AABB Annual Meeting Education Program 2014

October 25-28, 2014 | Pennsylvania Convention Center | Philadelphia, PA

Presentation Handouts

(9312-TC) Evaluating Antibodies for Clinical Significance, One Blood Group System at a Time: Round 3

October 27, 2014 ✧ 10:30 AM - 12:00 PM

Advancing Transfusion and Cellular Therapies Worldwide

Event Faculty List

Event Title: **(9312-TC)** Evaluating Antibodies for Clinical Significance, One Blood Group System at a Time: Round 3
Event Date: October 27, 2014
Event Time: 10:30 AM - 12:00 PM

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Anti-D: Simple Yet Complex

Evaluating Antibodies for Clinical Significance: One Blood Group at a Time
Round 3

AABB Annual Meeting
Philadelphia, PA
October 27, 2014

Susan T. Johnson, MSTM, MT(ASCP)SBB
Director, Clinical Education
BloodCenter of Wisconsin

Objectives

- Describe the immunologic response to RhD protein
- Compare & contrast *in-vivo* and *in-vitro* characteristics of alloanti-D, passive anti-D and autoanti-D
  - Discuss the clinical significance of anti-D in transfusion and pregnancy
- Discuss anti-D in Partial D individuals

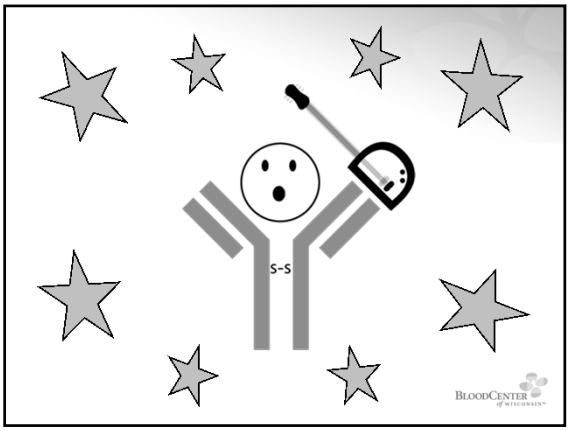
SIMPLE ☺

	D	C	c	E	e	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s	IAT
1	+	+	0	0	+	0	+	0	+	0	0	+	3
2	+	+	0	0	+	+	0	+	+	+	+	+	3
3	+	0	+	+	0	0	0	+	+	0	+	+	3
4	+	0	+	0	+	0	0	+	0	+	0	+	3
5	0	+	+	0	+	0	+	+	+	0	+	0	0
6	0	0	+	0	+	+	0	+	0	+	+	+	0
7	0	0	+	+	0	0	+	0	0	+	0	+	0
8	0	0	+	0	+	0	0	+	+	0	+	0	0
Auto													0

Anti-D

- Most studied of all antibodies to RBC antigens

"Rock Star" of Antibodies!

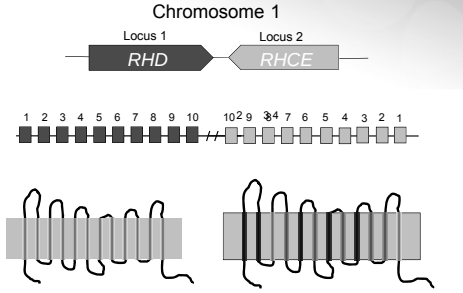


Erythroblastosis in 1943

- 12% of marriages paired an Rh Neg woman with an Rh Pos man
  - 5,000 – 10,000 babies died/year in USA (educated estimates)
  - "Once it occurred, all who followed would die…"
  - "Childbearing for parents, became a highly predictable recurring tragedy"

Excerpts from *Rh – The Intimate History of a Disease & Its Conquest*

RH Genes – Rh Positive



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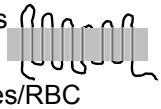
Rh Protein

- Only present on RBCs
- 417 amino acids
- Differ from RhCE by 31-35 amino acids
  - Dependant on *RHCE* allele
- Crosses membrane 12 times
  - 6 extracellular loops
- ~9,900 – 33,000 antigen sites/RBC (dependant on Rh phenotype)



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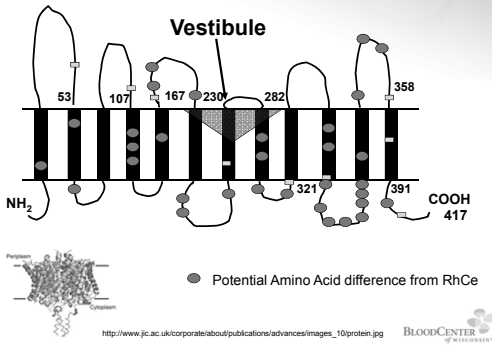
RhD Protein



http://www.jic.ac.uk/corporate/about/publications/advances/images_10/protein.jpg

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Immune Response

COMPLEX

Immune Response to RhD

- Antigen processed to immunogenic peptides by Antigen Presenting Cells (APCs) - dendritic cells
  - Peptides are loaded on MHC Class II molecules & presented to Helper T cells with receptors for the processed peptides
- B cell receptors are activated & produce anti-D

SJ Urbaniak, Transfusion clinique et biologique 13(2006) 19-22

Immune Response to RhD

- 1st exposure slow, up to 4 weeks
  - Short primary IgM response
- Memory lasts for years after immunizing event
- Response on re-stimulation
  - Strong IgG, often within 24 hrs
  - Peaks quickly (~ 6 days)

