CBCS 3RD Sem (M)

Unit 3 (III): Immunoglobulins

Basic Structure, Classes and Function, Antigenic Determinants

By-
Dr. Luna Phukan

What is Immunity

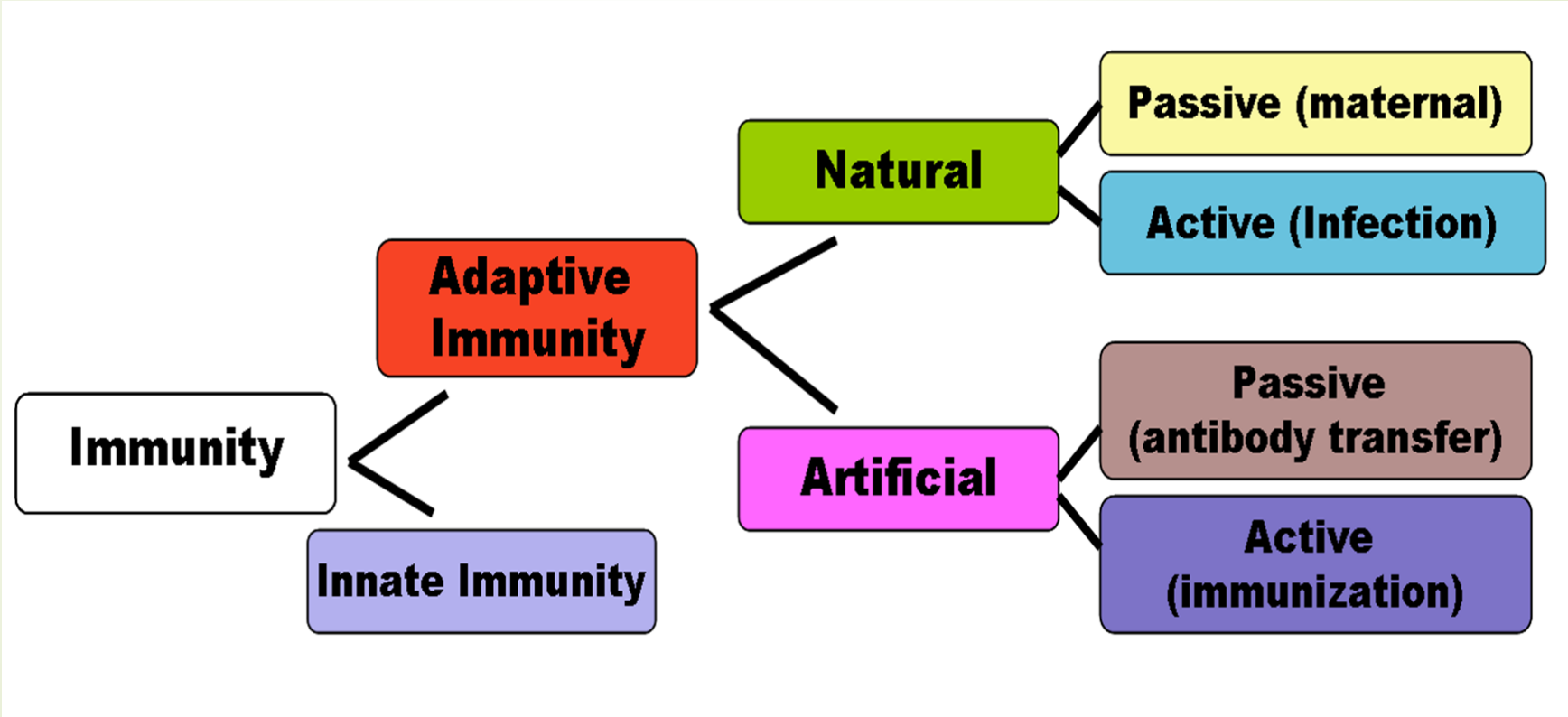
Immunity is the capability of multicellular organisms to resist harmful microorganisms. Immunity involves both specific and nonspecific components. The nonspecific components act as barriers or eliminators of a wide range of pathogens irrespective of their antigenic make-up. Other components of the immune system adapt themselves to each new disease encountered and can generate pathogen-specific immunity.

Immunity can be defined as a complex biological system endowed with the capacity to recognize and tolerate whatever belongs to the self, and to recognize and reject what is foreign (non-self).

