New York Blood Center Enterprises

RH You Ready?

Overview of a Blood Group System Through Cases

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Objectives

1. Describe important aspects of the RH blood group system revealed through a review of history.
2. Identify widely familiar and less familiar antigens of the RH blood group system.
3. Discuss circumstances in which RH antibodies present unique challenges in the blood bank.

Case 1 - 1937

- Baby delivered stillborn – erythroblastosis fetalis
- Mother required transfusion

At the time…
The cause of erythroblastosis fetalis (now called HDFN) was unknown

Case 1 - 1937

- Baby delivered stillborn – erythroblastosis fetalis
- Mother required transfusion
- As was custom at the time, **father donated blood** for transfusion
- Mother had **transfusion reaction**
- A sample collected after reacted with 41/54 (76%) of ABO-compatible RBCs
- Some months later, antibody no longer detectable

Levine & Stetson, 1939

- Published the case
- First described HDFN (then called erythroblastosis fetalis) cause as maternal antibody against paternal antigen
- Didn't name the antibody

At the time…

- No way to detect IgG antibodies
- What was detected was short-lived IgM

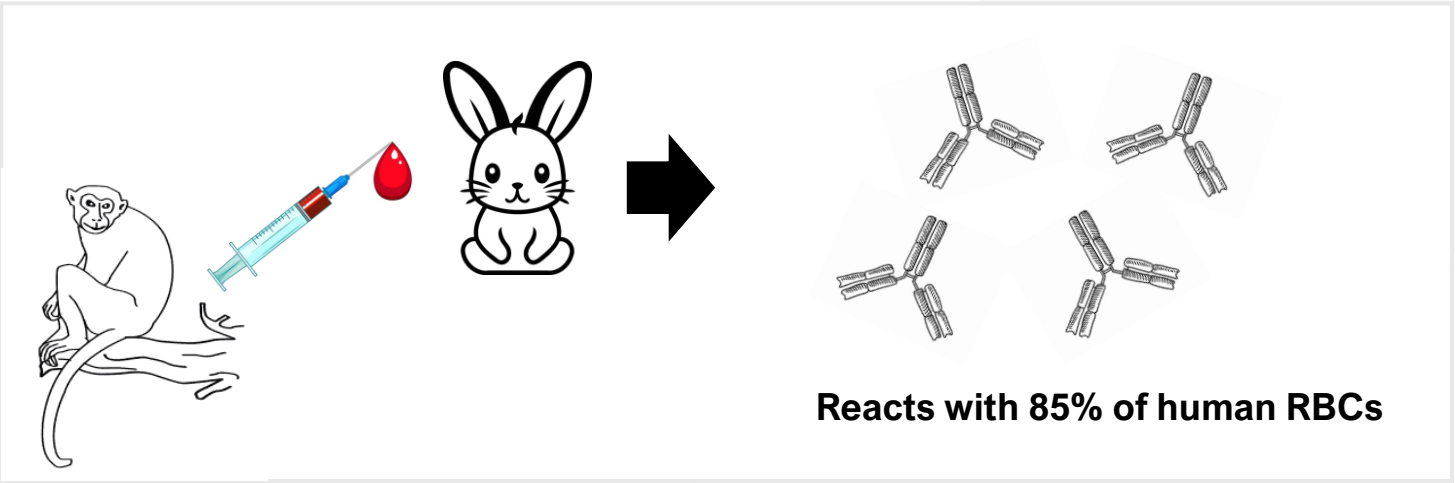
Authors noted that several cases appeared to have the same specificity.

Levine et al. *JAMA*. 1939;113:126–7.

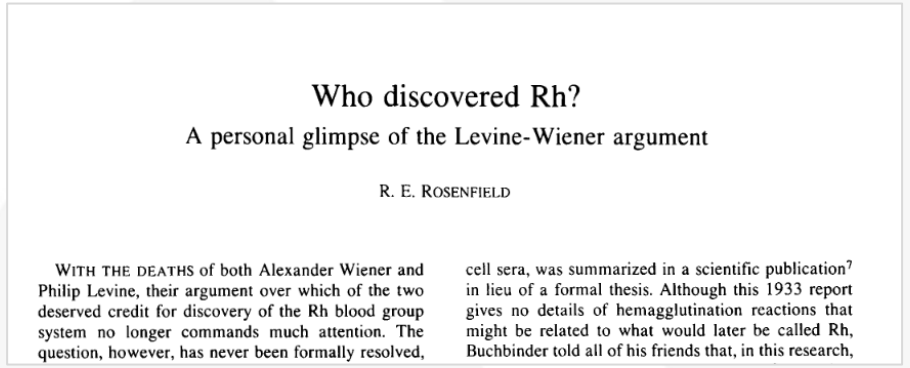
1940 Landsteiner & Wiener

- Studied antibody made by guinea pigs and rabbits when injected with blood from Rhesus monkeys
- Reacted with 85% of human RBCs tested
- They thought it was the same antibody described by Levine and Stetson
- They called it "**Anti-Rh**" (anti-D)

Rhesus macaque
https://primatesinc.com/resources/rhesus-macaques/



Reacts with 85% of human RBCs



Who discovered Rh?

