Antibody Structure and Function*

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Anatomy and Physiology of Antibodies
Antibodies are gamma-globulins

Fig. 16-1. Electrophoresis of serum from a rabbit intensively immunized with ovalbumin, before (-----) and after (-----) removal of Ab molecules by specific precipitation with ovalbumin. [From Tiselius, A., and Kabat, E. A. J Exp Med 69:119 (1939).]
Ig Domain Structures
Ig Light Chains

- Two types of Light chains are found in Ig of all animals, amino acid sequence differs
  - Kappa chains - Human 60% (mice 95%)
  - Lambda chains - Human 40% (mice 5%)
Ig Heavy Chains

• 5 classes of H chains in humans
• Similarities in amino acid sequence, but each class has a unique sequence
• H chains named with Greek letters corresponding with the class name, IgG, IgA, IgM, IgE, IgD (γ, α, μ, ε, δ)
• IgG has 4 subclasses, IgA has 2 subclasses
Domains

- Early studies showed regularity of structure of all the Ig classes
- Each 100-110 aa has a 60 aa S-S loop
- V domains code the paratope, binds Ag
- C domains code regions important for mediating secondary biological functions, ie binding Complement, crossing the placenta.
**DOMAIN FUNCTIONS OF HUMAN IgG**

<table>
<thead>
<tr>
<th>Domain(s)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_H + V_L$</td>
<td>Antigen Binding</td>
</tr>
<tr>
<td>$C_{H1} + C_L$</td>
<td>Spacer between antigen-binding and effector functions</td>
</tr>
<tr>
<td>$C_{H2}$</td>
<td>Binding C1q</td>
</tr>
<tr>
<td>$C_{H3}$</td>
<td>Control of catabolic rate</td>
</tr>
<tr>
<td>$C_{H1} + C_{H3}$</td>
<td>Interaction with Fc receptor on macrophage/monocyte</td>
</tr>
<tr>
<td></td>
<td>Bind to Protein A</td>
</tr>
</tbody>
</table>
Ig Variable and Hypervariable Regions

• Amino terminal aa sequence was shown to vary from one Light chain to another
• Kabat and Wu developed the Variability Plot to measure degree of variation
• Found 3 Hypervariable regions in both L and H chain V regions.
• These are epitope contact aa regions, CDR (complementarity determining regions)
Definition of Variability

• The ratio of the number of different amino acids at a given position to the frequency of the most common amino acid at that position is defined as VARIABILITY.
Variability Plot

![Variability Plot]

- Variability
- Amino Acid Position
- CDR1
- CDR2
- CDR3
Ig Hinge Regions

• Hinge regions on IgG, IgA and IgD are coded by distinct exons
• Short span of amino acids between 1st and 2nd C domains
• Rich in Cys and Pro
• Provides for flexibility of the molecule
• Is readily accessible to solvent and enzymes
Ig Classes or Isotypes

- There are 5 major classes or isotypes of Ig in Humans:
  - IgG, IgA, IgM, IgE, IgD
  - Ig\(\gamma\), Ig\(\alpha\), Ig\(\mu\), Ig\(\varepsilon\), Ig\(\delta\)

- There are 4 subclasses of IgG isotypes in Humans
  - IgG1, IgG2, IgG3, IgG4
Ig Allotypes

- Allelic variants of Ig Constant regions
- Co-dominant autosomal Mendelian genes
- Are allotypic variants of γ, α, and κ chains
  - (small amino acid changes in constant regions)
- Allotypes used forensically
- Speculated that allotypic differences may confer some biological advantage for some infectious agents
Ig Superfamily

• There are structural similarities to Ig of the molecules of numerous membrane bound glycoprotein molecules such as the MHC molecules and the T cell receptor molecules. T cell receptors and triggering will be covered in another lecture.
## Features of Ig Isotypes

