AABB Annual Meeting Education Program 2014

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

Presentation Handouts

(9303-TC) Serological Challenges in Perinatal Management

October 27, 2014 ✧ 8:30 AM - 10:00 AM

Advancing Transfusion and Cellular Therapies Worldwide

Event Faculty List

Event Title: **(9303-TC)** Serological Challenges in Perinatal Management
Event Date: October 27, 2014
Event Time: 8:30 AM - 10:00 AM

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Platelet Immunology Laboratory

Diagnostic Services Reference Lab
National Plateletpheresis Donor HLA/HPA Typing Lab

Speaker: Lynnette Beaudin, Charge Technologist

Debra Lane MD FRCPC
Medical Officer CBS Winnipeg

October 2014

Canadian Blood Services
it's in you to give

Human **P**latelet **A**ntigens (HPA)

- To date there are ~ 28 platelet specific alloantigens.
- Grouped as biallelic systems:
  - HPA-1a and -1b
  - HPA-2a and -2b
  - HPA-3a and -3b
  - HPA-4a and -4b
  - HPA-5a and -5b
  - HPA-15a and -15b

Canadian Blood Services
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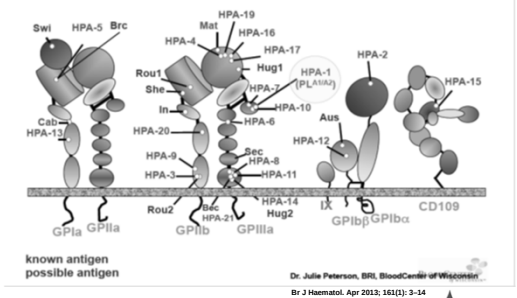
Alloantigens on Platelet Membrane Glycoproteins



Br J Haematol. Apr 2013; 161(1): 3–14

Canadian Blood Services
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Neonatal Allo-Immune Thrombocytopenia (NAIT)

- Maternal antibodies directed against fetal platelet antigen(s) cross the placenta and result in mild to severe fetal / neonatal thrombocytopenia.
- Intracranial Hemorrhage
  - Major cause of mortality and long-term morbidity
    - Blindness
    - Physical and mental disability

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Neonatal Allo-Immune Thrombocytopenia (NAIT)

- It can (and it does) occur in the first pregnancy. Approx. 30% of NAIT cases occur in the first pregnancy.
- Reported incidence of approximately 1/2000 live births.
- High rate of:
  - Re-occurrence in subsequent pregnancies.
  - Intrauterine Fetal Death (14%) and Spontaneous Abortion (14%)

Neonatal Allo-Immune Thrombocytopenia (NAIT)

- Most frequent antigens implicated in NAIT:
  - HPA-1a (Caucasian- 80-90% of cases) found on the GP IIb/**IIIa**.
    - Highly immunogenic
  - HPA-5b (Caucasian-5-15% of cases) found on GP **Ia**/IIa.
  - Remainder are caused by other HPA antibodies. Eg.
  - HPA-3a (Bak^a) (GPIIbIIIa)
  - HPA-15 (Gov) (CD 109)
- HLA role is controversial.

Case# 1
Patient: TW 2002

- 1st Pregnancy
- Full term infant
- Platelet count: 8 x 10^9/L.
- Generalized petechiae.
  - Transferred to NICU
  - Received IVIG
  - Received random platelets
  - April 2002

Platelet Management

- No HPA-1b/1b platelets available
- Easter Weekend
- No testing could be done immediately.
- Direct Donation- Mother donated 1 unit RBC and platelets.
- Platelet released untested Easter Monday with Authorization by Medical Director.

Patient: TW
Laboratory Testing 2002:

Maternal Samples: P02-0024

HPA Typing performed by Flow Cytometry.

Antibody Detection and Identification

- PAK12G Kit
- MAIPA (Monoclonal Antibody Immobilization of Platelet Antigens).
  - Platelet Antibody Detection

Platelet antibody kits and MAIPA detect antibodies against platelet-specific antigens without HLA interference.

Patient: TW
Laboratory Testing 2002:

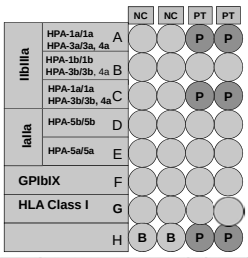
- Paternal Samples: P02-0025:
  - HPA Typing by Flow Cytometry
  - MAIPA Crossmatch with Maternal Serum/Plasma.
    - Platelet Isolation

PAK12G/PAKPlus

- Commercial ELISA Kit.
- Plasma/serum added to microtiter wells coated with glycoproteins.
- Incubation (30 - 35 min).
- Wash step
- Add goat anti-human IgG.(PAKPlus=IgG/A/M conjugate).
- Incubation (30-35min)
- Wash
- Color developer (PNPP)
- Incubation (30 minutes) in the dark.
- Read optical density on spectrophotometer. The intensity of color development is measured and interpreted.

PAK12G-ELISA

Anti-HPA-1a pattern of reactivity



MAIPA

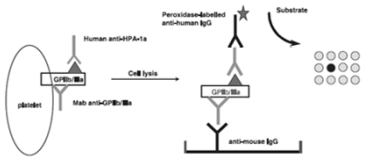
(Monoclonal Antibody Immobilization of Platelet Antigens)

- Labour intensive-In-house method.
  - Reagent preparation
  - Platelet isolation/panel preparation.
- Availability of monoclonal antibodies against various platelet glycoproteins
- Specificity depends on the availability of fully typed platelets.
- Detects antibodies against a platelet glycoprotein, not against a particular antigen: IIbIIIa, IbIX, IaIIa, IV, CD109

MAIPA

(Monoclonal Antibody Immobilization of Platelet Antigens)

Detection of HPA antibodies by MAIPA assay



Vox Sanguinis (2004) 87 (Suppl. 1), S82–S86

MAIPA

Monoclonals Used:

1.Monoclonal IIbIIIa- HPA-1, 3, 4
2.Monoclonal IaIIa- HPA-5
3.Monoclonal IV- CD36
4.Monoclonal IbIX- HPA-2
5.Monoclonal CD109- HPA-15

MAIPA-Platelet Crossmatching

– Mom vs Dad –
  - MoAbs GP IIbIIIa, IaIIa, IbIX, IV, and CD 109 dependent on HPA incompatibilities.
– Maternal serum against paternal platelets
– To detect platelet specific incompatibility.

