The Rh blood group system: a review

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The Rh blood group system is one of the most polymorphic and immunogenic systems known in humans. In the past decade, intense investigation has yielded considerable knowledge of the molecular background of this system. The genes encoding 2 distinct Rh proteins that carry C or c together with either E or e antigens, and the D antigen, have been cloned, and the molecular bases of many of the antigens and of the phenotypes have been determined. A related protein, the Rh glycoprotein is essential for assembly of the Rh protein complex in the erythrocyte membrane and for expression of Rh antigens. The purpose of this review is to provide an overview of several aspects of the Rh blood group system, including the confusing terminology, progress in molecular understanding, and how this developing knowledge can be used in the clinical setting. Extensive documentation is provided to enable the interested reader to obtain further information. (Blood. 2000;95:375-387)

Introduction

The Rh blood group system was first described 60 years ago. A woman had a severe transfusion reaction when she was transfused with blood from her husband following delivery of a stillborn child with erythroblastosis fetalis. Her serum agglutinated red blood cells (RBCs) from her husband and from 80% of Caucasian ABO-compatible donors.[1] The following year, Landsteiner and Wiener[2] found that sera from rabbits (and later guinea pigs) immunized with RBCs from *Macaca mulatta* (*Macacus rhesus* in the original paper) agglutinated 85% of human RBC samples. Initially, it was thought that the animal and human antibodies identified a common factor, Rh, on the surface of *rhesus* and human RBCs. It was soon realized that this was not the case.[3] Therefore, the original terms (Rh factor and anti-Rh) coined by Landsteiner and Wiener, although being misnomers, have continued in common usage. The heteroantibody was renamed anti-LW (after Landsteiner and Wiener), and the human alloantibody was renamed anti-D.[4]

The Rh blood group system is the most polymorphic of the human blood groups, consisting of at least 45 independent antigens and, next to ABO, is the most clinically significant in transfusion medicine. The ability to clone complementary DNA (cDNA) and sequence genes encoding the Rh proteins has led to an understanding of the molecular bases associated with some of the Rh antigens. Serologic detection of polymorphic blood group antigens and of phenotypes provides a valuable source of appropriate blood samples for study at the molecular level. This review summarizes our present understanding of the complexities of Rh blood group expression and how this knowledge impacts on clinical situations that arise through Rh blood group incompatibility.

Terminology

Several nomenclatures have been used to describe antigens, proteins, and genes in the Rh system. Throughout this review, we will use traditional terminology recommended by the International Society of Blood Transfusion (ISBT) committee for terminology of blood group antigens.[5] The numeric portion of the ISBT terminology for Rh antigens is based on the nomenclature described by Rosenfield et al.[6-9] *RH30* and *RH50* have been used to describe genes encoding Rh proteins (Rh30) and Rh glycoprotein (Rh50), respectively, where the numbers relate to the apparent molecular mass of the proteins on a SDS-polyacrylamide gel. Because Rh30 and Rh50 also relate to Go^a and FPTT antigens, respectively, we will use *RH* as a generic term for genes encoding either the RhD protein or the RhCcEe (also known as RhCE) protein and use *RHAG* for the gene encoding the Rh-associated glycoprotein (RhAG). The common Rh antigens: D, C or c, and E or e, were originally written in alphabetical order (CDE) but later, when it was recognized that C and E antigens are inherited en bloc, the order was changed to DCE. Although d antigen, which was thought to be antithetical to D, does not exist, the letter ''d'' is used to indicate the D-negative phenotype. The most frequently occurring forms of *RHCE* and *RHD* encode 8 haplotypes: Dce, dce, DCe, dCe, DcE, dcE, DCE, and dCE, known in short, respectively, as R_0, r, R_1, r′, R_2, r″, R_z, and r_y. The uppercase ''R'' is used when the D antigen is expressed, lowercase ''r'' when it is not. This notation has practical value in transfusion medicine as a means to communicate the Rh phenotype of a patient or donor. Rare deletion phentoypes use dashes in the notation to indicate a lack of antithetical antigens; eg, Dc−. RBCs lack E and e antigens, and D−− RBCs lack C, c, E, and e antigens. RBCs with the Rh_{null} phenotype do not express any of the Rh antigens.

The Rh complex

Biochemical studies, protein purification, and amino acid sequencing of Rh and RhAG are beyond the scope of this article but have been reviewed elsewhere.[10-16]

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Submitted June 11, 1999; accepted August 31, 1999.

Supported in part by a National Institutes of Health Specialized Center of Research (SCOR) grant in transfusion medicine and biology HL54 459.

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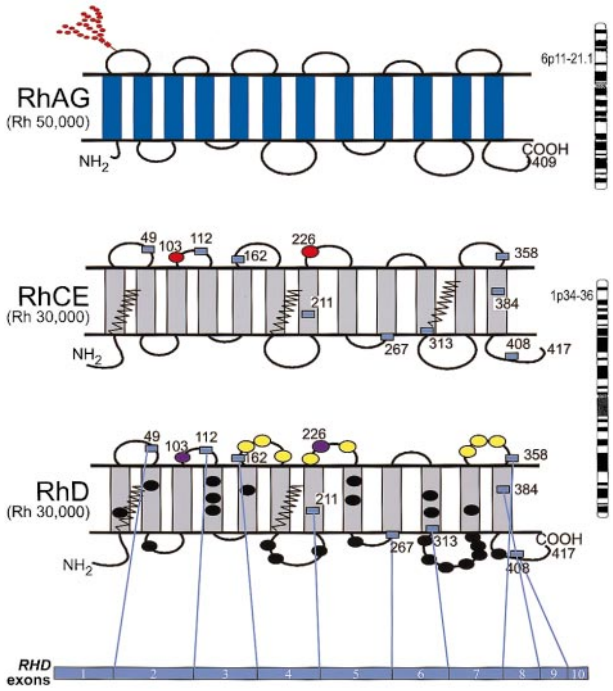

Figure 1. Model of topology for RhAG, RhCE, and RhD. RhAG (M_r 50 000) consists of 409 amino acids and is encoded by *RHAG* on chromosome 6p11-p21.1. RhCE and RhD (M_r 30 000) are predicted to have a similar topology and are encoded by *RHCE* and *RHD,* which are adjacent on chromosome 1p34-p36. The domain of the RhD protein encoded by each exon is depicted by numbered boxes, which represent the start and finish of each exon. Of the D-specific amino acids, 8 are on the exofacial surface (yellow ovals), and 24 are predicted to reside in the transmembrane and cytoplasmic domains (black ovals). Red ovals represent amino acids that are critical for C/c (Ser103Pro) and E/e (Pro226Ala) antigens; purple ovals represent Ser103 and Ala226 on RhD. The zigzag lines represent the Cys-Leu-Pro motifs that are probably involved in the palmitoylation sites. The N-glycan on the first loop of RhAG is indicated by the branched structure of red circles.

The Rh proteins carry Rh antigens but are only expressed on the erythrocyte surface if RhAG is also present. The amino acid sequence homology (approximately 40%) of the Rh and RhAG proteins indicates an ancestral relationship, and collectively they are referred to as the ''Rh protein family.'' Hydrophobicity profiles, immunochemical analyses, and data obtained through site-directed mutagenesis imply that Rh and RhAG proteins have 12 transmembrane spans with both the N-terminus and C-terminus oriented to the cytoplasm (Figure 1).[17-24]

''Rh accessory proteins'' is a collective term for other glycoproteins that are associated with the Rh protein family as defined by their absence or deficiency from Rh_{null} RBCs (see below and Table 1).[25] Together, the association of the Rh protein family and the Rh accessory proteins is called the ''Rh complex.''

Rh protein family

The complex of the Rh protein family is estimated by density ultracentrifugation to be 170 000 daltons[26] and to consist of a tetramer with 2 RhAG molecules and 2 RhCcEe or RhD protein molecules stabilized by both N-terminal and C-terminal domain associations.[18,19,26,27] The mode of association of this core complex with Rh-accessory proteins, some of which interact directly with the membrane skeleton, remains undefined.

RhD and RhCcEe proteins. The RhD protein expresses the D antigen, while the RhCcEe protein carries either C or c antigens (involving the second extracellular loop) together with E or e antigens (involving the fourth extracellular loop) on the same protein.[19,28-30] Characteristics of the RhD protein (synonyms: Rh30, Rh30B, Rh30D, D_{30}, Rh30 polypeptide [30 kd], RhXIII, Rh13) and of the RhCcEe protein (synonyms: Rh30, Rh30A, Rh30C [RhCE], Rh30 polypeptide [32 kd], RhIXb cDNA, [RhcE], Rh21 cDNA [RhcE], $R6A_{32}$, Rhce, RhCe, RhcE, RhCE, CcEe) are summarized in Table 1 and depicted in Figure 1. Analysis of the primary amino acid sequences (inferred from cDNAs) shows that the first 41 N-terminal amino acids of RhD and RhCE/e are identical.[20,31-33] and that RhD differs from the common forms of RhCE by only 30 to 35 amino acids along the entire protein.[20,28,31-33,35,36] Despite the high degree of homology, the various RhCcEe proteins do not express any D epitopes, and RhD protein does not express C or e antigens.

The Rh proteins are thought to interact with the membrane bilayer by palmitoylation,[26,37] where acylated palmitic acid residues are attached to cysteine side chains. These cysteine residues are predicted to be at the boundary of the cytosol and lipid bilayer (Figure 1). Cys-Leu-Pro motifs, flanked by charged amino acids (2 are on RhD and 3 are on RhCcEe) are likely candidates although 2 other cysteine residues (315 and 316) may be alternative sites.[20,26] This interaction may explain why alteration of membrane cholesterol concentration affects the accessibility of the D antigen.[38] The ability to label Rh proteins with ^{3}H-palmitate[26,37] indicates that a reversible coenzyme A and adenosine triphosphate (ATP)-dependent acylation-deacylation cycle occurs in mature RBC membranes, which is of unknown significance.

Rh-associated glycoprotein. The characteristics of RhAG (synonyms: Rh50, Rh glycoprotein Rh50A, D_{50}, MB-2D10 protein, $R6A_{45}$, GP50, GP50A) are summarized in Table 1 and depicted in Figure 1. One of the 2 potential N-glycan sites is glycosylated. A third site is predicted to be cytoplasmic and, therefore, not accessible for glycosylation.[17,39] The N-glycan carries ABH antigens,[12] but RhAG is not known to possess a protein-based blood group polymorphism. Based on the predicted amino acid sequence, RhAG shares 39.2% and 38.5% amino acid sequence identity with, respectively, the Rhce and RhD proteins.[17,20,31-33]

Table 1. Proteins in the Rh Complex in Normal RBC Membranes That May Be Absent or Reduced in Rh_{null} RBC Membranes

Protein	Antigens	Gene Location	M_r	Accession Numbers for cDNAs
Rh protein family				
RhD	D	1p36.13-p34.3[180]	30-32 kd	X63094, X63097,U66341
RhCcEe	Ce, CE, ce, cE	1p36.13-p34.3[180]	32-34 kd	X54534,M34015,U66340
RhAG	Carries MB2D10 epitope[58]	6p21.1-p11[17,75,174]	45-100 kd	X64594
Rh accessory proteins				
LW	LW	19p13.3[181]	37-47 kd	L27670, L27671
IAP	None known	3q13[55]	47-52 kd	Z25521
GPB	'N,' S, s, U	4q28-q31	20-25 kd	J02982
Band 3	Diego	17q12-q21[182]	90-100 kd	X77738, M27819

Expression of Rh proteins and RhAG during erythropoiesis. Rh antigens appear early during erythropoietic differentiation. Anti-D binds to approximately 3% of BFU-E (burst-forming unit, erythroid), 68% of CFU-E (colony-forming unit, erythrocyte), and to all of the more mature erythroid cells. However, the binding of anti-D to proerythroblasts, basophilic erythroblasts, polychromatophilic erythroblasts, and normoblasts was, respectively, 25%, 50%, 66%, and 75% compared with mature RBCs.[33] RhAG protein is detectable on CD34 progenitors isolated from cord blood, after culture for 3 to 5 days, while RhCcEe appears after 5 to 7 days, and RhD appears after 9 to 11 days of culture.[40] In the fetus, Rh antigens are expressed on RBCs from the 6-week conceptus.[41]

Possible function of Rh protein family. The function of the Rh complex remains unclear. Rh proteins have approximately 20% homology to the methylamine permease (Mep) transporters and ammonium transporters (Amt) in yeast, bacteria, and simple plants.[42] This family of transporters are uniporters that have evolved to concentrate ammonium salts from the surrounding environment. Higher animals use more complex nitrogen sources, and they eliminate toxic ammonium via the urea cycle and transport it in the form of glutamine and alanine. The role of the Rh complex as a dedicated ammonium transporter is unlikely, but the complex could cotransport ammonium with other cations; however, further study is needed. Matassi et al[43] report that RHAG shares greatest homology to MEP2, which behaves as an ammonium sensor and transporter in yeast.[44] Furthermore, the presence of RhAG homologs in Caenorhabditis elegans and Geodia cydonium infers they have roles that are not confined to RBCs.

