ABO Blood Group System

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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)

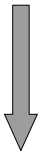
Transfuse any unit of RBC into any recipient:

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

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?

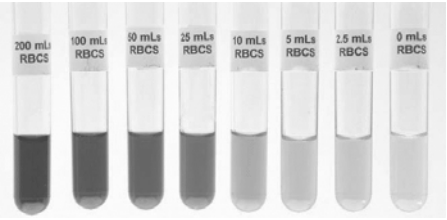
- ABO incompatible RBC → death
- *ABO incompatible xplant → hyperacute rejection*

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

Still we have problems

Hemolytic Transfusion Reactions



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

Hemolytic Transfusion Reactions

Acute HTRs

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

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 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		255 (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

RBC + IgM

↓

Complement activation

↓

Intravascular hemolysis

↓

Shock, renal failure, death

Hemolytic Transfusion Reactions

RBC + IgM

↓

Complement activation

↓

Intravascular hemolysis

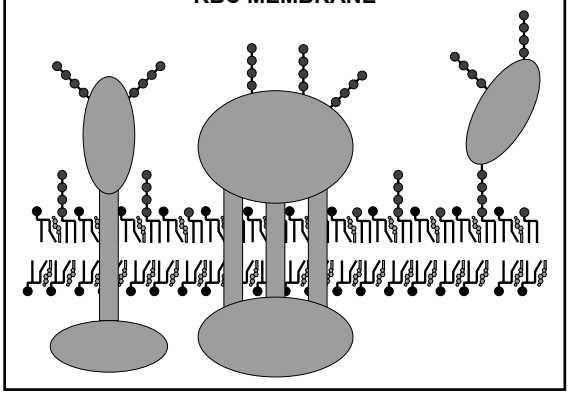
↓ "Magic happens"

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

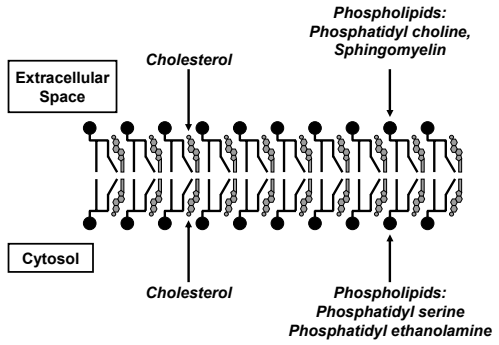
Lipid bilayer:
- **phospholipids, cholesterol**

Glycosphingolipids

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

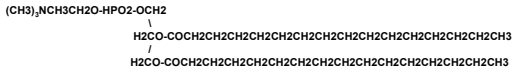
LIPID BILAYER (PHOSPHOLIPIDS)

STRUCTURE OF THE LIPID BILAYER

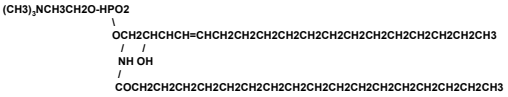


STRUCTURES OF PHOSPHOLIPIDS

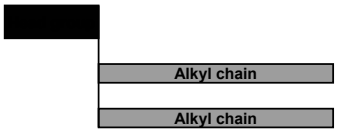
Phosphatidylcholine



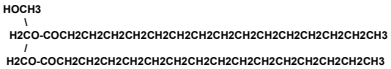
Sphingomyelin



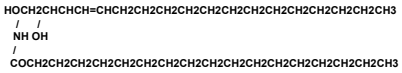
STRUCTURES OF PHOSPHOLIPIDS
Lipid tails



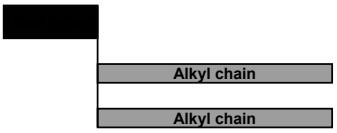
Diacylglyceride

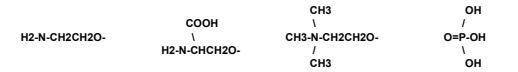


Ceramide



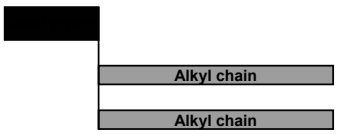
STRUCTURES OF PHOSPHOLIPIDS
Head groups





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

STRUCTURES OF PHOSPHOLIPIDS



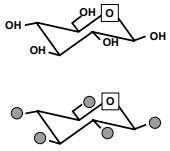
Choline + **Phosphate** + **Diacylglyceride** = **Phosphatidylcholine**

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

GLYCOSPHINGOLIPIDS (GLYCOLIPIDS)

MONOSACCHARIDE STRUCTURE

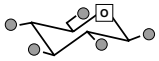
Glucose = Glc



Numbering
Axial vs. equatorial
Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