Immune Response to RhD Volunteer Studies

Gunson HH, Stratton F, Phillips PK. BJH,1976,32,317

Reference	No.	Rh Phenotype	Dose (ml)	Detectable Anti-D after 1st stimulus
Mollison (1969, 1970)	10	R_1r	1.0	10%
Woodrow (1975)	31	R_1r	1.0	23%
Samson & Mollison (1975)	12	R_1R_2	1.0	42%
Pollack (1971)	22	R_1r	500	82%
Gunson (1976)	43	R_2R_2	0.5-5.0	79%

50-80% of RhD- people given ≥1 unit of RhD+ blood make anti-D

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Immunogenicity of RhD Patient Studies

- 21-22% of RhD- patients given ≥1 unit of RhD+ blood make anti-D
  - Frohn et. al.,Transfusion 2003;43:893–898
    - Predicted incidence – 30.44%
    - Raw Data – 21%
  - Yazer et. al., Transfusion 2007;47:2197-2201
    - Raw Data – 22%

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Anti-D alloimmunization after D-incompatible platelet transfusions

KL O'Brien, RL. Haspel, L Uhl
Transfusion 2014;54:650-654.

- 14 year retrospective study
- 626 D- patients rec'd 2,770 D+ prestorage leukoreduced apheresis PLT transfusions (contain < 0.001 mL of RBCs)
  - 50 rec'd D+ RBCs & 28% made anti-D
  - 130 evaluable patients rec'd 565 SDP

No Anti-D!! No need for RhIG

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Anti-D
Serologic Characteristics

SIMPLE

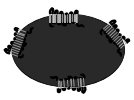
Serologic Characteristics of Anti-D

- Immune - IgG
  - Most IgG1, some IgG3
- Hyperimmunized individuals
  - IgG2 & IgG4, IgA & IgM

- Detectable antibody persists
- Rare non-RBC stimulated – IgM

In Vitro Detection of Anti-D

- IgM - R.T. and 37ºC
- IgG - some 37ºC, most IAT
- Enhanced by:
  - High protein media
  - Proteolytic enzymes
  - Polybrene
  - PEG
  - CAT
  - SP



- May rarely show dosage
- Rarely bind C in vitro

Case History

- 22 y/o pregnant female
- High Risk patient – MCA doppler↑
- History of Anti-D, -C, -Jk^a

Forward Type			Reverse Type		
Anti-A	Anti-B	Anti-D	A1 cells	A2 Cells	B cells
4	0	0	1	0	4

Antibody Identification – Test Tube Methods

	D	C	E	c	e	K	Fy^a	Fy^b	Jk^a	Jk^b	S	s	IS	37C	IAT
1	+	0	+	+	0	0	+	0	0	+	0	+	1	1	4
2	+	0	0	+	+	0	0	+	0	+	+	+	w	w	4
3	0	+	0	+	+	0	0	+	+	0	+	+	0	0	3
4	0	+	0	+	+	0	0	+	0	+	0	+	0	0	3
5	0	0	0	+	+	0	+	+	+	0	+	0	0	0	0√
6	0	0	0	+	+	+	0	+	+	0	+	+	0	0	0√
Auto													0	0	0√

Anti-D Titer 512
Anti-C Titer 8
Anti-Jk^a too weak to titrate

ABO Discrepancy Resolution

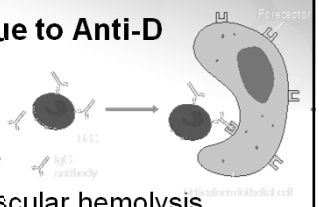
Forward Type			Reverse Type		
Anti-A	Anti-B	Anti-D	A1 cells	A2 Cells	B cells
4	0	0	1	0	4

	Anti-D	Anti-C	Anti-E	Anti-c	Anti-e
A1 Cells	4	0	3	4	0
A2 Cells	4	3	0	4	4

Anti-D *In Vivo*

SIMPLE

HTR due to Anti-D



- Most delayed
- Rare immediate
- Usually extravascular hemolysis
- Rarely bind C *in vivo*
  - Far distance between Ag sites

HDFN due to Anti-D

- Severe
- D is highly immunogenic
- 0.1-1.0 mL of D+ RBCs can result in anti-D sensitization

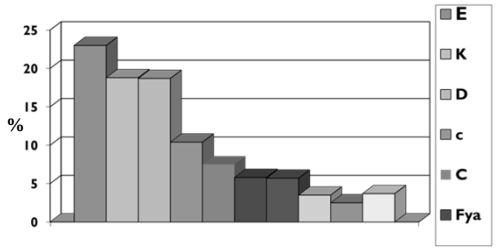
- Resulting anti-D is IgG
- RhD protein present on fetal RBCs by 6 weeks gestation

Anti-D in Pregnancy Prior to RhIG

- **16%** RhD negative, **ABO compatible** women make anti-D
- **<2%** RhD negative, **ABO incompatible** women make anti-D

MATERNAL ALLOIMMUNIZATION: FREQUENCY OF HDFN

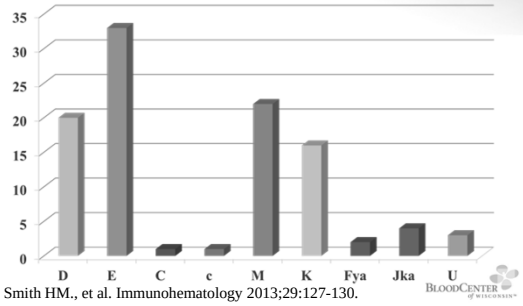
1388 sera from pregnant Dutch women (1999-2001)



Van der Schoot et al. Trans Med Rev 2003;17:31-44.

