid and Lomas-Francis[18]).

Why is D So Immunogenic?

Most blood group antigens differ from their antithetical partners by a single amino acid change (e.g., Jk^a/Jk^b, Fy^a/Fy^b, E/e, K/k, etc.). An important consideration in the immunogenicity of an antigen is the degree of foreignness to the host. RhD and RhCE differ by 32 to 35 amino acids, which explains why RhD, when seen by the immune system of a D– person, often induces a very robust immune response. Although only nine or ten of the changes are predicted to be extracellular, changes located in the transmembrane and cytoplasmic regions can also affect the topology of the protein in the membrane. Additionally, the large number of amino acid differences explains the numerous epitopes of the D antigen, estimated to range from 9 to more than 30.[19,20] Exposure to a foreign protein carrying this large number of amino acid changes results in the production of a polyclonal immune response directed at many different parts of the protein.

What Causes the Large Number of Variations in Expression of D?

D negative

The RBCs of D– individuals lack RhD protein because of deletion,[21] or, rarely, a mutation, of the *RHD* gene (Fig. 1). The D– phenotype is much more prevalent in Caucasians of European descent (15%–17%), less likely in individuals with African backgrounds (3%–5%), and rare in Asian populations (< 0.1%).[6] The D– phenotype has arisen numerous times, as evidenced by the different genetic events

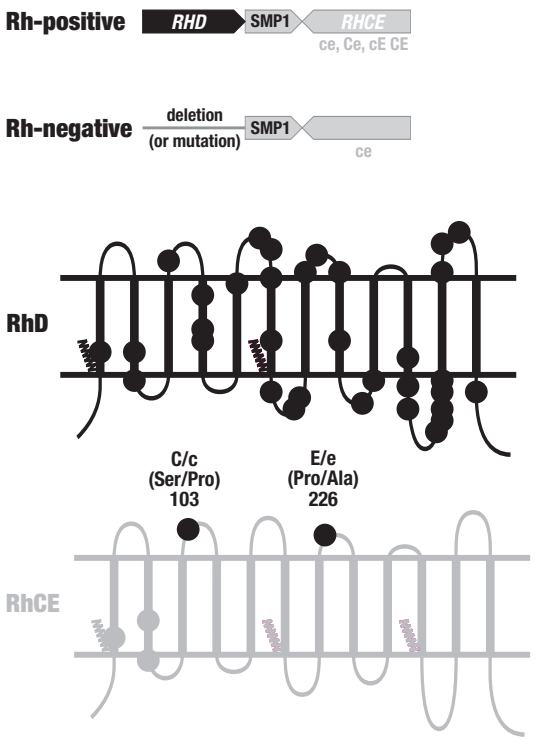

Fig. 1. Diagram of the *RHD* and *RHCE* gene locus (**top**) and of the Rh proteins in the RBC membrane (**bottom**).

The two *RH* genes have opposite orientation, with the 3′ends facing each other. *SMP,* a gene of unkown function, is located between them. Most D– Caucasian individuals have a complete deletion of *RHD.*

The RhD and RhCE proteins are predicted to have twelve transmembrane domains. Amino acid positions that differ between RhD and RhCE are shown as dark circles on RhD. The locations of the C/c and E/e polymorphisms are shown on RhCE.

responsible for the D– phenotype in different populations. In Caucasians, it is primarily the result of deletion of the entire *RHD* gene.[21] Some exceptions exist, including *RHD* genes that are not expressed because of a premature stop codon, nucleotide insertions, point mutations, or *RHD/CE* hybrids.[18,22] Because most of the exceptions arise on DCe (R_1) or DcE (R_2) backgrounds and result in the less common *Ce (r′)* or *cE (r″)* haplotypes, the presence of these unusual haplotypes is a useful marker for these exceptions.

In contrast to the complete *RHD* deletion found in Caucasians, D– phenotypes in African and Asian persons are often caused by inactive or silent *RHD* genes. Only 3 to 7 percent of South African black persons are D–, of which 66 percent have *RHD* genes that contain a 37-bp internal duplication resulting in a premature stop codon.[23] The 37-bp insert *RHD* pseudogene is also found in 24 percent of D– African Americans. Additionally, 15 percent of the D–

phenotypes in Africans result from a hybrid *RHD-CE-D* linked to ce^s, termed (C) ce^s, which is characterized by expression of VS, altered C and e (which may appear weakened), normal c, and no D antigen.[16] The weak C phenotype is only observed with polyclonal reagents; monoclonal reagents are strongly reactive with (C) ce^s RBCs. The prevalence of this haplotype in sickle cell patients can complicate transfusion because this C antigen is altered and these patients may make anti-C when stimulated. In D– African Americans, 54 percent completely lack the *RHD* gene, while the remaining have either the 37-bp insert *RHD* pseudogene or the hybrid *RHD-CE-D.*[23]

Asian D– phenotypes result from mutations in *RHD* most often associated with Ce, indicating that they originated on a *DCe (R_1)* haplotype. Many Asians whose RBCs type as D– are actually D_{el}.[24,25]

D_{el}

D_{el} refers to RBCs with a very low level of D antigen that is detectable only by adsorption and elution, hence the name. Because these RBCs type as D– (including testing by the IAT), they are often only recognized if they stimulate production of anti-D in a D– recipient.[26,27] This very low level D expression results from several different *RHD* mutations; currently there are five confirmed *DEL* alleles.[28] Because these are different mutations, the RBCs can vary in the amount of D antigen expressed and in the effect on D epitope expression. Those resulting from mutations in the intron 3 splice site (designated IVS3+1g>a) have altered D epitopes, as evidenced by epitope mapping and by the production of anti-D after emergency transfusion of D+ units. This mutation appears to cause a partial D_{el} phenotype.[28] Although investigation of the other four D_{el} backgrounds did not show epitope alteration,[28] this is not definitive because testing is limited by available reagents and not all D epitopes are known.

D_{el} RBCs are most often found in Asians, with the most frequent mutation being a 1227G>A change with skipping of exon 9.[25] However, a *DEL* allele encoding a M295I amino acid change, which has the highest level of D antigen expression,[28] has been found in 1 in 3700 Europeans.[22] The observation that D_{el} phenotypes may be infrequent, but not rare, along with two recent reports of donor RBCs with a D_{el} phenotype that stimulated anti-D in D– recipients,[26,27] have motivated discussions suggesting that these might be an emerging problem.[29] Some have suggested consideration of removing D_{el} RBCs from the D– donor pool by implementation of DNA-based testing methods.[30] Importantly, since all D_{el} samples reported to date express the C antigen and appear to be r′, the D– C+ phenotype is a useful marker for samples that potentially could be D_{el}.