Rh accessory proteins

The blood group antigens associated with the Rh family of proteins, the gene location, their molecular mass, number of copies per RBC, and selected accession numbers are summarized in Table 1.

LW glycoprotein. The LW glycoprotein (synonym: ICAM-4) is a single pass (type I) membrane protein with homology to intercellular adhesion molecules (ICAMs), which are ligands for β2 integrins. LW has been reported to be a ligand for the integrin LFA-1 (synonyms: αLβ2, CD11a/CD18).[45]

While the LW glycoprotein is absent from RBCs of LW(a−b−) and Rh_{null} individuals, expression of Rh antigens is normal on LW(a−b−) RBCs. LW antigens are more abundant on D-positive RBCs than on D-negative RBCs from adults, which led to the initial interpretation that anti-D and anti-LW were the same.[46,47] It is possible that the LW glycoprotein interacts preferentially with RhD as compared with RhCcEe; however, the nature of such an interaction awaits definition. Interestingly, LW antigens are expressed equally well on D-positive and D-negative RBCs from fetuses and newborns and more strongly than on RBCs from adults.[48,49]

Integrin-associated protein. Isoform 2 of integrin-associated protein (IAP; synonyms: CD47, BRIC 125 glycoprotein, AgOAB, 1D8) is present in the RBC membrane, where it is predicted to pass through the RBC membrane 5 times and have 6 potential N-glycan motifs.[50,51] IAP carries ABH antigens but no known protein-based blood group antigen. IAP occurs as different isoforms in various tissues where it binds to β3 integrins.[50,52] The IAP isoform in RBCs does not bind integrins but does bind thrombospondin[53] and may be involved in calcium transport, possibly as a gated channel.[54] While the amount of IAP is reduced in RBC membranes from Rh_{null} and D−− people, it is present in normal levels in lymphoblastoid cell lines from these people.[55-57]

Glycophorin B. Glycophorin B (GPB; synonyms: Ss sialoglycoprotein [SGP], δ-SGP, PAS-3) is a type I membrane glycoprotein that has several O-glycans but no N-glycan. The Rh complex appears to aid, but is not essential for, the correct insertion of GPB in RBC membranes. In S−s−U− RBCs that lack GPB, the Rh proteins are apparently normal, but RhAG has increased glycosylation, suggesting a slower migration through the endoplasmic reticulum and Golgi apparatus.[39] An interaction of GPB and RhAG may be required for full expression of the U antigen[58,59] and, to a lesser extent, S and s antigens (Table 2). Further, the known ability of GPB to form heterodimers with glycophorin A (GPA) may bridge the Rh complex with the band 3/GPA complex, forming a large unit in the RBC membrane.

Fy glycoprotein. A possible association between the Fy glycoprotein (synonyms: Duffy, DARC) and the Rh complex is indicated by the Fy5 antigen, which is absent from Fy(a−b−) and Rh_{null} RBCs.[60] However, Rh_{null} RBCs have normal Fy^a, Fy^b, Fy3, and Fy6 antigens, and Fy(a−b−) RBCs have normal Rh antigens. The specific requirements for expression of the Fy5 antigen remain unknown.

Band 3. Band 3 (synonyms: AE1, anion exchanger, solute carrier family 4 anion exchanger member 1) is a glycosylated protein that is predicted to pass through the RBC membrane 12 or 14 times and is the major anion transporter.[61,62] Unlike the proteins described above, band 3 is apparently normal in Rh_{null} RBCs; however, based on hemagglutination studies, antigens on Rh proteins and on band 3 are decreased in South-East Asian ovalocytic RBCs.[63] The molecular defect associated with South-East Asian ovalocytic RBCs results from a deletion of a segment of

Table 2. Comparison of amorph Rh_{null}, regulator Rh_{null}, and Rh_{mod} RBCs

Phenotype	Rh Proteins/Antigens	RhAG	LW	IAP	GPB Protein/Antigens	Obligate Heterozygotes	Altered Gene
Rh_{null}-amorph	Absent	Reduced (20%)	Absent	Reduced by 90%	Reduced by 50% S/s Normal U Weak	Express one Rh haplotype*	*RHCE* (*RHD* deleted)
Rh_{null}-regulator	Absent	Absent	Absent	Reduced	Reduced by 70%[183] S/s weak U Absent	Express both Rh haplotypes	*RHAG*
Rh_{mod}	Reduced (variable)	Absent or reduced (variable)	Absent or reduced	Reduced (variable)	Reduced (variable) S/s Normal U Normal/Weak	Express both Rh haplotypes	*RHAG*

*That is, appear as homozygotes.

DNA encoding 9 amino acids located at the boundary of the cytoplasmic N-terminal domain and membrane domain of band 3.[64-67] Recent evidence that the expression of endogenous and retrovirally expressed Rh antigens were enhanced following transduction of K562 cells with band 3 suggests that band 3 and Rh proteins associate in erythroid cells.[68]

Structure of *RH* and *RHAG* genes

The genes encoding RhD and RhCcEe are highly homologous, while the gene encoding RhAG is almost 40% homologous. The 3 genes are each composed of 10 exons; *RHCE* and *RHD* in tandem encompass 69 kilobases (kb) of DNA (Figure 2), while *RHAG* encompasses 32 kb. The RhD protein is encoded by *RHD* (synonyms: *RH30, RH30B, RH30D, RHXIII, RH13*); the RhCcEe protein is encoded by *RHCE* (synonyms: *RH30, RH30A, RH30C (RHCE), RHIXB, RH21*); and the RHAG glycoprotein is encoded by *RHAG* (synonyms: *RH50, RH50A*).

The intron-exon boundaries of the *RHCE* gene[21] and the complete nucleotide sequences of some *RHCE* and *RHD* introns have been described.[69-74] Selected GenBank accession numbers for cDNA are listed in Table 1, and those for introns are given in Figure 2. The intron-exon structure of the *RHAG* gene also has been defined and is remarkably similar to *RHCE* and *RHD*.[22,75-77] Several mutations in *RHAG* have been described that cause the regulator type of Rh deficiency syndrome (see below).

Evolution of the RH gene family

It was thought that Rh proteins were erythroid-specific and confined to higher vertebrates. However, the discovery of sequence-related *RHAG* homologs in invertebrates suggests otherwise. These homologs have been found as 2 different *RHAG*-like genes in *Caenorhabditis elegans* (a nematode; GenBank accession U64 847 and Z74 026)[78] and as at least 1 in *Geodia cydonium* (a marine sponge; GenBank accession Y12 397).[79] These genes are predicted to encode proteins with remarkably high (respectively, 46%, 39%, and 47%) amino acid identity to human RhAG. The highest homology is within the transmembrane domains, suggesting a conserved functional role for the RhAG protein family. Recent work has also demonstrated the presence of *RHAG* counterparts in mouse (GenBank accession AF065 395; AF057 524-27, AF012 430), macaque (AF058 917) and *RH* orthologs in chimpanzee (L37 048-50), gorilla (L37 052, L37 053), orangutan (AF012 425), gibbon (L37 051), baboon (AF012 426), macaque (L37 054 570 343), New World monkeys (AF012 427-9, AF021 845) and cow (U59 270).[77,80,81]

As the invertebrate homologs more strongly resemble human *RHAG* than human *RH*, it is likely that an ancient gene duplication event, estimated to have occurred 250 million to 346 million years ago, caused divergence of *RH* from *RHAG*.[75] Subsequent to the gene duplication, *RH* and *RHAG* underwent different evolutionary pathways.[43] A second gene duplication event, being the origin of the human *RHCE* and *RHD* genes, occurred much later in a primate ancestor 5 million to 12 million years ago. Based on the evolutionary rates of *RHAG* and *RH* genes in different species, it appears that *RHAG* evolved some 2.6 times slower than *RH*, suggesting that RhAG has a more important functional role than Rh proteins.[22,73,77,82]

The order of the Rh genes on chromosome 1 is probably *RHCE-RHD*.[83] (After submission of this manuscript, a paper was published that questions the order of the Rh genes on chromosome 1. Sequencing the intergenic region of the two RH genes suggests that the order may in fact be *RHD-RHCE*.[211]) The primordial human Rh haplotype is believed to be Dce, and the other 7 common Rh haplotypes most likely each arose from this gene complex by a single genetic event. The predominant Caucasian D-negative haplotype (dce) probably arose by a deletion of the *RHD* gene[84] from the *RHce/RHD* gene complex, whereas the DCe haplotype (the most common D-positive haplotype in Caucasians) arose by gene conversion with exon 1 and 2 from *RHD* replacing the same exons of *RHce*. The remaining haplotypes arose through point mutations (eg, the E/e polymorphism) or rare recombination events of the various haplotypes.[83]

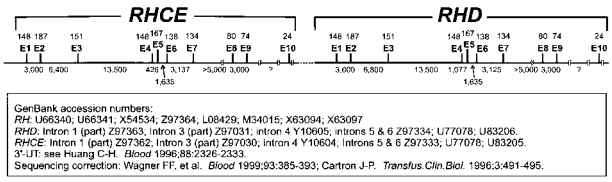

Figure 2. *RHCE-RHD* gene organization. Organization of exons (E) and introns of *RHCE* and *RHD* is shown. Exon sizes are indicated above the line as number of nucleotides, and intron sizes are indicated below the line. A c-specific short tandem repeat (STR) is located in intron 2 of *RHCE* and another in intron 6 of *RHD*. The information used to compile this figure came from the database accession numbers given in the figure.

Molecular basis of Rh antigens

Since the first descriptions of Rh cDNAs,[20,31-33] much effort has been expended in differentiating the molecular bases underlying the antigens of the Rh system. The different genetic mechanisms that give rise to the major clinically relevant Rh antigens are described within this section. These include gene deletion (D-negative phenotype); gene conversion (C/c polymorphism); antithetical missense mutations (E/e); and other missense mutations (VS and V). The *RH* genes appear to be a source of massive diversity, and combinations of these different genetic rearrangements abound among all racial groups. We have selected examples of Rh polymorphisms that are of clinical significance and have been defined at the molecular level. Figures 3-6 detail the molecular basis of published examples of Rh variants. Enthusiastic readers requiring more data regarding Rh variants should consult references.[16,25,85,86]

D antigen

The D antigen is a collection of conformation-dependent epitopes along the entire RhD protein. While in most D-negative Caucasians there is a deletion of *RHD*, in other populations (notably Japanese and African blacks) the D-negative phenotype is associated with a grossly normal *RHD*, and the reason for the lack of expression of the D antigen is not known (except in Africans; see later). Figure 3 depicts the molecular basis of some D-negative phenotypes.[70,74,84,87-89]

People whose RBCs have an altered form of RhD protein (partial D) may make alloanti-D. Such RBCs, depending on which D epitopes are altered, are agglutinated by a proportion of anti-D reagents. Figure 4 summarizes the molecular changes that are associated with partial D antigens.

Analysis of genes encoding the weak D phenotype (previously known as D^U) showed a normal *RHD* sequence but a severely reduced expression of *RHD* messenger RNA (mRNA), suggesting a defect at the level of transcription or pre-mRNA processing.[70,90]

