Title

IMMUNOGLOBULINS STRUCTURES

LEARNING OBJECTIVES:

The student should be able to:

- Identify chemical nature of the immunoglobulins.
- Describe the basic monomer structure of IgG immunoglobulins.
- Determine the number of heavy and light chain per each immunoglobulin molecule.
- Determine number of variable and constant regions of the light chain.
- Determine number of variable and constant regions of the heavy chain.
- Explain how all immunoglobulin chains are held together?
- Discuss the importance of the amino acids sequence.
- Determine the number of light and heavy chain domains for each immunoglobulin isotype.
- Define the term; paratope, Fc, Fab, Antigen binding site, hinge region, constant, hyper variable region, CDRs, carbohydrate moiety, joining chains (J-chains), Secretory component and domain.
- Describe the effects of proteolytic enzymes on the immunoglobulin structure (e.g., Papain, and pepsin).
- Describe the distinctive structural feature of each isotype.
- Define the terms affinity, and avidity.
- Explain the importance of identify the immunoglobulin isotype in clinical applications.

LECTURE REFRENCE:

1. TEXTBOOK: ROITT, BROSTOFF, MALE IMMUNOLOGY. 6th edition. Chapter 4. pg. 65-83.

2. TEXTBOOK: ABUL K. ABBAS. ANDREW H. LICHTMAN. CELLULAR AND MOLECULAR IMMUNOLOGY. 5TH EDITION. Chapter 9 .pg 189-214.

IMMUNOGLOBULINS STRUCTURE

INTRODUCTION

One of the most important characteristics of the immune system is the production of soluble proteins (glycoproteins), that migrate through the blood circulation into different parts of the body (thus present in the serum & tissue fluids) and some are present on the surface of the B-lymphocytes act as receptors for specific antigens. The antibodies perform many biological functions related to the protection and body's immunity against pathogens. These soluble proteins are the antibodies, and they 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 -globulin, while those migrate but slower, towards the anode is called γ -globulin] (Figure). 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).

A. Physiology

- 1. Immunoglobulins, or antibodies, are glycoproteins present in the gamma globulin fraction of serum.
- 2. The concentration of antibodies in the body is equally divided between the intravascular and extravascular (primarily lymphatic) compartments; 25% exchanges each day as the tissues are bathed in plasma proteins.

B. Immunology the Immunoglobulins are produced by B lymphocytes (B cells) or 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.

A. Basic unit (monomer) of the Immunoglobulin

- 1. The basic structure unit of an immunoglobulin molecule, called a **monomer**, consists of four polypeptide chains linked covalently by disulfide bonds (Figure-1).
- a. Polypeptide chains are unbranched polymers composed of amino acids.
- **b.** The sequence of amino acids in a polypeptide chains is what identifies a given protein and distinguishes it from other molecule.

2. The four-chain monomeric immunoglobulin structure is composed of two identical **heavy** (**H**) polypeptide chains and two identical **light** (**L**) polypeptide chains.



Figure-1 Basic unit (monomer) molecule, consisting of four polypeptide chains linked covalently by disulfide bonds (S-S) with interchain disulfide linkages as well. The loops correspond to domains within each chain. V = variable domain; C = constant domain; L = light chain; H = heavy chain; CHO = carbohydrate side chain. The diagram below shows the complementarity-determining (hypervariable) regions (CDRs) [shaded], and framework regions (FRs) [open] of each variable domain in the paratopic region.