Weak D (formerly D^u)

Weak D RBCs are historically defined as having reduced D antigen levels that require the IAT for detection. An estimated 0.2 to 1 percent of Caucasians have RBCs with weak D expression,[6] but the number of samples classified as weak D can depend on the characteristics of the typing reagent. Weak D RBCs were thought to simply have a reduction in the level of D antigen (i.e., a quantitative rather than a qualitative difference), based on the observation that individuals with weak D RBCs generally did not make anti-D when transfused with D+ RBCs.[31] From the work of Wagner et al.,[32] it is clear that the majority of weak D phenotypes have at least one amino acid change in RhD.

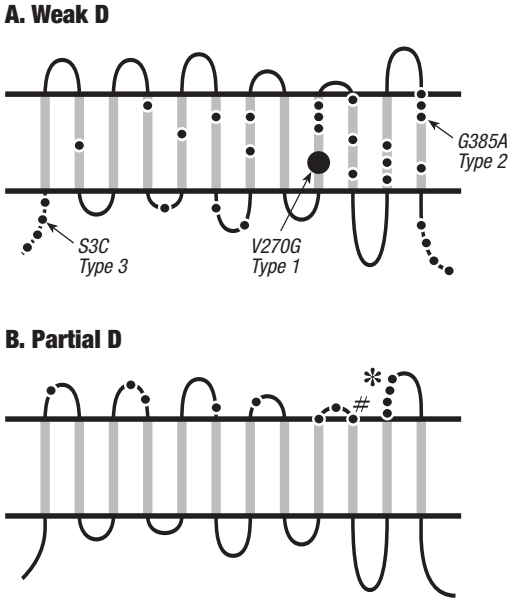

Fig. 2. Diagram of amino acid changes in RhD proteins. The location is shown as dark circles.

A. Weak D phenotypes. Amino acid changes that cause weak D expression are predicted to be located predominantly in transmembrane and cytoplasmic regions. **B.** Partial D phenotypes. Amino acid changes that cause partial D phenotyes are predicted to be located in the extracellular loops.

Table 1. Reactivity of FDA-licensed anti-D reagents with some Rh variant RBCs that resulted in D typing discrepancies

Reagent	IgM monoclonal	IgG	DVI IS/AHG	DBT	DHAR (Caucasian)	Crawford (Blacks)	Choice of reagent for
Gamma-clone	GAMA401	F8D8 monoclonal	Neg/Pos*	Pos	Pos	**Pos**	DONORS
Immucor Series 4	MS201	MS26 monoclonal	Neg/Pos	Pos	Pos	Neg	–
Immucor Series 5	Th28	MS26 monoclonal	Neg/Pos	Pos	Vary/Pos	Neg	–
Ortho BioClone	MAD2	Polyclonal	Neg/Pos	Neg/Pos	Neg/Neg	Neg	RECIPIENTS (No IAT)
Ortho Gel (ID-MTS)	MS201	–	Neg	Pos	Pos	Neg	–
Polyclonal	–	–	Neg/Pos	Neg/Pos	Neg/Neg	Neg/Neg	–

*Result following slash denotes anti-D test result by the IAT, as permitted by the manufacturer.

Because the changes are intracellular or are in the transmembrane regions of RhD and not on the outer surface of the RBC (Fig. 2A), these RBCs do not lack extracellular D epitopes.[32] They affect primarily the efficiency of insertion and, therefore, the quantity of RhD in the membrane. This is reflected in the reduced number of antigen sites on these RBCs and explains why the IAT is required for detection. Weak D expression is caused by a large number of different mutations (classified as Types 1 to 42[33]), with the most common being a Val270Gly substitution designated Type 1.[32] The majority of individuals with weak D phenotypes can safely receive D+ RBCs and will not make anti-D. Nevertheless, because amino acid changes located intracellularly or in the transmembrane regions have the potential to alter surface epitopes, it is still unclear which of the 42 different weak D types may have altered D epitopes. Indeed, individuals with weak D type 4.2 and 15 have been reported to make anti-D.[34] Therefore, they are better classified as partial D.[17]

Partial D (D categories or D mosaics)

RBCs with partial D antigens have historically been classified as such because the RBCs type as strongly D+ but the individuals make anti-D when exposed to normal D antigen. It was hypothesized that the RBCs of these individuals lack some part of RhD so that they can produce antibodies to the missing portion. Molecular analysis has shown that this hypothesis was correct and that the altered or missing portions of *RHD* are actually replaced by corresponding portions of *RHCE.* Some replacements involve single amino acids but, in contrast to weak D discussed above, these changes are predicted to be located in the extracellular loop regions of RhD (Fig. 2B). Others involve entire exons or large regions of the gene and the novel sequence of amino acids generates new antigens (e.g., D^{W}, BARC, Rh32) (reviewed in Westhoff[17] and Reid and Lomas-Francis[18]). Individuals with partial D antigens can make anti-D and, ideally, should receive D– donor RBCs. In practice, however, most are typed as D+ and are only recognized after the individual makes anti-D.

D epitopes expressed on Rhce proteins (D^{HAR}, ceCF, ceRT)

The discovery that some Rhce proteins carry D-specific amino acids that react with some monoclonal anti-D reagents adds an additional layer of complexity to D typing. Two examples, D^{HAR} (R_0^{Har}),[35,36] found in individuals of German ancestry, and Crawford (ceCF), found in individuals of African ancestry, deserve attention because of their strong reactivity (3+–4+) with some FDA-licensed monoclonal reagents and lack of reactivity with others (including the weak D test) (Table 1).

D^{HAR} RBCs can be characterized serologically by their strong reactivity with monoclonal anti-D clones GAMA401 (present in Gamma-clone) and MS201 (present in Immucor Series 4 and Ortho Gel) while showing no reactivity with MAD2 (present in Ortho tube) or with polyclonal reagents (including the weak D test). They can also react variably with Th28 (Immucor Series 5). These individuals do not have a *RHD* gene, but exon 5 of their *RHCE* gene is from *RHD* (Fig. 3). They can make anti-D when stimulated[35] and should be treated as D– for transfusion and Rh immune globulin prophylaxis and as D+ as donors.

