7. The Rh blood group

The Rh blood group is one of the most complex blood groups known in humans. From its discovery 60 years ago where it was named (in error) after the Rhesus monkey, it has become second in importance only to the ABO blood group in the field of transfusion medicine. It has remained of primary importance in obstetrics, being the main cause of hemolytic disease of the newborn (HDN).

The complexity of the Rh blood group antigens begins with the highly polymorphic genes that encode them. There are two genes, RHD and RHCE, that are closely linked. Numerous genetic rearrangements between them has produced hybrid Rh genes that encode a myriad of distinct Rh antigens. To date, 49 Rh antigens are known.

The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen will produce anti-D if they encounter the D antigen on transfused RBCs (causing a hemolytic transfusion reaction, HTR) or on fetal RBCs (causing HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be.

Despite the importance of the Rh antigens in blood transfusion and HDN, we can only speculate about the physiological function of the proteins, which may involve transporting ammonium across the RBC membrane and maintaining the integrity of the RBC membrane.

At a glance

Antigens of the Rh blood group.

Number of antigens	**49: D, C, E, c, and e are among the most significant**
Antigen specificity	**Protein** The sequence of amino acids determines the specificity of most of the Rh antigens.
Antigen-carrying molecules	**Proteins with unknown function** The RhD and RhCE proteins are both transmembrane, multipass proteins that are integral to the RBC membrane. The RhCE protein encodes the C/c antigen (in the 2nd extracellular loop) and the E/e antigen (in the 4th extracellular loop), plus many other Rh antigens e.g., C^w, C^x.

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	Unlike most cell surface molecules, the Rh proteins are not glycosylated (they do not contain oligosaccharides) but they are closely associated with a RBC membrane glycoprotein called RhAG. The function of the Rh-RhAG complex might involve transporting ammonium or carbon dioxide. The RhD protein encodes the D antigen.
Molecular basis	**Two genes, RHD and RHCE, encode the Rh antigens.** The Rh genes are 97% identical, and they are located next to each other on chromosome 1. The D/d polymorphism most commonly arises from a deletion of the entire RHD gene. The C/c polymorphism arises from four SNPs that cause four amino acid changes, one of which (S103P) determines the C or c antigen specificity. The E/e polymorphism arises from a single SNP (676G→C) that causes a single amino acid change (A226P).
Frequency of Rh antigens	**D**: 85% Caucasians, 92% Blacks, 99% Asians **C**: 68% Caucasians, 27% Blacks, 93% Asians **E**: 29% Caucasians, 22% Blacks, 39% Asians **c**: 80% Caucasians, 96% Blacks, 47% Asians **e**: 98% Caucasians, 98% Blacks, 96% Asians (1)
Frequency of Rh phenotypes	**Rh haplotype DCe**: most common in Caucasians (42%), Native Americans (44%), and Asians (70%) **Rh haplotype Dce**: most common in Blacks (44%) **Rh D-negative phenotype**: most common in Caucasians (15%), less common in Blacks (8%), and rare in Asians (1%) (1)

Antibodies produced against Rh antigens.

Antibody type	**Mainly IgG, some IgM** The majority of Rh antibodies are of the IgG type.
Antibody reactivity	**Capable of hemolysis** Rh antibodies rarely activate complement. They bind to RBCs and mark them up for destruction in the spleen (extravascular hemolysis).
Transfusion reaction	**Yes—typically delayed hemolytic transfusion reactions** Anti-D, anti-C, anti-e, and anti-c can cause severe hemolytic transfusion reactions. Hemolysis is typically extravascular (1).
Hemolytic disease of the newborn	**Yes—the most common cause of HDN**. The D antigen accounts for 50% of maternal alloimmunization (2). Anti-D and anti-c can cause severe disease. Anti-C, anti-E, and anti-e can cause mild to moderate disease.

Background information

History

In 1939, a mother who had just given birth to a still-born child needed a blood transfusion. The ABO blood group system had been discovered almost 40 years previously, and the importance of giving an ABO-compatible blood transfusion was well established. However, although the mother was transfused with ABO compatible blood from her husband, she still experienced an adverse reaction to the transfusion. Her serum was found to contain antibodies that agglutinated her husband's RBCs, even though they

were ABO compatible. The death of the mother's fetus and her adverse reaction to a blood transfusion from her husband was related. During the pregnancy, the mother had been exposed to an antigen on the fetal RBCs that was of paternal origin. Her immune system attacked this antigen, and the destruction of the fetal RBCs resulted in fetal death. The mother re-encountered the same paternal antigen when she received a blood transfusion from her husband. This time her immune system attacked the transfused RBCs, causing a hemolytic transfusion reaction. The antibodies responsible led to the discovery of the Rh blood group.

