Lec.7: Immunoglobulins

A**ntibody** (Ab), also known as an **immunoglobulin** (Ig), Y-shaped protein produced mainly by plasma cells that is used by the immune system to identify and neutralize pathogens such as bacteria and viruses.

The Immunoglobulins are produced by plasma cells in response to exposure to as antigen. They react specifically with that antigen in vivo or in vitro and are hence a part of the adaptive immune response-specifically, humoral immunity.

The antibodies belong to a class of protein called "globulins" due to their "globular structure". Antibodies present in the γ-globulin fraction of serum [when serum is subjected to electrophoresis (separation of proteins according to their charges in an electrical field), proteins which migrate faster to the anode (+ve) is called α−globulin, and β−globlulin, while those migrate but slower, towards the anode is called γ−globulin] .Later it was shown that antibody activity is present not only in the gamma-globulin fraction but also in a slightly more anodic area. Today antibodies are collectively known as immunoglobulins (Igs).

Antibodies can be found in blood plasma and in many body fluids (e.g., tears, saliva, colostrum).

IMMUNOGLOBULINS STRUCTURE

- Antibodies are Y-shaped protein.
- All antibodies share a common structure of four polypeptide chains, consisting of two identical **light (L) chains** and two identical **heavy (H) chains ,** the arms of the tip join with the tail by hinge region.
- Each light chain is bound to its partner heavy chain by a disulfide bond between corresponding cysteine residues, as well as by non covalent interactions between the VH and VL domains and the CH1 and CL domains. These bonds enable the formation of a closely associated heterodimer (H-L).
- Multiple disulfide bridges link the two heavy chains together about halfway down their length, and the C-terminal parts of the two heavy chains also

participate in non covalent bonding interactions between corresponding domains.

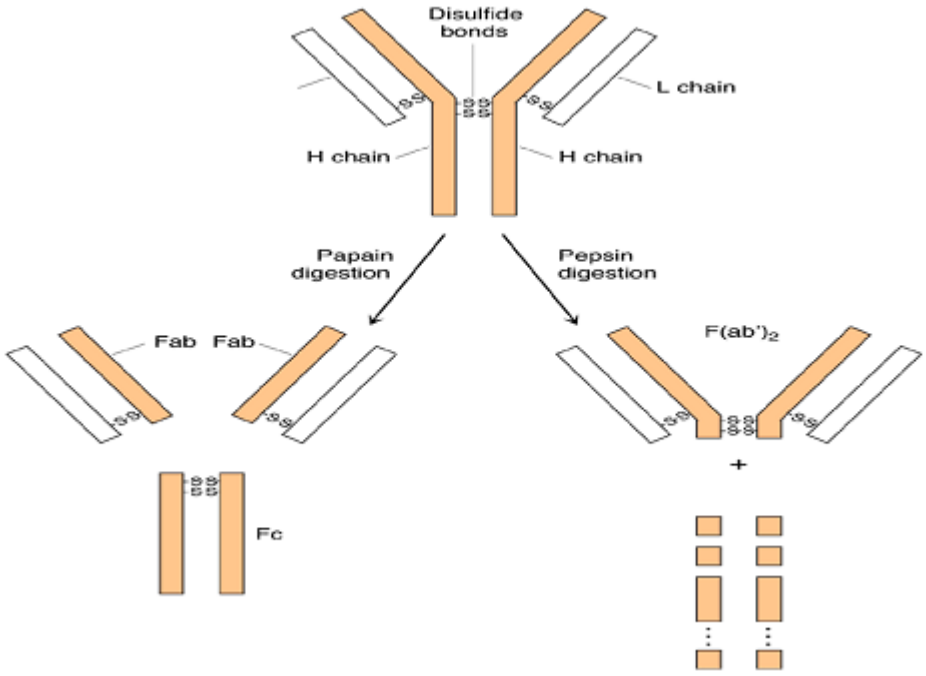
- The antibody molecule forms a Y shape with two identical antigen-binding regions at the tips of the Y.
- Each antigen-binding region is made up of amino acids derived from both the heavy- and the light chain amino-terminal domains .
- The heavy and light chains both contribute two domains to each arm of the Y, with the non–antigen-binding domain of each chain serving to extend the antigen-binding arm.
- The base of the Y consists of the C-terminal domains of the antibody heavy chain.