TW 2002 Lab Summary

- Platelet specific allo-antibody detected by ELISA (PAK kits and MAIPA) methods:
  – Anti-HPA-1a antibody.
- Positive crossmatch against paternal platelets (MAIPA) (Only IIbIIIa).
- Maternal phenotype: HPA-1a neg
- Paternal phenotype: HPA- 1a pos

Manitoba PROGESA

- Both Perinatal and Crossmatch labs are notified of patient's Anti-HPA-1a.
- Transfusion protocols are added to patient's electronic file.
  – Patient must received washed red cells.
  – Patient must receive HPA-1b/1b platelets.

INFANT TW					
DATE	PLATELET COUNTS			# of RD or APLT unit#	COMMENTS/RESULTS
	PRE	POST	AM		
31Mar02	12	34		1 Random	Issued at 0700
31Mar02	15	39		1 Random	Issued at 2234
01Apr02			18		
01Apr02	33	73		1 Directed	Issued at 1647
NAIT TESTING					
08Apr02			30		
10Apr02	No pre	167		HPA-1a neg	1- Plasma reduced HPA-1a neg PLT.
12Apr02			150		
16Apr02			89		
19Apr02			78		
26Apr02			76		

Next Steps

- Medical Director requested HPA-1b/1b platelets to be bled every Thursday in Winnipeg.
- Triggered a National Change within CBS.

National Platelet Donor Registry

Platelet Donor Registry (PDS)

What is it?

- National Donor Database:
  - Apheresis donors across Canada are typed for HLA and HPA-1.
- The PDS system allows us the ability to search for donors that:
  - Match patients' HLA antigens and/or HPA specific antigens.
  - Antigen negative to the patients' antibodies.

PDS- HPA-1b/1b Donors

- CBS goal is to build and maintain a national donor database of HPA-1b/1b donors.
- Availability of HPA-1b/1b platelet units.
- Registry can be utilized for other antibodies implicated in NAIT cases.

Patient: TW 2003

- 2nd Pregnancy
- Managed by high risk neonatology service
- Miscarried at 22 weeks

TW 2004

- 3rd Pregnancy
- Pregnancy managed by high risk neonatology service.
- TW received IVIG starting weekly at approx. 24 weeks.
- C-section delivery on 2004-11-26.
- 2- HPA-1b/1b platelets were on hand for delivery. None were transfused.

TW 2008

- Medical Director gets call from small rural hospital requesting platelets- Aug 2008
- Medical Director recognizes the name.
- Insisted patient be transferred to the city immediately for investigation.
- Came to city and was seen by Hematologist.
- AML Diagnosis

National Registry to the Rescue

- HPA-1b/1b donors were identified quickly due to the registry list.
- 5 doses of HPA-1b/1b platelets were sent from across the country for that weekend.
- TW induction chemotherapy did not have to wait.

Laboratory Testing 2008

TW Test Results:

- Platelet Antibody Detection and Identification
  - PAK12G Kit
- HLA Antibody Detection and Identification
  - LSA Class I Kit.
- HPA Genotyping (repeated by molecular method)
  - HPA 1-6, 15 Typing.

TW- Bone Marrow Transplant

- 13 Nov 2008- Sibling Transplant
- Sibling typed as HPA-1a/1b.
- Successful Transplant
- On 17 Dec 2008 patient TW now types as HPA-1a/1b.
- No longer requires HPA-1b/1b platelets

TW 2008

	ELISA Kit OD Reading	Antibody Specificity	HPA-1 Genotyping
19 Aug 2008	1.442	Anti-HPA- 1a	HPA-1b/1b
10 Sept 2008	1.927		
09 Oct 2008	1.551		
04 Nov 2008	1.588		
Allogeneic Transplant 2008-11-13			
24 Dec 2008	0.124	No antibodies detected	HPA-1a/1b
10 Feb 2009	0.095		
27 Mar 2009	0.217		

Case # 2 MC 2012

- 1st Pregnancy Jan 2010 - Uneventful
  - Pre-term identical twins- 31 weeks gestation.
  - Discharged March 2010
  - Normal platelet count of 218 and 221 at birth and through admission.
  - Follow up on Dec 2011- unremarkable.

Case # 2 MC 2012

- 2nd Pregnancy
- Pre-term identical twins – 36 weeks
- Platelet counts:
  - 29 x 10^9/L Twin A.
  - 25 x 10^9/L Twin B
- No evidence intracranial hemorrhage. No bleeding complications.
- Same father as 1st Pregnancy.