Maternal Alloimmunization in Large Tertiary-Care Facility

8894 Obstetric Patients in Baltimore (2007-2011)



Smith HM., et al. Immunohematology 2013;29:127-130.

Maternal Alloimmunization Large Tertiary-Care Facility Significance

- 5 infants required antigen negative units within 24 hours of birth
- 8 women required IUT
  - Anti-D was implicated in 7 of 8 cases
- Nearly all cases of HDFN had anti-D + other antibodies

Smith HM., et al. Immunohematology 2013;29:127-130.

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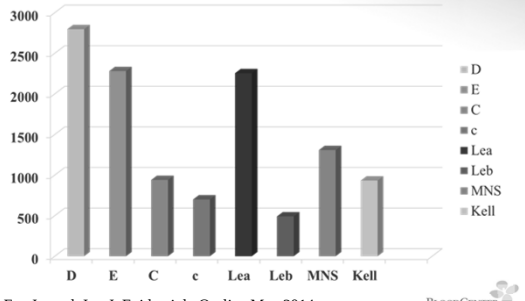
Maternal Alloimmunization in Sweden

1,022,569 Singleton Births in Sweden (1987-2002)



Fan J., et al. Int. J. Epidemiol. On-line May 2014.

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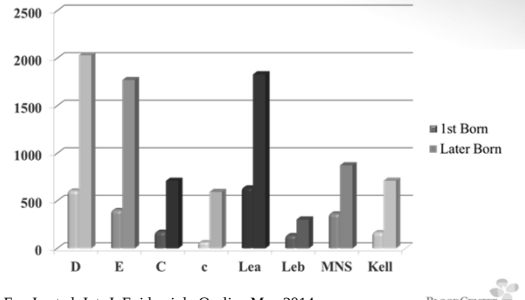
Association of Alloimmunization in First Born & Later Born Singleton Births

1,022,569 Singleton Births in Sweden (1987-2002)



Fan J., et al. Int. J. Epidemiol. On-line May 2014.

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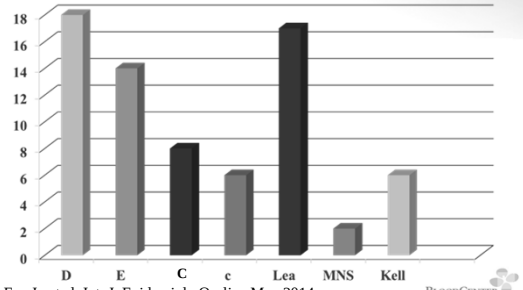
Association of Stillbirth & Alloimmunization

1,022,569 Singleton Births in Sweden (1987-2002)



Fan J., et al. Int. J. Epidemiol. On-line May 2014.

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Anti-D in Partial D

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Anti-D in Partial D

- DNB, DVI and DVII most common in European ancestry

	IS	D IAT	Ct. IAT
Anti-D	0	3	0

- Partial D more common in African ancestry
  - Often type as RhD positive at IS

	IS
Anti-D	3+

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Partial D
Missing Rh"Pieces" Present

Normal RhD antigen

Serological Weak D Phenotype Definition

- Anti-D reagent agglutinates RBCs weakly (≤ 2+) or not at all by test tube method at IS, but agglutinates moderate to strongly (2-4+) when IAT is performed

Serological Weak D Phenotype or Discrepant Results

	Anti-D	Control	Weak D IAT	Control IAT
Lab 1	0/w+	0	w-3+	0
Lab 2	2+			

Consider *RHD* Genotype

Weak D Types 1-3 do not make anti-D

Commentary, Sandler SG et al, Transfusion, in press
It's time to phase-in RHD genotyping for patients with a serological weak D phenotype

Anti-D in Partial D Significance

- Two reports in literature of anti-D causing hydrops fetalis in Partial DVI

Lacey PA, Caskey CR, Werner DJ, Moulds JJ
Transfusion 1983 Mar-Apr;23(2):91-4

M Cannon, R Pierce, EB Taber, J Schucker.
Obstet Gynecol 2003;102: 1143-5

It's significant!!

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Passive Anti-D

SIMPLE, YET COMPLEX

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Rh Immune Globulin (RhIG)

- Anti-D
  - 300ug (1,500 IU) & 50 ug doses
    - Rhogam®
    - Rhophylac®
    - HyperRHO®
    - WinRHO®

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Passive Anti-D Detection

	Rh					MNS				P1	Lewis		KEL		Duffy		Kidd		
	D	C	E	c	e	M	N	S	s	P1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IAT
1	+	+	0	0	+	+	+	+	0	+	0	+	+	+	+	+	+	0	2
2	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	2
3	0	0	0	+	+	+	0	+	+	+	0	+	0	+	0	+	+	+	0

- Increased detection
  - Column Agglutination Testing
  - SPRCA

Passive Anti-D Detection

- ~10% of antenatal dose is present in mom at 40 weeks delivery
  - ½ life of IgG is 25 days
- Factors influencing detection
  - Method used
  - Mom's BMI
  - Amount of FMH

