The Rh and RhAG blood group systems

S.T. Chou and C.M. Westhoff

History

Rh is the most well-recognized blood group system after ABO because of the immunogenicity of the principal antigen, D. Discovered more than 70 years ago, the D antigen is well known as the target in severe and potentially fatal hemolytic disease of the fetus and newborn (HDFN).[1] Today, HDFN caused by anti-D is much less frequently observed in developed countries owing to prevention by administration of Rh immunoglobulin (RhIgG; anti-D IgG) after delivery of a D+ infant.[2,3] Children of women of European extraction are more likely affected as D– is most common in Caucasians (15–17%), less common in African Blacks (3–5%), and uncommon or rare in Asians (<0.1%).[4] Thus, the D antigen status is not routinely tested for in some parts of Asia, principally China, where D– is considered a rare blood type. HDFN caused by anti-D is still seen where women have limited access to prenatal care and in countries where RhIG administration is not standard practice. Anti-D during pregnancy is also more often seen if RhIG is not administered during the third trimester of pregnancy in addition to after delivery. The policy of giving antepartum RhIG to all D– women has not been adopted in all countries.

The Rh system is a complex RBC antigen system that includes more than 50 different antigenic specificities (Table 1). "Rh-positive" and "Rh-negative" refer to the presence or absence of the D antigen. The five principal Rh antigens—D, C, c, E, and e—are responsible for the majority of clinically significant antibodies. Some of the others represent compound specificities, i.e., ce or f is an epitope on the Rhce protein that is not present on RBCs with RhcE or RhCe. Many of the antigens are found primarily in a population group or specific ethnic group; for example C^X has the highest prevalence in Finns, and V and VS are found primarily in African Black ethnic backgrounds (summarized in Reid and Lomas-Francis[5]).

Our understanding of the Rh system has been greatly advanced since the genes were cloned in the early 1990s. The sequence of *RHCE* was first reported in 1990,[6,7] and *RHD* was subsequently sequenced in 1992.[6,8] The different *RHCE* alleles responsible for the C or c and E or e antigens were clarified in 1994.[9] In the last decade, molecular genotyping has revealed that the genetic diversity of the *RH* locus greatly exceeds estimates predicted by serology. More than 170 *RHD* and more than 70 different *RHCE* alleles have been described, and new alleles are still being discovered. A directory of *RHD* alleles is maintained on the RhesusBase website, and *RHCE* alleles are found on the National Center for Biotechnology Information (NCBI) human blood group mutation Web site.[10] In an effort to standardize terminology, the International Society of Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Blood Group Terminology maintains a list of alleles that encode blood group antigens (see Web Resources).

Terminology and Nomenclature

The term "Rh" for Rhesus resulted from work by Landsteiner and Wiener, who found that antiserum produced by immunizing guinea pigs and rabbits with RBCs of the *Rhesus macaque* agglutinated 85 percent of human RBCs. Initially, they believed that a common factor "Rh" had been identified in animals and humans.[11] The antibody actually detected the LW antigen, which is present on D+ RBCs in greater amounts than on D– RBCs. Although misnomers, the terms Rh factor and anti-Rh continued in common usage to describe the human D antigen and the D antibody. Although the use of "Rhesus" is now discouraged, it has been pointed out (WA Flegel, personal communication) that "Rh" can be difficult to translate into other languages.

Over the years, four Rh system nomenclatures have been introduced (Table 1). Terminology established by Fisher-Race in England was based on the premise that three closely linked genes, C/c, E/e, and D, were responsible for the antigens, whereas the Wiener nomenclature (Rh-Hr) was based on the belief that a single gene encoded several blood group factors. The Rh system is encoded by two genes, *RHD* and *RHCE,* as predicted by Tippett.[12] Rosenfeld gave each Rh antigen a number based on the order of its discovery or assignment to the Rh system. The ISBT Working Party on Terminology for Red Cell Surface Antigens adopted a six-digit number for each RBC antigen.[13] The first three numbers represent the system (the number 004 was assigned to the Rh system), and the remaining three digits refer to the antigenic specificity (Table 1).