Patient: MC
Laboratory Testing 2012:

<u>Maternal Samples: P12-0008</u>

HPA-1-6, 15 HPA Genotyping

Antibody Detection and Identification

- PAKPlus Kit
- MAIPA (Monoclonal Antibody Immobilization of Platelet Antigens).
  - Platelet Antibody Detection

Patient: MC
Laboratory Testing 2012:

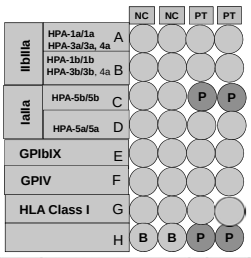
- Paternal Samples: P12-0009
  - HPA-1-6, 15 Genotyping
  - MAIPA Crossmatch with Maternal Serum/Plasma.
    - Platelet Isolation

Patient: MC
Platelet Genotyping Results

	Maternal P12-0008	Paternal P12-0009
HPA-1a	+	+
HPA-1b	-	-
HPA-2a	+	+
HPA-2b	-	+
HPA-3a	+	+
HPA-3b	+	+
HPA-5a	+	+
HPA-5b	-	+
HPA-15a	+	+
HPA-15b	+	-

PAK12G- ELISA

Anti-HPA-5b pattern of reactivity



MC 2012 Lab Summary

- Platelet specific alloantibody detected by ELISA (PAK kit and MAIPA) methods:
  - Anti-HPA-5b antibody.
- Positive crossmatch against paternal platelets (MAIPA) with IaIIa only.
- Maternal genotyping: HPA-5a/5a
  Paternal genotyping: HPA- 5a/5b

INFANTS MC		
DATE	PLATELET COUNTS	
	Twin A	Twin B
30Dec11	29	25
31Dec11 AM	35	31
31Dec11 PM	46	30
01Jan12	52	30
02Jan12	68	31
Last count:	123	95
NAIT TESTING- 2012-01-03		
• Transferred to NICU. • No platelet transfusions. • No IVIG given.		
• Platelet counts recovered. • Discharged Jan 2012		

NAIT Statistics

- Out of 459 NAIT cases investigated reviewed:
  - 78 cases had HPA specific allo-antibodies (17.0%)
    - 59 Anti-HPA-1a (12.8%)
    - 14 Anti-HPA-5b (3.0%)
    - 5 Anti-HPA-1b (1.8%)
  - In 6 cases (1.3%): Maternal sample typed as HPA-1b/1b with no detectable allo-antibody.
- Platelet antibody tests fail to detect antibodies in 15 – 20% of the NAIT cases.

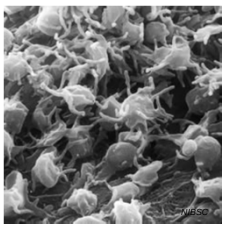
NAIT Diagnosis

Antibody	√	-
Platelet Incompatibility	√	√
Crossmatch	√	-
Clinical Picture	√	√
Diagnosis	NAIT	NAIT

Acknowledgments

- CBS- Platelet Immunology Lab, Winnipeg, Manitoba.
- CBS- Perinatal and Red Cell Serology Labs, Winnipeg, Manitoba.
- Debra Lane MD FRCPC-Medical Officer CBS Winnipeg.

Thank you!



Manitoba Case of Anti-Gerbich in Pregnancy- no HDFN

AABB Annual Meeting – October 2014

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1

JM

- Initial prenatal visit 2013-04-10
- P 6 G 9
- From the far north of Manitoba
- O Positive
- Screen cell 1 4+
  Screen cell 2 4+
- Transfused after last pregnancy

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2

Patient JM - Phenotype

- R1R2 K-

Fy^a+ Fy^b- Jk^a+ JK^b S+ M+N-

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3

JM

- Referred to Canadian Blood Services National Immunohematology Reference Laboratory
- Testing with unlicensed antisera confirmed Ge 2 -, Ge 3 -
- Sent for additional genotyping and sequencing to New York Blood Center

4

JM

- GE / GYPC testing for the presence / absence of exon 3 and genomic sequencing of exons 1, 2, 4

5

JM – Genotyping Results

- GE / GYPC testing for the presence / absence of exon 3 and genomic sequencing of exons 1, 2, 4
- No change in exons 1, 2, 4
- Exon 3 failed to amplify
- Genotype GE .01 – 03 I .01 – 03
  -homozygous for GE deletion exon 3
- Predicted Phenotype
  Ge: -2, -3, 4

6

JM - Siblings

- JM has 14 siblings
- 6 were tested
- 1 compatible (but also pregnant)

7

JM – Follow-up Titres

Date	Titre
2013-08-08	1
2013-08-20	16

- Marked increased titre sent to American Red Cross – Garratty's Laboratory for Monocyte Macrophage Assay (MMA)

8

JM - Monocyte Monolayer Assay (MMA)

- Ge: 3+ and Ge: -2, -3 reagent red cells were sensitized with the patients plasma then tested in MMA
- JM plasma + Ge: 3 rbc
- JM plasma + Ge: -2 -3 rbc

IAT*	MMA**
3 ½ +	7.8%
0	1.2%

*Indirect Antiglobulin Test

**% reactivity of Monocytes

9

MMA

- > 20% MMA suggests severe Hemolytic Disease of the Fetus & Newborn requiring transfusion

AJCP 1989; 92 : 89-92

10

MMA

- Later studies suggest that MMA may not be helpful in predicting HDFN

AJ Perinat 1993; 10 : 428-431

	Predictive Value
MMA > 20%	43%
MMA negative	100%

11

MMA may not be predictive in this case!

- Patient may be exposed to fetal rbc between 2013-08-03 and may change the antibody characteristic
- Serum was not available
- fresh serum added to patient serum may enhance result*
- Weaker results when the sample is greater than a week old

Transfusion 88; 28: 398-399

12

JM – 2013-09-05

- Mom titre 32
  - Hemoglobin 99
  - Directed donation was not pursued
- Partner was also phenotyped
  - O+ D+
  - Ge2+, Ge3+
  - M-, N-, K-, Fy^{b-}
- Fetal Mean Cerebral Artery Flow was median for gestational age

JM – Blood Availability

- One donor in Ottawa
- One donor in Winnipeg(amazing!)
  - Non-native
  - I had deferred him 15 years ago
  - Frequent donation made him iron defiecient
- Collected & frozen
- 1 unit came from the Ottawa group

JM

	anti- Ge 3
2013-09-22	32
2013-10-01	32

- Kell, Fyb, anti-N not ruled out

- Mother refused to come to Winnipeg for the delivery
- Blood kept in Winnipeg
- Baby delivered 2013-10-18 in a Northern Town
  - O Pos
  - DAT weak IgG
  - Hemoglobin 158.0
  - Bilirubin Total 150

