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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OFENGINEERING & TECHNOLOGY

LT3.Structure and function of Immunoglobulin

Content Outline

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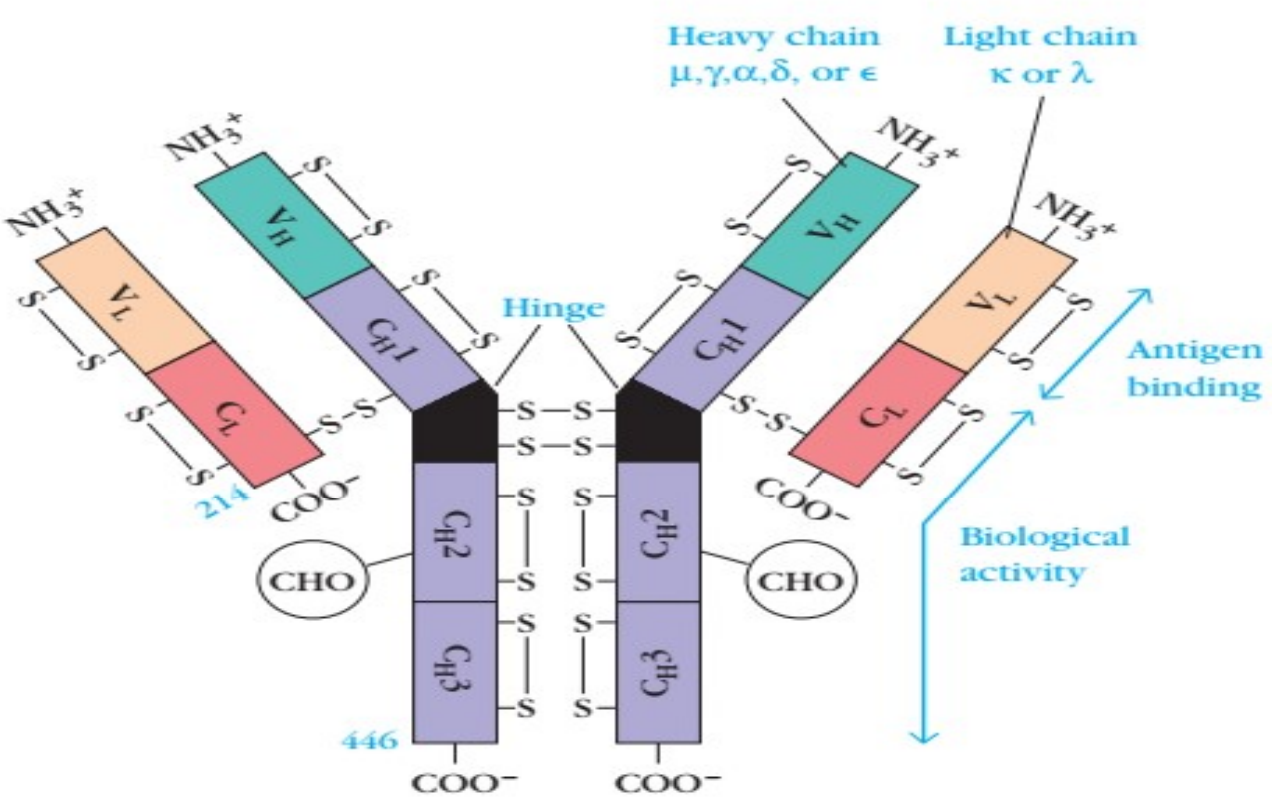
Structure of Immunoglobulin

Immunoglobulins are glycoprotein molecules that are produced by plasma cells in response to an immunogen and which function as antibodies. These are found in the blood plasma, lymph and secretions such as saliva, tears, and gastrointestinal fluid. Most antibodies are found in γ-globulin fraction of the serum. The structure of the antibodies was discovered by Rodney Robert Porter and Gerald M. Edelman.