Current terminology distinguishes the genes and the proteins from the antigens. Capital letters, with or without italics, are used when referring to the *RH* genes, *RHD* and *RHCE*. Alleles of *RHCE* are designated after an asterisk, i.e. *RHCE*ce, RHCE*Ce, RHCE*cE, RHCE*CE,* according to which antigens they encode. Alleles of *RHD* are designated *RHD*DVI, RHD*DIIIa, RHD*weak D type 2,* and so on, according to the partial or weak D encoded, and the alleles have also been given numbers by the ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology (see Table 2 for examples and Web Resources). Lastly, the Rh proteins are indicated without italics and with the "h" in lowercase, as RhD or RhCE, or according to the specific antigens they carry, Rhce, RhCe, RhcE, or RhCE.

Table 1. Rh and RhAG blood group systems: antigen terminology/nomenclature

ISBT	Rosenfeld	Fisher-Race DCE	Wiener	Other names
Rh				
004001	Rh1	D	Rh_0	
004002	Rh2	C	rh′	
004003	Rh3	E	rh″	
004004	Rh4	C	hr′	
004005	Rh5	E	hr″	
004006	Rh6	ce	Hr	
004007	Rh7	Ce	rh_i	
004008	Rh8	C^W	rh^W	Willis
004009	Rh9	C^X	rh^X	
004010	Rh10	V	hr^V	
004011	Rh11	E^W	rh^{W2}	
004012	Rh12	G	rh^G	
Rh13, 14, 15, 16 obsolete				
004017	Rh17		Hr_0	
004018	Rh18		Hr^S	Shabalala
004019	Rh19		hr^S	Shabalala
004020	Rh20	VS		
004021	Rh21	C^G		
004022	Rh22	CE	Rh	Jarvis
004023	Rh23	D^W		Wiel
Rh24 and Rh25 obsolete				
004026	Rh26	c-like		Deal
004027	Rh27	cE	rh_i	
004028	Rh28		hr^H	Hernandez
004029	Rh29			Total Rh
004030	Rh30	D^{Cor}		Gonzalez, Go[a]
004031	Rh31		hr^B	Bastiaan
004032	Rh32		R^N	
004033	Rh33			Har, D^{Har}
004034	Rh34		Hr^B	Bastiaan
004035	Rh35			1114
004036	Rh36			Be[a] (Berrens)
004037	Rh37			Evans
Rh38 obsolete (see Duclos below)				
004039	Rh39	C-like		
004040	Rh40			Tar, Targett
004041	Rh41	Ce-like		
004042	Rh42	Ce^S	rh_i^s	Cce^S, Thornton Crawford
004043	Rh43			
004044	Rh44			Nou
004045	Rh45			Riv
004046	Rh46			Sec
004047	Rh47			Dav
004048	Rh48			JAL
004049	Rh49			STEM
004050	Rh50			FPTT
004051	Rh51			MAR
004052	Rh52			BARC
004053	Rh53			JAHK
004054	Rh54			DAK
004055	Rh55			LOCR
004056	Rh56			CENR
	RH57			CEST
	Rh58			CELO
	Rh59			CEAG
RhAG				
030001	RhAG1	Duclos		Prev 901013
030002	RhAG2	Ol^a		Prev 700043
030003	RhAG3	DSLK		

Genetics and Inheritance

RH Genes and Rh Proteins

RH genes are inherited as codominant alleles. *RHD* encodes the D antigen and *RHCE* encodes CE antigens (ce, cE, Ce, or CE). Each gene has ten coding exons, and the RhD and RhCE proteins differ by 32 to 35 amino acids (depending on the specific CE allele). The large number of differences explains why D, when seen by the immune system of a D– person who lacks the protein, often induces a robust immune response. The large number of amino acid differences also explains the numerous epitopes of the D antigen, estimated to range from 9 to more than 30.[14] Although only nine or ten of the amino acid changes are predicted to be on the extracellular surface of the RBC membrane, changes located in the transmembrane and cytoplasmic regions also alter the topology and epitopes of the protein. Although early on it was assumed that only extracellular changes would prompt an immune response to Rh proteins, new antigens identified by an antibody response are also generated with intracellular changes. For example, the Leu245Val change in Rhce proteins is not extracellular, but this change results in expression of the novel RBC antigens V and VS, which are also serologic markers for an altered e antigen. Patients who are homozygous for altered *Rhce* alleles encoding partial e antigens type serologically as e+, but are at risk for anti-e production when exposed to conventional Rhce protein.