What are the different types of immunity

The immune system has two components: innate and adaptive immunity. The innate immunity is present in all metazoans, while the adaptive immunity only occurs in vertebrates.

The innate system relies on the recognition of certain foreign molecules to stimulate two types of innate immune responses: inflammatory responses and phagocytosis. The adaptive system, on the other hand, is composed of more advanced lymphatic cells that are programmed to distinguish between specific "non-self" substances in the presence of "self". The reaction to foreign substances is etymologically described as inflammation, meaning to set on fire.


Immunity
Innate Immunity
Adaptive Immunity
Natural
Passive (maternal)
Active (Infection)
Artificial
Passive (antibody transfer)
Active (immunization)

Immunoglobulin or Antibody: Introduction

Immunoglobulin (Ig)

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. The immunoglobulins derive their name from the finding that they migrate with globular proteins when antibody-containing serum is placed in an electrical field

. BASIC STRUCTURE OF IMMUNOGLOBULINS

The basic structure of the immunoglobulins is illustrated in figure 2. Although different immunoglobulins can differ structurally, they all are built from the same basic units.

A. Heavy and Light Chains

All immunoglobulins have a four chain structure as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD)

B. Disulfide bonds

1. Inter-chain disulfide bonds - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.

2. Intra-chain disulfide binds - Within each of the polypeptide chains there are also intra-chain disulfide bonds.

C. Variable (V) and Constant (C) Regions

When the amino acid sequences of many different heavy chains and light chains were compared, it became clear that both the heavy and light chain could be divided into two regions based on variability in the amino acid sequences. These are the:

1. Light Chain - VL (110 amino acids) and CL (110 amino acids)

2. Heavy Chain - VH (110 amino acids) and CH (330-440 amino acids)

D. Hinge Region

This is the region at which the arms of the antibody molecule forms a Y. It is called the hinge region because there is some flexibility in the molecule at this point.

E. Domains

Three dimensional images of the immunoglobulin molecule show that it is not straight as depicted in figure 2A. Rather, it is folded into globular regions each of which contains an intra-chain disulfide bond (figure 2B-D). These regions are called domains.

1. Light Chain Domains - VL and CL

2. Heavy Chain Domains - VH, CH1 - CH3 (or CH4)

F. Oligosaccharides

Carbohydrates are attached to the CH2 domain in most immunoglobulins. However, in some cases carbohydrates may also be attached at other locations.

IV. STRUCTURE OF THE VARIABLE REGION

A. Hypervariable (HVR) or complementarity determining regions (CDR)

Comparisons of the amino acid sequences of the variable regions of immunoglobulins show that most of the variability resides in three regions called the hypervariable regions or the complementarity determining regions as illustrated in figure 3. Antibodies with different specificities (i.e. different combining sites) have different complementarity determining regions while antibodies of the exact same specificity have identical complementarity determining regions (i.e. CDR is the antibody combining site). Complementarity determining regions are found in both the H and the L chains.

B. Framework regions

The regions between the complementarity determining regions in the variable region are called the framework regions (figure 3). Based on similarities and differences in the framework regions the immunoglobulin heavy and light chain variable regions can be divided into groups and subgroups. These represent the products of different variable region genes.

V. IMMUNOGLOBULIN FRAGMENTS: STRUCTURE/FUNCTION RELATIONSHIPS

Immunoglobulin fragments produced by proteolytic digestion have proven very useful in elucidating structure/function relationships in immunoglobulins.

A. Fab

Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain disulfide bond Figure 4. This results in the formation of two identical fragments that contain the light chain and the VH and CH1 domains of the heavy chain.

Antigen binding - These fragments were called the Fab fragments because they contained the antigen binding sites of the antibody. Each Fab fragment is monovalent whereas the original molecule was divalent. The combining site of the antibody is created by both VH and VL. An antibody is able to bind a particular antigenic determinant because it has a particular combination of VH and VL. Different combinations of a VH and VL result in antibodies that can bind a different antigenic determinants.

B. Fc

Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a CH2 and CH3 domain. This fragment was called Fc because it was easily crystallized.

effector functions - The effector functions of immunoglobulins are mediated by this part of the molecule. Different functions are mediated by the different domains in this fragment .Normally the ability of an antibody to carry out an effector function requires the prior binding of an antigen; however, there are exceptions to this rule.