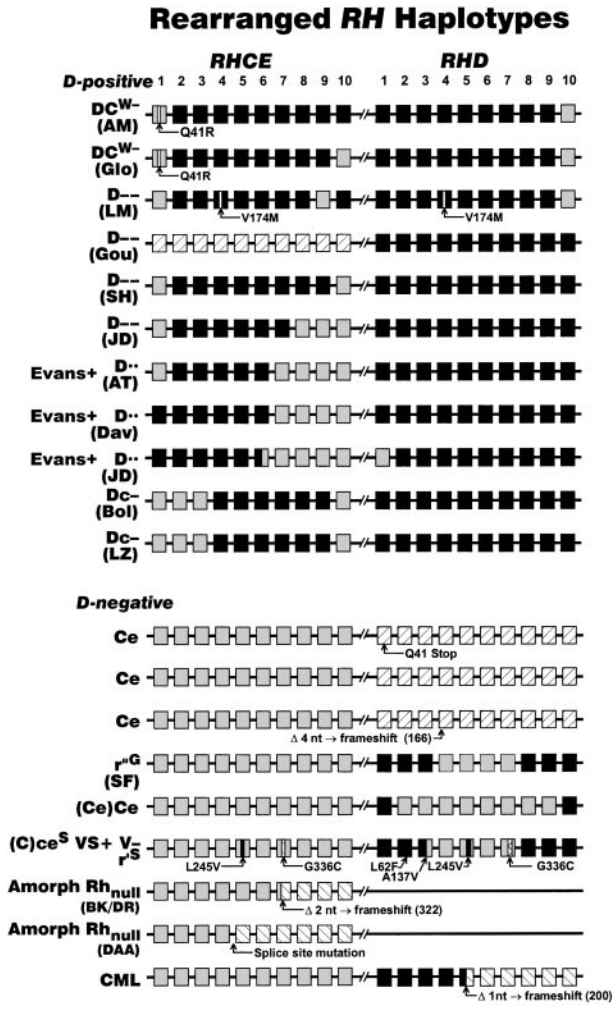

Figure 3. Rearrangements at the Rh locus giving rise to D-negative and Rh deletion haplotypes. The structures of the *RH* locus (located at 1p34-36) that has been defined in various D-negative phenotypes and rare Rh antigen deletion phenotypes are depicted. Each *RH* gene is represented as 10 boxes, each box representing an exon, where *RHCE* is shown as gray, *RHD* as black. Crosshatched boxes depict silent *RHD* alleles (eg, *RHD* Q41 X). The positions of microinsertions or deletions of DNA that cause or are indicative of D-negative phenotypes are shown as triangles. Because exon 8 of *RHCE* and *RHD* are of identical sequence and their origins are not possible to define, they are shaded according to the gene loci position. The significance of these rearrangements, and their impact in particular on molecular genotyping, is discussed within the text. Sources for the information in this figure: $DC^{W}-$ (AM)[184]; $DC^{W}-$ (Glo)[185]; D–– (LM)[186]; D–– (Gou)[186]; D–– (SH)[72]; D–– and Evans+ D•• (JD)[187]; Evans+ D•• (AT)[69]; Evans+ D•• (Dav)[186]; Dc– (Bol)[186]; Dc– (LZ)[188]; Ce[70,74,149]; $r^{w}G$ (SF)[105,140]; (Ce)Ce[184]; $(C)ce^{S}$ VS+ (Donor 1077) $V-r'^{S}$ [29;98;104]; Amorph Rh_{null} (BK/DR)[176,189]; Amorph Rh_{null} (DAA)[177]; CML.[179]

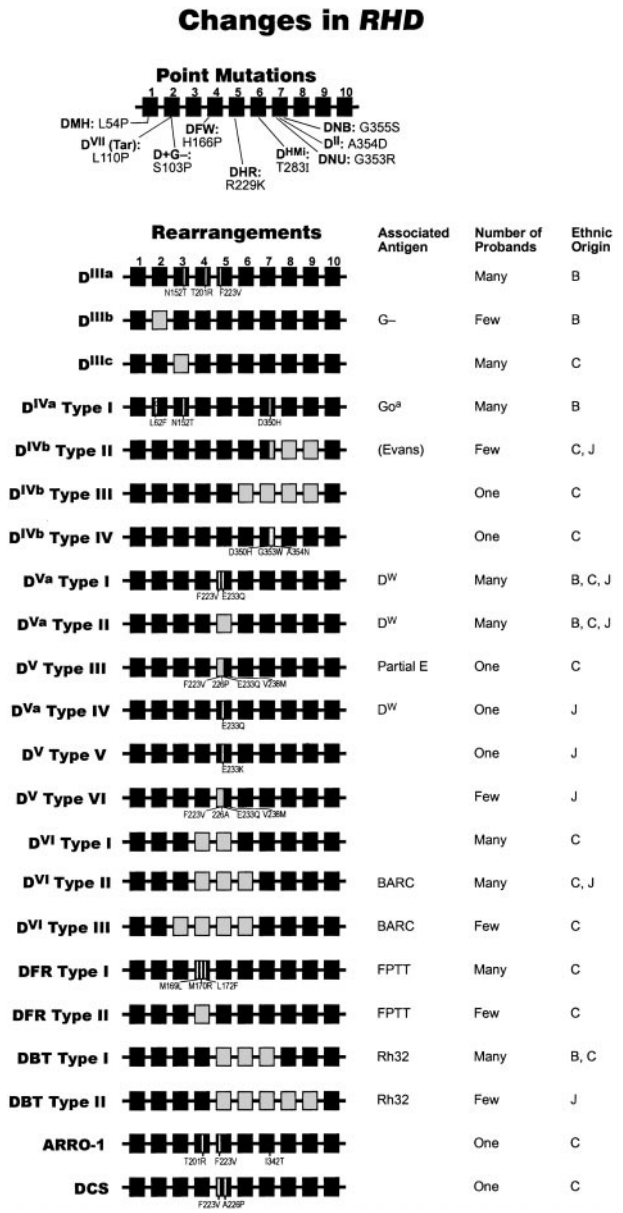

Figure 4. Molecular bases of partial D phenotypes. The different alleles of *RHD* that cause partial D phenotypes are depicted here graphically. The genetic structure of each partial D *RHD* 10-exon gene is shown, as are associated low-incidence antigen(s) and the estimated gene frequency. *RHD* (ie, wild type) exons are shown as black boxes; where they have been replaced by *RHCE* equivalents is shown as white boxes. Missense mutations are indicated within the exon where they occur. We have used the original Roman numeral notation (ie, D^{II} to D^{VII}) and the more recent 3-letter notation (eg, DFR, DBT) for the different D categories. Where partial D phenotypes have identical (or very similar) serologic profiles but different genetic backgrounds, we have adapted the classification originally described by Mouro et al[190] to describe different D^{VI} phenotypes (types I and II). Thus, we depict D^{IV} types I to IV, D^{V} types I to VI; D^{VI} types I to III, and DFR types I and II. We use D^{Va} to indicate the presence of the D^{W} antigen and D^{V} to represent samples that have a similar molecular background but that either do not express the D^{W} antigen or have not been tested for this antigen. Few = 1 to 10 examples. Many = 11 or more examples as indicated by serological testing. D^{VII} is common (1 in 900) in the German population.[191] Under "Ethnic Origin," B = black, C = Caucasian, and J = Japanese. The information used for the point mutations used in this figure are as follows: D+G– [106]; DNU and D^{II} [192]; D^{HMi} [92]; D^{VII} [193]; D^{Va} [71,194] DFW[195]; DHR.[196] The information used for the rearrangements in this figure was obtained from the following: D^{IIIa} [197]; D^{IIIb} [106]; D^{IIIc} [198]; D^{IVa} type I [194]; D^{IVb} type II[194]; D^{IVb} type III[92]; D^{IVb} type IV[195]; D^{Va} type I[194]; D^{Va} type II[194]; D^{V} type III[102]; D^{Va} type IV[156]; D^{V} type V[156]; D^{V} type VI[156]; D^{VI} type I[199,200]; D^{VI} type II[190]; D^{VI} type III[71]; DFR type I[194]; DFR type II[201]; DBT type I[202]; DBT type II[203]; ARRO-1[204]; DCS[205].

More recently, *RHD* transcripts from people whose RBCs express a weak form of the D antigen were found to have missense mutation(s) within the predicted transmembrane or cytoplasmic domains of RhD (Figure 5).[91,92] RBCs with some weak D antigens may not be agglutinated by all monoclonal anti-D. People whose RBCs express this type of weak D antigen do not make anti-D.

CcEe antigens

The RhC/c and RhE/e polymorphisms are caused by nucleotide substitutions in *RHCE*.[28,93] While 6 nucleotide substitutions causing 4 amino acid changes (Cys16Trp; Ile60Leu; Ser68Asn; Ser103Pro) are associated with the C to c polymorphism (Figure 6), only the Ser103Pro polymorphism strictly correlates with C/c

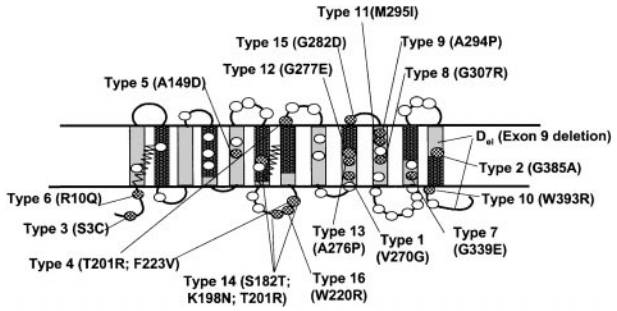

Figure 5. Molecular basis of weak D phenotypes. This figure depicts missense mutations in the *RHD* gene associated with weak D phenotypes.[92,153] The locations of these mutations on the predicted topology of the RhD protein are depicted as checkered ovals; the D-specific amino acids are shown as open ovals. Most of the missense mutations are located within nonconserved membrane spans (gray) and cytoplasmic regions. Regions of conserved Rh protein family sequence are indicated as black rectangles.

antigenicity.[94] However, Pro102 appears to be a critical part of the c antigen.[95,96] The presence of 2 adjacent proline residues (102 and 103) would be expected to form a relatively rigid structure that is resistant to changes in nearby amino acid residues and may explain the relatively low number of c variants as compared with other Rh antigens. It has been generally accepted that a single nucleotide substitution is sufficient for expression of the E to e polymorphism (Pro226Ala). However, variants of the e antigen have been described,[97] showing that the requirements for expression of the e antigen are not fully understood. For example, the presence of Val at residue 245 instead of Leu,[29,98,99] a deletion of Arg at amino acid residue 229,[100] or the presence of Cys (instead of Trp) at amino acid residue 16 [101] affects the expression of the e antigen. The molecular basis of partial E antigens (categories I, II, and III, and D^{V} type III) has been determined and are shown in Figures 4 and 6.[102,103]

VS and V antigens

The imultaneous presence of 2 low-incidence antigens (VS and V) occurs with a single amino acid substitution (Leu245Val) that is predicted to be within a transmembrane domain (Figure 6).[104] The V antigen (in the presence of VS) is not expressed when another transmembrane amino acid substitution is present at residue 336 (Gly→Cys) (Figure 3).[98,104] The membrane location of residues 245 and 336 illustrate that Rh antigen expression is affected significantly by nonexofacial amino acids and suggests that the prediction of some Rh epitope expression cannot be based solely on externalized residues.

G antigen

RhD and RhC proteins carry the G antigen, which is associated with residues in the second extracellular loop encoded by exon 2.[105,106] In D^{VI}cE (D^{VI} type I) RBCs, which are predicted to have a hybrid RhD (exons 1-3)–RHCE (exons 4 and 5)–RhD (exons 6-10) protein, the G antigen was not detected by 1 of 2 monoclonal anti-G.[107] Thus, it would appear that the G antigen is conformation-dependent and not solely dependent on the second external domain of RhC(e/E) or RhD proteins.

Rh variants

Rh-variant phenotypes arise through at least 4 mechanisms: (1) rearrangements of the tandemly arranged *RHCE* and/or *RHD* (Figures 3, 4, and 6); (2) point mutation(s) in either gene causing amino acid change(s), with subsequent loss of some epitopes and/or expression of a low-incidence antigen; (3) nonsense mutations, and (4) deletion of nucleotides causing a frameshift and premature stop codon. There is some evidence that there are recombination hot spots due to *Alu* IV elements in the *RH* genes.[72,108]

Rearranged *RHCE* genes, associated with D− − and D••, ablate expression of C, c, E, and e antigens, while the D antigen expression is exalted to the extent that immunoglobulin (Ig) G anti-D can agglutinate the RBCs in saline.[109] It is now clear that this increased expression is due to a large insert of *RHD* into *RHCE* in tandem with a *RHD* gene (Figure 3). In DC^{W}− and Dc− phenotypes, the region of the *RHCE* gene encoding the E/e antigen is replaced by an RhD equivalent with loss of E/e antigenicity (Figure 3). While these appear as RHCE deletion phenotypes at the protein level, they are encoded by rearranged *RHCE* and thus are *RHCE*-depleted.

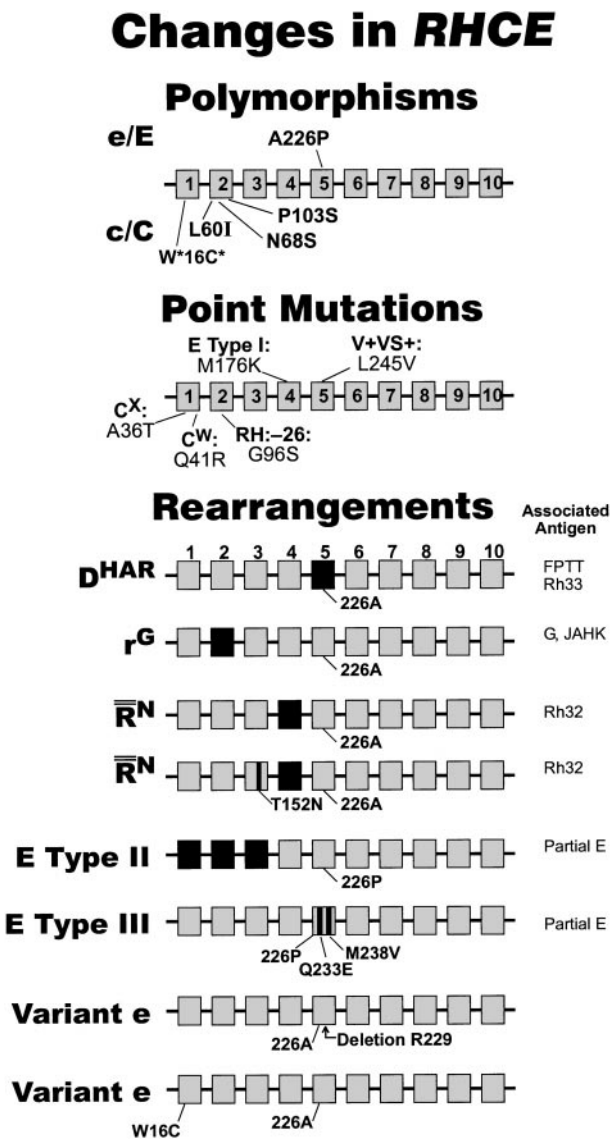

Figure 6. Changes in *RHCE*. Amino acids encoded by *RHCE* are shown by gray boxes, and those encoded by exons from *RHD* are shown by black boxes. The amino acids associated with E/e and C/c antigens[28,93] are shown at the top, and single amino acid changes associated with variant forms of RhCE are shown in the middle. The bottom portion of the figure shows rearrangements of the *RHCE* and associated antigens. Polymorphism that does not have a 100% correlation with expression of c and C antigens. The information depicted in this figure was obtained from the following sources: Point Mutations[35,98,99,104,185,206]; Rearrangements D^{HAR} [207]; r^{G}[208]; R^{N} [90]; E Cat II and III[185]; and Variant e[100,101].

