Slide 1

**Foundations of Public Health Immunology**

**Antibodies**
- Structure & Functions

Slide 2

**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

Slide 3

**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

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**SLIDE 1** Antibodies. This presentation will discuss the structure and functions of antibodies.

**SLIDE 2** We will focus on these topics related to humoral immunity. Emphasis for the quiz & activities will be on antibody structure & function, antibody classes, and antigen recognition.

**SLIDE 3** The humoral immune response is critical to defend the body from microbes- it is a specific protein (antibody) mediated response to an antigen. Antibodies are relatively large molecules & cannot pass through the cell membrane. Consequently, their role in the immune response is to neutralize extracellular microbes. Humoral immunity is most effective against bacteria, bacterial toxins, and viruses before these agents enter cells.
Slide 4

**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

SLIDE 4 The primary function of antibodies is to bind antigen. This function is carried out by the Fab region of the antibody. The Fab region supplies antigen specificity for the antibody. With the binding of the antibody to an antigen, this triggers secondary effector functions. These functions include complement activation, immune cell activation, opsonization, and antibody-dependent cellular cytotoxicity (ADCC).

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Slide 5

**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

SLIDE 5 These are important terms to know in conjunction with antibodies. For example, glycoprotein indicates that an antibody is composed of both protein and “sugar”. Immunoglobulin indicates that an antibody is an immune protein.

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Slide 6

**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

SLIDE 6 These are further terms to understand in conjunction with antibodies. Antibody specificity of an antiserum refers to the sum of the specificities of all the antibodies in a serum. Antiserums are composed of polyclonal antibodies that have been produced by different B-cells; therefore it is a "population effect". The reaction produced is due to all the different antibodies and not to just to one particular clone of antibodies.
Slide 7

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!

Slide 8

SLIDE 7 Antibodies exist free in body fluids (serum, lymph, tears, & saliva, for example) and membrane bound to B lymphocytes. When membrane bound, they function to capture antigen for which they have specificity, alter which the B lymphocytes will take the antigen into its cytoplasm for further processing.

SLIDE 8 Secreted antibodies use these secondary effector functions to fight the infection. Antibodies perform many functions as described on this slide. These functions include the activation and targeting of complement, promotion of inflammation, destruction of extracellular pathogens, virus neutralization, activation of mast cells in type 1 hypersensitivity reactions, and inactivation of enzymes and toxins. Antibodies are active against only extracellular pathogens because antibodies are too large to enter cells.

Slide 9

SLIDE 9 Opsonization (enhanced attachment) refers to the antibody molecules IgG, IgE and the complement proteins C3b and C4b (remember that the “b” fragments bind?!) attaching antigens to phagocytes. This allows for more efficient phagocytosis. ADCC stands for antibody dependent cellular cytotoxicity. Natural killer (NK) cells are capable of ADCC as they have receptors on their surface for the Fc portion of IgG. When IgG is made to epitopes on "foreign" membrane-bound cells, especially virus-infected cells and cancer cells, the Fab portions of the antibodies react with the "foreign" cell. NK cells then release enzymes to kill the abnormal cell. Neutrophils, eosinophils, & phagocytes also mediate ADCC.
Slide 10

**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.

**SLIDE 10** Ig genes rearrange during B cell development. The parts of genomic DNA with antibody genes in embryonic tissue, or adult non-B cells, are larger than those from mature B cells. This means that the DNA from mature B cells is rearranged during development resulting in the excision of some DNA. The removal of some DNA is done in a precise order. First the heavy chain rearranges: 1) D -> J 2) V -> DJ (then if a functional heavy chain [always IgM initially] results then the light chains rearrange also in order) 3) V -> J.

Slide 11

**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.

**SLIDE 11** One of the defining features of the immune system is that it adapts to improve its recognition of pathogens during an ongoing immune response. Somatic hypermutations are additional point mutations in the Ig heavy & light chains in the germinal center of B cells that can lead to increased affinity for the antigen.

Slide 12

**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.

**SLIDE 12** Plasma cells are the powerhouses. Structurally they are different from B cells, as they have an extensive Golgi apparatus to synthesize thousands of antibody proteins. This slide also includes a diagram of the immune response & plasma cell development. Plasma cells can produce up to 2000 antibody molecules a second!!
SLIDE 13 We now will concentrate on how the structure of an antibody (even at the genetic level) greatly impacts function. Antibodies are complex structures, with a Y shape, that specifically bind antigen & a constant region that has biologic activity. The arms of the Y have variable regions at the end which specifically will bind one antigen. This region is called the Fab region, for antigen binding. The Fc region is a constant region that connects the antibody-antigen to a result. For example, it will bind to a phagocyte so that the phagocyte can take care of the antigen and remove it from the body. This slide shows the immunoglobulin molecule. Notice the different fragments of the immunoglobulin including the heavy (H) chains and light (L) chains, the Fab regions, and Fc region.

SLIDE 14 Again, the Fab region will specifically recognize an epitope (a small part of the antigen). The Fc region then links the antigen to a biologic action that will clear the infection.

