Weak D and partial D: our experience in daily activity

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Dear Sir,

The *RH* genes *RHD* and *RHCE* encode two proteins that represent the clinically most important blood group system defined by the sequences of red cell membrane proteins. *RHD* and *RHCE*, encoding the Rh proteins (D and Cc/Ee, respectively), are organised in tandem on chromosome 1p34-p36 and probably derived from duplication of a common ancestral gene. Many *RH* genes carry point mutations, or have rearrangements and exchanges between *RHD* and *RHCE* which result from gene conversion events. *RHCE* encode hybrid proteins that have RhCE-specific amino acids in RhD, or RhD-specific residues in RhCE. These might generate new antigens in the Rh blood group system, and alter or weaken expression of the conventional antigens[1,2].

Reduced expression of D antigen occurs in an estimated 0.2%-1% of Caucasians. Historically, red blood cell antigens that react with anti-D only after extended testing with the indirect antiglobulin test are called weak D. Weak D expression primarily results from single point mutations in *RHD* which encode amino acid changes predicted to be intracellular or in the transmembrane regions of RhD. These affect the efficiency of insertion, and, therefore, the quantity of RhD protein in the membrane, reflected in the reduced number of D antigen sites on the red blood cells. Red blood cells with partial D antigen type as D-positive, but individuals often produce anti-D when stimulated by transfusion or pregnancy. Some partial D, similar to weak D, result from point mutations in *RHD* that cause single amino acid changes. But, in contrast to weak D, these changes are located on the extracellular regions and alter or create new epitopes[1,2].

Molecular methods for blood group genotyping became available more than 10 years ago and are useful methods to help to clarify immunogenetic doubts or to verify results[3]. Molecular *RHD* blood group typing is very efficient for managing donors and patients carrying any of the various molecular types of weak D and partial D. Weak D and partial D expression are caused by a large number of *RHD* alleles and variations of the antigen structure of RhD result either in a partial (partial D) or a weak D phenotype (weak D). Weak and partial D result in quantitative and qualitative changes in Rh protein expression respectively. The clinical relevance of these changes are, according to Flegel[1,2], that weak D subjects belonging to weak type 1, 2, 3, 4.0, 4.1 and 5 can be treated as Rh-positive and be transfused by Rh-positive red blood cells, while subjects with weak type 4.2-11 and 15 should be treated as Rh-negative and transfused with Rh-negative red blood cells. Partial D can produce different protein epitope expression and, therefore, induce specific antibody production. In this situation, partial D subjects should be considered Rh-negative and transfused with Rh-negative red blood cells[1,2].

In our daily practice, D antigens are determined serological agglutination tests according to the guidelines of Italian Society for Transfusion Medicine and Immunohaematology[4]. In particular, during routine Rhesus tests, a microtitre plate-based assay employing two different anti-D (D-Rapid, clone RUM-1 IgM and D-Fast, clone IgM; Immucor Gamma, Immucor, Inc. Norcross, GA, USA) is used. Thereafter, the D negative samples are tested for D^u with two different methods: a microtitre plate employing Anti-D Duo IgG-IgM (clone IgG/IgM clone Th28+MS26; Galileo Capture R ImmucorGamma) using solid phase capture and a gel matrix test employing one anti-D (Id-Dia Clone Anti-D; DiaMed GmbH, Switzerland). If the result is positive, the samples are tested with a gel matrix direct antiglobulin assay. The serological analysis for allelic D variant is based on "Partial Rh Typing" (ImmucorGamma) using six monoclonal IgG antisera and Capture-R Select (ImmucorGamma) in a solid phase method.

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Molecular biology analysis is performed using commercial kits from BAGene Health Care GmbH (Weak D-TYPE; Partial D-TYPE; BAG Health Care GmbH, Germany). The basic material for typing with BAGene DNA-SSP kits is purified DNA from peripheral blood mononuclear cells. The test is based on sequence-specific primers (SSP) - polymerase chain reaction (PCR). BAGene Partial D-TYPE allows for the molecular genetic determination of partial D such as *DII, DIII, DIV, DV, DVI, DVII, DAU, DBT, DFR, DHMi, DHMii, DNB* and *DHAR* (Rh33)[5], whereas BAGene Weak D-TYPE allows the molecular genetic determination of weak D types including *1*, *2*, *3*, *4.0/4.1*, *4.2*, *5*, *11*, *15* and *17*[5]. Both methods are based on the fact that primer extension, and hence successful PCR, relies on an exact match at the 3'-end of both primers. Therefore, only if the primers entirely match the target sequence is amplification obtained; this is subsequently visualised by agarose gel electrophoresis.

In 2010, a survey performed at our Unit, the Immunohaematology and Transfusion Medicine Unit of the "Paolo Giaccone" University Hospital in Palermo, revealed that out of 11 samples (from 8 males and 3 females) analysed and regarded by preliminary analysis as weak D, only eight were confirmed by complete serological analysis as weak D, whereas the other three samples did not give satisfactory results. A genetic protocol was, therefore, used, which gave the results of D weak type 1/DCS, type 11/DCS, and 5/DAR respectively. On the basis of this outcome, we re-evaluated the eight patients assessed as weak D by serological analysis getting D weak type 5/DCS as the most frequent result. These results were not influenced by the patients' gender or age.

Given the strong immunogenicity of the D antigen and the high rate of immunisation of D-negative individuals after the transfusion of D-positive red blood cells, the determination of *RHD* alleles is of special significance[1,2,5]. Immunisation of D-negative individuals can occur following transfusions of D-positive red blood cells and in D-negative pregnant women carrying D-positive foetuses[5]. The aim of this study was to use a genetic protocol to confirm and/or clarify D antigen doubts in order to prevent immunisation of patients. At first glance, serological analysis for D weak appeared to be trustworthy regarding common D weak phenotypes whereas analysis of non-common phenotypes was less satisfactory. In particular, D weak type 5 was easily identifiable by serological analysis whereas D weak types 1 and 11 seemed to display lower antibody affinity and was, therefore, less well identified. With respect to this problem, the use of the genetic protocol was decisive for obtaining correct results. The evidence of D partial results was confirmed by the presence of DCS and DAR variants. The genetic resolution of these variants is, so far, limited, and these two results are, therefore, taken in the context of D-positive results. However, given the low number of samples screened, this study cannot be decisive and other samples need to be analysed.

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Claudia Rizzo is PhD student at the Molecular Medicine PhD course (directed by Calogero Caruso) at Palermo University and this work is submitted in partial fulfillment of the requirement for her PhD degree.

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