Fragments of antibody

Enzymes such as papain and pepsin, each splits the immunoglobulin molecule into definable fragments:

- **Papain** split the monomeric basic unit into three fragments of approximately equal size at the hinge region.
  The hinge region is particularly susceptible to proteolytic cleavage by the enzyme papain. Papain cleavage resolves the antibody molecule into two identical fragments that retain the antigen-binding specificity of the original antibody (called **Fab regions**), and the remaining region of the molecule, which consists of the non antigen-binding portion. This latter region, which is identical for all antibodies of a given class, crystallizes easily and was thus called the **Fc region** (fragment *c*rystallizable).

- **Pepsin** digests most of the Fc fragment, leaving one large fragment termed the F(ab')2 fragment which consists of two Fab fragments joined by covalent bonds and has two antigen-binding sites; thus it is bivalent, possessing the ability to bind and precipitate an antigen.



Immunoglobulin fragments, by the effect of papain and pepsin enzymes

The antibody recognizes a unique molecule of the harmful agent, called an antigen, via the variable region. Each tip of the "Y" of an antibody contains a paratope(analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. The ability of an antibody to communicate with the other components of the immune system is mediated via its Fc region (located at the base of the "Y").

***The paratope** is the area of the immunoglobulin molecule which interacts specifically with the epitope of the antigen .

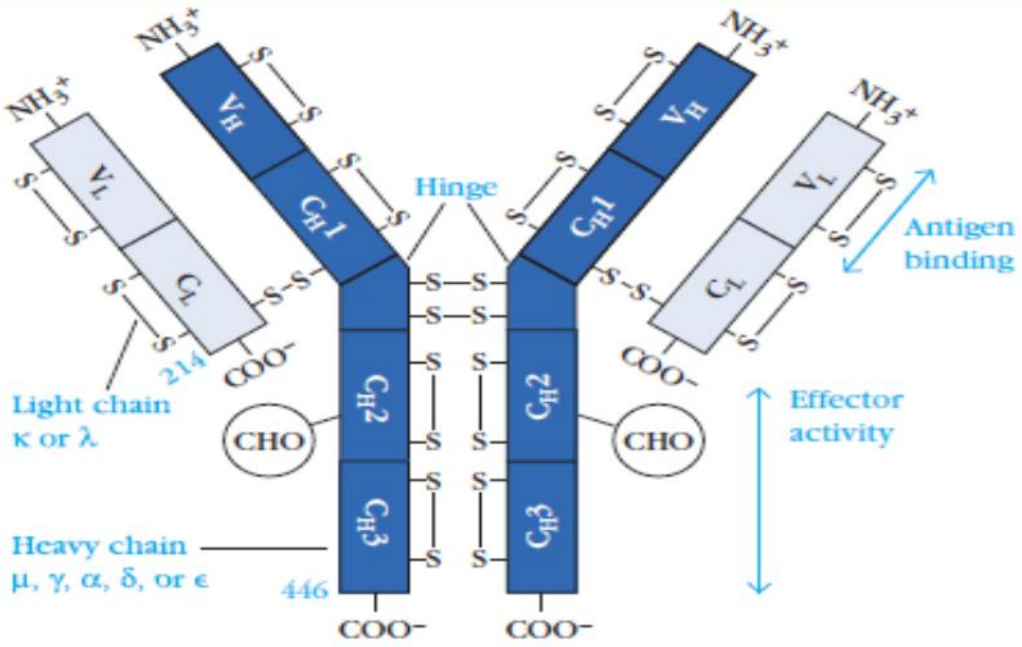
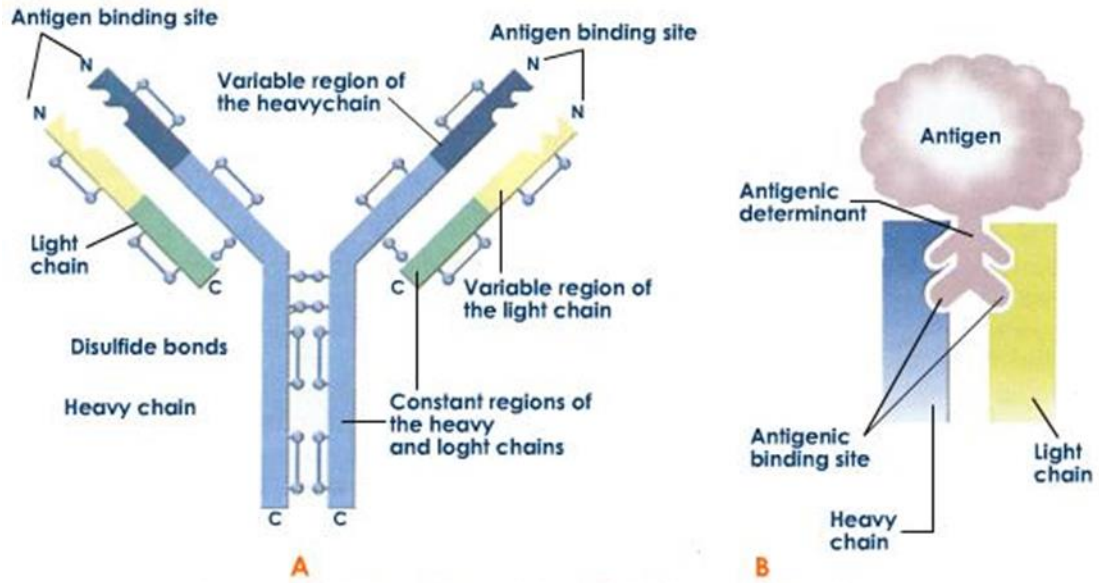
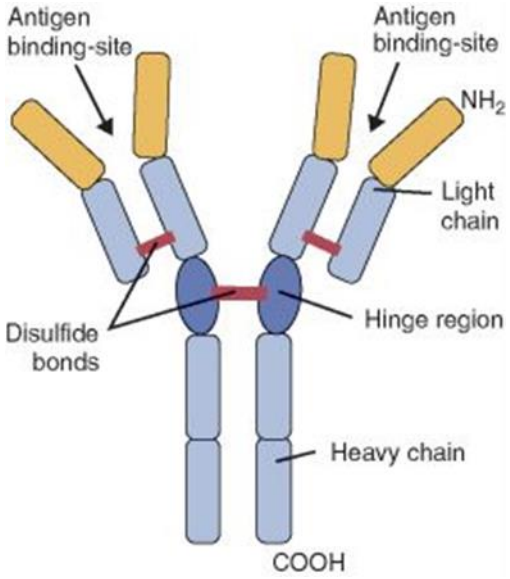


