ANTIBODY

ANTIBODY - AN INTRODUCTION

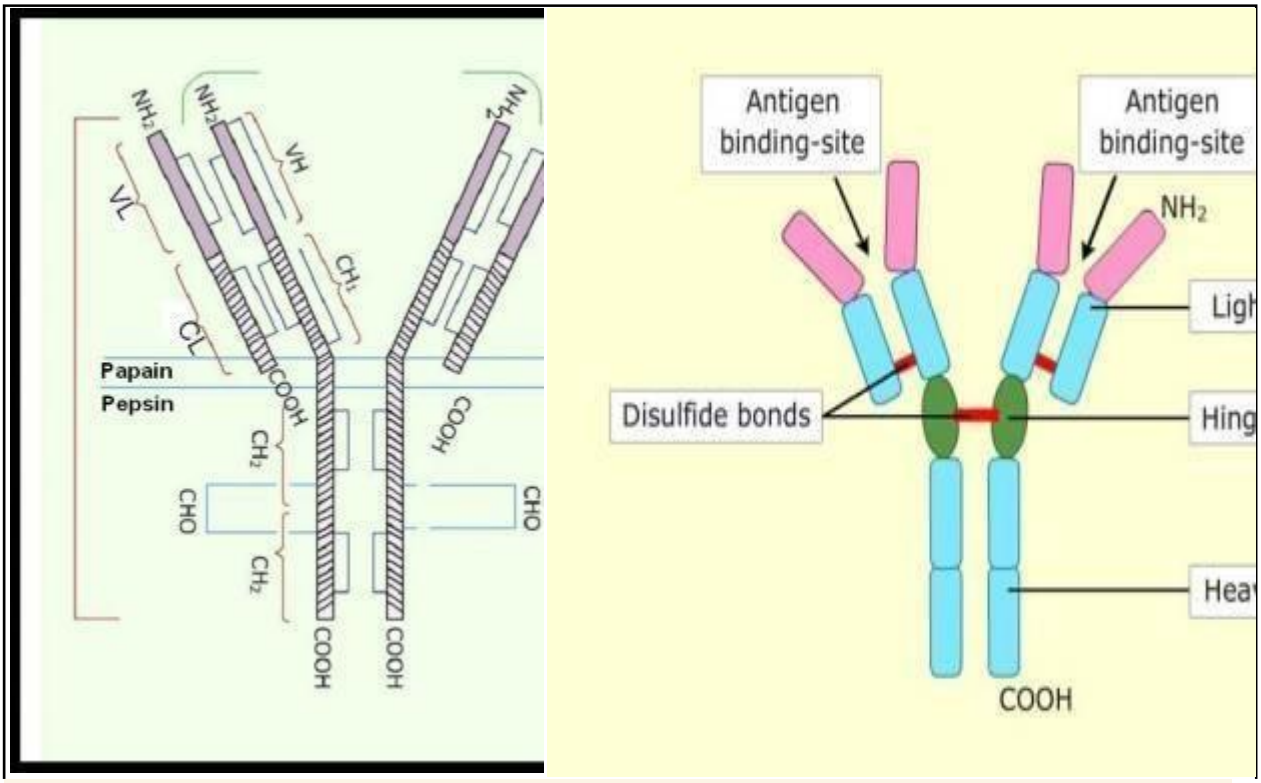
- An antibody is a specific substance produced in the body in response to an antigen.
- Antibodies are soluble B cell antigen proteins shed from B cell into surrounding fluid following antigenic stimulation.
- They bind specifically with antigen and try to destroy or eliminate from the body.
- Antibodies are found in many body fluids but more concentration in serum.
- The antigen and antibody react specifically in some detectable way.

NATURE

- Antibody molecules are glycoproteins.
- Tiselius and Kabat (1938) subjected immune serum to electrophoresis and separated its protein into four major fractions - serum albumin, alpha (α), beta (β) and gamma (γ) globulins.
- Most immunoglobulins are found in the gamma fraction and some are in beta fraction.
- Immunoglobulins are heterogeneous group of proteins and constitute about 20% of plasma proteins.
- Based on sedimentation studies using ultracentrifuge, it was found that most antibody molecules sediment at 7S (Mol. Weight 150000Da) and some at 19S (Mol. Weight 900 000Da). 'S' stands for Svedberg unit = a sedimentation constant of 1×10^{-13} seconds.
- Protein molecules that bind specifically with antigen are called *antibodies*.
- Proteins with antibody activity are called *immunoglobulins*.
- The term immunoglobulin is a structural and functional concept; antibody is a biological and functional concept.
- Immunoglobulins have been classified into five groups based on physicochemical and antigenic differences – IgG, IgM, IgA, IgD and IgE.