A personal glimpse of the Levine-Wiener argument

R. E. ROSENFIELD

WITH THE DEATHS of both Alexander Wiener and Philip Levine, their argument over which of the two deserved credit for discovery of the Rh blood group system no longer commands much attention. The question, however, has never been formally resolved,

cell sera, was summarized in a scientific publication[7] in lieu of a formal thesis. Although this 1933 report gives no details of hemagglutination reactions that might be related to what would later be called Rh, Buchbinder told all of his friends that, in this research,

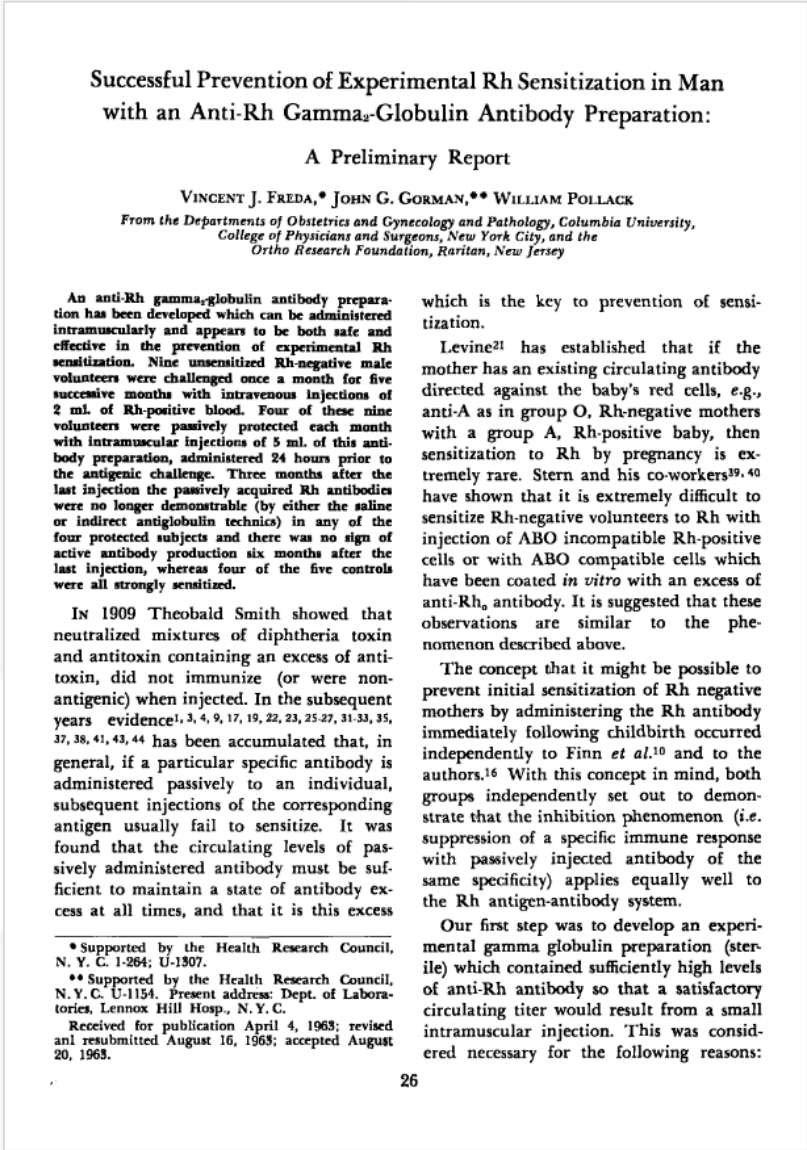
Landsteiner et al. *Proc Soc Exp Biol Med*. 1940;43:223. Rosenfield. *Transfusion.* 1989;29(4):355-357.

What do we learn from this brief history?

1. “Anti-Rh” (Anti-D) is clinically significant
   - Causes transfusion reactions
   - Causes HDFN
2. D antigen is highly immunogenic
   - Once thought that 80% of D- patients receiving D+ RBCs would develop anti-D
3. At the time, 9-10% of pregnancies were affected by anti-D

Rh Immune Globulin (RhIg)- 1968

- **How did we determine this might work?**
  - Recognition that ABO incompatibility offered some protection against anti-D formation
- **How was it studied?**
  - D- prison volunteers transfused with D+ blood
    - Some also administered prophylactic anti-D
- **How does it work?**
  - Antibody destroys/removes fetal cells from maternal circulation so she doesn't make antibody?
  - Antibody-mediated immune suppression?
  - Some other mechanism?