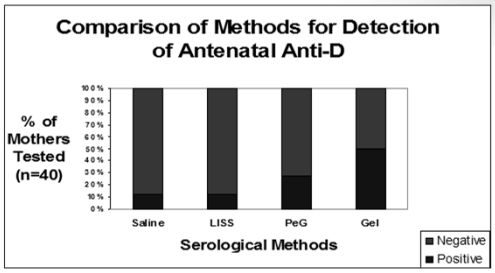
Comparison of Methods for Detection of Antenatal Anti-D

DA Klostermann, KE Puca, EA Scott, ST Johnson, Transfusion, Suppl. 2008

- D-negative mothers at delivery interviewed, those who met these criteria were consented:
  - Received 300 µg RhIG between 26 and 30 weeks gestation
  - no antibody detected on initial prenatal testing
  - singleton pregnancy
  - no complications during pregnancy
  - no additional doses of RhIG received

Comparison of Methods for Detection of Antenatal Anti-D

DA Klostermann, KE Puca, EA Scott, ST Johnson



Gel was able to detect anti-D after longer intervals from time of RhIG injection – ~65% after 70 days

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Comparison of Methods for Detection of Antenatal Anti-D

DA Klostermann, KE Puca, EA Scott, ST Johnson

Table 2. Detection Results by TPV Gain

Gain in TPV (ml)	n	Anti-D Detection Method			
		Saline n (%)	LISS n (%)	PeG® n (%)	Gel n (%)
100-199	1	1 (100%)	1 (100%)	1 (100%)	1 (100%)
200-299	20	3 (15%)	4 (20%)	8 (40%)	12 (60%)
300-399	16	1 (16%)	0	2 (13%)	7 (44%)
400-441	3	0	0	0	0
Total	40	5 (13%)	5 (13%)	11 (28%)	20 (50%)

Anti-D was detected more often in moms with TPV gain of <300 ml, irrespective of antibody detection method used

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Sensitivity of Common RBC Antibody Detection Methods in Detecting Rh-Immunoglobulin

Mikesell KV et al, Transfusion, Suppl. 2014, SP276

- Spiked pooled plasma with 50ng/ml of Rhogam®, Rhophylac®, HyperRHO®, WinRHO®
- Serial 2-fold dilutions
- Tested Echo®, Galileo®, Gel Test, PEG tube
- Echo® detected 5 dilutions (1.57ng/ml)
- Galileo® detected all 6 dilutions*
- Gel & PEG detected 3 or 4 dilutions (3.13 – 6.25ng/ml)

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Passive Anti-D Detection

AABB Technical Manual, 18th ed. 2014

- Titer is rarely >4
- RhIG is completely IgG
- Newly forming anti-D may have IgM component
- Issit suggested waiting 6 months

Antibody titration at birth is not a reliable indicator of passive vs. immune anti-D

Autoanti-D

SIMPLE

Autoanti-D

- Benign autoanti-D preceding alloanti-D
  - Immunized Patient
  - Reimmunized Individual
- Warm Autoimmune Hemolytic Anemia
  - Rare

Anti-D Reagents

COMPLEX

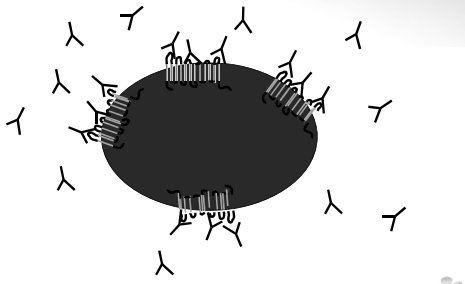
FDA Approved Reagent Anti-D - Tubes

	Anti-D	
Reagent	**IgM**	**IgG**
Gamma-Clone	GAMA401	F8D8
Immucor-4	MS201	MS26
Immucor-5	TH28	MS26
Ortho Bioclone Tube	MAD2	Human polyclonal
Biotest (Bio-Rad) - Blend	BS232	BS221 H41 11B7
Biotest (Bio-Rad)	BS226	
Quotient – Alpha	LDM1	
Quotient – Beta	LDM3	
Quotient – Delta	LDM1 ESD-M	
Quotient – Blend	LDM3	EDS1

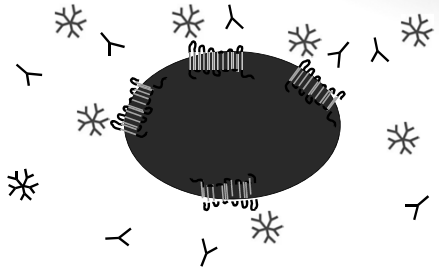
FDA Approved Reagent Anti-D - Other Methods

		Anti-D	
Anti-D	**Method**	**IgM**	**IgG**
Immucor – Series 4	Galileo Echo®/Neo®	MS201	MS26
Immucor– Series 5	Galileo Echo®/Neo®	TH28	MS26
Ortho	Gel/Provue®	MS201	
Diagast PK1	PK7200®/PK7300®	P3X61	
Diagast PK2	PK7200®/PK7300®	HM10	
Diagast (Monoclonal Blend)	PK7200®/PK7300®	P3X61 P3X21223B10	P3X290 P3X35
Erytype® S	Tango®	BS226	
Erytype	Tango®	BS232	
Erytype (Blend)	Tango®	BS221	H411B7
Grifols	DG Gel/Erytra®	P3x61	