Low-incidence antigens associated with partial D antigens. Low-incidence antigens associated with some partial D phenotypes are due to novel structures on the RBC surface and are useful markers for the identification of the partial D (Figure 4).[110] A few low-incidence antigens are associated with more than 1 molecular background, eg, the FPTT (Rh50) antigen is expressed on DFR, R_o^{Har}, and D^{IVa}(C)− phenotype RBCs; the Rh32 antigen is expressed on DBT and $\overline{\overline{R}}^N$ RBCs. The Evans antigen is expressed on D••, and a weak form of Evans is present on D^{IVb} RBCs. RBCs expressing Rh23 or Rh32 possess an antigen (Rh23/32) present on both phenotypes.[111] In these cases, it is likely that external surfaces of the altered proteins have localized similarities.

RhD epitope mapping

Partial D antigens were classically identified by testing the RBCs with well-characterized polyclonal anti-D made by other people with partial D phenotypes and, also, by testing the patient's anti-D against RBCs with known partial D antigens. Human monoclonal antibodies are now being used to classify partial D antigens in terms of expressed epitopes. The original model consisted of 8- and 9-epitope D (epD)[112,113] but has been expanded to consist of 16,[110] 30,[114] and 37 epitopes.[115] When using monoclonal anti-D to define D epitopes, it is important to perform the testing at the correct pH, temperature, ionic strength, and antibody concentration; to use RBCs that have been stored appropriately; and to include controls.[110,114] Most D epitopes are conformation-dependent and may be influenced by other proteins and lipids in the RBC membrane. Indeed, only 1 monoclonal anti-D has been described that reacts strongly by immunoblotting, implying that the epitope it recognizes may be linear.[116]

Predictions as to the location of various D epitopes have been based on which epitopes are absent from RBCs with a partial D for which the molecular basis is known.[117,118] However, the absence of a D epitope may not always be a direct result of the change in molecular structure, and the presence of Rh proteins encoded by *cis* and *trans* genes can effect the binding of certain monoclonal anti-D. For example, R_0^{Har} and D^{Va} do not have any RHD exons in common, but they have overlapping reactivity with monoclonal antibody anti-D, demonstrating the difficulty of correctly defining the molecular basis of D epitopes. A model proposed by Chang and Siegel[119] suggests that anti-D are essentially similar in that they react with the basic footprint of the D protein. In this model, a change in the footprint, induced by an amino acid substitution or a hybrid protein, is predicted to interfere with binding of anti-D. The involvement of certain residues for binding of monoclonal anti-D has been investigated by site-directed mutagenesis (SDM), which showed that incorporation of 3 D-specific amino acids (Asp350, Gly353, and Ala354) into an RhcE construct generated some epD3 and epD9 expression,[120,121] and incorporation of 9 exofacial D residues generated epitopes that were recognized by 40 of 50 monoclonal anti-D.[122] These data argue that at least some D epitopes are spatially distinct. However, SDM studies have not yet addressed the impact of amino acids located within the lipid bilayer or on the cytoplasmic side of the RBC membrane. Accurate determination of the contact points of interaction(s) between antigen and antibody awaits crystallographic data.

Clinical aspects

Clinical complications result from RBC destruction due to the interaction of an alloantibody with RBCs carrying the corresponding antigen. The D antigen is highly immunogenic and induces an immune response in 80% of D-negative persons when transfused with 200 mL of D-positive blood.[123] For this reason, in most countries D typing is performed routinely on every blood donor and transfusion recipient so that D-negative patients receive D-negative RBC products. Consequently, clinical complications due to mismatched transfusions are infrequent. In contrast, despite the use of immunosuppressive therapy with anti-D immunoglobulin prophylaxis, D alloimmunization in pregnancy still occurs.

Alloantibodies

Alloantibodies that recognize Rh antigens are usually IgG and react by the indirect antiglobulin test. This is a test in which RBCs are incubated in serum, washed to remove free immunoglobulin, and then exposed to an antiglobulin reagent that is formulated to detect the cell-bound IgG. The end point of the test is hemagglutination. Alloantibodies in the Rh blood group system can cause destruction of transfused RBCs and of fetal RBCs in hemolytic disease of the newborn (HDN). People whose RBCs have a rare deleted Rh phenotype (Rh_{null}, D− −) readily make alloantibodies. People with the Rh_{null} phenotype of amorph or regulator type can make anti-Rh29 (an antibody to ''total'' Rh), anti-Rh17 (an antibody to the RhCc/Ee protein), anti-D, anti-C, or a mixture of specificities. Transfusion of a patient with anti-Rh29 is a problem because only Rh_{null} RBCs will be compatible: People with the Rh_{null} phenotype are not only rare, but they have a compensated hemolytic anemia and are therefore unlikely to meet predonation criteria.[124] People with either the D− −, D••, DC^W−, or Dc− phenotype make anti-Rh17. A patient with anti-Rh17 also represents a transfusion conundrum because only RBCs with a deleted phenotype will be compatible.

Autoantibodies

An autoantibody is one that reacts with an antigen on the antibody maker's own RBCs. Autoantibodies that react optimally at 37°C are present in the serum of about 80% of patients with warm autoimmune hemolytic anemia.[125] Although most of these autoantibodies appear to be ''nonspecific,'' many have specificity to an Rh antigen, notably to e. Rarely is the specificity clear-cut, but the autoantibody commonly reacts more weakly with antigen-negative RBCs than with antigen-positive RBCs; however, in these cases, transfused antigen-negative RBCs only rarely survive better than antigen-positive RBCs.[123] Autoantibodies in serum from patients with warm autoimmune hemolytic anemia may be nonreactive only with Rh_{null} and D− − RBCs (autoanti-Rh17), or only with Rh_{null} RBCs (autoanti-Rh29). In such cases, antigen-negative blood will not be available, and transfusion with antigen-positive RBCs should not be withheld if the patient has life-threatening anemia.[125,126] In most cases, the autoantibody is equally reactive with all RBCs tested—whether from donors or antibody detection/identification kits. Thus, in the clinical setting, it is important to perform tests to ensure that the patient's serum does not have potentially clinically significant alloantibodies underlying the autoantibodies before transfusing incompatible RBCs. Detection and identification of such antibodies is required to prevent transfusion reactions but is beyond the scope of this review. For more information, see a current textbook on laboratory aspects of transfusion medicine.[125-127]

Partial and weak D phenotypes

As described earlier, people whose RBCs have a weak D phenotype (quantitative D variant) do not make anti-D, whereas people whose RBCs have a partial D phenotype (qualitative D variant with or without weakening of the D antigen) can make alloanti-D. This presents a different problem depending on whether the person is a donor or a patient. For donors, detection of weak and partial D antigens would eliminate the possibility of immunization should such blood be transfused to a true D-negative patient. However, historical data show that weakly expressed D antigens are most unlikely to be immunogenic. For transfusion recipients and pregnant women, it is common practice to use a procedure that will classify RBCs with a weak D antigen or some partial D antigens as D-negative. Thus, blood donated from such a person should be labeled as D-positive (Rh-positive), but the same person should be listed as D-negative (Rh-negative) when they are recipients in need of transfusion. The transfusion recipient will receive D-negative RBC products, and the pregnant woman will receive prophylactic Rh immunoglobulin, thereby preventing alloimmunization. Although a pregnant woman with the D^{VI} partial phenotype may make alloanti-D, this has rarely caused a clinical problem to a D-positive fetus.[128] In the autologous transfusion setting (in which the person is both the donor and patient), the above policy can cause confusion because partial D RBCs may be typed as D-positive at the donor center but D-negative at the hospital. In practice, it is difficult to distinguish RBCs with the D^{VI} phenotype from other weak D; however, this now can be accomplished by immunoblotting with the unique anti-D, LOR-15C9.[129]

Rh and hemolytic disease of the newborn

HDN is caused by maternal IgG antibody crossing the placenta, binding to the fetal antigen-positive RBCs, and initiating their destruction, thereby causing anemia. Prior to the use of prophylactic Rh immunoglobulin, anti-D frequently caused fetal brain damage due to increased levels of bilirubin (kernicterus) and even death (erythroblastosis fetalis). Despite the widespread use of prophylactic Rh immunoglobulin, a significant number of women still become alloimmunized during pregnancy for a variety of reasons, including nonadministration of Rh immunoglobulin, unrecognized miscarriage, leakage of fetal RBCs into the maternal circulation late in pregnancy, and exposure to maternal D-positive RBCs while in utero (grandmother effect).[130]

The D antigen accounts for about 50% of cases of maternal alloimmunization; the remainder is due mainly to incompatibility to K, c, C/G, E, and Fy^a antigens and to low incidence antigens in Rh, MNS, and Diego blood group systems.[131-133] Therefore, feto-maternal Rh incompatibility still represents the major cause of HDN. Ultraviolet phototherapy and, occasionally, exchange transfusion or even intrauterine transfusion may be required. Invasive procedures are used as a ''last option'' in monitoring and treating HDN, because they may cause further leakage of fetal RBCs into the maternal circulation. Measures such as determination of the optical density of amniotic fluid and functional assays (ADCC, MMA, chemiluminescence) have been used to monitor at-risk pregnancies and to identify cases requiring treatment (for review, see Zupanska[134]). With current molecular technology, it is possible to perform analyses on fetally derived DNA to predict the blood type of a fetus.

Interestingly, a fetus that is ABO incompatible with the maternal anti-A/B is less likely to have HDN due to anti-D, presumably due to rapid removal of the ABO-incompatible RBCs by the naturally occurring anti-A/B. Also, because the number of copies of the D antigen per RBC is higher in the R_2 haplotype (range, 14 000 to 16 000) than in the R_1 haplotype (range, 9000 to 14 600), fetuses whose RBCs are R_2 have more severe anemia than their R_1 counterparts.[123] There is also evidence that male fetuses have more severe HDN than female fetuses.[135]

Rh immunoglobulin prophylaxis in the prevention of HDN. The immunologic mechanism responsible for preventing production of maternal anti-D following administration of prophylactic Rh immunoglobulin may be due, at least in part, to antigen blocking and central inhibition of the immune response by negative feedback in the spleen (for review, see Bowman[130]). In some instances, recommendations have been made to administer anti-D to partial D-phenotype mothers (eg, D^{VI} and DBT phenotypes) following the birth of D-positive babies.[110,136] In Europe, anti-D reagents are selected to deliberately type D^{VI} mothers as D-negative and, thus, ensure that such mothers would automatically receive prophylactic Rh immunoglobulin therapy following pregnancy.

Prophylactic Rh immunoglobulin preparations for this purpose are usually for intramuscular injection. However, products approved also for intravascular injection are used for the treatment of idiopathic thrombocytopenia.[137,138]

Legislative restrictions for immunization of D-negative volunteers with accredited D-positive RBCs are partly responsible for the declining source of polyclonal anti-D for prophylaxis. Thus, clinical trials have explored the possibility of using human monoclonal anti-D to prevent anti-D alloimmunization;[139] however, the in vivo use of monoclonal antibodies derived from EBV-transformed cells remains controversial. It is possible that recombinant forms of anti-D can be prepared as an injectable prophylactic product.

