Hemolytic Transfusion Reactions
ABO Blood Group System

October 21, 2005

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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)

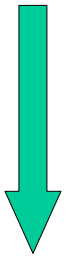
Transfuse any unit of RBC into any recipient:

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

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

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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

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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
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We go to extraordinary lengths to prevent this:

Every donor and donor unit it ABO typed every time

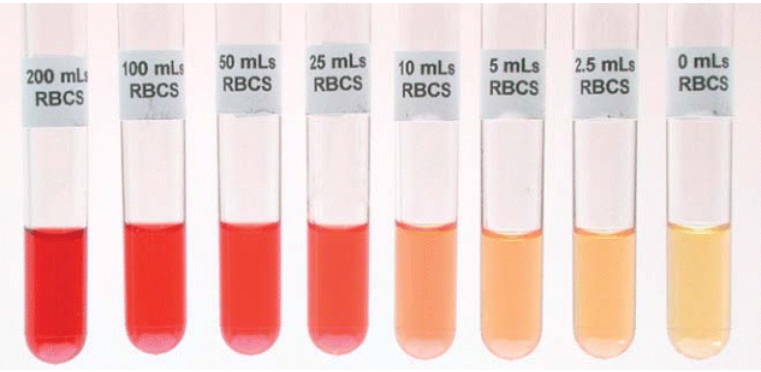
Every recipient is ABO typed every time

The front and back type must agree

Lots of barriers and requirements from phlebotomy to transfusion

Still we have problems

Hemolytic Transfusion Reactions

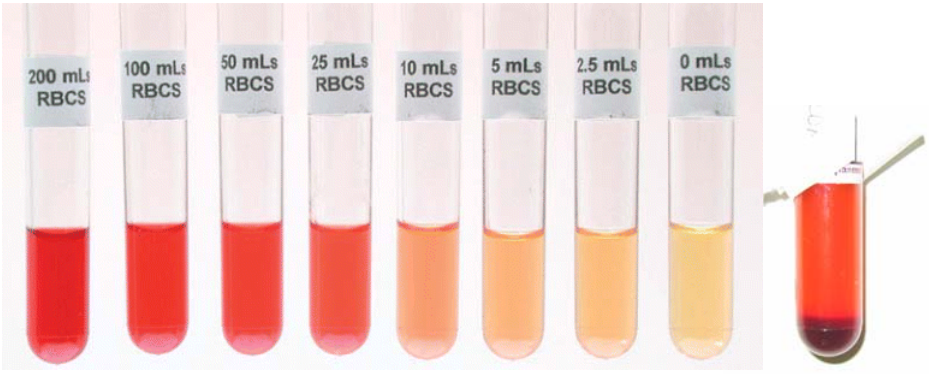


Elliott et al. Visualizing the hemolytic transfusion reaction. Transfusion 43: 297, 2003.

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

Delayed HTRs
- IgG-mediated
- 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-incompatible 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

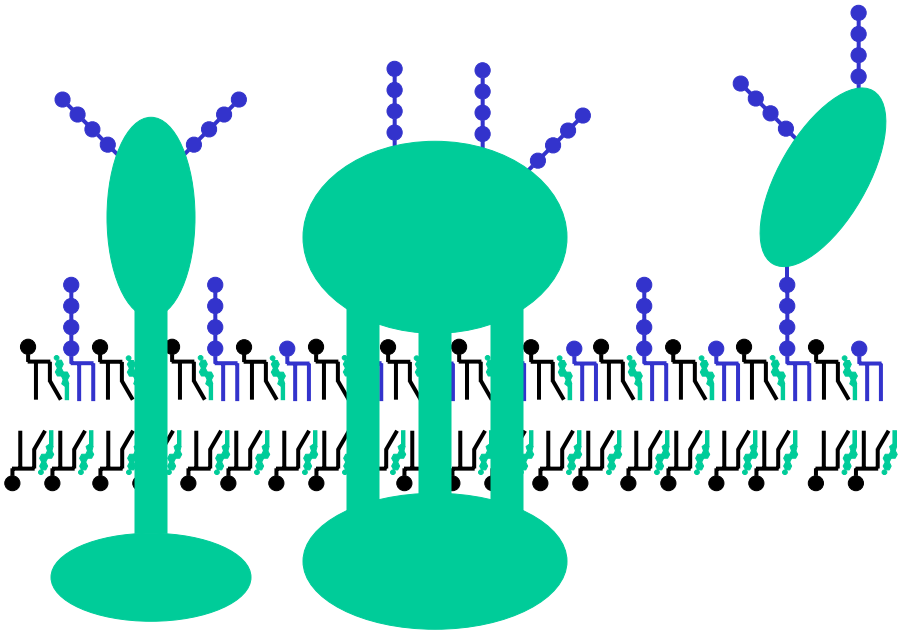
↓ "Magic happens"

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

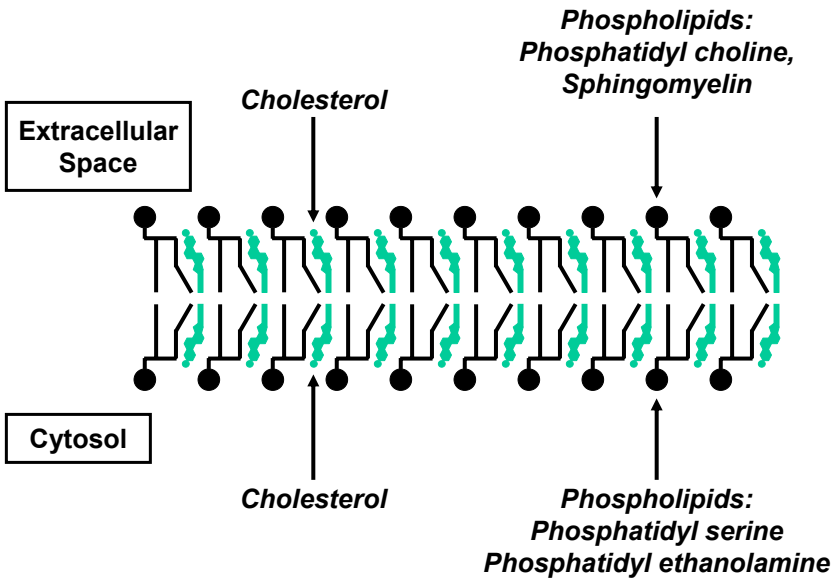
Lipid bilayer:
- **phospholipids, cholesterol**

