Antibodies and Antigens in the Blood Bank

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Outline

• Antibodies structure, classes and functions
• Most important Abs in the blood bank
• effective roles of Abs
• Zeta potential
• factors to influence agglutination in vitro
Why we study RBC Ag and Ab?

To avoid the immunological reaction between Ag on donor RBC and the naturally occurring Abs present in patient serum
Antibodies

• Proteins that recognize and bind to a particular Ag

• Belong to a group of serum proteins; immunoglobulins (Ig)

• Found in all body fluids; plasma, saliva, tears .. etc
Antibodies

• five classes in each individual;

• each class of Ab has at least 2 Ag binding sites (Paratopes)
Antibody structure
Antibody structure

• Basic structure unit in all classes:
  - 2 identical light chain
  - 2 identical heavy chain
• holds together by Covalent disulphide bonding
Antibody structure

Variable regions:
2 sections at the end of the ‘Y’ arm
Contain Ag binding sites (Fab)
Identical within the same Ab, dif form Ab to another
Antibody structure

- **Constant region**: lower part of Y arm

- **Fc region**: binds to Fc receptors on cells or activate complement pathways

   ![Cell lysis graphic]
Antibody classes

- classification based on dif in heavy chains
- 5 types of heavy chains .. 5 classes;
  - $\mu$ ; IgM
  - $\alpha$ ; IgA
  - $\gamma$ ; IgG
  - $\epsilon$ ; IgE
  - $\delta$ ; IgD
Antibody classes

• differ in their distribution, physiochemical properties, concentration and function,

• within some classes there is also subclasses
Antibody classes
Antibody fragments

- Ab when treated with certain enzymes cleaved into; FC and Fab
- they reflect the 2 main roles of Ab;
  - Fab: bind specifically to Ag
  - Fc: stimulates its elimination
Antibodies in the Blood Bank

- the most imp classes; IgG and IgM
- they can destroy transfused Ag-positive RBC, cause anaemia and transfusion reaction
IgM antibodies

- form most of anti-A and anti-B antibodies

- IgM Ab against A/B blood gp are Naturally occurring few months after birth

- probably as a result of exposure to ABH Ag-like substance in the diet or environment
IgM antibodies

• very effective in destroying Ag bec **can fix complement**

• pentamer molecule (5 Ig unites)

• so capable of binding up to 10 epitopes ??

• Pentavalent Ab .. Why ?
IgG antibody

• known as immune Ab
• formed in response to transfusion with non-self (incompatible) RBC
• monomer
• can cross the placenta
• 4 sub-classes (IgG1-4)
# IgM vs. IgG

<table>
<thead>
<tr>
<th></th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of heavy chain</strong></td>
<td>Mu</td>
<td>Gamma</td>
</tr>
<tr>
<td><strong>MW (kd)</strong></td>
<td>900</td>
<td>150</td>
</tr>
<tr>
<td><strong>Placental transfer</strong></td>
<td>No</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Complement fixation</strong></td>
<td>++++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Production</strong></td>
<td>Natural</td>
<td>Immune</td>
</tr>
<tr>
<td><strong>Temperature required</strong></td>
<td>Cold Ab (22C)</td>
<td>Warm Ab (37C)</td>
</tr>
</tbody>
</table>
IgM vs. IgG
Effector roles of Ab

- Induction of physical changes
- Cell lysis
- Phagocytosis
- Inflammation
Effector roles of Ab

• Induction of physical changes:

Ag-Ab binding to the surface of the RBC result in their clumping together

or Agglutination
Cell lysis

1. IgM and IgG activate complements → serious of reactions resulted in cell lysis (C3b)

2. ADCC; antibody-dependant-cellular-cytotoxicity (only IgG)
ADCC

- binding of FC region of IgG to certain receptor of lymphocytes
- activation of this lymphocytes results in releasing of its toxins
- toxins results in RBC lysis
- only mediated by IgG
Consequences of RBC Ag-Ab reaction
Consequences of RBC Ag-Ab reaction

Agglutination

Lysis by phagocytosis
Ag-Ab reaction in the blood bank

- to determine blood gp
- Ab screening
- compatibility testing

Detected by haemolysis or agglutination
Haemagglutination

- the major technique used in blood bank

- heam: blood  agglutination: clumping
  = red cell agglutination
haemagglutination

- Agglutination of RBC by Ab occur in 2 steps:
  1 - sensitization
  2 - agglutination
Sensitization

- first stage of agglutination; Ab bind to Ag

Stage 1 of agglutination:
binding of antibody to antigen
Agglutination

- when sensitized cells clump together resulting in the formation of visible agglutination
No agglutination

agglutination
Complete/incomplete Ab

• only IgM can bring about the 2 stages of Agglutination so the reaction is visible  
  (Complete Ab)

• IgG can bring sensitisation to RBC but not Agglutination  
  (incomplete Ab)
Zeta potential

• RBC carry –ve charge on their surface resulted in repulsive force (Zeta potential)

• cause RBC to stay apart (imp in vivo)

• in vitro we need to zeta potential to facilitate Agglutination
IgG can not form bridge because it is small in size.
Zeta potential

IgM can form bridges despite zeta potential because of its larger size.
Zeta potential
Factors influence agglutination reaction

- To help IgG in accomplishing the second stage of agglutination
- To enhance IgG and IgM agglutination
Centrifugation

- reaction timing by bringing reactants together
- allow RBC to overcome repulsive force
- places Ag and Ab in close proximity
Enhancement media

• required for IgG but not necessary for IgM

• increase reactivity of IgG by reducing zeta potential

• eg; albumin, low ionic strength solution (liss)
  polyethylene glycol, proteolytic enzymes and
  anti-human globulin reagent (AHG)
Addition of enzymes

1. Serum/plasma + cells
   - Red cells carry an overall negative charge
   - Cloud of positively charged ions surrounds red cell
   - Zeta potential keeps red cells apart
   - Sensitizing IgG antibodies

2. Serum/cell mixture + enzyme
   - (a) Addition of enzyme allows red cells to move closer together
   - (b) Zeta potential reduced
   - (c) IgG antibody molecules can reach adjacent red cells and cause agglutination
Other factors

• concentration of the reaction
• pH
• Temperature
• ionic strength
• Ab isotype
• RBC Ag dosage
Effect of pH

The ideal pH range is 6.5-7.5 similar of the pH of normal plasma or serum
Temperature

• dif Ab classes exhibit optimal reactivity at dif Tem.

• IgM react best at > 22°C (cold Ab)

• IgG usually require 37°C (warm Ab)
Detection of Ag-Ab reaction

- obtaining the right sample (most imp)

- dif tests may require dif sample types

- eg; measurement of complement fixation require serum sample

- complement activity requires presence of divalent ions Ca+2, Mg+2 which are chelated by some anticoagulants
Take home messages

Technical are more important!

No! Fundamentals are!
✓ 5 classes of Abs (at least 2 Paratobes in each) differ in their heavy chain

✓ Light and heavy chains linked together by Covalent disulphide

✓ functions of variable, constant;
  Fab: bind specifically to Ag
  Fc: stimulates its elimination

✓ Main differences bet. IgM and IgG
  IgM; naturally, bind to up to 5 Ag, cold can fix complement and reach the 2nd step of agg
  IgG; immune, only 2 Ag, warm Ab, cant reach the 2nd stage of Agg by it self

✓ 2 steps of agglutination; sensitization and agglutination

✓ Complete/ incomplete Abs ?

✓ Factors influence agglutinations;
  centrifugation, Liss, AHG, enzymes ...etc
Thanks for your attention..

And happy to answer your questions ???