Hemolytic Transfusion Reactions
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
Landsteiner Experiment
1900

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

<table>
<thead>
<tr>
<th>Blood group</th>
<th>RBC</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>anti-B</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>“none”</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>anti-A, anti-B</td>
</tr>
</tbody>
</table>

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 $\rightarrow$ death  
*ABO incompatible xplant* $\rightarrow$ *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
Why do we care?
  ABO incompatible RBC $\rightarrow$ death
  $ABO$ incompatible xplant $\rightarrow$ 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

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

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*

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC-incompatible</td>
<td>237</td>
<td>1/38,000</td>
</tr>
<tr>
<td>ABC-compatible</td>
<td>221</td>
<td>1/41,000</td>
</tr>
<tr>
<td>Total†</td>
<td>462</td>
<td>1/19,000</td>
</tr>
<tr>
<td>Adjusted total‡</td>
<td>659</td>
<td>1/14,000</td>
</tr>
<tr>
<td>Fatal reaction</td>
<td>5</td>
<td>1/1,800,000</td>
</tr>
</tbody>
</table>

* 9,000,000 transfusions were performed during this period.
† Includes 4 cases in which ABC compatibility was not reported.
‡ Adjusted to correct for estimated underreported/undetected ABC-compatible erroneous transfusions. A compatible-to-incompatible ratio of 1.76 was used.


Hemolytic Transfusion Reactions

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

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>No adverse effect</td>
<td>111</td>
<td>47</td>
</tr>
<tr>
<td>Acute hemolytic reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic†</td>
<td>96</td>
<td>41</td>
</tr>
<tr>
<td>Laboratory only</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Fatal</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Low-grade fever only</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Death due to underlying condition</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td>100</td>
</tr>
</tbody>
</table>

* Nonfatal.
Hemolytic Transfusion Reactions

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

<table>
<thead>
<tr>
<th>Nature of error</th>
<th>Number (%)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-blood bank error alone</td>
<td>259 (56)</td>
<td></td>
</tr>
<tr>
<td>Identification error</td>
<td>171 (37)</td>
<td></td>
</tr>
<tr>
<td>Phlebotomy error</td>
<td>62 (13)</td>
<td></td>
</tr>
<tr>
<td>Incorrect order sent</td>
<td>22 (5)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (1)</td>
<td></td>
</tr>
<tr>
<td>Blood bank error alone</td>
<td>136 (29)</td>
<td></td>
</tr>
<tr>
<td>Tossed wrong sample</td>
<td>39 (8)</td>
<td></td>
</tr>
<tr>
<td>Testing error, technical</td>
<td>36 (7)</td>
<td></td>
</tr>
<tr>
<td>Wrong unit issued</td>
<td>12 (4)</td>
<td></td>
</tr>
<tr>
<td>Testing error, clerical/transcription</td>
<td>16 (3)</td>
<td></td>
</tr>
<tr>
<td>Wrong unit tagged</td>
<td>16 (3)</td>
<td></td>
</tr>
<tr>
<td>Clerical error, recorded on wrong slip</td>
<td>11 (2)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (1)</td>
<td></td>
</tr>
<tr>
<td>Compound error</td>
<td>67 (15)</td>
<td></td>
</tr>
<tr>
<td>Wrong unit issued, identification error</td>
<td>48 (10)</td>
<td></td>
</tr>
<tr>
<td>Wrong unit tagged, not detected</td>
<td>5 (1)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>13 (3)</td>
<td></td>
</tr>
<tr>
<td>Could not be determined*</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Total</td>
<td>462 (100)</td>
<td></td>
</tr>
</tbody>
</table>

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


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

<table>
<thead>
<tr>
<th>Method of discovery</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As a result of reaction</td>
<td>90 (26)</td>
</tr>
<tr>
<td>At bedside</td>
<td>66 (21)</td>
</tr>
<tr>
<td>Subsequent blood request</td>
<td>68 (22)</td>
</tr>
<tr>
<td>Supervisory review</td>
<td>17 (5)</td>
</tr>
<tr>
<td>Other</td>
<td>75 (24)</td>
</tr>
<tr>
<td>Total</td>
<td>316 (100)</td>
</tr>
</tbody>
</table>