Glycosphingolipids

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

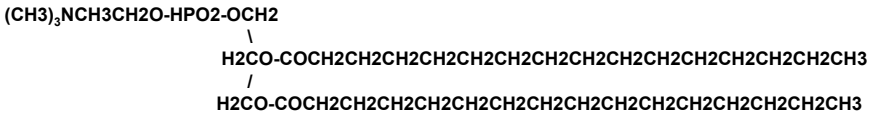
LIPID BILAYER (PHOSPHOLIPIDS)

STRUCTURE OF THE LIPID BILAYER

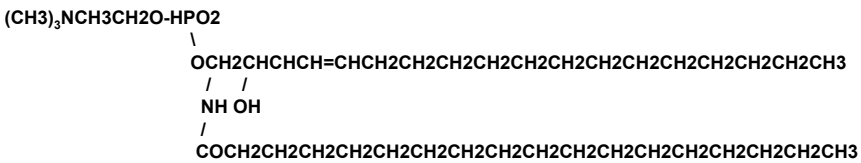


STRUCTURES OF PHOSPHOLIPIDS

Phosphatidylcholine



Sphingomyelin



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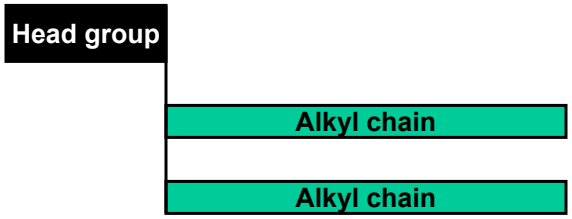
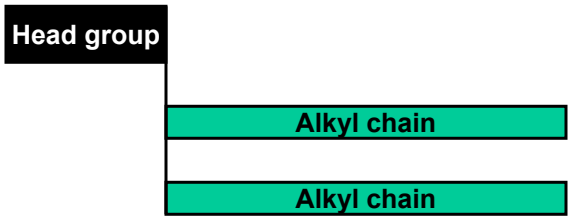
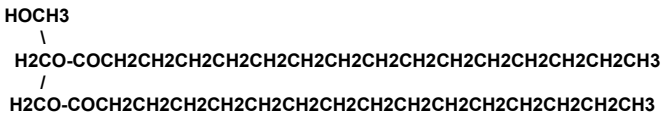


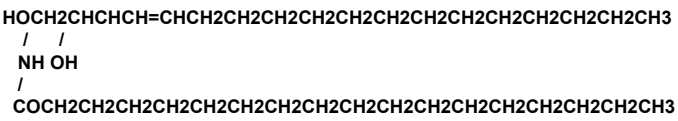
STRUCTURES OF PHOSPHOLIPIDS
Lipid tails



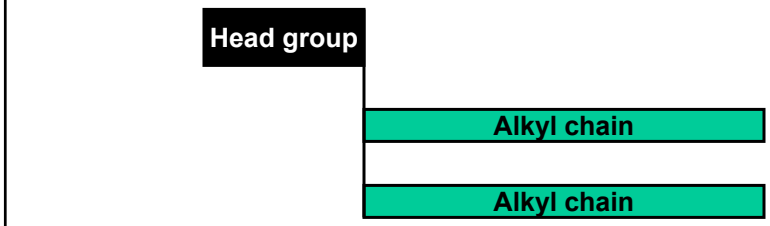
Diacylglyceride

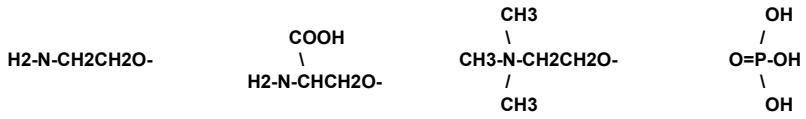


Ceramide



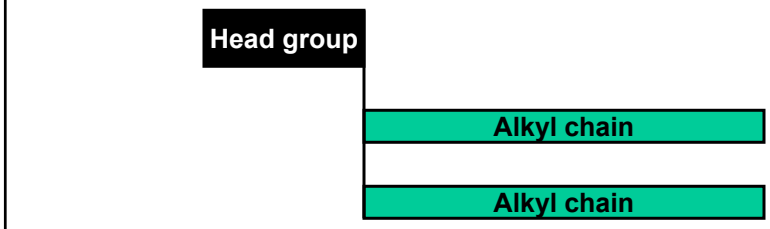
STRUCTURES OF PHOSPHOLIPIDS
Head groups





Ethanolamine | Serine | Choline | Phosphate

STRUCTURES OF PHOSPHOLIPIDS



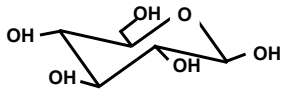
Choline + Phosphate + Diacylglyceride = Phosphatidylcholine

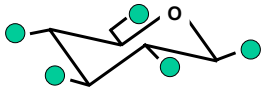
Choline + Phosphate + Ceramide = Sphingomyelin

GLYCOSPHINGOLIPIDS (GLYCOLIPIDS)

MONOSACCHARIDE STRUCTURE

Glucose = Glc

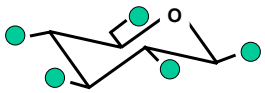




Numbering
Axial vs. equatorial
Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

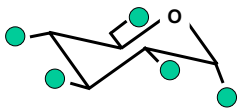
β-Glc



Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

α-Glc



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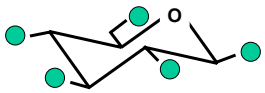
MONOSACCHARIDE STRUCTURE