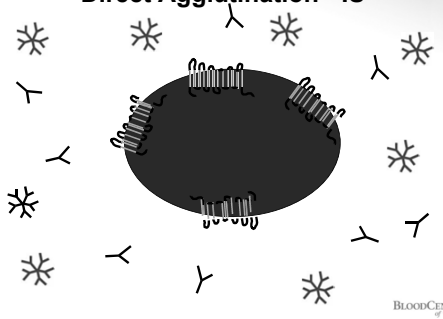
Human IgG Anti-D



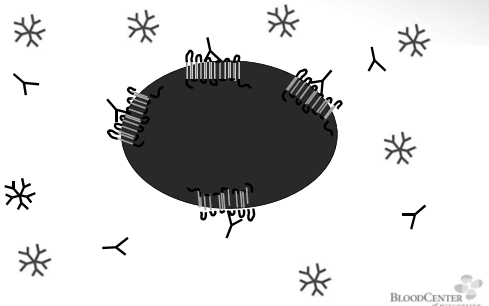
MONOCLONAL IgM/IgG ANTI-D



MONOCLONAL IgM/IgG ANTI-D #1
Direct Agglutination - IS



MONOCLONAL IgM/IgG ANTI-D #1
Weak D Test - IAT



Objectives

- Describe the immunologic response to RhD protein
- Compare & contrast *in-vivo* and *in-vitro* characteristics of alloanti-D, passive anti-D and autoanti-D
  - Discuss the clinical significance of anti-D in transfusion and pregnancy
- Discuss anti-D in Partial D individuals

Thank You

sue.johnson@bcw.edu

Evaluating Antibodies for Clinical Significance

One Blood Group System at a Time: ROUND 3

E. Ann Steiner, MT(ASCP)SBB
Transfusion Technical Specialist
Ortho Clinical Diagnostics

Disclaimer Information:

I am an employee of Ortho Clinical Diagnostics

I have no conflict of interest in regards to the content of this presentation

AABB 2012

What Makes an Antibody Clinically Significant?
George Garratty, PhD, FRCPath

Clinical Decision Making: Red Blood Cell Alloantibodies
Beth Shaz, MD

CLINICALLY SIGNIFICANT ALLO-ANTIBODIES

- *In Vivo*
  - Predict what could occur
  - Analyze what is occurring
- *In Vitro*
  - Pre-transfusion testing – antibody screen, XM, ID panel, et. al.
  - Diagnostic studies – DAT, eluate, et. al.

CLINICALLY SIGNIFICANT ALLO-ANTIBODIES

PreTx to predict what could occur *In Vivo*

- Transfusion reaction:
  - Severe: morbidity/mortality, effects on heart, renal, coagulation system, etc.
  - Moderate: anemia, lethargy, additional transfusions
  - Mild: lab findings only: bili, hgb, etc.?
- No reaction – immunized at next TS

CLINICALLY SIGNIFICANT ALLO-ANTIBODIES

Definition:

- Allo: does not react with autologous rbc's
- Clinical: *In vivo* reactivity
- Significant: Deleterious affect on the patient
  - Varies with patient's underlying condition
  - Could be primary or secondary to rbc destruction, e.g., anemia vs. renal/coagulation
  - Could be immunized and at high(er) future risk
  - Could be indirect: missed diagnosis, delay to scheduled surgery, increased cost of medical care

PATHOGENESIS REVIEW

Characteristics of antibody that affect *in vivo* behavior, i.e., severe reaction vs. no reaction:

- **Ability to activate complement; type of complement present on RBCs**
- **Quantity of RBC-bound IgG/complement**
- **Characteristics and quantity of target RBC antigen(s)**
- **Temperature of reactivity vs temperature of patient**
- **Immunoglobulin class and subclass of IgG**
- **Individual patient factors, e.g., immune system function (meds, diagnosis, age), inflammation, etc.**

CHARACTERISTICS OF PATHOGENESIS APPLIED TO *IN VITRO* TESTING

- **Activate Complement: Fresh serum and Poly AHG**
- **Quantity bound IgG/Complement: Sensitivity**
  - **Time; temperature; enhancements; IAT; reactant ratio**
- **Target RBC Antigen's:**
  - **Phenotype of screening cells**
  - **Age, storage, dosage, etc. of screening cells**
- **Subclass of IgG**
- **Individual patient factors, e.g., immune system function (meds, diagnosis, age), reason for anemia/transfusion, etc.**

AABB 2012

Accessing these presentations:

- **AABB Live Learning Center**
- **Not in attendance:**
  - **Nominal fee required**

Antibodies to P1PK and GLOB blood group system antigens: Important or not?

Jill R. Storry, Ph.D.
Associate Professor
Chair, ISBT Working Party on Red Cell Immunogenetics and Blood Group nomenclature

AABB meeting
27 October 2014
Philadelphia

P1PK and GLOB antigens

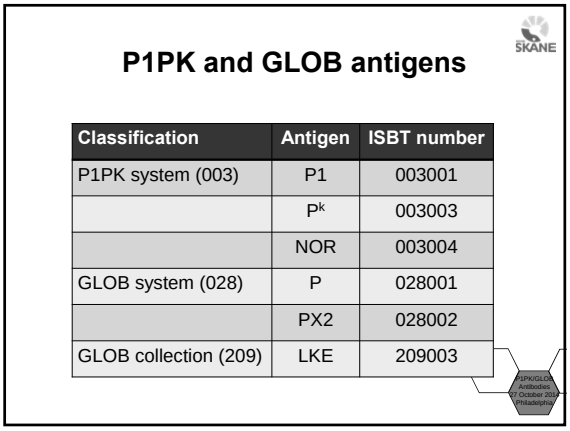
Classification	Antigen	ISBT number
P1PK system (003)	P1	003001
	P^k	003003
	NOR	003004
GLOB system (028)	P	028001
	PX2	028002
GLOB collection (209)	LKE	209003