It was wrongly thought that the agglutinating antibodies produced in the mother's serum in response to her husbands RBCs were the same specificity as antibodies produced in various animals' serum in response to RBCs from the Rhesus monkey. In error, the paternal antigen was named the Rhesus factor. By the time it was discovered that the mother's antibodies were produced against a different antigen, the rhesus blood group terminology was being widely used. Therefore, instead of changing the name, it was abbreviated to the Rh blood group.

Remarkably, only 20 years after the discovery of Rh incompatability in pregnancy, effective treatment became available. Today, the Rh status of mothers-to-be is checked during pregnancy to identify those at risk of HDN. In addition, all blood transfusions are matched for the Rh status.

Nomenclature

- Number of Rh antigens: 49
- ISBT symbol: Rh
- ISBT number: 004
- Gene symbols: RHD and RHCE
- Gene names: Rhesus blood group, D antigen; and, Rhesus blood group, CcEe antigens

Basic biochemistry

Common Rh phenotypes

The most common Rh haplotype in Caucasians, Asians, and Native Americans is DCe. In Blacks, the Dce haplotype is slightly more common (1).

In Caucasians, the Rh D-negative phenotype results from a deletion of the RHD gene. About 15% of Caucasians are Rh D-negative.

In Africans, there are three molecular backgrounds that give rise to the Rh D-phenotype which is found in 8% of the population. One is the RHD gene deletion that is common in Caucasians. The other two mechanisms are inheriting a RHD pseudogene (contains a duplication of nucleotides that introduces a premature stop codon) or inheriting a RHD

hybrid gene (contains nucleotide sequences from the RHCE gene, produces no D antigen and abnormal C antigen) (3)

Uncommon Rh phenotypes

The D antigen contains over 30 epitopes. Variations of the D phenotype arise when these epitopes are only weakly expressed ("weak D phenotype") or when some are missing ("partial D phenotype").

Weak D: all D antigen epitopes are present but are underexpressed

"Weak D" is a Rh phenotype found in less than 1% of Caucasians and is only slightly more common in African Americans (2). It is typically caused by a single amino acid switch in the transmembrane region of the RhD protein. This disrupts how the RhD protein is inserted into the RBC membrane, reducing the level of expression of RhD. In most cases, adequate levels of D antigen are present and because there has been no change in D epitopes, the formation of anti-D is prevented. Therefore, individuals with the weak D phenotype can receive Rh D-positive blood.

Partial D: some D antigen epitopes are missing

In contrast, people who have been identified as having the "partial D" phenotype should not receive Rh D-positive blood but in practice, people with partial D are difficult to identify. This phenotype is usually caused by the creation of a hybrid RhD and RhCE protein. The hybrid protein is similar enough to RhD to be correctly inserted in the RBC membrane, but it lacks several epitopes found on the complete RhD protein. If a person with the partial D phenotype encounters the complete D antigen on transfused RBCs, they may form anti-D and suffer from a transfusion reaction.

Expression of Rh antigens

The Rh antigens are expressed as part of a protein complex in the RBC membrane. This complex is only expressed in cells of the erythroid line, and therefore Rh antigens are only expressed in RBCs. The composition of the complex is unknown, but it is thought to be a tetramer, consisting of two molecules of Rh-associated glycoprotein (RhAG) and two molecules of Rh proteins. The Rh proteins may be RhD (carrying the D antigen) or RhCE (carrying the C or c antigen and the E or e antigen). It is unknown whether both RhCE and RhD can be in a single complex, but in D-negative individuals the complex would only contain RhCE.

RhAG must be present to direct the Rh antigens to the RBC membrane. If it is missing, none of the Rh antigens are expressed. RHAG is related to the Rh proteins, sharing about 35% of their primary sequence and is the same type of transmembrane protein. However, it is not polymorphic and does not carry Rh antigens itself (3).

Function of Rh proteins

The Rh antigens are thought to play a role in maintaining the integrity of the RBC membrane—RBCs which lack Rh antigens have an abnormal shape.

Individuals with the rare Rh_{null} phenotype caused by the deletion of RHAG have RBCs that do not express any of the Rh antigens because they cannot be targeted to the RBC membrane. The absence of the Rh complex alters the RBC shape, increases its osmotic fragility, and shortens its lifespan, resulting in a hemolytic anemia that is usually mild in nature. These patients are at risk of adverse transfusion reactions because they may produce antibodies against several of the Rh antigens.