Prenatal Rh genotyping

When a pregnant woman has a potentially clinically significant alloantibody and the father of the fetus is phenotypically heterozygous for the gene encoding the corresponding antigen (or is unknown), prenatal determination can be considered. The potential benefits of identifying a fetus whose RBCs are predicted to be antigen-negative is enormous in that the need for further invasive techniques is diminished. Fetal DNA can be obtained from amniocytes, chorionic villi, vaginal swabs, and mother's blood (see later). Following cloning and sequencing of *RHCE* and *RHD*, many polymerase chain reaction (PCR)-based tests to analyze DNA prepared from amniocytes have been reported (for recent review, see Flegel[86]). However, the genetic diversity of the Rh genes, particularly among blacks and Japanese, has reduced the clinical utility of this approach because false-negative and false-positive results can occur. Prenatal diagnosis of fetal *RHD* status exploits structural differences between the *RHD* and *RHCE* genes and is based on the assumption that D-negative individuals have a deleted *RHD* gene. As the knowledge regarding the molecular basis of partial D antigens evolved, use of multiplex,[70,86,140,141] heteroduplex,[142,143] and multiple sequence-specific PCR reactions[144,145] have replaced the single exon genotyping assays[32,146,147] in an attempt to avoid ''false-negative'' typing of a fetus with a partial D antigen. However, because HDN in a fetus whose RBCs have a partial D antigen is rare,[148] the clinical value of RHD multiplex analysis may only have marginal added value.

All current RhD genotyping assays will mistype people whose RBCs are D-negative and yet carry an intact, nonfunctional *RHD*.

Such people have been described in Caucasians (rare),[70] African blacks (common),[89,144] and Asians (common).[74,144] Molecular genotyping will have limited clinical utility in populations where the presence of nonexpressed *RHD* is frequent. The molecular backgrounds of these D-negative phenotypes are beginning to emerge: In 2 Caucasians expressing the dCe phenotype, 1 had an in-frame stop codon in exon 1 of the *RHD* gene[70] and the other a deletion of 4 nucleotides in exon 4.[149] Very recently, the molecular basis of the major silent *RHD* allele (named *RHDψ*) found in persons of African ancestry has been defined.[150] *RHDψ* has a 37–base pair insertion of DNA, being a duplication of the intron 3/exon 4 boundary, and has missense mutations in exon 5 and a nonsense and missense mutation in exon 6. The D_{el} phenotype (ie, D antigen is detectable only by adsorption-elution tests) was thought to have a deletion of *RHD*;[151,152] however, a deletion of 1013 base pairs encompassing intron 8, exon 9, and intron 9 has been observed (Figure 5).[153]

Clearly, knowledge of the ethnic group of both parents is helpful in the selection of appropriate genotyping tests. Wherever possible, to limit the gene pool, concurrent analyses of maternal and paternal blood group phenotypes and genotypes should be performed. It is worth noting that samples that have been used for clinical automated instruments are often contaminated with blood from previous tests.[144,154]

As molecular analysis becomes more common, it is worth remembering that some D variants may be more common than previously thought. An example of this is the hybrid gene encoding the D^{IIIa} phenotype, which has recently been shown to be present in 18% of blacks in New York and 28% of blacks from Brazil.[155] Furthermore, a similar pattern of reactivity may be obtained with monoclonal anti-D in tests with RBCs from people with different Rh genes. This is illustrated by the large number of molecular events associated with D^{Va} (or D^{V}-like) samples as defined by the pattern of reactivity with monoclonal anti-D.[156] Not all of the molecular events give rise to the D^{W} antigen, whose presence on RBCs is required for D^{Va} categorization.[110]

Although hemolytic disease due to Rh antibodies other than anti-D is less frequent, PCR-based tests have been designed to define *RHCE* alleles using fetally derived DNA.[157-159] Most of these are relatively straightforward; however, genotyping C in the presence of D is difficult because *RHC(E/e)* and *RHD* have identical sequences in exons 1 and 2. RhC typing is possible by exploitation of a polymorphism in intron 2 of *RHCE*, which involves a 109-base pair insert of DNA in *RHC(E/e)* but not *Rhc(E/e)* or *RHD*.[94,159]

Noninvasive prenatal Rh genotyping. It is now possible to obtain fetally derived DNA using noninvasive procedures. Fetally derived RHD has been detected using nested PCR analysis on genomic DNA (gDNA) extracted from maternal peripheral blood or plasma[160-163] or from transcervical samples.[164-166] An alternative approach uses cDNA templates derived by reverse transcriptase–PCR from maternal peripheral blood and detection of fetal RhD mRNA targets.[167] All noninvasive procedures have limited value because there is no suitable way to assess the presence of fetal cells in a given sample and, thus, negative results cannot be interpreted with confidence. Nevertheless, the fact that fetally derived Rh mRNAs and gDNA can be detected in maternal blood indicates that this area of prenatal diagnosis may soon have an impact. However, it is possible that fetal-nucleated RBCs are the most pertinent target cell type for noninvasive diagnosis[168,169] because other fetally derived CD34+ cells have been detected in maternal blood for as long as 27 years postpartum[169] and thus could interfere with analyses in women who have had multiple pregnancies.

Rh and other disease states

Rh_{null} disease. RBCs from people who have the Rh_{null} phenotype (synonyms: Rh_{null} syndrome, Rh_{null} disease) lack Rh proteins and, thus, Rh antigens. This phenotype is rare (approximately 1 in 6×10^6 individuals)[170] and most often results from a consanguineous mating. The syndrome is associated with stomatocytosis, spherocytosis, increased osmotic fragility, altered phospholipid asymmetry, altered cell volume, defective cation fluxes, and elevated Na^+/K^+ ATPase activity.[13,14,171-173] Rh_{null} RBCs may have a shortened in vivo survival, and the person may have a mild compensated hemolytic anemia.

There are 2 types of Rh_{null}, amorph and regulator, that historically were classified based on their pattern of inheritance. It is now known that the amorph type is the result of a molecular change in *RHCE* in tandem with a deleted *RHD* (Figure 3), whereas the regulator type is associated with a molecular defect in *RHAG* (Figure 7).[22,76,174-177] Table 2 summarizes the characteristics of the 2 types of Rh_{null} phenotypes and compares them with the Rh_{mod} phenotype, in which the Rh antigens are suppressed. While RhAG is apparently critical for the correct assembly of the Rh proteins in the RBC membrane, RhAG by itself can form stable complexes, albeit in reduced quantity, in the absence of Rh proteins.[22,58]

Myeloid leukemia

Patients with acute or chronic myeloid leukemia, myeloid metaplasia, polycythemia, or myelofibrosis occasionally have 2 populations of RBCs of different Rh type. In some cases, a loss of Rh antigens is associated with chromosome aberrations.[178] Recent analysis of blood from a D-positive patient with CML who became D-negative for the 3 years that she was studied revealed a single base deletion in exon 4 of *RHD* that occurred by somatic mutation.[179]

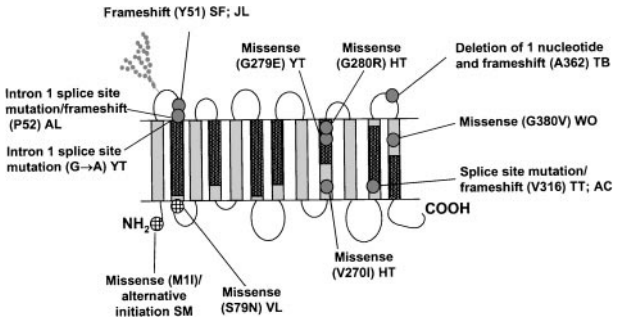

Figure 7. Localization of molecular defects on RhAG. The regulator type of Rh_{null} is associated with 2 mutant *RHAG* genes (homozygote or double heterozygote). The mutations include splice site/frameshift alterations and missense mutations (gray circles). The missense changes predominantly occur within conserved Rh protein family domains (black rectangles), within membrane-spanning regions. It is thought that missense mutations affect either RhAG-RhAG associations/RhAG-Rh protein assocations, resulting in an absence of the Rh protein family from mature RBC membranes. The Rh_{mod} phenotype is associated with missense mutations (cross-hatched circles), which lead to a marked reduction of the RhAG-Rh protein complex in mature RBC membranes. The initials refer to the probands. The information used in this figure was obtained from the following: SM[209]; SF, JL[174]; AL[177]; YT[27,175]; VL[174]; HT [210]; TT, AC[22]; TB[174]; WO[210].

Discussion

Considerable progress has been made in our understanding of the molecular basis of Rh and other blood group antigens in the past 10 years. Despite this, our knowledge concerning the function of many of the components in the RBC remains speculative. The Rh protein complex is a prime example of this; it is a major red cell protein of considerable clinical importance, yet our understanding of its functional significance in human RBCs and other animals relies almost entirely on circumstantial evidence.

The Rh blood group system consists of numerous antigens that are located on variant forms of RhD and RhCE proteins. These proteins form a core complex in the erythrocyte membrane with a glycosylated homolog (RhAG) and are only expressed when it is present. RhD, RhCE, and RhAG associate with other membrane proteins (LW, IAP, GPB, Duffy, and band 3) to form a large complex. Although the function(s) of these proteins has not been defined, it is possible that the complex forms a concerted transporter. The genes encoding the Rh proteins (*RHCE* and *RHD*) are highly homologous and adjacent on the short arm of chromosome 1, while the gene encoding RhAG (*RHAG*) is nearly 40% homologous and is located on the short arm of chromosome 6. Although the molecular basis associated with many of the Rh antigens is known, the actual epitopes have not been defined, but it is apparent that most of the Rh antigens are conformation-dependent. The molecular knowledge is increasingly being used in the clinical setting. However, the allelic diversity in this system is a potential problem for reliable genotyping by PCR-based assays. Hemagglutination is still a powerful, practical, and economical test with a specificity and sensitivity that is appropriate for clinical applications. However, the use of hemagglutination in conjunction with molecular techniques undoubtedly will lead to insights that can enhance approaches for the treatment of Rh incompatibility. Further understanding of the immunologic responses to the Rh antigens will be of importance in the treatment of hemolytic disease, and detailed epitope maps involving serologic, molecular, and protein crystallographic studies of the Rh proteins will contribute to this objective.

Acknowledgments

We thank Christine Lomas-Francis, Narla Mohandas, Olga Blumenfeld, Geoff Daniels, Michael J. A. Tanner, Jill Storry, and Karina Yazdanbakhsh for reading the manuscript and giving helpful suggestions. Neil Avent thanks Willy Flegel, Giorgio Matassi, and Tim Kemp for providing manuscripts prior to publication. We also thank Robert Ratner for preparing the manuscript and cataloging the references.