Baby

- Not transfused
- DAT IgG 1+
  anti-complement negative
- Units kept in Winnipeg
- Baby confirmed Ge 2, 3 positive by NIRL in Ottawa
- Baby followed 6 weeks
  - Bilirubins decreased
  - No phototherapy or exchange

16

Where in the World is Antigen Diego?- The Challenges of Anti-Di^b

Jackie Naphin MLT
Debra Lane MD FRCPC
2014
AABB-Philadelphia

Canadian Blood Services
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Famous Diegos

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Objectives

- Discuss four cases of anti-Di^b from northern Manitoba Canada
- Compare with literature review (2006) of 27 world wide cases
- Identify challenges of Anti-Di^b in Hemolytic Disease of the Fetus and Newborn
- Suggest possible solutions

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Diego Blood Group

- The gene for Diego found on human chromosome 17
  - 17q21.31
- A single amino acid substitution (Proline for Leucine) at amino acid 854 determines Di^b rather than Di^a
- Consists of a pair of antigens called Di^a/Di^b carried on the Band 3 glycoprotein
- Band 3 is anchored to the membrane by ankyrin and protein 4.2

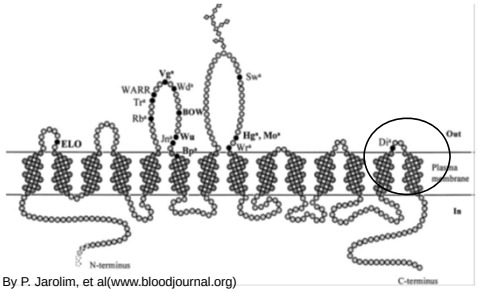
Br J Biomed Sci 1997; 54:16-54
Applied Blood Group Serology,4th edition,1998:581-608

Band 3

- Band 3 glycoprotein is also the Anion Exchanger 1(AE1)
- The AE1 glycoprotein is expressed only in RBCs and in some kidney cells
- Band 3 may be involved in the removal of senescent or defective RBC's and RBC's infected with falciparum

The Blood group Antigen FactsBook , 3rd Edition,Reid

Antigens of the Diego blood group system. Schematic representation of the membrane domain of band 3 based on the structural predictions



By P. Jarolim, et al(www.bloodjournal.org)

- **Di(a-b+)** found in 99.9% Caucasians, Black persons, 90% Asians
- **Di(a+b+)** found in 10% Asians
- **Di(a+b-)** found in 49% Kaingang people-Brazil, Venezuela 14-36%, North American Aboriginal populations 4%
- **Di(a-b-)** found in 1 case only

Reid ME et al The Blood Group Antigen Facts Book Second ed. 2004

Antibodies to Di^a/Di^b

- Anti-Di^a was first described in 1955 by Layrisse (named after the Venezuelan Diego family- 4 generations investigated)[1]
- Chown and Lewis (Winnipeg) concluded that what may be rare in one population may be frequent in another
- No published anti-Di^b cases until 1967 while Thompson was investigating 2 cases of delayed transfusion reaction in Mexican-Indian patients[2]

1. Science 1961; 34:1077-8
2. Vox Sang 1967;13;314-8

Anti-Di^b

- IgG mostly in nature
- Usually formed during pregnancy or post transfusion

Hemolytic Disease of Fetus and Newborn (HDFN) with anti-Di^{b}

- Reports indicate symptoms may vary from case to case ie: no symptoms to severe jaundice, even death
- Cord/Infant samples
  - ✓ positive DAT in most cases
  - ✓ increased bilirubin
  - ✓ decreased hemoglobin

Treatments

- Phototherapy
- IVT
- Exchange Transfusions
- Transfusion
- Use of IVIG therapy
- Any combination of above

Canadian Aboriginal Cases

- All the Mothers in our cases are of Aboriginal descent
- Only 1 case mentioned in the literature review from Canada
- We managed 2 cases in the same year

Patient BK

BK 1996

- Anti-Ec titre performed with cells that were probable Di^b positive – 4096
- No Di^b negative cells available to separate the titres
- Two IVTs performed during pregnancy using divided unit from brother

BK

- Assessment at 32 weeks showed lungs were mature
- Decision to deliver at 34 weeks by CS
- Baby managed with phototherapy and 2 exchange transfusions

Patient - RW

RW 2004

- 5 previous pregnancies
  - 4 live births
  - 1 spontaneous abortion
- 6th pregnancy identified an anti-Di^{b} titre of 64
- Spontaneous abortion occurred

RW-7th Pregnancy

- Live birth anti-Di^{b} titre 128
- Baby's DAT was 3+
- Treated with phototherapy

RW 8th Pregnancy

- Maximal titre was 128
- Two directed units were collected from mother (took six attempts- mother failed to show to donate)
- Baby's DAT 3+
- Treated with phototherapy and exchange at 28 hours
- Directed unit from the mother was used for the exchange

RW- 9th Pregnancy

- Maximum titre of 256
- - IVT performed,
  - no hydrops observed at 34 weeks
  - Mother delivered by C-section at 37 weeks
- Baby was exchanged at 3 hours and treated with phototherapy

Patient AC

AC 2004

- Previous 6 pregnancies resulted in live births and 1 therapeutic abortion
- Patient was transfused in 2001 post surgery

AC Pregnancy 7

- Anti-Di^b titre of 8
  - Anti-K + Anti-S not ruled out
- None of the siblings were compatible
- Di^b negative units imported from US frozen and placed in rare inventory2004
- Baby tested DAT 1+ possible Di^b positive - no antisera to confirm
- Baby did not require treatment

AC Pregnancy 8

- Anti-Di^b titre 8
- Patient induced at 39 weeks due to gestational diabetes
- Baby tested DAT 3+ possible Di^b positive – still no antisera to confirm
- No treatment post delivery