Successful Prevention of Experimental Rh Sensitization in Man with an Anti-Rh Gamma$_2$-Globulin Antibody Preparation:

A Preliminary Report

VINCENT J. FREDA,* JOHN G. GORMAN,** WILLIAM POLLACK

From the Departments of Obstetrics and Gynecology and Pathology, Columbia University, College of Physicians and Surgeons, New York City, and the Ortho Research Foundation, Raritan, New Jersey

An anti-Rh gamma$_2$-globulin antibody preparation has been developed which can be administered intramuscularly and appears to be both safe and effective in the prevention of experimental Rh sensitization. Nine unsensitized Rh-negative male volunteers were challenged once a month for five successive months with intravenous injections of 2 ml. of Rh-positive blood. Four of these nine volunteers were passively protected each month with intramuscular injections of 5 ml. of this antibody preparation, administered 24 hours prior to the antigenic challenge. Three months after the last injection the passively acquired Rh antibodies were no longer demonstrable (by either the saline or indirect antiglobulin technics) in any of the four protected subjects and there was no sign of active antibody production six months after the last injection, whereas four of the five controls were all strongly sensitized.

IN 1909 Theobald Smith showed that neutralized mixtures of diphtheria toxin and antitoxin containing an excess of antitoxin, did not immunize (or were nonantigenic) when injected. In the subsequent years evidence[1, 3, 4, 9, 17, 19, 22, 23, 25-27, 31-33, 35, 37, 38, 41, 43, 44] has been accumulated that, in general, if a particular specific antibody is administered passively to an individual, subsequent injections of the corresponding antigen usually fail to sensitize. It was found that the circulating levels of passively administered antibody must be sufficient to maintain a state of antibody excess at all times, and that it is this excess which is the key to prevention of sensitization.

Levine[21] has established that if the mother has an existing circulating antibody directed against the baby's red cells, *e.g.*, anti-A as in group O, Rh-negative mothers with a group A, Rh-positive baby, then sensitization to Rh by pregnancy is extremely rare. Stern and his co-workers[39, 40] have shown that it is extremely difficult to sensitize Rh-negative volunteers to Rh with injection of ABO incompatible Rh-positive cells or with ABO compatible cells which have been coated *in vitro* with an excess of anti-Rh$_0$ antibody. It is suggested that these observations are similar to the phenomenon described above.

The concept that it might be possible to prevent initial sensitization of Rh negative mothers by administering the Rh antibody immediately following childbirth occurred independently to Finn *et al.*[10] and to the authors.[16] With this concept in mind, both groups independently set out to demonstrate that the inhibition phenomenon (*i.e.* suppression of a specific immune response with passively injected antibody of the same specificity) applies equally well to the Rh antigen-antibody system.

Our first step was to develop an experimental gamma globulin preparation (sterile) which contained sufficiently high levels of anti-Rh antibody so that a satisfactory circulating titer would result from a small intramuscular injection. This was considered necessary for the following reasons:

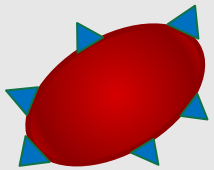
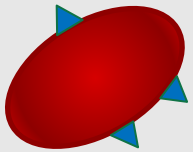
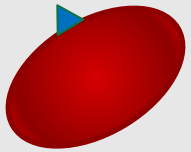
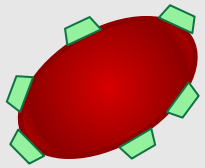
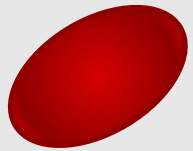
* Supported by the Health Research Council, N. Y. C. 1-264; U-1307.

** Supported by the Health Research Council, N. Y. C. U-1154. Present address: Dept. of Laboratories, Lennox Hill Hosp., N. Y. C.

Received for publication April 4, 1963; revised and resubmitted August 16, 1965; accepted August 20, 1963.

Freda et al. *Transfusion.* 1964;4:26-32.

