ANTIGEN CHARACTERISTICS

General Characteristics of Immunogens and Antigens

- An immune response is triggered by **immunogens,** macromolecules capable of triggering an adaptive immune response by inducing the formation of **antibodies** or sensitized T cells in an immunocompetent host (a host capable of recognizing and responding to a foreign antigen).
- **Immunogens** can specifically react with corresponding antibodies or sensitized T lymphocytes**.**
- **Immunogenicity– is the ability to induce humoral and /or cell mediated immune response.**
- In contrast, an **antigen** is a substance that stimulates antibody formation and has the ability to bind to an antibody or a T lymphocyte antigen receptor but may not be able to evoke an immune response initially.

SEVENTH LECTURE

- Foreign substances can be immunogenic or antigenic (capable of provoking a humoral and/or cell-mediated immune response) if their membrane or molecular components contain(s) structures recognized as foreign by the immune system.
- These structures are called antigenic determinants, or epitopes.
- An **epitope,** as part of an antigen, reacts specifically with an antibody or T lymphocyte receptor.
- Not all surfaces act as antigenic determinants. Only prominent determinants on the surface of a protein are normally recognized by the immune system and some of these are much more immunogenic than others. An immune response is directed against specific determinants and resultant antibodies will bind to them, with much of the remaining molecule being immunogenic.

- **Antigenicity– is the ability to combine with the final products of the humoral and/or cell mediated immune response.**
- lower molecular weight particles, **haptens,** can bind to an antibody but must be attached to a macromolecule as a carrier to stimulate a specific immune response. In reality, all immunogens are antigens but not all antigens are immunogens. The two terms, *immunogens* and *antigens,* are frequently used interchangeably without making a distinction between the two terms.

Autoantigens

The evolution of a recognition system that can recognize and destroy non self material must also have safeguards to prevent damage to self antigens. The body's immune system usually exercises tolerance to self antigens but, in some situations, antibodies may be produced in response to normal self antigens. This failure to recognize self antigens can result in autoantibodies directed at hormones, such as thyroglobulin

- The cellular membrane of mammalian cells consists chemically of proteins, phospholipids, cholesterol, and traces of polysaccharide.
- Polysaccharides (carbohydrates) in the form of glycoproteins or glycolipids can be found attached to the lipid and protein molecules of the membrane. When antigen-bearing cells, such as red blood cells (RBCs), from one person, a donor, are transfused into another person, a recipient, they can be immunogenic. Outer surfaces of bacteria, such as the capsule or the cell wall, as well as the surface structures of other microorganisms, can also be immunogenic.
- Cellular antigens of importance to immunologists include histocompatibility antigens, autoantigens, and blood group antigens.
- The normal immune system responds to foreignness by producing antibodies. For this reason, microbial antigens are also important to immunologists in the study of the immunologic manifestations of infectious disease.

- Proteins are excellent antigens because of their high molecular weight and structural complexity.
- Lipids are considered inferior antigens because of their relative simplicity and lack of structural stability. However, when lipids are linked to proteins or polysaccharides, they may function as antigens.
- Nucleic acids are poor antigens because of relative simplicity, molecular flexibility, and rapid degradation. Anti–nucleic acid antibodies can be produced by artificially stabilizing them and linking them to an immunogenic carrier.
- Carbohydrates (polysaccharides) by themselves are considered too small to function as antigens. In the case of erythrocyte blood group antigens, protein or lipid carriers may contribute to the necessary size and the polysaccharides present in the form of side chains confer immunologic specificity.

CHEMICAL NATURE OF ANTIGENS

Antigens, or immunogens, are usually large organic molecules that are proteins or large polysaccharides and, rarely, if ever, lipids. Antigens, especially cell surface or membrane-bound antigens, can be composed of combinations of biochemical classes (e.g., glycoproteins, glycolipids). For example, histocompatibility HLAs are glycoprotein in nature and are found on the surface membranes of nucleated body cells composed of solid tissue and most circulating blood cells (e.g., granulocytes, monocytes, lymphocytes, thrombocytes).

Blood Group Antigens

Blood group substances are widely distributed throughout the tissues, blood cells, and body fluids. When foreign RBC antigens are introduced to a host, a transfusion reaction or hemolytic disease of the fetus and newborn can result . In addition, certain antigens, especially those of the Rh system, are integral structural components of the erythrocyte (RBC) membrane. If these antigens are missing, the erythrocyte membrane is defective and results in hemolytic anemia. When antigens do not form part of the essential membrane structure (e.g., A, B, and H antigens), the absence of antigen has no effect on membrane integrity.

