



hr Problems

American Red Cross

Is this a discussion of your Human
Resource nightmares?

We are actually talking about
the Rh Blood Group System!

Hr and hr Antigens

Hr_o (Rh17)

Hr or Hr^S (Rh18)

Hr^B (Rh34)

These antigens are extremely high prevalence with an incidence of >99.9%. The antigens are lacking on *Rh-deletion* haplotypes.

hr^B (Rh31)

hr^S (Rh19)

These antigens have an incidence of about 98%, just like the e antigen (Rh5). These antigens are considered e variants. Since the antigens are not as prevalent, we encounter antibodies to these antigens on a more frequent basis.

The History of hr^S and hr^B

The antigen hr^S was first identified in 1960. The serum of a Bantu woman, Mrs. Shabalala contained an antibody which reacted with all cells that possessed E or e antigen. The antibody which remained after adsorption with R₂R₂ cells was named anti-hr^S.

The antigen hr^B was not identified until 1972 and was found in a South African woman named Mrs. Baastian. Reactivity was very similar to anti-hr^S.

Partial Expression of the e Antigen

“In the majority of, but not quite all cases, e and anti-e in Whites, are straightforward. The antibody can be formed by E+e- (usually R2R2) people and when made, reacts with all e+ samples. In Blacks, the story is rather different for some of them, who have red cells that type as e+, make antibodies that closely resemble anti-e (or anti-f or anti-rh_i), yet which do not react with the antibody makers’ own red cells. In this respect, it seems that the e antigen must involve many epitopes and that the state of lacking some of them, that is having red cells that carry partial e, is more common in Blacks than in Whites.”

Applied Blood Group Serology, 4th edition; Peter D Issitt, David J. Anstee; Chapter 12, pg 368

Can we think of partial e antigen the same way as partial D antigen?

No we can't!

- Partial D has very specific definitions and the epitopes are well defined.
- Partial e seems to be made in a more individualistic manner. The antigens and antibodies are much more difficult to define.

Partial Expression of the e Antigen

The e antigen must involve many epitopes and the state of having red cells that carry partial e antigen is more common in African-Americans than in Whites.

Cells that lack one epitope of e are more than likely to lack *more* than one epitope. This brings in a lot of variation when looking at a partial e antigen.

An added complication is that not all red cell samples called hr^S- or hr^B-, or all antibodies called anti-hr^S or anti-hr^B, are as alike as the names imply. Examples frozen in Reference and research laboratories over the years have revealed that many examples of “anti-hr^B” are incompatible with cells identified as “hr^B-”.

The safest way to report these antibodies without extensive serological and molecular testing is **“anti-e-like”**.



Laboratory Management of e-like antibodies

American Red Cross

Could my patient have an anti-e-like antibody?

- The patient would type as e+ .
- Anti-e (or anti-f or anti-rh_i) is identified in the serum.
- The DAT is negative.
 - *CAUTION: the patient's DAT may be positive due to an autoantibody, a transfusion reaction (is anti-e identified in the eluate too?), or just because nothing is easy in the Blood Bank!*
- The patient's race would most likely be African-American.

What about transfusion recommendations?

- Is your patient E+? Your answer is easy:
Transfuse e- units (R2R2)
- If your patient has anti-E already, the answer is more difficult. Precise information about the clinical significance of anti-hr^B and anti-hr^S is limited. Even more limited is the availability of compatible units for these patients. Throw in the additional complication that many of these patient's red cells also possess a partial D antigen (and so they can make allo-anti-D as well), and transfusion becomes very difficult. In an emergency, transfuse a patient with anti-D, -E, and -e-like antibodies with D-, E- e+ units.

But we need compatible units!!!

- Molecular testing can identify patients and blood donors with altered Rhce/RhD.
- The American Rare Donor Program keeps a file of all the donors for whom they have molecular testing results which indicate partial e phenotype. If a request comes in to the ARDP, they can match donor to patient based on the exact molecular types of each. The ARDP notifies each member Blood Center who has compatible donors in order for them to ship products where they are needed or recruit their donors.
- Unfortunately this is not a quick process, and patients may go untransfused or need incompatible blood.

Case study – could this be an anti-e-like antibody?

A selected cell panel of E-, K-, Js(a-) cells was tested. Looking at the strength of the serum reactivity at PEG/IAT, we noted stronger reactions with the test cells compared to the reactions with the autocontrol. The eluate was reactive with all cells tested.