### TABLE 4.2. The Most Important Features of Immunoglobulin Isotypes

<table>
<thead>
<tr>
<th>Feature</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgD</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>150,000</td>
<td>160,000</td>
<td>900,000</td>
<td>180,000</td>
<td>200,000</td>
</tr>
<tr>
<td>Additional protein subunits</td>
<td>—</td>
<td>J and S</td>
<td>J</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Approximate concentration in serum (mg/mL)</td>
<td>12</td>
<td>1.8</td>
<td>1</td>
<td>0–0.04</td>
<td>0.00002</td>
</tr>
<tr>
<td>Percent of total Ig</td>
<td>80</td>
<td>13</td>
<td>6</td>
<td>0.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Distribution</td>
<td>~Equal: intravascular and extravascular</td>
<td>Intravascular and secretions</td>
<td>Mostly intravascular</td>
<td>Present on lymphocyte surface</td>
<td>Present on basophils and mast cells present in saliva and nasal secretions</td>
</tr>
<tr>
<td>Half-life (days)</td>
<td>23</td>
<td>5.5</td>
<td>5</td>
<td>2.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Placental passage</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Presence in secretion</td>
<td>—</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Presence in milk</td>
<td>+</td>
<td>+</td>
<td>0 to trace</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Activation of complement</td>
<td>+</td>
<td>—</td>
<td>+++</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Binding to Fc receptors on macrophages, polymorphonuclear cells, and NK(^a) cells</td>
<td>++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Relative agglutinating capacity</td>
<td>1</td>
<td>++</td>
<td>+++</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Antiviral activity</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Antibacterial activity (gram negative)</td>
<td>+++</td>
<td>++ (with lysozyme)</td>
<td>+++ (with complement)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Antitoxin activity</td>
<td>+++</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Allergic activity</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1 1</td>
</tr>
</tbody>
</table>

\(^a\) Natural killer
IgG Structural Features

IgM (pentamer)

IgA (dimer)

155 kDa
IgG Structural Features II
IgG Biological Properties

- Agglutination and Precipitation
- Antibody dependent cell mediated cytotoxicity
- Complement Activation and Opsonization
- Placental passage (only isotype to do so)
- Toxin/Viral Neutralization
- Bacterial immobilization
- Long serum half-life (~23 days)
Effect of Opsonizing Ab and C’

- IgG antibodies are efficient activators of the Complement system.
- Bacterial antigen-antibody interactions trigger a series of enzymes collectively known as Complement.
- Some of the by-products of these reactions can act as opsonins and other components are chemotactic (attract phagocytic cells).
- Net effect is greater uptake of pathogenic bacteria and clearance by phagocytic cells.

Figure 10.2. Effect of opsonizing antibody and complement on rate of clearance of virulent bacteria from the blood. The uncoated bacteria are phagocytosed rather slowly (innate immunity) but on coating with antibody, adherence to phagocytes is increased many-fold (acquired immunity). The adherence is somewhat less effective in animals temporarily depleted of complement.
Antibody Dependent Cell-Mediated Cytotoxicity (ADCC)
<table>
<thead>
<tr>
<th>Important Differences Between Human IgG Subclasses</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence (% total IgG)</td>
<td>70</td>
<td>20</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Half-life</td>
<td>23</td>
<td>23</td>
<td>7</td>
<td>23</td>
</tr>
<tr>
<td>Complement binding</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>—</td>
</tr>
<tr>
<td>Placental passage</td>
<td>++</td>
<td>±</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Binding of monocytes</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>±</td>
</tr>
</tbody>
</table>
IgG is Recycled by Fc Receptor Protector (FcRp)

Receptor recycling assists in **increased half-life** of IgG in circulation/serum
Clinical Tidbit: IgG and Placental Passage

• **Rh antigens**, also called Rhesus antigens, are transmembrane proteins expressed at the surface of erythrocytes (involved in carbon dioxide and/or ammonia transport across the plasma membrane).