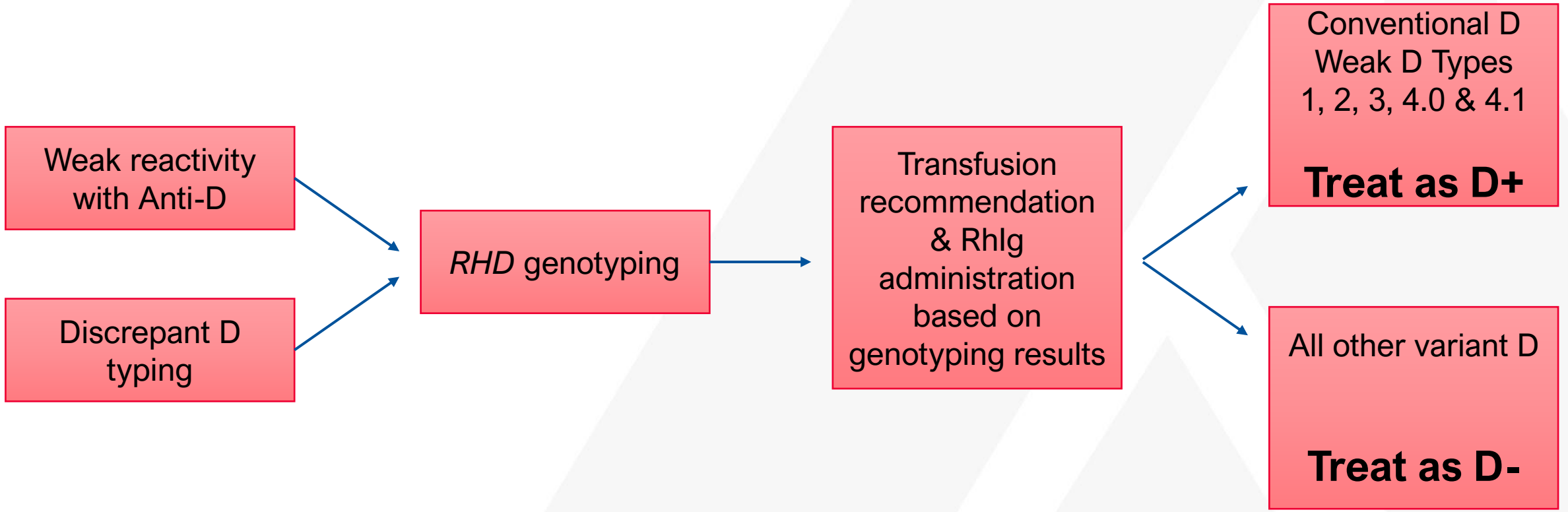
D^{u} you know about D variant expression?

	Conventional D	Weak D	D_{el}	Partial D	Negative
					
At risk of alloanti-D?	No	**Weak D Types 1, 2, 3, 4.0, 4.1** No	Depends on specific alleles present	Yes	Yes
Transfuse	D+	D+	Depends	D-	D-
RhIG candidate?	No	No	Depends	Yes	Yes

Routine serology can't: Detect D_{el} phenotype
Differentiate weak from partial D

Sandler et al. *Transfusion.* 2015;55(3):680-689.
Flegel et al. *Transfusion*. 2020;60(4):855-859.
Westhoff et al. *Blood Transfus.* 2019;17(2):91-93.

Recommended Approach to Patient* D Typing



* Especially patients of childbearing potential

Does this approach work?

Brian Easley shared:

- Algorithm used to determine when to *RHD* genotype
- How often *RHD* genotyping detected Variant D
- Treatment decisions as a result
  - How many patients treated as D+
  - How many patient treated as D-

Reaction Strength by Method					Reaction Strength by Method				
D Clone-Gel	D4 Clone-Hemagg	D5 Clone-Hemagg	Molecular	Race/Ethnicity	D Clone-Gel	D4 Clone-Hemagg	D5 Clone-Hemagg	Molecular	Race/Ethnicity
2	0	0	Weak D Type 2	Caucasian		2	2	Weak Partial 4.0	Other
2	0	0	Weak D Type 1	Caucasian	3	0	0	Partial D	Caucasian
3	2	1	Partial D	*African American		0	0	Weak D Type 1	Caucasian
2	0	0	Weak D Type 2	Caucasian		0	0	Weak D Type 2	Caucasian
3	0	0	Weak D Type 2	Caucasian		1	2	RHD Positive	African American
	2	1	Weak D Type 1	Caucasian	4	2	2	Weak D Type 1	African American
	2	1	Partial D	African American		0	0	Weak D Type 1	Caucasian
3	1	0	Weak D Type 1	Caucasian		1	0	Partial D	African American
3	1	1	Weak D Type 3	Caucasian	2	0	0	Weak D Type 2	Caucasian
3	2	1	Weak Partial 4.0	African American	3	2	2	RHD Positive	Hispanic/Latino
3	1	1	Weak D Type 3	Caucasian		2	2	RHD Positive	Caucasian
3	1	0	Weak Partial 4.0	African American		1	1	Weak D Type 3	Caucasian
	1	2	Weak Partial 4.0	African American	3	1	0	Weak D Type 1	Caucasian
	1	0	Partial D	*Caucasian		2	1	Partial D	African American
3	2	2	RHD Positive	Caucasian		1	0	Partial D	Caucasian
	1	0	Weak D Type 1	Caucasian		2	2	RHD Positive	Caucasian
	2	2	RHD Positive	Mixed Race		1	1	Weak D Type 1	Caucasian
	2	2	Weak Partial 4.0	Caucasian	3	1	0	Partial D	African American
3	1	2	Partial D	Caucasian	4	1	1	Weak Partial 4.0	*African American
	1	1	Weak Partial 4.0	African American		1	0	Weak D Type 1	Caucasian
	3	1	RHD Positive	Caucasian		0	0	Weak Partial 4.0	African American
3	3	1	Weak Partial 4.0	*African American	4	1	0	Weak Partial 4.0	Multiracial
3	2	2	RHD Positive	Caucasian		2	1	Partial D	African American
	1	0	Partial D	*African American		2	2	RHD Positive	African American
	1	0	Weak D Type 1	Caucasian	3	2	1	RHD Positive	African American
3	1	0	Weak D Type 3	Caucasian		2	1	Partial D	Caucasian
	3	1	Partial D	African American	3	2	2	Partial D	Caucasian
3	1	0	Weak Partial 4.0	Caucasian	3	2	1	Weak Partial 4.0	African American
	0	0	Weak Partial 4.0	African American	3	1	0	Weak D Type 1	Caucasian
	2	1	Weak D Type 33	African American	3	1	0	Weak D Type 1	Caucasian
	0	0	Weak D Type 2	Caucasian		1	1	Weak Partial 4.0	Unknown
4	0	0	Weak D Type 2	Hispanic/Latino		1	0	Partial D	African American
4	3	1	Weak Partial 4.0	African American		1	1	Partial D	African American
3	2	1	Partial D	African American		1	0	Weak D Type 1	Caucasian
3	1	1	Partial D	African American		1	1	Partial D	Caucasian
	1	1	Weak D Type 1	Caucasian		2	2	RHD Positive	African American
	2	2	RHD Positive	African American		1	0	Partial D	Caucasian
	0	0	Weak D Type 1	*Caucasian		1	0	Weak Partial 4.0	Caucasian