Patient's Name L.H. SELECTED PANEL 2:53 pm, 4/11/2014
 Patient's Number 12345 American Red Cross, Missouri-Illinois Region
 Date: Collected 02/19/2014 Date: Tested 02/19/2014 4050 Lindell, St. Louis, MO 63108
 Technologist: UCM

Supplier Lot #	Donor/Vial#	RhHr					Kell					Duf	Kid	Lew	P	MN			Lut	X	Additional Antigens	Patient's Plasma Test Results				
		D	C	c	E	e	f	V	W	K	k	K	k	J		J	F	F	J	J		L	L	L	L	X
1 Imm-10F 10323	B2065 2	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	Bg(a-)	15 IgG	1+
2 Imm-10F 10323	E294 5	0	+	+	0	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	Bg(a-)	0 2+	1+
3 Imm-10F 10323	V234 8	0	0	+	0	+	+	0	0	+	0	+	0	+	+	0	0	+	+	0	0	+	0	Bg(a-)	0 2+	1+
4 Imm-10F 10323	B6449 1	+	+	0	0	+	0	0	0	+	+	+	0	+	+	0	0	0	+	0	+	0	+	Bg(a-)	0 2+	1+
5 Ortho A RA976	83095 8	0	0	+	0	+	+	0	0	+	0	+	+	0	+	0	0	+	+	+	0	0	+	Bg+	0 2+	1+
6 Imm-16 09309	C4638 3	+	0	+	+	0	0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	Bg(a+)		+
																								auto	0 + ^m	

Adsorption studies

Adsorptions were performed using PEG and untreated allogeneic cells.

Following adsorptions, an apparent anti-e was detected in the R2R2 adsorbed serum.

What do we need to do to determine if the patient has developed an allo-anti-e-like antibody or whether the specificity is simply a component of the autoantibody?

American Red Cross Washington, DC 20006	Allogeneic Adsorption	Missouri-Illinois Region 4050 Lindell St. Louis, MO 63108
Patient Name: <u>L.H.</u>	Serum Adsorption: <input checked="" type="checkbox"/> PEG <input type="checkbox"/> Enzyme-Treated: F P <input checked="" type="checkbox"/> Untreated	REAGENT PREPARATION: Lot Number: <u>DIF-2014-2</u>
Identification Number: <u>12345</u>	Number: 37°C <u>1</u> 4°C	Expiration Date: <u>04032014</u>
Sample Date: <u>02192014</u>	Eluate Adsorption: <input type="checkbox"/> Enzyme-Treated: F P <input type="checkbox"/> Untreated	Prepared by/Date: <u>RGP 04/03/2014</u>
Test Date: <u>02192014</u>	Number: 37°C	Reviewed by/Date: <u>mlr 04032014</u>
Technologist: <u>VCW</u>		

	D	C	E	c	e	f	V	C*	M	N	S	s	P1	Le*	Le ^b	Lu ^a	Lu ^b	K	k	Kp*	Js*	Fy ^a	Fy ^b	Jk*	Jk ^b						
R ₁ R ₁ W201114000463000	+	+	0	0	+				+	0	+	0						0				+	0	+	+						
R ₂ R ₂ W20111402381200F	+	0	+	+	0				0	+	0	+						0				+	+	0	+						
rr W201114016892004	0	0	0	+	+				+	0	+	+						0				0	+	+	0						
<u>R₁R₁ads</u>																															
I09309	2	+	+	0	0	+			0	+	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	0	0	+	0	+	
I09309	14	0	0	0	+	+			0	0	+	+	+	+	0	+	+	+	0	+	0	0	0	+	0	+	0	+	0	+	
<u>R₂R₂ads</u>																															
I09309	3	+	0	+	+	0			0	0	+	0	+	0	+	0	+	0	+	0	0	0	0	+	+	0	0	+	+	0	
ORA976	8	0	0	0	+	+	+	0	0	+	+	+	0	+	0	0	0	+	0	+	0	0	+	0	0	+	0	0	+	+	
I09316	2	+	0	+	+	0			0	0	+	+	0	+	0	+	0	+	0	+	0	0	+	0	+	+	0	+	+	0	
I10323	1	+	+	0	0	+			0	0	0	+	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	
I10323	2	+	+	0	0	+			0	+	+	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+	0	+	+	0	
<u>rrads</u>																															
I09322	2	+	+	0	0	+			0	0	+	+	0	+	+	0	+	0	+	0	0	0	0	+	0	+	0	+	+	0	
I09322	17	0	0	0	+	+			0	0	0	+	+	+	+	0	+	0	+	0	0	+	0	+	+	0	+	+	0		

American Red Cross Biomedical Services
Form: Allogeneic Adsorptions

Page 1 of 1
16.4.Zfrm001 W2011 v-1.1

Initial panel

All cells but one were reactive with the initial panel at PEG-IgG. The results showed some variability in the strength of reaction.