The *RHCE* gene encodes C/c and E/e antigens on a single protein. C and c differ by four amino acids, whereas the E and e antigens differ by only one amino acid. Although the common alleles are *RHCE*ce, RHCE*Ce,* and *RHCE*cE,* a large number of alleles with additional polymorphisms are found in different ethnic groups and are discussed in a later section.

Molecular Basis of Rh Antigens

D Antigen

D Negative (Rh-Negative)

Several genetic mechanisms are responsible for the D– phenotype, but deletion of the *RHD* gene is the primary background.[15] Some exceptions include silenced *RHD* as a result of point mutations, nucleotide insertions, premature stop codons, or *RHD-CE* hybrids. In African Blacks, 66 percent of D– individuals have a 37-bp insertion in *RHD* causing a premature stop codon, whereas 15 percent carry a hybrid *RHD-CE-D* characterized by expression of weak C but no D antigen.[16] The rare D– phenotype in Asians is often attributable to point mutations in *RHD,* although 10 to 30 percent of Asians who type as D– have very low levels of D; this phenotype is termed D_{el}.[17]

D Positive (Rh-Positive)

Most people with D+ RBC phenotypes have conventional RhD protein, but more than 150 different alleles encoding changes in the amino acid sequence of the

Table 2A. Examples of *RHD* alleles†

Phenotype(s) or category	Allele designation	Nucleotide changes	Amino acid changes
D	*RHD*01*		
RH:1	*RHD*		
Partial D examples			
DII	*RHD*02*		
	*RHD*DII*	1061C>A	Ala354Asp
DIIIa	*RHD*03.01*	186G>T	Leu62Phe
RH:54 (DAK+)	*RHD*DIIIa*	410C>T	Ala137Val
		455A>C	Asn152Thr
		602C>G	Thr201Arg
		667T>G	Phe223Val
DIIIb	*RHD*03.02*	Hybrid with CE exon 2	Ile60Leu
RH:–12	*RHD*DIIIb*	*RHD*D-CE(2)-D*	Ser68Asn
G–			Ser103Pro
Weak D example			
Type 1	*RHD*01W.1*	809T>G	Val270Gly
	*RHD*weak D type 1*		
D– examples			
D–	*RHD*01N.01*		Complete deletion
D–	*RHD*04N.01* *RHD*Pseudogene* *RHD* Ψ*	37-bp insert, 609G>A, 645G>C, 677T>G, 674C>T, 807T>G	inactive

W in the allele name indicates a weak phenotype.

N in the allele name indicates a null phenotype.

†ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology

protein, or changes in the conserved nucleotides at the ends of coding exons required for efficient splicing of pre-mRNA to generate mature transcripts, have been reported. These result in weak D, partial D, and D_{el} phenotypes.

Weak D

Although common D phenotypes have 10,000 to 30,000 antigen sites per RBC, weak D RBCs have less than 100 to 5000 sites (summarized in Reid and Lomas-Francis[5]). Historically, weak D RBCs were defined as requiring the IAT for detection, but with the introduction of monoclonal antibodies this depends on the specific reagent used to test the RBCs. Weak D phenotypes are most often associated with single nucleotide changes in *RHD*.[18] These encode amino acid changes that negatively affect insertion of the protein in the membrane or negatively affect efficient splicing of the coding exons, resulting in reduced expression of D antigen on the RBCs. To date, more than 70 different mutations causing weak D expression have been reported, the most common encoding a Val270Gly change called weak D type 1.[18] The different weak D phenotypes have been given number designation, i.e., 1 through 73 to date. (summarized by Wagner, FF. RhesusBase Web site; see Web Resources)

Additionally, less D antigen is present in cells that also have C antigen, such that an R_2R_2 individual (DcE/DcE) has more D antigen sites than an R_1R_1 individual (DCe/DCe). A weak D phenotype can occur when Ce (r′) is present in *trans* to *RHD,* and depression of an already weak D type by Ce (r′) can result in an apparent D– (rr′, r′r′, or r″r′) or D_{el} phenotype in a sample that is in reality R (weak D)/r′(Ce).

