Foundations of Public Health
Immunology

Antibodies
Structure & Functions
Objectives

• Identify the primary and secondary effector functions of antibodies
• Describe the principles of antibody diversity and maturation of B cells into plasma cells
• Identify the structure and function of antibody molecules
• Identify the function of each antibody class
• Describe antigen-antibody binding & antigen recognition
Humoral Immunity

• A major component of **acquired immunity**, also called Antibody Mediated Immunity (AMI)

• B lymphocytes produce antibodies that target antigens (microbes)

• **Principal defense against extracellular microbes & their toxins**
Antibodies

• **Definition:** type of glycoprotein molecule produced by mature B cells
  • Also called immunoglobulin (Ig)
  • Basic structure forms a Y shape

• **Primary function is to bind antigens**
  • Often with high specificity & affinity

• There are five classes of antibodies: IgM, IgG, IgA, IgD, & IgE
Important Terms:

- **Antibody**: Implies a function (binding to antigen)
- **Glycoprotein**: chemical description
- **Gammaglobulin**: physical characteristic (electrophoretic mobility)
- **Immunoglobulin**: implies general function
- **Isotype**: “class” of antibody (IgG)
  - Subclasses: IgG (1-4) and IgA (1 & 2)
- **Idiotype**: specificity for antigen (Fab region)
- **Fc receptors**: receptors on cells which bind Ab

Top photo: Normal serum electrophoresis immunoblot with IgG polyclonal “smear” (lane 2).

Middle photo: Myeloma patient w/ tumors producing significant amounts of monoclonal IgG, as shown by immunoblot electrophoresis (thick condensed band of IgG) & elevated lambda light chain (lane 6).

Bottom photo: Myeloma patient with elevated gamma globulin, as shown by serum electrophoresis.
Medically Important Terms:

- **Allotype**: Differences between individuals (alleles)
- **Antiserum**: serum containing a variety of antibodies specific for certain antigens (sometimes used therapeutically)
- **Polyclonal antibody**: mixture of antibodies made by several different clones of plasma cells, slightly different specificities for Ag
- **Monoclonal antibody**: antibodies made by a single clone of plasma cells, same specificity for Ag; a laboratory technique
Antibody: The Basics

• Each person has **millions of different antibodies**

• Each antibody has **unique** antigen binding sites (high specificity)

• Antibodies can remain bound to the B cell surface (known as the BCR complex) to recognize antigen

• Or antibodies can be secreted to perform **effector functions**!
Secondary Effector Functions

- Activate and focus complement & promote inflammation
- Protective against EXTRACELLULAR pathogens (too large to pass cell membrane)
- Neutralize viruses (secondary infection)
Secondary Effector Functions Cont’d

- **Bactericidal** with and without complement
- Activate mast cells & Type 1 hypersensitivity rxns (IgE – allergy)
- **Inactivate enzymes & toxins**
- **Enhance phagocytosis**: opsonization
- Promote killing of cells: ADCC
- Agglutinate Antigen (IgM)
- Important **diagnostic**, research, & epidemiologic tools!!
B Cells to Plasma Cells

- New B cells are pre-programmed to recognize an antigen before exposure
- Random gene-splicing reactions occur early in the development of each B cell (somatic recombination)
- Produces a B cell receptor or Fab that has a unique 3-D shape to fit the matching epitope

Fig 4-10: Somatic recombination of immunoglobulin genes leads to diversity
**B Cells to Plasma Cells**

- B cell receives **first signal** for action, when antigen binds to the B cell receptor (Fab region)
- **Second signals** activate the B cell to proliferate, once cytokines are released from T cell help
- **Additional mutations** can occur in the **Fab region** at this time (**somatic hypermutation**) to increase antibody diversity
- If new mutated antibody has a **better fit** to the antigen, the B cell receives stronger signals to mature into a plasma cell
Plasma Cells

- Plasma cell becomes a **factory to produce antibodies**
- Antibodies all have **same specificity** for a single antigen
- Plasma cell **secretes thousands of antibodies** per second
Antibody Structure

- Composed of **two identical light** (light green) and **two identical heavy chains** (dark green)
- **Fab** region binds antigen
- **Fc** region leads to an action
Structure = Function

• Fab has **variable** amino-terminal regions which **specifically recognize** epitope

• Fc region determines **what action** is taken once an antigen is bound, including:
  • Complement activation
  • Cellular activation (engage killer cells)
  • Opsonization (enhanced phagocytosis)
  • ADCC (antibody-dependent cellular cytotoxicity)
Antibody Molecule

- The Fc region is responsible for the biological activity of an antibody
Importance of Antibody Structure

• Antibodies are complex structures
• Variability allows for binding a diverse array of antigens (primary function)
• Fc region packs a punch – allows the antibody to interact with other immune cells & complement to enhance the immune response against the antigen
• In effect, the antibody directly links the antigen to an immune action against it
Focus on Isotypes & Idiotypes

• **One cell – one antibody rule**
  • Genetic variability “programs” the B cell

• **Isotypes**
  • Determined by C region of the H chain
  • Structural and functional differences
  • **5 classes**: IgG, IgA, IgM, IgD, IgE

• **Idiotypes**
  • Differences in *Fab* region
  • B cell **only** makes one *idiotype* specific to one Ag determinant
  • B cell can still make other *isotypes* of the same specificity later (affinity maturation)
Antibody Classes (Isotypes)
IgM