1. Heavy and light chains (H chains)

H chains Heavy chains: They have a molecular weight 50-75 kDa (400 amino-acids) approximately twice of that of the light chain (**Figrue-1**). The amino acid differences in the carboxyl terminal portion of the H chains identify five different classes (isotypes). These classes (isotypes) are: $IgG = (\gamma)$, $IgA = (\alpha)$, $IgM = (\mu)$, $IgD = (\delta)$, and $IgE = (\epsilon)$. Some of these five main classes of the immunoglobulins are also subdivided into subclasses such as IgG, IgA, and IgM, and the subdivision is based on the greater similarity of amino acid sequence shown by subclasses of the same class than is shown by different classes. These subclasses of the IgG are $IgG1 = \gamma 1$, $IgG2 = \gamma 2$, $IgG3 = \gamma 3$, and $IgG4 = \gamma 4$, and those of the IgA are $IgA1 = \alpha 1$, $IgA2 = \alpha 2$, and those of the IgM are $IgM1 = \mu 1$, $IgM2 = \mu 2$).

a. Size. H chains have a molecular weight of 50 to 75 kDa approximately twice that of the L chains, and contain about 450 amino acids twice the number of the L chains.

b. Isotypes (classes)

- 1. Amino acid differences in the carboxy terminal portion of the H chains identify five antigenically distinct H chains isotypes includes, **IgG**, **IgA**, **IgM**, **IgD**, **and IgE**.
- 2. The H chain isotypes form the basis for the five classes of immunoglobulin molecules

c. Subclasses

- 1. H chain classes γ , α , and μ are subdivided into subclasses of molecules (Table). The subdivision is based on the greater similarity of amino acid sequence shown by subclasses of the same class than is shown by different classes.
- 2. The **H** chain subclasses correspond to immunoglobulin subclasses: $\gamma 1$ corresponds to IgG1; $\alpha 1$ to IgA1, and so forth.

2. Light chains (L chains)

- **a.** Size. L chains have a molecular weight of approximately 23 kDa and are composed of about 212 amino acids.
- b. Types
- (1) L chains are of two types κ and λ based on their structural (antigenic) differences.
- (2) All immunoglobulins classes have both κ and λ chains. However a given immunoglobulin molecule may contain either two identical κ chains or identical λ chains, but never a κ chain and λ chain combined.
- (3) The proportion of κ to λ chains in human immunoglobulin molecules is about 3:2.
- (4) There is no isotopic variation in κ chains. However, there are four distinct λ chains, giving rise to four distinct **subtypes** (so called to avoid confusion with the H chain subclasses that distinguish immunoglobulin isotypes). All four λ subtypes are present in each of the immunoglobulin classes.

C. Bonds. Disulfide (-S-S-) bonds hold together the four polypeptide chains in immunoglobulin molecules, and are of two types.

1. Interchain bonds occur between H chains (H-H), between H and L chains (H-L), and between L chains (L-L).

a. H-H bonds

(1) H-H bonds occur primarily in the hinge region of the immunoglobulin molecule, but they are also occurring in the carboxy terminal portion of the H chain.

(2) They can vary in number from 1 to 15, depending on the class and subclass of the immunoglobulin molecule.

b. H-L bonds. Only one disulfide bond connects H and L chains. H-L bonding occurs in most immunoglobulin molecules; IgA2 lacks an H-L bond.

c. L-L bonds. Single L-L bonds occur in IgA2. L-L bonds can also occur under pathologic conditions (e.g., in Bence Jones protein).

2. Intrachain bonds occur within an individual chain, and are stronger than intrachain bonds.

d. The number of intrachain disulfide bonds varies:

- (1) L chains have two.
- (2) Human γ , α , and δ H chains have four.
- (3) Human μ , and ϵ H chains have five.
- e. The intrachain bonds divide each immunoglobulin molecule into domains.
- f. D. Variable (V) and constant (C) regions
- 1. Each H chain and each L chain has a variable region and a constant region.
- **a.** The **variable region**, which lies in the amino, or N, terminal portion of the molecule, shows a wide variation in amino acid sequence.
- **b.** The **constant region**, in the carboxy, or C, terminal portion of the molecule, demonstrates an unvarying amino acid sequence, except for minor inherited differences.
- 2. The variable regions are associated with appropriate constant region such that:
 - **a.** No variable H chain region will occur in a L chain, and vice versa.
 - **b.** However, a particular variable H chain sequence may occur in more than one H chain class (e.g., γ , α , μ , and so forth).
- **3.** This permits **class switching**, which occur during an immune response, when B cells change their production form IgM for example to IgG.

