

D^u PHENOTYPE - A REVIEW

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D^u is the phenotypic term used to denote a weakened expression of the D antigen. D^u originally defined as those red cells reacting with anti-D only when a more sensitive indirect antiglobulin test was used. D^u phenotype can arise from three different genetic situations. a) A person may inherit a gene coding for weakened quantitative expression of D antigen. b) One gene may interact with another to modify and weaken the expression of the D antigen. c) A gene may not code for the total material that makes up the antigen. The frequency of D^u antigen is relatively low less than 1%. D^u is a poor immunogen, however, accelerated destruction of D^u red cells can result if transfused to a person already making anti-D. Hence D^u donor units are currently labelled as Rah positive. D^u recipients are labelled as Rah negative. Newborn of Rah negative mother are tested for D&D^u and Rh Ig is recommended for mothers of D positive or D^u positive infants in order to prevent potential immunisation. The terms D^u variant or partial D^u are recommended when there is both a qualitative and quantitative difference noted in the D antigen.

INTRODUCTION

There are 23 genetically independent blood group systems which together comprise 191 different inherited antigens¹. The antigens of two of these system (LE, CH/RG) are not intrinsic to the red cell but are acquired from plasma, of the remaining 21 systems, three (ABO, H,P) are defined by carbohydrate structure and 18 by protein structure (Lowe 1993 cited by Anstee¹).

Rh blood group system was discovered in 1939. It was the 4th system to be discovered. The first three were ABO in 1901, MNS in 1927, P in 1927¹.

The Rh blood group system is the most polymorphic system of red cells. Until now, 44 different Rh system antigens have been identified (numerical notation 1-51), 7 having been withdrawn². The Rh system antigens are associated with a non-glycosylated human red cell (RBC) membrane protein of molecular mass 30 Kda (Rh 30 polypeptide) and a glycoprotein of 40 to 100 ADa (Rh glycoprotein)³. This Rh 50 glycoprotein and Rh 30 polypeptides form a complex essential for Rh antigen expression and erythrocyte membrane integrity⁴.

There are three schemes for Rh antigen nomenclature shown in the table-1⁵.

Fisher Race Nomenclature or Three Locus Theory:

There are three closely linked loci each with a primary set of allelic genes (D and d, C, c, E and e). These three loci are believed to be closely linked that crossing over occurs very rarely and the three Rh genes are inherited as a complex. In terms of nomenclature, the Rh antigens are therefore named

C,D,E,c,d and e. These can be assembled in eight different ways Cde, cde, cDE, cDe, CDE, cdE, DdE and Cde. The antigen d (and it's corresponding antibody, antid) has never been discovered and is thought not to exist. The symbol "d" is used, however, to denote the absence of D.

Table-1: Three systems of Rh antigen nomenclature

Fisher Race (1994)	Weiner (1951)	Rosenfield (1962)
D	Rho	Rh1
C	rh	Rh2
E	rh	Rh3
c	hr	Rh4
E	hr	Rh5

The Wiener Nomenclature or One Locus Theory:

Rh antigens are determined by allelic genes at a single locus rather than three genes.

In comparing the two theories, it becomes obvious that the only practical difference between the two is that Fisher and Race envision a complex gene, whereas Wiener envisions a complex antigen.

The Rosenfield Nomenclature:

It proposes a numerical terminology for the Rh groups based on a proposal by Murray in 1994 to eliminate certain difficulties in classification using the Fisher-Race and Wiener nomenclature and to pave the way for the storage of such information in computers. The nomenclature proposes a system that describes reaction with particular anti-sera, is free of any genetic implication, and gives equal importance to positive and negative reactions.

The antigens are numbered in chronological order of discovery, and the system has certain obvious advantages. In practical, however, it is

difficult to apply; for example, a phenotype given as Rh; 1,2,3,4,5,6,7,-8,9,-10,-11,12 and so on, depending on how many further antigens had been tested for instant recognition of the involved antigens (Note the minus sign designates absence of the antigen). If an antigen is not tested for, it's number does not appear in the sequence.