• Red blood cells that express surface antigen D (RhD antigen) are called “Rh positive”.

• About 15% of the population have no RhD antigens (“Rh negative”).
Clinical Tidbit: IgG and Placental Passage

- A Rh negative mother who carries a Rh positive fetus runs the risk of producing immune antibodies to the Rh antigens on the fetal RBC. The exposure during primary pregnancy is minimized. However, the mother may generate Rh antibodies after birth if the mother comes into contact with fetal blood cells during placenta rupture.

- Upon subsequent pregnancies, the next Rh positive fetus will be at risk since the mother will retain a low level of circulating antibodies against the Rh antigen. Destruction of fetal erythrocytes will ensue by passive immune transfer of maternal antibodies to fetus, resulting in erythroblastosis fetalis (hemolytic disease of the newborn).
Clinical Tidbit: IgG and Placental Passage

- It is of great clinical importance to identify Rh mismatched mother and fetus; typically an indirect agglutination test is performed to identify isohemmagglutination.

- If positive, the mother is clinically treated immediately after giving birth with anti-Rh antibodies (Rh immune globulin (RhIG) or Rhogam) which reacts with the fetal RBC. Ensuing antibody-antigen complexes are removed prior to maternal recognition of foreign Rh antigen.
Clinical Correlation

- Hemolytic disease of the fetus/newborn. Maternal IgG antibodies specific for RhD are actively transported across the placenta, opsonize fetal RhD+ RBC for phagocytosis by liver cells, fetal hematocrit drops to dangerous or fatal levels.
IgG Neutralization

• **TOXIN NEUTRALIZATION:**
  – Bacterial **toxins** bind to specific cellular receptors to gain entry to the cell and then exert toxic effects intracellularly.
  
  – The strategy to protect the host from toxins is to make a variety of antibodies specific for different epitopes on the toxin to immobilize it in the form of an antigen-antibody aggregate, thus preventing the toxin from reaching the cell receptor.
  
  – The Ab-Ag aggregates can be easily phagocytosed (via Fc receptors) and the toxins degraded and rendered non-toxic by acid proteases in the phagosomes.
  
  – Note: this is the basis for using antigenically similar **TOXOIDS** to make vaccines that will elicit a strong immune response with no toxic effects.
IgG Neutralization

**BACTERIAL IMMOBILIZATION**
- Motile bacteria have movement arrested by IgG antibodies by cross-linking flagella or clumping them via flagella. The antibody functions like handcuffs in stopping the waving of flagella. The result is that the bacteria are less invasive and less efficient in spreading through tissue.

**VIRAL NEUTRALIZATION**
- Most viruses utilize some form of cellular receptor for initial binding to gain entry into the cell. IgG antibodies specific for viral structures that bind to cell receptors inhibit or eliminate initial binding to the cell, thereby protecting the cell from viral entry. The binding of IgG also facilitates phagocytosis of the organism, targeting for removal and destruction.
Review IgG

IgG Anatomy: Gamma Heavy Chains, κ/λ Light Chains

IgG Physiology: Agglutination, Placental Passage, Opsonization, ADCC, Complement Binding, Toxin/Viral Neutralization, Bacterial Immobilization, Recycled to allow greater serum half-life
IgM Structural Features

IgM is a pentameric molecule, linked by disulfide bonds.

IgM has an associated J chain.