4. Hypervariable regions

- **a.** Certain areas within the variable region are highly variable in amino acid sequences.
- (1) These hypervariable regions, often called complementarity-determining regions (CDRs) or "hot spots", occur at relatively constant amino acid positions in an otherwise relatively invariant molecule.
- (2) They are short polypeptide segments lying near amino acid positions 30, 50, and 95 in the variable regions of both L and H chains.
 - **b.** The CDRs are important in the structure of the antigen-binding site.
 - **c.** L chains have three CDRs [CDR1 (hv1), CDR2 (hv2), and CDR3 (hv3)] and the **H** chains have four, although only three of the four have been associated with antibody activity.
 - d. There are extreme variability in the amino acids found in the CDR regions.
 - e. The intervening peptide sequences, called **framework regions** (FRs), are relatively constant in amino acid sequence.

E. Domains

- **1.** Each immunoglobulin chain consists of a series of globular regions, or **domains**, enclosed by disulfide bonds.
- **a.** Each **H chain** has four or five domains, one in the variable region (V_H) and three or four in the constant region (C_H1 , C_H2 , C_H3 , and C_H4).
- (1) The γ , α , and δ H chains have four domains (one variable and three constant).
- (2) The μ , and ϵ H chains have five domains (one variable and four constant).
- **b.** Each **L** chain has two domains, one in the variable region (V_L) and one in the constant region (C_L) .
- 2. Domains consist of about 110 amino acid residues.
- **a.** Peptide loops of 60 to 70 amino acid residues enclosed by intrachain bonds represent the central portion of each domain.
- **b.** The amino acid sequences of the loops show a high degree of homology (e.g., the sequence is very similar).

F. Antigen-binding site (paratope)

- 1. The **paratope** is the area of the immunoglobulin molecule which interacts specifically with the **epitope** of the antigen (**Figure-2**, and 3).
- 2. The paratope is formed by only a very small portion of the entire immunoglobulin molecule.
- **a.** Folding of the polypeptide chains brings the CDR portions of the V_H and V_L domains into close proximity.
- b. This folding creates a three-dimensional structure which is complementary to the epitope.



Figure-2 antigen binding site and hypervariable regions



Figure-3 the variable region

G. Fragments. Enzymes such as **papain and pepsin**, each splits the immunoglobulin molecule into definable fragments (**Figure-4**).

- 1. Papain split the monomeric basic unit into three fragments of approximately equal size at the hinge region.
- **a.** Two **Fab** (antigen-binding) fragments each contain an entire L chain and the amino terminal half of the H chain.
- (1) The Fab portion of the H chain is called the **fd** fragment; it contains the V_H and $C_H 1$ domains.
- (2) A Fab fragment is **monovalent**; that is, it possesses only one antigen-binding site. Therefore, unlike bivalent molecules, a Fab fragment cannot facilitate antigen precipitation.
- **b.** One **Fc** (Crystallizable) fragment contains the carboxy terminal portion of the H chain.
- (1) This portion of the molecule has several properties:
 - (a) It binds the C1q component of complement.
 - (b) It contains carbohydrate.
 - (c) It dictates whether a given immunoglobulin can, for example, cross the placenta (IgG) or bind to mast cells (IgE).
 - (d) It is responsible for opsonization of bacteria and other cellular elements.



Figure-4 Immunoglobulin fragments, by the effect of papain and pepsin enzymes

Phagocytic cells have membrane receptors for the Fc portions of several different immunoglobulins. These Fc receptors (FcRs) have the following specificity:

- (e) **FcyRI** binds the Fc portion of IgG1, IgG3, and IgG4.
- (f) FcyRII binds the Fc portion of all IgG subclasses.
- (g) **FcyRIII** binds the Fc portion of IgG1 and IgG3.
- (h) FcαR, found on neutrophils, binds IgA.
- (i) FcERI, and FcERII, found on monocytes, mast cells, and basophils, binds IgE.

