

## The Rh Antigen D: A Review for Clinicians

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**Introduction:** In transfusion medicine, after the ABO blood groups, the D antigen is the most significant. A high proportion of people whose red blood cells (RBCs) lack D will make anti-D if exposed to the D antigen by pregnancy or transfusion. Accordingly, all D- patients, especially girls and women who may become pregnant, should be transfused with D- RBCs. The D antigen is in the Rh blood group system, which with 49 distinct antigens is the most polymorphic blood group system. This document reviews fundamental information for the D antigen.

**History and terminology:** The D antigen originally was found in 85% of Caucasians typed with serum from a woman whose baby had *erythroblastosis fetalis*. The D antigen, or Rh as it first was called, originally was thought to be the antigen now called LW that initially was defined by Landsteiner and Wiener (hence the renaming to LW) using antibodies produced in rabbits and guinea-pigs immunized with rhesus (hence Rh) monkey RBCs. Consequently, D often is called the Rh antigen, and the terms Rh+ and Rh- refer, respectively, to the presence or absence of D.

The nomenclature for antigens in the Rh system is diverse: a single letter (D, E, c), a symbol with a superscript (C<sup>w</sup>, Go<sup>a</sup>, hr<sup>B</sup>), letters (Evans, Tar, DAK, BARC), and numerical (Rh17, Rh32) notations all are used.<sup>1</sup> The D antigen on the RhD protein is encoded by the RHD gene (*RHD*). The *RHD* is homologous and adjacent to *RHCE* on chromosome 1 (1p36.11). The names used for the D antigen, phenotypes, protein, and gene, and the antigen prevalence in three ethnicities are given in Table I.

**The D antigen:** The D antigen is unique among blood groups because it expresses at least 30 epitopes distributed along the extracellular portions of the RhD protein. Thus a change, or changes, in the amino acid sequence of RhD may not ablate the entire D antigen but can cause epitope loss, giving rise to variant forms of D antigen (known as partial D). In Cauca-

### SUMMARY: The Rh Antigen D

- The Rh antigen D is highly immunogenic and clinically significant in transfusion and pregnancy. As many as 80% of D- recipients of a unit of D+ RBCs will form anti-D.
- Ideally, D- patients should be transfused with D- RBC components.
- A patient whose RBCs lack some D epitopes but still type D+ can make anti-D and should be transfused with D- RBC components. These RBCs type D+ with some anti-D from some clones and D- with other anti-D from others.
- With certain D variant types, differences in testing protocols used by hospital transfusion services and blood collection facilities can lead to the same person being classified as a D- patient but a D+ donor.

sians, most D- phenotypes arise from a deletion of *RHD*, but other mechanisms have been identified, particularly in black Africans and Japanese.<sup>2,3</sup> D- individuals lack the entire RhD protein on their RBCs. This is thought to be a major factor in the highly immunogenic nature of D antigen. RBCs from most people are either D+ (Rh+) or D- (Rh-), but variants of D exist as three main groups: weak D, partial D, and partial weak D. These are encoded by >100 RHD alleles.

**Weak D antigen:** There is no discrete serological distinction between normal D and weak D expression. Instead, there is a spectrum of test reaction strengths. In most cases, RBCs with a weak expression of D antigens are not agglutinated by routinely used anti-D reagents unless the indirect antiglobulin test (IAT) is used. Weak D expression is caused by mutations in the transmembrane or internal portions of RhD, resulting in RBCs with a full complement of D epitopes but fewer D antigen sites per RBC. It is believed that patients with weak D cannot make anti-D, and thus need not be transfused with D- blood or receive prenatal Rh-IgG prophylaxis.

**Partial D antigen and partial weak D antigen:** Rare D+ people have RBCs that lack one or more epitope(s) of the D antigen and, if immunized, may make anti-D to the missing epitope(s). They usually are detected either when their RBCs type D+ and allo anti-D is present in their plasma, or by the detection of a low-prevalence Rh antigen. The anti-D they produce will agglutinate all RBCs with a normal expression of D. There are many types (or categories) of partial D antigens (*e.g.*, DIIIa, DV, DBT), each with a unique molecular basis. Partial D antigens occur as a consequence of novel mutations in RhD, or from the replacement of RhD-specific amino acid(s) with RhCE-specific amino acid(s).<sup>1,3</sup>

RBCs with a partial D antigen usually are agglutinated by some – but not all – monoclonal anti-D reagents in a distinct pattern, typically with strength equivalent to that obtained with normal D+ RBCs using the same reagent. For this reason,

Table I. Terminology for the D blood group antigen, phenotypes, protein and gene, and prevalence of D in three ethnicities

Antigen Name(s)	Phenotypes	Protein	Gene	Prevalence of D		
				Asians	Blacks	Caucasians
D (RH1, Rh <sub>0</sub> )	D+ (Rh+) D- (Rh-)	RhD	<i>RHD</i>	99	92	85



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**Table II. Clinically relevant information about the D antigen**