P1PK and GLOB antigens

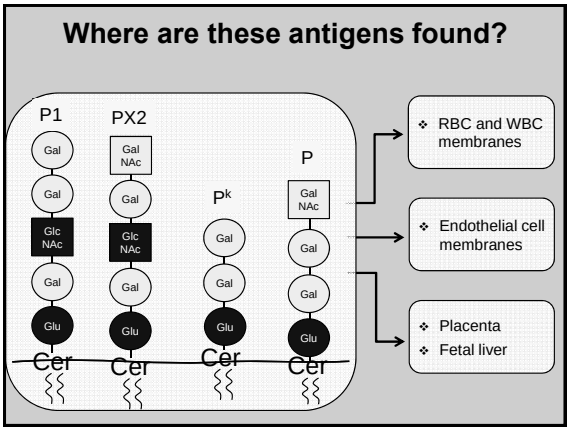
Classification	Antigen	ISBT number
P1PK system (003)	P1	003001
	P^k	003003
	NOR	003004
GLOB system (028)	P	028001
	PX2	028002
GLOB collection (209)	LKE	209003

P1PK and GLOB antigens

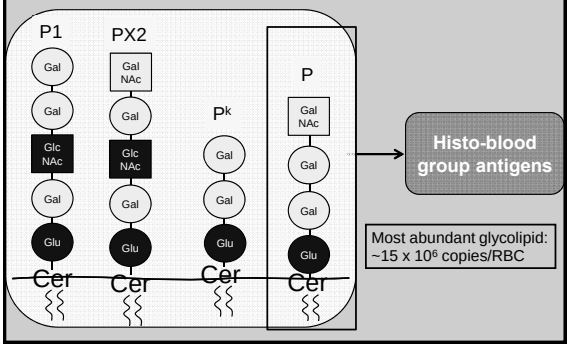
Classification	Antigen	ISBT number
P1PK system (003)	P1	003001
	P^k	003003
	NOR	003004
GLOB system (028)	P	028001
	PX2	028002
GLOB collection (209)	LKE	209003



Where are these antigens found?



Where are these antigens found?



Why are the P1PK/GLOB blood groups and their null phenotypes interesting?

- Involved in a number of medical conditions
  - 5th (childhood) disease
  - Urinary tract infections
  - Intravascular haemolysis due to Donath-Landsteiner antibodies
  - Recurrent spontaneous abortion
- Involved in pathogenesis of many microorganisms
  - Parvovirus B19, HIV
  - Some P-fimbriated *E.coli*
  - Verotoxins (shiga-like toxins)
- Important in transfusion medicine
  - Hemolytic transfusion reactions
  - HDN (?)

Blood group serology

SKÅNE

Phenotype	Prevalence	Antigens	Antibodies
P_1	~80%	P1 P (P^k, PX2)	-
P_2	~20%	P (P^k, PX2)	anti-P1
P_1^k	1-5:10^6	P1 P^k	anti-P, PX2
P_2^k	1-5:10^6	P^k	anti-P1, P, PX2
p	1-5:10^6	-	anti-P, P1, P^k
LKE	98%	LKE	-

"Naturally occuring" antibodies

What stimulates the naturally occuring antibodies?

- Gut bacterial flora have similar sugar structures
- Antibodies are produced to environmental micro-organisms (like anti-A and anti-B)
- Defence mechanism?

Antibody characteristics

Antibody	Antibody class	Preferred temp.	Bind C´	HTR	HDFN	Spontaneous abortion
anti-P1	IgM	RT	Rare	No (Rare)	No	No
Anti-NOR*	IgM	RT	No	ND	ND	No
anti-P	IgM & IgG	RT→37C	Yes	Yes	No to mild	Yes
anti-P, P1, P^k	IgM & IgG	RT→37C	Yes	Yes	No to mild	Yes
Anti-PX2	IgM & IgG	RT→37C	ND	ND	ND	ND
Anti-LKE	IgM & IgG	RT→37C	Yes	Yes (Rare)	No	No

ND = no data; * polyagglutinin

Autoantibodies

- Autoanti-P^k has been described
- Autoanti-P may occur as a biphasic hemolysin in PCH following viral infections in young children
  - Detected by the Donath-Landsteiner test

Other useful serological tips

- All antibodies are greatly enhanced by treatement of tests RBCs with proteases
- Anti-P1 can be neutralised with P1 substance, isolated from pigeon egg-white or hydatid cyst fluid
  - Very specific

Association of anti-P(P1P^k) with spontaneous abortion

"Cytotoxic IgM and IgG3 antibodies directed against P and/or Pk antigens are associated with a higher than normal rate of spontaneous abortion in women with the rare p [Tj(a–)], P1k, and P2k phenotypes."

The Blood Group Antigen Factsbook, 3rd edition.

IMMUNOHEMATOLOGY

Transfusion 2003

Additional molecular bases of the clinically important p blood group phenotype

Åsa Hellberg, Rudi Steffensen, Vered Yahalom, Birgitta Nilsson Sojka, Hans Erik Heier, Cyril Levene, Joyce Poole, and Martin L. Olsson

known to be second cousins. For the remaining 14 donors no known familial connections were revealed. Seventeen of the 29 Swedes are women. Evaluation of their obstetrical history revealed that 11 of them had gone through a total of at least 31 spontaneous abortions (range, 1–7 per woman). Eighteen live births in 9 women were registered (range, 1–4 children per woman) of which 1 was assisted by intense plasmapheresis between pregnancy weeks 5 and 28 following 5 previous spontaneous abortions.