PANOCELL-20 Master List

IMMUCOR, INC. Norcross, GA 30071 USA
U.S. License No: 886 Lot No: 09322

MO-IL Regional Red Cross
4050 Lindell Blvd.
St. Louis, MO 63108

Exp. Date: 2014/05/09

NAME A.M. NO. 45678
INSTITUTION _____
BLOOD GROUP _____ ANTIBODY IDENTITY _____
TECH TER DATE 12282013

SYSTEM	Rh - Hr.							Kell							Duff	Kidd	Lewis	P	MN		Lutheran		Xg	Special Antigen Type	PATIENT'S TEST RESULTS		
	Donor	D	C	c	E	e	V	C ^w	K	k	Kp ^a	Kp ^b	Js	N					S	Lu ^a	Lu ^b	Xg ^a			15	15 ⁺	1
1	R1wR1 B3785	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+					
2	R1R1 B1938	+	+	0	0	+	0	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0				1	
3	R1R1 B8269	+	+	0	0	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0				2		
4	R1R1 B7126	+	+	0	0	+	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+				3	
5	R1R1 B8629	+	+	0	0	+	0	0	0	+	0	+	0	+	0	0	+	0	0	0	+	+				4	
6	R2R2 C5505	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0				5	
7	R2R2 C5389	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	Co(b+)			6	
8	R2R2 C2644	+	0	+	+	0	0	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0				7	
9	R2R2 C3804	+	0	+	+	0	0	0	0	+	0	+	0	0	+	0	+	0	+	0	+	0				8	
10	R1R1 B8627	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	Bg(a+)			9	
		D	C	c	E	e	V	C ^w	K	k	Kp ^a	Kp ^b	Js	Duff	Kidd	Lewis	P	N	S	Lu ^a	Lu ^b	Xg ^a				10	
11	r ^r E277	0	+	+	0	+	0	0	0	+	+	0	+	0	+	0	+	+	+	+	+	+	Lu:14			11	
12	r ^r F334	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	+	+	+	+	+				12	
13	rr G1565	0	0	+	0	+	0	0	+	+	0	+	0	+	+	0	+	+	+	+	0	0				13	
14	r ^r E458	0	+	+	0	+	0	0	+	+	0	+	0	+	0	+	0	0	+	0	+	+				14	
15	rr G842	0	0	+	0	+	0	0	+	+	0	+	0	0	+	0	+	+	+	+	0	+	+	+	+	15	
16	rr H555	0	0	+	0	+	0	0	0	+	0	+	0	0	+	0	0	+	+	0	0	+	+	+	+	16	
17	rr H378	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	+	+	17	
18	rr N3209	0	0	+	0	+	0	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	18	
19	rr N531	0	0	+	0	+	0	0	0	+	0	+	0	+	+	+	+	+	0	+	+	+	+	+	+	19	
20	Ror D1041	+	0	+	0	+	+	0	0	+	0	+	0	0	+	0	0	0	+	+	+	+	+	+	+	20	
	PATIENT'S CELLS																										

**Now
what do
we do?**

In those instances where a patient's serum is known to contain anti-D, it may be desirable to perform antibody screening tests with D- red cells. The panel cells 11,12 and 13 can be used together to form a D- negative antibody screening reagent.

*Indicates those cells that are not used in the initial panel.

REVERSE GROUPING

A ₁			
A ₂			



Phenotypically similar selected cells

Often when we are puzzled, we will test cells that are phenotypically similar to the patient. These can show us whether we are looking at multiple antibodies or a high prevalence antibody.

In this case, two cells were positive and one was negative. We've now noticed that both our negative cells are R2R2.

Patient's Name A.M.
 Patient's Number 45678
 Date: Collected 12282013 Date: Tested 12282013

SELECTED PANEL
 American Red Cross, Missouri-Illinois Region
 4050 Lindell, St. Louis, MO 63108

Technologist: TER

Supplier Lot #	Donor/ Vial#	RhHr					MN			P	Lew	Lut	Kell				Duf	Kid	X	Additional Antigens	Patient's Plasma Test Results														
		D	C	E	c	e	f	V	w	M	N	S	s	P	L	e	L	u	u		K	k	J	J	F	F	J	J	X	g	a	g	a	g	a
1 Ortho A RA976	312351 4	+	0	0	+	+	+	0	+	0	0	+	+	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	Bg-						
2 Ortho A RA976	310921 9	0	0	0	+	+	+	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	0	+	+	Bg+						
3 Imm-20 06275	C1498 8	+	0	+	+	0	0	0	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	Bg(a-)						
<u>Patient's Phenotype</u>		<u>+</u>	<u>0</u>	<u>+</u>	<u>+</u>	<u>+</u>			<u>+</u>	<u>+</u>	<u>0</u>	<u>+</u>	<u>+</u>	<u>0</u>	<u>0</u>		<u>0</u>					<u>0</u>	<u>0</u>	<u>+</u>	<u>0</u>										

Confirmation of alloantibodies

Additional cells confirm the presence of anti-Fy^a, and anti-Jk^b in the patient's serum along with the anti-e-like antibody.

