P. G. Semester- III,

(CC-10),Vertebrate Immunology,

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ANTIBODIES: Introduction & Definition.

Antibody: An immunoglobulin, a specialized immune protein, produced because of the introduction of an antigen into the body, and which possesses the remarkable ability to combine with the very antigen that triggered its production. In other words **,** Antibodies are glycoproteins, termed as immunoglobulins (Igs), which are produced in response to an immune reaction and specifically bind to **antigens** responsible for initiating the reaction.

They are classified into five major categories based on their physicochemical and biological properties.

Immunoglobulins are also present on B cell membrane and hence serve as membrane bound antibody. Serum antibodies produced by the pharmablast cell in response to a particular entity belong to heterogeneous category because of multiple B cell epitope, therefore circulating antibodies are of polyclonal type.

HISTORY OF ANTIBODIES DISCOVERY:

Edward Jenner in 1798 was the pioneer to establish the concept of vaccination. Smallpox was the dreaded disease at that time, and he inoculated a small boy with fluid from cowpox pustule to get him immunity against this disease. After a century, Emil Von Behring showed that

serum of animals immunized against Diphtheria could cure other animals. He and his colleague were awarded a noble prize for this discovery in 1901. In 1900 Paul Ehlrich, first proposed the model for an antibody molecule wherein its branched structure had multiple sites for binding the antigen and complements. It was later in 1948, Astrid Fagraeus described that antibodies arose from plasma B cells of the body and then in 1957 Frank Burnet and David Talmage proposed a clonal selection theory to explain how antibodies specific to an antigen are made by these cells. In 1959 Gerald Edelman and Rodney Porter independently published the molecular structure of antibodies.

Basic Structure of Immunologobulins :

The basic structure of immunoglobulin was first deciphered by Porter and Edelman in the early 1950s. Immunoglobulin molecule is composed of four polypeptide chains with two identical heavy chains and two identical light chains – designated as H_2 and L_2, respectively. The polypeptide chains, H and L are linked to each other by disulphide bond. Further, the identical heavy chains are inter-bridged by a disulphide bond giving a Y shaped structure.