Basic structure of Immunoglobulins

Immunoglobulins are heterodimers containing four peptide chains as their basic unit. They are composed of two identical light chains (23kD) and two identical heavy chains (50-70kD). Each light chain is bound to a heavy chain by a disulfide bond, and by noncovalent interactions as salt linkages, hydrogen bonds, and hydrophobic bonds, to form a heterodimer (H-L). Similar noncovalent interactions and disulfide bridges link the two identical heavy and light (H-L) chain combinations to each other to form the basic four-chain (H-L)2 antibody structure, a dimer of dimers. 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: **Light Chain** - VL (110 amino acids) and CL (110 amino acids) **Heavy Chain** - VH (110 amino acids) and CH (330-440 amino acids). The variable region on both heavy and light chain is responsible for difference in sepecificity of different antibody. Immunoglobulins also has hinge 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. To the immunoglobulin molecules carbohydrate molecules are also attached and hence it is a glycoprotein. The site of attachment for catbohydrate is restrictrd to constant region of immunoglobulin. Glycosylation of immunoglobulin is believed to increase the solubility of immunoglobulin and enhance the interaction between antibody and the complement system and between antibodies and Fc receptors.



Basic structure of immunoglobulins (*The amino-terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the other domains) VL = Variable region on light chain, VH = variable region on heavy chain, CH = constant region on heavy chain, CL = Constant region on light chain.*

STRUCTURE OF THE VARIABLE REGION OF IMMUNOGLOBULINS

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. The three heavy-chain and three light-chain CDR regions are located on the loops that connect the strands of the VH and VL domains . 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. Hypervariable regions form the antigen binding site of the antibody molecule.

B. Framework regions

The regions between the complementarity determining regions in the variable region are called the framework regions i.e. it separates the variable regions. Based on similarities and differences in the framework regions the immunoglobulin heavy and light chain variable regions can be divided into groups and subgroups. Framework region exhibit far less variability than the hypervariable region. The wide range of specificities exhibited by antibodies is due to variations in the length and amino acid sequence of the six CDRs in each Fab fragment. The framework region acts as a scaffold that supports these six loops i.e it holds the variable regions in position to contact inhibition.

Immunoglobulin fragments: structure/ Function relationship

A.Fab

Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain disulfide bond into three fragments. This results in the formation of two identical fragments that contain the light chain and the VH and CH1 domains of the heavy chain. Each of the two fragments has MW = 45000 and had antigen-binding activity and were called Fab fragments. 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 different antigenic determinants