Patient's Name A.M.
 Patient's Number 45678
 Date: Collected 12/28/2013 Date: Tested 12/28/2013

SELECTED PANEL
 American Red Cross, Missouri-Illinois Region
 4050 Lindell, St. Louis, MO 63108

6:44 pm, 4/16/2014
 Technologist: TER

Supplier Lot #	Donor/Vial#	RhHr					MN			P	Lew		Lut		Kell				Duf		Kid		X	Additional Antigens	Patient's Plasma Test Results							
		D	C	E	c	e	f	V	w	M	N	S	s	P	L	L	L	L	K	K	J	J	F		F	J	J	X	X	Pcc	154	
1 Ortho A RA975	313663 3	+	0	+	+	0	0	0	+	0	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	Bg-		1+			
2 Ortho B RB390	312263 15	+	0	+	+	0	0	0	+	0	+	+	+	0	+	+	0	+	0	+	+	0	+	0	+	+	Bg-		1+			
3 Imm-Pano 07292	C589 2	+	0	+	+	0	0	0	+	0	+	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+			2+			
4 Imm-20 OOD 03225	C4362 9	+	0	+	+	0	0	0	+	0	+	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	Bg(a-)		2+			

Is this anti-hr^B or anti-hr^S?

We know the patient is e+ with an anti-e-like antibody which does not react with his own red cells.

We have several examples of anti-hr^B frozen, so we use them to type the patient's red cells.

Unfortunately we do not have any ABO compatible anti-hr^S.

IMMUNOHEMATOLOGY STUDIES

Name A.M.		Ref # XX-13		Date of Specimen 12282013		<input type="checkbox"/> Plasma		<input type="checkbox"/> Pretransfusion Sample		<input type="checkbox"/> Post Transfusion Sample																									
ID Number 45678				Facility Hospital																															
Last Transfused on 2011		Date Started 12282013		Tech TER		WID 1 2 3 4 5 6 7 8 <input type="checkbox"/> 011.1642 <input type="checkbox"/> 011.1414 <input type="checkbox"/> 011.6233																													
	Cell Typings										Serum Grouping					DAT																			
	A	B	A,B	H	D	Rh Ct	C	E	c	e	Ct*	Weak D	O	Auto	A ₁	A ₂	B	Poly	IgG	c'	Sal Ct														
IS																						NA													
5' RT																						NA													
* Ct = Rh phenotype control, use only when required by manufacturer's instructions Cell Treatments: EGA = EDTA Glycine-Acid; CDP=Chloroquine Diphosphate																																			
Other Antigens:		hr ^B		hr ^B		hr ^B																													
Result	Neat		1+		W+		2+																												
	Cell Sep.																																		
Cell treatment		<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP	<input type="checkbox"/> EGA <input type="checkbox"/> CDP														
Manufacturer		Sample 1		Sample 2		Sample 3																													
Lot Number																																			
Expiration																																			
Manufacturers: I = Immucor; O = Ortho; A = American Red Cross (in house or Diagnostic Manufacturing Division); B = Biorad; Q = Alba Bioscience/Quotient																																			
Routine											Eluate		Last Wash		Crossmatching:																				
		15'		15'		IS		LISS		LISS		PEG		GEL		IqG		PEG		IqG		PEG													

Molecular Confirmation and Transfusion Recommendations

- The patient's cells were submitted for molecular characterization of the e antigen. The cells were confirmed as hr^S- and the exact molecular variation of the patient's antigen was determined.
- If you recall our patient's phenotype, they were E+. We were unable to rule out anti-K, so we recommended the transfusion of e-, K-, Fy(a-), Jk(b-) units. Of course these are still quite rare, but not as hard to find as E-, hr^S- units.

Conclusions

Generally when a patient has an autoantibody, we don't recommend antigen negative units for transfusion, as this may immunize them to the alternate antigen. In the case of an apparent auto-anti-e, patient history including race needs to be strongly considered. Additional testing must be performed to show whether the specificity is autoantibody or alloantibody.

Conclusions

Patients with e-like alloantibodies who type e+ should be tested molecularly to characterize their e antigen. This is particularly important for E- patients who may become immunized to both E and e.

Conclusions

It is always best to avoid transfusion if you have a patient with anti-e-like antibody. In an emergency, transfusion of e+ red blood cells may be necessary.

Questions