900 kDa
IgM Biological Properties

• Efficient bacterial/viral agglutinator, potentially 10 paratopes/molecule
• Has a half-life of 5-10 days in serum
• Most efficient Ig for mediating Complement fixation
• Very effective at toxin neutralization
• Isohemagglutinin-naturally present Ab reactive with A/B blood groups, barrier to random transfusion & transplantation
IgM Antigen Binding

Efficient for agglutination, complement fixation, neutralization.
Blood Groups, IgM and Isohemagglutinins

• **ABO Blood Groups.** The ABO blood groups were first identified in 1901. They represent important antigens to be accounted for to assure safe blood transfusions.
• The ABO antigens represent carbohydrate moieties present on erythrocytes.
• Individuals naturally develop IgM antibodies (called *isoantibodies*) specific for ABO antigens that they do not express (cross-reactive to bacterial polysaccharides).
• If the individual receives a transfusion of blood that contains non-compatible ABO antigens, isoantibodies will cause agglutination of the donor cells.
• This process is referred to as *isoheamagglutination*; the antigens are sometimes called *isoheamagglutinins*.
## Blood Groups and Isohemagglutinins

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Erythrocyte Antigens</th>
<th>Serum Antibodies</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
<td>AA or AO</td>
</tr>
<tr>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
<td>BB or BO</td>
</tr>
<tr>
<td>AB</td>
<td>A and B</td>
<td>Neither</td>
<td>AB</td>
</tr>
<tr>
<td>O</td>
<td>Neither</td>
<td>Anti-A and anti-B</td>
<td>OO</td>
</tr>
</tbody>
</table>

Sugar moieties found on RBCs:

- O = universal donor

**Diagram:**

- Glucose
- Lipid or protein
- N-Acetylglucosamine
- O antigen
- N-Acetylgalactosamine
- A antigen
- B antigen
Review IgM

IgM Anatomy: Pentameric, high molecular weight

IgM Physiology: Agglutination, no Placental Passage, Opsonization, Complement fixation, Toxin/Viral Neutralization, Isohemagglutination
IgA Structural Features

160 kDa x2
IgA Subclasses

Two subclasses: IgA1 and IgA2.
- Differ in disulfide links between heavy and light chains.

- IgA has J chain.
- IgA has hinge region.
- IgA has associated secretory component.
Biological Properties of IgA Antibodies

• IgA is abundant on mucosal surfaces as a “First Line Defense”.
• IgA is bactericidal for Gram negative organisms in the presence of Lysozyme.
• IgA is an efficient viral agglutinator, preventing viral attachment to epithelial cell viral receptors.
IgA Secretion Mechanism

Plasma cell

Dimeric IgA

Poly Ig receptor

Epithelial cells

Lumen

Secretory component
Review IgA

IgA Anatomy: Dimer

IgA Physiology: Mucosal surfaces, bactericidal, viral agglutinator, does not fix complement
IgD Structural Features

180 kDa
Biological Properties of IgD Antibodies

- IgD is not found in significant amounts in serum; primarily found on surface of B cells.
- Principal function involves initial Ag triggering of B cells while bound to the membrane on the surface of B cells.
IgE Structural Features

200 kDa
Biological Properties of IgE Antibodies

- IgE mediates Type I hypersensitivity reactions.
- IgE antibodies bind to Fcε receptors on Mast cells.
- Ag binding with the IgE antibody induces degranulation, secretion of histamine, heparin, and other pharmacologic agents.
- High IgE levels occur during “large” parasitic infections (eg. helminths).
IgE Cross-linking Leads to Mast Cell Triggering and Degranulation

IgE Anti-DNP

DIVALENT DNP HAPten

Antibody to IgE Receptor

IgE RECEPTOR

Degranulation

MAST CELL

Degranulation

MAST CELL
Coda

• The function of antibodies, like the rest of the body, is only understood with a firm foundation in (molecular) anatomy.

• The 5 Ig isotypes each mediate specific biological effects, due to different C region amino acid sequences in their respective H chain.