B. Fc fragments

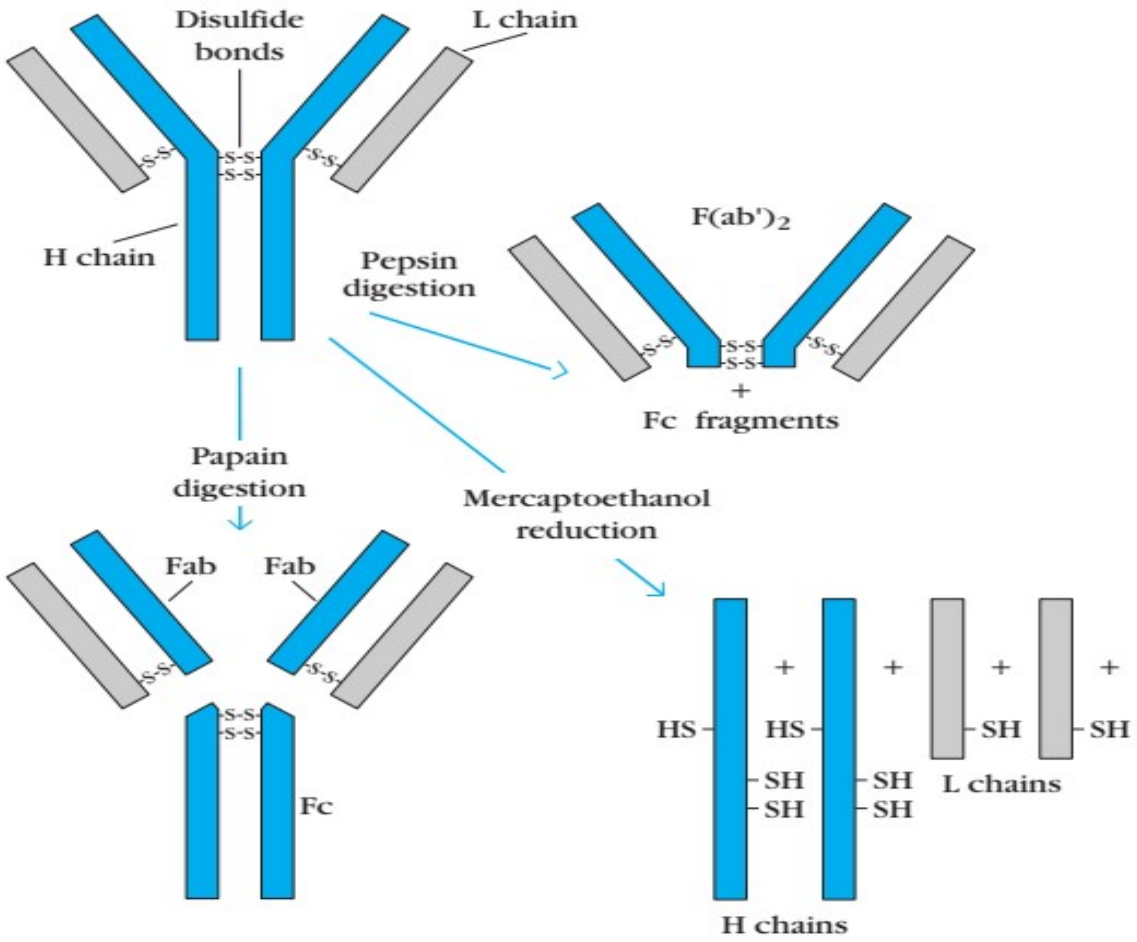
The third fragmen had no antigen binding site and had MW = 50000, was called Fc fragments (because it was found to crystallize during cold storage, Fc= fragments crystallizable). Fc contains the remainder of the two heavy chains each containing a CH2 and CH3 domain.

C. F (ab)2 fragments

Pepsin splits IgG into an Fc fragment and a single dimeric $F(ab)_2$ that can cross-link as well as bind antigens. The F(ab')2 binds antigen but it does not mediate the effector functions of antibodies. The Fc fragment was not recovered from pepsin digestion because it had been digested into multiple fragments.

D. Mercaptoethanol and alkylation digestion

Treatment with mercaptoethanol and alkylating agent irreversibly cleaves disulphide bonds of immunoglobulin. Each IgG molecule contains two 50,000-MW polypeptide chains, designated as heavy (H) chains, and two 25,000-MW chains, designated as light (L) chains.



Different digestion procedures and fragments of immunoglobulin after treatment

Definitions of Paratopes, epitopes, idiotypes and isotypes

Paratope : Immunoglobulin-antigen interactions typically take place between the *paratope*, the site on the Ig at which the antigen binds,

***Epitope* :** It is the site on the antigen that is bound to paratope on antibody

Cross-reactivity: The ability of the same antibody to bind divergent antigens that share equivalent or similar epitopes.

Idiotype: Individual determinant(s), termed *idiotype(s)*, are contained within V domains. An idiotype is a shared characteristic between a group of immunoglobulin or T cell receptor molecules based upon the antigen binding specificity and therefore structure of their variable region

Isotype: Common determinants, termed *isotypes*, are specific for the constant portion of the antibody and allow grouping of immunoglobulins into recognized classes, with each class defining an individual type of C domain. It is a mechanism that causes the production of **antibodies** to change from IgM. or IgD to the other **antibody isotypes**, IgE, IgA or IgG, that have defined roles in the immune system. In immunology, the "immunoglobulin isotype" refers to the genetic variations or differences in the constant region of the heavy chain of the Ig (immunoglobulins) classes and sub-classes. In humans, there are nine isotypes

