Immunohematology Case Studies 2017 - 2

Sandra J. Nance, MS, MT(ASCP)SBB
American Red Cross Biomedical Services
University of Pennsylvania
American Rare Donor Program
Sandra.Nance@redcross.org

Clinical History

History:

- 25 y.o. female
- No record of transfusion
- 2 live births, no clinical issues noted in chart
- 1st pregnancy - received prenatal and postnatal Rh Immune Globulin, child D type not known
- 2nd pregnancy - received prenatal Rh immune globulin, second child typed O negative, no postnatal Rh Immune Globulin administered
- Currently 28 weeks pregnant – being seen in doctor's office for routine sample draw and prenatal Rh Immune Globulin

Serologic History

- Type O Negative
- Red cell antibody screen in Gel AHG method (with anti-IgG) at 28 weeks in last pregnancy
- Antibody screen not performed at the time of delivery of the second child

Current Sample Presentation Data

ABO/Rh: O Negative

DAT: Not performed

Antibody Screen Method: Gel AHG with anti-IgG

Antibody Screen Results: Positive 2 of 3 RBCs tested

Antibody Identification Method: Gel AHG with anti-IgG

Antibody Identification Preliminary Results:

- Anti-D and anti-C by referring hospital
- All other antibodies to common antigens ruled out

Patient received Rh Immune Globulin right after the current sample was drawn in the Dr's office

Challenge with the Current Presentation

- O negative pregnant woman has an apparent anti-D even though she received Rh immune globulin appropriately with first child and second child was D negative
- Anti-C was also identified by referring hospital
- Is this really anti-G which presents as anti-D and anti-C? Or has she been sensitized to D and C?
- Other possibilities are:
  - Anti-D and anti-G
  - Anti-C and anti-G
  - Anti-D and anti-C and anti-G

Challenge with the Current Presentation

- This is the only sample that is available to clearly delineate the specificity since she received Rh Immune Globulin after the sample was drawn
- Clinical question is, should she have received the Rh Immune Globulin?

Referral Laboratory Testing

#	D	C	E	c	e		IS*	Alb 37	Anti-IgG
1	+	0	+	+	0		0	1+	2+
2	+	0	+	+	0		0	1+	2+
3	0	+	0	+	+		0	+w	2+
4	0	+	0	+	+		0	+w	2+
5	0	0	0	+	+		0	0	0
6	0	0	0	+	+		0	0	0

At AHG phase, the reactivity is the same (2+) with D+ or C+ RBCs
At 37C, slightly different reactivity is noted between RBCs #1, 2 and RBCs #3, 4,
Is the 37C reactivity difference showing anti-D and anti-C OR anti-G with the G antigen expressed less well on C+c+ RBCs OR is it not significantly different
Patient's RBCs type D- C- E- c+ e+

*IS= Immediate Spin

Interim Antibody Identification Possible Answers and Next Steps

- Reactivity appears to be anti-D and anti-C only
- Anti-G is possible, further testing to be done to rule in or rule out
- IRL confirmed that appropriate Rh Immune globulin prophylaxis prenatally and postnatally in each of her two prior pregnancies
- Assume that current sample in the IRL is only one that will be informative since Rh Immune Globulin given after sample was drawn
    - Action step for IRL is to check with Dr office to ensure sample drawn before Rh Immune Globulin administered

Anti-G Identification Studies

Tests to identify anti-G and rule-in or rule-out the presence of concomitant anti-D and/or anti-C generally include adsorption/elution studies. These steps include:

- Serum or plasma is used to adsorb onto D- C+ G+ RBC
  - Adsorb until fresh adsorbing RBC does not react with adsorbed serum/plasma, save RBCs from 1st adsorption
  - Elution is performed on the RBCs from the 1st adsorption
  - Test adsorbed serum to identify anti-D (if present)
- Eluate from above RBCs adsorbed onto D+ C- G+ RBC
  - Adsorb until fresh adsorbing RBC does not react with adsorbed eluate, save RBCs from 1st adsorption
  - Elution is performed on the RBCs from the 1st adsorption
  - This will identify anti-G (if present)
  - Test adsorbed eluate for presence of anti-C
- Final Eluate is tested with 2 D+ C- and 2 D- C+ RBCs:
  - if all RBCs reactive, anti-G is present
  - if both negative, anti-G is not present

Further Referral Laboratory Testing

#	D	C	E	c	e		r' Ads	r' El/Ads	Ro Eluate
1	+	0	+	+	0		0	0	2+
2	+	0	+	+	0		0	0	2+
3	0	+	0	+	+		0	0	2+
4	0	+	0	+	+		0	0	2+
5	0	0	0	+	+		0	0	0
6	0	0	0	+	+		0	0	0

r' Ads= Serum adsorbed with D- C+ G+ RBCs until no reactivity with adsorbing RBCs

r' El/Ads = First set of Ro adsorbing RBCs eluted, then eluate adsorbed onto D+ C- G+ RBCs until no reactivity with adsorbing RBC

Ro Eluate= eluate from r' eluate adsorbed to Ro RBCs and eluate made

Further Work - Interpretation

Serum adsorbed to completion with D- C+ G+ RBCs

- negative with D+ RBCs, no anti-D present

Eluate from D- C+ G+ adsorbing RBCS adsorbed to completion with D+ C- G+ RBCs

- negative with C+ RBCs, no anti-C present

Eluate from D+ C- G+ adsorbing RBCs

positive with D+ C- G+ RBCS

positive with D- C+ G+ RBCs

negative with D- C- G- RBCs

Anti-G identified

#	D	C	E	c	e		r' Ads	r' El/ Ads	Ro Eluate
1	+	0	+	+	0		0	0	2+
2	+	0	+	+	0		0	0	2+
3	0	+	0	+	+		0	0	2+
4	0	+	0	+	+		0	0	2+
5	0	0	0	+	+		0	0	0
6	0	0	0	+	+		0	0	0

Further Testing

Type Father's RBCs

Father's RBCs typed D+ C+ E+ c+ e+

Test r^G RBC (D- C- G+) if available

Positive, consistent with adsorption/elution studies

Titer the anti-G with RBCs similar to potential type of baby (D+ C+) throughout the pregnancy

28 week sample – Titer of 4

32 week sample – Titer of 4

36 week sample – Titer of 4

Further Testing Options

Father's sample could be genotyped to determine his *RH* alleles

- Most common is *DCe/DcE*
- Most likely is *DCE/dce*

Why?