Patient EK

EK Pregnancy 4

- Known Anti-E
- Directed units were collected from Mother during pregnancy
- She lived in neighbouring province, 3 flights away
- Anti-Di^b discovered titre of 8
- Infant tested DAT positive - no treatment required

EK- Pregnancy 5

- Initial anti-Di^b titre was16 but fell to 8
- Units on hand from previous pregnancy and also from patient AC in rare inventory
- Mother wanted to deliver at home but was advised to deliver in Winnipeg
- Infant tested DAT positive – no treatment required
- In hindsight, we should have let her deliver at home to be closer to her children

Literature review

- 27 cases to date in the literature
- Case study and review was performed by Mochizuki which concluded that a maternal high titre of anti-Di^b greater than 64 is associated with an increased risk of severe hyperbilirubinemia
- Our 4 patients confirm this conclusion

Transfusion 2006;Hemolytic disease of the newborn due to anti-Dib: a case study and review of the literature, Mochizuki, K; 46:454-460

TABLE 1. Reported cases of HDN due to Diego^b mismatch

Number	Year reported	First author	Country or ethnicity	Gravida*	Para*	Maternal history of transfusion	Anti-Di^b titer by IAT	Target cell for titration	IgG subclass	Diego blood type or DAT of newborn	Treatment for newborn	Coexistence of other antibody
1	1967	Thompson[1]	Mexican Indian		5	+	64	Di(a–b+)		ND†	NO	
2	1967	Thompson[1]	Mexican Indian		9	–	128	Di(a–b+)		ND	NO	
3	1970	Feller[28]	Colombia	2	2	–	256	Di(a–b+)		Di(a+b+)	NO	
4	1971	Gottlieb[29]	Mexican Indian	4	4	+	ND			Di(a? b+)	PHOTO	Lu^a
5	1971	Nakajima[6]	Japan	2	2	+	256			DAT(+)	ET‡	c, E
6	1976	Ishimori[5]	Japan		2	+	64			Di(a+b+)	ET‡ + BT	c, E, Fy^a, HLA
7	1977	Buchanan[30]	Canadian Indian	2	2	+	16	Di(a–b+)		Di(a+b+)	NO	
8	1978	Nakajima[6]	Japan		2	+	16			DAT(+)	NO	
9	1978	Takagi[7]	Japan	2	1, twin I	+	64			ND	ET‡ + BT	C, e
10	1978	Takagi[7]	Japan	2	1, twin II	+	64			ND	PHOTO + BT	C, e
11	1979	Orlina[26]	Mexican Indian	1	1	+	128	Di(a–b+)		Di(a+b+)	ET‡ + BT	Jk^a
12	1980	Ikemoto[8]	Japan		0, one of twins	ND	1	Di(a–b+)		Di(a? b+)	NOII	
13	1981	Shinomiya[9]	Japan	2	1	+	1024	Di(a–b+)		Di(a+b+)	ET‡ + PHOTO + BT	
14	1981	Uchikawa[10]	Japan	1	1	+	64	Di(a–b+)		Di(a+b+)	ET‡ + PHOTO	
15	1983	Ikeda[11]	Japan	0	0	+	64			Di(a+b+)	ET (1st‡, 2nd§) + PHOTO	
16	1987	Goto[12]	Japan	0	0	+	4096			Di(a+b+)	ET (1st‡, 2nd§) + PHOTO	
17	1990	Yamamoto[19]	Japan	2	1	+	1024	Di(a–b+)	IgG1 + IgG3	Di(a+b+)	ET§$^{\parallel}$ + BT	
18	1991	Habash[31]	Chile	0	0	–	8	Di(a–b+)	IgG3	DAT(+)	NO	
19	1993	Chen[20]	Taiwan	3	2	–	512	Di(a–b+)		DAT(+)	PHOTO	
20	1996	Suda[14]	Japan	2	2	+	64			Di(a+b+)	NO	E
21	2000	Iwamoto[15]	Japan	0	0	+	48			Di(a+b+)	PHOTO + BT	
22	2000	Kiryu[16]	Japan	1		–	8			Di(a+b+)	PHOTO	
23	2003	Donato[18]	South American	2	1	–	512			Di(a? b+)	PHOTO	
24	2003	Win[17]	Bangladesh	Multi	Multi	+	4			DAT(+)	NO	E, c
25	2003	Lenkiewicz[32]	Poland	2	2	+	64	Di(a–b+)	IgG3	Di(a+b+)	NO	
26	2004	Ichikawa[18]	Japan	1	1	–	512§	Di(a–b+)		Di(a? b+)	ET‡ + IVGG + PHOTO	
27		Present case	Japan	1	1	–	128	Di(a–b+)	IgG3	Di(a+b+)	IVGG + PHOTO	M

* Does not include the last pregnancy and delivery.
† ND = not described.
‡ With (proven/probably) mismatched RBCs.
§ With matched RBCs.
‖ Personal communication.

Transfusion 2006;46:454-460

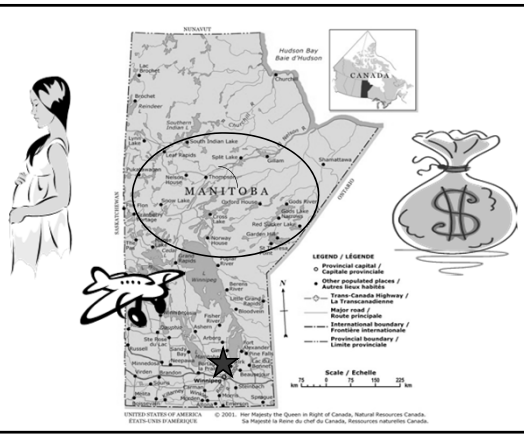
Summary: Mochizuki

- If titres are less than 64, no treatment is required
- Our Aboriginal cases confirm Mochizuki's review

Transfusion 2006;46:454-460

Challenges

- Antibody may go unidentified due to unavailable Di^b negative cells on panels provided by vendors
- Local lab has no access to Anti-Di^b antisera to type units or patients
- Waiting for titre to rise may not give enough time to collect units from the mother
- Difficulty finding rare units



Dream Solutions

- Convince vendors of panel cells to include Di^b negative cells for exclusions
- Licensed antisera
- Funding to transport possible donors

Acknowledgments

- CBS Winnipeg Perinatal Laboratory
- CBS Winnipeg Crossmatch Laboratory
- CBS Ottawa National Immunohematology Reference Lab
- CBS Winnipeg Clinic Staff
- Dr. Debra Lane

HAPPY ENDING

Challenges for the Blood Supplier in Providing Blood for Perinatal Patients with Antibodies to Common Antigens: A Case of Anti-Ge3

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Why Did you Move To Canada?

