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

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

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 idioype specific to one Ag determinant
  - B cell can still make other isotypes of the same specificity later (affinity maturation)
**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!!!
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)

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 10⁸ 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 idiotypic? 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?