Crawford (Rh43) is found in persons of African heritage and was first described in 1980.[37] The antigen was present on the RBCs of 1 in 950 random African Americans in the southeastern part of the United States. Most Crawford+ samples were from persons with an r'^{S} haplotype, but exceptions were seen. The Crawford allele, *ceCF,* encodes the same amino acid changes found in ce^{S} (W16C, L245V) but also carries an additional Q233E change, which is a D-specific residue on the ce^{S} background.[38,39] The D-specific residue (233E) is responsible for strong direct agglutination (3+) with the GAMA401 clone but it does not react with

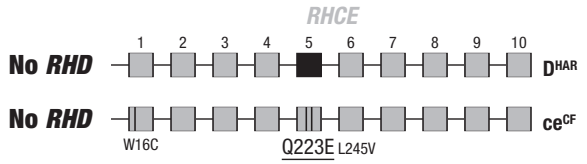

Fig. 3. Diagram of the *RHCE* genes that encode proteins reactive with some D typing reagents. The ten exons are represented by grey boxes, and the *RHD*-specific exon 5, or D-specific residues (position 223 and 245) are shown.

the anti-D present in Ortho, Immucor, and polyclonal reagents (Table 1). These individuals make anti-D when stimulated (our unpublished observations) and should be treated as D– for transfusion and Rh immune globulin prophylaxis. Although Crawford+ RBCs have not yet been reported to stimulate production of anti-D, most would agree that individuals with this phenotype are better classified as D+ as donors.

Lastly, a Rhce protein with a R154T mutation, designated ceRT, demonstrates weak reactivity with some anti-D monoclonal reagents, and the reactivity is enhanced at lower temperatures. Interestingly, this variant does not carry any D-specific amino acid but mimics a D-epitope (epD6) structure.[40]

D Typing Discrepancies

Multiple factors conspire to complicate D typing in the United States. These include the numerous methods used (i.e., slide, tube, solid phase, gel, and automated analyzers using enzyme-treated RBCs) as well as variations in the phases of testing; dissimilar monoclonal antibodies present in manufacturers' FDA-licensed reagents that can differ in reactivity with variant D antigens; and the large number of different *RHD* genes present in populations, which affects both the level of expression and, potentially, the structure and epitopes of the D antigen. To date, there are more than 100 different *RHD* genes known, including 42 that encode different forms of weak D, 40 that result in expression of different partial D antigens, 5 or 6 *DEL,* and several *RHCE* genes that encode D epitopes on the Rhce protein. If one takes into account differences in methods, antibody clones, and the variability of D expression, discrepancies are bound to occur and the surprise should probably be that they are not encountered more often.

Methods

Multiple methods for D typing are used in various facilities in the United States. A 2001 to 2004 College of American Pathologists' (CAP) survey of North American D Testing Practices[41] showed an increase in the use of gel technology (1.1%–7.7%) but multiple methods will continue to be used. Regarding weak D, although testing is the standard practice for donor D determination, there are wide variations in practices in hospital transfusion services. Data from a 1999 CAP survey[42] revealed that weak D testing was performed in 58 percent of responding facilities, although that number has likely dropped since then. A striking feature of the survey was the absence of a standard of practice regarding the D phenotype of units transfused to recipients testing positive for weak D. Forty-four percent indicated that they would give D– RBCs, while 42 percent would give D+ RBCs. About 10 percent would give D– donor RBCs if the woman was of childbearing age. These statistics may reflect uncertainty regarding the significance of weak D and also the conservation and limited availability of D– donor units.

Reagents

Early reagents developed for D antigen testing exploited antibodies produced in D-sensitized women or hyperimmunized volunteers. These polyclonal antibodies were potent and effective because they recognized numerous epitopes of D. Some were IgM antibodies causing direct agglutination but most were IgG. IgG antibodies are unable to cross-link D antigens on adjacent RBCs and cause direct agglutination, probably because of the number of sites and the lack of mobility of the protein. IgG reagents were often subjected to either chemical modification or addition of potentiating agents, with the goal of enhancing cross-linking to produce direct agglutination.

With the advent of monoclonal antibody technology in the 1980s came the promise of freedom from reliance on human source material for anti-D. The fusion of specific antibody-producing B cells with immortalized cell lines allows production of antibodies in cell culture and a potentially inexhaustible source. A large number of IgM, direct-agglutinating, anti-D monoclonals were generated, although it was soon realized that a single monoclonal anti-D specific for a single D epitope did not detect all D+ RBCs. Therefore, D typing reagents in the United States are a blend of monoclonal IgM reactive at room temperature and monoclonal or polyclonal IgG reactive by the IAT for the determination of weak D. The U.S. market offers four different reagents for tube testing and one for gel (Table 1). All but two contain different IgM clones, so

the reactivity of each with variant D antigens may differ. The FDA requires only that manufacturers specify reactivity with category DIV, DV, and DVI RBCs and only limited studies have been carried out with these U.S. reagents to characterize reactivity with other D variants.[43] Additional data are needed concerning the reactivity of molecularly characterized variant D antigens with FDA-licensed reagents. The RBCs used for these studies must be well characterized at the molecular level, as the same category D RBCs may have different genetic backgrounds that can impact the data.

Table 1 compares the reactivity of different manufacturers' reagents with some of the more frequently encountered RhD and RhCE variants that cause D typing discrepancies referred to our laboratory for investigation by molecular techniques. This serologic information, along with knowledge of the ethnic background of the donor or patient, can be very helpful in resolving the discrepancy. Additionally, although this comparison only includes four different variant D, it suggests that the Gamma reagent used in the donor setting would successfully exclude DVI, DBT, D^{HAR}, and Crawford phenotype RBCs from the D– donor pool by typing them as D+. This comparison also suggests that the Ortho tube reagent best serves these individuals in the hospital or prenatal setting by classifying the RBCs as D– for transfusion and Rh immune globulin purposes. (Note the importance of eliminating the antihuman globulin [AHG] testing phase on recipients to achieve the desired interpretation for DVI and DBT.)

The suggestion that there should be different anti-D reagents used for donor D determination and for typing patients is not new. This concept was avidly debated in the United States in the early days of implementation of monoclonal antibody reagents.[44] Discussions primarily focused on category VI RBCs, the most common D variant in Caucasians. Because DVI RBCs can stimulate production of anti-D in a D– individual, the reagent for typing donors should be reactive with these RBCs. However, since these individuals often make anti-D when exposed to conventional D through transfusion or pregnancy, the reagent for use in typing patients' samples would be selected to not react with category VI RBCs. At that time, however, it was felt that this was not practical and that being called D+ as a donor and D– as a patient was problematic.[44] Blends were judged to be the best answer; this is reflected in the current reagents available in the United States. In all of them, the IgM anti-D component does not react with DVI RBCs but the IgG component reacts with these RBCs in the AHG phase of testing (Table 1). This has prompted the movement away from weak D testing in the hospital and prenatal setting to better serve individuals with DVI RBCs by classifying them as D–. This movement was slow to develop, however, and many hospital transfusion service laboratories were still performing the weak D test in the 1999 CAP survey,[42] a decade after implementation of monoclonal reagents.