Objectives

1. Describe reasons for planning for blood availability with pregnancy and delivery in alloimmunized women
2. Detail some important challenges when rare blood is needed for transfusion support
3. Comments on the biology of glycophorin C & D and the Gerbich antigens
4. Present a case of prenatal anti-Ge3 with associated hemolytic disease of the fetus and newborn – highlights points made in 1 & 2

No Disclosures

Canadian Blood Services

- CBS: provides the blood supply in all provinces and territories outside of Quebec
- Supply blood products to over 850 facilities
  - > 800,000 whole blood units collected
  - ~ 78,000 whole blood buffy coat platelets
  - ~ 40,000 apheresis plasma donations
  - ~ 40,000 apheresis platelet donations
- ~ 600,000 donors donate per year

Rationale: Blood On Hand for Alloimmunized Pregnant Women

- Post-Partum Hemorrhage (PPH)
- Hemolytic disease of the fetus and newborn (HDFN)
  - Intrauterine transfusion
  - Exchange transfusion

PPH Requiring Transfusion

Table 4. Transfusion data

Blood products	Number of patients (%)	Median units (range)
Packed red blood cells	104 (100)	3 (1–26)
Fresh frozen plasma	44 (42.3)	4 (1–22)
Platelets	19 (18.2)	5 (1–20)
Cryoprecipitate	10 (9.6)	5.5 (1–15)
Recombinant Factor VII	1 (1)	1

- Retrospective cohort study June 1, 2000 and June 30, 2005 - 33,631 deliveries
  - The overall incidence of primary PPH was 3.2%
  - 10% of PPH patients required RBC transfusion

M. Balki et. al. *J Obstet Gynaecol Can* 2008;30(11):1002–1007

Table 2. Antenatal risk factors for postpartum hemorrhage requiring blood transfusion

Risk factors	n (%)
Previous uterine surgery	22 (21.2)
Antepartum hemorrhage	21 (20.1)
Multiple gestation	18 (17.3)
Macrosomia	17 (16.3)
Abnormal placentation	17 (16.3)
Pregnancy induced hypertension	14 (13.5)
Chorioamnionitis	9 (8.7)
Blood disorders/anticoagulation	8 (7.7)
History of PPH	5 (4.8)

- Risk factors could be identified antenatally in 61%
- 39% could only be identified only intrapartum
  - Uterine atony and retained products of conception
- Uncommon for blood center to be aware of risks

M. Balki et. al. *J Obstet Gynaecol Can* 2008;30(11):1002–1007

Non-ABO, Non-RhD - HDFN

- Presence of clinically relevant maternal red blood cell antibodies other than anti-D is rare
  - Prevalence 0.15 - 1.1% in population studies
- 2-4% severe enough to require intrauterine and/or neonatal exchange transfusions
- Anti-K, anti-c and to a lesser extent other Rh antibodies (C, Cw, E and e) cause the vast majority of cases of severe non-RhD HDFN

J. Koelewijn et. al. BJOG 2009;116:655–664

Can we Predict Severity of HDFN Based on Antibody Specificity?

Table 1. Probability of causing severe HDFN associated with RBC antibodies[2,16,*]

Blood group	Highest likelihood of severe HDFN	Rare cases of severe HDFN	Usually associated with mild disease	Not a cause of HDFN
MNS		M, S, s, U, Mi^a, Vw, Mur, Mt^a, Hut, Hil, M^1, Far, s^D, En^a, MUT	M, S, s, U, Mi^a, Mit	N
Rh	D, c	C, E, f, Ce, C^w, C^x, E^w, G, Hr$_0$, Hr, Rh29, Go^a, Rh32, Be^a, Evans, Tar, Rh42, Sec, JAL, STEM	E, e, f, C^x, D^w, Rh29, Riv, LOCR	
Lutheran			Lu^a (rare), Lu^b	
Kell	K	k, Kp^a, Kp^b, Ku, Js^a, Js^b, UP, K11, K22	Ku, Js^a, K11	K23, K24
Lewis				Le^a, Le^b
Duffy		Fy^a	Fy^b (rare), Fy3 (rare)	
Kidd		Jk^a	Jk^b (rare), Jk3	
Other		Di^a, Wr^a, Rd, Co^a, Co3, PP1P^k Vel, MAM Bi, Kg, JONES, HJK, RITT	Di^b, Co3, Co^b, Ge2 (rare), Ge3, Js^a Lan, At^a, Jr^a JFV, HOFM	P1, Wr^b, Yt^a, Yt^b, Sc1, Sc2, CH/RG, CROM, KN, JMH, I Jr^a HLA: Bg^a, Bg^b, Bg^c

* For some of the antibodies listed, the information is based on a very small number of examples, sometimes only one, resulting in overlap between categories.