SLIDE 15 Diagram of an antibody molecule. Note the constant portion (Fc) of the antibody.
**Slide 16**

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

**Slide 17**

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)

There is a great diversity of antigens; and, the antibodies must be able to respond to this diversity. Normally, once a lymphocyte has been stimulated to produce antibodies, it produces a single clone of antibodies against a particular antigen. This is called clonal selection and can be thought of as the “One Cell- One Ab Rule”. This process is accomplished through several processes including somatic hypermutation and gene recombination. Somatic hypermutation refers to frequently occurring point mutations that occur within the heavy and light chains of immunoglobulins found in germinal center B-cells. Gene recombination refers to the recombining of genes that occurs in the variable regions of antigen receptors during lymphocyte development. Isotypes are also known as the “class” of antibody (IgM, IgG, IgA, IgD, IgE). One B cell can make more than one isotype (we will discuss isotype switching later). However, B cells have been genetically programmed to only make a single idiotype, or Fab region. This provides the acquired immune response with very high specificity for an antigen. So even though the B cell can switch and make IgM and IgG antibodies, they will both have the same Fab region & bind the same antigen.
SLIDE 18 Diagram of each of the five classes of antibodies. Especially note the size and structural differences among the five isotypes.

SLIDE 19 IgM is the first antibody produced following an infection. IgM antibodies are the largest type of antibody. IgM does not have high affinity for antigen, and is not long lasting. IgM antibody levels in patient sera can be used to diagnose recent infections.

SLIDE 20 IgG antibodies are found in all body fluids. IgG are the smallest but most abundant of the antibodies, normally comprising about 75% to 80% of all the antibodies in the body. They are the most important antibodies for fighting bacterial and viral infections, as they are neutralizing and remain elevated for years. IgG antibodies are the only type of antibody that can cross the placenta. Therefore, the IgG antibodies of a pregnant woman can also help protect the fetus.
**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 an important line of defense to prevent entry of antigens along mucous membrane barriers

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**SLIDE 21** IgA antibodies are found mainly in mucosal areas of the body, such as the nose, breathing passages, digestive tract, ears, eyes, and vagina. IgA protects body surfaces that are exposed to the outside from foreign organisms and substances, and is found in saliva and tears. IgA accounts for approximately 10% to 15% of the antibodies usually present in the body. A small percentage of people do not make IgA antibodies.

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**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

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**SLIDE 22** IgE antibodies are found in the lungs, skin, and mucous membranes. They cause the body to react against foreign substances such as pollen, fungus spores, and animal dander. People with allergies often have elevated levels of IgE in their serum.

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**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!!!

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**SLIDE 23** IgD antibodies are found in small amounts in the tissues that line the abdominal or chest cavity of the body. Although the function of IgD antibodies is not well-understood, they appear to play a role in allergic reactions to some substances such as milk, some medications, and some poisons.
### Slide 24

**This slide provides the major characteristics of the immunoglobulin classes.** For example, IgG is the most common immunoglobulin. IgA is the immunoglobulin that is secreted. IgM is the immunoglobulin produced earliest in immune responses. IgD is present on most lymphocyte surfaces. IgE is commonly found during parasitic and allergic reactions.

### Slide 25

**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)

**SLIDE 25** The humoral immune response is very important in maintaining a healthy body. This response is designed to improve over time, so that when antigen is encountered again, the response will be so rapid, you may not notice any symptoms!

### Slide 26

**Basis of Humoral Immunity**
- Antigens enter the bloodstream...
- 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

**SLIDE 26** A diagram of both humoral & cell-mediated immune responses alter a pathogen enters the body. This slide describes the transfer of processed antigen between immune cells. Antigen-presenting cells (APCs) engulf antigens and then present the processed antigen to T-cells. These primed T-cells then activate B-cells which proceed to mature into plasma cells and memory B-cells.
Slide 27

Introduction to Antigen Recognition

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

SLIDE 27 These are important topics concerning the antigen-antibody interaction that will be covered in the succeeding slides. Antigen recognition is extremely important for B cells, antibodies, and T cells.

Slide 28

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

SLIDE 28 A few characteristics of antigens are shown here, to give you an idea of what the antibody will bind to.

Slide 29

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

SLIDE 29 An immunodominant epitope is a specific region of an antigen to which most antibodies are formed or to which most T-cells respond. It can involve an exposed region that lacks rigid structures and suggests that antigen-antibody 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

SLIDE 30 The variable regions and diversity of B cells allows for a huge stockpile of weapons to fight millions & millions of antigens, even the new diseases.

Epitopes & Antibodies

SLIDE 31 This Diagram shows that a single antigen can have multiple epitopes on its surface that different antibodies bind. Notice that the antigenic determinants (epitopes) are all shaped differently.

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

SLIDE 32 Antibodies can recognize an antigen in its native configuration or its overall epitope shape in both fluid or on cell surfaces. In contrast, T-cell receptors (TCR) can only recognize antigen in association with the major histocompatibility (MHC) proteins on cell surfaces and are responsive to fewer epitopes.
**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
Slide 36

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

Slide 37

In Summary
- Understand the structure & functions of Abs
- Know the Ab classes, function of each type
- Understand mechanism of antigen-antibody binding

Slide 38

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?