Development of Anti-G, Anti-C and Anti-Jk(b) in a 22-Year-Old Mother during Her Fourth Pregnancy

Torsten J. Schulze^a Meike Goebel^a Erwin A. Scharberg^b Peter Bugert^a Karin Janetzko^a

^a Institute of Transfusion Medicine and Immunology, Medical Faculty Mannheim, Heidelberg University, Red Cross Blood Service Baden-Württemberg – Hessen, Mannheim,

^b Institute of Transfusion Medicine and Immunohematology, Red Cross Blood Service Baden-Württemberg – Hessen, Baden Baden, Germany

Keywords

Anti-G · Pregnancy · Antibodies · HDN

Summary

Background: Anti-G antibodies are rarely found since anti-D, in combination with anti-C, are difficult to discriminate from anti-G antibodies in routine testing. **Case Report:** A 22-year-old, gravida-3, para-1, woman with blood group A Rh D neg ccddee and known antibody anti-Jk(b), gave birth to her second child. While anti-Jk(b) could not be detected at birth, a new anti-C was found. Antibody screening tests (IAT) were performed using gel cards and rare G positive rGr erythrocytes. Genotyping for RHD and RHCE was performed using PCR-SSP. **Results:** The child's blood group was A Rh D neg Ccddee. Genotyping revealed Cde/cde haplotypes. The erythrocytes of the new-born showed a positive direct antiglobulin test with IgG; anti-D and anti-C could be eluted. Erythrocytes with the rare phenotype rGr were reactive with the serum of the mother. **Conclusion:** The presence of anti-D and anti-C in the eluate from then new-born's Ccddee erythrocytes proved anti-G or anti-G in combination with anti-D. When anti-C and anti-D are seen during a pregnancy, possibly anti-G is present. This observation is of relevance since women with anti-G can still develop anti-D and require rhesus prophylaxis.

position 103 of either protein, as characterized by Faas et al. [1, 2]. Rhesus G was first described in 1958 by Allen and Tippett [3]. The authors found that erythrocytes bearing the C and/or D antigen exhibit the G antigen. It was also discovered that erythrocytes that do not carry either antigen usually do not express the G antigen. Expression of G is at maximum when both D and C antigens are present [4]. In accordance to the frequency of C and D antigens, G is present in 84% of all Caucasians. Anti-D and anti-C as well as anti-G antibodies have been reported in pregnancies of Rh D neg (ccddee) women and can also lead to severe haemolytic disease of the new-born (HDN) [5–10]. Additional clinical relevance can arise when rhesus prophylaxis is not administered such as in case that anti-G is erroneously considered to be anti-C in combination with anti-D [10]. However, all of these women are still able to form anti-D.

Material and Methods

Rhesus antigens Ccddee were determined using Mono-Type® monoclonal antibodies in tube test with two different clones (Medion-Grifols, Langen, Germany). The clones are anti-C: MS-24, MS-273, anti-c: MS-33, MS-35; anti-E: MS-258, MS-12, and anti-e: MS-62, MS-16/MS-21. Indirect antiglobulin test (IAT) and direct antiglobulin test (DAT) were performed in a gel card system ID System® (DiaMed-Diagnostika, Munich, Germany). Elution was performed using chloroform technique. Anti-G was confirmed using G-expressing erythrocytes with the rare phenotype rGr. Rh genotyping was performed using a commercial PCR-SSP system (RBC READY GENE CDE®-SSP; Inno-Train Diagnostik GmbH; Kronberg/Ts., Germany).

Introduction

The rhesus G antigen (Rh12) is encoded within the RHD and RHCE genes, more precisely, within the latter in the C encoding gene. G is not expressed when serine is replaced by proline on

Case Report

A 22-year-old gravida-3, para-1, woman with blood group A Rh D neg ccddee and known anti-Jk(b) was monitored for antibody titres before giving birth to her second child. During her third pregnancy in 2008 anti-D

Table 1. Progression of antibody development

Date	Detectable antibody	Pregnancy
May 23, 2008	anti-D (titre 8) or rhesus prophylaxis	3rd
October 4, 2010	rhesus prophylaxis	
November 3, 2010	anti-D (titre 8) or rhesus prophylaxis	4th, 26th week
December 8, 2010	anti-D (titre 8) or rhesus prophylaxis anti-C (titre 1) or already anti-G anti-Jk(b) (titre 1)	4th, 31st week
January 14, 2011	anti-D (titre 32) or rhesus prophylaxis anti-C (titre 2) or already anti-G anti-Jk(b) n.d.*	4th, 36th week
February 16, 2011	anti-D (titre 16) or rhesus prophylaxis anti-C (titre 2) anti-Jk(b) n.d.* anti-G (titre 1)	4th, 41st week

*n.d. = Not detectable.

