Hemolytic Transfusion Reactions
ABO Blood Group System

October 16, 2006

Steven L. Spitalnik, M.D.

Learning Objectives

0. What is the difference between serum and plasma?

1. What are the carbohydrate differences between the A, B, and H blood group antigens?
2. What class of enzyme is responsible for synthesizing the ABH antigens?
3. Understanding the structure and composition of RBCs, the structure of IgM antibodies, and the function of the complement system, what changes in urine and blood would you expect to see due to a hemolytic transfusion reaction resulting from ABO incompatibility?

Holy Grail of Transfusion Medicine

Manipulate the composition of blood:

With complete control

Without adverse consequences

Transfusion Medicine

Transfusion of "products":
RBC, Plt, WBC, PBSC, FFP

Infusion of recombinant proteins:
FVIII, FVIIa, ATIII

Prescription of "drugs":
Epo, G-CSF, GM-CSF

Removal of "evil humors":
Apheresis of cells and solutes

Holy Grail of RBC Transfusion Therapy (corollary)

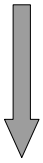
Transfuse any unit of RBC into any recipient:

With perfect acquisition of the desired effect:
- Normalizing Hct
- Diminishing Hgb SS levels
- Improving O2 delivery

Without adverse consequences:
- Transfusion transmitted diseases (e.g. HIV)
- Transfusion reactions
- Missing the therapeutic target
- Volume overload

Hemolytic Transfusion Reactions

Incompatible transfusion



DIC, renal dysfunction, shock, death

Landsteiner Experiment 1900

Mix serum and RBC from random individuals
Incubate at RT
Observe for RBC agglutination

Blood group	RBC	Serum
A	**A**	**anti-B**
B	**B**	**anti-A**
AB	**AB**	**"none"**
O	**O**	**anti-A, anti-B**

Modern interpretation: "All" humans have "naturally-occurring" IgM antibodies to the carbohydrate ABO antigens they lack

Landsteiner Experiment 1900

Why do we care?
- ABO incompatible RBC → death
- *ABO incompatible xplant → hyperacute rejection*

We go to extraordinary lengths to prevent this:
- Every donor and donor unit it ABO typed every time
- Every recipient is ABO typed every time
- The front and back type must agree
- Lots of barriers and requirements from phlebotomy to transfusion

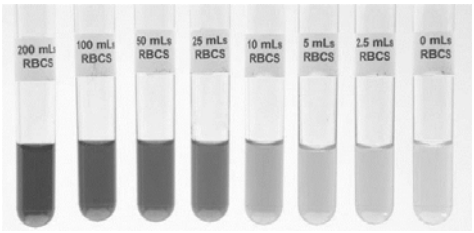
Landsteiner Experiment 1900

Why do we care?
- ABO incompatible RBC → death
- *ABO incompatible xplant → hyperacute rejection*

We go to extraordinary lengths to prevent this:
- Every donor and donor unit it ABO typed every time
- Every recipient is ABO typed every time
- The front and back type must agree
- Lots of barriers and requirements from phlebotomy to transfusion

Still we have problems

Hemolytic Transfusion Reactions

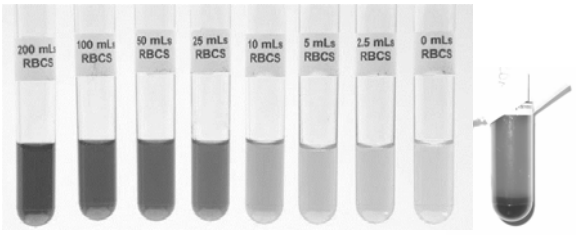


Elliott et al. Visualizing the hemolytic transfusion reaction. Transfusion 43: 297, 2003.