References

1. Levine P, Stetson RE. An unusual case of intragroup agglutination. JAMA. 1939;113:126-127.
2. Landsteiner K, Wiener AS. An agglutinable factor in human blood recognized by immune sera for rhesus blood. Proc Soc Exp Biol Med. 1940;43:223.
3. Fisk RT, Foord AG. Observations on the Rh agglutinogen of human blood. Am J Clin Pathol. 1942;12:545-552.
4. Levine P, Celano MJ, Wallace J, Sanger R. A human 'D-like' antibody. Nature. 1963;198:596-597.
5. Daniels GL, Anstee DJ, Cartron J-P, et al. Blood group terminology 1995: ISBT working party on terminology for red cell surface antigens. Vox Sang. 1995;69:265-279.
6. Rosenfield RE, Allen FH Jr, Swisher SN, Kochwa S. A review of Rh serology and presentation of a new terminology. Transfusion. 1962;2:287-312.
7. Rosenfield RE, Allen FH Jr, Rubinstein P. Genetic model for the Rh blood-group system. Proc Natl Acad Sci U S A. 1973;70:1303-1307.
8. Allen FH Jr, Rosenfield RE. Review of Rh serology: eight new antigens in nine years. Haematologia. 1972;6:113-120.
9. Rosenfield RE, Allen FH Jr, Swisher SN, Kochwa S. Rh nomenclature. Transfusion. 1979;19:487.
10. Moore S, Woodrow CF, McClelland DB. Isolation of membrane components associated with human red cell antigens Rh(D), (c), (E) and Fy. Nature. 1982;295:529-531.
11. Gahmberg CG. Molecular identification of the human Rh_O (D) antigen. FEBS Lett. 1982;140:93-97.
12. Moore S, Green C. The identification of specific Rhesus polypeptide blood group ABH-active glycoprotein complexes in the human red-cell membrane. Biochem J. 1987;244:735-741.
13. Agre P, Cartron J-P. Molecular biology of the Rh antigens. Blood. 1991;78:551-563.
14. Anstee DJ, Tanner MJ. Biochemical aspects of the blood group Rh (rhesus) antigens. Baillieres Clin Haematol. 1993;6:401-422.
15. Moore S, Gahmberg CG. Identification of Rh polypeptide and Rh polypeptide/Rh glycoprotein complexes. Biotest Bull. 1997;5:409-413.
16. Huang C-H. Molecular insights into the Rh protein family and associated antigens. Curr Opin Hematol. 1997;4:94-103.
17. Ridgwell K, Spurr NK, Laguda B, MacGeoch C, Avent ND, Tanner MJ. Isolation of cDNA clones for a 50 kDa glycoprotein of the human erythrocyte membrane associated with Rh (rhesus) blood-group antigen expression. Biochem J. 1992;287:223-228.
18. Eyers SA, Ridgwell K, Mawby WJ, Tanner MJ. Topology and organization of human Rh (rhesus) blood group-related polypeptides. J Biol Chem. 1994;269:6417-6423.
19. Avent ND, Liu W, Warner KM, et al. Immunochemical analysis of the human erythrocyte Rh polypeptides. J Biol Chem. 1996;271:14,233-14,239.
20. Avent ND, Ridgwell K, Tanner MJA, Anstee DJ. cDNA cloning of a 30 kDa erythrocyte membrane protein associated with Rh (Rhesus)-blood-group-antigen expression. Biochem J. 1990;271:821-825.
21. Chérif-Zahar B, Le Van Kim C, Rouillac C, Raynal V, Cartron J-P, Colin Y. Organization of the gene (*RHCE*) encoding the human blood group RhCcEe antigens and characterization of the promoter region. Genomics. 1994;19:68-74.
22. Huang C-H. The human Rh50 glycoprotein gene—structural organization and associated splicing defect resulting in Rh_{null} disease. J Biol Chem. 1998;273:2207-2213.
23. Avent ND, Butcher SK, Liu W, et al. Localization of the C termini of the Rh (rhesus) polypeptides to the cytoplasmic face of the human erythrocyte membrane. J Biol Chem. 1992;267:15,134-15,139.
24. Hermand P, Mouro I, Huet M, et al. Immunochemical characterization of rhesus proteins with antibodies raised against synthetic peptides. Blood. 1993;82:669-676.
25. Cartron JP, Bailly P, Le Van Kim C, et al. Insights into the structure and function of membrane polypeptides carrying blood group antigens. Vox Sang. 1998;74(suppl 2):29-64.
26. Hartel-Schenk S, Agre P. Mammalian red cell membrane Rh polypeptides are selectively palmitoylated subunits of a macromolecular complex. J Biol Chem. 1992;267:5569-5574.
27. Huang CH, Liu Z, Cheng GJ, Chen Y. Rh50 glycoprotein gene and Rh_{null} disease: a silent splice donor is *trans* to a Gly_{279}—>Glu missense mutation in the conserved transmembrane segment. Blood. 1998;92:1776-1784.
28. Mouro I, Colin Y, Chérif-Zahar B, Cartron J-P, Le Van Kim C. Molecular genetic basis of the human Rhesus blood group system. Nature Genet. 1993;5:62-65.
29. Blunt T, Daniels G, Carritt B. Serotype switching in a partially deleted RHD gene. Vox Sang. 1994;67:397-401.
30. Smythe JS, Avent ND, Judson PA, Parsons SF, Martin PG, Anstee DJ. Expression of *RHD* and *RHCE* gene products using retroviral transduction of K562 cells establishes the molecular basis of Rh blood group antigens. Blood. 1996;87:2968-2973.
31. Le Van Kim C, Mouro I, Chérif-Zahar B, et al. Molecular cloning and primary structure of the human blood group RhD polypeptide. Proc Natl Acad Sci U S A. 1992;89:10,925-10,929.
32. Arce MA, Thompson ES, Wagner S, Coyne KE, Ferdman BA, Lublin DM. Molecular cloning of RhD cDNA derived from a gene present in RhD-positive, but not RhD-negative individuals. Blood. 1993;82:651-655.
33. Chérif-Zahar B, Bloy C, Le Van Kim C, et al. Molecular cloning and protein structure of a human blood group Rh polypeptide. Proc Natl Acad Sci U S A. 1990;87:6243-6247.
34. Suyama K, Goldstein J, Aebersold R, Kent S. Regarding the size of Rh proteins. Blood. 1991;77:411.
35. Mouro I, Colin Y, Sistonen P, Le Pennec PY, Cartron J-P, Le Van Kim C. Molecular basis of the RhC^W (Rh8) and RhC^X (Rh9) blood group specificities. Blood. 1995;86:1196-1201.

36. Kajii E, Umenishi F, Iwamoto S, Ikemoto S. Isolation of a new cDNA clone encoding an Rh polypeptide associated with the Rh blood group system. Hum Genet. 1993;91:157-162.
37. de Vetten MP, Agre P. The Rh polypeptide is a major fatty acid-acylated erythrocyte membrane protein. J Biol Chem. 1988;263:18,193-18,196.
38. Basu MK, Flamm M, Schachter D, Bertles JF, Maniatis A. Effects of modulating erythrocyte membrane cholesterol on Rh_o(D) antigen expression. Biochem Biophys Res Commun. 1980;95:887-893.
39. Ridgwell K, Eyers SA, Mawby WJ, Anstee DJ, Tanner MJ. Studies on the glycoprotein associated with Rh (rhesus) blood group antigen expression in the human red blood cell membrane. J Biol Chem. 1994;269:6410-6416.
40. Southcott MJG, Tanner MJ, Anstee DJ. The expression of human blood group antigens during erythropoiesis in a cell culture system. Blood. 1999;93:4425-4435.
41. Chown B. On a search for Rhesus antibodies in very young foetuses. Arch Dis Child. 1955;30: 232-233.
42. Marini AM, Urrestarazu A, Beauwens R, André B. The Rh (rhesus) blood group polypeptides are related to NH_4^+ transporters. Trends Biochem Sci. 1997;22:460-461.
43. Matassi G, Chérif-Zahar B, Pesole G, Raynal V, Cartron JP. The members of the *RH* gene family (*RH50* and *RH30*) followed different evolutionary pathways. J Mol Evol. 1999;48:151-159.
44. Lorenz MC, Heitman J. The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae.* EMBO J. 1998;17: 1236-1247.
45. Bailly P, Tontti E, Hermand P, Cartron JP, Gahmberg CG. The red cell LW blood group protein is an intercellular adhesion molecule which binds to CD11/CD18 leukocyte integrins. Eur J Immunol. 1995;25:3316-3320.
46. Issitt PD, Anstee DJ. Applied Blood Group Serology. Durham, NC: Montgomery Scientific Publications; 1998.
47. Daniels G. Human Blood Groups. Oxford, England: Blackwell Science Ltd; 1995.
48. Giles CM. The LW blood group: a review. Immunol Commun. 1980;9:225-242.
49. Mallinson G, Martin PG, Anstee DJ, et al. Identification and partial characterization of the human erythrocyte membrane component(s) that express the antigens of the LW blood-group system. Biochem J. 1986;234:649-652.
50. Brown E, Hooper L, Ho T, Gresham H. Integrin-associated protein: a 50-kD plasma membrane antigen physically and functionally associated with integrins. J Cell Biol. 1990;111:2785-2794.
51. Mawby WJ, Holmes CH, Anstee DJ, Spring FA, Tanner MJ. Isolation and characterization of CD47 glycoprotein: a multispanning membrane protein which is the same as integrin- associated protein (IAP) and the ovarian tumour marker OA3. Biochem J. 1994;304:525-530.
52. Reinhold MI, Lindberg FP, Plas D, Reynolds S, Peters MG, Brown EJ. In vivo expression of alternatively spliced forms of integrin-associated protein (CD47). J Cell Sci. 1995;108:3419-3425.
53. Gao AG, Lindberg FP, Finn MB, Blystone SD, Brown EJ, Frazier WA. Integrin-associated protein is a receptor for the C-terminal domain of thrombospondin. J Biol Chem. 1996;271:21-24.
54. Schwartz MA, Brown EJ, Fazeli B. A 50-kDa integrin-associated protein is required for integrin-regulated calcium entry in endothelial cells. J Biol Chem. 1993;268:19,931-19,934.
55. Miller YE, Daniels GL, Jones C, Palmer DK. Identification of a cell-surface antigen produced by a gene on human chromosome 3 (cen-q22) and not expressed by Rhnull cells. Am J Hum Genet. 1987;41:1061-1070.
56. Avent N, Judson PA, Parsons SF, et al. Monoclonal antibodies that recognize different membrane proteins that are deficient in Rhnull human erythrocytes: one group of antibodies reacts with a variety of cells and tissues whereas the other group is erythroid-specific. Biochem J. 1988;251:499-505.
57. Gardner B, Anstee DJ, Mawby WJ, Tanner MJ, von dem Borne AE. The abundance and organization of polypeptides associated with antigens of the Rh blood group system. Transfus Med. 1991;1:77-85.
58. Mallinson G, Anstee DJ, Avent ND, et al. Murine monoclonal antibody MB-2D10 recognizes Rh-related glycoproteins in the human red cell membrane. Transfusion. 1990;30:222-225.
59. Ballas SK, Reilly PA, Murphy DL. The blood group U antigen is not located on glycophorin B. Biochim Biophys Acta. 1986;884:337-343.
60. Colledge KI, Pezzulich M, Marsh WL. Anti-Fy5, and antibody disclosing a probable association between the Rhesus and Duffy blood group genes. Vox Sang. 1973;24:193-199.
61. Tanner MJ. Molecular and cellular biology of the erythrocyte anion exchanger (AE1). Semin Hematol. 1993;30:34-57.
62. Fujinaga J, Tang X-B, Casey JR. Topology of the membrane domain of human erythrocyte anion exchange protein, AE1. J Biol Chem. 1999;274: 6626-6633.
63. Booth PB, Serjeantson S, Woodfield DG, Amato D. Selective depression of blood group antigens associated with hereditary ovalocytosis among Melanesians. Vox Sang. 1977;32:99-110.
64. Tanner MJ, Bruce L, Martin PG, Rearden DM, Jones GL. Melanesian hereditary ovalocytes have a deletion in red cell band 3. Blood. 1991; 78:2785-2786.
65. Schofield AE, Reardon DM, Tanner MJ. Defective anion transport activity of the abnormal band 3 in hereditary ovalocytic red blood cells. Nature. 1992;355:836-838.
66. Liu S-C, Palek J, Yi SJ, et al. Molecular basis of altered red blood cell membrane properties in Southeast Asian ovalocytosis: role of the mutant band 3 protein in band 3 oligomerization and retention by the membrane skeleton. Blood. 1995: 86;349-358.
67. Mohandas N, Winardi R, Knowles D, et al. Molecular basis for membrane rigidity of hereditary ovalocytosis: a novel mechanism involving the cytoplasmic domain of band 3. J Clin Invest. 1992;89:686-692.
68. Beckmann R, Smythe JS, Anstee DJ, Tanner MJA. Functional cell surface expression of band 3, the human red blood cell anion exchange protein (AE1), in K562 erythroleukemia cells: band 3 enhances the cell surface reactivity of Rh antigens. Blood. 1998;92:4428-4438.
69. Huang C-H, Chen Y, Reid M, Ghosh S. Genetic recombination at the human *RH* locus: a family study of the red cell-Evans phenotype reveals a transfer of exons 2-6 from the *RHD* to the *RHCE* gene. Am J Hum Genet. 1996;59:825-833.
70. Avent ND, Martin PG, Armstrong-Fisher SS, et al. Evidence of genetic diversity underlying Rh D^-, weak D (D^u), and partial D phenotypes as determined by multiplex polymerase chain reaction analysis of the *RHD* gene. Blood. 1997;89:2568-2577.
71. Wagner FF, Gassner C, Müller TH, Schönitzer D, Schuhnter F, Flegel WA. Three molecular structures cause rhesus D category VI phenotypes with distinct immunohematological features. Blood. 1998;91:2157-2168.
72. Kemp TJ, Poulter M, Carritt B. A recombination hot spot in the Rh genes revealed by analysis of unrelated donors with the rare D—phenotype. Am J Hum Genet. 1996;59:1066-1073.
73. Westhoff CM, Wylie DE. Investigation of the RH locus in gorillas and chimpanzees. J Mol Evol. 1996;42:658-668.
74. Okuda H, Kawano M, Iwamoto S, et al. The *RHD* gene is highly detectable in RhD-negative Japanese donors. J Clin Invest. 1997;100:373-379.
75. Matassi G, Chérif-Zahar B, Raynal V, Rouger P, Cartron JP. Organization of the human *RH50A* gene (RHAG) and evolution of base composition of the RH gene family. Genomics. 1998;47:286-293.
76. Iwamoto S, Omi T, Yamasaki M, Okuda H, Kawano M, Kajii E. Identification of 5' flanking sequence of RH50 gene and the core region for erythroid-specific expression. Biochem Biophys Res Commun. 1998;243:233-240.
77. Kitano T, Sumiyama K, Shiroishi T, Saitou N. Conserved evolution of the Rh50 gene compared to its homologous Rh blood group gene. Biochem Biophys Res Commun. 1998;249:78-85.
78. Wilson R, Ainscough R, Anderson K, et al. 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans.* Nature. 1994;368:32-38.
79. Seack J, Pancer Z, Muller IM, Muller WE. Molecular cloning and primary structure of a Rhesus (Rh)-like protein from the marine sponge *Geodia cydonium.* Immunogenetics. 1997;46:493-498.
80. Apoil PA, Blancher A. Sequences and evolution of mammalian *RH* gene transcripts and proteins. Immunogenetics. 1999;49:15-25.
81. Apoil PA, Roubinet F, Blancher A. Gorilla *RH*-like genes and antigens. Immunogenetics. 1999;49: 125-133.
82. Blancher A, Klein J, Socha WW. Molecular Biology and Evolution of Blood Group and MHC Antigens in Primates. Berlin: Springer-Verlag; 1997.
83. Carritt B, Kemp TJ, Poulter M. Evolution of the human RH (rhesus) blood group genes: a 50 year old prediction (partially) fulfilled. Hum Mol Genet. 1997;6:843-850.
84. Colin Y, Chérif-Zahar B, Le Van Kim C, Raynal V, Van Huffel V, Cartron J-P. Genetic basis of the RhD-positive and RhD-negative blood group polymorphism as determined by Southern analysis. Blood. 1991;78:2747-2752.
85. Avent ND. The Rhesus blood group system: insights from recent advances in molecular biology. Transfus Med Rev. In press.
86. Flegel WA, Wagner FF, Müller TH, Gassner C. Rh phenotype prediction by DNA typing and its application to practice. Transfus Med. 1998;8:281-302.
87. Umenishi F, Kajii E, Ikemoto S. Molecular analysis of Rh polypeptides in a family with RhD-positive and RhD-negative phenotypes. Biochem J. 1994;299:207-211.
88. Hyland CA, Wolter LC, Saul A. Three unrelated Rh D gene polymorphisms identified among blood donors with Rhesus CCee (r'r') phenotypes. Blood. 1994;84:321-324.
89. Daniels G, Green C, Smart E. Differences between RhD-negative Africans and RhD-negative Europeans. Lancet. 1997;350:862-863.
90. Rouillac C, Gane P, Cartron J, Le Pennec PY, Cartron JP, Colin Y. Molecular basis of the altered antigenic expression of RhD in weak D (D^u) and RhC/e in R^N phenotypes. Blood. 1996;87:4853-4861.
91. Legler TJ, Maas JH, Blaschke V, et al. *RHD* genotyping in weak D phenotypes by multiple polymerase chain reactions. Transfusion. 1998; 38:434-440.
92. Wagner FF, Gassner C, Müller TH, Schönitzer D, Schunter F, Flegel WA. Molecular basis of weak D phenotypes. Blood. 1999;93:385-393.
93. Simsek S, de Jong CA, Cuijpers HT, et al. Sequence analysis of cDNA derived from reticulocyte mRNAs coding for Rh polypeptides and demonstration of of E/e and C/c polymorphisms. Vox Sang. 1994;67:203-209.

