Immunohematology
(Introduction)

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References:
- Blood Groups and Red Cell Antigens (Laura Dean)
- Cellular and molecular immunology, 8th edition

Modified from “Serotonin” version
Introduction

...to replace blood lost by hemorrhage or to correct defects caused by inadequate production of blood cells

• The major problem is the immune response of the recipient

• ABO alloantigens are the most important...not only on RBCs

• If the individual lacks blood group antigen, he will produce naturally IgM to that antigen...if given blood with that antigen: complement activation & transfusion reaction
Blood type and cross match

• Before a blood transfusion, two blood tests known as a "type and cross match" are done

...the recipient's **blood type** is determined, i.e., their ABO type and Rh D status

...donor blood may still be incompatible because it contains other antigens that are not routinely typed but may still cause a problem if the recipient's serum contains antibodies that will target them

...therefore, a "**cross match**" is done to ensure that the donor RBCs actually do match against the recipient's serum...see next slide
Cross match

- Small amount of the recipient's serum is mixed with a small amount of the donor RBCs

...the mixture is then examined under a microscope
...if incompatible, the donor RBCs are agglutinated by antibodies in the recipient's serum
Immune-mediated transfusion reactions

- Destruction of incompatible RBCs = hemolytic transfusion reaction
  ...acute or after days (delayed)
  can be life-threatening

- Destruction of incompatible donor white blood cells → febrile non-hemolytic transfusion reaction (FNHTR)
  mild and resolve by itself

- Destruction of incompatible donor platelets → post-transfusion purpura

Chills, fever, shaking, and aching are common to all these reactions.
It is better to use a specific blood component rather than whole blood. Also, all WBCs are now removed from donated blood to reduce infections and WBC incompatibility.
Hemolytic transfusion reactions

• Acute type:
  ...within 24 hours, often during the transfusion

  Intravascular hemolysis:
  - Most severe
  - Strong activation of complement...may cause shock
    ...and high fever due to TNF, IL-1, etc.
  - Hemoglobin released into plasma and excreted in urine (hemoglobinuria)...acute tubular necrosis (AKI)
  - Jaundice (bilirubin in blood)
  - Large amounts of tissue factor from destroyed RBCs ...DIC
  - Mainly against ABO antigens
    ...few other antigens (of the Kidd & P blood group systems

  Extravascular hemolysis:
  - RBCs are removed from the body by macrophages of liver & spleen
    - especially due to IgG Fc bound to their receptors on macrophages
  - the Rh blood group mediate this type of RBC removal
  - may also: Abs bound to C3b then bind C3b receptors on macrophages
    ...such antibodies include those directed against antigens of the ABO, Duffy, and Kidd blood groups

*slower & more controlled than intravascular hemolysis
...less released Hb in blood or urine and less bilirubin
less jaundice
...mainly chills & fever
Hemolytic transfusion reactions

• **Delayed type:**
  ...
  ...after 1-14 days

...the hemolysis is "delayed" because the antibodies are only present in low amounts initially

  ...although the recipient is also previously sensitized

...by the time a cross match is done, the level of antibody in the recipient's plasma is too low to cause agglutination, making this type of reaction difficult to prevent

...with time ➔ extravascular hemolysis

-Much less severe than acute reactions
-Especially against Kidd & Rh antigens
Other immune-mediated transfusion reactions

• **Transfusion associated lung injury (TRALI)**
  ...rare and occasionally fatal transfusion
  ...characterized by a sudden onset of shortness of breath
  ...antibodies from donor’s plasma attack WBCs of recipient
    ...these WBCs will accumulate in lung vasculature and secrete inflammatory mediators with resulting pulmonary edema
ABO Blood Group Antigens

• Carbohydrates
  ...attached to membrane proteins or lipids
  ...synthesized by polymorphic **glycosyltransferase** enzymes
  ...a common core glycan

