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The Second Example of Alloanti-D In a Weak D Type 33 Individual

Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

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Background

The RH blood group system contains the most clinically significant blood groups, after ABO. The RhD and RhCcEe proteins are encoded by two highly homologous genes, *RHD* and *RHCE* respectively. Currently there are 54 antigens in the RH blood group system, of which RhD is the most clinically significant, with alloanti-D causing both haemolytic disease of the newborn and haemolytic transfusion reactions.

The RhD blood group phenotype can be further categorised as: Partial D, weak D, DEL and D-. These phenotypes are the products of over 150 partial D, weak D, DEL and RhD_{null} alleles currently recognised (not including 'sub-alleles') [www.isbtweb.org/working-parties/red-cell-immunogenetics-and-blood-group-terminology/]. Alleles are assigned to partial or weak D groups largely dependent on the predicted ability or inability to develop alloanti-D when exposed to RhD+ red cells

Aims

Samples from a 68 year old female patient diagnosed with rectal carcinoma, requiring blood for anterior resection of the tumour, were referred for investigation due to the patient having apparent alloanti-D, whilst having been historically typed as RhD+ and transfused RhD+ units.

Methods

Routine Rh typing was initially performed by AutoVue Innova, further RhD typing by tube agglutination using Bioscot anti-D reagents (BS226 and BS232) and extended RhD typing was performed using the Quotient Advanced RhD Typing Kit. In total the patient's cells were tested with 16 different monoclonal anti-D reagents.

Methods

Antibody investigation was performed by standard BioRad IAT using untreated and papain-treated panel cells. Antibody titration was performed by BioRad IAT (DCe/dce cell). Eluate was prepared using Immucor Elu-Kit II and investigated by BioRad IAT. Cord cells were used to exclude the presence of anti-LW.

All exons of both *RHD* and *RHCE* were sequenced by Sanger sequencing at the IBGRL.

Results

The patient's cells typed as O RhD+ C+ c+ E- e+. All anti-D reagents gave 4+ reactions with the patient's cells. Anti-D was identified in the patient's plasma (IAT titre of 8). Autologous control and DAT were negative. An eluate prepared from the patient's cells contained no detectable anti-D. The anti-D present was considered to be alloanti-D.

Sequencing confirmed the presence of normal *RHCE*Ce/ce*. However, *RHD* sequencing revealed that the patient was homozygous (or hemizygous) for a 520G>A mutation. This mutation results in a V174M transition and is characteristic of Weak D type 33 (*RHD*01W.33*) [Lin *et al*., 2003].

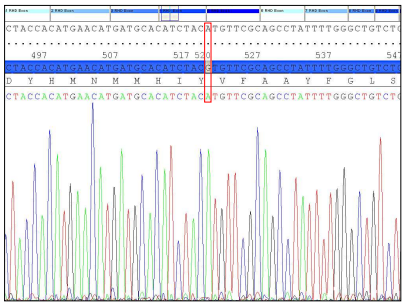

Fig. 1. *RHD* Sequencing identifiying *RHD*01W.33*.

Conclusion

We report the second example of alloanti-D in a weak D type 33 individual. The first was reported in 2011 [Bruce *et al*., 2011], in an apparent normal RhD+ individual. Autoanti-D has also been reported in a weak D type 33 female [Pham *et al*., 2013]. Other reported cases of individuals with "weak D" types producing alloanti-D include weak D types 4.2, 11, 15 and 21. Such cases indicate the need to re-evaluate the system for naming RhD variant phenotypes and *RHD* variant alleles to improve clarity of the nomenclature.

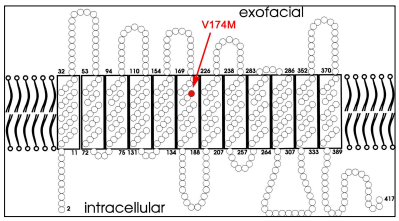

Fig. 2. Although predicted to be within a transmembrane region of the RhD peptide, the V174M mutation causes loss of D epitopes.

References

Lin IL, Shih MC, Hsieh MH, Liu TC, Chang SE, Lin CL, Chang JG. Molecular basis of weak D in Taiwanese. *Ann Hematol* 2003;**82**:617-620.

Bruce D, Rounding L, Barnes S, Grimsley S, Poole J. Immune Alloanti-D in a Patient With Weak D Type 33 Genotype. *Transfusion Medicine* 2011;**21**(Suppl.1):15.

Pham BN, Roussel M, Gien D, Ripaux M, Carine C, Le Pennec PY, Andre-Botte C. Molecular analysis of patients with weak D and serologic analysis of those with anti-D (excluding type 1 and type 2). *Immunohematology* 2013;**29**:55-62.

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