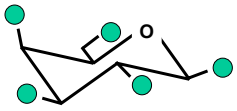
β-Glc



Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

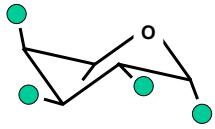
β-Gal



Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

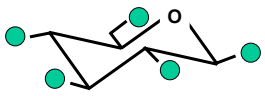
L-α-Fuc



Fucose = 6-deoxy-L-Gal

MONOSACCHARIDE STRUCTURE

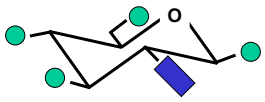
β-Glc



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

MONOSACCHARIDE STRUCTURE

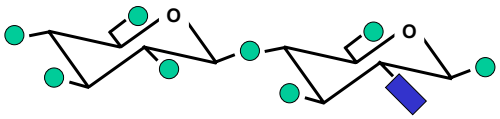
β-GlcNAc



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

CARBOHYDRATE STRUCTURES

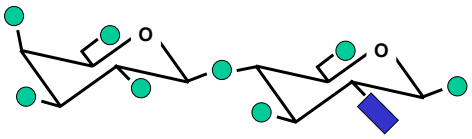
Disaccharides



Glc(β1-4)GlcNAc

CARBOHYDRATE STRUCTURES

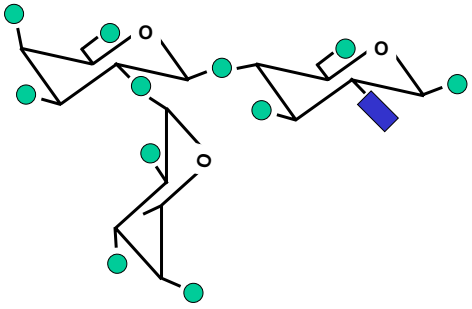
Type 2 Chain



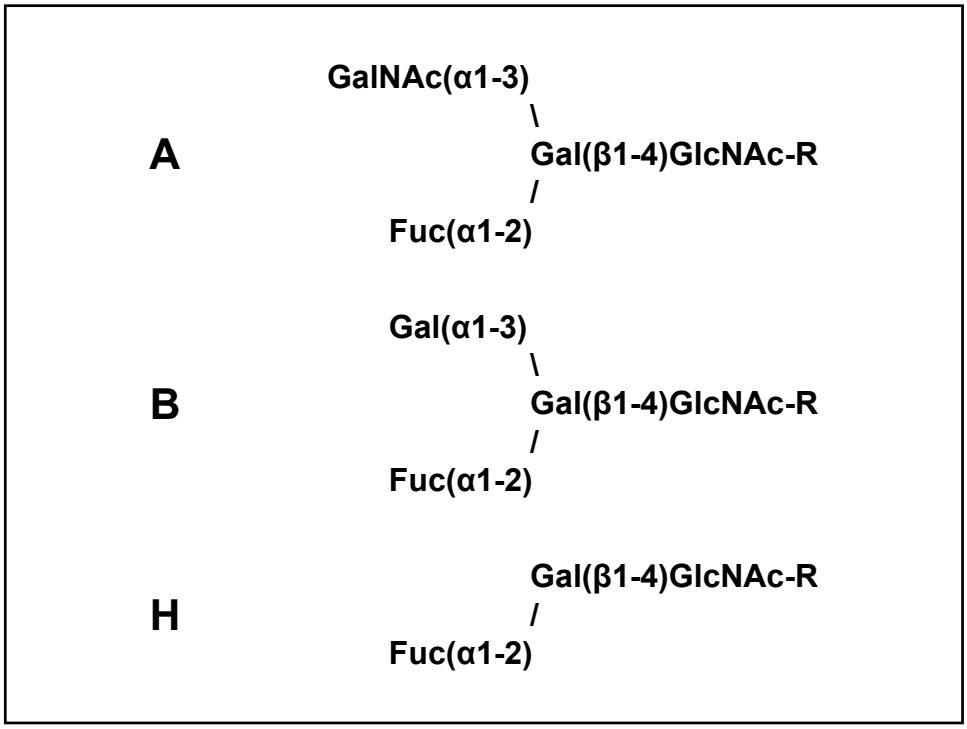
Gal(β1-4)GlcNAc

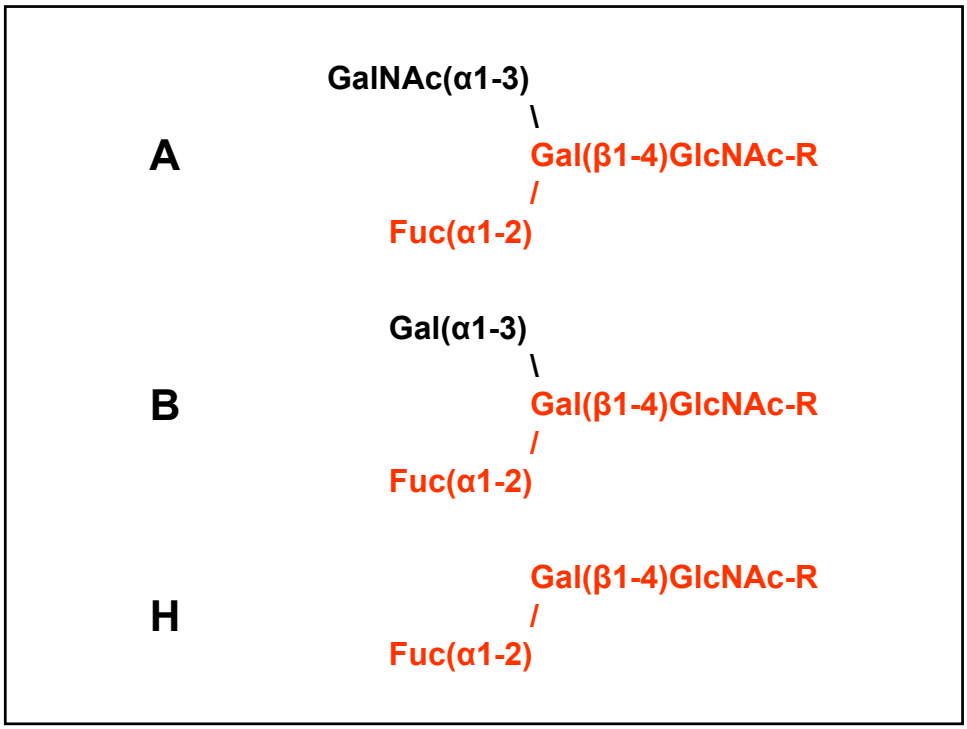
CARBOHYDRATE STRUCTURES

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)