STRUCTURE OF IMMUNOGLOBULINS

- The immunoglobulins are a large group of closely related heterogeneous glycoproteins.
- An immunoglobulin molecule is about 160 KDa and composed of 2 part of peptide chains of different sizes.
- The larger chains are called heavy (H) chains (50-60 KDa) and smaller chains are called light (L) chains (25 KDa).
- Both the light chains and both the heavy chains are identical.
- The carboxy (C) terminus domains of the heavy chains are inserted into the lipid bilayer of the B cells surface membrane.
- The light chains are only half the length of heavy chains and they are linked to heavy chains at its amino (N) terminus by disulphide (-S-S-) bonds, giving 'Y' shaped appearance.
- Two heavy chains are also joined together by disulphide bonds.
- The tail of 'Y' is F_C (crystalisable fragment can be crystallized in the cold) region is attached to the B cell and the arms called F_{ab} region which bind antigen.
- The antigen binding sites are formed by the groove created between light and heavy chains at their N-terminus.



HEAVY CHAINS

- In general there are five types of Ig heavy chain denoted by the Greek letters: α, δ, ε, γ, and μ , which determines the class of antibody such as IgA, IgD, IgE, IgG, and IgM antibodies respectively.
- Distinct heavy chains differ in size and composition, α and γ contain approximately 450 amino acids, while μ and ε have approximately 550 amino acids.
- Each heavy chain has two regions, the constant region and the variable region.
- The constant region is identical in all antibodies of the same isotype, but differs in antibodies of different isotypes.
- Heavy chains γ, α and δ have a constant region composed of three domains, and a hinge region. Heavy chains μ and ε have a constant region composed of four domains.
- The variable region of the heavy chain differs in antibodies produced by different B cells, but they are same for all antibodies produced by a single B cell or B cell clone.
- The variable region of each heavy chain is approximately 110 amino acids long and is composed of a single Ig domain .

LIGHT CHAINS

- Two light chains are either κ (Kappa) or λ (lambda) but never both in one immunoglobulin.
- The names were derived from Koragold (κ) and Lapori (λ) who originally described. Each light chain consists of two domains of 110 amino acids.
- The amino acid sequence of C-terminus domain is constant (C_L) where as sequence of N-terminus is variable (V_L) and some regions show great variability, known as hyper variable regions or complementarity determining regions (CDRs) or hot spots, each containing 6-10 amino acids. Between the CDRs there are intervening peptide sequences called frame work regions.
- Three regions show maximum variability i.e. at position 24-34, 50 to 56 and 89-97.
- The amino acid sequence in V_L domain of each light chain is different. Molecular weight of each light chain is 25KDa.
- The presence of κ or λ light chain varies with species. For example, cattle and horses have 95 % λ, rat and mice have over 95 % κ chain, monkeys have 50 % of each and humans have 60 % κ chains.

HINGE REGION

- *Fab* regions of immunoglobulin which bind antigen can swing around central molecule and it is possible as they are hinged.
- The hinge region is about 12 amino acid long and located between C_H1 and C_H2 and the sequence is unique for each immunoglobulin class and subclass. But the μ and ε heavy chains do not have hinge region.
- The hinge region of IgD lack in cysteine residue thus there is no inter chain links.
- This region is hydrophilic and rich in proline residues.

- When digested with proteolytic enzyme *papain*, it cleaves at hinge region by hydrolysis of peptide bonds and produce 3 fragments, *2 'Fab' fragments (sedimentation coefficient is 3.5S) and one F_C fragment.*
- When digested with *pepsin*, the site of cleavage is towards carboxy terminal side of disulphide bond and produce two Fab fragments held together [referred as $F(ab)_2$] with sedimentation coefficient of 5S and F_C fragment is digested into multiple fragments.

IMMUNOGLOBULIN CLASSES

- The five different forms of immunoglobulin molecules are common to all members in a particular species and referred as immunoglobulin *isotypes or class*.
- Different forms of the immunoglobulin isotypes are class *subisotype or subclass*. They are,
    - IgG
    - IgM
    - IgA
    - IgE
    - IgD

IMMUNOGLOBULIN G(IgG)

- This is the major source of immunoglobulin secreted by plasma cells (plasma cells develop from B cells on antigenic stimulation and secretes immunoglobulins) found in the spleen, lymph nodes and bone marrow.
- IgG is 7S immunoglobulin with molecular weight of 160 KDa found highest concentration in blood.
- It constitutes about 80 % of all immunoglobulin and about 20 % of plasma proteins.
- They have lowest electrophoretic mobility and found in γ fraction.