Hemolytic Transfusion Reaction

On 10/8/04 two teenage boys, each with sickle cell disease, were each receiving RBC exchange transfusions in the therapeutic apheresis unit. One patient was B+ and one was A+. All serological testing was done correctly and the correct units were released from the Blood Bank. The nurse mistakenly began transfusing one unit of B+ RBC into the A+ 15 year old patient. Virtually immediately he began having symptoms of a sickle cell crisis (severe headache, chest pain, palpitations, mild respiratory distress). The nurse recognized that the patient was having a reaction to the transfusion, stopped the transfusion, and immediately contacted the pathology resident and attending. Fluids, solumedrol, benedryl, and lasix were administered.

Hemolytic Transfusion Reactions



Hemolytic Transfusion Reactions

Hemolytic Transfusion Reactions

Incompatible transfusion

↓

DIC, renal dysfunction, shock, death

Similar to sepsis or "cytokine storm"

Hemolytic Transfusion Reactions

Acute HTRs

Delayed HTRs

Hemolytic Transfusion Reactions

IgM-mediated
- Acute
- ABO
- Clinical course: severe, significant mortality
- Malpractice

IgG-mediated
- Delayed (or acute)
- Rh
- Clinical course: mild-severe, low mortality
- Adverse outcome

Hemolytic Transfusion Reactions

Acute HTRs
- ~14 x 10^6 RBC transfused/year in USA
- ~1000 clinically significant ABO incompatible transfusions
- ~10 deaths in US from ABO HTRs
- Risk of death: ~1/10^6 per transfusion

Hemolytic Transfusion Reactions

TABLE 1. Frequency of erroneous administration of RBCs in New York State, 1990 through 1999*

	Number	Frequency
ABO-incompatible	237	1/38,000
ABO-compatible	221	1/41,000
Total†	462	1/19,000
Adjusted total‡	659	1/14,000
Fatal reaction	5	1/1,800,000

* 9,000,000 transfusions were performed during this period.
† Includes 4 cases in which ABO compatibility was not reported.
‡ Adjusted to correct for estimated underreported/undetected ABO-compatible erroneous transfusions. A compatible-to-in-compatible ratio of 1.78 was used.

Linden et al. Transfusion errors in New York State: an analysis of 10 years' experience. Transfusion 40:1207-1213, 2000.

Hemolytic Transfusion Reactions

TABLE 2. Outcomes after receipt of ABO-incompatible RBCs in New York State, 1990 through 1999

Outcome	Number	Percentage
No adverse effect	111	47
Acute hemolytic reaction		
Symptomatic*	96	41
Laboratory only	16	7
Fatal	5	2
Low-grade fever only	1	0.4
Death due to underlying condition	8	3
Total	237	100

* Nonfatal.

Linden et al. Transfusion errors in New York State: an analysis of 10 years' experience. Transfusion 40:1207-1213, 2000.

Hemolytic Transfusion Reactions

TABLE 3. Sources of transfusion-associated errors in New York State, 1990 through 1999

Nature of error	Number (%)	Number (%)
Non-blood bank error alone		259 (56)
Identification error	171 (37)	
Phlebotomy error	62 (13)	
Incorrect order sent	22 (5)	
Other	4 (1)	
Blood bank error alone		135 (29)
Tested wrong sample	39 (8)	
Testing error, technical	34 (7)	
Wrong unit issued	17 (4)	
Testing error, clerical/transcription	16 (3)	
Wrong unit tagged	14 (3)	
Clerical error, recorded on wrong slip	11 (2)	
Other	4 (1)	
Compound error		67 (15)
Wrong unit issued, identification error	48 (10)	
Wrong unit tagged, not detected	6 (1)	
Other	13 (3)	
Could not be determined*	1 (0.2)	1 (0.2)
Total		462 (100)

* Change in blood type. Could not be determined whether blood bank or phlebotomy error.

Linden et al. Transfusion errors in New York State: an analysis of 10 years' experience. Transfusion 40:1207-1213, 2000.

Hemolytic Transfusion Reactions

TABLE 4. Method of discovery* of transfusion-associated errors in New York State, 1990 through 1999

Method of discovery	Number (%)
As a result of reaction	90 (28)
At bedside	66 (21)
Subsequent blood request	68 (22)
Supervisory review	17 (5)
Other	75 (24)
Total	316 (100)

* Where known or reported, RBC-containing components only.

