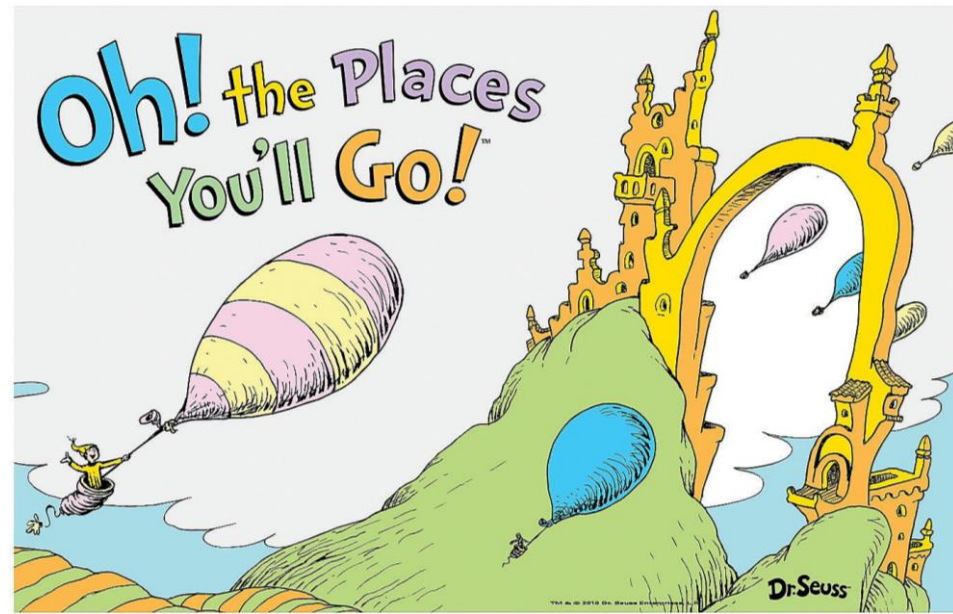




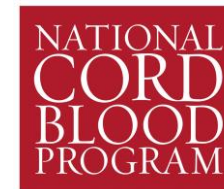
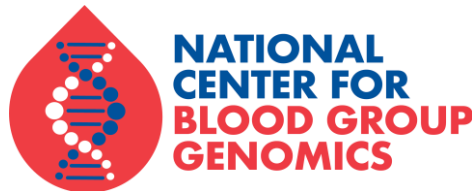
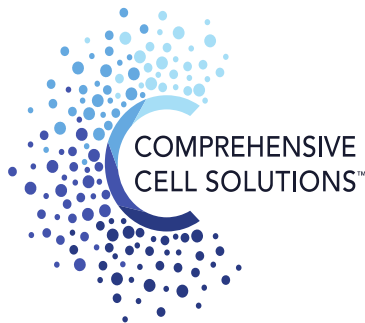
Oh, the Places You'll Go! The Many Paths to Antibody Identification

Julie Kirkegaard
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New York *Blood Center Enterprises*

EXPANDING OUR ORGANIZATION TO MEET CLINICAL, CELLULAR AND TRANSFUSION PRODUCT AND SERVICE NEEDS FOR PATIENTS. NOW PROVIDING ALMOST ONE MILLION BLOOD PRODUCTS, OVER 450,000 LABORATORY AND MULTI-ASSAY INFECTIOUS DISEASE TESTS AND OVER 12,500 SPECIALTY CLINICAL PROCEDURES ANNUALLY TO HOSPITALS NATIONWIDE.