D_{el}

RBCs with a D_{el} phenotype express very low levels of D antigen detected serologically only by adsorption and elution with anti-D. D_{el} is most commonly found in Asians, who comprise 10 to 30 percent of individuals who type as D– with standard serologic testing. D_{el} is less often found in Europeans (0.027%). In Asians, the D_{el} phenotype most often results from a nucleotide change 1227G>A, which does not affect the amino acid sequence but interferes with efficient splicing of exon 9, whereas a single amino change, Met295Ile, is the most common mutation in Europeans.[17,18,19] D_{el} RBCs are primarily diagnosed by RHD genotyping or adsorption and elution studies because the RBCs type as D–.

Partial D

RBCs with partial D type serologically as D+, but individuals can produce anti-D when stimulated by transfusion or pregnancy. Because the D antigen consists of numerous conformation-dependent epitopes (epD), a person who lacks one or more D epitopes can form alloantibodies to the missing portion(s). More than 30 D epitopes have been defined with monoclonal antibodies, designated epD1 through epD9 with further subdivisions of each.[14] Point mutations in *RHD* causing single amino acid changes, or more often hybrid genes in which portions of *RHD* are replaced by the corresponding regions of *RHCE,* cause partial D. The novel sequences of the hybrid protein resulting from regions of RhD joined to RhCE result in the loss of D epitopes and may generate new low prevalence or cause the loss of high prevalence antigens.

Elevated D or D– – Haplotypes

Deletion phenotypes, designated D– –, Dc–, and DC^W–, (the dashes represent missing antigens) can have enhanced expression of D antigen and no, weak, or altered C/c and E/e antigens.[20] Many are caused by replacement of portions of *RHCE* by *RHD,* such that the additional *RHD* sequences in *RHCE,* along with a normal *RHD,* account for the increased D expression. However, some are caused by mutations that alter or silence *RHCE*. For example, a silenced *RHCE*cE* designated *RHCE*cE(907del C)* encodes a stop codon and is associated with the D– – haplotype and the absence of c and E antigen expression in Hispanics.[21]

Table 2B. Examples of RHCE alleles.† The nucleotides and amino acids diagnostic for the presence of *RHCE*ce* are indicated shaded as the reference allele and the changes diagnostic for each altered allele are shown.

Phenotype	Allele	Nucleotide Changes	Amino acid changes	Also reported as
ce‡	*RHCE*01*			
RH:4 or c	*RHCE*c*	307C	Pro103	
RH:5 or e	*RHCE*e*	676G	Ala226	
RH:6 or ce or f	*RHCE*ce*			
	*RHCE*01.01*	48G>C	Trp16Cys	
	*RHCE*ce.01*			
	*RHCE*01.02*	48G>C	Trp16Cys	
	*RHCE*ce.02*	1025C>T	Thr342Ile	*RHCE*ceTI*
Ce§	*RHCE*02*			
RH:2 or C	*RHCE*C*	48G>C	Trp16Cys	
		178C>A	Leu60Ile	
		203A>G	Asn68Ser	
		307C>T	Pro103Ser	
RH:5 or e	*RHCE*e*	676G	Ala226	
RH:7 or Ce	*RHCE*Ce*			
cE‖	*RHCE*03*	676G>C	Ala226Pro	
RH:4 or c	*RHCE*c*			
RH:5 or e	*RHCE*E*			
RH:27 or Ce	*RHCE*cE*			
CE**	*RHCE*04*	48G>C	Trp16Cys	
RH:2 or C	*RHCE*C*	178C>A	Leu60Ile	
RH:3 or E	*RHCE*E*	203A>G	Asn68Ser	
RH:22	*RHCE*CE*	307C>T	Pro103Ser	
		676G>C	Ala226Pro	