A
GalNAc(α1-3)
Gal(β1-4)GlcNAc-R
Fuc(α1-2)
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Gal(α1-3)
Gal(β1-4)GlcNAc-R
Fuc(α1-2)
H
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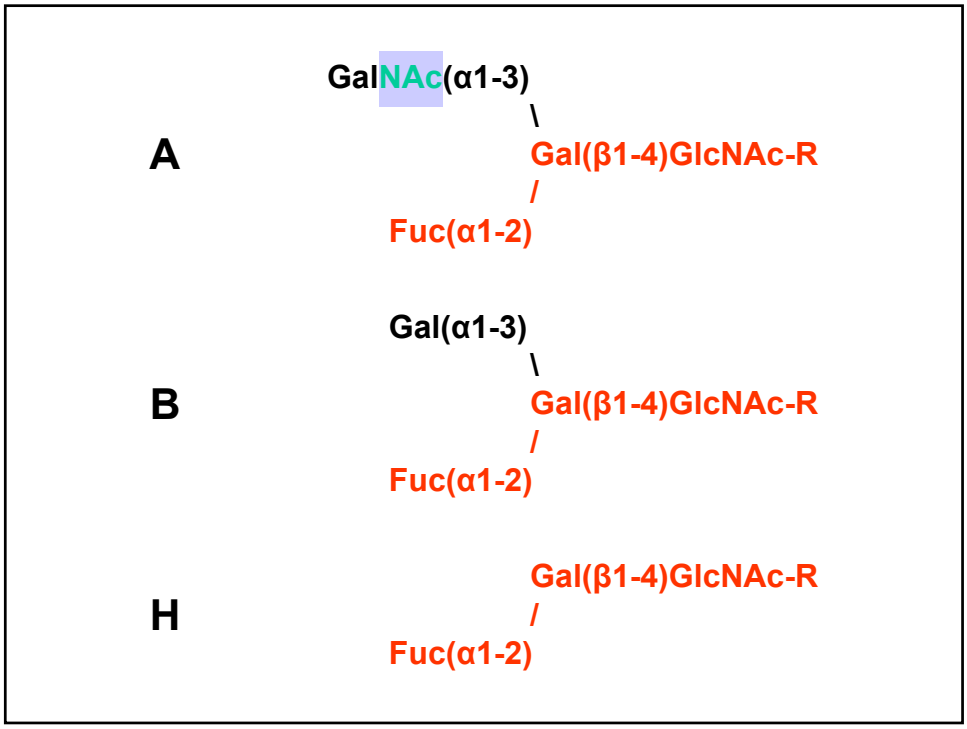
Fuc(α1-2)



GLYCOCONJUGATE BIOSYNTHESIS
Glycosidic bonds
Glycosyltransferase

Nucleotide-sugar + Acceptor → Glycosylated acceptor + Nucleotide

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

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer — Ceramide

UDP-Glc ↓ *Glucosylceramide synthase*

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer — Ceramide

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Glc(β1-1')Cer — Glucosylceramide

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer — Ceramide

↓

Glc(β1-1')Cer — Glucosylceramide

UDP-Gal ↓ *Lactosylceramide synthase*

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer — Ceramide

↓

Glc(β1-1')Cer — Glucosylceramide

UDP-Gal ↓ *Lactosylceramide synthase*

Gal(β1-4)Glc(β1-1')Cer — Lactosylceramide

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer — Ceramide

↓

Glc(β1-1')Cer — Glucosylceramide

↓

Gal(β1-4)Glc(β1-1')Cer — Lactosylceramide

UDP-GlcNAc ↓ *Lacto (β1-3)GlcNAc transferase*

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer — Ceramide

↓

Glc(β1-1')Cer — Glucosylceramide

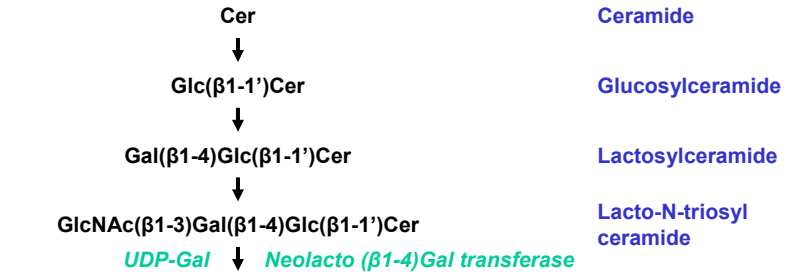
↓

Gal(β1-4)Glc(β1-1')Cer — Lactosylceramide

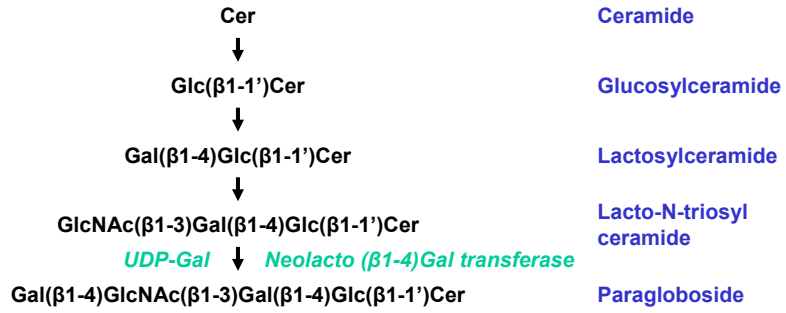
UDP-GlcNAc ↓ *Lacto (β1-3)GlcNAc transferase*

GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer — Lacto-N-triosyl ceramide

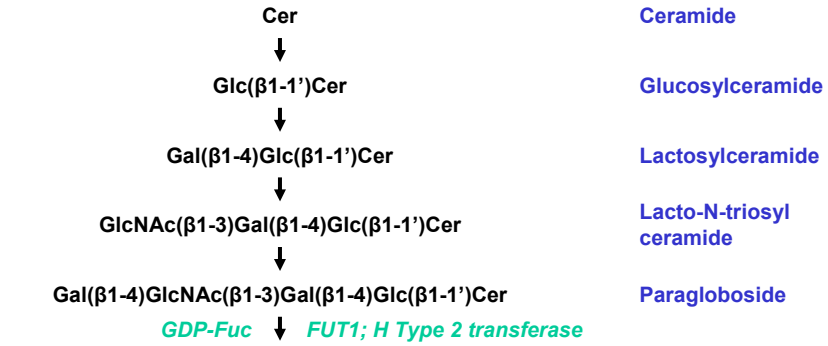
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID



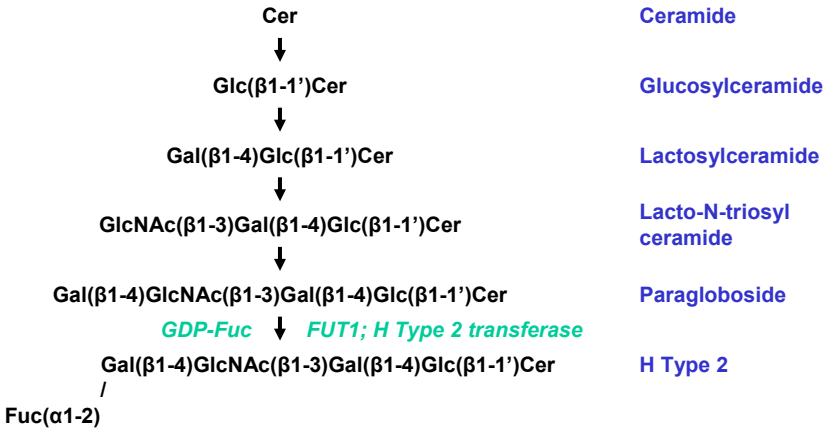
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID



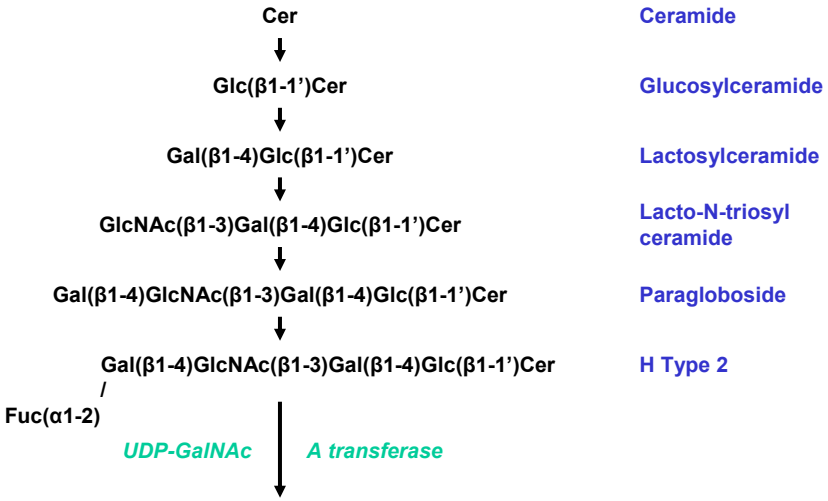
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID



BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID



BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID



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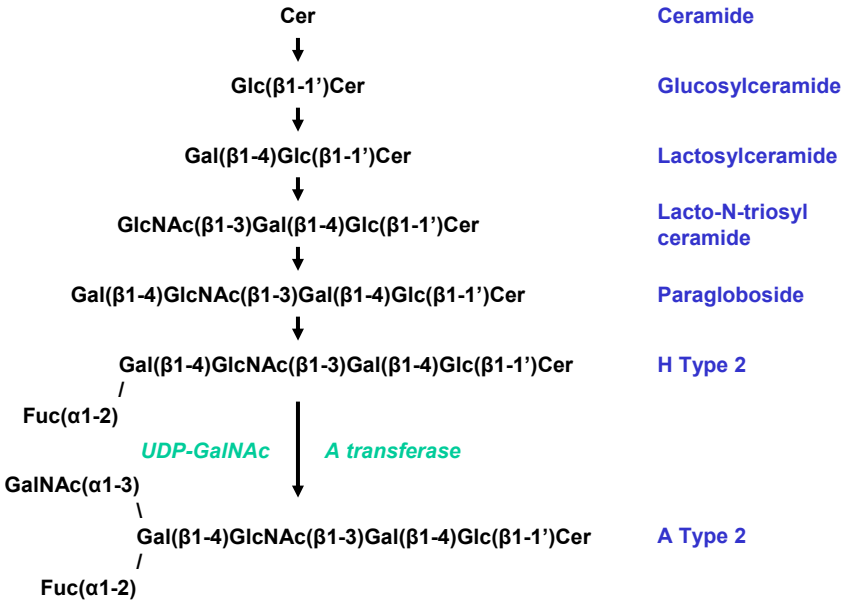

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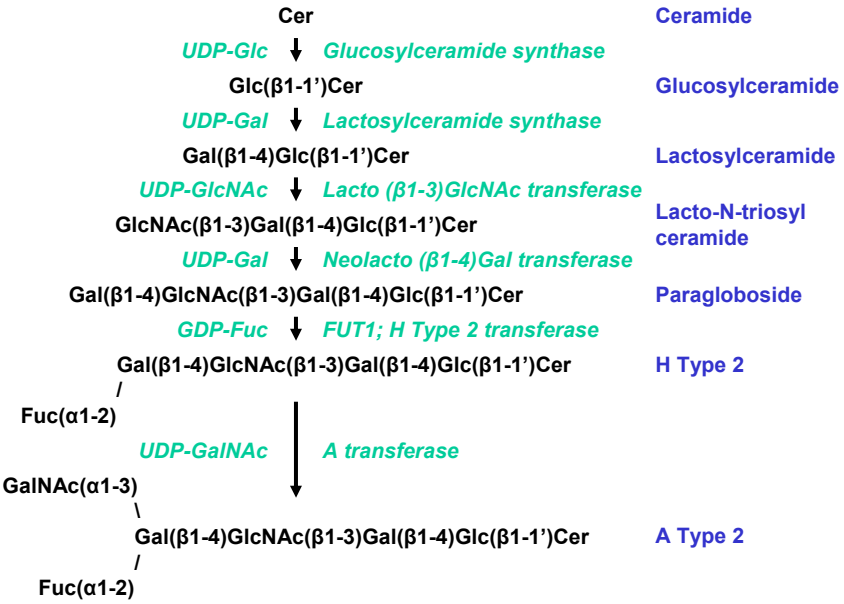
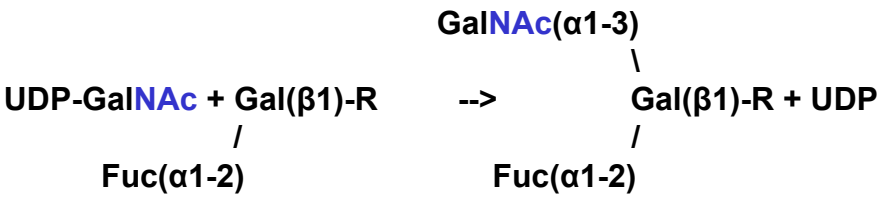


CHARACTERISTICS OF THE A AND B TRANSFERASES

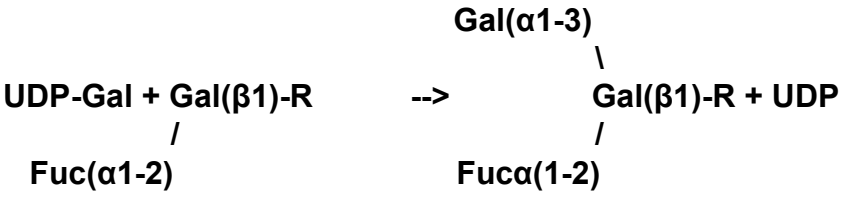
- 354 amino acids
- Type II membrane glycoprotein
- Golgi localization
- A and B transferases are highly homologous
- Require Mn+2 for enzymatic activity
- GT6 family of glycosyltransferases (CAZy): http://afmb.cnrs-mrs.fr/CAZY/
- 7 coding exons
- Chromosome 9 q34