C. F (ab ') 2

Treatment of immunoglobulins with pepsin results in cleavage of the heavy chain after the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites. This fragment was called F(ab')2 because it is divalent. The Fc region of the molecule is digested into small peptides by pepsin. The F(ab')2 binds antigen but it does not mediate the effector functions of antibodies.

HUMAN IMMUNOGLOBULIN CLASSES, SUBCLASSES, TYPES AND SUBTYPES

A. Immunoglobulin classes

The immunoglobulins can be divided into five different classes, based on differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a given class will have very similar heavy chain constant regions. These differences can be detected by sequence studies or more commonly by serological means (i.e. by the use of antibodies directed to these differences).

1. **IgG - Gamma heavy chains**
2. **IgM - Mu heavy chains**
3. **IgA - Alpha heavy chains**
4. **IgD - Delta heavy chains**
5. **IgE - Epsilon heavy chains**

B. Immunoglobulin Subclasses

The classes of immunoglobulins can de divided into subclasses based on small differences in the amino acid sequences in the constant region of the heavy chains. All immunoglobulins within a subclass will have very similar heavy chain constant region amino acid sequences. Again these differences are most commonly detected by serological means.

1. IgG Subclasses

a) IgG1 - Gamma 1 heavy chains

b) IgG2 - Gamma 2 heavy chains

c) IgG3 - Gamma 3 heavy chains

d) IgG4 - Gamma 4 heavy chains

2. IgA Subclasses

a) IgA1 - Alpha 1 heavy chains

b) IgA2 - Alpha 2 heavy chains

C. Immunoglobulin Types

Immunoglobulins can also be classified by the type of light chain that they have. Light chain typ are based on differences in the amino acid sequence in the constant region of the light chain. These differences are detected by serological means.

1. Kappa light chains

2. Lambda light chains

D. Immunoglobulin Subtypes

The light chains can also be divided into subtypes based on differences in the amino acid sequences in the constant region of the light chain.

1. Lambda subtypes

a) Lambda 1

b) Lambda 2c) Lambda 3

d) Lambda 4

E. Nomenclature

Immunoglobulins are named based on the class, or subclass of the heavy chain and type or subtype of light chain. Unless it is stated precisely, you should assume that all subclass, types and subtypes are present. IgG means that all subclasses and types are present.

F. Heterogeneity

Immunoglobulins considered as a population of molecules are normally very heterogeneous because they are composed of different classes and subclasses each of which has different types and subtypes of light chains. In addition, different immunoglobulin molecules can have different antigen binding properties because of different VH and VL regions.

STRUCTURE AND SOME PROPERTIES OF IG CLASSES AND SUBCLASSES

A. IgG

1. Structure

The structures of the IgG subclasses are presented in figure 7. All IgG's are monomers (7S immunoglobulin). The subclasses differ in the number of disulfide bonds and length of the hinge region.

2. Properties

IgG is the most versatile immunoglobulin because it is capable of carrying out all of the functions of immunoglobulin molecules.

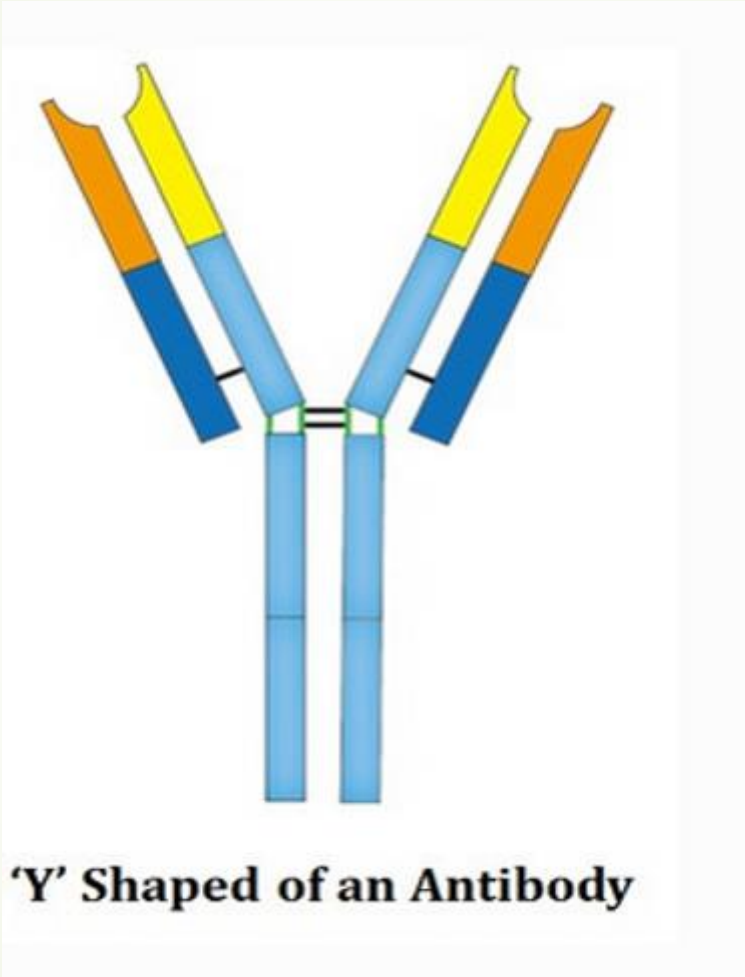
a) IgG is the major Ig in serum - 75% of serum Ig is IgG