†ISBT Working Party on Red Cell Immunogenetics and Blood Group Terminology.
‡ce=01 §Ce=02 ‖cE=03 **CE=04

D and D-like Epitopes on Rhce

One further complication of serologic D status determination is the expression of D epitopes by the protein product of the *RHCE* gene in the absence of *RHD*. Two significant examples are D^{HAR}, found in individuals of German ancestry,[22,23] and Crawford (ceCF), found in individuals of African ancestry.[24] These two are notable because the RBCs show strong reactivity with some monoclonal anti-D reagents (3+ to 4+), but are nonreactive with others. Other amino acid changes in Rhce mimic a D-epitope structure, designated ceSL and ceRT, and such RBCs react weakly to some but not all monoclonal anti-D.[25,26] Importantly, the phenotypes discussed here lack the RhD protein, and individuals with them can be readily sensitized if transfused with D+ RBCs.[22,27]

Frequency of Altered RHD

Approximately 2 percent of Europeans carry altered *RHD,* and those most often encountered will vary by geographic location, which reflects population diversity in the region. In our experience, weak D is found primarily in European and Asian backgrounds. The incidence of partial *RHD* alleles in individuals with African backgrounds has not been determined but seems to be significantly higher than in European populations.

C/c and E/e Antigens

The common *RHCE* alleles encode the C or c and E or e antigens. Of the four amino acid changes (Cys16Trp; Ile60Leu; Ser68Asn; Ser103Pro) associated with the C to c polymorphism, Ser103Pro correlates with C/c antigen expression. The E to e polymorphism is caused by a single amino acid substitution, Pro226Ala. Numerous changes in *RHCE* cause quantitative and qualitative changes in C/c or E/e antigen expression, with altered C and e most often encountered. More than 70 different *RHCE* alleles are associated with altered, weak, or no expression of the principal antigens.

Compound Antigens (ce, Ce, cE, and CE)

Compound antigens define epitopes that depend on conformational changes that result from amino acids associated with *both* C/c and E/e. These were previously referred to as *cis* products, to indicate the antigens were expressed from the same haplotype, but it is now known that these are expressed on a single protein. These include ce or f, Ce or rh_i, cE or Rh27, and the rare CE or Rh22. Serologically, RBCs with *Dce/DCE* or *DcE/DCe* haplotypes would be identical when tested with common Rh antisera, but only the former sample would react with anti-f.

Altered or Variant C and e Antigens

In Caucasians, altered C is not common but when encountered is primarily associated with amino acid changes Gln41Arg or Ala36Thr, and expression of the C^W and C^X antigens, respectively. RBCs that express C^W and C^X, in the absence of C *in trans* have weak expression of C antigen. Altered C is also associated with RhCE proteins expressing novel antigens, including JAHK (Ser122Leu), and JAL (Arg114Trp). The RBCs type as C+, but patients can make anti-C when exposed to conventional C antigen.

Altered *RHCE* alleles are relatively common in individuals with African ancestry. In this population, the most common cause for altered C is an *RHDIIIa-CE(4-7)-D* hybrid gene, which does not encode a D antigen, but encodes altered C antigen on a hybrid protein background (Fig. 1). Approximately 25 percent of African Americans have this hybrid.[16] In most cases, the RBCs type C+ with commercial monoclonal reagents, and the altered C goes undetected. Most of these patients coinherit an altered e antigen and have a V–VS+ phenotype; together this haplotype is referred to as (C)ceS.[28] Because altered *RHCE* alleles are not distinguished by routine serologic tests, homozygous patients often make anti-C and anti-e alloantibodies after transfusion. Additionally, homozygous individuals typically lack high-prevalence antigens, designated hr^B and hr^S.

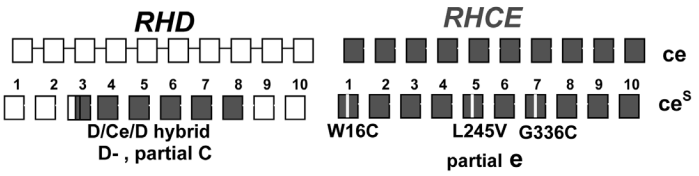

Figure 1: Representation of the *RH* gene associated with altered C antigen. The *RHD* and *RHCE* genes are shown as 10 boxes representing the coding exons. Amino acid changes are indicated by their single letter designation and the position of the amino acid in the protein is given.