CHARACTERISTICS OF THE A AND B TRANSFERASES

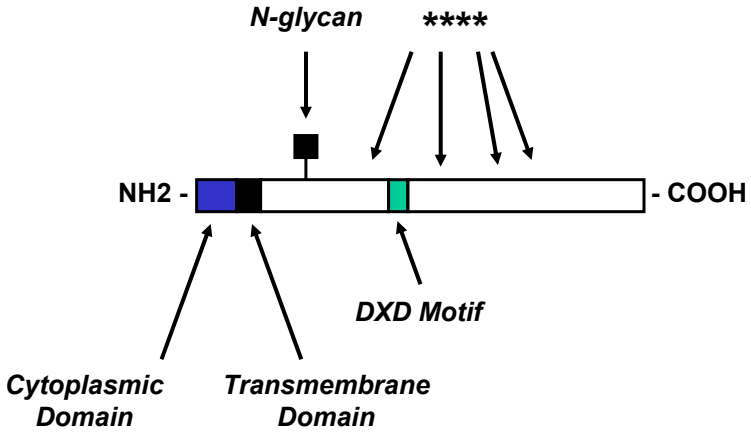
A: (α1-3) GalNAc-transferase (EC 2.4.1.40)



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

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

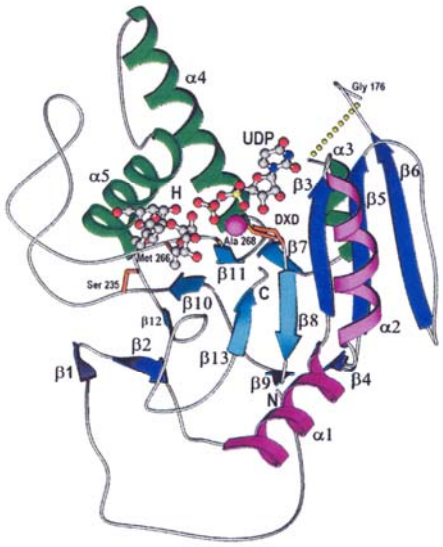
Four Critical Residues

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

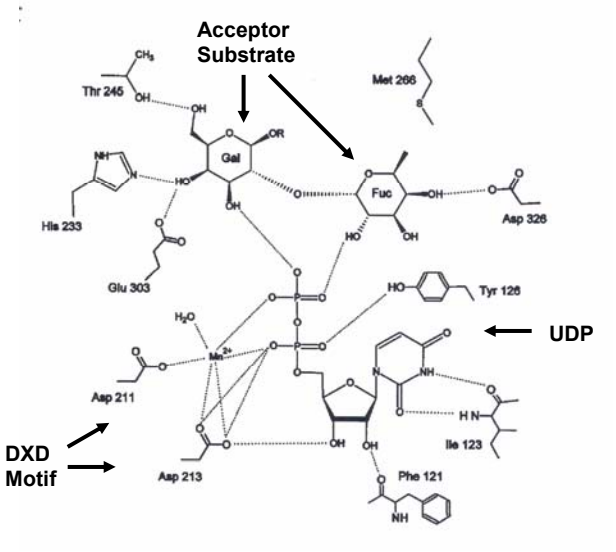
Yamamoto et al. J Biol Chem 265:19257, 1990

CRYSTAL STRUCTURE OF THE B TRANSFERASE



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

ACTIVE SITE OF THE B TRANSFERASE



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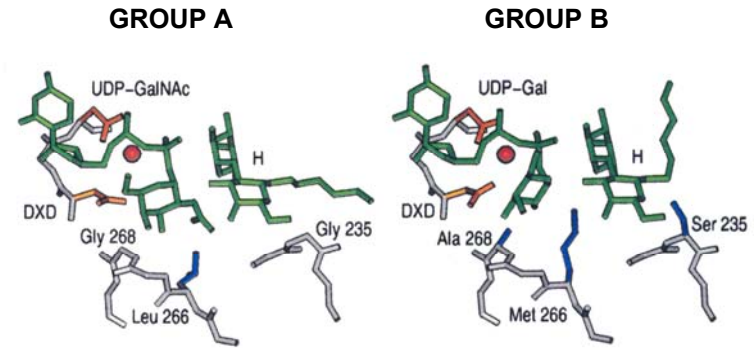

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Hemolytic Transfusion Reactions

RBC + IgM

↓

Complement activation

↓

Intravascular hemolysis

↓

Shock, renal failure, death

Hemolytic Transfusion Reactions

RBC + IgM

↓

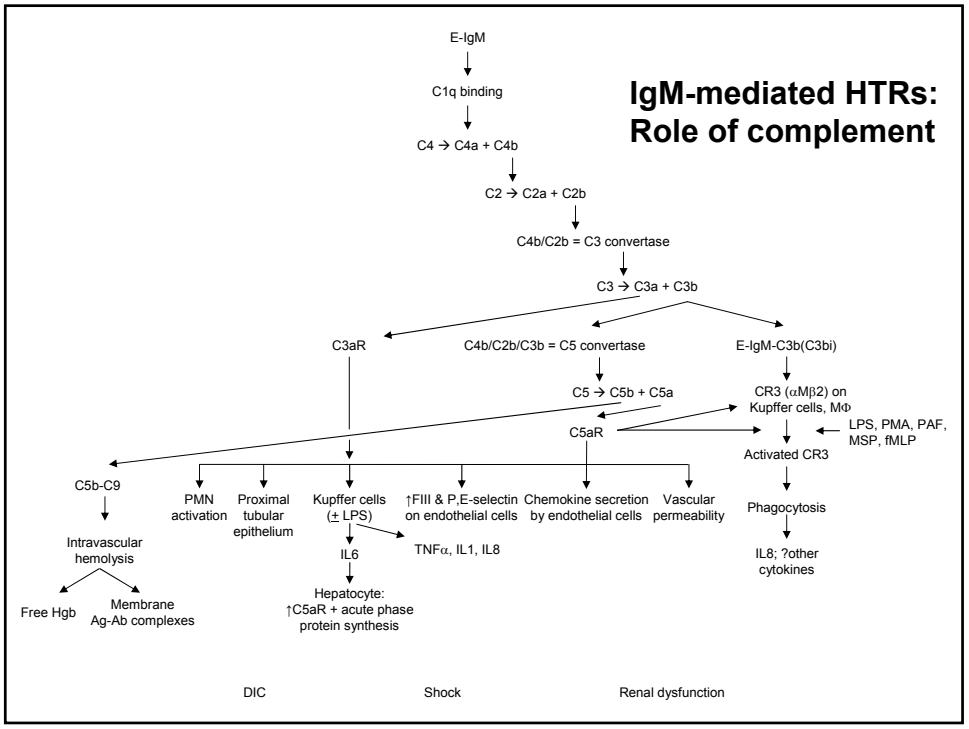
Complement activation

↓

Intravascular hemolysis

↓ "Magic happens"

