Immunohematology Case Studies 2020-3

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Clinical History

Female, Caucasian, 34 years

Pregnant for the first time, 13 weeks

No transfusion in history

Weak reactivity was seen in the antibody screen

Serologic History

ABO or RhD typing unknown

Current Sample Presentation Data

ABO/Rh: O RhD positive

D+C-E+c+e+ (R_2r)

Antibody Screen Method: LISS, gel card

Antibody Screen Results: positive (1 out of 3 cells)

Antibody Identification Method: LISS, gel card

Initial screening panel

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Lua	Lub	Jka	Jkb	M	N	S	s	Lea	Leb	P1		LISS gel	
I	+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+		w+	
II	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	w		0	
III	0	0	0	+	+	0	+	+	+	+	0	+	0	+	+	+	+	+	+	0	+		0	

Initial panel

	D	C	E	c	e	C w	K	k	Fy a	Fy b	Lu a	Lu b	Jk a	Jk b	M	N	S	s	Le a	Le b	P 1	Additional typing	LISS gel
1	+	+	0	0	+	+	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+		0
2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+		w
3	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	w		0
4	0	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	0	+	0	+	0		0
5	0	0	+	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	+	0	+		0
6	0	0	0	+	+	0	+	+	+	0	0	+	0	+	+	+	+	0	0	+	0		0
7	0	0	0	+	+	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+		0
8	+	0	0	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	0	0	+	V+	2+
9	0	0	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	0	+	s		0
10	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+		0
11	+	+	0	0	+	0	0	+	+	0	0	+	+	0	+	+	0	+	0	+	+		0
auto																							0

Challenge with the Current Presentation

Weak positive reactions are seen with several cells tested, and a strong reaction with one cell.

No clear pattern can be distinguished.

Initial panel

	D	C	E	c	e	C w	K	k	Fy a	Fy b	Lu a	Lu b	Jk a	Jk b	M	N	S	s	Le a	Le b	P 1	Additional typing	LISS gel
1	+	+	0	0	+	+	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+		0
2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+		w
3	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0	w		0
4	0	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	0	+	0	+	0		0
5	0	0	+	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	+	0	+		0
6	0	0	0	+	+	0	+	+	+	0	0	+	0	+	+	+	+	0	0	+	0		0
7	0	0	0	+	+	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+	+		0
8	+	0	0	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	0	0	+	V+	2+
9	0	0	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	0	+	s		0
10	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0	+		0
11	+	+	0	0	+	0	0	+	+	0	0	+	+	0	+	+	0	+	0	+	+		0
auto																							0

Challenge with the Current Presentation

Weak positive reactions are seen with several cells tested, and a strong reaction with one cell.

No clear pattern can be distinguished.

The presence of all relevant antibody specificities can be ruled out twice.

An enzyme (papain) treated panel is also tested (direct agglutination, no anti-IgG used). No reactivity was found.

Additional panel

	D	C	E	c	e	C w	K	k	Fy a	Fy b	Lu a	Lu b	Jk a	Jk b	M	N	S	s	Le a	Le b	Additional typing	LISS gel
1	+	+	0	0	+	+	0	+	+	0	0	+	+	0	0	+	0	+	0	+		0
2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+		w
3	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	0		0
4	0	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	0	+	0	+		0
5	0	0	+	+	+	0	0	+	+	+	0	+	0	+	+	+	0	+	+	0		0
6	0	0	0	+	+	0	+	+	+	0	0	+	0	+	+	+	+	0	0	+		0
7	0	0	0	+	+	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+		0
8	+	0	0	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	0	0	Js(a+)	3+
9	0	0	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	+	0	+		0
10	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	0		0
11	0	0	0	+	+	0	0	+	+	0	0	+	+	0	+	+	0	+	0	+		0
auto																						0

Interim Antibody Identification

Several weak and 2 strong reactions are seen but no pattern can be seen.

The presence of the most common specificities can be ruled out (twice).

The 2 cells that show a strong reaction, both carry a low frequency antigen (LFA). Antigens that are almost exclusively expressed in donors of African origin.

Possibly one or more antibody specificities present directed against low frequency antigens (LFA).

Several antibody specificities against LFA's can cause HDFN, therefore further investigation is needed.

Further Work, LFA panel

To investigate the presence of antibodies against specific low-frequency antigens the following panel is tested.