H antigen
(= fucosylated glycan)
ABO Blood Group Antigens, cont’d

• OO individuals are said to be blood type O

• AA and AO individuals are blood type A

• BB and BO individuals are blood type B

• AB individuals are blood type AB

• What is Bombay blood group???
ABO Blood Group Antigens, cont’d

<table>
<thead>
<tr>
<th>Red blood cell type</th>
<th>Group A</th>
<th>Group B</th>
<th>Group AB</th>
<th>Group O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td><img src="image1.png" alt="Type A" /></td>
<td><img src="image2.png" alt="Type B" /></td>
<td><img src="image3.png" alt="Type AB" /></td>
<td><img src="image4.png" alt="Type O" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibodies present</th>
<th>Anti-B</th>
<th>Anti-A</th>
<th>None</th>
<th>Anti-A and Anti-B</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image5.png" alt="Anti-B" /></td>
<td><img src="image6.png" alt="Anti-A" /></td>
<td><img src="image7.png" alt="None" /></td>
<td><img src="image8.png" alt="Anti-A and Anti-B" /></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antigens present</th>
<th>A antigen</th>
<th>B antigen</th>
<th>A and B antigen</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image9.png" alt="A antigen" /></td>
<td><img src="image10.png" alt="B antigen" /></td>
<td><img src="image11.png" alt="A and B antigen" /></td>
<td><img src="image7.png" alt="None" /></td>
<td></td>
</tr>
</tbody>
</table>

Universal recipients

Universal donors
• ABO typing and solid organ transplants

...These antigens are also expressed on endothelial cells

...hyperacute rejection may occur

• ABO incompatibility between mother and fetus

..generally does not cause problems

...Why?
Other blood group antigens, *Rhesus (Rh) Antigen*

- Non-glycosylated, hydrophobic cell surface proteins

- Rh proteins are encoded by 2 genes...one of which is important here
  ...RhD $\Rightarrow$ 15% of the population have deleted or altered RhD allele
  ...these people are not tolerant to RhD antigen
  ...will make antibodies if exposed
## Other Rh antigens

<table>
<thead>
<tr>
<th>Number of antigens</th>
<th>49: D, C, E, c, and e are among the most significant</th>
</tr>
</thead>
</table>

The RhD and RhCE proteins are both transmembrane, multipass proteins that are integral to the RBC membrane. The RhCE protein encodes the C/c antigen (in the 2nd extracellular loop) and the E/e antigen (in the 4th extracellular loop), plus many other Rh antigens e.g., $C^w$, $C^x$.

Anti-D, anti-C, anti-e, and anti-c can cause severe hemolytic transfusion reactions.
Rhesus (Rh) Antigen, clinical significance

• If Rh-negative mother carries Rh-positive fetus...
  ...she will be sensitized by his RBCs...usually during birth
  ...IgM will be formed and will be class-switched to IgG
  ...next pregnancy: IgG will cross the placenta into the Rh-positive fetus
  ...hemolytic disease of the newborn (HDN) will result... = erythroblastosis fetalis

*Prevention: by administration of anti-RhD antibodies to the mother within 72 hours of birth of the first Rh-positive baby

- other Rh antigens, such as c, C, E, and e, can also cause problems
- HDN can also be caused by incompatibility of the ABO blood group
  ...usually less severe than Rh-caused
Coombs test

= antiglobulin test or AGT
...we use anti-human globulin (anti-Ig, “Coombs reagent”) in the final step and check if agglutination of RBCs occurs (visual detection)

*Direct...for diagnosing HDN
  or
  Indirect...for preventing HDN

*Can be also used for diagnosing autoimmune hemolytic anemia
Coombs test, cont’d

• Direct:
RBCs from fetus + anti-Ig from the lab → if agglutination occurs: (+ test)

• Indirect:
Serum from mother + RBCs that are Rh+ from the lab + anti-Ig from the lab → if agglutination occurs: (+ test)
Other blood group antigens

- Kell antigens
- Duffy antigens
- Kidd antigens
- MNS antigens
- Lewis antigens
Thank You