Reporting Variable Reactivity With Anti-D Typing Reagents

How does the laboratory report the D type when testing results differ between various manufacturers' reagents or between transfusion service and donor center D typing? Key to assigning D antigen status should be whether the patient is a blood donor or a transfusion recipient. DNA-based testing is very useful to confirm the molecular basis underlying D typing discrepancies, but is not always necessary if a thorough serologic workup is performed. Most agree that serologically or molecularly confirmed D variants should be considered D+ as blood donors but D– as recipients. Historically it has caused consternation within the profession to label an individual as D+ in one situation and D– in another. Laboratories fear appearing indecisive and do not want to confuse the patient or donor, the physician, or the nursing staff. However, as we move toward an age of well-informed medical care consumers, with the promise of designer health care algorithms and treatments based on genetic polymorphisms, any *RHD* polymorphism that results in altered D antigen expression is relevant and should be part of the medical record. To begin to move in this direction, one colleague has modified the acceptable Rh typing interpretation fields in the hospital computer system to include POS, NEG, and DEP. DEP is translated to "Negative*" in this hospital setting, with the following explanatory remark. *"*The Rh type is **dependent** on reagents used, tests performed, and/or technical performance. Patient may have been previously reported as Rh Positive or Rh Negative. For Transfusion Service testing, the patient will be treated as Rh Negative, a candidate for Rh Immune Globulin, and will receive Rh Negative blood. As a Blood Donor, patient will be treated as Rh Positive."** (B. Sipherd, personal communication, 2005)

European D Testing

In Europe, weak D testing is not performed; two different IgM monoclonal anti-D reagents are used for

initial D typing. For testing recipients, at least one must *not* react with category DVI RBCs, so that these individuals are properly classified as D– for transfusion and Rh immune globulin prophylaxis. Many more monoclonal anti-D reagents are available in Europe and most do not use the same clones that are licensed in the United States. The wider availability of monoclonals and the fact that a weak D test is not performed are important considerations when reviewing data from recent European publications detailing the frequency of D+ donors not detected by routine testing. One cannot directly extrapolate to the incidence in the United States when the anti-D clones and methods differ.

D Typing Concerns

Issues of concern for the determination of the D status of donors differ from those for transfusion recipients. In donors, the issue is one of detection of any and all D antigen expression. In recipients, it is one of detection of D antigens with altered D epitopes.

Donors

Ideally, tests to determine a donor's D status would detect all RBCs with any amount of D antigen or D epitope expression as D+. Unfortunately, some RBCs with the weak D phenotype may be "missed" serologically, and there are no serologic reagents to detect D_{el} RBCs. It had been suggested that the number of antigen sites, termed the *Rhesus index,* might be a way to distinguish which RBCs would be immunogenic when transfused to D– recipients, with a possible cutoff of 300 to 400 antigen sites required.[45] Antigen dose is one consideration that determines immunogenicity, but the large number of RBCs associated with a unit of blood will compensate for a low number of antigen sites. RBCs with less than approximately 30 sites stimulate anti-D.[27,28] Molecular screening for the presence of the *RHD* gene would detect these and testing could be done as *pools,*[31] lending support for future implementation of DNA testing methods in donor centers. However, the absolute association of the C phenotype with the *DEL* alleles described to date, as well as the strong association of either C or E phenotypes with weak D types that might not be detected serologically, suggest that elimination of apparent *r′* (D– C+) or *r″* (D– E+) donors from the D– donor pool would be an effective approach for those concerned about screening out donor units capable of immunizing D– recipients. Alternatively, molecular testing could target specifically this group of D– donors to test for the presence of the *RHD* gene. Indeed, molecular screening for the presence of *RHD* in D– donors who phenotype as D– and C+ or E+ has been implemented in some Central European blood centers.[46]

Patients

Ideally, tests to determine a patient's D status would distinguish those with RBCs that lack, or have altered, D epitopes (and are at risk of immunization to conventional D) from those that carry mutations that simply reduce expression levels of D (and do not confer such risk). Unfortunately, standard serologic anti-D reagents cannot discriminate partial D RBCs. Nor can they identify weak D RBCs that, similar to Type 4.2 and Type 15,[34] have altered D epitopes manifest as production of anti-D when transfused with conventional D+ RBCs. On a positive note, our long history of transfusing patients who have weak D RBCs with conventional D+ donor blood strongly suggests that weak D Types 1, 2, and 3, which comprise at least 90 percent of weak D individuals, do not make anti-D, as others have emphasized.[30] It is also important to put the "D dilemma" into perspective. We routinely accept that 10 percent of recipients are potentially exposed to c and K antigens. Severe anemia and hemolytic disease of the fetus and newborn have been reported due to maternal anti-c or -K stimulated by transfusion. Most believe that routine matching for c and K is not justified even in females of childbearing age. Perhaps it might be appropriate to revisit this policy; however, the question is one of clinical allocation of resources. Importantly, is D different, in that we will not tolerate any anti-D immunizations?

Will DNA-Based Testing Be the Answer for D Typing?

DNA-based testing strategies can, by sampling multiple regions of the *RH* genes, determine a specific weak D type or partial D category or the presence of D_{el}. Currently, this can be cumbersome, often requiring complete gene sequencing. The development of automated, high-throughput platforms that sample many regions of both *RHD* and *RHCE,* along with detailed algorithms for accurate interpretation, are needed. The significance of many of the other weak D types (Types 4–42) has not been determined because serologic evidence, in the form of immunization and production of antibodies in recipients, is not available.

Because direct experimentation and deliberate immunization cannot be used to answer the question of which RBCs lack, or have altered, D and which mutations simply reduce expression levels of D, it is important to investigate D+ patients who make anti-D. Although anti-D immunization is probably still infrequent or rare, as pointed out previously,[30] the observation that 32 percent of participants in the 1999 CAP survey had observed at least one case per year of a weak D individual who had anti-D and 21 percent had encountered two or more cases might suggest otherwise.[42] To gather the appropriate information relevant to the test methods and anti-D reagents used in the United States, it will be important for laboratories to investigate the following:

1. D– patients who receive D– products but produce anti-D.
2. Products labeled D– that may have stimulated anti-D.
3. D+ patients who make anti-D.

This should ideally include serologic workups combined with molecular DNA testing. It is the powerful combination of serology with genetics that will define our future as we move into the postgenomic era of DNA-based testing methods.