Adjuvant

The response to immunization can be enhanced by a number of agents, collectively called adjuvants. One of the best-known emulsifying agents in vaccine studies is Freund's complete adjuvant. An **adjuvant** is a substance, distinct from antigen, that enhances T cell activation by promoting the accumulation of APCs at a site of antigen exposure and by enhancing the expression of costimulators and cytokines by the APCs.

Degradability

For an antigen to be recognized as foreign by an individual's immune system, sufficient antigens to stimulate an immune response must be present. Foreign molecules are rapidly destroyed and thus cannot provide adequate antigenic exposure. In the case of vaccination, an adequate dose of vaccine at appropriate intervals must be administered for an immune response to be stimulated.

Molecular Weight

The higher the MW, the better the molecule will function as an antigen. The number of antigenic determinants on a molecule is directly related to its size. For example, proteins are effective antigens because of a large MW.

Although large foreign molecules (MW 10,000 daltons) [Da] are better antigens, **haptens,** which are tiny molecules, can bind to a larger carrier molecule and behave as antigens. If a hapten is chemically linked to a large molecule, a new surface structure is formed on the large molecule, which may function as an antigenic determinant.

Factors affecting on immunogenicity

A. Contribution of the Immunogen

Important factors in the effective functioning of antigens include foreignness, degradability, molecular weight (MW), structural stability, and complexity.

Foreignness

Foreignness is the degree to which antigenic determinants are recognized as non self by an individual's immune system. The immunogenicity of a molecule depends to a great extent on its degree of foreignness. For example, if a transplant recipient receives a donor organ with several major HLA differences, the organ is perceived as foreign and is subsequently rejected by the recipient. Normally, an individual's immune system does not respond to self antigens.

B. Contribution of the Biological System

1. Genetic Factors

Some substances are immunogenic in one species but not in another. Similarly, some substances are immunogenic in one individual but not in others (i.e. responders and non-responders). The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells or they may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

2.Age

Age can also influence immunogenicity. Usually the very young and the very old have a diminished ability to mount and immune response in response to an immunogen.

C. Method of Administration

1. Dose

The dose of administration of an immunogen can influence its immunogenicity. There is a dose of antigen above or below which the immune response will not be optimal.

2. Route

Generally the subcutaneous route is better than the intravenous or intragastric routes. The route of antigen administration can also alter the nature of the response

3. Adjuvants

Substances that can enhance the immune response to an immunogen are called adjuvants. The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

Structural Stability

If a molecule is an effective antigen, structural stability is mandatory.

If a structure is unstable (e.g., gelatin), the molecule will be a poor antigen. Similarly, totally inert molecules are poor antigens. Ther structural stability of an antigen is important in cases where the goal is to elicit a patient antibody response when adminstering a vaccine.

Complexity

The more complex an antigen, the greater is its effectiveness. Complex proteins are better antigens than large repeating polymers such as lipids, carbohydrates, and nucleic acids, which are relatively poor antigens.

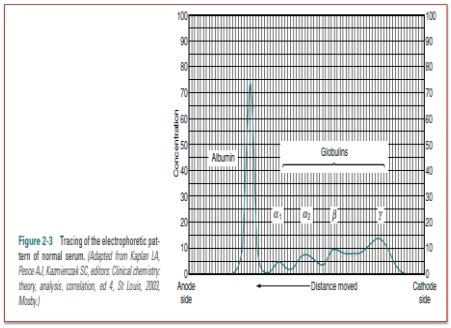

Figure 2-3 Tracing of the electrophoretic pattern of normal serum. *(Adapted from Kaplan LA, Pesce AJ, Kazmierczak SC, editors: Clinical chemistry: theory, analysis, correlation, ed 4, St Louis, 2003, Mosby.)*

Antibodies

GENERAL CHARACTERISTICS OF ANTIBODIES

- Antibodies are specific proteins referred to as immunoglobulins.
- Many antibodies can be isolated in the gamma globulin fraction of protein by electrophoresis separation (Fig. 2-3). The term *immunoglobulin* (Ig) has replaced gamma globulin because not all antibodies have gamma electrophoretic mobility.
- Antibodies can be found in blood plasma and in many body fluids (e.g., tears, saliva, colostrum).
- The primary function of an antibody in body defenses is to combine with antigen, which may be enough to neutralize bacterial toxins or some viruses. A secondary interaction of an antibody molecule with another effector agent (e.g., complement) is usually required to dispose of larger antigens (e.g., bacteria).