Linden et al. Transfusion errors in New York State: an analysis of 10 years' experience. Transfusion 40:1207-1213, 2000.

Hemolytic Transfusion Reactions

RBC + IgM

↓

Complement activation

↓

Intravascular hemolysis

↓

Shock, renal failure, death

Hemolytic Transfusion Reactions

RBC + IgM

↓

Complement activation

↓

Intravascular hemolysis

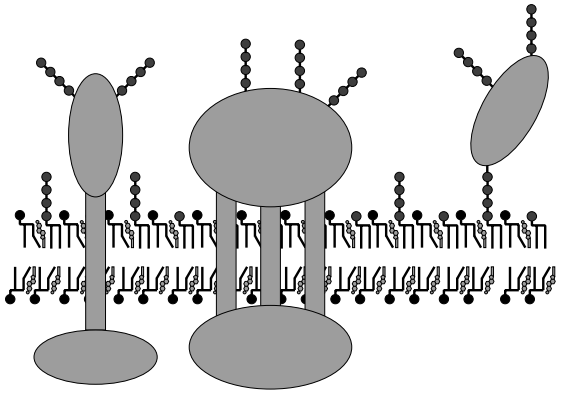
↓ "Magic happens"

Shock, renal failure, death

Red Blood Cells (RBC): Basic stuff

Biconcave disk
Membrane structure
Cytoplasm: Hgb, LDH, K
No internal membranes
No nucleus
No RNA
No synthetic capacity
Terminally differentiated

RBC MEMBRANE



CONSTITUENTS OF THE RBC MEMBRANE

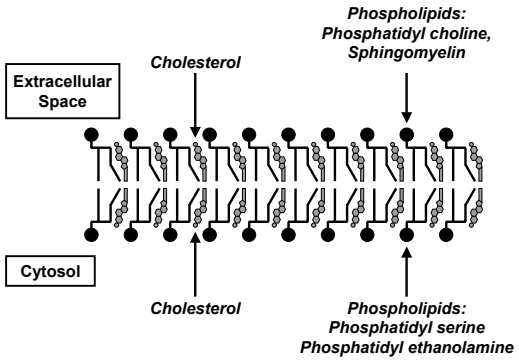
Lipid bilayer:
- **phospholipids, cholesterol**

Glycosphingolipids

Proteins:
- **Transmembrane proteins (RhD)**
- **Transmembrane glycoproteins:**
  - **Single span (Glycophorin A)**
  - **Multispan (Band 3)**
  - **GPI-anchored (DAF)**

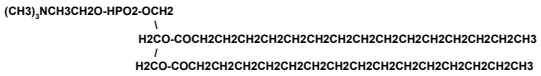
LIPID BILAYER (PHOSPHOLIPIDS)