Objectives

- Describe some possible causes of panreactivity in a patient's plasma
- List some techniques that can be used to identify the causes of panreactivity in a patient's plasma
- List some benefits of obtaining a patient's genotype

Case Background

- 32 year old female African Descent
- Diagnosis: Pregnant C-section
- Hgb 9.6 g/dL
- Uncomplicated pregnancy. No blood products received
- Antibody screen negative 7 months ago
- Submitting facility reports the patient currently types as A Pos. All cells tested in Gel are 4+ and auto control is negative
- Sample submitted to IRL for antibody identification
- No units requested



ABO/Rh

ABO Group						Rh Type	
	Anti-A	Anti-B	Anti-A1	A ₁ Cells	B Cells	Anti-D	Control
IS	4+	0	4+	0	4+	3+	0

Direct Antiglobulin Test			
Poly	IgG	C'	Saline
(0) [√]	(0) [√]	(0) [√]	(0)

IRL confirms patient's ABO and Rh type as A positive. DAT is negative.

Antibody Screen Testing

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	5' RT	P-√EG IAT
I	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	@ ^s	
II	R ₂ R ₂	+	0	+	+	0	0	+	+	0	+	0	+	+	0	+	0	0	@ ^w	
III	rr	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	r	@ ^s	
Auto																		0	(0) ✓	

- Plasma is reactive with all screening cells but nonreactive with autologous cells
- Consistent with what hospital was reporting

@=weakly agglutinated after washing and prior to antiglobulin addition.

- Typically associated with carryover reactivity due to colder reacting antibodies or IgM antibodies.

Cold Antibody Screen Testing

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	30' RT	30' 4 C	
I	R ₁ R ₁	+	+	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	2+	3+
II	R ₂ R ₂	+	0	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	0	2+	3+
III	rr	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	2+	3+	
Auto																			0	3+	
A ₁																			1 ^w	3+	
A ₂																			2 ^s	3+	
O																			2 ^s	3+	

- Cold autoantibody present in patient's plasma

Plasma Interpretation

Causes of panreactive plasma:

- Autoantibody
 - Warm, cold or combination
- Monoclonal antibody therapy
- Multiple antibodies
- Antibody to high prevalence antigen
- Unusual antibody due to gene inheritance



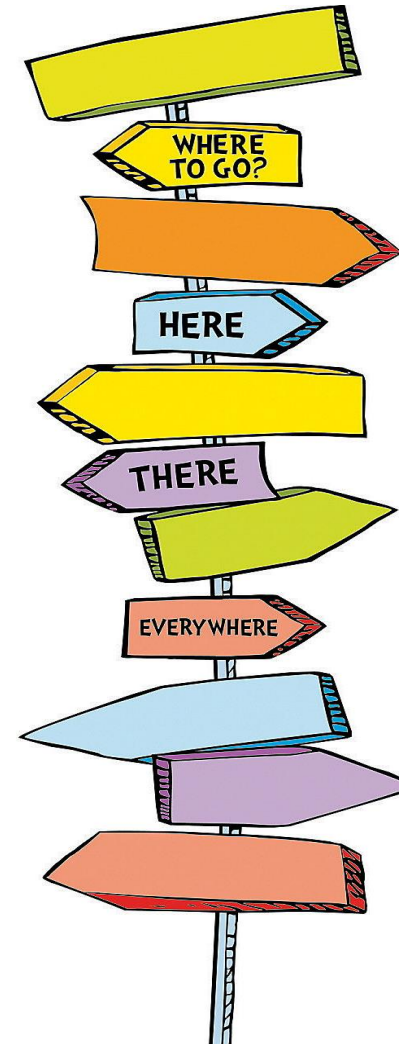
Next Steps

What do we know:

- DAT and auto control are negative
 - Unlikely to be warm reactive autoantibody
- No report of monoclonal antibody therapy or disease associated with use of monoclonal antibody therapy
 - Reactivity unlikely to be due to monoclonal antibody
- Patient is pregnant
 - Possibility of cold autoantibody
 - Possibility of anti-Le^a and anti-Le^b
- Possibility of multiple alloantibodies
 - Phenotype or genotype is useful
- Patient is African American
 - Possibility of high incidence antibodies: anti-U, anti-Js^b
 - Possibility of unusual RH genotype and associated unusual alloantibodies
 - ❖ Need RH genotype investigated

Next Steps

“ You have brains in you head.
You have feet in your shoes.
You can steer yourself any direction
you choose.”