b) IgG is the major Ig in extra vascular spaces

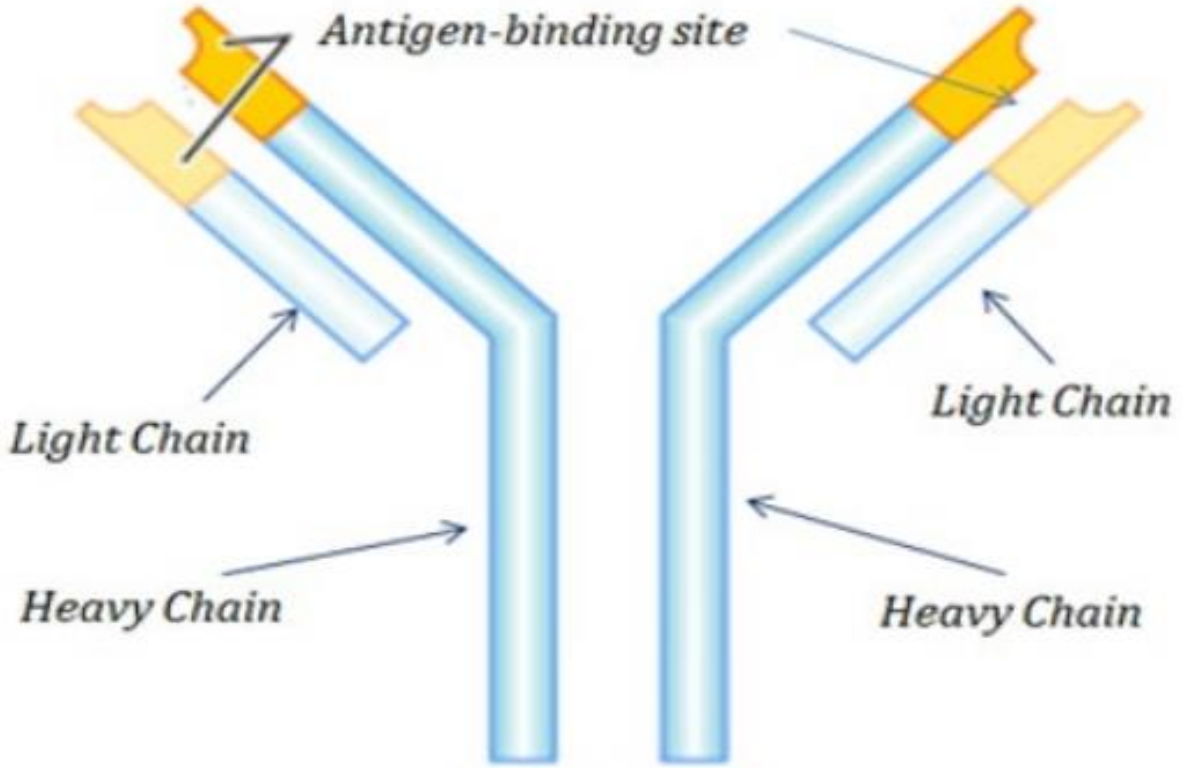
c) Placental transfer - IgG is the only class of Ig that crosses the placenta. Transfer is mediated by a receptor on placental cells for the Fc region of IgG. Not all subclasses cross equally well; IgG2 does not cross well.

d) Fixes complement - Not all subclasses fix equally well; IgG4 does not fix complement

e) Binding to cells - Macrophages, monocytes, PMNs and some lymphocytes have Fc receptors for the Fc region of IgG. Not all subclasses bind equally well; IgG2 and IgG4 do not bind to Fc receptors. A consequence of binding to the Fc receptors on PMNs, monocytes and macrophages is that the cell can now internalize the antigen better. The antibody has prepared the antigen for eating by the phagocytic cells. The term opsonin is used to describe substances that enhance phagocytosis. IgG is a good opsonin. Binding of IgG to Fc receptors on other types of cells results in the activation of other functions.