Fig. 1. Genotyping for RHD and RHCE using a commercial PCR-SSP system (CDE-SSP; InnoTrain Diagnostik). **A** RHD typing (exons 1–10, except 8) indicated a D negative genotype. Positive result in the second reaction (exon 2) is explained by the presence of the RHC allele. **B** Detection of RHCE alleles in reactions 1–4 (1: C; 2: c; 3: E; 4: e) indicated a Ce/cE genotype. Further variant RHD alleles (reactions 5–8: DVII, DHMI, DAU, DNB) were negative. Thus, the RH genotype was Cde/cde.

was first discovered with a titre of 8 (table 1). However, it cannot be definitely stated whether anti-D was due to active immunisation of the patient or due to rhesus prophylaxis without notification within the patient's record. Rhesus prophylaxis was then administered during the fourth pregnancy. In the course of this pregnancy, in addition anti-C and anti-Jk(b) appeared within gestation week 31, the latter was not present in further analysis over the time. Anti-C titre remained discrete at 1 and in gestation weeks 36 and 41 at 2. In the gestation week 36 the anti-D titre rose from 8 to 32 and remained at 16 at the final analysis in week 41.

The new-born was well and did not present any signs of haemolysis or icterus. The child's blood group was A Rh D neg Ccddee, Jk(b) pos. The rhesus phenotype Ccddee of the child was confirmed using PCR-SSP (fig. 1). The child's genotype was Rh D negative.

Routine diagnostics of the new-born included the antibody screening (IAT) in which no antibodies in the serum could be detected. The DAT was positive for IgG. From the neonatal red blood cells, an anti-D and an anti-C

could be eluted. Anti-Jk(b) could not be eluted from the child's red blood cells.

Finally, serological testing with D-negative, G-positive red blood cells with the rare phenotype rGr confirmed the specificity of anti-G antibody. Titre differences between anti-C and anti-G indicated the existence an additional anti-C antibody.

Anti-D titres in the mother's serum may still be within the expected range due to rhesus prophylaxis administered 4 months before birth.

Conclusion

Anti-D and anti-C are frequent antibodies. In a recent Danish study, anti-D accounted for 46% of all pregnancy-related antibodies and anti-C for no more than 1%. However, of all 122 multiple cases with antibody combinations, the majority (52, i.e., 43%) accounted for a combination of anti-D and anti-C [11]. Both are known to cause severe HDN [12, 13]. Since both antibodies often occur in combination, identification of an anti-G that comprises both epitopes on the D and C antigen is often unrecognised [5, 7, 10]. Even combinations of anti-C and anti-G, in the absence of anti-D, are found to cause severe HDN [8, 9]. However, little data exists on the presence of anti-G on its own during pregnancies [6]. This antibody is rarely diagnosed and may occur more often in pregnancies due to the high frequency of C and D in the population. However, women that present anti-G antibodies can develop additional anti-D. An accurate differentiation between anti-C plus anti-D and anti-G is of high importance in pregnancies. When anti-D is absent, rhesus prophylaxis must be administered.

Disclosure Statement

The authors declare no conflict of interest.

References

- 1 Faas BH, Beckers EA, Simsek S, Overbeeke MA, Pepper R, van Rhenen DJ, von dem Borne AE, van der Schoot CE: Involvement of Ser103 of the Rh polypeptides in G epitope formation. *Transfusion* 1996;36:506–511.
- 2 Faas BH, Beuling EA, Ligthart PC, van Rhenen DJ, van der Schoot CE: Partial expression of RHc on the RHD polypeptide. *Transfusion* 2001;41:1136–1142.
- 3 Allen FH Jr, Tippet PA: A new Rh blood type which reveals the Rh antigen G. *Vox Sang* 1958;3:321–330.
- 4 Skov F: Observations of the number of available G (rhG, Rh12) antigen sites on red cells. *Vox Sang* 1976;31:124–130.
- 5 Shirey RS, Mirabella DC, Lumadue JA, Ness PM: Differentiation of anti-D, -C, and -G: clinical relevance in alloimmunized pregnancies. *Transfusion* 1997;37:493–496.
- 6 Cash K, Brown T, Strupp A, Uehlinger J: Anti-G in a pregnant patient. *Transfusion* 1999;39:531–533.
- 7 Maley M, Babb R, Chapman CE, Fitzgerald J, Cavanagh G: Identification and quantification of anti-D, -C and -G in alloimmunized pregnant women. *Transfus Med* 2001;11:443–446.
- 8 Palfi M, Gunnarsson C: The frequency of anti-C + anti-G in the absence of anti-D in alloimmunized pregnancies. *Transfus Med* 2001;11:207–210.
- 9 Trevett TN Jr, Moise KJ Jr: Twin pregnancy complicated by severe hemolytic disease of the fetus and newborn due to anti-G and anti-C. *Obstet Gynecol* 2005;106:1178–1180.
- 10 Muller CL, Schucker JL, Boctor FN: When anti-G and anti-C antibodies masquerade as anti-D antibody. *J Matern Fetal Neonatal Med* 2011;24:193–194.
- 11 Nordvall M, Dziegiel M, Hegaard HK, Bidstrup M, Jonsbo F, Christensen B, Hedegaard M: Red blood cell antibodies in pregnancy and their clinical consequences: synergistic effects of multiple specificities. *Transfusion* 2009;49:2070–2075.
- 12 Bowman JM, Pollock JM, Manning FA, Harman CR: Severe anti-C hemolytic disease of the newborn. *Am J Obstet Gynecol* 1992;166:1239–1243.
- 13 Duerbeck NB, Seeds JW: Rhesus immunization in pregnancy: a review. *Obstet Gynecol Surv* 1993;48:801–810.