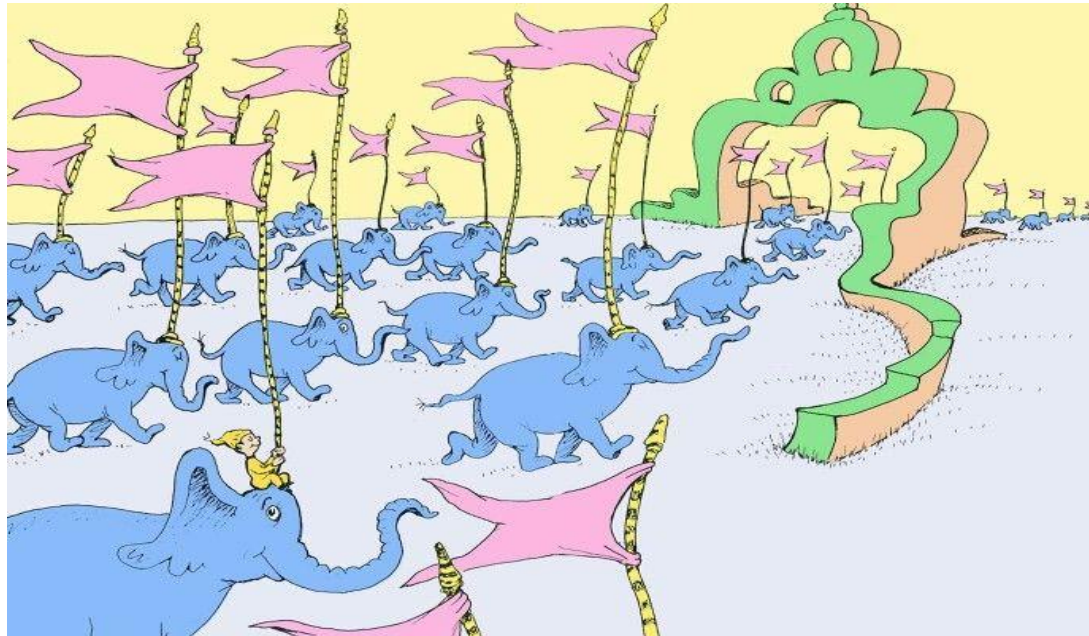
Oh, the Places You'll Go! Dr. Suess

Plasma Testing with Selected Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	5' RT	PEG IAT
1	Vel -	+	+	0	+	+	0	+	+	0	+	0	+	0	+	+	+	+	0	@
2	Ge -	+	+	0	+	+	+	+	0	+	+	+	0	+	+	0	+	0	@	
3	PP1P ^k -	+	+	0	0	+	0	+	0	+	0	0	+	+	+	+	+	0	@	
4	Adult I-	0	0	+	+	+												0	@	
5	Cord cell	+	+	0	+	+	0		+	+	+	0			0	+	+	+	0	@

Patient Phenotype

	Rh					Kell	Duffy		Kidd		MNS		Lewis	
	D	C	E	c	e	K	Fy ^a	Fy ^b	Jk ^a	Jk ^b	S	s	Le ^a	Le ^b
Pt. Cells	+	0	0	+	+	0	0	0	+	0	0	+	0	0



Plasma testing with Enzyme Treated Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	DTT-Tx PEG IAT
1	R ₁ R ₁	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+	+	+	@
2	R ₂ R ₂	+	0	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	1+ ^w
3	rr	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	@

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	Ficin- TX PEG IAT
1	rr V-VS-	0	0	0	+	+	0	+	0	0	+	0	0	+	0	+	0	+	@
2	R ₀ V+VS+	+	0	0	+	+	0	+	0	0	+	0	0	+	+	0	0	+	@
3	R ₀	+	0	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	@