Rh antigens may also be involved in the transport of ammonium across the RBC membrane. Interestingly, the first member of a family of water channels (aquaporins) and the first member of a family of urea transporters were both found in blood group proteins (the Colton blood group and Kidd blood group, respectively).

Clinical significance of Rh antibodies

The Rh antigens are highly immunogenic, and most of the Rh antibodies should be considered as potential causes of hemolytic transfusion reactions and HDN.

Whereas most blood types are determined by red cell antigens that differ by one or two amino acids, the Rh blood group contains the D antigen which differs from the C/c and E/e antigens by 35 amino acids. This large difference in amino acids is the reason why the Rh antigens are potent at stimulating an immune response (4).

The majority of antibodies formed against the Rh antigens are of the IgG type. They are capable of causing significant HTR and HDN. Rh antibodies rarely, if ever, bind complement, and therefore RBC destruction is mediated almost exclusively via macrophages in the spleen (extravascular hemolysis).

There are a few examples of Rh alloantibodies that are naturally occurring and are of the IgM type, but they are in the minority.

Transfusion reactions

Anti-D, anti-C, anti-E, and anti-e have all been involved in hemolytic transfusion reactions, particularly delayed reactions (5).

Routine blood typing for Rh D status in both blood donors and transfusion recipients has reduced the incidence of transfusion reactions caused by anti-D. But sensitization to other Rh antigens can be a problem in transfusion medicine, particularly in patients with sickle cell anemia (SCA). SCA is more common in Blacks, and the treatment of SCA involves blood transfusions. Blacks are also more likely to express variants of the Rh e antigen, and therefore produce anti-e, along with other Rh alloantibodies, which increases the difficulty in finding Rh-compatible blood donors.

Hemolytic disease of the newborn

Anti-D causes the most severe form of HDN and it used to be a major cause of fetal death. Since the introduction of anti-D immunoglobulin along with careful monitoring of at-risk pregnancies, the prevalence of HDN because of Rh D incompatibility has decreased dramatically. However, all cases cannot be prevented, and RhD alloimmunization remains a major cause of disease (6).

Other Rh alloantibodies that are capable of causing severe HDN include anti-c (7, 8), which clinically is the most important Rh antigen after the D antigen.

Moderate disease can be caused by anti-C^w (9) and anti-C^x (10). Rh alloantibodies that are typically associated with mild HDN include anti-C (relatively common) (11), anti-E (12), and anti-e (13).

Molecular information

Gene

The Rh locus is located on the long arm of chromosome 1 (on 1p36-p34). It contains the RHD and RHCE genes, which lie in tandem. The RHD and RHCE genes are structural homologs and result from a duplication of a common gene ancestor.

RHD and RHCE each contain 10 exons and span a ~75-kb DNA sequence. The RHD gene is flanked by two 9-kb, highly homologous sequences called "Rhesus boxes" (14, 15). It is thought that unequal homologous recombination confined to the Rhesus boxes is a common cause of the deletion of the RHD gene, which is found in up to 40% of the population.

View the sequences of RHD and RHCE alleles at the
dbRBC Sequence Alignment Viewer

Protein

The RHD and RHCE genes each encode a transmembrane protein over 400 residues in length that traverses the RBC membrane 12 times. The RhD protein only differs from the common form of the RhCE protein by about 35 amino acids.

The RhD protein bears the D antigen which has over 30 epitopes. The RhCE protein carries the epitope for the C or c antigen on the second extracellular loop, and the epitope for the E or e antigen on the fourth extracellular loop. A number of nucleotide substitutions in the RHCE gene in turn cause a number of amino acid changes in the RhCE protein, but two polymorphisms are thought to be key in producing the polymorphic antigens on this protein, i.e., the S103P polymorphism (produces the C or c

antigen, respectively), and the P226A polymorphism (produces the E or e antigen, respectively).

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NCBI Resources

The D antigen and CcEe antigens in OMIM

The RHD locus in Entrez Gene | MapViewer | PubMed Central | PubMed

The RHCE locus in Entrez Gene | MapViewer | PubMed Central | PubMed

Alleles of the RHD and RHCE loci in the dbRBC Sequence Alignment Viewer (To view this site, your browser needs to allow pop-ups)

Other Resources

Read more about the Rh blood group in the Blood Group Antigen Gene Mutation Database