HDFN ABID CASE #5: ANSWERS

1. The outside laboratory identified anti-D + anti-C. Note that the anti-C titer is greater than the anti-D titer. Is there any other possibility other than anti-D plus anti-C? What testing would prove this?

The patient could have anti-G. G is an antigen on most individual's D proteins, as well as on most C-bearing CE proteins. Therefore anti-G produces reactions that appear to represent anti-D plus anti-C when reacted with most D positive and/or C positive RBCs. Anti-G typically reacts more strongly with D-C+ cells than it does with D+C- cells which is the tipoff that it is present. This is shown by the titration results for r'r vs. Ror cells. The distinction between anti-G and anti-D + anti-C can be made by adsorption studies. Since G is present on most D-carrying proteins AND C-carrying proteins the anti-C-like activity can be adsorbed by D-pos, C-neg cells and vice versa. An eluate from such adsorbing cells can also be shown to react with the D or C antigen that was NOT on the adsorbing cell. Note that any combination of anti-G with anti-D or anti-C can exist so careful interpretation of such tests is necessary.

2. What antibody specificity(ies) is(are) suggested by the adsorption results shown above? Explain your answer.

The adsorption results are consistent with anti-G in the absence of anti-D or anti-C. The reactions of anti-D plus anti-C after adsorption are compared to those of anti-G in the following table.

Phenotype of adsorbing cell	*Reactions of plasma adsorbed by cells listed in the left column*			
	anti-D plus anti-C		*anti-G*	
	Ror	*r'r*	*Ror*	*r'r*
Ror	*0*	+	*0*	*0*
r'r	+	*0*	*0*	*0*

3. Is any further workup needed to prove it? Are there any problems with the way in which the tests were performed?

Unfortunately appropriate controls were not performed with these tests. Adsorption inevitably causes some level of dilution of the serum, and controls are needed to demonstrate that the loss of activity is not due to dilution alone. This could have been done by performing an adsorption with rr (D-neg, C-neg) RBCs. After adsorption with such cells one would expect the anti-D and anti-C reactivity to persist.

An alternative, very elegant procedure developed by Vos (Vox Sang., 1960) is to perform the following sequence:

1. *Adsorb the serum with dCe/dce (r'r) cells.*
2. *Prepare an eluate from the adsorbing cells.*
3. *Adsorb the eluate with Dce/dce (Ror) cells.*
4. *Prepare an eluate from the second adsorbing cells.*
5. *Test the adsorbed serum, the adsorbed eluate (first adsorption supernatant), and both eluates with r'r and Ror cells.*

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Reactions of the adsorbed serum and eluates in the case of various antibody combinations are shown in the table below.

Combination of antibodies in the original serum	*Reactions with Ror and r'r cells*							
	Serum ads. with r'r cells		*Eluate 1*		*Eluate 1 ads. with Ror cells*		*Eluate 2*	
	Ror	*r'r*	*Ror*	*r'r*	*Ror*	*r'r*	*Ror*	*r'r*
Anti-G	0	0	+	+	0	0	+	+
Anti-D + anti-C	+	0	0	+	0	+	0	0
Anti-D + anti-G	+	0	+	+	0	0	+	+
Anti-C + anti-G	0	0	+	+	0	+	+	+
Anti-D + anti-C + anti-G	+	0	+	+	0	+	+	+

Rare cells exist which have G in the absence of D or C antigens, and which have D but not G. Consistent reactions with such cells demonstrate anti-G specificity. Anti-G specificity is demonstrated in the above sequence without access to such rare cells, but it is technically challenging, particularly if the antibodies are weak.

4. Is this patient a candidate for antenatal Rh immune globulin?

If anti-G is the only antibody, she is not immunized against the D antigen, so she remains an RhIG candidate. Determination of RhIG candidacy is reason to make these complicated distinctions.

5. Is this patient at risk for a hemolytic transfusion reactions? *Yes* HDFN? *Yes*

6. What antibody specificity(ies) is(are) demonstrated now?

The patient now is making anti-D as well as the anti-G since an r'r cell (D-C+) is no longer able to remove the anti-D activity. Anti-C is not ruled out.

7. What would you advise the patient's physician?

The patient is no longer a candidate for Rh immune globulin and should be treated as any other patient with anti-D. Although our laboratory regards 128 as the critical titer for anti-D, we begin non-invasive monitoring of the fetus with middle cerebral artery blood velocity studies when the titer reaches 32. In this case early delivery was performed as soon as there was evidence of fetal compromise.