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, Pit, 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>&quot;none&quot;</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 → 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

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**

Incompatible transfusion

DIC, renal dysfunction, shock, death

Similar to sepsis or “cytokine storm”

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

Delayed HTRs
- IgG-mediated
- Rh
  - Clinical course: mild-severe, low mortality
  - Adverse outcome

~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>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO-incompatible</td>
<td>237</td>
<td>1/36,000</td>
</tr>
<tr>
<td>ABO-compatible</td>
<td>221</td>
<td>1/41,000</td>
</tr>
<tr>
<td>Total</td>
<td>462</td>
<td>1/10,000</td>
</tr>
<tr>
<td>Adjusted total</td>
<td>659</td>
<td>1/11,800</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 ABO compatibility was not reported.
‡ Adjusted to correct for estimated underreported and undetected ABO-compatible erroneous transfusions. A compatible-to-incompatible ratio of 1:70 was used.


Hemolytic Transfusion Reactions

**TABLE 2.** Outcomes after receipt of ABO-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>19</td>
<td>7%</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>96</td>
<td>41%</td>
</tr>
<tr>
<td>Laboratory only</td>
<td>10</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.** Causes of transfusion-associated errors in New York State, 1990 through 1999

<table>
<thead>
<tr>
<th>Error Type</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrong blood type</td>
<td>222 (56%)</td>
</tr>
<tr>
<td>Non-heparin anticoagulant</td>
<td>213 (53%)</td>
</tr>
<tr>
<td>Blood bank error</td>
<td>180 (44%)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (1%)</td>
</tr>
<tr>
<td>Wrong bank error</td>
<td>109 (27%)</td>
</tr>
<tr>
<td>Wrong blood group</td>
<td>83 (20%)</td>
</tr>
<tr>
<td>Wrong group antigen</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>Wrong group antigen + wrong blood group</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>Wound care error</td>
<td>47 (12%)</td>
</tr>
<tr>
<td>Transfusion error</td>
<td>42 (10%)</td>
</tr>
<tr>
<td>Wrong blood group + wrong blood group</td>
<td>23 (6%)</td>
</tr>
<tr>
<td>Other</td>
<td>16 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>392 (100%)</td>
</tr>
</tbody>
</table>

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


Hemolytic Transfusion Reactions

**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>50 (30%)</td>
</tr>
<tr>
<td>At bedside</td>
<td>68 (22%)</td>
</tr>
<tr>
<td>Subsequent blood request</td>
<td>55 (19%)</td>
</tr>
<tr>
<td>Transfusion team</td>
<td>11 (5%)</td>
</tr>
<tr>
<td>Other</td>
<td>76 (24%)</td>
</tr>
<tr>
<td>Total</td>
<td>316 (100%)</td>
</tr>
</tbody>
</table>

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

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

LIPID BILAYER
(PHOSPHOLIPIDS)

LIPID BILAYER
(PHOSPHOLIPIDS)

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)

STRUCTURES OF PHOSPHOLIPIDS

Phosphatidylcholine

\[
\begin{align*}
&\text{H}_2\text{C}=\text{O} \quad \text{H}_2\text{CO}-\text{C}=\text{O} \\
&\text{CH}_3 \quad \text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2 \quad \text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2 \quad \text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2
\end{align*}
\]

Sphingomyelin

\[
\begin{align*}
&\text{H}_2\text{CO} \quad \text{H}_2\text{CO} \\
&\text{CH}_3 \quad \text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2 \quad \text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2 \quad \text{CH}_2\text{CH}_2\text{CH}=\text{CH}_2 \quad \text{NH}_2 \quad \text{OH}
\end{align*}
\]
**STRUCTURES OF PHOSPHOLIPIDS**

- Head group
- Alkyl chain
- Alkyl chain
- 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

β-Gal

Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

α-Glc

Anomerity: α vs. β

MONOSACCHARIDE STRUCTURE

L-α-Fuc

Fucose = 6-deoxy-L-Gal

MONOSACCHARIDE STRUCTURE

β-Glc

Epimers: Gal vs. Glc

MONOSACCHARIDE STRUCTURE

β-Glc

Amino sugars
N-acetyl-glucosamine = GlcNAc
N-acetyl = CH3CONH-
**MONOSACCHARIDE STRUCTURE**

\( \beta\)-GlcNAc

---

**CARBOHYDRATE STRUCTURES**

**Type 2 H**

Fuc(\(\alpha\)-1-2)Gal(\(\beta\)-1-4)GlcNAc

---

**CARBOHYDRATE STRUCTURES**

**Disaccharides**

Glc(\(\beta\)-1-4)GlcNAc

---

**CARBOHYDRATE STRUCTURES**

**Type 2 Chain**

Gal(\(\beta\)-1-4)GlcNAc

---

**A**

Gal(\(\beta\)-1-4)GlcNAc-R

\ Fuc(\(\alpha\)-1-2)

Gal(\(\alpha\)-1-3)