94. Avent ND, Daniels GL, Martin PG, Green CA, Finning KM, Warner KM. Molecular investigation of the Rh C/c polymorphism [abstract]. Transfus Med. 1997;7(suppl 1):18.
95. Blancher A, Socha WW. The Rhesus system. In: Blancher A, Klein J, Socha WW, eds. Molecular Biology and Evolution of Blood Group and MHC Antigens in Primates. Berlin: Springer-Verlag; 1997:147-218.
96. Westhoff CM, Silberstein LE, Wylie DE, Apoil P, Blancher A. Characterization of the Rh locus of the New World capuchin (*Cebus apella*) monkey [abstract]. Transfusion 1998;38(suppl):102S.
97. Issitt PD. An invited review: the Rh antigen e, its variants, and some closely related serological observations. Immunohematology. 1991;7:29-36.
98. Faas BHW, Beckers EAM, Wildoer P, et al. Molecular background of VS and weak C expression in blacks. Transfusion. 1997;37:38-44.
99. Steers F, Wallace M, Johnson P, Carritt B, Daniels G. Denaturing gradient gel electrophoresis: a novel method for determining Rh phenotype from genomic DNA. Br J Haematol. 1996;94: 417-421.
100. Huang C-H, Reid ME, Chen Y, Novaretti M. Deletion of Arg229 in RhCE polypeptide alters expression of RhE and CE-associated Rh6 [abstract]. Blood. 1997;90(suppl 1):272a.
101. Westhoff CM, Silberstein LE, Sipherd B, et al. Altered "e" antigen expression associated with ^{16}Cys in exon 1 of the RHce gene [abstract]. Transfusion. 1998;38(Suppl):64S.
102. Avent ND, Finning KM, Liu W, Scott ML. Molecular biology of partial D phenotypes. Transfus Clin Biol. 1996;3:511-516.
103. Noizat-Pirenne F, Mouro I, Gane P, et al. Heterogeneity of blood group RhE variants revealed by serological analysis and molecular alteration of the *RHCE* gene and transcript. Br J Haematol. 1998;103:429-436.
104. Daniels GL, Faas BHW, Green CA, et al. The VS and V blood group polymorphisms in Africans: a serological and molecular analysis. Transfusion. 1998;38:951-958.
105. Faas BHW, Beckers EAM, Simsek S, et al. Involvement of Ser103 of the Rh polypeptides in G epitope formation. Transfusion. 1996;36:506-511.
106. Rouillac C, Le Van Kim C, Blancher A, Roubinet F, Cartron J-P, Colin Y. Lack of G blood group antigen in D^{IIIb} erythrocytes is associated with segmental DNA exchange between *RH* genes. Br J Haematol. 1995;89:424-426.
107. Lomas C, Mougey R. Rh antigen D: variable expression in D^{VI} phenotypes; a possible subdivision of category VI by a low frequency antigen [abstract]. Transfusion. 1989;29(suppl):14S.
108. Matassi G, Chérif-Zahar B, Mouro I, Cartron JP. Characterization of the recombination hot spot involved in the genomic rearrangement leading to the hybrid *D-CE-D* gene in the D^{VI} phenotype. Am J Hum Genet. 1997;60:808-817.
109. Race RR, Sanger R, Selwyn JG. A probable deletion in a human Rh chromosome. Nature. 1950; 166:520-521.
110. Tippett P, Lomas-Francis C, Wallace M. The Rh antigen D: partial D antigens and associated low incidence antigens. Vox Sang. 1996;70:123-131.
111. Reid ME, Sausais L, Zaroulis CG, Mohandas K, Coghlan G, Lomas-Francis C. Two examples of an inseparable antibody that reacts equally well with $D^{W}+$ and Rh32+ red blood cells. Vox Sang. 1998;75:230-233.
112. Lomas C, Tippett P, Thompson KM, Melamed MD, Hughes-Jones NC. Demonstration of seven epitopes on the Rh antigen D using human monoclonal anti-D antibodies and red cells from D categories. Vox Sang. 1989;57:261-264.
113. Lomas C, McColl K, Tippett P. Further complexities of the Rh antigen D disclosed by testing category DII cells with monoclonal anti-D. Transfus Med. 1993;3:67-69.
114. Jones J, Scott ML, Voak D. Monoclonal anti-D specificity and Rh D structure: criteria for selection of monoclonal anti-D reagents for routine typing of patients and donors. Transfus Med. 1995;5: 171-184.
115. Scott M. Rh serology—Coordinator's report. Transfus Clin Biol. 1996;3:333-337.
116. Apoil PA, Reid ME, Halverson G, et al. A human monoclonal anti-D antibody which detects a non-conformation-dependent epitope on the RhD protein by immunoblotting. Br J Haematol. 1997;98: 365-374.
117. Cartron J-P, Rouillac C, Le Van Kim C, Mouro I, Colin Y. Tentative model for the mapping of D epitopes on the RhD polypeptide. Transfus Clin Biol. 1996;3:497-503.
118. Scott ML, Voak D, Jones JW, et al. A structural model for 30 Rh D epitopes based on serological and DNA sequence data from partial D phenotypes. Transfus Clin Biol. 1996;3:391-396.
119. Chang TY, Siegel DL. Genetic and immunological properties of phage-displayed human anti-Rh(D) antibodies: implications for Rh(D) epitope topology. Blood. 1998;91:3066-3078.
120. Liu W, Smythe JS, Scott ML, Jones JW, Voak D, Avent ND. Site-directed mutagenesis of the human D antigen: definition of D epitopes on the sixth external domain of the D protein expressed on K562 cells. Transfus. 1999;39:17-25.
121. Zhu A, Haller S, Li H, Chaudhuri A, Blancher A, Suyama K. Use of RhD fusion protein expressed on K562 cell surface in the study of molecular basis for D antigenic epitopes. J Biol Chem. 1999;274:5731-5737.
122. Liu W, Avent ND, Jones JW, Scott ML, Voak D. D epitope localisation using site directed mutagenesis and expression of Rh mutant constructs in K562 cells. Blood. In press.
123. Mollison PL, Engelfriet CP, Contreras M. Blood Transfusion in Clinical Medicine. Oxford, England: Blackwell Science; 1997.
124. Standards Committee of American Association of Blood Banks. Standards for Blood Banks and Transfusion Services. Bethesda, MD: American Associations of Blood Banks; 1999.
125. Petz LD, Garratty G. Acquired Immune Hemolytic Anemias. New York: Churchill Livingstone; 1980.
126. Sloan SR, Silberstein LE. Transfusion in the face of autoantibodies. In: Reid ME, Nance SJ, eds. Red Cell Transfusion: A Practical Guide. Totowa, NJ: Humana Press; 1998:55-70.
127. Vengelen-Tyler V. Technical Manual. Bethesda, MD: American Association of Blood Banks; 1999.
128. Lacey PA, Caskey CR, Werner DJ, Moulds JJ. Fatal hemolytic disease of a newborn due to anti-D in an Rh-positive Du variant mother. Transfusion. 1983;23:91-94.
129. Reid ME, Halverson GR, Roubinet F, Apoil PA, Blancher A. Use of LOR-15C9 monoclonal anti-D to differentiate erythrocytes with the partial D^{VI} antigen from those with other partial D antigens or weak D antigens. Immunohematology. 1998; 14:89-93.
130. Bowman JM. The development and use of polyclonal prophylactic anti-D IgG. Biotest Bull. 1997; 5:503-510.
131. Giblett ER. Blood group alloantibodies: an assessment of some laboratory practices. Transfusion. 1977;4:299-308.
132. Hoeltge GA, Domen RE, Rybicki LA, Schaffer PA. Multiple red cell transfusions and alloimmunization: experience with 6996 antibodies detected in a total of 159,262 patients from 1985 to 1993. Arch Pathol Lab Med. 1995;119:42-45.
133. Heddle NM, Klama L, Frassetto R, O'Hoski P, Leaman B. A retrospective study to determine the risk of red cell alloimmunization and transfusion during pregnancy. Transfusion. 1993;33:217-220.
134. Zupanska B. Assays to predict the clinical significance of blood group antibodies. Curr Opin Hematol. 1998;5:412-416.
135. Ulm B, Svolba G, Ulm MR, Bernaschek G, Panzer S. Male fetuses are particularly affected by maternal alloimmunization to D antigen. Transfusion. 1999;39:169-173.
136. Lubenko A, Contreras M, Habash J. Should anti-Rh immunoglobulin be given D variant women? Br J Haematol. 1989;72:429-433.
137. Bussel JB, Graziano JN, Kimberly RP, Pahwa S, Aledort LM. Intravenous anti-D treatment of immune thrombocytopenic purpura: analysis of efficacy, toxicity, and mechanism of effect [comments]. Blood. 1991;77;1884-1893.
138. Scaradavou A, Woo B, Woloski BMR, et al. Intravenous anti-D treatment of immune thrombocytopenic purpura: experience in 272 patients. Blood. 1997;89:2689-2700.
139. Kumpel BM, Goodrick MJ, Pamphilon DH, et al. Human Rh D monoclonal antibodies (BRAD-3 and BRAD-5) cause accelerated clearance of Rh D^{+} red blood cells and suppression of Rh D immunization in Rh D^{-} volunteers. Blood. 1995;86: 1701-1709.
140. Maaskant-van Wijk PA, Faas BH, de Ruijter JA, et al. Genotyping of *RHD* by multiplex polymerase chain reaction analysis of six *RHD*-specific exons. Transfusion. 1998;38:1015-1021.
141. Pope J, Navarrete C, Warwick R, Contreras M. Multiplex PCR analysis of RhD gene. Lancet. 1995;346:375-376.
142. Stoerker J, Hurwitz C, Rose NC, Silberstein LE, Highsmith WE. Heteroduplex generator in analysis of Rh blood group alleles. Clin Chem. 1996; 42:356-360.
143. Rose NC, Hurwitz C, Silberstein L, Andovalu R, Stoerker J. Prenatal analysis of rhesus CcDEe blood groups by heteroduplex generator. Am J Obstet Gynecol. 1997;176:1084-1089.
144. Aubin JT, Kim CL, Mouro I, et al. Specificity and sensitivity of RHD genotyping methods by PCR-based DNA amplification. Br J Haematol. 1997; 98:356-364.
145. Gassner C, Schmarda A, Kilga-Nogler S, et al. *RHD/CE* typing by polymerase chain reaction using sequence-specific primers. Transfusion. 1997;37:1020-1026.
146. Bennett PR, Le Van Kim C, Colin Y, et al. Prenatal determination of fetal RhD type by DNA amplification. N Engl J Med. 1993;329:607-610.
147. Wolter LC, Hyland CA, Saul A. Rhesus D: genotyping using polymerase chain reaction. Blood. 1993;82:1682-1683.
148. Mayne K, Bowell P, Woodward T, Sibley C, Lomas C, Tippett P. Rh immunization by the partial D antigen of category D^{Va}. Br J Haematol. 1990; 76:537-539.
149. Andrews KT, Wolter LC, Saul A, Hyland CA. The RhD-trait in a white patient with the RhCCee phenotype attributed to a four-nucleotide deletion in the RHD gene. Blood. 1998;92:1839-1840.
150. Singleton BK, Green CA, Avent ND, et al. An *RHD* pseudogene containing a 37 bp duplication and a nonsense mutation is present in most Africans with the Rh D-negative blood group phenotype. Blood. 2000;95:12-18.
151. Fukumori Y, Hori Y, Ohnoki S, et al. Further analysis of Del (D-elute) using polymerase chain reaction (PCR) with RHD gene-specific primers. Transfus Med. 1997;7:227-231.
152. Sun C-F, Chou CS, Lai NC, Wang WT. RHD gene polymorphisms among RhD-negative Chinese in Taiwan. Vox Sang. 1998;75:52-57.
153. Chang JG, Wang JC, Yang TY, et al. Human Rh_{Del} is caused by a deletion of 1,013 bp between

introns 8 and 9 including exon 9 of RHD gene. Blood. 1998;92:2602-2604.