Allotype: Determinants common to subsets of individuals within a species, yet differing between other members of that species, are termed *allotypes* and define inherited polymorphisms that result from gene alleles

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 be 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 types 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 2

c) Lambda 3

d) Lambda 4

STRUCTURE AND SOME PROPERTIES OF IG CLASSES AND SUBCLASSES

Immunoglobulins G (IgG)

IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin. The IgG molecule consists of two heavy chains and two light chains. There are four human IgG subclasses, distinguished by differences in -chain sequence and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4. All Ig G antibodies are monomer.

Properties of IgG

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;

b) 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.

IgM

1. Structure

IgM accounts for 5%–10% of the total serum immunoglobulin, with an average serum concentration of 1.5 mg/ml. 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 extra 20 amino acids at the C-terminus to anchor it into the membrane. 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. 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

1. Structure

IgA constitutes only 10%–15% of the total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears, and mucus of the bronchial, genitourinary, and digestive tracts. Serum IgA is a monomer but IgA found in secretions is a dimer. 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. The secretory piece helps IgA to be transported across mucosa and also protects it from degradation in the secretions.

2. 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

IgD exists only as a monomer. It has a serum concentration of 30 g/ml and constitutes about 0.2% of the total immunoglobulin in serum.

2. Propertiess

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.

GENERAL FUNCTIONS OF IMMUNOGLOBULINS

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)

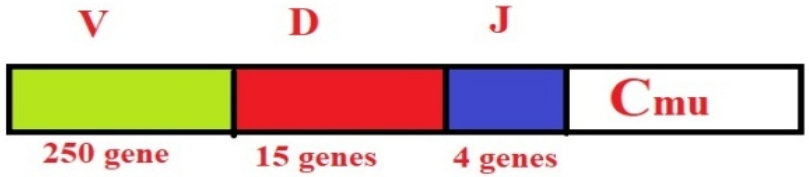
2. Binding to various cell types - Phagocytic cells, lymphocytes, platelets, mast cells, and basophils have receptors that bind immunoglobulins. This binding can activate the cells to perform some function. Some immunoglobulins also bind to receptors on placental trophoblasts, which results in transfer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

Molecular basis of Immunoglobulin formation and diversity

•Ig heavy and light chains are each encoded by a separate multigene family, and the individual V and C domains are each encoded by independent elements: V(D)J gene segments for the V domain and individual exons for the C domains. The primary sequence of the V domain is functionally divided into three hypervariable intervals, termed complementarity determining regions (CDRs) that are situated between four regions of stable sequence termed frameworks (FRs). The variable regions of the gene for light (L) chains of immunoglobulins are formed by the association of V and J genes. In case of heavy chains V,D, and J genes comes together.

•The only allowed pairing during recombination event of different gene segments are V with D, and D with J. The site specific recombination event is mediated by two recombination activating gene (RAG-1 and RAG-2), which are almost exclusively expressed in developing lymphocytes. This combinatorial association of different gene segments greatly increases the diversity of the antibody repertoire. For example, 56,250 different kind of H genes can be formed from 250 V genes, 15 D genes, and 5 J gene that can be joined in three frames.

The variable regions of the heavy chain is encoded by –V, D- and J-segment genes



Test your understanding

The basic structure of antibodies are_____
a) Y-shaped
b) X-shaped
c) Linear
d) Hyperbolic

What is the name of the hypervariable region of immunoglobin, which is responsible for its diversity?
a. CDR
b. Hinge region
c. Epitopes
d. Agretope

Which of the following amino acid is found in the hinge region?
a) Alanine
b) Aspargine
c) Proline and cysteine
d) Phenylalanine

Name the class of immunoglobulin which has a pentameric structure?
a) IgE
b) IgG
c) IgA
d) IgM

References & Further reading

Further reading

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