'Y' Shaped of an Antibody



Heavy (H) and Light (L) Chains in an Antibody

B. IgM

1. Structure

The structure of IgM is presented in figure 8. IgM normally exists as a pentamer (19S immunoglobulin) but it can also exist as a monomer. In the pentameric form all heavy chains are identical and all light chains are identical. Thus, the valence is theoretically 10. IgM has an extra domain on the mu chain (CH4) and it has another protein covalently bound via a S-S bond called the J chain. This chain functions in polymerization of the molecule into a pentamer.

2. Properties

a) IgM is the third most common serum Ig.

b) IgM is the first Ig to be made by the fetus and the first Ig to be made by a virgin B cells when it is stimulated by antigen.

c) As a consequence of its pentameric structure, IgM is a good complement fixing Ig. Thus, IgM antibodies are very efficient in leading to the lysis of microorganisms.

d) As a consequence of its structure, IgM is also a good agglutinating Ig . Thus, IgM antibodies are very good in clumping microorganisms for eventual elimination from the body.

e) IgM binds to some cells via Fc receptors.

f) B cell surface Ig

Surface IgM exists as a monomer and lacks J chain but it has an extra 20 amino acids at the C-terminus to anchor it into the membrane (figure 9). Cell surface IgM functions as a receptor for antigen on B cells. Surface IgM is noncovalently associated with two additional proteins in the membrane of the B cell called Ig-alpha and Ig-beta as indicated in figure 10. These additional proteins act as signal transducing molecules since the cytoplasmic tail of the Ig molecule itself is too short to transduce a signal. Contact between surface immunoglobulin and an antigen is required before a signal can be transduced by the Ig-alpha and Ig-beta chains. In the case of T-independent antigens, contact between the antigen and surface immunoglobulin is sufficient to activate B cells to differentiate into antibody secreting plasma cells. However, for T-dependent antigens, a second signal provided by helper T cells is required before B cells are activated.

. IgA

Structure

Serum IgA is a monomer but IgA found in secretions is a dimer as presented in Figure 11. When IgA exits as a dimer, a J chain is associated with it.

When IgA is found in secretions is also has another protein associated with it called the secretory piece or T piece; sIgA is sometimes referred to as 11S immunoglobulin. Unlike the remainder of the IgA which is made in the plasma cell, the secretory piece is made in epithelial cells and is added to the IgA as it passes into the secretions (Figure 12). The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions.

Properties

a) IgA is the 2nd most common serum Ig.

b) IgA is the major class of Ig in secretions - tears, saliva, colostrum, mucus. Since it is found in secretions secretory IgA is important in local (mucosal) immunity.

c) Normally IgA does not fix complement, unless aggregated.

d) IgA can binding to some cells - PMN's and some lymphocytes.

D. IgD

1. Structure

The structure of IgD is presented in the Figure 13. IgD exists only as a monomer.

2. Properties

a) IgD is found in low levels in serum; its role in serum uncertain.

b) IgD is primarily found on B cell surfaces where it functions as a receptor for antigen. IgD on the surface of B cells has extra amino acids at C-terminal end for anchoring to the membrane. It also associates with the Ig-alpha and Ig-beta chains.

c) IgD does not bind complement.

E. IgE

1. Structure

The structure of IgE is presented in Figure 14. IgE exists as a monomer and has an extra domain in the constant region.

2. Properties

a) IgE is the least common serum Ig since it binds very tightly to Fc receptors on basophils and mast cells even before interacting with antigen.

b) Involved in allergic reactions - As a consequence of its binding to basophils an mast cells, IgE is involved in allergic reactions. Binding of the allergen to the IgE on the cells results in the release of various pharmacological mediators that result in allergic symptoms.

c) IgE also plays a role in parasitic helminth diseases. Since serum IgE levels rise in parasitic diseases, measuring IgE levels is helpful in diagnosing parasitic infections. Eosinophils have Fc receptors for IgE and binding of eosinophils to IgE-coated helminths results in killing of the parasite.

d) IgE does not fix complement.

