Multiple red cell antibodies and an unexpected RhD variant in a pregnant woman

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Background

The D antigen is the most immunogenic antigen in the Rh system. Generally people are characterised as either D positive or D negative, however, some individuals are described as D variant carriers due to genetic mutations that affect quality or quantity of the D antigen expression. DIV is a D variant antigen with highest frequency amongst the African population. D variants originate due to mutations resulting in amino-acid substitution on the extracellular and trans-membrane loops (Daniels, 2013). Serologically DIV lacks epD 1, 2, 3 and 9. In comparison to other D variant phenotypes the D antigen expression in DIV is higher than on other D variants. Serologically, two forms of DIV are recognised; all DIVa; are Go^a positive and DIVb are considered Go^a negative (Scott, 2002).

Molecularly, 5 DIV types are recognised and can be identified using Sequence-Specific Primer Polymerase Chain Reaction (SSP-PCR). DIV type 1 to 5 share a single missense mutation in exon 7 (1048G>C) which replaces aspartic acid with histidine (D350H) in the 6 extracellular loop of RhD protein. DIV type 1.0 is differentiated by the presence of 3 missense mutation (186G>T, 410C>T, 455A>C), which replace amino acids in the trans-membrane regions. DIV type 3 to 5 share missense mutation that substitute glycine with tryptophan (G353W) and alanine with aspargine (A354N) in the 6 extracellular loop of RhD protein (Reid, et al., 2012; Von Zabern, et al., 2013). The amino-acid changes on the extracellular loop promote an allo-immunisation against D positive red cell units.

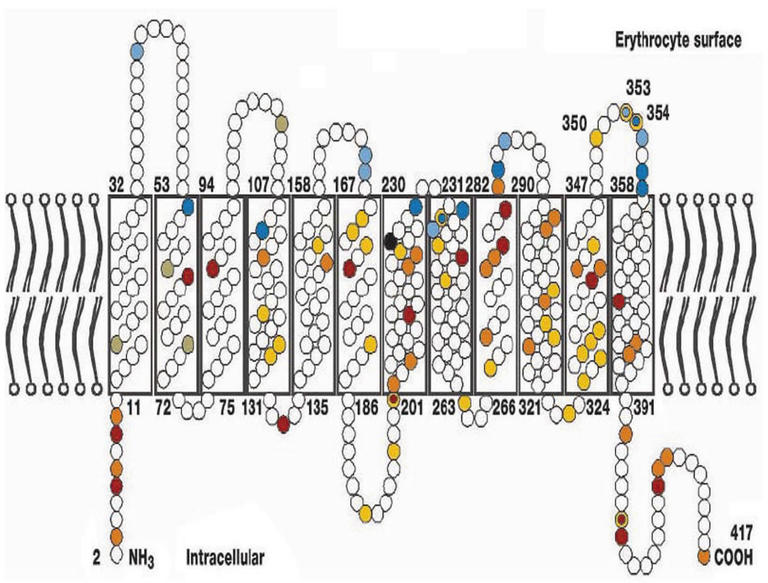

Figure 1. RhD is a transmembrane protein with 12 trans-membrane and 6 extracellular loops. All DIV variant subtypes are characterised by D350H substitution on the 6 extracellular loop. The DIV type 1 is differentiated by lack of amino acid substitution on position 353 and 354.

Patient's clinical history

A sample was referred to the Red Cell Immunohaematology Laboratory from the, Coombe Women & Infants University Hospital on a 36 year pregnant woman from Nigeria. The patient had two previous pregnancies and no history of transfusion.

The patient's phenotype was O RhD+ E- K- S- Fy^a- Jk^b-. A CR1-related antibody and a weak anti-Jk^b were detected initially. The anti-Jk^b was too weak to titre, and during the course of pregnancy became undetectable. At 38 weeks gestation an apparent anti-D was detected in a seemingly RhD+ individual. Here we report the presence of CR1-related antibody, anti-Jk^b and anti-D in an RhD variant patient.

Methods

Serological investigation was performed by the RCI Laboratory in IBTS:

- ABO/D blood group was performed using AutoVue Innova system and manual tube technique.
- Direct antiglobulin test (DAT) was performed using Bio-Rad ID gelcard method.
- CR-1 related antibody was neutralised using KNIR (NHSBT, IBGRL) blood recombinant protein.
- Serological investigation was performed using BIO-RAD column agglutination technique by indirect agglutination test on neat and KNIR neutralised plasma.
- Soluble recombinant LW^a (Imusyn, Hanover) blood protein was used to exclude possible anti-Lw^a .
- BIO-RAD papanised red cells were set up against the neat and Lw^a neutralised plasma using AHG column agglutination BIO-RAD gelcards.
- RhD variant analysis was performed using RhD variant investigation kit (Quotient, Edinburgh).

Molecular investigation was performed by the Blood Group Genetics Laboratory in IBTS

- RhD variant molecular analysis was performed using SSP-PCR Ready-Gene CDE Kit (Inno-Train, Germany).

Results

The patient was referred to the RCI lab for antibody investigation throughout the pregnancy. The patient presented with CR-1 related antibody causing pan-reactivity with IAT panel only. The DAT was negative with IgG and C3d and no reactivity was observed in the enzyme treated panel. Following neutralisation with KNIR recombinant blood group protein an underlying anti-Jk^b has been detected. On every occasion the anti-Jk^b was too weak to titre.

At week 38, an unexpected anti-D pattern was observed in the enzyme panel. The KNIR neutralisation allowed for exclusion studies using additional selected red cells.

The neat and Lw^a neutralised plasma tested using the BIO-RAD enzyme-IAT technique confirmed the presence of anti-D. The RhD variant investigation kit suggested a variant RhD type- DIV. The serological findings were confirmed following molecular investigation.

Conclusion

CR1-related antigens are expressed on complement receptor 1 on the surface of the red cells. These antibodies are 'high titre low avidity' in nature and not considered to be clinically significant. Pan-reactivity observed with CR1-related antibodies masks the presence of underlying antibodies (Petty, et al., 1997). Following elimination of the CR1-related antibody, the underlying anti-Jk^b detected was serologically weak and not considered a risk factor for haemolytic disease.

An anti-D was detected with enzyme treated cells. The possibility of an auto-antibody mimicking anti-D was excluded as the auto-test control and DAT were negative. Anti-LW^a which is known to mimic anti-D reactivity was investigated and excluded using soluble recombinant LW^a protein (Miola, et al., 2013). The RhD variant serological investigation kit indicated a DIV variant. Prophylaxis anti-D was not administrated to the patient.

The DIV type was confirmed using SSP-PCR. The detected mutation was characteristic of a variant allele *RHD*04*01 (RHD*DIV.1)*. An allo-anti-D was discovered two days prior to planned caesarean section and RhD negative red cell units needed to be sourced. The patient's transfusion protocol changed to D- E- C- Jk^b- CMV-. Aditionaly S- Fy^a- units were also sourced to prevent further allo-immunisation. This investigation highlights the fact that apparent RhD Positive individuals may in fact be RhD variant and the importance of pre-transfusion testing prior to delivery.

Infant's clinical and laboratory presentation

ABO blood group B RhD+, C+ c+ E- e+ K- Jk(a+b+). Haemoglobin levels were taken at birth (12.3 g/dL) and up to 17 days (10.6 g/dL) post-delivery. Total bilirubin was also analysed during this period; 59 µmol/L at birth which rose as high as 174 µmol/L on day 2 but dropped to 86 µmol/L on day 17.
The baby has since been diagnosed with sickle cell disease but to date has not received transfusion support.

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