Plasma reactivity not affected by enzyme treatment of cells

Plasma Testing with Selected Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	5' RT	PEG IAT	* IAT
1	R ₀ V+	+	0	0	+	+	0	+	0	0	+	0	0	+	0	+	0	0	r	@	(+)
2	R1r	+	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	0	r	@	(0)✓
3	R ₀ V+VS+	+	0	0	+	+	0	+	0	0	+	0	0	+	+	0	0	+	r	@	(+)
4	R ₀	+	0	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	r	@	(+)
5	R ₀	+	0	0	+	+	0	+	0	0	+	0	0	0	+	+	0	+	<u>±</u>	@	(0)✓
6	R ₀ Js(b-)	+	0	0	+	+	0	+	0	0	+	0	0	+	0	+	0	0	<u>±</u>	@	(+)

* = strict prewarming technique 30' at 37C

Plasma Testing with Le(a-b-) Cells

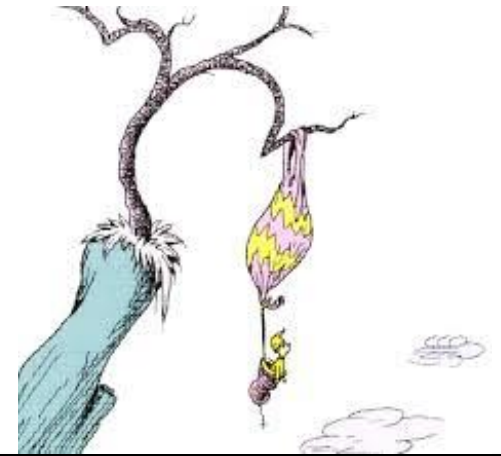
		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results			
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	5' RT	PEG IAT	* IAT	
1	rr	0	0	0	+	+	0	+	0	+	+	0	0	0	+	0	+	0	0	0	@	(+)
2	R ₁ R ₁	+	+	0	0	+	0	+	+	0	0	+	0	0	+	0	0	+	0	0	@	(0)✓
3	rr	0	0	0	+	+	0	+	0	+	0	+	0	0	+	+	0	+	0	0	@	(+)
4	R ₂ R ₂	+	0	+	+	0	0	+	0	0	+	0	0	0	+	+	0	+	0	0	(0)✓	

* = strict prewarming technique 30' at 37C

Lewis Neutralization and Cold Autoadsorption

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Lewis substance neutralization	Saline control	2 X autoadsorption @ 4C
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	PEG IAT	PEG IAT	PEG IAT
1	R ₀	+	0	0	+	+	0	+	0	0	+	0	0	0	+	+	0	+	@	@	(+)
2	rr	0	0	0	+	+	+	+	0	+	0	+	0	+	+	+	0	+	@	@	NT
3	R ₀	+	0	0	+	+	0	+	0	0	+	0	0	+	0	+	0	0	@	@	@

Plasma reactivity is not removed by Lewis neutralization or cold autoadsorption



Blood Group	Antigen	Results	Comments
Rh	c	+	little c+ (partial)
	C	0	
	e	+	Little e+ (partial)
	E	0	
	V	+	
	VS	+	
Kell	K	0	
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	0	
	Jsb	+	
Duffy	Fya	0	
	Fyb	(0)*	Not at risk for anti-Fyb
Kidd	Jka	+	
	Jkb	0	
MNS	M	+	
	N	+	
	S	0	
	s	+	
	U	+	
Lutheran	Lua	0	
	Lub	+	
Diego	Dia	0	
	Dib	+	
Colton	Coa	+	
	Cob	0	
Dombrock	Doa	0	
	Dob	+	
	Hy	+	
	Joa	+	
Landsteiner-Wiener	LWa	+	
	LWb	0	
Scianna	Sc1	+	
	Sc2	0	

Human Erythrocyte Antigen (HEA) Phenotype by DNA Analysis Report

- Sample contains GATA mutation resulting in loss of Fy^b expression on RBCs
 - Individuals not expected to make anti-Fy^b
- Sample homozygous for c.733C>G associated with partial c and e
 - Patient could produce allo anti-c, anti-e and anti-f