CLINICAL IMPLICATIONS OF HUMAN IMMUNOGLOBULIN CLASSES

IgG

1. Increases in:

a) Chronic granulomatous infections

b) Infections of all types

c) Hyperimmunization

d) Liver disease

e) Malnutrition (severe)

f) Dysproteinemia

g) Disease associated with hypersensitivity granulomas, dermatologic disorders, and IgG myeloma

h) Rheumatoid arthritis

Decreases in:

a) Agammaglobulinemia

b) Lymphoid aplasia

c) Selective IgG, IgA deficiency

d) IgA myeloma

e) Bence Jones proteinemia

f) Chronic lymphoblastic leukemia

IgM

1. Increases (in adults) in:

a) Waldenström's macroglobulinemia

b) Trypanosomiasis

c) Actinomycosis

d) Carrión's disease (bartonellosis)

e) Malaria

f) Infectious mononucleosis

g) Lupus erythematosus

h) Rheumatoid arthritis

I) Dysgammaglobulinemia (certain cases)

Note: In the newborn, a level of IgM above 20 ng./dl is an indication of in utero stimulation of the immune system and stimulation by the rubella virus, the cytomegalovirus, syphilis, or toxoplasmosis.

2. Decreases in:

a) Agammaglobulinemia

b) Lymphoproliferative disorders (certain cases)

c) Lymphoid aplasia

d) IgG and IgA myeloma

e) Dysgammaglobulinemia

f) Chronic lymphoblastic leukemia

Age

Increases in:

a) Wiskott-Aldrich syndrome

b) Cirrhosis of the liver (most cases)

c) Certain stages of collagen and other autoimmune disorders such as rheumatoid arthritis and lupus erythematosus

d) Chronic infections not based on immunologic deficiencies

e) IgA myeloma

. Decreases in:

a) Hereditary ataxia telangiectasia

b) Immunologic deficiency states (e.g., dysgammaglobulinemia, congenital and acquired agammaglobulinemia, and hypogammaglobulinemia)

c) Malabsorption syndromes

d) Lymphoid aplasia

e) IgG myeloma

f) Acute lymphoblastic leukemia

g) Chronic lymphoblastic leukemia

IgD

1. Increases in:

a) Chronic infections

b) IgD myelomas

IgE

1. Increases in:

a) Atopic skin diseases such as eczema
b) Hay fever
c) Asthma
d) Anaphylactic shock
e) IgE-myeloma

2. Decreases in:

a) Congenital agammaglobulinemia
b) Hypogammaglobulinemia due to faulty metabolism or synthesis of immunoglobulins

Function of immunoglobulins

A. **Antigen binding**

Immunoglobulins bind specifically to one or a few closely related antigens. Each immunoglobulin actually binds to a specific antigenic determinant. Antigen binding by antibodies is the primary function of antibodies and can result in protection of the host. The valency of antibody refers to the number of antigenic determinants that an individual antibody molecule can bind. The valency of all antibodies is at least two and in some instances more.

B. Effector Functions

Frequently the binding of an antibody to an antigen has no direct biological effect. Rather, the significant biological effects are a consequence of secondary "effector functions" of antibodies. The immunoglobulins mediate a variety of these effector functions. Usually the ability to carry out a particular effector function requires that the antibody bind to its antigen. Not every immunoglobulin will mediate all effector functions. Such effector functions include:

1. Fixation of complement - This results in lysis of cells and release of biologically active molecules (see chapter two)

2. Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer of the immunoglobulin across the placenta. As a result, the transferred maternal antibodies provide immunity to the fetus and newborn