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

Hemolytic Transfusion Reactions

RBC + IgM
↓
Complement activation
↓
Intravascular hemolysis
↓
Shock, renal failure, death

"Magic happens"
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
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**

Extracellular Space

**Cholesterol**

Phospholipids: Phosphatidyl choline, Sphingomyelin

Cytosol

**Cholesterol**

Phospholipids: Phosphatidyl serine, Phosphatidyl ethanolamine

**STRUCTURES OF PHOSPHOLIPIDS**

**Phosphatidylcholine**

(CH3)2NCH3CH2O-P-OH2

\[ \text{H}_2\text{CO-COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]

\[ \text{H}_2\text{CO-COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]

**Sphingomyelin**

(CH3)2NCH3CH2O-P-OH2

\[ \text{OCH}_2\text{CHCH}=\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]

\[ \text{H}_2\text{CO-COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]

\[ \text{NH}_2\text{OH} \]

\[ \text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]
STRUCTURES OF PHOSPHOLIPIDS

**Head group**

- Alkyl chain
- Alkyl chain

**Lipid tails**

**Head group**

- Alkyl chain
- Alkyl chain

**Diacylglyceride**

\[ \text{HOCH}_3 \]
\[ \text{H}_2\text{CO-COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]
\[ \text{H}_2\text{CO-COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]

**Ceramide**

\[ \text{HOCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]
\[ \text{NH} \text{ OH} \]
\[ \text{COCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 \]
STRUCTURES OF PHOSPHOLIPIDS

Head groups

- **Head group**
  - Alkyl chain
  - Alkyl chain

- Ethanolamine
- Serine
- Choline
- Phosphate

- 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**

\[ \beta\text{-GlcNAc} \]

**Amino sugars**

N-acetyl-glucosamine = GlcNAc

N-acetyl = CH3CONH-

**CARBOHYDRATE STRUCTURES**

**Disaccharides**

\[ \text{Glc(\(\beta\)1-4)GlcNAc} \]
CARBOHYDRATE STRUCTURES

Type 2 Chain

Gal(β1-4)GlcNAc

CARBOHYDRATE STRUCTURES

Type 2 H

Fuc(α1-2)Gal(β1-4)GlcNAc
A
---
\( \text{GalNAc(}\alpha_1-3) \)
/ \
\( \text{Gal(}\beta_1-4)\text{GlcNAc-R} \)
/  
\( \text{Fuc(}\alpha_1-2) \)
/ \
\( \text{Gal(}\alpha_1-3) \)
/ \
B
---
\( \text{Gal(}\beta_1-4)\text{GlcNAc-R} \)
/  
\( \text{Fuc(}\alpha_1-2) \)
/ \
H
---
\( \text{Gal(}\beta_1-4)\text{GlcNAc-R} \)
/  
\( \text{Fuc(}\alpha_1-2) \)
GLYCOCONJUGATE BIOSYNTHESIS
Glycosidic bonds
Glycosyltransferase

Nucleotide-sugar + Acceptor → Glycosylated acceptor + Nucleotide
GLYCOCONJUGATE BIOSYNTHESIS
Glycosidic bonds
Glycosyltransferase

Nucleotide-sugar + Acceptor → Glycosylated acceptor + Nucleotide

UDP-GalNAc
GDP-Fuc
CMP-sialic acid

Carbohydrate
Lipid
Protein
Other

Oligosaccharide
Glycolipid
Glycoprotein
Other glycan

UDP
GDP
CMP

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer  Ceramide
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer  →  Ceramide

UDP-Glc  \downarrow  Glucosylceramide synthase

Glc(β1-1')Cer  →  Glucosylceramide
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer \[\rightarrow\] Ceramide