Anti-hr^B and anti-hr^S can be clinically significant,[29,30] and finding compatible blood can be very difficult because of differing *RH* gene backgrounds.[31,32] As an additional complication, altered *RHCE*ce* are often inherited with partial *RHD* (DIII, DAU, DAR, etc.). The inheritance of partial D with altered *RHCE*ce* may lead to production of antibodies sometimes identified as anti-Hr^B (RH34) and anti-Hr (RH18).

G Antigen

The G antigen is found on RBCs expressing C or D with a 103Ser residue present on RhD, RhCe, or RhCE. Serologically, anti-G reacts as though it were anti-D plus anti-C. Anti-G explains the D– person transfused with D–C+ blood, or the D– woman who delivered a D–C+ child, who subsequently appears to have made anti-D. To distinguish anti-D, -C, and -G, adsorption and elution studies can be performed.[33] Their results determine the need for RhIG prophylaxis for obstetric patients with anti-G to prevent additional immunization and formation of anti-D.

Antibodies in the Rh System

Most Rh antigens are immunogenic and have the potential to cause clinically significant HDFN or transfusion reactions. Rh alloimmunization occurs by exposure to foreign RBCs during pregnancy or after transfusion and may persist for many years. Even if serum antibody drops below detectable levels, subsequent exposure to the antigen can produce a rapid secondary immune response. Outside of the ABO system, the D antigen is the most immunogenic antigen, followed by c and E. Anti-c can cause severe HDFN, whereas anti-C, -E, and -e are associated with mild HDFN. Because Rh alloimmunization is IgG-mediated, transfusion reactions are primarily extravascular. The patient may have fever, jaundice, icteric sclera, dark urine, or a drop in hemoglobin level. Antigen-negative RBCs should be provided to any patient with a history of Rh antibody sensitization.

Rh antibodies are often found together. It is not unusual for a patient with a single Rh antibody to become alloimmunized to additional Rh antigens if further exposed. For example, a DCe/DCe (R_1R_1) patient with anti-E has usually been exposed to the c antigen as well. Anti-c may be present at the time anti-E is identified, but may be weak and undetectable. Therefore, to prevent delayed transfusion reactions, some advocate avoiding c+ blood for patients alloimmunized to the E antigen. In contrast, pursuing anti-E in serum containing anti-c is not necessary as the patient will likely have been exposed to c without being exposed to E. Moreover, the vast majority of c– donor blood will be negative for E.

Rh Antibodies in Patients with Sickle Cell Disease

Some sickle cell transfusion programs perform Rh typing to match patients and donors for D, C, and E (in addition to K) to prevent alloimmunization.[34,35] The prevalence of *RH* alleles that encode altered or variant D, C, and e antigens in African ethnic groups often underlies the production of complex Rh alloantibody specificities in chronically transfused patients with sickle cell disease (SCD). Many altered Rh protein epitopes cannot be distinguished serologically, and are only identified by RH genotyping once a patient develops apparent autoantibodies to Rh antigens. Some common examples include the partial C antigen encoded by the r′S allele, i.e. (C)ceS, and partial DIIIa; the RBCs react 3+–4+ with monoclonal reagents and are not detected as altered. Anti-hr^S, -hr^B, -Hr^B, and -Hr can be difficult to identify and can cause clinically significant hemolytic transfusion reactions including transfusion fatalities.[30]

Other Rh Antibodies

In addition to the SCD population, D– – and Rh_{null} individuals also develop alloantibodies to high-prevalence Rh antigens with Rh17 and Rh29 specificity, respectively. Lastly, autoantibodies to high-prevalence Rh antigens often occur in the sera of patients with warm autoimmune hemolytic anemia and in some cases of drug-induced autoimmune hemolytic anemia.[36] The biologic explanation for this apparent specificity or cross-reactivity remains to be determined.