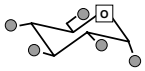
β-Glc



Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

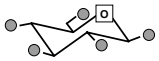
α-Glc



Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

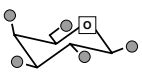
β-Glc



Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

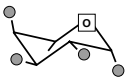
β-Gal



Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

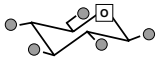
L-α-Fuc



Fucose = 6-deoxy-L-Gal

MONOSACCHARIDE STRUCTURE

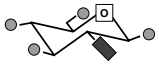
β-Glc



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

MONOSACCHARIDE STRUCTURE

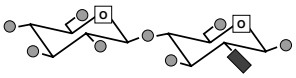
β-GlcNAc



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

CARBOHYDRATE STRUCTURES

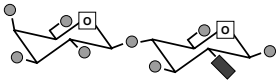
Disaccharides



Glc(β1-4)GlcNAc

CARBOHYDRATE STRUCTURES

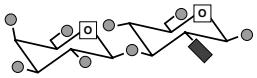
Type 2 Chain



Gal(β1-4)GlcNAc

CARBOHYDRATE STRUCTURES

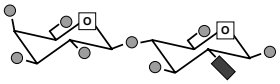
Type 1 Chain



Gal(β1-3)GlcNAc

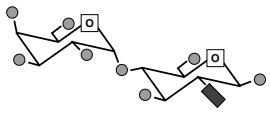
CARBOHYDRATE STRUCTURES

Type 2 Chain



Gal(β1-4)GlcNAc

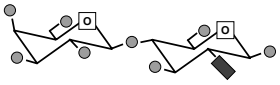
CARBOHYDRATE STRUCTURES



Gal(α1-4)GlcNAc

CARBOHYDRATE STRUCTURES

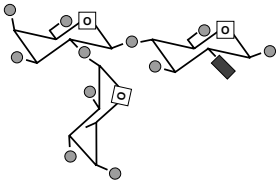
Type 2 Chain



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

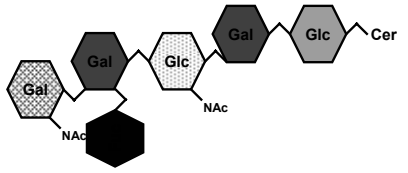
Nucleotide-sugar + Acceptor → Glycosylated acceptor + Nucleotide

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

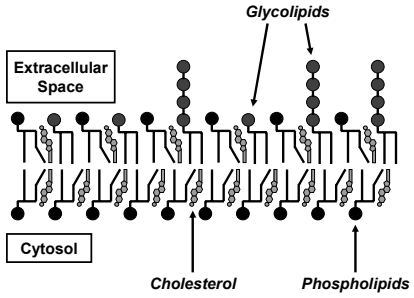
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

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 (Fuc(α1-2) /)	H Type 2
UDP-GalNAc ↓ A transferase	
GalNAc(α1-3) \ Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer (Fuc(α1-2) /)	A Type 2

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID



A TYPE 2

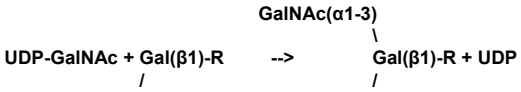


CHARACTERISTICS OF THE A AND B TRANSFERASES

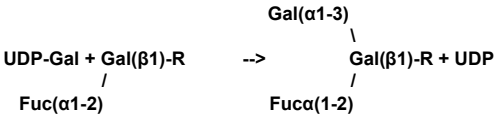
- 354 amino acids
- Type II membrane glycoprotein
- Golgi localization
- A and B transferases are highly homologous
- Require Mn+2 for enzymatic activity
- GT6 family of glycosyltransferases (CAZy): http://afmb.cnrs-mrs.fr/CAZY/
- 7 coding exons
- Chromosome 9 q34

CHARACTERISTICS OF THE A AND B TRANSFERASES

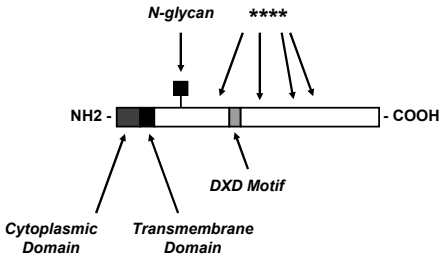
A: (α1-3) GalNAc-transferase (EC 2.4.1.40)



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

STRUCTURE OF THE A AND B TRANSFERASES

Four Critical Residues

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

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

STRUCTURE OF THE A AND B TRANSFERASES

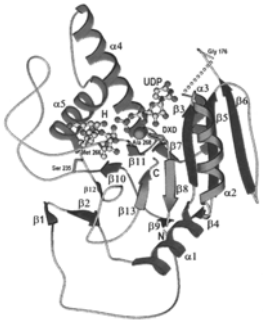
Four Critical Residues

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

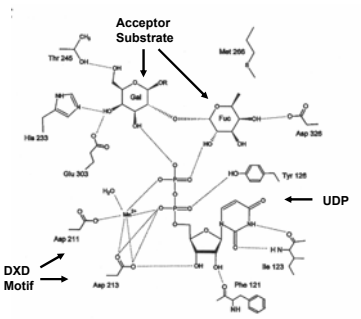
Yamamoto et al. J Biol Chem 265:19257, 1990

CRYSTAL STRUCTURE OF THE B TRANSFERASE



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

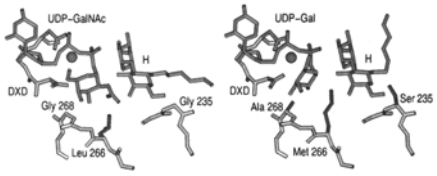
ACTIVE SITE OF THE B TRANSFERASE



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

ACTIVE SITES OF THE A AND B TRANSFERASES

GROUP A **GROUP B**



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

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	**Great Britain**
W. Morgan	**Great Britain**
V. Ginsburg	**NIH**
R. Oriol	**France**
S. Hakomori	**Seattle**
H. Clausen	**Seattle**
F. Yamamoto	**Seattle**