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Connie M. Westhoff, SBB, PhD, Scientific Director, Molecular Blood Group and Platelet Antigen Testing Laboratory, American Red Cross Blood Services, Penn-Jersey Region, and Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA.

Letter From the Editors

Ortho-Clinical Diagnostics Sponsorship*

It is with great pleasure that I announce that Ortho-Clinical Diagnostics has once again, for the 15th year, supported the publication of an issue of *Immunohematology.* This has been an ongoing practice since Volume 6, Number 4, 1990. The third issue (September) has become "The Ortho Issue" to the staff of *Immunohematology* and we would like to thank Ortho for its continuing dedication to the education and support of blood bankers around the world.

During my 26 years as editor of Immunohematology, I have always had great support and encouragement from the management and staff of Ortho-Clinical Diagnostics. I would personally like to express my gratitude for our many years of partnership and for the help they have always given over the years with articles, funds, and distribution of the journal. I hope that this partnership will continue with the new editors.

Delores Mallory
Outgoing Editor-in-Chief

Cindy Flickinger
Managing Editor

*Note: This letter should have been published in Volume 21, Number 3, 2005, the issue sponsored by Ortho-Clinical Diagnostics. Our sincere apologies.

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.

Letter From the Editors

Thank You to Contributors to the 2005 Issues

The journal depends on readers, authors, editorial board, peer reviewers, and our Penn-Jersey staff. We wish we could thank all of you personally, but doing so is not practical. Instead, we thank each of you as members of an honored group.

First and foremost, we thank the authors for their reviews, scientific articles, case reports, book reviews, and letters to the editors that come not only from the United States but from many countries of the world. This has given the journal an international flavor.

Our editorial board is a prestigious one and we depend on them, not only for peer reviews, but for guidance in policy and suggestions for improvements. Special thanks go to our medical editors, who review every article for medical content, and to Christine Lomas-Francis, MSc, our technical editor, who reads every article for technical content. The current board is listed by name in the front of each issue of the journal.

Our peer reviewers did a wonderful job in 2005. In each December issue we list them by name with thanks to each.

James P. AuBuchon, MD
Theresa Boyd, MD
Tony S. Casina, MT(ASCP)SBB
Brian Curtis, MT(ASCP)SBB
Geoffrey Daniels, PhD
Richard Davey, MD
Brenda Grossman, MD
Gregory Halverson, MT(ASCP)SBB
Janis R. Hamilton, MT(ASCP)SBB
Teresa Harris, MT(ASCP)SBB
A.J. Hibbard, MD
Connie Howard, MT(ASCP)SBB
Sue Johnson, MT(ASCP)SBB
Cassandra Josephson, MD
Kathy Kaherl, MT(ASCP)SBB
Melanie Kennedy, MD
Cathy Litty, MD
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Marilyn K. Moulds, MT(ASCP)SBB
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Joyce Poole, FIBMS
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Vivien E. Powell, MSc, FIBMS
Dawn Rumsey, ART(CSLT)
Kathleen Sazama, MD, JD
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Ira Shulman, MD
Frank Stearns, PhD
Jill Storry, PhD, FIBMS
David Stroncek, MD
Marilyn Telen, MD
Phyllis Walker, MT(ASCP)SBB
Connie M. Westhoff, PhD

We also want to thank the office staff at Penn-Jersey, Marge Manigly and Judy Abrams, for their help in preparing the journal for press. They manage the manuscript submissions, keep up with subscriptions, and many other behind-the-scenes tasks. We also thank Lucy Oppenheim, our copy editor; and Paul Duquette, our electronic publisher.

Finally, thanks go to our readers, whose enthusiasm and interest in the journal make it all worthwhile.

Delores Mallory
Editor-in-Chief

Cindy Flickinger
Managing Editor

Letter From the Outgoing Editor-in-Chief

Thank You

This is my last letter from the editor after 21 years. I wish I could thank individually everyone who made this journal the success it is, but that is not possible. Every author who wrote an article, every secretary who helped put *Immunohematology* together, every peer reviewer, every editor, and every financial contributor made it successful. I am profoundly grateful to each one of them.

In the 20th anniversary issues, we talked about how we got started and who helped along the way and we thanked them. Twenty-one years ago the American Red Cross was publishing a newsletter, *The Red Cell Free Press,* for the reference laboratories and I felt that the articles were good enough to be published as a peer-reviewed journal. *Immunohematology* was born and over these 21 years there have been thousands of people to thank, but I would like to honor three who have made a significant impact on the journal.

Immunohematology and I owe a great deal to Dr. S. Gerald Sandler, who never let me wander from the path of the purpose of the journal. Never publish a paper on ovarian surgery, for example. He was medical director of the American Red Cross when we started the journal 21 years ago and was always encouraging. He could always find the funding and would find a paper when we needed one. He was medical editor for 17 years and examined every article for medical accuracy as well as editing every article. He is still a peer reviewer. When we thought we had lost our chance to be cited by *Index Medicus,* I asked Dr. Sandler to take on the task. He did and we are. Not only that, our back articles will be included, which is wonderful for all of our faithful authors. *Immunohematology* would not be without Dr. S. Gerald Sandler.

Mary McGinniss was managing editor for 19 years and brought many talents to *Immunohematology.* Her background as a world-renowned immunohematologist and writer was perfect for us. She read every table and figure and made sure all the numbers added up so that the papers in *Immunohematology* had virtually no errors, spelling or otherwise. She is also one of the best scientific writers I've met and she enjoyed helping new and foreign authors whose papers might otherwise not have been published. She read and greatly improved every letter, paper, and obituary written by this editor. *Immunohematology* would not be the first-class journal it is today without Mary McGinniss.

Finally, and in many ways representing many of you, I want to honor Dr. Marion Reid. Marion was there 21 years ago when we started *Immunohematology.* She is a founding editor and a peer reviewer and she comes to every editorial breakfast at the American Association of Blood Banks with suggestions for articles, new reviewers, and editors. It has taken a long time for the journal to become cited by *Index Medicus,* but our loyal authors continued to support us with articles and our most loyal was Dr. Reid, who has sent more articles than any other author. *Immunohematology* would not have the quality it has today without Dr. Marion Reid.

I wish I could say thank you to each of you, but I hope you know that over the 21 years I have enjoyed every minute of this wonderful journey. I have learned one thing that I will pass on. Write it down or it is lost. When you write it down, send it to *Immunohematology.*

Delores Mallory
Editor-in-Chief

Letter From the Outgoing Editor-in-Chief

Changing of the Guard

It is with great pleasure that I introduce the new Editors-in-Chief of *Immunohematology.* Sandra J. Nance, MS MT(ASCP)SBB, and Connie M. Westhoff, PhD MT(ASCP)SBB, both of the American Red Cross Blood Services, will be assuming the leadership of *Immunohematology* with the first issue of 2006. To that end, over the last year they have assembled a new and vital editorial staff and are making changes that will continue to move the journal forward.