2. Pepsin digests most of the Fc fragment, leaving one large fragment termed the $F(ab')_2$ fragment.

- **a.** The F (ab')₂ fragment consists of two Fab fragments joined by covalent bonds.
- **b.** The F (ab')₂ fragment has two antigen-binding sites; thus it is **bivalent**, possessing the ability to bind and precipitate an antigen.

H. Hinge region. It is the portion of the H chain between the C_{H1} and C_{H2} domains.

- 1. The hinge region is considered a separated domain because it is not homologous to any of the other domains.
- **2.** In this region, interchain disulfide bonds form between the arms of the Fab fragments, preventing them from folding and thus rendering this portion of the molecule highly susceptible to fragmentation by enzymatic attack.

a. Papain cleaves the molecule on one side of the disulfide bonds, acting directly on the hinge region.

b. Pepsin acts on the opposite side of the disulfide bonds, degrading the molecule just below the hinge region.

3. The hinge region is highly flexible and allows for movement of the Fab arms in relation to each other.

I. Carbohydrate moiety. Immunoglobulins are glycoproteins, from 3% to 13% of the immunoglobulin molecule being composed of **oligosaccharides.**

- 1. The oligosaccharides are a **side chains**, and are usually attached to the immunoglobulin at one or more location in the constant region of H chains (the C_H) region.
 - **a.** N-Glycosidic bonds usually link an N-acetyglucosamine in the carbohydrate moiety to an asparagines residue in the polypeptide chain at the C_H region.
 - **b.** Linkage occurs in the Golgi complex via N-acetylglucosamine-asparagine transglycosylase, a transferase enzyme.

2. The **half-life** of antibodies in the circulation depends on the status of the oligosaccharide side chains.

a. The oligosaccharide terminates in galactose to which sialic acid is frequently attached.b. When antibodies in the circulation have the sialic acid removed by enzymes such as neuraminidase, they become susceptible to degradation in the liver.

- (1) The terminal galactose binds to a receptor on hepatocytes and the entire molecule is internalized by the cell.
- (2) Degradation then occurs due to the proteolytic enzymes in the lysosomes of the cell.

J. J chains. These small proteins connect the two or more basic units in polymeric immunoglobulins (e.g., IgM, and some IgA). They are named **J chains** because of their **joining** function.

- **1.** A **J** chain is glycoprotein chain with a molecular weight of 15 kDa.
- 2. It is covalently linked to the carboxyl terminal portions of α and μ H chains. J chain is synthesized by B lymphocytes.
- **3.** J chain is thought to be involved in the synthesis, polymerization, and /or secretion of IgA and IgM polymers.
- **4.** It is synthesized by B lymphocytes during maturation and is incorporated into polymeric immunoglobulins before they are secreted from the plasma cell.
- K. Secretory component (secretory piece) is unique to IgA immunoglobulin.

L. The sedimentation coefficient (sedimentation constant) and the S value (Svedberg unit)

- **1.** The **sedimentation coefficient** expresses the rate at which a particular sediments during ultracentrifugation.
- **a.** The rate depends on the size, shape, and density of the particle, as well as the density and viscosity of the medium and the speed of centrifugation.
- **b.** Thus the sedimentation coefficient gives an **estimate of molecular weight**.
- 2. The sedimentation coefficient is expressed in Svedberg units (S).
- 3. The S value of immunoglobulins range from 7S to 19S.

QUESTINOING AND ANSWERING STUDY

What are the immunoglobulins?

The body glycoproteins which possess the ability to specifically recognize & bind to specific non-self (foreign = antigen) molecules are called antibodies. Antibodies are a heterogeneous mixture [mixtures of different classes of Igs (isotypes) and different subclasses, and each Ig with different specificity (different specificities)] of structurally related glycoproteins.

Plasma or serum glycoproteins are conjugated proteins, proteins that associated with a non-protein, e.g., carbohydrate residues, traditionally separated by solubility characteristic into water soluble glycoproteins (Albumins), and water insoluble glycoproteins (globulins). All serum globulins (water insoluble) with antibody activity are referred to as immunoglobulins (Igs).