STRUCTURE OF THE LIPID BILAYER

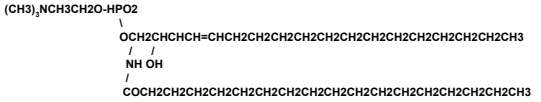


STRUCTURES OF PHOSPHOLIPIDS

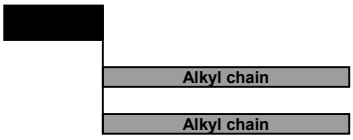
Phosphatidylcholine



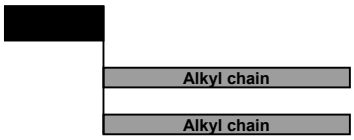
Sphingomyelin



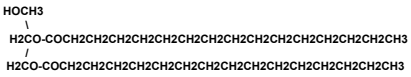
STRUCTURES OF PHOSPHOLIPIDS



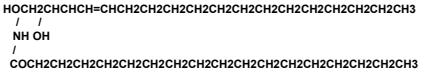
STRUCTURES OF PHOSPHOLIPIDS
Lipid tails



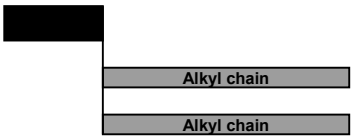
Diacylglyceride

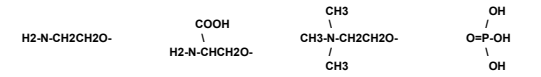


Ceramide



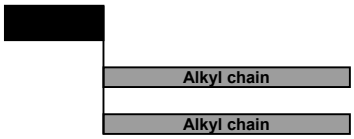
STRUCTURES OF PHOSPHOLIPIDS
Head groups





Ethanolamine **Serine** **Choline** **Phosphate**

STRUCTURES OF PHOSPHOLIPIDS



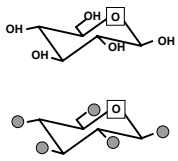
Choline + **Phosphate** + **Diacylglyceride** = **Phosphatidylcholine**

Choline + **Phosphate** + **Ceramide** = **Sphingomyelin**

GLYCOSPHINGOLIPIDS (GLYCOLIPIDS)

MONOSACCHARIDE STRUCTURE

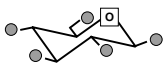
Glucose = Glc



Numbering
Axial vs. equatorial
Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

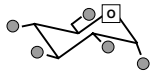
β-Glc



Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

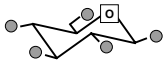
α-Glc



Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

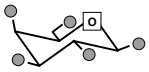
β-Glc



Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

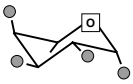
β-Gal



Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

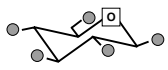
L-α-Fuc



Fucose = 6-deoxy-L-Gal

MONOSACCHARIDE STRUCTURE

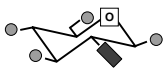
β-Glc



Amino sugars
N-acetyl-glucosamine = GlcNAc
N-acetyl = CH3CONH-

MONOSACCHARIDE STRUCTURE

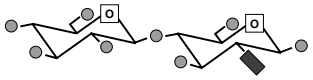
β-GlcNAc



Amino sugars
N-acetyl-glucosamine = GlcNAc
N-acetyl = CH3CONH- = ▬

CARBOHYDRATE STRUCTURES

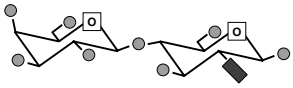
Disaccharides



Glc(β1-4)GlcNAc

CARBOHYDRATE STRUCTURES

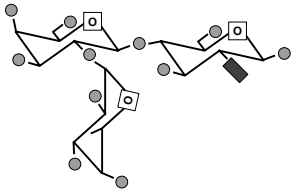
Type 2 Chain



Gal(β1-4)GlcNAc

CARBOHYDRATE STRUCTURES

Type 2 H



Fuc(α1-2)Gal(β1-4)GlcNAc

A

GalNAc(α1-3)
****
Gal(β1-4)GlcNAc-R
/
Fuc(α1-2)

B

Gal(α1-3)
****
Gal(β1-4)GlcNAc-R
/
Fuc(α1-2)

H

Gal(β1-4)GlcNAc-R
/
Fuc(α1-2)

GLYCOCONJUGATE BIOSYNTHESIS
Glycosidic bonds
Glycosyltransferase

Nucleotide-sugar + Acceptor → Glycosylated acceptor + Nucleotide

UDP-GalNAc	Carbohydrate	Oligosaccharide	UDP
GDP-Fuc	Lipid	Glycolipid	GDP
CMP-sialic acid	Protein	Glycoprotein	CMP
	Other	Other glycan	