- 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.

- **Antibodies Share a Common Structure of Two Light Chains and Two Heavy Chains**
- All antibodies share a common structure of four polypeptide chains (Figure 3-20), consisting of two identical **light (L) chains** and two identical **heavy (H) chains**.
- 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.
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- Each Fab region and Fc region of antibodies mediates its own particular functions during an antibody response to an antigen.
- The Fab regions bind to the antigen, and the Fc region of the antigen-coupled antibody binds to **Fc receptors** on phagocytic or cytolytic cells, or to immune effector molecules.
- In this way, antibodies serve as physiological bridges between an antigen present on a pathogen, and the cells or molecules that will ultimately destroy it. A family of Fc receptors exists; each Fc receptor is expressed on a different array of cells and binds to a different class of antibodies

- Figure 3-21 further shows that the overall structure of the antibody molecule consists of three relatively compact regions, joined by a more flexible **hinge** region.
- The hinge region is particularly susceptible to proteolytic cleavage by the enzyme **papain**. Papain cleavage resolves the antibody molecule into two identical *f*ragments that retain the *a*ntigen-*b*inding specificity of the original antibody (shown as **Fab regions** in Figure 3-21), 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** (*f*ragment *c*rystallizable).

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**. As more light-chain sequences were generated, it became apparent that the _ chain constant region sequences could be further subdivided into four subtypes—λ-1, λ-2, λ-3, and λ-4 based on amino acid substitutions at a few positions.

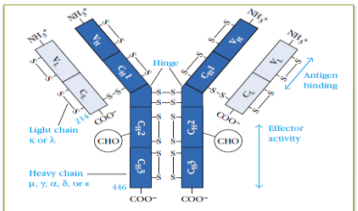

FIGURE 3-20 Schematic diagram of the structure of immunoglobulins derived from amino acid sequence analysis. Each heavy (dark blue) and light (light blue) chain in an immunoglobulin molecule contains an amino-terminal variable (V) region that consists of 100 to 110 amino acids and differs from one antibody 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.

There are Five Major Classes of Antibody Heavy Chains

- Using antibodies directed toward the constant region of immunoglobulins and amino acid sequencing of immunoglobulins derived from plasmacytoma tumor cells, investigators discovered that the sequences of the heavy-chain constant regions fall into five basic patterns.
- These five basic sequences have been named with Greek letters: (mu),(delta), (gamma), (epsilon), and (alpha).
- 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).

- 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**.

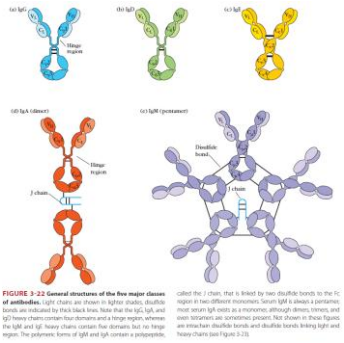

FIGURE 3-22 General structures of the five major classes of antibodies. Light chains are shown in lighter shades; disulfide bonds are indicated by thick black lines. Note that the IgG, IgA, and IgD heavy chains contain four domains and a hinge region, whereas the IgM and IgE heavy chains contain five domains but no hinge region. The polymeric forms of IgM and IgA contain a polypeptide, called the J chain, that is linked by two disulfide bonds to the Fc region in two different monomers. Serum IgM is always a pentamer; most serum IgA exists as a monomer, although dimers, trimers, and even tetramers are sometimes present. Not shown in these figures are intrachain disulfide bonds and disulfide bonds linking light and heavy chains (see Figure 3-23).

- Minor differences in the amino acid sequences of groups of _ and _ heavy chains led to further sub classification of these heavy chains into **sub-isotypes** and their corresponding antibodies therefore into **subclasses** (Table 3-2).
- There are two sub-isotypes of the _ heavy chain, _1 and _2, and thus two IgA subclasses, IgA1 and IgA2. Similarly, there are four sub-isotypes of _ heavy chains, _1, _2, _3, and _4, with the corresponding formation of the four subclasses of IgG: IgG1, IgG2, IgG3, and IgG4. In mice, the four _ chain sub isotypes are _1, _2_, _2_, and _3, and the corresponding subclasses of mouse IgG antibodies are IgG1, IgG2a, IgG2b, and IgG3, respectively.
- Further analysis revealed that the exact number, and precise positions of the disulfide bonds between the heavy chains of antibodies, vary among antibodies of different classes and subclasses (Figure 3-22).

EIGHTH LECTURE