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## 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 re-naming 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 ( $C^W$ ,  $Go^a$ ,  $hr^B$ ), 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 Caucasians, 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

### SUMMARY

- 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.

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, 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+),

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

(continued on next page)

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 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>3</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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