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

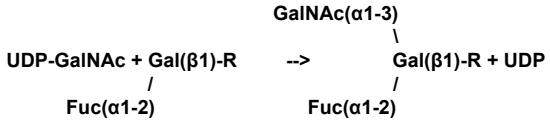
Cer	Ceramide
UDP-Glc ↓ *Glucosylceramide synthase*	
Glc(β1-1')Cer	Glucosylceramide
UDP-Gal ↓ *Lactosylceramide synthase*	
Gal(β1-4)Glc(β1-1')Cer	Lactosylceramide
UDP-GlcNAc ↓ *Lacto (β1-3)GlcNAc transferase*	
GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer	Lacto-N-triosyl ceramide
UDP-Gal ↓ *Neolacto (β1-4)Gal transferase*	
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer	Paragloboside
GDP-Fuc ↓ *FUT1; H Type 2 transferase*	
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer	H Type 2
Fuc(α1-2) /	
UDP-GalNAc ↓ *A transferase*	
GalNAc(α1-3) \	
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer	A Type 2
Fuc(α1-2) /	

CHARACTERISTICS OF THE A AND B TRANSFERASES

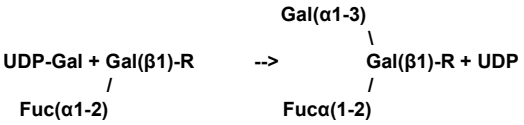
- 354 amino acids
- Type II membrane glycoprotein
- Golgi localization
- A and B transferases are highly homologous
- Require Mn+2 for enzymatic activity
- GT6 family of glycosyltransferases (CAZy): http://afmb.cnrs-mrs.fr/CAZY/
- 7 coding exons
- Chromosome 9 q34

CHARACTERISTICS OF THE A AND B TRANSFERASES

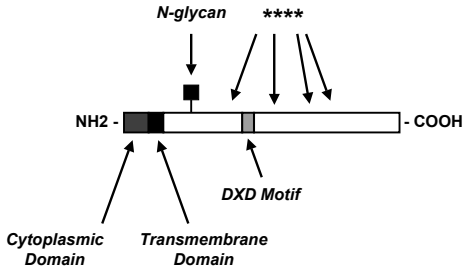
A: (α1-3) GalNAc-transferase (EC 2.4.1.40)



B: (α1-3) Gal-transferase (EC 2.4.1.37)



STRUCTURE OF THE A AND B TRANSFERASES



Yamamoto et al. Nature 345:229, 1990

STRUCTURE OF THE A AND B TRANSFERASES

Four Critical Residues

Transferase	Amino acid number			
	176	235	266	268
A	R	G	L	G
B	G	S	M	A
"AABB"	R	G	M	A

Yamamoto et al. J Biol Chem 265:19257, 1990

STRUCTURE OF THE A AND B TRANSFERASES

Four Critical Residues

Transferase "genotype"	Transferase "phenotype"
AAAA	A
AAAB	A
AABA	AB
AABB	B
BBAA	A
BBBB	B

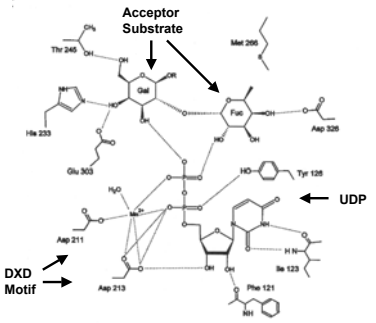
Conclusion: The last two critical residues (aa 266 and 268) are very important in determining specificity

Yamamoto et al. J Biol Chem 265:19257, 1990

CRYSTAL STRUCTURE OF THE B TRANSFERASE

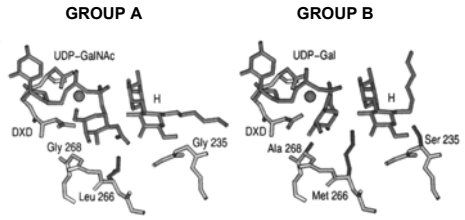
Patenaude et al. Nature Struct Biol 9:685, 2002