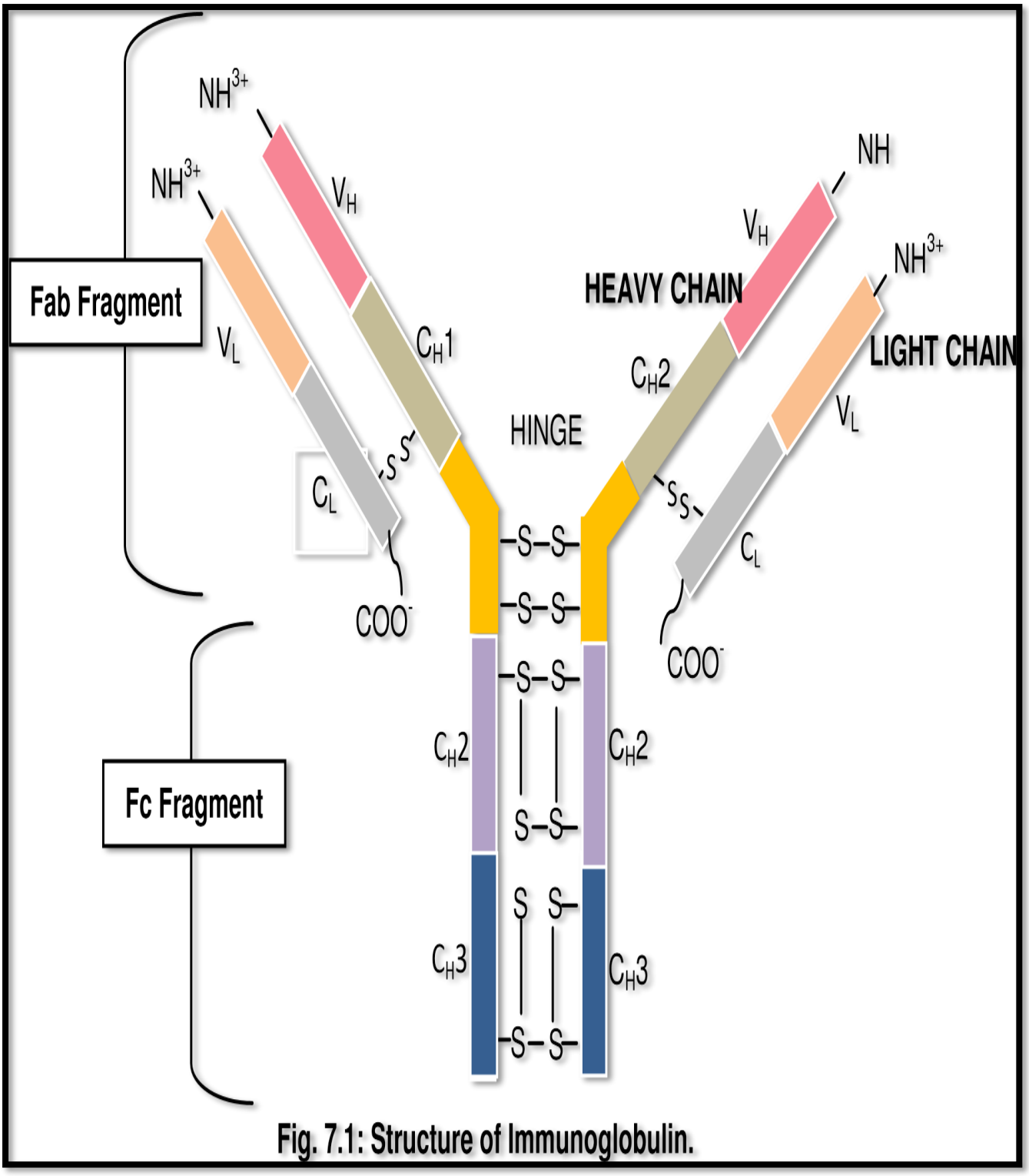


Fig. 7.1: Structure of Immunoglobulin.

The heavy and light polypeptide chain of the immunoglobulin is divided into different regions based on the similarity in the composition of amino acids: -

Variable region: From the amino-terminal, about 100-110 amino acids sequences in both heavy and light chains are highly variable among different immunoglobulins. Hence, the region is known as Light chain variable region (V_L) and Heavy chain variable region (V_H) respectively. This is about half the length of L_2 Chain. This region is also known as **Fab Fragment** or Fab arm. They function as the antigen binding fragment/ portion of immunoglobulins. The Fab domains have two variable regions ad two constant regions. The variable regions which have three hypervariable regions are called **Complementarily Determining Region (CDR)** which provide specific antigen recognition sites and binding.

Constant region: The remaining portion towards the carboxyl terminal is known as Constant region. They are abbreviated as C_L in light chain and C_H in heavy chain. The C_L polypeptide chains are composed of two different types. They are designated as *Kappa* (κ) which constitutes 60% and *Lambda* (λ) 40% of the region. In heavy chain, the constant region is abbreviated as C_H1 the segment closer to the amino-end, C_H3 segment nearer to carboxyl end and C_H2 segment between the two. The composition of polypeptides in C_H region determines the class of antibodies. C_H is about 330 amino acids for λ, δ, α and
440 for μ, ε class of immunoglobulins. This region is also called as **Fc Fragment** or "crystallizable fragment". They function as mediators in phagocytosis, triggering of inflammation and other immunological response. Antibodies are glycosylated proteins with the position and extent of glycosylation varying between isotypes. The glycosylation is present in the Fc region.

Hinge region: Hinge region is an extended portion between C_H1 and C_H2 of λ, δ and α heavy chain that are rich in proline residues. However, this region is absent in μ and ε class of immunoglobulins. The prominent

amino acids of the hinge region are proline and cysteine. The cysteine residue facilitates in formation of disulphide bond between the identical heavy chains and presence of rich proline residue in the hinge region imparts flexibility to the Fab arm.