Because first child reported to be D+

second child reported to be D-C-E-

Mother's type is D- C- E- c+ e+

Children of this pairing have a 50% likelihood to be D+ (or G+)

Note: Some labs use titers with different phenotypes of RBCs to differentiate anti-D, -C and –G, this author does not advocate this method

Updated Clinical Information

As indicated previously, patient received Rh Immune Globulin at 28 weeks

- Exactly what was needed since the patient was shown not to have anti-D

Third pregnancy monitored by titer only

- No change in titer throughout the pregnancy (4)

Delivered baby at 39 weeks

- No clinical problems
- Cord blood typed D+ C+

Mother received postnatal Rh Immune Globulin

Conclusions

Crossmatches with D- C- units will ensure a rare r^G unit is not selected for transfusion should the mother or baby require it

Rh Immune Globulin should be given in cases like this one where anti-D is not identified with:

Anti-G only or

Anti-G and anti-C

Summary of Case Challenges

Apparent anti-D and anti-C in a pregnant patient with history of being treated appropriately with Rh Immune Globulin

Only the current sample could be evaluated by IRL due to possible serologic interference of the RH Immune Globulin administered after sample was drawn

Father's predicted *DCE/dce,* somewhat uncommon for phenotype of D+ C+ E+ c+ e+

Lessons Learned by the Case

Research unusual cases thoroughly

Think of possible alternative explanations

In cases of Anti-D and Anti-C:

- Transfusion therapy easy D- C- , no need to look for anti-G
- In cases of pregnancy, important to look for presence of anti-D to know whether Rh Immune Globulin should be given

Allelic pairings are not always the most common

Dad's phenotype was D+ C+ E+ C+ e+ and likely *DCE/dce*

What is Known about G (RH12)

- Anti-G reacts with RBCs that have D, C or both, with rare exceptions
- The G antigen is encoded by Ser103 in *RHD* and by C allele in *RHCE*
  - *Occurrence rate: Caucasians 84%, Blacks 92%, Asians 100%*
- r^G gene produces G, very weak C detected by about 33% of anti-C from D+ samples, weak e, and low frequency antigen JAHK
- r''^G produces G, E and possibly very weak C
- Anti-G can be found in sera from D- C-, D+ G-, and some DIIIb people with anti-D

Daniels, G Human Blood Groups 2nd Ed, 2002 pages 228-229.
Reid et al Blood Group Antigens Facts Book, 3rd Edition 2012

Previously Published Report
Palfi and Gunnarsson

Sera from 27 alloimmunized women, initially identified as containing anti-D + anti-C, were analysed by adsorption/elution studies in the presence of polyethylene glycol using Ror (D+C-G+) and r'r(D-C+G+) red blood cells (RBC)

- 15/27 samples were tested by adsorption in the presence of PEG and subsequently warm elution, using $r^{G}r$ (D-C-G+) RBC
    - Anti-G + anti-C, without anti-D, were identified in 4/27 samples (14.8%) and none of the newborn children needed postpartum treatment.
    - Anti-D+G occurred in 25.9%
    - Anti-D+C occurred in 11.1%
    - Anti-D+C+G occurred in 48.1%
    - Overall, anti-G was detected in 24/27 samples (88.9%)

Recommendation from publication:

Pregnant women shown to have anti-G+C but not anti-D should receive Rh immune globulin.

Additionally, the finding of apparent anti-D+C during pregnancy in D-negative spouses may lead to paternity testing and therefore a correct antibody identification is necessary

Palfi M, Gunnarsson C. The frequency of anti-C+ anti-G in the absence of anti-D in alloimmunized pregnancies. Transfus Med. 2001;11:207–10

Previously Published Report Shirey et al

A pregnant woman, para 1 gravida 4, who had received Rh immune globulin at appropriate intervals during her previous pregnancies was reported to have anti-D (titer = 4) and anti-C (titer = 32). Differential adsorption and elution studies showed that the patient had anti-C and anti-G, but not anti-D.

This case prompted retrospective examination of the sera from six other women with anti-D and anti-C who were referred to a high-risk pregnancy clinic

- Two had anti-D, -C, and –G
- Three had anti-D and -G, but not anti-C
- One had anti-C and -G, but not anti-D

CONCLUSION:

Cases of pregnant women with anti-C and -G, but not anti-D, are not infrequent. Studies to differentiate anti-D, -C, and -G should be performed on alloimmunized pregnant women presumptively identified as having anti-D and anti-C when the medical history (Rh immune globulin prophylactic therapy) and/or titer values (e.g., anti-C titer higher than anti-D titer) suggest that anti-D may not actually be present. Rh immune globulin has not failed in these patients, and they should receive this therapy during pregnancy to prevent immunization to D.

Shirey RS, Mirabella DC, Lumadue JA, Ness PM. Differentiation of anti-D, -C, and -G: Clinical relevance in alloimmunized pregnancies. Transfusion. 1997;37:493–6.

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