FIGURE 3-20 Schematic diagram of the structure of immunoglobulins derived from amino acid sequence analysis. Each heavy (dark blue) and light (light blue) chain in an immunoglobulin molecule contains an amino-terminal variable (V) region that consists of 100 to 110 amino acids and differs from one antibody to the next. The remainder of each chain in the molecule—the constant (C) regions—exhibits limited variation that defines the two light-chain subtypes and the five heavy-chain subclasses. Some heavy chains (γ, δ, and α) also contain a proline-rich hinge region. The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the carboxy-terminal domains. The μ and ε heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule. CHO denotes a carbohydrate group linked to the heavy chain.



Immunoglobulins : A. Structure, B. Antigen binding site



There are Two Major Classes of Antibody Light Chains

- Amino acid sequencing of antibody light chains revealed that the amino-terminal half (approximately 110 amino acids) of the light chain was extremely variable, whereas the sequence of the carboxyl-terminal half could be classified into one of two major sequence types.
- The N terminal half of light chains is thus referred to as the **variable,** or **VL**, region of the light chain, and the less variable part of the sequence is termed the **constant,** or **CL,** region.
- The two major light chain constant region sequences are referred to as κ _ **(kappa)** or λ _ **(lambda) chains**.
- In humans, the light chains are fairly evenly divided between the two light-chain classes; 60% of human light chains are κ- whereas only 40%are λ-light-chain type.
- Further analysis of light-chain sequences demonstrated that, even within the variable regions of the light chain, there were regions of hypervariability. Since these **hypervariable** regions could be shown to interact with the bound antigen, they were renamed the **complementarity-determining regions**, or **CDRs**.

There are Five Major Classes of Antibody Heavy Chains

- According to the amino acid sequencing of the heavy-chain constant regions fall into five basic patterns.
- These five basic sequences have been named with Greek letters: (mu, μ) ,(delta, δ), (gamma, γ), (epsilon, ε), and (alpha, α).
- Each different heavy-chain region is referred to as an **isotype**, and the isotype of the heavy chain constant chains of a given antibody molecule determines its **class**. Thus, antibodies with a heavy chain of the μ _ isotype are of the IgM class; those with a heavy chainδ are IgD; those with γ _, IgG; those with ε_, IgE; and those with α _, IgA. The length of the constant

region of the heavy chains is either 330 amino acid residues (for _ γ , δ , and α _ chains) or 440 amino acids (for _ ε and μ chains).