Plasma or serum "glycoproteins" are traditionally separated by solubility characteristics into albumins (water soluble) and globulins (water insoluble) and may be further separated by migration in an electric field, a process called electrophoresis.

Albumin [from albus: white (a protein that liver cells normally secrete into the blood in large amount)] : Any of several simple, water soluble proteins (without the addition of salt and that, unlike a globulin, stays in solution on half saturation with ammonium sulfate, usually serum albumin is meant) that are coagulated by heat and are found in egg white, blood, milk, various animal tissues, and many plant juices and tissues.

Globulin: A category of protein (any of a class of simple proteins), comprising those that are soluble in dilute salt solutions & are precipitated by half saturation with ammonium sulfate, in distinct form the albumins (water soluble), which require complete saturation to precipitate them. Globulins are found extensively in blood, milk, muscle, and plant seeds. Typical globulins were once considered to be insoluble in water in the absence of salt, but the globulins have since been subdivided into the euglobulins, which posses this property, and the pseudoglobuins, which dissolve even in the absence of salt. Generally globulins are insoluble in pure water, soluble in dilute salt solution, and coagulable by heat.

Alpha: The first letter of the Greek alphabet.

Alpha globulin: Any of the globulins (water insoluble proteins) of plasma which have the greatest electrophoretic mobility of the globulins (water insoluble proteins) in neutral or alkaline solutions (pH 8.6).

Beta: The second letter of the Greek alphabet.

Beta globulin: Any of the globulins (water insoluble plasma proteins) of blood plasma characterized by electrophoretic mobility intermediate between those of alpha (fast) and gamma (slow). Gamma: The third letter of the Greek alphabet.

Human Gamma globulin: Has two definitions:

- 1. A group of insoluble plasma proteins characterized by very slow electrophoretic mobility in alkaline buffers. Most antibodies [glycoproteins (globulins)] are found in the gamma globulin fraction (water insoluble, very slow electrophoretically mobility), antibody activity is present in not only in the gamma fraction but also in a slightly more anodic area (anode "+ve" area), and gamma globulins are thus usually identified with immunoglobulins.
- 2. Immunoglobulin:
 - Gamma-A globulin: IMMUNOGLOBULIN A.
 - Gamma-D globulin: IMMUNOGLOBULIN D.
 - Gamma-E globulin: IMMUNOGLOBULIN E.
 - Gamma-G globulin: IMMUNOGLOBULIN G.
 - Gamma-M globulin: IMMUNOGLOBULIN M.



Figure-5 Immunoglobulin E



Figure-6 Secretory IgA (sIgA) molecule



Figure-7 Immunoglobulin M

IgD



Figure-8 Immunoglobulin D

| Isotype | Structure | Placental transfer | Binds mast cell surfaces | Binds phagocytic cell surfaces | Activates complement | Additional features |
|---------|-----------|--------------------|--------------------------------|--------------------------------------|----------------------|--|
| lgM | | - | - | - | + | First Ab in development and response. |
| lgD | B-cell | - | - | - | - | B-cell receptor. |
| lgG | ۲r | + | - | + | + | Involved in opsonization and ADCC. Four subclasses; IgG1, IgG2, IgG3, IgG4. |
| lgE | | - | + | - | - | Involved in allergic responses. |
| lgA | ₩ ≽≪< | - | - | - | - | Two subclasses; IgA1, IgA2. Also found as dimer (sIgA) in secretions. |



Body glycoproteins that possess the ability to specifically recognize and bind to specific entity on a non-self (foreign / antigen):

- **1.** A mixture of heterogeneous shapes and specificities but structurally related glycoproteins.
- 2. A globulin (a water insoluble) protein, a form of glycoprotein.
- 3. Not water soluble (albumin) proteins, a form of glycoprotein.
- **4.** All serum globulins with antibody activities are referred as immunoglobulins
- 5. Conjugated proteins with carbohydrate residues.
- 6. Globulins that separated from albumin by their characteristic of water insolubility.
- 7. Found in the third portion after serum electrophoresis (migration of serum or plasma proteins in an electric field) this third portions is called gamma (third Greek alphabet, the first and second alphabet is called alpha, and beta) globulin portion, which is the slowest in migration towards the anode (+ve) comparing to the alpha or beta portion.