LATEST GENETIC THEORY:

The Rh system antigens are products of genes situated at chromosome 1. These genes are closely aligned and named RHD and RHCE and each comprises 10 exons. The individuals with D positive red cells have both RhD and RhCE genes, while most of the individuals with D negative red cells have only one gene, RHCE, RhCE is the name whose alleles include RHCE, RHcE, Rhce and RHCE; most individuals with D negative red cells are homozygous for Rhce. RhCE is the name whose alleles include RHCE, RHcE, Rhce and RHCE; most individuals with D negative red cells are homozygous for Rhce. In D negative persons, a lack of D antigen almost always represents deletion of RHD, although rare individuals with D negative red cells have one or more detectable but non-functional RHD genes⁶.

The Rh blood group system is the next most important to the ABO system in term of it's clinical significance in blood transfusion⁷.

Of the five common Rh system antigens (D,C,c,E and e) D is the most important in transfusion medicine, being highly immunogenic², while the C and E loci of the Rh system are weakly immunogenic. The hemolytic transfusion reactions caused by these minor antigens are typically less severe⁸.

DISCOVERY:

Stratton in 1946 coined the term D^u to describe red cells with a weakened form of the D antigen (cited by Jones et al⁷).

Later, Race et al in 1948 and Renton and Stratton in 1950 studied this antigen further and showed that it was an inherited characteristic. They found that D^u red cells were not agglutinated directly by anti-Rh0(D) serum, but required subsequent antiglobulin addition to show the presence of this antigen (Cunningham et al)⁹.

VARIETIES:

Several varieties of the D^u phenotype have been described.

“High-grade” D^{us} : These RBCs directly agglutinate with selected high-protein potentiated incomplete anti-D reagents.

“Low-grades” D^{us}. These RBCs have a strict requirement for antiglobulin (Cunningham et al)⁹.

GENETIC MECHANISM:

There are three different genetic situations from which D^u phenotype can arise¹⁰:

1. A person may inherit a gene coding for a weakened quantitative expression of D.
2. One gene may interact with another to modify and weaken the expression of D antigen.
3. A gene may not code for the total material that makes up the D antigen.

Race and Sanger in 1948 states that the D^u phenotype produced by an Rh gene coding for a quantitatively weaker reactive D antigen is more common in blacks. In this case, the Ro/r genotype CD^ue/cde appears as the product of a deviant of gene Ro(cDe). Transmission of a gene for the weakened expression of D is less common in whites, but it is more common in the genotypes cD^ue/cde and cD^ue/cde as products of the deviants of genes R1 (Cde) and R2 (cDE) respectively. These inherited form of weak D are generally referred to as low grade D^u. They give negative or very weak reactions with direct agglutination tests and may only be detectable by indirect antiglobulin tests.

The second way or gene interaction is observed most often when the D gene is accompanied by the Cde haplotype represented on the red cells of the genotype Cde/Cde (R1r1) or cDe/Cde(Ror'). The expression of D, carried by one gene complex, can be depressed, for unknown reasons, by the actions of the C antigen carried on the opposite paired gene complex (Cepillini et al 1955 cited by Gunson and Smith¹³).

The D gene can express normally in the next generation if it is not paired with a modifying C antigen on the opposite chromosome. The D^u phenotype is an example of the trans position effect. Most of the gene interaction forms of D^u are referred to as high-grade D^u. The high-grade D^u red cells are more easily agglutinated by the anti-D typing sera as compared with the low grade D^u cells. C gene in cis position (i.e. on the same chromosome) may also weaken the expression of D On the other hand, E is cis position enhances the expression of D¹⁴.

The inherited and gene interaction form of D^u phenotype involves a quantitative variation of D. Normal D+red cells of the R1r and R2R2 phenotypes bear about 10,000 and 30,000 D antigen sites per

cell respectively¹⁴. In contrast, on D^u red cells, D antigen sites number from 300 to 9000 per cell¹³.