AF Eder. *Immunohematology* 2006;22:188–195

PPH and Non-RhD HDFN

- From a blood supplier point of view are an annoying combination of:
  - Rare
  - Hard to predict
  - Possibly very serious
- "Most of the time nothing happens, except the time you fail to prepare"
- Suppliers need to do what they can to make even rare blood available if possible
- While simultaneously limiting wastage of rare resources

Sources of Rare Blood

- Recruit donors for liquid unit
  - 42 day outdate
  - Can draw before due date and will last until after planned delivery date
  - Delay of 36 hours to a week or more depending on donor availability
- Donors need to be identifiable, findable, eligible, available, and willing
- If recruited when not really needed, are not available for 56 days for others

Frozen Red Cells

- Frozen / thawed red cells (at CBS)
  - Stored frozen at <-80C for up to 10 years
  - 1.5 – 2 hours to thaw, then transport time
  - Thawed units stored at 1 – 6 C with 24 hour outdate
  - **On hand so no need to find donor**
- Difficult to use to support delivery
  - No specific date of transfusion
  - 24 hour outdate
  - Additional documentation may be required
  - Delay to reach hospital (thaw and transport time), particularly for more remote sites

The best-laid schemes o' mice an' men

- Canadian Blood Services has an approximately 2% breakage / wastage rate for frozen – thawed red cell units
- Other reports in the literature describe ~2% breakage rate without transport, and ~6% with transport

CR. Valeri, G. Ragno. *Transfusion*. 2005 May;45(5):822-3 J. Lecak, KL. Scott, JP Acker. *Transfusion* 45 (3S): 80A-91A 2005

Did Someone Order the Blood?

- It is common for us to receive requests late in the pregnancy, and have little time to plan for blood
- Some have not receive prenatal care
  - Manitoba health administrative databases
  - Index of prenatal care use was calculated for each singleton live birth from 1991 to 2000 (N = 149,291)
  - Wide variation inadequate prenatal care 1.1% - 21.5%
- Some have received care but need for rare blood not reported to blood supplier

MI Heaman et al. *J Obstet Gynaecol Can*; 2007 Oct;29(10):806-16.

Summary: Challenges of Providing Rare Blood for Prenatal Cases

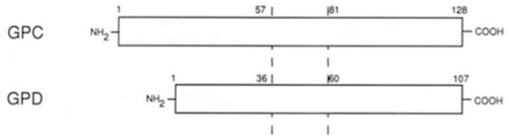
- Clinical scenarios requiring transfusion (PPH, HDFN) are rare, but can be life threatening
  - Don't want to under or over plan
- Do not always have advanced notice of need
- No specific transfusion date – difficult to plan
- Donor recruitment can be difficult
- Frozen blood takes time to thaw, has a short out-date, may require additional communication, can be lost in processing, and is lost if not used

Glycophorin C & D

- Glycophorin C & D are transmembrane red cell sialoglycoproteins
- Significant contributors to the overall negative charge of red cells that prevents aggregation
- Host the Gerbich antigens
- Help maintain normal RBC shape
- Likely function in a form of red cell apotosis

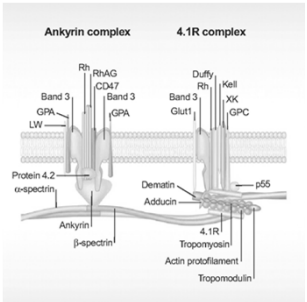
Glocophorin C & D

- GPC and GPD are encoded by a single gene
  - Chromosome 2q14-q21
- GPD contains a truncated amino terminal domain, the remaining polypeptide is identical to that of GPC



JA Chasis and N MohandasBlood, *Blood*. Vol80, No8 (October 15). 1992: pp 1869-1879

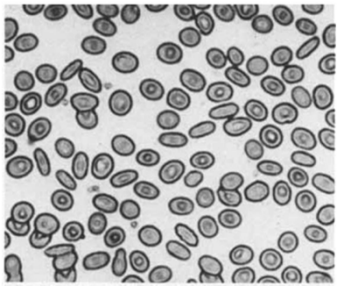
A schematic representation of red cell membrane.



Mohandas N , and Gallagher P G *Blood* 2008;112:3939-3948

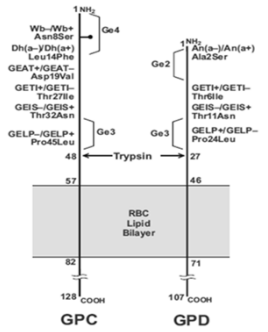
blood

Ge -2,-3,–4 - Elliptocytes



G. L. Daniels et al. *Vox Sang*. 50: 117-121 (1986)

Gerbich Antigens



P.S. Walker, M.E. Reid. *Immunohematology* 2010; 26(2):60-65.

Gerbich Phenotypes and Antibodies

Table 2. Gerbich-negative phenotypes

Traditional phenotype name	ISBT phenotype name	Antibodies	Compatible with
Yus	GE:–2,3,4	Anti-Ge2	GE:–2,3,4, GE:–2–3,4, and GE:–2,–3,–4
Gerbich	GE:–2,–3,4	Anti-Ge3 or anti-Ge2	GE:–2,–3,4 and GE:–2,–3,–4 (if anti-Ge2 then compatible with GE:–2,3,4)
Leach	GE:–2,–3,–4	Anti-Ge4, anti-Ge3, or anti-Ge2	GE:–2,–3,–4 only

P.S. Walker, M.E. Reid. Immunohematology 2010; 26(2):60-65

Anti-Gerbich Antibodies

Gerbich blood group system

Clinical significance of alloanti-Ge2

Transfusion reaction	No to moderate/immediate/delayed
HDN	Positive DAT but no clinical HDN

Clinical significance of alloanti-Ge3

Transfusion reaction	No to moderate, immediate or delayed
HDN	Positive DAT to severe[a]

Reid ME and Lomas-Francis C (2003) The Blood Group Antigen Facts Book, 2nd edn. London: Academic Press.