Clinical Considerations

Determination of D status

The *AABB Standards for Blood Banks and Transfusion Services*[37] requires donor blood to be tested for weak D expression and to be labeled as Rh-positive if the test is positive. Hospital transfusion services are also required to confirm the D-negative status before the unit is released for transfusion to avoid transfusion of D+ RBCs to D– recipients. It is recognized that some very weak D antigens are not detected, and standard typing procedures (including IAT) do not detect D_{el} RBCs. Isolated cases have been reported in which very weak D and D_{el} donor units have caused anti-D alloimmunization in D– recipients.[38–41]

When determining the D type of a patient, detecting weak D expression is not necessary unless testing RBCs of an infant of a D– mother at risk for D immunization. DVI is the most common partial D found in Caucasians, and anti-D produced by women with partial DVI has resulted in

fatal hemolytic disease.[42] Therefore, current FDA-licensed monoclonal IgM reagents are selected to be nonreactive with partial DVI RBCs in direct tests. Performing only a direct test and eliminating the indirect test for female children and women of child-bearing age avoids the risk of sensitization by classifying DVI as D– for transfusion and RhIG prophylaxis.

Patients who have RBCs expressing the common type 1, 2, or 3 weak D phenotype usually do not make anti-D and can safely receive D+ blood. Patients with the rare weak D types 11 and 15 have become alloimmunized, suggesting they have altered D epitopes.[38] The risk for anti-D production in other uncommon weak D types is unknown. In contrast to weak D, patients with partial D RBCs form alloanti-D directed at the missing D epitopes and should receive D– blood and RhIG when indicated. Unfortunately, serologic reagents frequently do not distinguish partial D status. Many partial D RBCs, including DIIIa, the most common partial D in African Americans, type strongly D-positive in direct tests and are only recognized after producing anti-D.

Sickle Cell Disease and Altered *RH* Alleles

RBC alloimmunization remains a serious complication of transfusion for patients with SCD, with more than 25 to 30 percent of chronically transfused SCD patients developing RBC antibodies. Altered D, C, and e antigens often underlie the complex RH alloantibodies SCD patients exhibit after transfusion. Although randomized controlled trials have not been performed, extended RBC antigen-matching (including D, C, E, and K) has been shown in numerous single-institutional and prospective multicenter experiences to significantly reduce the incidence of alloantibody production in SCD.[43–45] Therefore, many centers determine the pretransfusion RBC serologic phenotype and provide RBCs that are antigen-matched for D, C, E, and K. Because the high alloimmunization rates are also believed to be caused by antigenic disparity between African Americans and Caucasians, some programs actively recruit African American donors to supply blood specifically for patients with SCD.[34,35] Although these approaches reduce the incidence of alloantibody production, some patients still become sensitized to Rh antigens (D, C, e, and hr^B, Hr^B, hr^S, Hr^S), indicating they were not Rh antigen matched.

RH Genotyping

RBC genotyping methods were introduced to transfusion medicine a decade ago after cloning of the genes made genetic testing for blood groups possible. Genotyping methods include amplification of target gene sequences by PCR followed by analysis using RFLP, real-time PCR, or sequence-specific primer PCR. More recently, mass-scale genotyping technologies have been developed by several manufacturers to perform high-throughput blood group prediction.[46,47] There is nearly complete concordance between the genotype and serologic phenotype, and studies have included samples from diverse backgrounds. However, the complexities and numerous alleles in the Rh blood group system have precluded high-throughput genotyping for *RH* other than for the common nonvariant forms of C/c and E/e antigens. The detection of numerous silencing mutations is required for accurate typing; several regions of the genes must be sampled to detect multiple alleles, and new alleles are continuously being identified.[48]

Currently, *RH* genotyping is primarily restricted to specialized immunohematology laboratories. One application of *RH* genotyping is to find compatible donors in the American Rare Donor Program (ARDP) for patients with antibodies to high-prevalence Rh antigens.[32] Genotyping can also be used to determine the *RHD* status of a fetus by amniocentesis or chorionic villus sampling. More recently, noninvasive techniques have been used to collect cell-free, fetal-derived DNA from maternal plasma for *RHD* genotype prediction with a high-throughput method.[49,50] Once high-throughput platforms encompassing all the Rh variants are developed and are cost effective, *RH* genotyping can complement serologic testing for typing transfused patients, *RHD* zygosity determination, fetal D typing, resolution of D status, and finding compatible blood for patients with SCD.