Sandy is currently the director of the Immunohematology Reference Laboratory (IRL), director of the American Rare Donor Program, the IRL division director, Atlantic Division, and an adjunct assistant professor at the University of Pennsylvania.

Sandy has been closely associated with *Immunohematology* as a contributing author, an editor, a peer reviewer, and the financial manager for many years and will bring a deep understanding to setting the future direction of the journal to meet the needs of the readers and authors.

Her professional background is outstanding. She has been a member of the AABB since 1978, has chaired the Annual Seminar Committee, Scientific Program Committee, and, currently, the Awards Committee, as well as serving on nominating committees and the Board of Directors. She is on the Editorial Board for *Transfusion* and has authored or coauthored more than 100 published articles and presented more than 100 papers at national meetings.

Sandy, who has a master's degree in pathology from the University of Maryland and an SBB from Johns Hopkins, was active in the Pennsylvania Association of Blood Banks and in the American Society of Clinical Pathologists, serving as vice chair of the Teleconference Committee. She chairs the International Society of Blood Transfusion Rare Donor Working Party.

Sandy received the AABB John Elliott Memorial Award in 1996, the ASCP Excellence in Management Award in 2002, and the Ron Dubin Memorial Award from the New York City Supervisor in 2005. She coordinates the Resident-Fellow program at the American Red Cross.

Connie is the scientific director, Molecular Blood Group and Platelet Antigen Testing Laboratory, Penn-Jersey Region, and adjunct research professor of the Department of Pathology and Laboratory Medicine at the University of Pennsylvania, where she maintains a basic research program investigating the structure and function of the Rh proteins.

Connie has contributed to *Immunohematology* for the past 15 years as an author and peer reviewer and her experience as a researcher and scientific director will add much to the future direction of the journal. She has been a member of the AABB since 1978, serving as a member of the board of directors, and has chaired the Scientific Section Coordinating Committee since 2002. She serves as reviewer for the National Blood Foundation (NBF) grant proposals and will chair the NBF Grant Review Committee in 2006. Connie has reviewed manuscripts for *Transfusion, Vox Sanguinis,* and *Blood,* and has authored or coauthored more than 25 published papers as well as presenting at more than 40 seminars and conferences in the past 4 years.

Connie, who received her PhD in molecular genetics from the University of Nebraska, has received several honors and awards, including the Katherine Beattie Award from the Michigan Association of Blood Banks, and has taught at the University of Pennsylvania Medical School since 1998.

Congratulations and welcome to the new Editors-in-Chief! They are undoubtedly superbly qualified for the job! Please give them your unqualified support!

Delores Mallory

Letter From the Incoming Editorial Staff

With Special Thanks to Delores

We take this opportunity to celebrate the years of leadership that Delores Mallory has given to *Immunohematology,* Journal of Blood Group Serology and Education. Delores became the editor-in-chief in 1984 and has held that post through this year, for a total of 21 years!

One of us (GM) first met Delores when she was at Hoxworth and Delores was in Dayton. Delores' gregarious style was evident then as it is in her position as editor-in-chief. One could see that she truly enjoyed "blood banking"—discussing the difficult cases, learning new techniques, and educating technologists and physicians. Her enthusiasm was infectious (let's not screen this out of the blood supply) and she made everyone feel very comfortable discussing immunohematology together—from technologist to researcher to physician.

Through her leadership, the journal made the transition from a newsletter, *Red Cell Free Press,* to the peer-reviewed quarterly publication *Immunohematology* in 1988 and to academic journal status indexed by *Excerpta Medica* in 1992. This has been a dynamic evolutionary process.

The editorial board expanded to encourage first-time manuscript writers to publish. And that it did! Delores has helped many fledgling authors on their way—from requesting that a manuscript be written, to offering her time and help in its writing, to moving it through the review and revision process and to its final publication. Going forward, we will continue that tradition.

Delores has a gift for networking and "coercing" leaders in the blood bank world to write review articles on timely topics. She also has encouraged medical and technical experts in the field to become peer reviewers for submitted manuscripts. With Delores' influence, *Immunohematology* has contributing authors and peer reviewers from all over the world. Her enthusiasm and zest for blood banking and for the educational purpose of *Immunohematology* have driven the success of this journal, so much so that it is currently cited in *Index Medicus.*

In spite of a heavy travel schedule since retirement, Delores has continued to perform duties as editor-in-chief and to lead the journal as a volunteer. She has been paramount in knowing the "perfect" peer reviewer for each manuscript and in offering her help in moving the issues to press. She truly has *Immunohematology* in her veins. We plan to celebrate her success and contributions to the journal at the AABB conference in Miami Beach in the fall of 2006, provided she does not have travel plans that interfere.

We wish her the best as she moves forward with her life and we thank her for all of her time and effort in making *Immunohematology* the successful journal that it is today.

Sandra J. Nance
Geri Meny, MD
Connie Westhoff

IN MEMORIAM

John Maxwell Bowman, MD

John Maxwell Bowman, MD, was born May 24, 1925, and died May 22, 2005, in Winnipeg, Manitoba, Canada.

Dr. Bowman graduated from the University of Manitoba Medical College in 1949, finished his internship in medicine, and went into rural general practice in Oakville, Manitoba for several years. He then took postgraduate training at Children's Hospital of Winnipeg, Winnipeg General Hospital's Newborn Service and Rh Laboratory, and the Babies Hospital of the Columbia Presbyterian Medical Center of New York City. Certified in pediatrics in 1956, he spent a year lecturing and studying in clinical pediatrics at Queens University before returning to Winnipeg in 1957, where he joined his brother, Dr. William Bowman, in the Department of Pediatrics of Manitoba Medical Clinic. He also became a parttime associate of Dr. Bruce Chown of the Rh Laboratory and a member of the Department of Pediatrics of the University of Manitoba. In 1961 he became medical director of the Rh Laboratory. In 1967, he became a full Professor of Pediatrics of the University of Manitoba, holding both positions until his retirement in 1996. He served as medical director of the Manitoba Red Cross Blood Services until 1982.

Through his work at the Rh Laboratory, he became a world expert in the treatment and prevention of HDN. His research touched hundreds of lives directly and hundreds of thousands of lives indirectly. One of his accomplishments was the development of Rh immune globulin used in Canada and North America (WinRho) to prevent HDN. Dr. Bowman helped form the Rh Institute, which furthered research in this area and which continues to support research at the University of Manitoba. For his work in research, treatment, and prevention of HDN, Dr. Bowman was made an Officer of the Order of Canada in 1983. Dr. Bowman greatly enjoyed teaching and lecturing and gave his last lecture in Saskatoon, 3 days before he died.