154. Reed W, Lee T-H, Busch MP, Vichinsky EP. Sample suitability for the detection of minor leukocyte populations by polymerase chain reaction (PCR) [abstract]. Transfusion. 1997;37(suppl):107S.
155. Rios M, Storry JR, Hue-Roye K, Reid ME, Castilho LL, Pelegrino JJ. Incidence of partial D, D^{IIIa}, in Black donors as determined by PCR-RFLP analysis [abstract]. Transfusion. 1998;38(suppl): 63S.
156. Omi T, Takahashi J, Tsudo N, et al. The genomic organization of the partial D category D^{Va}: the presence of a new partial D associated with the D^{Va} phenotype. Biochem Biophys Res Commun. 1999;254:786-794.
157. Le Van Kim C, Mouro I, Brossard Y, Chavinie J, Cartron J-P, Colin Y. PCR-based determination of Rhc and RhE status of fetuses at risk of Rhc and RhE haemolytic disease. Br J Haematol. 1994; 88:193-195.
158. Faas BHW, Simsek S, Bleeker PM, et al. Rh E/e genotyping by allele-specific primer amplification. Blood. 1995;85:829-832.
159. Poulter M, Kemp TJ, Carritt B. DNA-based rhesus typing: simultaneous determination of RHC and RHD status using the polymerase chain reaction. Vox Sang. 1996;70:164-168.
160. Lo YM, Bowell PJ, Selinger M, et al. Prenatal determination of fetal RhD status by analysis of peripheral blood of rhesus negative mothers. Lancet. 1993;341:1147-1148.
161. Lo YMD, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet. 1997;350:485-487.
162. Lo YMD, Hjelm NM, Fidler C, et al. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med. 1998;339:1734-1738.
163. Faas BH, Beuling EA, Christiaens GC, von dem Borne AE, van der Schoot CE. Detection of fetal RHD-specific sequences in maternal plasma. Lancet. 1998;352:1196.
164. Adinolfi M, Sherlock J, Kemp T, et al. Prenatal detection of fetal RhD DNA sequences in transcervical samples. Lancet. 1995;345:318-319.
165. Bennett PR, Overton TG, Lighten AD, Fisk NM. Rhesus D typing. Lancet. 1995;345:661-662.
166. Kingdom J, Sherlock J, Rodeck C, Adinolfi M. Detection of trophoblast cells in transcervical samples collected by lavage or cytobrush. Obstet Gynecol. 1995;86:283-288.
167. Hamlington J, Cunningham J, Mason G, Mueller R, Miller D. Prenatal detection of rhesus D genotype. Lancet. 1997;349:540.
168. Cheung M-C, Goldberg JD, Kan YW. Prenatal diagnosis of sickle cell anaemia and thalassaemia by analysis of fetal cells in maternal blood. Nat Genet. 1996;14:264-268.
169. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci U S A. 1996;93:705-708.
170. Seidl S, Spielmann W, Martin H. Two siblings with Rh_{null} disease. Vox Sang. 1972;23:182-189.
171. Ballas SK, Clark MR, Mohandas N, et al. Red cell membrane and cation deficiency in Rh null syndrome. Blood. 1989;63:1046-1055.
172. Nash R, Shojania AM. Hematological aspect of Rh deficiency syndrome: a case report and a review of the literature. Am J Hematol. 1987;24: 267-275.
173. Kuypers F, van Linde-Sibenius-Trip M, Roelofsen B, Tanner MJ, Anstee DJ, Op den Kamp JA. Rh_{null} human erythrocytes have an abnormal membrane phospholipid organization. Biochem J. 1984;221:931-934.
174. Chérif-Zahar B, Raynal V, Gane P, et al. Candidate gene acting as a suppressor of the *RH* locus in most cases of Rh-deficiency. Nat Genet. 1996; 12:168-173.
175. Hyland CA, Chérif-Zahar B, Cowley N, et al. A novel single missense mutation identified along the RH50 gene in a composite heterozygous Rh_{null} blood donor of the regulator type. Blood. 1998;91:1458-1463.
176. Huang C-H, Chen Y, Reid ME, Seidl C. Rh_{null} disease: the amorph type results from a novel double mutation in RhCe gene on D-negative background. Blood. 1988;92:664-671.
177. Chérif-Zahar B, Matassi G, Raynal V, et al. Rh-deficiency of the regulator type caused by splicing mutations in the human *RH50* gene. Blood. 1998;92:2535-2540.
178. Reid ME, Bird GW. Associations between human red cell blood group antigens and disease. Transfus Med Rev. 1990;4:47-55.
179. Chérif-Zahar B, Bony V, Steffensen R, et al. Shift from Rh-positive to Rh-negative phenotype caused by a somatic mutation within the RHD gene in a patient with chronic myelocytic leukaemia. Br J Haematol. 1998;102:1263-1270.
180. Chérif-Zahar B, Mattei MG, Le Van Kim C, Bailly P, Cartron J-P, Colin Y. Localization of the human Rh blood group gene structure to chromosome region 1p34.3-1p36.1 by in situ hybridization. Hum Genet. 1991;86:398-400.
181. Hermand P, Gane P, Mattei MG, Sistonen P, Cartron J-P, Bailly P. Molecular basis and expression of the LW^a/LW^b blood group polymorphism. Blood. 1995;86:1590-1594.
182. Zelinski T, Coghlan G, White L, Philipps S. The Diego blood group locus is located on chromosome 17q. Genomics. 1993;17:665-666.
183. Dahr W, Kordowicz M, Moulds J, Gielen W, Lebeck L, Krüger J. Characterization of the Ss sialoglycoprotein and its antigens in Rh_{null} erythrocytes. Blut. 1987;54:13-24.
184. Huang C-H. Alteration of *RH* gene structure and expression in human dCCee and DC^W- red blood cells: phenotypic homozygosity versus genotypic heterozygosity. Blood. 1996;88:2326-2333.
185. Noizat-Pirenne F, Mouro I, Gane P, et al. Molecular analysis of selected Rh variants. Transfus Clin Biol. 1996;3:517-519.
186. Chérif-Zahar B, Raynal V, Cartron JP. Lack of RHCE-encoded proteins in the D—phenotype may result from homologous recombination between the two *RH* genes. Blood. 1996;88:1518-1520.
187. Cheng G-J, Chen Y, Reid ME, Huang C-H. Evans antigen: a new hybrid structure occurring on background of *D*•• and *D*—Rh complexes. Vox Sang. In press.
188. Huang C-H, Liu PZ, Cheng JG. Molecular biology and genetics of the Rh blood group system. Semin Hematol. In press.
189. Chérif-Zahar B, Matassi G, Raynal V, et al. Molecular defects of the *RHCE* gene in Rh-deficient individuals of the amorph type. Blood. 1998;92: 639-646.
190. Mouro I, Le Van Kim C, Rouillac C, et al. Rearrangements of the blood group RhD gene associated with the D^{VI} category phenotype. Blood. 1994;83:1129-1135.
191. Flegel WA, Wagner FF. The frequency of RHD protein variants in Caucasians [abstract]. Transfus Clin Biol. 1996;3:10S.
192. Avent ND, Jones JW, Liu W, et al. Molecular basis of the D variant phenotypes DNU and D^{II} allows localization of critical amino acids required for expression of Rh D epitopes epD3, 4 and 9 to the sixth external domain of the Rh D protein. Br J Haematol. 1997;97:366-371.
193. Rouillac C, Le Van Kim C, Beolet M, Cartron J-P, Colin Y. Leu110Pro substitution in the RhD polypeptide is responsible for the D^{VII} category blood group phenotype. Am J Hematol. 1995;49:87-88.
194. Rouillac C, Colin Y, Hughes-Jones NC, et al. Transcript analysis of D category phenotypes predicts hybrid Rh D-CE-D proteins associated with alteration of D epitopes. Blood. 1995;85:2937-2944.
195. Wagner FF, Gassner C, Eicher N, Lonicer C, Flegel WA. Characterization of D category IV type IV, DFW, and DNB [abstract]. Transfusion. 1998; 38(suppl):63S.
196. Jones JW, Finning K, Mattock R, et al. The serological profile and molecular basis of a new partial D phenotype, DHR. Vox Sang. 1997;73:252-256.
197. Huang C-H, Chen Y, Reid M. Human D^{IIIa} erythrocytes: RhD protein is associated with multiple dispersed amino acid variations. Am J Hematol. 1997;55:139-145.
198. Beckers EA, Faas BH, Ligthart P, et al. Characterization of the hybrid RHD gene leading to the partial D category IIIc phenotype. Transfusion. 1996;36:567-574.
199. Avent ND, Liu W, Jones JW, et al. Molecular analysis of Rh transcripts and polypeptides from individuals expressing the D^{VI} variant phenotype: an *RHD* gene deletion event does not generate all D^{VI}ccEe phenotypes. Blood. 1997;89:1779-1786.
200. Huang C-H. Human D^{VI} category erythrocytes: correlation of the phenotype with a novel hybrid RhD-CE-D gene but not an internally deleted RhD gene. Blood. 1997;89:1834-1835.
201. Faas BHW, Beckers EAM, Maaskant-van Wijk PA, Overbeeke MAM, Van Rhenen DJ, Von dem Borne AEGK. Molecular characterization of qualitative Rh variants. Biotest Bull. 1997;5:439-449.
202. Beckers EAM, Faas BHW, Simsek S, et al. The genetic basis of a new partial D antigen: D^{DBT}. Br J Haematol. 1996;93:720-727.
203. Huang C-H, Chen Y, Reid ME, Okubo Y. Evidence for a separate genetic origin of the partial D phenotype DBT in a Japanese family. Transfusion. In press.
204. Hemker M, Ligthart PC, Faas BHW, et al. ARRO-I: a new partial D phenotype involving exon 4 and 5 [abstact]. Vox Sang. 1998;74(suppl 1):1331.
205. Pisacka M, Vytisková J, Hejná J, Gassner C. A new variant of Rh(D) antigen: revealed by reactions of anti-ep12 monoclonal antibodies and lacking exon 5 D-specific reaction of exon-scanning RHD/CE PCR-SSP [abstract]. Vox Sang. 1998;74(suppl 1):1332.
206. Faas BHW, Ligthart PC, Lomas-Francis C, Overbeeke MAM, von dem Borne AEG, van der Schoot CE. Involvement of Gly96 in the formation of the Rh26 epitope. Transfusion. 1997;37:1123-1130.
207. Beckers EAM, Faas BHW, Von dem Borne AEGK, Overbeeke MAM, Van Rhenen DJ, van der Schoot CE. The R_0^{Har}Rh:33 phenotype results from substitution of exon 5 of the *RHCE* gene by the corresponding exon of the *RHD* gene. Br J Haematol. 1996;92:751-757.
208. Mouro I, Colin Y, Gane P, et al. Molecular analysis of blood group Rh transcripts from a r^Gr variant. Br J Haematol. 1996;93:472-474.
209. Huang CH, Cheng GJ, Reid ME, Chen Y. Rh_{mod} syndrome: a family study of the translation-initiator mutation in the Rh50 glycoprotein gene. Am J Hum Genet. 1999;64:108-117.
210. Huang C-H, Cheng G, Liu Z, et al. Molecular basis for Rh_{null} syndrome: identification of three new missense mutations in the Rh50 glycoprotein gene. Am J Hematol. In press.
211. Okuda H, Suganuma H, Tsudo N, Omi T, Iwamoto S, Kajii E. Sequence analysis of the spacer region between the *RHD* and *RHCE* genes. Biochem Biophys Res Commun. 1999;263:378-383.