Antigenic Determinants

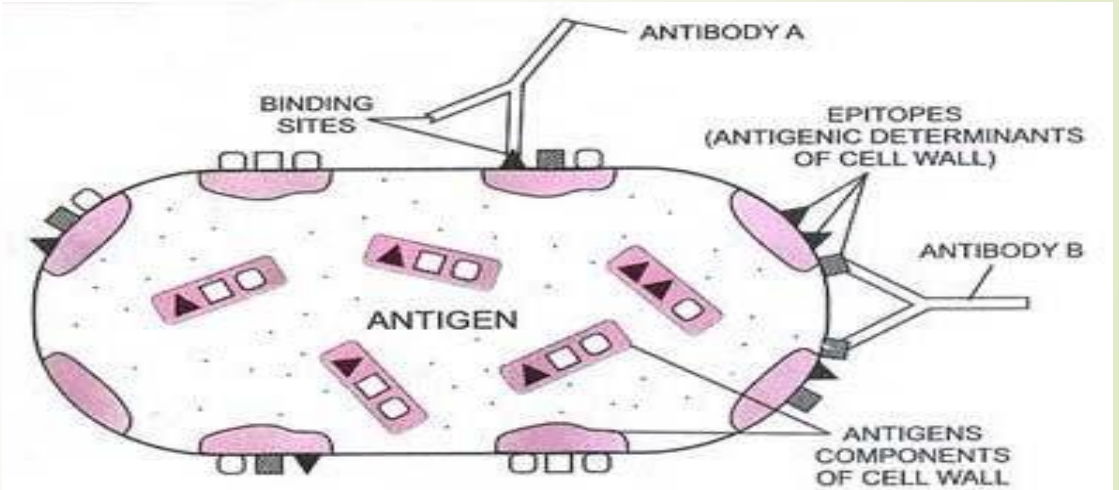


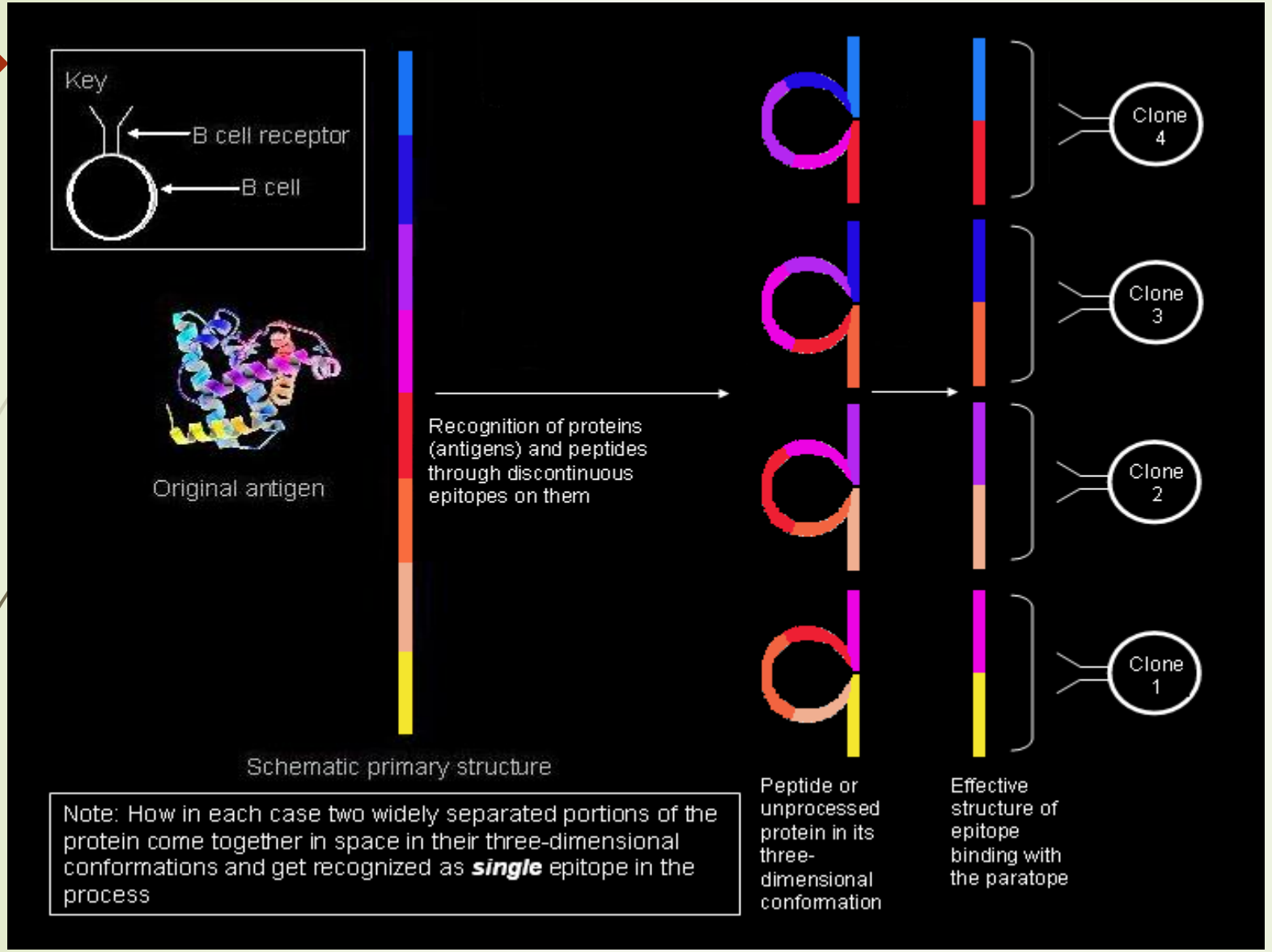
Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.\