ACTIVE SITE OF THE B TRANSFERASE



Patenaude et al. Nature Struct Biol 9:685, 2002

ACTIVE SITES OF THE A AND B TRANSFERASES



Patenaude et al. Nature Struct Biol 9:685, 2002

Hemolytic Transfusion Reactions

RBC + IgM

↓

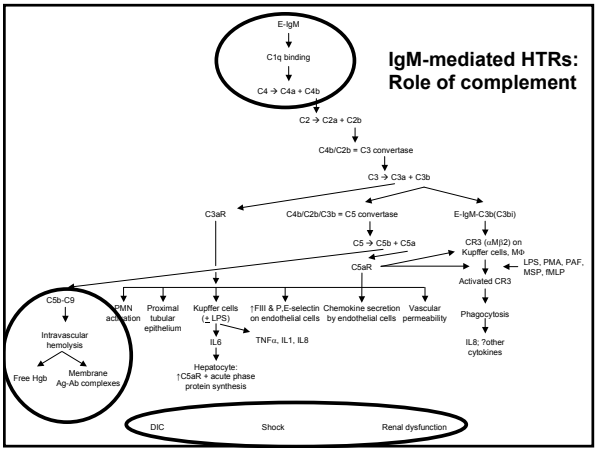
Complement activation

↓

Intravascular hemolysis

↓ "Magic happens"

Shock, renal failure, death



Initiating Event

Ag-Ab interaction

Final Common Pathways

Renal dysfunction
DIC
Shock
Death

Proximal Consequences

Complement activation
RBC lysis
Hemoglobinemia
Fc receptors
Complement receptors
Phagocytosis
SIRPα – CD47 interaction
Cytokine release
Acute phase reaction
Coagulation cascade
Δ's in renal blood flow
NO function

Modifiers

Acute or chronic illness
DAF, CD59, etc.
Complement levels
Renal disease
Hypoperfusion
Hypoxia (systemic or local)
Haptoglobin levels
SS Hgb disease
Bystander hemolysis
Gender
Cytokine, complement, and FcγR polymorphisms

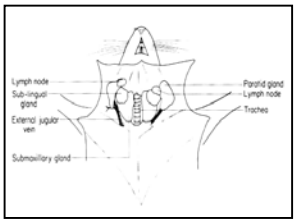
Hemolytic Transfusion Reactions

Current treatment:

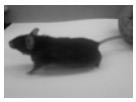
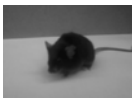
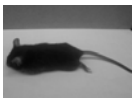
- Prevention
- Steroids, fluid, mannitol, IVIg
- Flagellation (self and other)
- Prayer

Mouse model: methods

- Passively immunize with Mab
- Anesthetize; expose jugulars
- Infuse compatible or incompatible mRBC
- 1 minute later, withdraw aliquot from opposite jugular vein (t=0 h)
- 1-24 h later, exsanguinate and sacrifice (t=1-24 h)
- Determine %RBC survival
- Perform autopsy



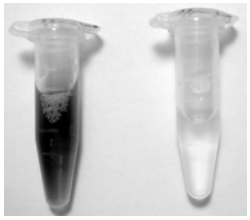
Transfusion of 1-2 "units" of incompatible mRBC into passively immunized mice results in physical illness 24 hr post-transfusion

Not immunized, Antigen(+) RBC "Compatible"	Immunized, Antigen(-) RBC "Compatible"	Immunized, Antigen(+) RBC "Incompatible"
		
Normal; sleek fur; lively movements	Still, but arousable; mildly hunched; mild piloerection	Lying on stomach; back legs splayed; panting; unable to move
~100% RBC survival	~100% RBC survival	~8% RBC survival

Transfusion of 1-2 "units" of incompatible hGPA-Tg mRBC into immunized mice is fatal

Immunization	RBC	Mouse survival (at 24 hr)
Anti-hGPA	WT	5/5
None	hGPA-Tg	5/5
Anti-hGPA	hGPA-Tg	0/5
Anti-HEL	hGPA-Tg	5/5

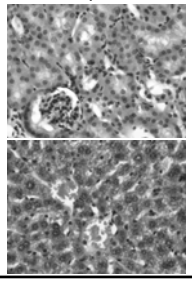
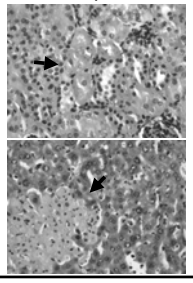
Passive immunization with an IgM anti-hGPA Mab, leads to intravascular hemolysis of transfused incompatible mRBC with gross hemoglobinuria