Asso
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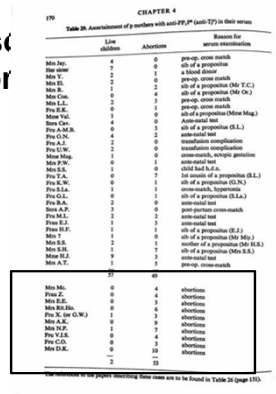

CHAPTER 4

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Table 20. Ascertainment of p-mothers with anti-P1,P^k (anti-Tj^a) in their serum

	Live children	Abortions	Reason for serum examination
Mrs Jez.	4	0	pre-op, cross match
Her sister	7	0	sib of a propositus
Mrs Y.	2	1	a blood donor
Mrs El.	2	0	pre-op, cross match
Mrs B.	1	2	sib of a propositus (Mr T.C.)
Mrs Cou.	0	4	sib of a propositus (Mr Or.)
Mrs L.L.	2	3	pre-op., cross match
Pru E.K.	0	1	pre-op, cross match
Mme Val.	1	0	sib of a propositus (Mme Mag.)
Sters Cav.	4	3	Azoo-natal test
Pru A.M.B.	0	3	sib of a propositus (S.L.)
Pru G.N.	4	2	azoo-natal test
Pru A.J.	2	0	transfusion complication
Pru U.W.	2	0	transfusion complication
Mme Mag.	1	0	cross-match, ectopic gestation
Mrs F.W.	0	1	azoo-natal test
Mrs P.N.	1	0	child had h.d.n.
Pru T.A.	0	7	1st cousin of a propositus (S.L.)
Pru K.W.	0	1	sib of a propositus (G.N.)
Pru S.Le.	1	1	cross-match, hyperemia
Pru G.L.	0	1	sib of a propositus (S.Le.)
Pru B.A.	2	0	azoo-natal test
Sters A.P.	3	0	post-partum cross-match
Pru M.L.	2	2	azoo-natal test
Frau E.J.	1	3	azoo-natal test
Frau H.F.	1	1	sib of a propositus (E.J.)
Mrs ?	1	0	sib of a propositus (Mr Mip.)
Mrs S.S.	2	1	mother of a propositus (Mr H.S.)
Mrs S.H.	1	7	sib of a propositus (Mrs S.S.)
Mme H.J.	9	3	azoo-natal test
Mrs A.T.	1	5	pre-op, cross-match
	77	49	
Mrs Mc.	0	4	abortions
Frau Z.	0	4	abortions
Mrs E.E.	0	3	abortions
Mrs Rit.Ho.	0	6	abortions
Frs X. (or G.W.)	1	3	abortions
Mrs A.K.	0	9	abortions
Mrs N.P.	1	7	abortions
Pru V.H.	0	4	abortions
Pru C.O.	0	3	abortions
Mrs D.K.	0	10	abortions
	—	—	
	2	53	

The references to the papers describing these cases are to be found in Table 26 (page 135).

Race & Sanger, Blood groups in man;5th edition

Is anti-PX2 clinically relevant?

Description of index case

- **Blood group phenotype**
  Patient is A_1B but has globoside deficiency of P_1^k phenotype.
  Found to be homozygous for 811G>A (Hellberg *et al*., J. Biol. Chem., 2002).
- **Known antibodies in plasma**
  As expected a strongly reactive, naturally-occurring anti-P
- **Diseases**
  Dialysis since long time. Now also diagnosed with cancer.
  Palliative transfusions ordered by clinicians.

Description of index case

Transfusion history

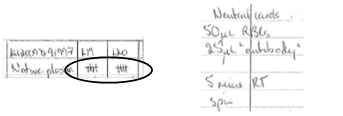
- Previously given several units of group B blood of p phenotype.
- Crossmatch initially negative and blood received apparently uneventfully.
- Another two units requested but now crossmatch positive.
- Common alloantibodies against known polymorphic antigens ruled out.

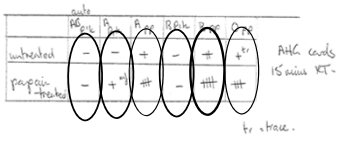
Materials and Methods

- Samples
  - Blood from 8 P^k individuals (7 P_1^k and one P_2^k) of various geographic/ethnic origins.
  - Investigated in the reference laboratories in Paris or Lund.
  - Mutations in their *B3GALNT1* genes confirmed phenotype (Lund)
  - Native and papain-treated test RBCs of multiple donors with the p phenotype and various ABO groups
  - Test RBCs of Bombay phenotype
- Methods
  - Various serological routine assays (gelcards fromDiaMed)
  - Flow cytometry including competitive inhibition and anti-x2 (clone TH2)
  - Thin layer chromatography and ELISA for glycolipid analysis

Results: Serological findings

P_1^k plasma of index case against a panel of test RBCs



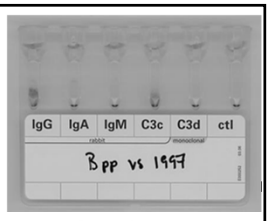


Step 1:
Adsorbing his plasma onto a pool of random group O RBC

Purpose: To adsorb out his anti-P.