Shock, renal failure, death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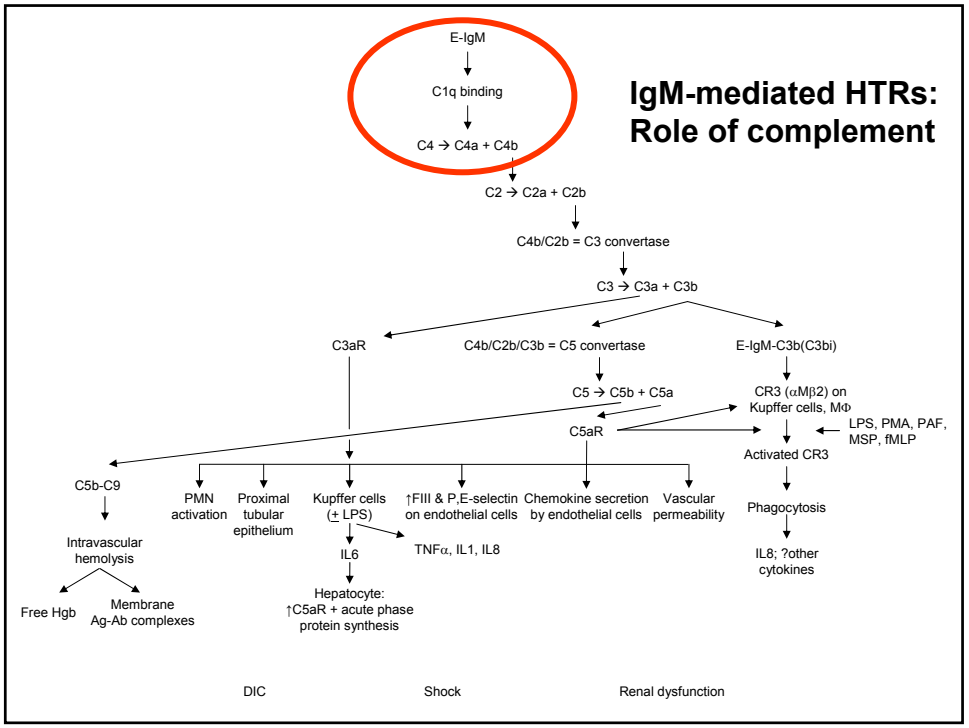
Role of complement
E-IgM
C1q binding
C4 → C4a + C4b
C2 → C2a + C2b
C4b/C2b = C3 convertase
C3 → C3a + C3b
C3aR
C4b/C2b/C3b = C5 convertase
E-IgM-C3b(C3bi)
C5 → C5b + C5a
CR3 (αMβ2) on Kupffer cells, MΦ
C5aR
LPS, PMA, PAF, MSP, fMLP
Activated CR3
C5b-C9
PMN activation
Proximal tubular epithelium
Kupffer cells (± LPS)
↑FIII & P,E-selectin on endothelial cells
Chemokine secretion by endothelial cells
Vascular permeability
Phagocytosis
Intravascular hemolysis
IL6
TNFα, IL1, IL8
IL8; ?other cytokines
Free Hgb
Membrane Ag-Ab complexes
Hepatocyte: ↑C5aR + acute phase protein synthesis
DIC
Shock
Renal dysfunction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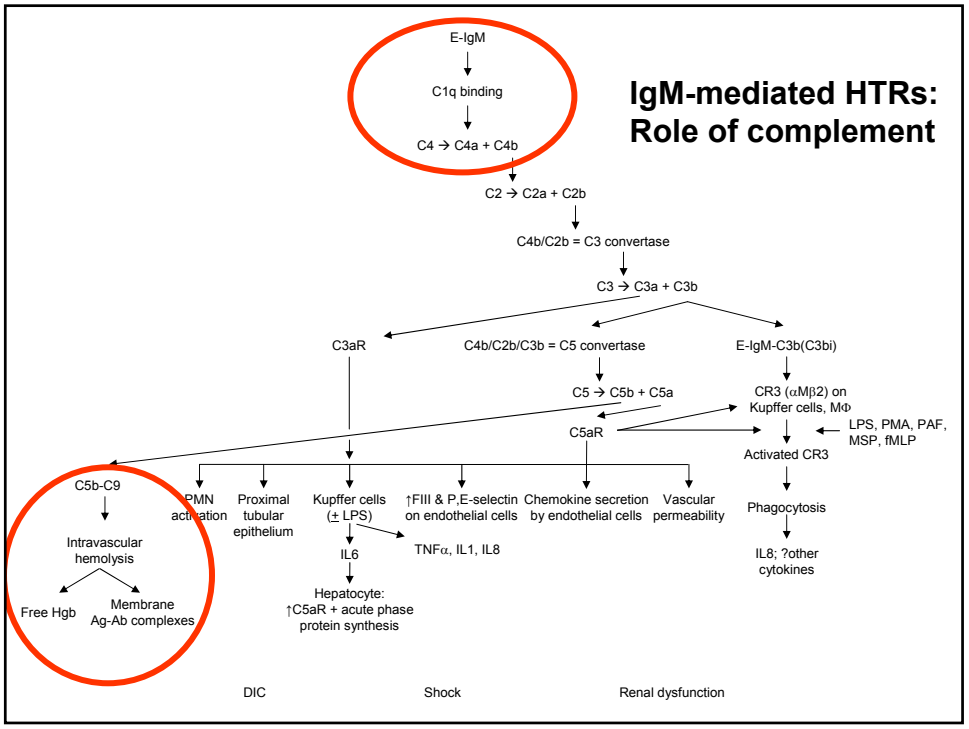
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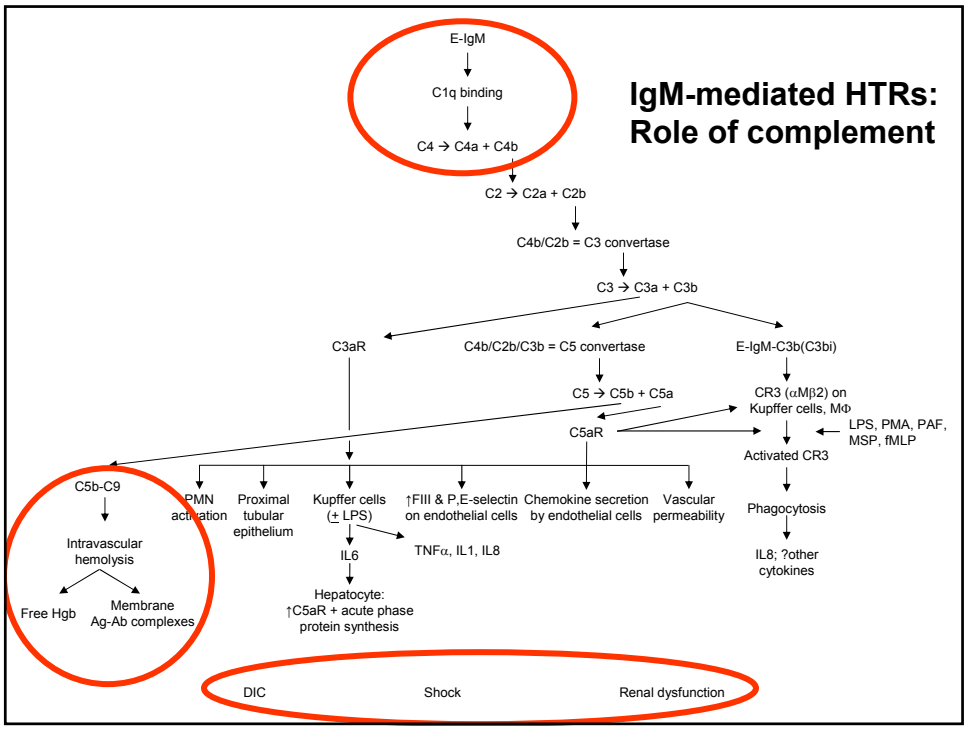
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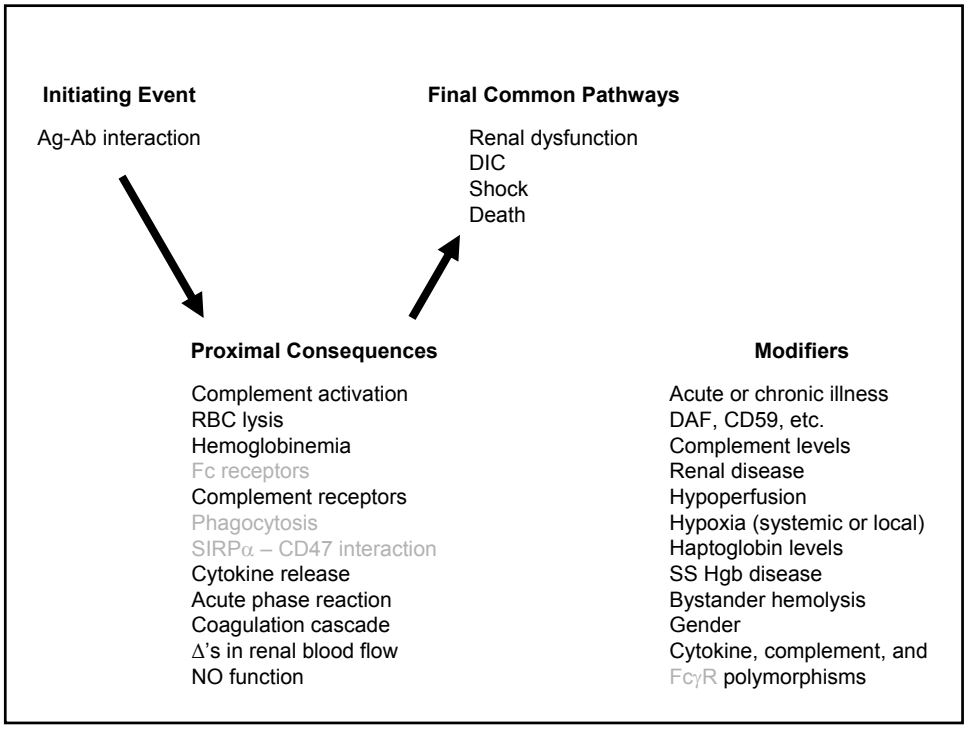