  – Review Chart: Features of Ig Isotypes!
<table>
<thead>
<tr>
<th>Structure</th>
<th>Pentamer</th>
<th>Monomer</th>
<th>Monomer</th>
<th>Monomer</th>
<th>Monomer, Dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy Chain Designation</td>
<td>μ</td>
<td>δ</td>
<td>γ</td>
<td>ε</td>
<td>α</td>
</tr>
<tr>
<td>Molecular Weight (kDa)</td>
<td>970</td>
<td>184</td>
<td>146-165</td>
<td>188</td>
<td>160 x 2</td>
</tr>
<tr>
<td>Serum concentration (mg/ml)</td>
<td>1.5</td>
<td>0.03</td>
<td>0.5-10.0</td>
<td>&lt;0.0001</td>
<td>0.5-3.0</td>
</tr>
<tr>
<td>Serum half-life (days)</td>
<td>5-10</td>
<td>3</td>
<td>7-23</td>
<td>2.5</td>
<td>6</td>
</tr>
<tr>
<td>J chain</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Complement activation</td>
<td>Strong</td>
<td>No</td>
<td>Yes, except IgG4</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Bacterial toxin neutralization</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Antiviral activity</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Binding to mast cells and basophils</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
Which immunoglobulin isotype has the highest molecular weight?
A. IgM
B. IgD
C. IgG
D. IgE
E. IgA

Option A (IgM) is correct. IgM is found on the surface of B lymphocytes, and secreted as a pentameric molecule of approximately 970 kdaltons. IgM is extremely effective at fixing complement, and is an effective agglutininator of particulate antigens because of the high number (10) of antigen specific binding sites.
A 34 year old caucasian woman gives birth to a first child who is identified as being Rh-positive. Although the mother is Rh-negative, she was not treated after birth with Rhogram (anti-Rh antibodies). During the next pregnancy, the fetus is found to have erythroblastosis fetalis. Which isotype of maternal immunoglobulin is responsible for the hemolytic disease of the newborn?

A. IgM  
B. IgD  
C. IgE  
D. IgG  
E. IgA

Option D (IgG) is correct. A Rh negative mother who carries a Rh positive fetus runs the risk of producing immune antibodies to the Rh antigens on the fetal RBC. The exposure during primary pregnancy is minimized; the mother can generate anti-Rh antibodies after birth if she contacts fetal blood cells during placenta rupture. Upon subsequent pregnancies, the next Rh positive fetus will be at risk since the mother will retain a low level of circulating antibodies against the Rh antigen. Maternal IgG antibodies specific for RhD are actively transported across the placenta, opsonize fetal RhD+ RBC for phagocytosis by liver cells, fetal hematocrit drops to dangerous or fatal levels. Only IgG has significant placental passage to cause this effect.
A person having Type O blood is best described as:
A. Having erythrocyte antigens A and B, and having anti-A and anti-B antibodies.
B. Having erythrocyte antigens A and B, but not having anti-A and anti-B antibodies.
C. Having no erythrocyte antigens, but not having anti-A and anti-B antibodies.
D. Having no erythrocyte antigens, and having anti-A and anti-B antibodies.

Option D (Having no erythrocyte antigens, and having anti-A and anti-B antibodies) is correct. The ABO blood groups represent important carbohydrate moieties present on erythrocytes to be accounted for to assure safe blood transfusions. Individuals naturally develop antibodies (called isoantibodies) specific for ABO antigens that they do not express (due to cross-reactivity with bacterial polysaccharides). If the individual receives a transfusion of blood that contains non-compatible ABO antigens, isoantibodies will cause agglutination of the donor cells, a process referred to as isohemagglutination; the antigens are sometimes called isohemagglutinins.
Which organ serves as both a primary and secondary lymphoid organ?

A. Peyer’s Patches
B. Tonsils
C. Appendix
D. Thymus
E. Bone Marrow

Option E (Bone Marrow) is correct. Islands of haemopoietic progenitor cells in the adult bone marrow give rise directly to polymorphonuclear cells, mononcytes, dendritic cells, B lymphocytes and precursor T lymphocytes. Although the bone marrow is technically a primary lymphoid organ, recirculation due to vascularization enable entry of circulating leukocytes from peripheral tissue, thus also allowing bone marrow to serve a secondary organ function.