1

Grupp O, P+



3

4

	P-	P+	P-	P+	P-	P-	P+	P-	P+	P-
Ab / RBC	AB P_1^k (saliva)	AB	A P_1^k	A	A p	B P_1^k	B	B p	O	O p
RBC origin	European (RW)	random	Israeli	random	Sweden (IN)	Arabic (AA)	random		random	Swedish
A4GALT mutation	-	n.t.	-	n.t.	1487>A	-	n.t.		n.t.	1487>A
B3GALNT1 mutation	811G>A	n.t.		n.t.	-	37_38insA	n.t.	-	n.t.	-

Hemagglutination tests: Extended testing against p RBCs

P_1^k plasma number		1	2	3	4	5	6	7	8
ABO phenotype		O	A	AB	B	O	A	A	A
P^k phenotype		P_1^k	P_1^k	P_1^k	P_2^k	P_1^k	P_1^k	P_1^k	P_1^k
		Reactivity with p RBCs							
Untreated	20°C (n)	0 (1)	4+ (2)	2-3+ (3)	0/1+ (2)	not tested			
Papain-treated	37°C (n)	4+ (1)	3+ (2)	2-4+ (3)	0/3+ (2)	0 (6)	1+ (4)	H (6)	1+ (4)
	20°C (n)					1+ (4)	1+ (5)	2+ (6)	3+(20)
	4°C (n)								

Sera from 8 unrelated P^k individuals were tested against up to 20 native and papain-treated p RBC samples at different temperatures.

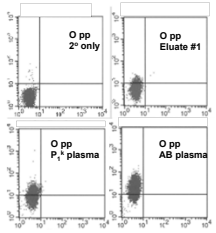
Conclusion: All P^k individuals tested had antibodies reactive with p RBCs

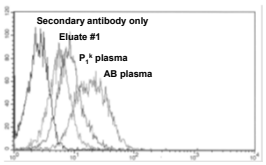
n = number of p RBCs tested is given in brackets after strength of reactivity
H = hemolysis

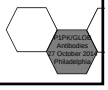
Can antibodies from P_1^k plasma inhibit monoclonal anti-x2 binding?

- Aim: To see if P_1^k plasma or eluate could block the binding of MAb anti-x2
- Therefore, group O pp RBCs were pre-incubated with either P_1^k plasma or an eluate containing the additional antibody specificity.
- Pooled AB plasma was used as a control for unspecific inhibition.
- Following this incubation, the RBCs were washed and then labelled with MAb anti-x2 (TH2) and finally PE-labelled rat anti-mouse Ig kappa as secondary antibody.

Antibodies from P_1^k plasma inhibit monoclonal anti-x2 binding







aaBB Annual Meeting and CTTXPO 2013 Denver
14 October 2013

Identification of the Genetic Basis of PX2, a Recently Reported Glycolipid Blood Group Antigen

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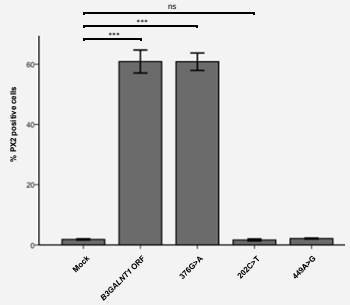
PX2 antigen expression on transfected MEG-01 cells



Conclusions

- These data indicate that the weak/variable crossmatch reactivity observed in tests of Pk plasma with p RBCs is due to the occurrence of a naturally-occurring antibody to an antigen found in elevated amounts on p RBCs.
- x2 glycolipid is known to be elevated on p RBCs
- The index case appears to have boosted antibodies against p RBCs following transfusion

Conclusions

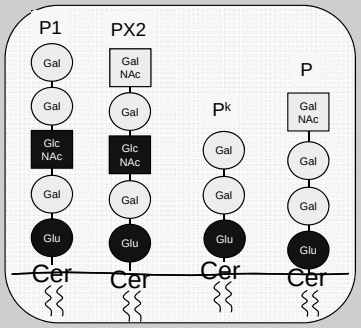
- These data show that ***B3GALNT1*** encodes the glycosyltransferase responsible for **PX2 antigen** synthesis
  - The enzyme is able to add **β1,3GalNAc** onto both paragloboside (PX2) and P^k (P)
  - Naturally-occurring globoside-deficient mutants with P_1^k or P_2^k phenotype lack both P and PX2 antigens on the surface of RBCs
  - All P_1^k and P_2^k individuals tested made both anti-P and anti-PX2
- **P_1^k** or **P_2^k RBC units** should be selected for transfusion to P^k patients
  - p RBCs are typically incompatible with P^k plasma
  - even if the clinical significance for anti-PX2 is not yet known, p RBCs may pose a risk due to their high PX2 level

P1PK and GLOB blood group antigens

Blood group system	P1PK	-	GLOB
Collection	-	GLOB	-
ISBT number	003	209	028
Antigens	P1, Pk	LKE	P
Null phenotype	p	LKE neg	P_1^k, P_2^k
Antibodies related to the null phenotype	Anti-P^k Anti-P1 Anti-P	Anti-LKE	Anti-P (Anti-P1)
Enzyme	α4GalT	α2SialylT	β3GalNacT1
Gene	*A4GALT*	?	*B3GALNT1*
Chromosome	22	?	3
Exons	4	?	5?

PK/GLOB Antibodies 27 October 2014 Philadelphia

Thanks to:



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