Glc(β1-1')Cer \[\rightarrow\] Glucosylceramide

UDP-Gal \[\rightarrow\] Lactosylceramide synthase

Glc(β1-1')Cer \[\rightarrow\] Glucosylceramide

UDP-Gal \[\rightarrow\] Lactosylceramide synthase

Gal(β1-4)Glc(β1-1')Cer \[\rightarrow\] Lactosylceramide
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

\[
\begin{align*}
\text{Cer} & \quad \text{Ceramide} \\
\downarrow & \\
\text{Glc(β1-1')Cer} & \quad \text{Glucosylceramide} \\
\downarrow & \\
\text{Gal(β1-4)Glc(β1-1')Cer} & \quad \text{Lactosylceramide} \\
\text{UDP-GlcNAc} & \quad \text{Lacto (β1-3)GlcNAc transferase}
\end{align*}
\]
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer → Ceramide
Glc(β1-1')Cer → Glucosylceramide
Gal(β1-4)Glc(β1-1')Cer → Lactosylceramide
GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer → Lacto-N-triosyl ceramide
UDP-Gal + Neolacto (β1-4)Gal transferase

Cer → Ceramide
Glc(β1-1')Cer → Glucosylceramide
Gal(β1-4)Glc(β1-1')Cer → Lactosylceramide
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
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer → Ceramide
↓
Glc(β1-1’)Cer → Glucosylceramide
↓
Gal(β1-4)Glc(β1-1’)Cer → Lactosylceramide
↓
GlcNAc(β1-3)Gal(β1-4)Glc(β1-1’)Cer → Lacto-N-triosyl ceramide
↓
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1’)Cer → Paragloboside

GDP-Fuc → FUT1; H Type 2 transferase

BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Cer → Ceramide
↓
Glc(β1-1’)Cer → Glucosylceramide
↓
Gal(β1-4)Glc(β1-1’)Cer → Lactosylceramide
↓
GlcNAc(β1-3)Gal(β1-4)Glc(β1-1’)Cer → Lacto-N-triosyl ceramide
↓
Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1’)Cer → Paragloboside

GDP-Fuc → FUT1; H Type 2 transferase

Fuc(α1-2)
BIOSYNTHESIS OF BLOOD GROUP A GLYCOLIPID

Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer / Fuc(α1-2)

UDP-GalNAc A transferase

Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer

GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer

Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer / H Type 2

Glc(β1-1')Cer

Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer

GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer

Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer

Gal(β1-4)Glc(β1-1')Cer

Glc(β1-1')Cer

UDP-GalNAc A transferase

GalNAc(α1-3)

Glc(β1-1')Cer

Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer / A Type 2

Fuc(α1-2)
**BIOSYNTHESIS OF BLOOD GROUP A GLYCOSLIPID**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cer</td>
<td>Glucosylceramide synthase</td>
</tr>
<tr>
<td>UDP-Glc down Glc(β1-1')Cer</td>
<td>Glucosylceramide</td>
</tr>
<tr>
<td>UDP-Gal down Lactosylceramide synthase</td>
<td>Lactosylceramide</td>
</tr>
<tr>
<td>Gal(β1-4)Glc(β1-1')Cer</td>
<td>Lacto(β1-3)GlcNAc transferase</td>
</tr>
<tr>
<td>UDP-GlcNAc down Lacto(β1-3)GlcNAc transferase</td>
<td>Lacto-N-triosyl ceramide</td>
</tr>
<tr>
<td>GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer</td>
<td>Paragloboside</td>
</tr>
<tr>
<td>UDP-Gal down Neolacto(β1-4)Gal transferase</td>
<td>FUT1; H Type 2 transferase</td>
</tr>
<tr>
<td>Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer</td>
<td>H Type 2</td>
</tr>
<tr>
<td>GDP-Fuc down Paragloboside</td>
<td></td>
</tr>
<tr>
<td>Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc(β1-1')Cer</td>
<td></td>
</tr>
<tr>
<td>Fuc(α1-2)</td>
<td></td>
</tr>
</tbody>
</table>

**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):**
- **7 coding exons**
- **Chromosome 9 q34**
CHARACTERISTICS OF THE A AND B TRANSFERASES

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

\[
\text{GalNAc}(\alpha 1-3) \\
\text{UDP-GalNAc} + \text{Gal}(\beta 1)-\text{R} \rightarrow \text{Gal}(\beta 1)-\text{R} + \text{UDP} \\
\text{Fuc}(\alpha 1-2) \quad \text{Fuc}(\alpha 1-2)
\]