RhAG System

RHAG is the ancestral gene from which *RHCE* and *RHD* arose. It resides on chromosome 6 and encodes the Rh-associated glycoprotein and the antigens of the RhAG blood group system. The RhAG protein consists of 409 amino acids and associates with the Rh proteins in the membrane to form the Rh-core complex.[51]

Antigens

The RhAG protein does not express the common Rh antigens, but it has recently been shown to carry several antigens,[52] and it has been assigned system 30 by ISBT (Table 1). Two high-frequency antigens, Duclos and DSLK, and one low-frequency antigen, Ol^a, had serologic characteristics suggestive of expression on RhAG. This was confirmed by gene sequencing and expression of recombinant RhAG in HEK 293 cells. The Duclos-negative patient was homozygous for a nucleotide 316C>G change, encoding Gln106Glu. The DSLK-negative patient was homozygous for nucleotide 490A>C, encoding Lys164Gln. Two Ol(a+) family members of a Norwegian family were heterozygous for 680C>T encoding Ser227Leu. Duclos is *RHAG1,* Ol^a is *RHAG2,* and DSLK is provisionally *RHAG3*.

Rh_{null}

RhAG protein must be present for the expression of Rh antigens. Rh_{null} RBCs lack expression of Rh antigens, and the phenotype most often results from mutations in *RHAG,* previously termed "regulator" Rh_{null}. Less often, Rh_{null} individuals have inactivating or silencing mutations in *RHCE* and deletion of *RHD* previously referred to as "amorph" Rh_{null}.[33]

The Rh-core complex also interacts with band 3, glycophorins A and B, LW, and CD47 and is believed to contribute to the RBC membrane structure because Rh_{null} RBCs demonstrate abnormal morphology. The function of the Rh blood group proteins is not known, but RhAG is an ammonia transporter, and the recent crystalization of the human RhAG homolog from the kidney, RhCG, confirms the relationship of human Rh proteins to ammonia transporters.[53]

Summary Perspective

The Rh/RhAG blood group systems are some of the most complex, and in the past two decades major insights have been gained into the molecular basis of the Rh system. The 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 system. 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.[12] Their work is the foundation of our understanding today, as new genetic information builds on the serologic backbone. Serologic reactivity is still the basis for blood groups and blood transfusion practice because serology defines an antigen. That will not change as we use DNA-based testing methods because it is important to remember that without a serologic relationship, a variation in a blood group allele at the DNA level is just another SNP (single nucleotide polymorphism), and these occur once in every 100 to 300 bp in the human genome. These polymorphisms are only of academic interest until associated with a phenotype and found to be relevant to transfusion medicine by stimulation of an antibody.

Molecular testing will be a powerful adjunct to serologic methods and improve transfusion safety and outcomes, particularly for the chronically transfused population. Which of the numerous alleles in the Rh system are clinically significant is yet to be fully determined. Ultimately, with the development of mass-scale *RH* genotyping technology, genetically matching donor units with the patients could significantly reduce alloimmunization, and *RHD* genotyping will provide a definitive D classification for donors and patients. The availability of cost-effective, reliable high-throughput genotyping platforms is needed for this to become incorporated into clinical transfusion medicine practice.

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Web Resources

International Society of Blood Transfusion (ISBT) Working Party on Red Cell Immunogenetics and Blood Group Terminology. http://ibgrl.blood.co.uk/ISBTPages/ISBTHome.htm. Accessed 1/4/2011.
RhesusBase. http://www.uni-ulm.de/~fwagner/RH/RB/. Accessed 1/4/2011.
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Stella T. Chou, MD, Assistant Professor of Pediatrics, Division of Hematology, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, 3615 Civic Center Boulevard, ARC 316D, Philadelphia, PA 19104, and Connie M. Westhoff, SBB, PhD, Director of Immunohematology and Genomics, New York Blood Center, 310 East 67th Street, New York, NY 10065.