• **First antibody produced** in primary response to antigen
• Produced by newborn babies
• Indicates infection after birth
• **Largest** Ab molecule, pentamer has 10 antigen binding sites
• 5-10% of total serum Ig
• Fixes complement to initiate classical pathway
**IgG**

- **Second antibody produced** in immune response to antigen
- **Most abundant** antibody in sera & bodily fluids, ~75%
- Neutralizing antibody that may provide **long-term immunity**
- Passes through placenta to protect fetus *in utero* (the only Ig class that can)

**Note:** Immunity is often not life-long! Antibody titers may decrease over time. Consequently, booster shots are often necessary for some vaccines to re-build antibody titers during your lifetime! The length of immunity is related to the agent, as well as your individual immune response.
IgA

- Monomeric form, present in serum 10-15%
- Dimeric form, *secretory* IgA
- Predominant isotype in external secretions
- Present in mucous, saliva, tears, and breast milk
- Provides important line of defense to prevent entry of antigens along mucous membrane barriers
IgE

- Very low concentration in serum, less than 1%
- IgE **binds to mast cells**, containing granules & histamine, which will be released when encounters antigens
- Symptoms of asthma, hay fever, & other allergies result from this action
- Also, very important in the **defense against parasitic infections**
IgD

- Very low concentration in serum, less than 1%
- **Major membrane-bound** Ig on mature B cells (IgM also)
- Membrane-bound antibodies recognize antigens to stimulate humoral immune responses
- Remember, not all antibodies are secreted!!!
<table>
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<th>IgM</th>
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- IgG: Most abundant Ig of internal body fluids particularly extravascular where it combats microorganisms and their toxins.
- IgA: Major Ig in sero-mucous secretions where it defends external body surfaces.
- IgM: Very effective agglutinator; produced early in immune response – effective first-line defence vs. bacteremia.
- IgD: Most, if not all present on lymphocyte surface.
Basis of Humoral Immunity

• Five classes of antibodies to **protect different areas** of the body

• The **structure** of antibodies allows them to recognize antigen & then trigger a response to fight the microbe

• Primary & secondary immune responses are critical in the **development of immunologic memory** and to initially defeat the invader (covered next week)
• Antigens enter the blood stream...
• T lymphocytes (& B cells, not shown) recognize the antigen
• Signals humoral immune response to produce antibodies
• Plasma cells, the manufacturers, make & secrete antibodies
• Antibodies coat the bacteria
Introduction to Antigen Recognition

• From the antibody’s perspective:
  • Recognition of antigens
  • Antigen-antibody interactions
Antigen Recognition

• How does the antibody bind the antigen?
• Antigens made of proteins, rather than polysaccharides or lipids, usually elicit the best response
• Antibodies target a particular region of the antigen, called the epitope
Immunodominant Epitope

- Antigen molecules have regions of differing antigenicity: most Abs are formed to the **region of highest antigenicity** (also the site to which T lymphocytes respond)
  - Exposed regions lacking rigid structures
  - Suggests that Ag – Ab binding requires flexibility for maximum fit
Antigen Recognition

• Each antibody has **at least 2 sites** to bind antigen (the arms of the Y), IgM antibodies can bind more.

• Antibodies may bind to several epitopes on a single antigen.

• It is estimated that you **B cells can recognize 10^8 different epitopes**.
Epitopes & Antibodies
**Antigen Recognition**

- **Antibodies** recognize antigen in native configuration (overall shape of the epitope) **in solution or on cell surfaces**
- T lymphocytes (TCR) recognizes Ag only in association with MHC proteins on cell surface, fewer epitopes
- T lymphocytes have more restrictions than B cells & antibodies!!
- After antibodies bind antigen, the **secondary effector functions** come into play
Antigen-Antibody Binding

- Formation of numerous reversible, noncovalent attraction between Ag epitope and hypervariable regions at Fab end
  - Hydrogen bonds, Van der Walls forces & hydrophobic interactions
  - Requires complementary configuration and close fit
Antibody Affinity

- Strength of a **single** Ab – Ag bond
- Sum of attractive & repulsive forces at a single antigenic determinant and combining site
- **High affinity binding is superior**, creates a better fit (see figure, ag-ab bond is better because rectangular shape of epitope closely resembles ab)
- Affinity maturation, improving the fit of Abs, occurs as the immune response progresses
Antibody Avidity

- Overall strength of binding of a **multivalent** antibody to multivalent Ag
- Greater than the sum of all affinities
- For example, the avidity of IgM antibody (with 10 binding sites) is usually greater than IgG for the same antigen
Antibody Cross-Reactivity

- Except there is one small problem:
- Epitopes are **shared** by more than one antigen
- A proportion of antibodies will bind with several Ags
- Not a perfect system – but sometimes results in cross-protection to closely related microbes!!
- Also impacts diagnostic assays that are used to determine cause of infection
In Summary

• Understand the structure & functions of Abs
• Know the Ab classes, function of each type
• Understand mechanism of antigen-antibody binding
Self-Test Questions

• What is the humoral immunity?
• What is a plasma cell? What is its main function?
• Describe the structure of an antibody. What region is biologically active?
• What is an isotype? An idiotype? Name the 5 isotypes of antibodies.
• What is the first antibody class made in an immune response?
• What is an epitope? What types of bonds are involved in antigen-antibody interactions?
• What is antibody affinity?