IMMUNE HEMATOLOGIC DISEASE

Anti-Ge3 causes late-onset hemolytic disease of the newborn: the fourth case in three Hispanic families

- Mothers of Hispanic origin with anti-Ge3
- Healthy neonate born at or near term
  - Otherwise uncomplicated pregnancy
  - Normal hemoglobin and reticulocyte count
  - Positive DAT, anti-Ge3 on eluate
  - Elevated bilirubin requiring phototherapy
- Severe anemia day 10-40 requiring RBC transfusion (difficulty obtaining red cells)
- 2 Treated with IVIG and EPO

LL Pate et al. *Transfusion* 2013;53:2152-21570

Anti-Ge3 and HDFN

- Demonstrated anti-GPC antibody induces cell death
  - Erythroleukemic cell line K562
  - Erythroid progenitors derived from cord blood stem cells
- "This process remains only partially characterized…"
  - Involves the exposure of phosphatidylserine (PS) at the extracellular leaflet of the membrane
- Anti-Ge3 therefore likely induces phosphatidylserine exposure on fetal RBCs and induces their death
  - Different mechanism than anti-D
  - It is therefore important that this pathway is unraveled as it may play a fundamental part in RBC turnover

ND Avent. *Transfusion* 2013;53:2111

D Wang et al. *Transfusion* 2013;53:2134-2140

Anti-Ge3 Prenatal Case

- 27 y/o group A, G3P2 Hispanic female
  - 1st child normal pregnancy and delivery
  - 2nd child neonatal jaundice not requiring therapy
- 3rd pregnancy no prenatal care until 32-weeks
- Our reference lab received a sample from this visit with a difficult to characterize antibody
  - Reactive (4+) by saline or PEG IAT with all RBCs tested
  - Auto-control negative
  - Non-reactive with Ge:–2,–3 and Ge:–2,–3,–4 RBCs by saline and PEG-IAT
  - Reacted with Ge:–2,3 RBCs
  - Consistent with anti-Ge3.

Ge Genotype Results

- Genomic DNA analysis revealed a deletion of GE exon 3
- Sequencing of exons 2 & 4 found no changes
- Consistent with her serologically determined Ge:–2,–3, 4 phenoype
- Deletion of exon 3 results in the Gerbich (Ge-2,-3,4) phenotype

P.S. Walker, M.E. Reid.
Immunohematology 2010; 26(2):60-65.

Anti-Ge3 Prenatal Case

- NIRL contacted blood center with new anti-Ge3 antibody on a prenatal sample
  - Hospital did not request blood, IRL waited for final
- Started working with hospital to plan for blood, but mom delivered before plans completed
- Uncomplicated delivery at 38 weeks
- Baby was blood group A+, 3.8-kg healthy male
  - Hemoglobin 148g/L (14.8 g/dL)
  - Total bilirubin 66µmol/L (3.9 mg/dL)
  - Positive Direct Antiglobulin Test

Anti-Ge3 Prenatal Case

- Hemoglobin dropped from 148 to 133 g/L (14.8 to 13.3 g/dL), and
- Total bilirubin rose from 66 to 89 µmol/L (3.9 to 5.2 mg/dL) in a few hours
- Neonatal bilirubin normal range:
  - 1 day old: Less than 103 µmol/L or 6.0 mg/dL
  - 2 day old: Less than 170 µmol/L or 10.0 mg/dL
  - Initiate phototherapy: 257 µmol/L or 15.0 mg/dL
- Due to anti-Ge antibody, decided to treat

ML Porter, BL Dennis. *Am Fam Physician.* 2002 Feb 15;65(4):599-607.

Anti-Ge3 Prenatal Case

- Phototherapy, IV fluids, and IVIG 1g/kg X 1 dose
- Total bilirubin peaked at 273µmol/L (16 mg/dL) on day 4
- D/C day 6 with hemoglobin 90g/L (9.0g/dL)
- Readmit day 10 with hemoglobin 57g/L (5.7g/dL)
  - Absolute retic count 64 x10^9/L (Normal)
  - Total bilirubin 59µmol/L (3.6 mg/dL) (Normal)
- DAT (+), antibody screen (+)
- Ge3- blood was requested for urgent transfusion

http://en.wikipedia.org/wiki/Canada
Image in Public Domain

Anti-Ge3 Prenatal Case

- No liquid units available
- Frozen units in Ottawa and Winnipeg
  - Units all from donors with recent donations
- Decided to bring in 1 unit from Ottawa and thaw it for transfusion in the morning
- Make plans for mom to donate
- The unit arrived safely but…
- Technical failure with thaw procedure, unit lost

Anti-Ge3 Prenatal Case

- Many flights per day between Toronto and Ottawa
- Brought in another unit, thawed successfully same day
- Compatible thawed Ge:–2,–3,4 RBCs were transfused to the patient uneventfully
- Hemoglobin increased to 121g/L (12.1d/dL)

Anti-Ge3 Prenatal Case

- Over several weeks his hemoglobin dropped slowly
  - Total bilirubin did not rise
  - Reticulocyte count remained inappropriately low
- At 6 weeks his hemoglobin was 104g/L (10.4g/dL)
  - But reticulocyte count was 171x 10^9/L (elevated)
- No further transfusions required
- Patient was clinically well throughout
- Likely another case of severe HDFN from anti-Ge3

Conclusions: Prenatal Red Cell Support

- Planning for red cell transfusion support for moms with rare antibodies is complex
  - Lack of a set transfusion date makes planning difficult, and liquid units may not be available
  - Frozen red cells have a short outdate, delay transfusion related to transport and thaw, and can break
  - Early and frequent communication between the hospital, the IRL, and the blood supplier is critical

Conclusions: Gerbich

- Gerbich antigens are on GPC and GPD
  - GPC is important in maintaining normal red cell morphology, and lack of GPC is associated with HE
  - Anti-GPC antibodies involved in a form of red cell apotosis
- Anti-Ge antibodies are clinically significant
  - Anti-Ge2 and Ge3 have been associated with HTRs
  - Anti-Ge2 is associated with + DAT but not HDFN
  - Anti-Ge3 can be associated with HDFN with late onset anemia out of proportion to hyperbilirubinemia