D Phenotypes	Changes in Amino Acid(s) in RhD	D Antigen expression	Tests Used to Detect D Antigen	IN PATIENT			IN DONOR
				Can make anti-D through transfusion or pregnancy	Type of RBC components for transfusion	Rh IgG prophylaxis recommended	Can cause immunization if transfused to D- recipient
D+	None	Normal	Direct agglutination	No	D+ (Rh+) (or D-)	No	Yes
Partial D	Extracellular	Altered (some D epitopes present, some absent)	Direct agglutination & IAT (depending on reagent used)	Yes	D- (Rh-) or matched partial D*	Yes	Yes
Partial weak D	Extracellular (sometimes also transmembrane and intracellular)	Altered (some D epitopes present, some absent) and weak or variable	Direct agglutination & IAT (depending on reagent used)	Yes	D- (Rh-) or matched partial weak D*	Yes	Unlikely, but possible
Weak D	Transmembrane or intracellular	Normal but weak	IAT	No	D+ (Rh+) (or D-)	No	Unlikely, but possible
D-	RhD absent	Absent	IAT	Yes	D- (Rh-)*	Yes	No

\*When D- RBCs are in short supply, D+ RBCs may be transfused until the patient makes anti-D, especially to patients who cannot become pregnant, e.g. males and postmenopausal women. This is particularly likely when the patient has multiple alloantibodies.

RBCs with a partial D antigen may type as D+ with one anti-D reagent (containing a reactive clone) but D- with another (containing a non-reactive clone). When compared to RBCs with normal D expression (D+), RBCs with a partial weak D antigen are agglutinated by reactive monoclonal anti-D more weakly or variably (*i.e.*, some anti-D react strongly and some react weakly). This variable reactivity of anti-D with RBCs expressing a partial D antigen can lead to confusion (see below), and mistyping.

As RBCs of either type of partial D antigen (partial D and partial weak D) lack D epitopes, patients with any of the various partial D can make alloanti-D. Thus, it is recommended that these D+ patients be transfused with D- RBC components and receive prenatal Rh-IgG prophylaxis. Clinically relevant generalizations about D, weak D, and the partial D antigens are summarized in Table II.

**Testing for D:** D is detected by testing RBCs with anti-D reagents using hemagglutination. Commercial reagents are strongly reactive and agglutinate D+ RBCs in direct tests. Partial D antigens usually are detectable by direct testing with a proportion of anti-D reagents. For RBCs with a weak expression of D antigen, an indirect antiglobulin test usually is needed to detect the D.<sup>5</sup>

Differences in test procedures for D between donors and recipients can result in a person with weak D being classified as D+ (Rh+) as a blood donor, but as D- (Rh-) as a transfusion recipient or pregnant female. RBCs from *donors* that type D- in direct testing are tested by IAT, but IAT testing is not required for *patients*. As a conservative approach, many transfusion services treat patients whose RBCs are agglutinated weakly (2+ or weaker) by anti-D in direct testing as D-. In autologous donations, this presents a discrepancy, because pretransfusion testing would classify the patient's weak D RBCs as D-, but the donor center would label the autologous unit as D+. Testing the patient's RBCs with anti-D by IAT should resolve the discrepancy and confirm the weak D status.

It can be difficult to distinguish weak partial D from weak D and may depend on the patient making anti-D or require

molecular analysis. Similarly, in prenatal cases, DNA testing can be used to identify D+ women who could make anti-D.

**Rh complex in the RBC membrane:** The protein (RhD) carrying D is predicted to pass through the RBC lipid bilayer 12 times. RhD is part of a complex in the RBC membrane that includes the homologous RhCE protein, which carries different combinations of two of the four other clinically relevant Rh antigens (C or c and E or e), and the Rh-Associated Glycoprotein (RhAG). RhAG is required for the expression of Rh antigens. The RhD/RhCE/RhAG (or RhCE/RhAG in D-negative people) complex is part of a larger macromolecular complex that includes CD47, the LW glycoprotein, glycophorin B, protein 4.2 and ankyrin (reviewed in Reid & Mohandas<sup>6</sup>). Much information about the Rh complex in the RBC membrane came from the study of Rh<sub>null</sub> RBCs, which lack RhD, RhCE, RhAG, CD47, and LW glycoprotein. Mutations in *RhCE* or *RhAG* lead to unusual phenotypes, including the Rh<sub>null</sub> phenotype.

**Rh<sub>null</sub> phenotype:** The Rh<sub>null</sub> phenotype is extremely rare but easy to identify because Rh<sub>null</sub> RBCs lack all Rh antigens. Rh<sub>null</sub> RBCs are not agglutinated by anti-D, anti-C, anti-E, anti-c, or anti-e. There are two types of Rh<sub>null</sub>: the regulator type and the amorph type. The regulator type is a consequence of an altered RhAG, while the amorph type is caused by a silenced *RHCE* in a person who has a silenced *RHD* gene (and thus lacks RhD in their RBCs). Both types of Rh<sub>null</sub> pose transfusion problems because the patient readily makes anti-Rh29 (anti-total Rh) and must be transfused with very rare Rh<sub>null</sub> blood. However, not only is the phenotype rare but RBCs with this phenotype have shortened survival and they have a compensated hemolytic anemia. Thus, Rh<sub>null</sub> individuals may not meet blood donation criteria.

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