Hemolytic Transfusion Reactions

Current treatment:

Prevention

Steroids, fluid, mannitol, IVIg

Hemolytic Transfusion Reactions

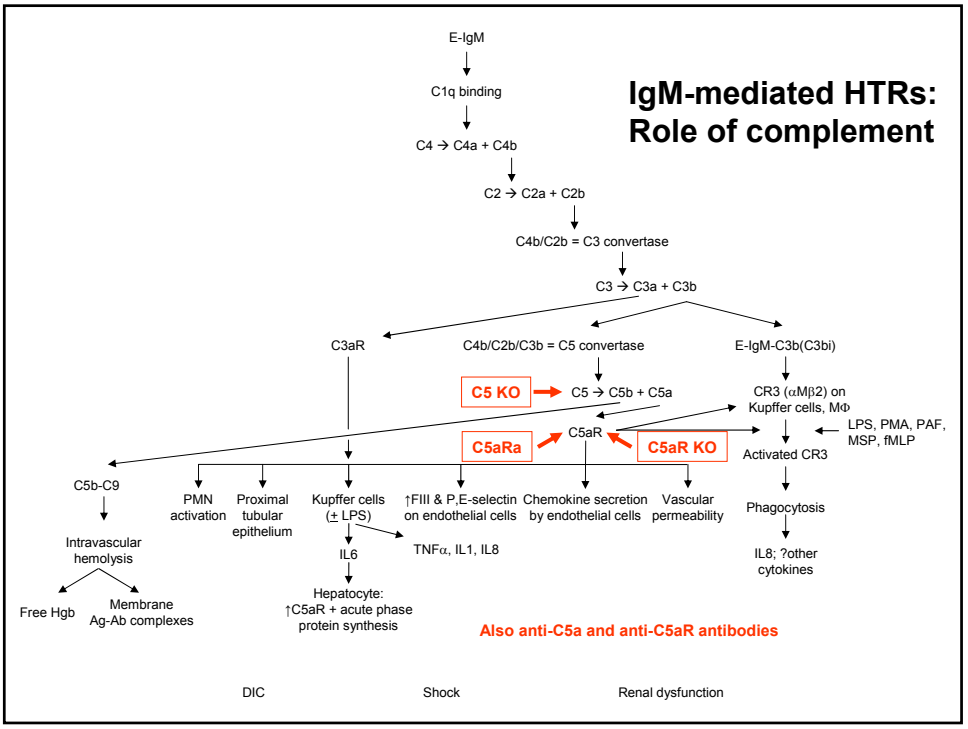
Current treatment:

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

Hemolytic Transfusion Reactions

Potential future treatment options:

- Etanercept (Enbrel): soluble TNFα receptor
- Infliximab (Remicade): anti-TNFα
- Anakinra (Kineret): recombinant IL1ra
- Activated Protein C
- Complement inhibitors
- 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

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**