The individuals of D^u phenotype (inherited + gene interaction form) are unable to make alloimmune anti-D, which suggest that their red cells carry all epitopes of D. In contrast, a small subset of persons with D + red cells are able to make anti-D that reacts with all normal D + red cells but not with their own or with those of other individuals of the same unusual D + phenotype. Such persons are described as having partial D on their red cells, previously called D variants¹⁶. Actually anti-D production by a D + patient was first reported by Argall et al (1953) and then by Unger et al 1959 (cited by Jones et al)¹⁵. Jones et al¹⁵ describes that the RhD antigen comprises a mosaic of at least 30 epitopes expressed on a 30KD non-glycosylated RhD polypeptide. The equivalent Rh Ce/Ee polypeptide expressing the RhC/c and E/e antigens differs in only 36 of the 417 amino acids residues. Partial D individuals have been described who fail to express a number of D epitopes.

In partial D phenotypes, one or more epitopes of D are missing and such persons can produce alloimmune anti-D against the epitope(s) of D that their red cells lack. This is a qualitative difference in the D antigen and sometimes leads to a quantitative difference (partial D^u phenotype, a third mechanism for production of D^u phenotype².

The Classification of Partial D or D Variants:

The initial classification grouped cells lacking a part of the D antigen into one of four categories using the Wiener notation of Rho (Rh^A, Rh^B, Rh^C and Rh^D), absent component e.g. D replaced by small d as RhABcd or absence of all components written as Rhabcde. This was later numbered as Rh13, Rh14, Rh15 and Rh16.

Tippett and Sanger in¹⁴ expanded the four original component classifications to six and named them as categories I, II, III, IV, V and VI and then addition subdivision a, b and c. A category VII is proposed by Lomas et al 1986. Category I is considered obsolete and is excluded. Rhesus D category VI(DVI) is the clinically most important partial D^u.

Change of Terminology:

D^u originally defined those red cells reacting with anti-D only when a more sensitive indirect antiglobulin test was used. But with the use of more potent anti-sera (monoclonal reagents certain previously D^u labelled now classified as D-positive. A particular sample could thus be designated D-

positive, high-grade D^u, low-grade D^u or D-negative dependent on the reagents used for testing used for testing. So there is, disagreement on definition and classification, keeping this in view, Agree et al⁸ suggests that term D^u should be replaced by weak D. Also Moore (1983) suggests replacement of term D^u by weak D as former suggests a definite antigen, but actually reflects a decrease in its number of the normal D antigen.

Clinical Significance of D^u (weak D) and Controversies Related to it:

It is generally agreed that it is not necessary or even desirable to test recipients for D^u. The use of IAT for RhD (D^u) typing can be dangerous as patients with have been falsely recorded as D positive when controls have been omitted or wrongly interpreted. If a mistyped Rh-negative female patient was then transfused with RhD positive blood, the consequences due to the serious risk of alloimmunization would be more serious than if the test had not been performed.

Likewise, it is unnecessary to perform a “D^u test” on samples from pregnant women, the administration of anti-D immunoglobulin either ante or post-natally to a woman with a weak D phenotype will cause no harm and could be of benefit to those whose RBCs contain a “D category” antigen.

It is not necessary to test infants or cord blood samples for weak D as the immunogenicity of D^u is low and the immunising dose by a fetomaternal hemorrhage can never be large. The risk of material sensitisation is extremely low indeed no cases have been reliably documented although some anecdotal reports were published a long time ago when reagents were not as good as they are at present. The area where most uncertainties remain is whether or not donors need to be tested for D^u²⁰.

Schmidt et al in 1962 (cited by Jones et al¹³ investigated the ability of D^u blood to stimulate production of anti-D in negative recipients. Of the 45 recipients injected with small amounts of D^u blood, none-produced anti-D, although one made anti-E and another anti-K. Keeping in view the experiment of Schmidt et al and use of monoclonal reagents for typing RhD, Contreras¹⁹ states that if these weak D antigens do not cause antibody stimulation, there is little reason to spend excessive time and money in their detection.

Contreras²⁰ further states that it is upto us, as user to demand from the manufactures, the provision of high standard potent reagents that will not force us to resort to the IAT to enable detection of moderately weak forms of D.

In the Netherlands, VanRhenin and Overbeeke 1989 (cited by Jones et al)¹³ recommended the discontinuation of use of IAT for D^u testing of donors, on the basis of investigation of Schmidt et al (Cited by Jones¹³) that demonstrated weak D (D^u) was not immunogenic. Similarly, at the North London Blood Transfusion Centre, the use of IAT to type donor blood for D has been stopped²⁰.

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