Easley et al. *Transfusion.* 2025;65(S2):274A-275A.

What Makes the RH System So Complex

- Variable expression of RH antigens (weak, partial, D_{el} expression of D)
- Some phenotypes don't fit neatly into these categories
  - Weak/partial
  - Del/partial
- For some variant alleles, affect on phenotype is unknown

For other RH antigens (C, E, c, e), weak, partial and even "el" expression have been described

Objectives

1. Describe important aspects of the RH blood group system revealed through a review of history.
2. Identify widely familiar and less familiar antigens of the RH blood group system.
3. Discuss circumstances in which RH antibodies present unique challenges in the blood bank.

Antigens in the RH system

- **Common:** D, C, E, c, e
- **Sometimes also listed on antigrams:** f, C^{w}, V
- **You may have also heard of:** G
- **There are so many others:** 56 antigens in all!
  - Most are either high prevalence or low prevalence

Case 2:

Patient:

- 35-year-old female
- Type and crossmatch for 2 units ordered for upcoming surgery
- History of transfusion 2 years prior
  - Group A, Rh positive
  - AUS (antibody of undetermined specificity)
    - Weak +/- reactivity with 4-5 reagent RBCs
  - Requires full XM per facility policy

Today's results

ABO/Rh		**A, Rh positive**
Antibody Screen		Gel
	SCI	0
	SCII	0
Antibody Screen Interpretation		**Negative**

Crossmatch Results

	IAT	DAT
Unit #1	0	
Unit #2	2+	0
Unit #3	0	
Unit #4	2+	
Unit #5	2+	
Unit #6	2+	
Unit #7	2+	

Panel

		Rh					Kell		Duffy		Kidd		MNS				Results
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	gel
1	R_1R_1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0
2	R_1R_1	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0
3	R_2R_2	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0
4	R_0r	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	2+
5	r'r	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	2+
6	r"r	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	2+
7	rr	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	2+
8	rr	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	2+
9	rr	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	2+
10	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	0
11	R_0r	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	2+
Auto																	0

Blood Group	Antigen	Result
Rh	c	+
	C	+
	e	+
	E	+
	V	0
	VS	0
Kell	K	0
	k	+
	Kp^a	0
	Kp^b	+
	Js^a	0
	Js^b	+
Duffy	Fy^a	+
	Fy^b	+
Kidd	Jk^a	+
	Jk^b	0
MNS	M	+
	N	+
	S	+
	s	+
	U	+
Lutheran	Lu^a	0
	Lu^b	+
Diego	Di^a	0
	Di^b	+
Colton	Co^a	+
	Co^b	0
Dombrock	Do^a	+
	Do^b	+
	Hy	+
	Jo^a	+
Landsteiner--Wiener	LW^a	+
	LW^b	0
Scianna	Sc1	+
	Sc2	0

Patient Genotype on File!

- Luckily, patient was genotyped at the time AUS was detected
- C+,c+,E+,e+; **K-**,k+; Fy(a+b+); **Jk**(a+**b-**); S+,s+
- Of "common" antibodies, can only make anti-K & anti-Jk^b
  - Both of these were ruled out on panel

What's going on?