	D	C	E	c	e	Cw	K	k	Jsa	Jsb	Fya	Fyb	Jka	Jkb	M	N	S	s	Additional typing		LISS gel	
1	0	0	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	V+		0	
2	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	-	+	-	Js(a+)		3+	
3	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	-	-	+	VS+		W	
4	+	0	0	+	+	0	0	+	0	+	0	0	+	0	-	+	-	+	He+, Js(a+)		0	
5	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	+	+	+	V+		0	
6	+	0	0	+	+	0	0	+	0	+	0	+	+	0	-	+	-	+	Go(a+)		0	
7	0	0	0	+	+	0	0	+	0	+	0	0	+	+	+	+	-	+	Js(a+)		2+	
8	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	He+		3+	
9	+	0	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	VS+		0	

Further Work, LFA panel

	D	C	E	c	e	C w	K	k	Js a	Js b	Fy a	Fy b	Jk a	Jk b	M	N	S	s	Aditional typing		LISS gel	
1	0	0	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	~~V+~~		0	
2	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	0	+	0	Js(a+)		3+	
3	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	VS+		W	
4	+	0	0	+	+	0	0	+	0	+	0	0	+	0	0	+	0	+	~~He+~~, ~~Js(a+)~~		0	
5	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	+	+	+	V+		0	
6	+	0	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	~~Go~~(a+)		0	
7	0	0	0	+	+	0	0	+	0	+	0	0	+	+	+	+	0	+	Js(a+)		2+	
8	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	He+		3+	
9	+	0	0	+	+	0	0	+	0	+	+	0	0	+	0	+	0	+	~~VS+~~		0	

3 strongly reactive cells, and one weak reaction.

Challenge with the Current Presentation

The follow-up panel showed several reactions (strong and weak).

Maybe we are dealing with two antibody specificities.

One that is strongly reactive and another one that is only weakly reactive.

The presence of antibodies against the LFA's He, V, VS, Go^a and Js^a can be ruled out.

Further Work

An antibody identification at a low temperature is performed to look for cold reactive antibodies.

Follow-up Panel at 16°C

	D	C	E	c	e	C w	K	k	Fy a	Fy b	Lu a	Lu b	Jk a	Jk b	M	N	S	s	Le a	Le b	P1		NaCl 16oC
1	+	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+		0
2	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	0	0		2+
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+		0
4	+	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	+		1+
5	0	+	0	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	+	0	0		w+
6	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0	+	+		w+
7	0	0	0	+	+	0	0	+	+	0	0	+	0	+	0	+	0	+	0	+	+		0
8	+	0	0	+	+	0	0	+	0	0	0	+	0	+	0	+	0	0	+	0	+		0
9	0	0	0	+	+	0	0	+	+	0	0	+	0	+	+	0	+	+	0	+	0		1+
10	0	0	0	+	+	0	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+		0
Cord	0	0	0	+	+	0									+	+						ii	w+
auto																							0

Interim Antibody Identification

Several reactions are seen and the pattern fits with a weak anti-M.

The presence of other “cold-reactive” antibodies (anti-P1, -Le^{a}, -Le^{b}) can be ruled out.

M antigen typing

	Patient red cells	M+N+, (single dose) positive control	M-N+, negative control
Anti M reagent	0	4+	0
Control reagent	0		

The presence of anti-M was confirmed.

Interim Antibody Identification

When we look back at the weak reactions of the first panels we can see that all found weak reactions are with M+ N- panel RBCs.

All positive reactions combined

	D	C	E	c	e	C w	K	k	Fy a	Fy b	Lu a	Lu b	Jk a	Jk b	M	N	S	s	Le a	Le b	P 1	Additional typing	LISS gel
S-I	+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+		w+
I-2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+		w
I-8	+	0	0	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	0	0	+	V+	2+
A-2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+		w
A-8	+	0	0	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	0	0	0	Js(a+)	3+
L-2	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	0	+	0	0	+	+	Js(a+)	3+
L-3	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	0	+	VS+	w
L-7	0	0	0	+	+	0	0	+	0	+	0	0	+	+	+	+	0	+	0	+	0	Js(a+)	2+
L-8	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	0	+	He+	3+

S=screening, I=initial panel, A=aditional panel, L=LFA panel

Interim Antibody Identification

When we look back at the weak reactions of the first panels we can see that all found weak reactions are with M+ N- panel RBCs.