An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells. For example, the epitope is the specific piece of the antigen to which an antibody binds. The part of an antibody that binds to the epitope is called a paratope. Although epitopes are usually non-self proteins, sequences derived from the host that can be recognized (as in the case of autoimmune diseases) are also epitopes.

The epitopes of protein antigens are divided into two categories, conformational epitopes and linear epitopes, based on their structure and interaction with the paratope.

Conformational and linear epitopes interact with the paratope based on the 3-D conformation adopted by the epitope, which is determined by the surface features of the involved epitope residues and the shape or tertiary structure of other segments of the antigen.

A conformational epitope is formed by the 3-D conformation adopted by the interaction of discontiguous amino acid residues.



Recognition of conformational epitopes by B cells. Note how the segments widely separated in the primary structure have come in contact in the three-dimensional tertiary structure forming part of the same epitope

In contrast, a linear epitope is formed by the 3-D conformation adopted by the interaction of contiguous amino acid residues. A linear epitope is not determined solely by the primary structure of the involved amino acids. Residues that flank such amino acid residues, as well as more distant amino acid residues of the antigen affect the ability of the primary structure residues to adopt the epitope's 3-D conformation

The proportion of epitopes that are conformational is unknown.[

Function

T cell epitope are presented on the surface of an antigen-presenting cell, where they are bound to MHC molecules. In humans, professional antigen-presenting cells are specialized to present MHC class II peptides, whereas most nucleated somatic cells present MHC class I peptides.

T cell epitopes presented by MHC class I molecules are typically peptides between 8 and 11 amino acids in length, whereas MHC class II molecules present longer peptides, 13–17 amino acids in length, and non-classical MHC molecules also present non-peptidic epitopes such as glycolipids

Cross-activity

Epitopes are sometimes cross-reactive. This property is exploited by the immune system in regulation by anti-idiotypic antibodies (originally proposed by Nobel laureate Niels Kaj Jerne). If an antibody binds to an antigen's epitope, the paratope could become the epitope for another antibody that will then bind to it. If this second antibody is of IgM class, its binding can upregulate the immune response; if the second antibody is of IgG class, its binding can downregulate the immune response.

Epitope mapping

Main article: Epitope mapping

Epitopes can be mapped using protein microarrays, and with the ELISpot or ELISA techniques. Another technique involves high-throughput mutagenesis, an epitope mapping strategy developed to improve rapid mapping of conformational epitopes on structurally complex proteins.

MHC class I and II epitopes can be reliably predicted by computational means alone,although not all in-silico T cell epitope prediction algorithms are equivalent in their accuracy.

Epitope tags

Epitopes are often used in proteomics and the study of other gene products. Using recombinant DNA techniques genetic sequences coding for epitopes that are recognized by common antibodies can be fused to the gene

Following synthesis, the resulting epitope tag allows the antibody to find the protein or other gene product enabling lab techniques for localisation, purification, and further molecular characterization. Common epitopes used for this purpose are Myc-tag, HA-tag, FLAG-tag, GST-tag, 6xHis,V5-tag and Peptides can also be bound by proteins that form covalent bonds to the peptide, allowing irreversible immobilization

These strategies have also been successfully applied to the development of "epitope-focused" vaccine design.

Neoantigenic determinant

A neoantigenic determinant is an epitope on a neoantigen, which is a newly formed antigen that has not been previously recognized by the immune system.

Neoantigens are often associated with tumor antigens and are found in oncogenic cells

Neoantigens and, by extension, neoantigenic determinants can be formed when a protein undergoes further modification within a biochemical pathway such as glycosylation, phosphorylation or proteolysis.

This, by altering the structure of the protein, can produce new epitopes that are called neoantigenic determinants as they give rise to new antigenic determinants. Recognition requires separate, specific antibodies.

Thank You