How antibody molecules can separate (purified) from serum, plasma, or other natural fluids (saliva, urine, and tearsetc)?

Antibodies can be separated by two-steps procedure.

- 1. Precipitation with 40-50% saturation of ammonium sulfate. Antibodies can be separated from biological fluid by adding a concentration of ammonium sulfate that ranges from 40-50% saturation. Thus albumin and most small molecules remain in solution, so that partially purified antibody can be collected in a pellet by centrifugation.
- 2. The antibody-containing pellet is redissolved in buffer and then purified by chromatography [The process of separating substances according to the differences in their partition coefficients (the ratio between concentrations of a substance in two phases at equilibrium) between two phases (solid-liquid, liquid-liquid, or liquid-gas). The term chromatography derives from the fact that colored substances were originally separated as colored bands that moved at different speeds down a column]. Homogeneous (similar : same class) antibodies can be isolated from other proteins in the pellet by :
- **A.** Size (gel filtration chromatography), large molecules flow more rapidly through this column and emerge first because a smaller volume is accessible to them.
- **B.** Charge (ion exchange chromatography), proteins can be separated on the basis of their net charge. If a protein has a net positive charge at pH7, it will usually bind to a column of beads containing carboxylate groups, whereas a negatively charged protein will not. A positively charged protein bound to such a column can then be eluted (released) by increasing the concentration of sodium chloride or another salt in the eluting buffer. Sodium ions compete with positively charged groups on the protein for binding to the column. Proteins that have a low density of net positive charge will tend to emerge first, followed by those having a higher charge density.
- C. Specific binding to an antibody-binding molecule such as staphylococcal protein A.
- **D.** Affinity chromatography: when the antibody of interest in the biologic fluid is specific for a known antigen, the antigen can be immobilized (fixed so it can not move) on a column matrix and used to bind the antibody.

In all cases antibodies can be removed from the column matrix by a suitable change in buffer conditions.

- **A.** In the case of affinity chromatography, release often involves temporary and reversible denaturation with a salt solution such as magnesium chloride or with a change in pH.
- **B.** Many such chromatographic procedures, especially gel filtration and ion exchange chromatography, are now routinely run under high pressure using special column matrices to achieve rapid and highly resolved separations. This technique is called high-pressure liquid chromatography (HPLC).

Which type of immune cells produce immunoglobulins?

Immunoglobulins are produced by B-lymphocytes (plasma cells).

What is the main function of the immunoglobulin?

The main function of the immunoglobulin is to:

- 1. Specifically recognize and bind to a unique structural entity on microbial toxins (antigens).
- **2.** Perform a common biological function (effector function) after combining with the antigen e.g., binding to Fc receptors on some immune cells.

What is the serum, antiserum and serology?

When blood or plasma forms a clot (a solid or semisolid mass), antibodies remain in the residual fluid called "serum" (the clear yellowish fluid obtained from coagulated blood). A sample of serum that contains a large number of antibodies that bind specific antigen is commonly called an antiserum. The study of antibodies and their reactions with microbial toxins (antigens) is called "serology".

Where these immunoglobulins (antibodies) are found?

Immunoglobulins are found:

- **1.** Within cytoplasmic membrane-bound compartments (endoplasmic reticulum and Golgi apparatus), and on the surface of B lymphocytes as a cell surface receptor.
- 2. In the plasma and lymph, and to lesser extent, in the interstitial fluid of the tissues where the secreted antibody from B cells accumulates.
- **3.** On the surface of certain immune effector cells, such as mononuclear phagocytes, natural killer (NK) cells, and mast cells, which do not synthesize antibody molecules?
- 4. In the secretory fluids such as mucus and milk, into which certain antibody molecules are specifically transported.

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