IN MEMORIAM

Professor Sir John V. Dacie, MD

Professor Sir John Vivian Dacie, MD, was born in Putney, England, on July 20, 1912, and died on February 12, 2005, in London, England, at the age of 92. He was one of the most distinguished hematologists of the 20th century.

Sir John Dacie was educated at King's College School, Wimbledon, and studied medicine at King's College, London, and King's College Hospital. From 1943 to 1945 he was a Lieutenant Colonel in the Royal Army Medical Corps. In 1946, he was appointed as Senior Lecturer in Haematology in the Department of Clinical Pathology at the Postgraduate Medical School at Hammersmith Hospital, London, and in 1957 he was appointed by the London University to the first Chair of Haematology to be established in the United Kingdom. He remained at the Hammersmith Hospital as Professor of Haematology until his retirement in 1977. After obtaining his MD in 1952, he was elected a Fellow of the Royal College of Physicians in 1956 and a Fellow of the Royal Society in 1967, and he was knighted in 1976. He was a member and, often, president of many societies. He received many honors in his lifetime.

His tremendous knowledge of hemolytic anemia began when, as a student, he closely examined blood films and recorded abnormal information. His first published paper, in 1938 was based on his desire to establish an accurate quantitative method for performing an osmotic fragility test. Sir John Dacie published 180 scientific papers in his lifetime.

His work was the first in many areas: unstable hemoglobin at the β and α chain amino acid sites, reported first in 1971; many aspects of paroxysmal nocturnal hemoglobinuria; and microangiopatic hemolytic anemia in 1962. Along with White, he developed the method used for concentrating bone marrow on the slide for films still used today. Sir John Dacie became involved in the early investigation of pernicious anemia and vitamin B12; wrote knowledgably on the diagnosis and management of hemophilia and was co-author on the Biggs paper describing "Christmas" (Factor IX) disease; he worked with the research, diagnosis, and treatment of leukemia and lymphoma patients, and of course, in all areas of the hemolytic anemias.

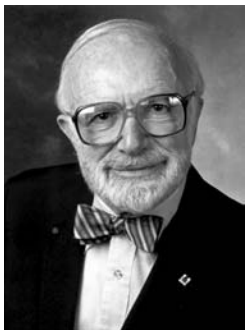
In addition to the scientific papers Sir John Dacie wrote, his major contributions to his fellow scientists are these monumental works: *The Haemolytic Anaemias* and *Practical Haematology.* The first edition of the *The Haemolytic Anaemias* was published in 1954; the third edition was published in five volumes between 1985 and 1999—a tribute to his exacting scholarly style. In 1950 he published a short paper, *Practical Haematology.* Later coauthored with Dr. Martin Lewis, the book *Practical Haematology* has been translated into several languages and is the standard text in many countries.

Sir John Dacie was an avid lepidopterist. He collected and classified butterflies and moths as a hobby, both at home and abroad. He was very proud to have become a member of the British Entomological Society, with three papers published in the Entomologists Record.

Immunohematologists deeply appreciate Sir John Dacie's many contributions and mourn his passing.

IN MEMORIAM

Tibor J. Greenwalt, MD



Dr. Tibor J. Greenwalt was born January 23, 1914, in Budapest, Hungary, and died July 17, 2005, in Cincinnati, Ohio, USA at the age of 91. He immigrated to the United States in 1920 at the age of six.

Dr. Greenwalt received his undergraduate and medical degrees from New York University, the latter in 1937. He studied hematology at New England Medical Center and served in the army in India during World War II. After the war, he became the Medical Director at what is now the Blood Center of Wisconsin in Milwaukee. Dr. Greenwalt also directed the hematology clinic at Milwaukee General Hospital and was on the faculty of Marquette University School of Medicine. He then became National Director of the American Red Cross Blood Program in Washington, DC, from the early 1960s to the late 1970s. He was Clinical Professor of Medicine at Georgetown University and consultant and lecturer at the National Naval Medical Center and the National Institutes of Health. From 1979 to 1984 he was the Director of Hoxworth Blood Center and he was the Director of Research from 1997 to 2003. In 2003 he became Emeritus Director of Research and was also Emeritus Professor of Internal Medicine and Pathology at the University of Cincinnati Medical Center.

While at the American Red Cross, he was responsible for directing research into hepatitis and for developing the first WBC filters. While at Hoxworth Blood Center, his studies included long-term storage of RBCs, resulting in new storage solutions. He was responsible for establishing the national registry of rare donors at the national organizations of both the American Red Cross and the American Association of Blood Banks.

During his long and distinguished career, Dr. Greenwalt served as vice president of the American Association of Blood Banks, of which he was a founding member. He was a founding editor of *Transfusion,* the major journal of blood banking in the world. He published more than 200 major books and scientific papers and had recently completed two scientific papers. He was elected to the Institute of Medicine of the National Academy of Science. In 2004, he received the highest honor of the American Association of Blood Banks, the Karl Landsteiner Memorial Award, and a lectureship was also named after him.

Dr. Greenwalt will be missed by his family, friends, and colleagues.

IN MEMORIAM

Professor J. J. van Loghem

After a long period of ill-health, Professor J.J. (Joghem) van Loghem died on 3rd August 2005. He specialized in Internal Medicine at the Wilhelmina Gasthuis in Amsterdam which, at that time, was one of the two academic hospitals of Amsterdam University.

In 1945 he joined the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB) as a medical officer on the reserve of the Dutch Army. He was appointed as head of the Department of Blood Group Serology with the special task of setting up typing for the Rhesus factor D, the existence of which was unknown in the Netherlands during World War II. To learn this skill he visited the laboratory of the famous Drs. Race and Sanger at the Lister Institute in London. Thus began his career in blood group serology and immunohaematology, and his friendship with many colleagues in the UK and all over the world.

In 1950, Van Loghem succeeded Dr. J. Spaander as director of the CLB, a position that he held until his retirement in 1979. Under his direction the CLB grew rapidly to become one of the largest and most famous institutes of its kind in the world, its strength being the amalgamation of different disciplines such as transfusion medicine, haematology, pathology, blood protein chemistry, and the physiology and pathology of coagulation of the blood and their clinical application.

In 1952, Van Loghem became Reader in Immunohaematology at Amsterdam University and, in 1959, became Professor of Immunopathology (extra-ordinarius), the first chair for this subject in Europe. As a result, the University Laboratory of Experimental and Clinical Immunology was integrated in the CLB.