**B**

Gal(\(\beta\)-1-4)GlcNAc-R

\ Fuc(\(\alpha\)-1-2)

**H**

Gal(\(\beta\)-1-4)GlcNAc-R

\ Fuc(\(\alpha\)-1-2)

---

**A**

Gal(\(\beta\)-1-4)GlcNAc-R

\ Fuc(\(\alpha\)-1-2)

Gal(\(\alpha\)-1-3)

**B**

Gal(\(\beta\)-1-4)GlcNAc-R

\ Fuc(\(\alpha\)-1-2)

**H**

Gal(\(\beta\)-1-4)GlcNAc-R

\ Fuc(\(\alpha\)-1-2)
A: \( \text{Gal}^{\alpha 1-3} \text{gal}^{\beta 1-4} \text{glcNAc-R} / \text{fuc}^{\alpha 1-2} \text{gal}^{\alpha 1-3} \text{gal}^{\beta 1-4} \text{glcNAc-R} / \text{fuc}^{\alpha 1-2} \text{gal}^{\beta 1-4} \text{glcNAc-R} / \text{fuc}^{\alpha 1-2} \)

B: \( \text{Gal}^{\beta 1-4} \text{glcNAc-R} / \text{fuc}^{\alpha 1-2} \text{Gal}^{\beta 1-4} \text{glcNAc-R} / \text{fuc}^{\alpha 1-2} \text{Gal}^{\beta 1-4} \text{glcNAc-R} / \text{fuc}^{\alpha 1-2} \)

H: \( \text{Gal}^{\beta 1-4} \text{glcNAc-R} / \text{fuc}^{\alpha 1-2} \)
**BIOSYNTHESIS OF BLOOD GROUP A GLYCOSYLATED**

<table>
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<tr>
<th>Ceramide</th>
<th>Glc(GlcNAc)GalCer</th>
<th>Lactosylceramide</th>
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<tr>
<td>GlcNAcGal[1-3]Glc[1-1′]Cer</td>
<td>Lacto-N-triosyl ceramide</td>
<td></td>
</tr>
<tr>
<td>GDP-Fuc</td>
<td>FUT1; H Type 2 transferase</td>
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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
- 7 coding exons
- Chromosome 9 q34
A: (α1-3) GalNAc-transferase (EC 2.4.1.40)

GalNAc(α1-3)

UDP-GalNAc + Gal(β1)-R \rightarrow Gal(β1)-R + UDP
Fuc(α1-2)
Fuc(α1-2)

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

Gal(α1-3)

UDP-Gal + Gal(β1)-R \rightarrow Gal(β1)-R + UDP
Fuc(α1-2)
Fuc(α1-2)

CHARACTERISTICS OF THE A AND B TRANSFERASES

STRUCTURE OF THE A AND B TRANSFERASES

Four Critical Residues

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


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


CRYSTAL STRUCTURE OF THE B TRANSFERASE

Hemolytic Transfusion Reactions

RBC + IgM

Complement activation

Intravascular hemolysis

“Magic happens”

Shock, renal failure, death
IgM-mediated HTRs: Role of complement

DIC Shock Renal dysfunction

E-IgM

C1q binding

C5 Æ C5b + C5a

C2 Æ C2a + C2b

C4b/C2b = C3 convertase

C4b/C2b/C3b = C5 convertase

C3 Æ C3a + C3b

C4 Æ C4a + C4b

E-IgM-C3b(C3bi)

IL8; ?other cytokines

Phagocytosis

Activated CR3

CR3 (αMβ2) on Kupffer cells, MΦ

LPS, PMA, PAF, MSP, fMLP

C5b-C9

Intravascular hemolysis

Free Hgb Membrane Ag-Ab complexes

C5aR

Chemokine secretion by endothelial cells

Vascular permeability ↑

FIII & P, E-selectin on endothelial cells

Kupffer cells (+ LPS)

IL6

Hepatocyte: ↑ C5aR + acute phase protein synthesis

Proximal tubular epithelium

PMN activation

TNFα, IL1, IL8

C3aR

Current treatment:

Prevention

Steroids, fluid, mannitol, IVlg

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

Current treatment:

Prevention

Steroids, fluid, mannitol, IVlg

Flagellation (self and other)

Prayer

Initiating Event

Ag-Ab interaction

Final Common Pathways

Renal dysfunction

DIC Shock Death

Proximal Consequences

Complement activation

RBC lysis

Hemoglobinemia

Fc receptors

Phagocytosis

SIRPα – CD47 interaction

Cytokine release

Acute phase reaction

Coagulation cascade

J’s in renal blood flow

NO function

Modifiers

Acute or chronic illness

DAF, C3b, etc.

Complement levels

Renal disease

Hypoproteinemia

Hypoxia (systemic or local)

Haptoglobin levels

SS High disease

Bystander hemolysis

Gender

Cytokine, complement, and FcR polymorphism
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 transplants
- Mild HDN, if any

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- K. Landsteiner: Vienna and Rockefeller
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- H. Clausen: Seattle
- F. Yamamoto: Seattle
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