Plasma Test Rare Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results	
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	5' RT	PEG IAT
1	Rh _{null}	0	0	0	0	0	0	+	+	+	+	+	0	0	+	+	+	+	0	(0)✓
2	At(a-)	+	0	0	+	+	0		0	+	+	0	+	0	0	+	0	+	0	@
3	-D-	+	0	0	0	0	0	+	+	0	+	+	0	+	+	+	0	+	(+)	@
4	Rh:-46	+	+	0	0	+	0	+	+	+	0	0	+	0	+	0	0	+	0	@
5	Rh:-34	+w	0	0	+	+	0		0	0	+			+	+	0		0	1+	
6	• D •	+	0	0	0	0	0	+	0	+	+	+	+	+	+	+	+	+	1+w	@

Patient can make anti-C, -E, -K, -Fy^a, -Jk^b, -S, -Le^a, -Le^b

Plasma Testing with More Rare Cells

		Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results		
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le^b	M	N	S	s	5' RT	PEG IAT	
1	Rh _{null}	0	0	0	0	0	0	+	+	+	+	+	+	NT	NT	+	0	+	+	0	(0)✓
2	Rh _{null}	0	0	0	0	0	0	+	+	+	+	+	0	+	+	+	+	+	+	0	(0)✓
3	Rh _{null}	0	0	0	0	0	0	+	+	+	+	+	0	0	+	0	+	0	0	0	(0)✓



- Panreactive antibody is in the Rh system
- Cannot rule out all the alloantibodies patient can make

Alloabsorptions to Remove Antibody to High

		Rh					Kell		Duffy		Kidd		Lewis		MNS				rr x 3 @ 37C	R ₁ R ₁ x 3 @ 37C	R ₂ R ₂ x 3 @ 37C
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	PEG IAT	PEG IAT	PEG IAT
1	R ₁ R ₁	+	+	0	0	+	+	+	+	0	0	+	+	0	+	+	0	+	(0)✓	(0)✓	1+S
2	R ₂ R ₂	+	0	+	+	0	0	+	+	+	+	0	+	0	0	+	+	+	(0)✓	(0)✓	(0)✓
3	rr	0	0	0	+	+	0	+	0	+	+	0	0	0	+	0	+	0	(0)✓	(0)✓	2+
4	R ₁ R ₁	+	+	0	0	+	0	+	+	0	0	+	+	0	+	+	0	+	(0)✓	(0)✓	1+S
5	R ₂ R ₁	+	+	+	0	+	+	+	+	0	+	0	0	+	0	+	0	+	(0)✓	(0)✓	1+S
6	R ₂ R ₂	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	+	(0)✓	(0)✓	(0)✓
7	rr	0	0	0	+	+	+	+	+	+	+	0	0	+	+	0	+	0	(0)✓	(0)✓	2+

- rr and R₁R₁ cells removed all reactivity. No alloantibodies left behind.
- R₂R₂ cells left apparent anti-e behind

Rh Genotype Results from NYBC

Testing performed

- RHD: Automated RHD BeadChip Prototype, Zygoty determination by hybrid box detection
- RHCE: Automated RHCE BeadChip Prototype, PCR-RFLP for RHCE exon 2 (254C>G)

RH alleles

- *RHD***DAR* homozygote
 - Uncommon/rare altered RH haplotype and is at risk for allo anti-D
- *RHCE***ceAR* homozygote
 - amino acid changes associated with partial c, partial e, and a V+^W VS-, hr^{B+}, hr^{S-} phenotype

Rh Genotype Results from NYBC

Predicted Rh Phenotype:

- D+(partial), C-, E-, c+(partial), e+(partial), V+^W VS-, hr^B+, hr^S-

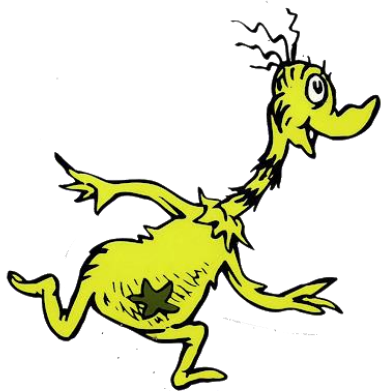
Comments:

- Patient is homozygous for uncommon/rare altered RH haplotype
- Patient is at risk for allo anti-D, e-like or ce(-f), -hr^S, and -Hr as well as anti-C and -E in the RH system
- Since RH genotype is rare, patient should donate autologous units for future transfusion needs
- Family members (full siblings) should be encouraged to get tested.

R₂R₂ Adsorbed plasma x 3 @ 37C

		Rh					Kell		Duffy		Kidd		Lewis		MNS				5" RT	PEG IAT
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s		
1	hr ^S -	+	0	0	+	+	0	+	0	NT	+	0	0	+	+	0	0	NT	0	(0)✓
2	hr ^S -	+	0	0	+	+	0	+	0	0	+	0	0	+	0	+	+	+	0	(0)✓
3	R ₂ R ₂	+	+	+	0	0	0	+	+	0	+	+	0	+	+	+	0	+	0	(0)✓

• Apparent anti-e left behind by R₂R₂ adsorbing cells is really anti-hr^S



Workup Conclusions

ABO Rh	A Pos
DAT	Negative
Plasma	Probable Anti-Hr Anti-hr ^S No additional common alloantibodies
Transfusion Recommendation	Autologous units, hr ^{S-} , Rh _{null} , or RhCE-depleted phenotypes
Units Provided	None needed

Hr antigen and anti-Hr

Hr antigen

- ISBT number is RH18
- Reported in 1960 in Bantu proband Mrs. Shabalala
- Hr antigen present on all RBCs except hr^S- , Rh_{null} and RhCE-depleted phenotypes: $RHCE^*ceAR$, $RHCE^*ceEK$, $RHCE^*ceBI$, $RHCE^*ceSM$

Anti-Hr

- Made by hr^S- people
- May be part of immune response of people with RH-depleted phenotypes
- Transfusion reactions: no to fatal
- HDFN: moderate

hr^S antigen and anti-hr^S

hr^S antigen

- ISBT number is RH19
- Reported in 1960 in Bantu proband Mrs. Shabalala
- Occurrence all populations 98%. R₂R₂ RBCs lack hr^S
 - 1% of Blacks are hr^S- as 1% of e+ Bantu people are hr^S-

Anti-hr^S

- Reacts preferentially with haplotypes containing ce
 - May be mistaken for anti-f
- Antibodies made by hr^S- people are not necessarily anti-hr^S
 - Unless tested with appropriate rare e variant cells, more correctly called anti-e like
- Transfusion reactions: no to fatal if with anti-Hr
- HDFN: little evidence in the absence of anti-Hr of causing HDFN



Remaining Questions...

What about the baby?

- Group O +
- Negative DAT
- No issues reported
- Discharged with mom
- IRL never got a sample on baby

Objectives

- Describe some possible causes of panreactivity in a patient's plasma
 - Autoantibody – warm, cold or both
 - Monoclonal antibody therapy
 - Multiple antibodies
 - Antibody to high prevalence antigen
- List some techniques that can be used to identify the causes of panreactivity in a patient's plasma
 - Alloadsorption or autoadsorption of patient's plasma
 - Testing cells similar to patient's phenotype
 - Neutralization of patient's plasma
 - Testing cells negative for high incidence antigens/null phenotype cells
 - Use of enzyme treated cells
- List some benefits of obtaining a patient's genotype
 - Determining which antigens are lacking on the patient's red cells and therefore which antibodies the patient could potentially produce
 - Identifying rare or unusual phenotypes
 - Determining if plasma reactivity is autoantibody or alloantibody
 - Resolving antigen typing discrepancies

References

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