Types of Immunoglobulins

The circulating antibodies provide **humoral immunity** which does not last longer unlike cellular immunity and also does not act on small quantities of antigens. These proteins are involved in immunological reaction and are therefore called **immune-globulins.** They are classified into five major categories based on their physicochemical and biological properties. Despite their diversity, due to their common property, they have the ability to cross react with same antigen indicating and all have antibody activity. Immunoglobulins are also present on B cell membrane and hence serve as membrane bound antibody. Serum antibodies produced by the pharmablast cell in response to a particular entity belong to heterogeneous category because of multiple B cell epitope, therefore circulating antibodies are of polyclonal type.

All the classes of immunoglobulins possess κ/λ type of light chain. Therefore, depending on the characteristics of heavy chain Igs are classified as follows and tabulated in Table 7.1:

Immunoglobulin G (γ-Globulin G or IgG)

IgG contains γ type heavy chain. IgGs are about 150 MW (kDa) having a half life of about 20-21 days. IgGs are the largest group of immunoglobulin class constituting of about 80%. They possess binding valency of 2.

Immunoglobulin M (γ-Globulin M or IgM)

IgM contains μ type heavy chain. IgMs are about 900 MW (kDa) having a half life of about 5 days. IgM constitutes about 5%-10% of the total immunoglobulin class. They have the highest binding valency about 5 or 10.

Immunoglobulin A (γ-Globulin A or IgA)

IgA contains α type heavy chain. IgAs are about 160 MW (kDa) having a half life of about 6 days. IgA are the second largest group of immunoglobulin class constituting of about 10%-15%. In external secretions such as breast milk, saliva, tears and mucus of the bronchial, genitourinary and digestive tracts, IgA is the most predominant immunoglobulin type. They have binding valency of 2.

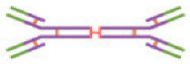
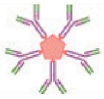
Immunoglobulin E (γ-Globulin E or IgE)

IgE contains ε type heavy chain. IgEs are about 200 MW (kDa) having a half life of about 2-3 days. IgA are the rarest group of immunoglobulin class constituting of only 0.002%. They have binding valency of 2.

Immunoglobulin D (γ-Globulin D or IgD)

IgD contains δ type heavy chain. IgDs are about 185 MW (kDa) having a half life of about 3 days. IgD constitutes about 0.2% of the total immunoglobulin class. They have the binding valency of 2.

The five classes of immunoglobulins:

Name	Properties	Structure
IgA	Found in mucous, saliva, tears, and breast milk. Protects against pathogens.	
IgD	Part of the B cell receptor. Activates basophils and mast cells.	
IgE	Protects against parasitic worms. Responsible for allergic reactions.	
IgG	Secreted by plasma cells in the blood. Able to cross the placenta into the fetus.	
IgM	May be attached to the surface of a B cell or secreted into the blood. Responsible for early stages of immunity.	

THE IMMUNOGLOBULIN MOLECULE IS FLEXIBLE ::

The hinge region that links the FC and Fab portions of the antibody molecule in reality is a flexible theatre allowing independent movement of the two Fab arms, rather than a rigid hinge. This feature has been demonstrated under electron microscope when antibodies get bound to **haptens**. This is a small molecule of typically about the size of tyrosine side chain. This can be recognized by the antibodies but are unable to stimulate the production of anti -hapten antibodies, only when they link to larger protein carrier. Under an electron microscope, it is possible to visualize the dimers, trimers, tetramers and so on which are formed when an antigen made up of identical hapten molecules joined by a flexible region, can link together with two or more anti- antibody. The shapes formed by such complexes demonstrate that antibody molecules are flexible at the hinge region, some flexibility is also found at the junction between the V and C domain also. This allows bending and rotation of the V domain relative to the C domain. This range of motion has led to junction between V and C domain and is called **molecular ball and socket joint**. Flexibility at both the hinge and the V and C junction enables both arms of the antibody molecule to bind two sites that are distant apart eg: sites on bacterial cell wall polysaccharides. This flexibility also helps the antibodies to interact with antibody binding proteins that mediate immune effector mechanism.

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