- History of AUS
- **Today's screen:** NEGATIVE
- **Panel:** 7/11 RBCs POSITIVE
- **Crossmatches:** 5/7 XMs POSITIVE
- ALL antibodies against common RBC antigens ruled out

- f antigen expressed when c & e are on the same protein (genetically, in *cis*)

Haplotypes		
D+	R_1	DCe
	R_2	DcE
	R_0	**Dce**
	R_Z	DCE
D-	r’	dCe
	r”	dcE
	r	**dce**
	r^y	dCE

Individuals with these haplotypes will express f antigen

Panel

		Rh					Kell		Duffy		Kidd		MNS				Results
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	gel
1	R_1R_1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0
2	R_1R_1	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0
3	R_2R_2	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	0
4	R_0r	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	2+
5	r'r	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	2+
6	r"r	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	2+
7	rr	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	2+
8	rr	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	2+
9	rr	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	2+
10	R_1R_1	+	+	0	0	+	0	+	+	0	+	0	+	+	+	0	0
11	R_0r	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	2+
Auto																	0

RBCs that express f (notice R_0 and r haplotypes)

What about the negative antibody screen?

		Rh					Kell		Duffy		Kidd		MNS				Results
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	gel
SCI	R_1R_1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	0
SCII	R_2R_2	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	0

- 2-cell screens always consist of R_1R_1 (DCe/DCe) and R_2R_2 (DcE/DcE) RBCs
  - No f!
- 3-cell screens always consist of R_1R_1 (DCe/DCe), R_2R_2 (DcE/DcE), and rr (dce/dce) RBCs
  - SCIII f+

What about the genotype?

- Patient phenotype: D+,C+,c+,E+,e+
- Likely R_1R_2 (f-negative)

Blood Group	Antigen	Result
Rh	c	+
	C	+
	e	+
	E	+
	V	0
	VS	0

		D	C	c	E	e
Who makes anti-f? f-negative individuals!	R_1R_1 (DCe/Dce)	+	+	0	0	+
	R_2R_2 (DcE/DcE)	+	0	+	+	0
	R_1R_2 (DCe/DcE)	+	+	+	+	+

There is no commercial anti-f reagent;

f status is inferred based on other Rh typings!

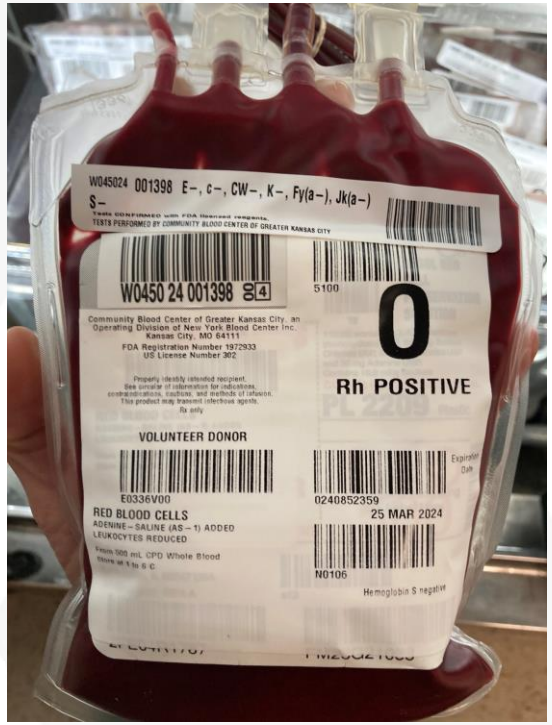
f… ollowing up on those incompatible crossmatches…

	ABO/Rh	IAT XM	Presumed haplotypes	D	C	c	E	e	f
Unit #1	A, Rh pos	0	R_1R_1 (DCe/DCe)	+	+	0	0	+	0
Unit #2	A, Rh pos	2+	R_2r (DcE/dce)	+	0	+	+	+	+
Unit #3	A, Rh pos	0	R_1R_2 (DCe/DcE)	+	+	+	+	+	0
Unit #4	A, Rh pos	2+	R_0r (Dce/dce)	+	0	+	0	+	+
Unit #5	A, Rh neg	2+	rr (dce/dce)	0	0	+	0	+	+
Unit #6	A, Rh neg	2+	rr (dce/dce)	0	0	+	0	+	+
Unit #7	A, Rh neg	2+	rr (dce/dce)	0	0	+	0	+	+

f…inding the right units

f-negative units, XM compatible at IAT

- No anti-f antisera available commercially
- f is not on licensed commercial genotyping platforms
- f status is inferred
  - Easiest way to order f-negative units is to order c- or e-



What about other compound antigens?

f = compound antigen expressed when c and e in *cis*

Ce (RH7) = compound antigen expressed when C and e in *cis*
CE (RH22) = compound antigen expressed when C and E in *cis*
cE (RH27) = compound antigen expressed when c and E in *cis*

V antigen

- In most populations, low prevalence antigen
- Polymorphic antigen in Black population
- Encoded by variant *RHCE* alleles
  - Expression of V may indicate partial c and/or partial e
  - Expression of V may indicate negative for high prevalence RH antigen

Who makes anti-V?