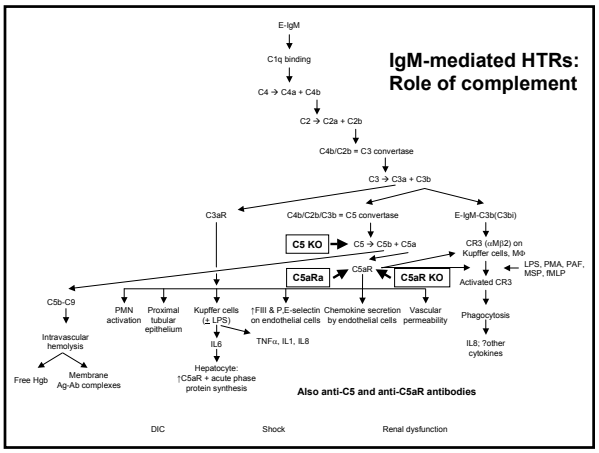


Transfusion of 1-2 "units" of incompatible mRBC into passively immunized mice results in "cytokine storm" 24 hr post-transfusion

	Cytokines (24 hr post-transfusion)				
	TNFα	IFNγ	MCP-1	IL10	IL6
Not immunized, hGPA-Tg RBC "Compatible"	0	0	165	0	383
Immunized, WT RBC "Compatible"	17 24	3 3	441 798	0 0	209 269
Immunized, hGPA-Tg RBC "Incompatible"	301 171	219 130	>5000 >5000	1621 359	>5000 >5000

Transfusion of 1-2 "units" of incompatible mRBC into passively immunized mice results in renal and liver pathology 24 hr post-transfusion

Immunized, WT RBC "Compatible"	Immunized, hGPA-Tg RBC "Incompatible"
	



Hemolytic Transfusion Reactions

Potential future treatment options:

- Etanercept (Enbrel): soluble TNFα receptor
- Infliximab (Remicade): anti-TNFα
- Anakinra (Kineret): recombinant IL1ra
- Eculizumab: anti-C5
- Activated Protein C
- Other complement inhibitors
- Other inhibitors of the inflammatory response
- etc.

Hemolytic Transfusion Reactions
Summary

How the patient presents:

fever and chills, hemoglobinuria, back pain,
sense of impending doom, dyspnea, renal failure, DIC

What should be done:

1. Stop the transfusion
2. Call your attending; contact Blood Bank
3. Clerical check
4. Blood sample and blood products sent to Blood Bank:
   - Clerical check
   - Re-check ABO type of patient and RBC
   - Hemolysis?
   - DAT
5. Urinalysis
6. Maintain urine output
7. Manage DIC, if necessary
8. Supportive care

ABO Histo-blood group system
Summary

Carbohydrate antigens

Glycolipids & glycoproteins

Indirect gene product

500,000 copies/RBC

On many tissues ("histoblood group Ag")

No known function

"Naturally occurring" IgM

T-independent

Direct agglutinin

C5b-9 membrane attack complex

Intravascular hemolysis

Acute hemolytic transfusion reaction

Hyperacute rejection of solid-organ tranplants

Mild HDN, if any

Other Types of Transfusion Reactions

IgG-mediated HTRs (i.e. "delayed type")

Febrile transfusion reactions
Allergic transfusion reactions
Anaphylactic transfusion reactions
Transfusion-associated acute lung injury (i.e. TRALI)
Transfusion-associated circulatory overload (i.e. TACO)

ACKNOWLEDGEMENTS

ABO Glycolipids

K. Landsteiner	**Vienna and Rockefeller**
E. Kabat	**Columbia University**
W. Watkins	**London**
W. Morgan	**London**
V. Ginsburg	**NIH**
R. Oriol	**Paris**
S. Hakomori	**Seattle**
H. Clausen	**Seattle**
F. Yamamoto	**Seattle**
M. Palcic	**Edmonton**