So the anti M could be the cause of the weak reactions found at 37^{o}C.

Also, the strong reactions are all M positive!

But some of the of these cells are M+N+ (single dose), and a strong reaction is not expected with such a weakly reactive anti-M.

Interim Antibody Identification

All cells that show a strong reaction, are M positive but all those cells are probably from donors from African origin.

They all have a low frequency antigen that has a higher frequency in people from African origin and some of them have the Fy(a-b-) phenotype.

All positive reactions combined

	D	C	E	c	e	Cw	K	k	Fya	Fyb	Lua	Lub	Jka	Jkb	M	N	S	s	Lea	Leb	P1	Additional typing	LISS gel
S-I	+	+	0	0	+	+	0	+	+	0	0	+	+	0	+	0	+	0	0	+	+		w+
I-2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+		w
I-8	+	0	0	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	0	0	+	V+	2+
A-2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+		w
A-8	+	0	0	+	+	0	0	+	0	0	0	+	+	+	+	+	0	+	0	0	0	Js(a+)	3+
L-2	+	+	0	0	+	0	0	+	0	+	0	0	+	+	+	0	+	0	0	+	+	Js(a+)	3+
L-3	0	0	0	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	0	+	VS+	w
L-7	0	0	0	+	+	0	0	+	0	+	0	0	+	+	+	+	0	+	0	+	0	Js(a+)	2+
L-8	+	0	0	+	+	0	0	+	0	+	0	+	+	0	+	+	+	+	0	0	+	He+	3+

S=screening, I=initial panel, A=additional panel, L=LFA panel

LFA's

In donors of African descent the frequency of the following LFA's is different from that in Caucasians.

%	Caucasians	Africans
He	0	3-7
V	<1	30
VS	<1	26-40
Go^a	0	2
Js^a	<1	20

Fy antigens

In donors of African descent the frequency of the Duffy antigens is different.

%	Caucasians	Africans
Fy^a	66	10
Fy^b	83	23
Fy(a+b-)	17	9
Fy(a+b+)	49	1
Fy(a-b+)	34	22
Fy(a-b-)	0	68

Interim Antibody Identification

All cells that show a strong reaction are M positive but all those cells are from donors with an African origin.

They all have a low frequency antigen that has a higher frequency in people from African origin and some of them have the Fy(a-b-) phenotype.

Is the M antigen in donors of African origin different from that in Caucasians?

M_1 antigen

M_1 is an “alternate M antigen” that has a frequency of up to 24% in people from African origin, in comparison with 4% in Caucasians.

The alterations, in the glycosylation of the GPA are genetically inherited. Anti-M_1 is claimed to be a sole specificity, distinct of anti-M.

This can be compared to anti-A and anti-A1.

In this case we probably have the combination of anti-M_1 with or without anti-M. The difference between these is difficult to make.

Anti-M and pregnancy

Although rare, anti-M can cause Hemolytic Disease of the Fetus and Newborn (HDFN).

This will only be the case if the antibodies are of IgG class.

In this case the plasma was treated with DTT to look for the presence of IgG anti-M.

The plasma was non reactive after treatment with DTT, so no IgG was detectable.

In this case no HDFN was expected.

Clinical significance of anti-M_1

There is no information on the clinical significance of anti-M_1 for transfusion or as a cause for Hemolytic Disease of the Fetus and Newborn (HDFN).

Conclusions

This case shows a pregnant woman who had anti M_1 and weakly-reactive anti-M antibodies. The presence of these antibodies could be best shown at 16^{o}C.

The strong reactions at 37^{o}C can be explained by the serologically typed “rare” phenotypes of the test cells.

Selection of M negative red cells for transfusion is dependent on the reactivity of the antibodies at 37^{o}C, pre-warm and all stages performed at 37^{o}C.

Summary of Case Challenges

A case of unexplained strong reactions next to weak reactions caused by two specificities with overlapping reactivity.

Knowledge of uncommon phenotypes in certain donors can help determine the specificity of the antibody and explain the unexpected differences in reaction strength.

Lessons Learned by the Case

- The presence of anti-M is often more likely detected when test are performed at a lower temperature (16^{o}C)

- The presence of a M_1+ phenotype in a panel of reagent RBCs can give unexpected strong reactions in the presence of weak anti-M

- Particular blood group phenotypes can provide a strong indication of the most likely ethnic origin of the donor, this information can help the technician to interpret the different reactions found