- Patients exposed to donor V+ blood
  - R_0 (D+, C-, E-) units from Black donors often transfused to patients with SCD requiring C-E-K-

Occurrence	
Caucasians	1%
Blacks	30%

Blood Group Antigen FactsBook

Other Rh antigens….

- **High prevalence:** Hr, Hr_0, many others
- **Low prevalence:** Go^a, VS, C^W, many others

Key concept:

Variant RH Alleles

- may encode low prevalence antigens (like V, VS, C^W, etc.)
- may encode proteins missing a high prevalence antigen (like Hr-, Hr_0-, etc.)
- may encode partial common Rh antigens (like partial e, partial C, etc.)

RH Genotyping helps

Objectives

1. Describe important aspects of the RH blood group system revealed through a review of history.
2. Identify widely familiar and less familiar antigens of the RH blood group system.
3. Discuss circumstances in which RH antibodies present unique challenges in the blood bank.

Case 3:

Patient: 18-year-old female

Diagnosis: Sickle cell disease, on chronic transfusion program

Group B, Rh positive

RBC exchange with C-E-K- units every ~5-6 weeks

New sample arrives, and patient now has clear **anti-D**

What questions do you want to ask?

What's the autocontrol result?

- Positive, but weaker than other D+ reagent RBCs

When was the last transfusion?

- 4 weeks ago

Has the patient been RH genotyped?

- No

Is there evidence of decreased survival of transfused RBCs?

- Unknown for now

Anti-D

- Autoantibody in D+ patient?
- Alloantibody, patient is D+ partial?

What makes antibodies with Rh specificity so difficult?

1. Auto vs alloantibody (especially with recent transfusion)
2. Serology results (with monoclonal antisera) may be strongly reactive with partial antigens
3. Drugs can cause Rh antibodies??!!
4. Evidence that Rh variants in donors can cause antibodies in transfused patients
   - Anti-C detected in C-negative patient only receiving C-negative units

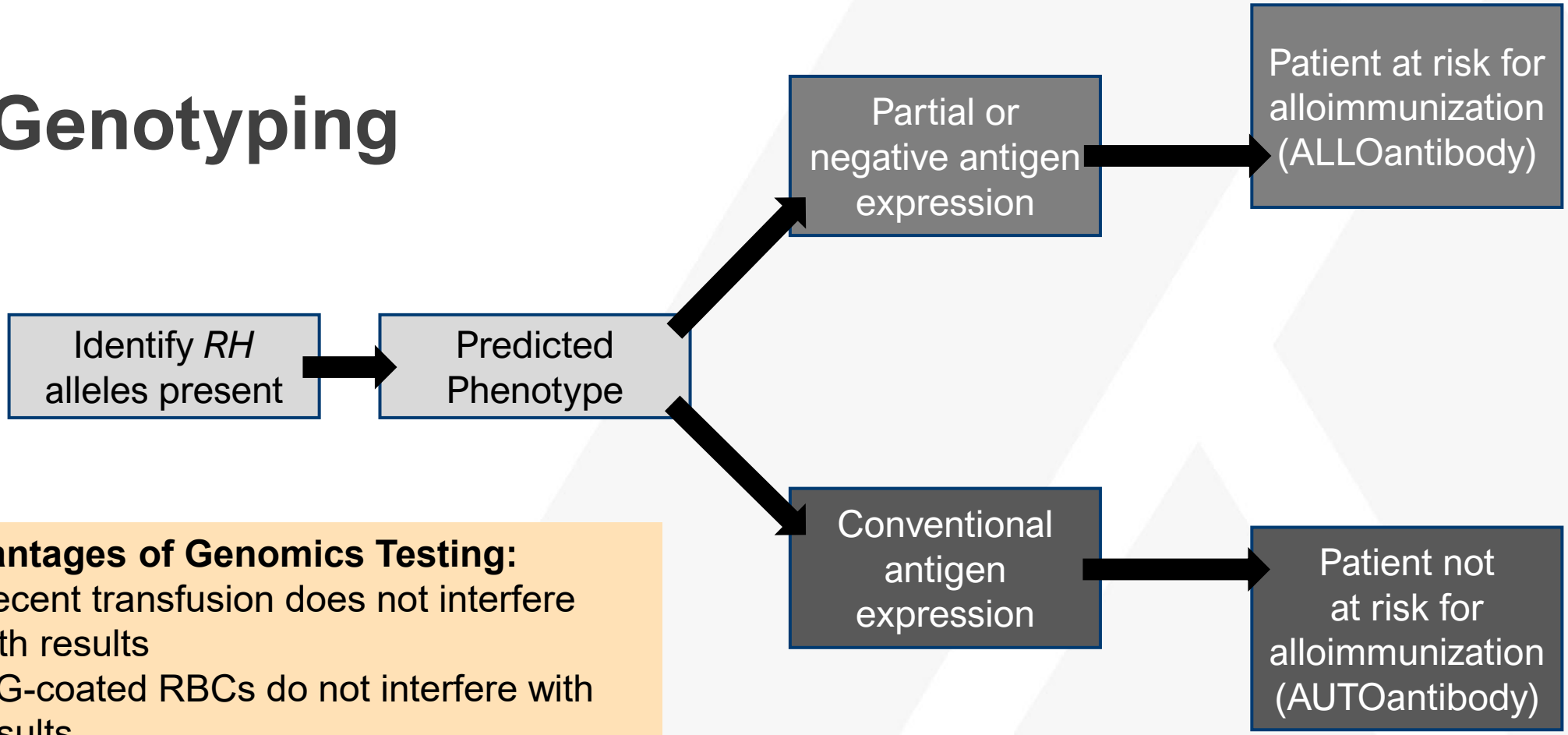
Also consider:

- Patients are often recently transfused!
- Patients can have both allo- and autoantibodies!
- Patients & donors can both have variant Rh

Kirkegaard et al. *Transfusion.* 2023;63(10):1962-1968.

Differentiating Allo- From Autoantibody

RH Genotyping



Advantages of Genomics Testing:

- Recent transfusion does not interfere with results
- IgG-coated RBCs do not interfere with results

Back to the case

RHD genotyping results:

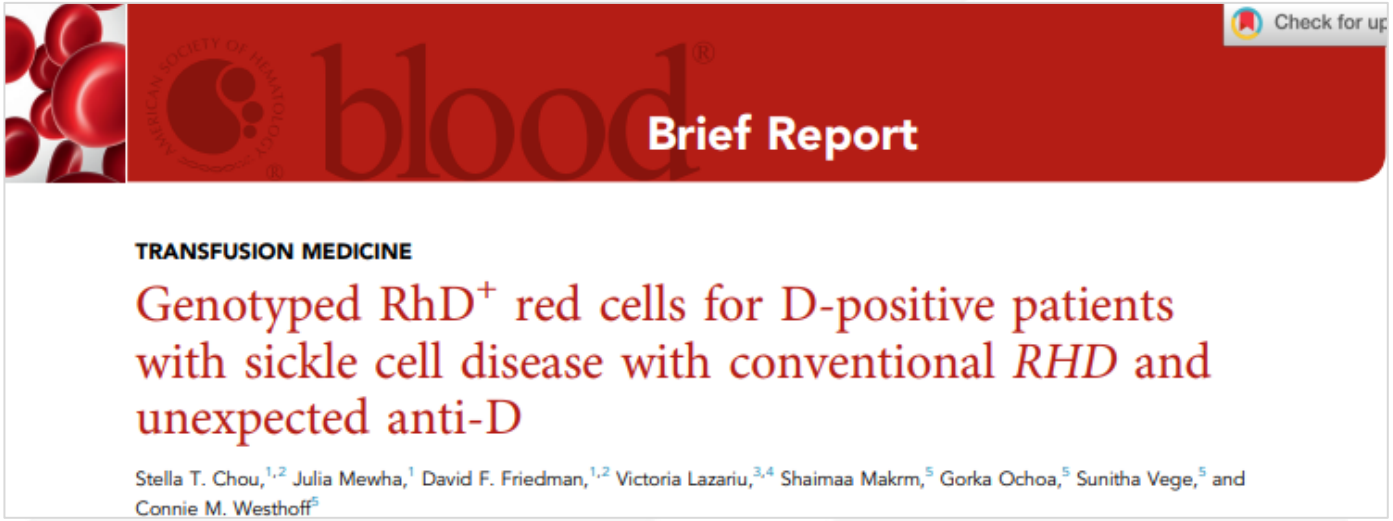
RHD / RHDIIIa-CE(4-7)-D

RHCE genotyping results:

ce / ce(733G)

Per genotyping results, patient not at risk for alloanti-D

But we should keep giving D- units forever, right???



Exciting work by Children's Hospital of Philadelphia and New York Blood Center

- 5 patients
  - SCD
  - History of anti-D (no longer detectable)
  - Genotyped – confirmed conventional D
- Received increasing number of genotyped D-positive units
  - Ensure not transfused D variants
- No anti-D restimulation

Chou et al. *Blood.* 2024;144(19):2045-2049.

Objectives

1. Describe important aspects of the RH blood group system revealed through a review of history.
2. Identify widely familiar and less familiar antigens of the RH blood group system.
3. Discuss circumstances in which RH antibodies present unique challenges in the blood bank.

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Thank you!

Questions?

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