It is not possible to mention all Van Loghem's functions, but the most important were the following: president of the Emma Hospital for sick children of Amsterdam; vice-president of the 'Society of Amsterdam University'; president of the 'Society of the Netherlands Cancer Institute'; member of the Board of the Foundation for Medical Research; member of the Central Blood Transfusion Committee of the Netherlands Red Cross; member of the Study group 'Medical Research' of the Royal Netherlands Academy of Arts and Sciences; founder and president of the Dutch Society for Immunology; president of the International Society of Blood Transfusion from 1962 to 1966; advisor of the League of Red Cross Societies for blood transfusion problems; and member of the Committee of Clinical Immunology of the International Union of Immunological Societies.

Van Loghem was a member of the Editorial Board of many important international scientific journals in the field of transfusion medicine and immunology. He founded, in 1951, the 'Bulletin of the CLB', which became the journal *Vox Sanguinis* in 1953 and, in 1956, the official journal of the International Society of Blood Transfusion. He served as Editor-in-Chief from 1956 to1960. It remained one of his special interests until the end of his life.

He received many national and international awards, such as the Cross of Merit of the Dutch Red Cross and the prestigious Karl Landsteiner Memorial Award of the American Association of Blood Banks.

He was made Officer in the Order of Oranje Nassau and, later, Knight in the Order of the Dutch Lion (both Dutch Royal distinctions) and he became Doctor Honoris Causa at the Universities of Leyden and Turin.

Van Loghem was an honorary member of several societies, such as the Dutch Society of Immunology, the International Society of Blood Transfusion, the International Society of Forensic Genetics, and the

South African Society for Blood Transfusion, among others. He was a fellow of the International Society of Haematology.

Although much interested in all aspects of immunology, Van Loghem's main field of research was immunohaematology. His work led to the detection of several new red cell blood groups. This contributed to our knowledge of the complex serological and biochemical characteristics of red cell autoantibodies and the association with their clinical significance. He made important contributions to leucocyte and platelet serology. One of his most important findings was the detection of the platelet antigen, Zw^a, now called human platelet antigen (HPA)-1a, and the fact that it occurred on no other cells of the peripheral blood. The antigen, and others of its kind, proved to be of great clinical importance and are, even now, a subject of study because of their possible association with susceptibility for cardiovascular disease. One of Van Loghem's main interests was the aetiology of autoimmunity and he was the first to suggest that 'ideopathic' autoimmunity may be caused by a viral infection. His name appears in the list of more than 200 articles in international scientific papers. Joghem was surrounded by a group of devoted co-workers, including his wife Erna, who participated in his research.

Joghem was also an excellent lecturer, not only on scientific subjects, but he was also famous for his witty and interesting after-dinner speeches.

He had an incomparable gift of encouraging co-workers and students to become enthusiastic research workers and he created opportunities for them to work under his direction. More than 60 PhD theses were completed under his guidance. Furthermore, colleagues from all over the world came to the CLB for a training period to increase their knowledge and skills.

In addition to immunology, Joghem was greatly interested in art, in particular contemporary art. He thought that the creativity in art would stimulate creativity in science. He began buying contemporary art to decorate the CLB and thus was the first in the Netherlands to form an art collection for an institute. His example was followed by many institutions, for example the Academic Medical Center in Amsterdam. He became such an expert on modern art that he was invited to become a member of the acquisition committee of the Municipal Museum of Amsterdam.

Joghem will be much missed by his wife Erna, by his family and by friends from all over the world. For us, his death is a great personal loss.

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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^a, -Fy^b, -K, -k, -Kp^a, -Js^b, -Do^b, -Wr^b, 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^a. Some are directly agglutinating (anti-M, -Wr^b, and -Rh17), and a few have been humanized into the IgM isoform and are directly agglutinating (anti-Js^b and -Fy^a). 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.

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

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.

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.**

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 2006 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 Cellular and Molecular Medicine. 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.

UPCOMING MEETINGS

February 4 Medical Technologists South Regional Seminar (CBBS)
The Medical Technologists South Regional Seminar, ***"Getting It Right!"*** sponsored by the California Blood Bank Society (CBBS) will be held on February 4, 2006, at the University of California, Los Angeles (UCLA), in Los Angeles, California. For more information, **contact** the CBBS central office at (520) 749-6908 or refer to the Web site at http://www.cbbsweb.org.

February 11 Medical Technologists North Regional Seminar (CBBS)
The Medical Technologists North Regional Seminar, ***"Getting It Right!"*** sponsored by the California Blood Bank Society (CBBS) will be held on February 11, 2006, at the American Red Cross in Oakland, California. For more information, **contact** the CBBS central office at (520) 749-6908 or refer to the Web site at http://www.cbbsweb.org.

March 7–8 Kentucky Association of Blood Banks (KABB)
The 2006 Kentucky Association of Blood Banks (KABB) Spring Meeting will be held on March 7 and 8, 2006, at the Campbell House Crowne Plaza Hotel, in Lexington, Kentucky. This meeting is held in conjunction with the Kentucky Society for Clinical Laboratory Science. For more information, **contact** Donna Ratliff at donna.ratliff@kctcs.edu or refer to the Web sites at http://www.kabb.org or http://www.kscls.org.

April 26–29 California Blood Bank Society (CBBS)
The 53rd annual California Blood Bank Society (CBBS) conference will be held April 26 through 29, 2006, at the Hyatt Regency Lake Tahoe, in Incline Village, Nevada. For more information, **contact** the CBBS central office at (520) 749-6908 or refer to the Web site at http://www.cbbsweb.org.

April 28–30 American Red Cross Immunohematology Reference Laboratory (IRL) Conference 2006
The American Red Cross Immunohematology Reference Laboratory (IRL) Conference 2006 will be held April 28 through 30, 2006, at the Hawthorne Suites—Orlando Airport, in Orlando, Florida. For more information, **contact** Cindy Flickinger at (215) 451-4909 or flickingerc@usa.redcross.org.

May 1–4 South Central Association of Blood Banks (SCABB)
The 48th annual South Central Association of Blood Banks (SCABB) meeting and exhibit will be held May 1 through 4, 2006, at the Wyndham DFW Hotel and Arlington Convention Center in Arlington, Texas. For more information, refer to the Web site at http://www.scabb.org.

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.

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

REMEMBER: THE PASSWORD IS "2000" For 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!

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

Subject Index

Contributors to Volume 21

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
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Additional information can be found by visiting the following Web sites: www.ascp.org, www.caahep.org, and www.aabb.org

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