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

\[
\text{Gal}(\alpha 1-3) \\
\text{UDP-Gal} + \text{Gal}(\beta 1)-\text{R} \rightarrow \text{Gal}(\beta 1)-\text{R} + \text{UDP} \\
\text{Fuc}(\alpha 1-2) \quad \text{Fuc}(\alpha 1-2)
\]

STRUCTURE OF THE A AND B TRANSFERASES

\[
\text{N-glycan} \\
\text{Cytoplasmic Domain} \\
\text{Transmembrane Domain} \\
\text{DXD Motif} \\
\text{NH}_2 - \quad \text{****} \\
\text{- COOH}
\]

### STRUCTURE OF THE A AND B TRANSFERASES

#### Four Critical Residues

<table>
<thead>
<tr>
<th>Transferase</th>
<th>Amino acid number</th>
<th>176</th>
<th>235</th>
<th>266</th>
<th>268</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
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<td>R</td>
<td>G</td>
<td>L</td>
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<td>G</td>
<td>S</td>
<td>M</td>
<td>A</td>
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<tr>
<td>“AABB”</td>
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<td>R</td>
<td>G</td>
<td>M</td>
<td>A</td>
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</tbody>
</table>

STRUCTURE OF THE A AND B TRANSFERASES

Four Critical Residues

<table>
<thead>
<tr>
<th>Transferase “genotype”</th>
<th>Transferase “phenotype”</th>
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</thead>
<tbody>
<tr>
<td>AAAA</td>
<td>A</td>
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<tr>
<td>AAAB</td>
<td>A</td>
</tr>
<tr>
<td>AABA</td>
<td>AB</td>
</tr>
<tr>
<td>AABB</td>
<td>B</td>
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<tr>
<td>BBAA</td>
<td>A</td>
</tr>
<tr>
<td>BBBB</td>
<td>B</td>
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</tbody>
</table>

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


CRYSTAL STRUCTURE OF THE B TRANSFERASE

ACTIVE SITE OF THE B TRANSFERASE


ACTIVE SITES OF THE A AND B TRANSFERASES

GROUP A

GROUP B

Hemolytic Transfusion Reactions

RBC + IgM

Complement activation

Intravascular hemolysis

Shock, renal failure, death

“Magic happens”
IgM-mediated HTRs: 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
- C5 → C5a + C5b
- C5aR
- E-IgM-C3b(C3bi)
- IL-8; ?other cytokines
- Phagocytosis
- Activated CR3
- C3b-C9
- PMN activation
- Proximal tubular epithelium
- Kupffer cells (⊥ LPS)
- Inflammatory cytokines
- C3aR
- DIC
- Shock
- Renal dysfunction

C5b-C9
- Intravascular hemolysis
- Free Hgb
- Membrane Ag-Ab complexes

C5a
- Intravascular hemolysis

C3a + C3b
- Membrane Ag-Ab complexes

C3aR
- Chemokine secretion by endothelial cells
- Vascular permeability
- ↑ FIII & P, E-selectin
- Hepatocyte: ↑ C5aR + acute phase protein synthesis
- Proximal tubular epithelium
- PMN activation
- Kupffer cells (⊥ LPS)
- Inflammatory cytokines
- TNFα, IL-1, IL-8

C5a
- Membrane Ag-Ab complexes
- DIC
- Shock
- Renal dysfunction
IgM-mediated HTRs: Role of complement

C1q binding
C4 → C4a + C4b
C2 → C2a + C2b
C4b/C2b + C3 convertase
C3 → C3a + C3b

C3αR
C4b/C2b/C3b = C5 convertase
C5 → C5b + C5a
C5αR
C5b-C9
Intravascular hemolysis
Free Hgb
Membrane Ag-Ab complexes
DIC
Shock
Renal dysfunction

C1q binding
C4 → C4a + C4b
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C5 → C5b + C5a
C5αR
C5b-C9
Intravascular hemolysis
Free Hgb
Membrane Ag-Ab complexes
DIC
Shock
Renal dysfunction
Initiating Event: Ag-Ab interaction

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

Final Common Pathways:
- Renal dysfunction
- DIC
- Shock
- Death

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
Current treatment:
  Prevention
  Steroids, fluid, mannitol, IVIg
  Flagellation (self and other)
  Prayer

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
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 transplants
Mild HDN, if any

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ABO Glycolipids

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