- Minor differences in the amino acid sequences of groups of _ and _ heavy chains led to further sub classification of these heavy chains into sub-class.
- There are two sub-class of the IgA (IgA1 and IgA2). Similarly, there are four sub-class of _ heavy chains of IgG(IgG1, IgG2, IgG3, and IgG4).

Immunoglobulin M: A Polymeric Molecule

- The Pentameric Nature of IgM. Serum IgM is basically composed of five subunits (monomeric subunits, IgMs), each one of them composed of two light chains (k or l) and two heavy chains (μ). The heavy chains are larger than those of IgG by about 20,000 daltons.
- Immunoglobulin M accounts for about 10% of the Ig pool and is largely confined to the intravascular pool because of its large size.
- This antibody is produced early in an immune response and is largely confined to the blood.
- The J chain, a third polypeptide chain, can be revealed by adequate methodology in IgM molecules. This is a small polypeptide chain of 15,000 daltons, also found in polymeric IgA molecules. One single J chain is found in any polymeric IgM or IgA molecule, regardless of how many monomeric subunits are involved in the polymerization. It has been postulated that this chain plays some role in the polymerization process.
- In humans, IgM is found in smaller concentrations than IgG or IgA.

Immunoglobulin G: Monomeric Molecule

- The major immunoglobulin in normal serum is IgG. It diffuses more readily than other immunoglobulins into the extravascular spaces and neutralizes toxins or binds to microorganisms in extravascular spaces.
- IgG can cross the placenta. In addition, when IgG complexes are formed, complement can be activated. IgG accounts for 70% to 75% of the total Ig pool.

Immunoglobulin A:

- Immunoglobulin A represents 15% to 20% of the total circulatory Ig pool. It is the predominant immunoglobulin in secretions such as tears, saliva, colostrum, milk, and intestinal fluids.
- Serum IgA is molecularly heterogeneous, composed of a mixture of monomeric, dimeric, and larger polymeric molecules. In a normal individual, over 70–90% of serum IgA is monomeric. Monomeric IgA is similar to IgG, and composed of two heavy chains (α) and two light chains (k or l). The dimeric and polymeric forms of IgA found in circulation are covalently bonded synthetic products containing J chains.
- IgA is the predominant immunoglobulin in secretions. Secretory IgA molecules are most frequently dimeric, contain J chains as do all polymeric immunoglobulin molecules.
- IgA is synthesized largely by plasma cells located on body surfaces. If produced by cells in the intestinal wall, IgA may pass directly into the intestinal lumen or diffuse into the blood circulation. As IgA is transported through intestinal epithelial cells or hepatocytes, it binds to a glycoprotein called the secretory component. The secretory piece protects IgA from digestion by gastrointestinal proteolytic enzymes. It forms a complex molecule termed secretory IgA, which is critical in protecting body surfaces against invading microorganisms because of its presence in seromucous secretions (e.g., tears, saliva, nasal fluids, colostrum).

The Minor Immunoglobulin Classes: IgE and IgD

Immunoglobulin E

- IgE is a monomeric immunoglobulins
- IgE is a trace plasma protein found in the blood plasma of unparasitized individuals (MW, 188,000 Da).
- IgE is crucial because it mediates some types of hypersensitivity (allergic) reactions, allergies, and anaphylaxis and is generally responsible for an

individual's immunity to invading parasites. In allergic individuals, if those IgE molecules have a given antibody specificity and react with the antigen while attached to the basophil or mast cell membranes, they will trigger the release of histamine and other substances that cause the symptoms of allergic reactions.

- The IgE molecule is unique in that it binds strongly to a receptor on mast cells and basophils and, together with antigen, mediates the release of histamines and heparin from these cells.

Immunoglobulin D

- Immunoglobulin D is found in very low concentrations in plasma, accounting for less than 1% of the total Ig pool.
- IgD is extremely susceptible to proteolysis and is primarily a cell membrane Ig found on the surface of B lymphocytes in association with IgM.