Structure

- It has two heavy γ chains and two light chains of either κ or λ types but not both. Fc region has CH^1, CH^2 and CH^3 domains.
- It is smallest of all immunoglobulins and can easily escape the blood vessels into the area of inflammation and distribution in extra vascular and intravascular compartments.
- It is found in almost all tissue fluids and secretion except CSF.
- It is the only immunoglobulin can pass placental barriers and found in newborn because of passive immunization.
- Based on antigenic and structural differences in heavy chains the subclasses of IgG in humans are IgG1 (65-70%), IgG2 (23-28%), IgG3 (4-7 %) and IgG4 (3-4%) and numbers were given in accordance with their decreasing average serum concentration.
- Inter chain disulphide bindings vary between two heavy chains at their hinge region. It is less in IgG1 and more in IgG2, IgG4 and IgG3 in order. In dogs and cats also, IgG have been classified into IgG1, IgG2, IgG3 and IgG4. In cattle, IgG is classified into IgG1, IgG2 and IgG3.
- In horse it is IgG1, IgG2, IgG3, IgG4, IgG5 and IgG6. In sheep, they are classified as IgG1, IgG2 and IgG3.
- IgG contain about 3 % carbohydrate. Half-life varies with species.
- IgG has high affinity.

Functions

- Act as antitoxins
- Form antiviral antibodies
- Act as precipitins
- Act as complement fixing antibodies.
- Provide passive immunity in new born animals or birds
- It is produced later to IgM but provide long lasting immunity.
- C_H3 domains of F_C region bind to macrophages for biological activity.

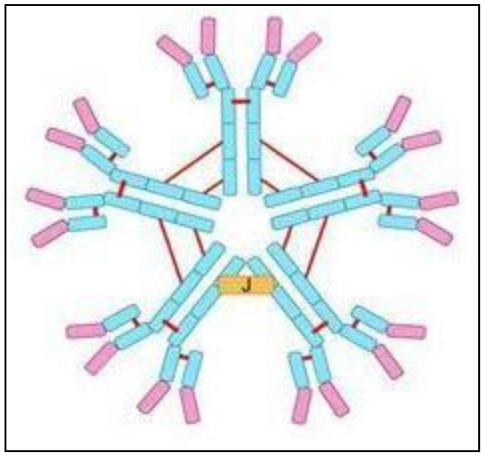
IgM (MACROGLOBULIN)

- It is also secreted by plasma cells in spleen, bone marrow and lymph nodes.
- It has sedimentation co-efficient of 19S (900 KDa) and constitutes about 5-8% of all immunoglobulins and giving rise to second highest concentration in mammalian serum.

Structure

- On B cell surface, IgM is single monomer (180KDa) but the secreted IgM is a pentamer (occasionally hexamer).

- Five monomers of IgM are linked in a circular fashion by disulphide bonds between heavy chins and one-addition chain rich in cysteine and asparagine called *J Chain* (15 KDa) joing two monomers to complete the circle.
- *J chain* is about 118-125 amino acid long.
- Each IgM monomer has two heavy μ chains and 2 light chains of either κ or λ.
- Each heavy chain has an additional domain (C_H4) at the C terminus of F_C region.
- IgM do not have hinge region and contain about 12 % Carbohydrate.
- IgM is predominantly present intavascularly (80%) and theoretically the pentamer IgM has valence of 10 (each subunit posses two antigens binding sites) but practically found to be 5 probably due in steric hindrance.
- IgM found in serum (pentamer) can be disrupted by treating with 2-mercaptoethanol (0.12M) into monomers, as disulphide bounds joining the monomers are broken. This treatment differentiates IgM antibodies from IgG antibodies.
- IgM is the first antibody produced in primary response to antigen and half-life is relatively shorter than IgG. Based on peptide mapping and complement fixing activity IgM is classified into IgM1 and IgM2 in humans but not in other animal species. IgM has high avidity.



Functions

- Biologically more active than IgG. A single molecule of IgM can cause immune hemolysis where as 1,000 IgG are required for the same effect.
- IgM is 500-1000 times more effective than IgG in opsonization, 100 times more effective in bactericidal action and 20 times more effective in agglutination.
- The complement-binding site is present in C_H4 and C_H3 domains. It acts as complement fixing antibody. It is more active than IgG.
- It is the first antibody produced in primary immune response.

IMMUNOGLOBULIN A (Ig A)

- It is produced by plasma cells present mainly in the intestinal tract, respiratory tract, urinary tract, mammary gland and skin.
- Serum concentration is usually lower than IgM (except in humans).
- It is the major Immunoglobulin found in colostrums, saliva and tears.
- IgA has sedimentation coefficient of 7S with molecular weight of 160 KDa to 360 KDa.

Structure

- IgA molecule has two α heavy chains containing 3 constant domains (C_H1, C_H2, C_H3) and either two κ or two λ light chains.
- IgA occurs in two forms.
    - Serum IgA
    - Secretary IgA
- *Serum IgA* is a monomer (7S with molecular weight 160 KDa).
- But *secretary IgA (SIgA)* usually found in mucosal surfaces and in secretions is a dimer.
- It is formed by two 7S IgA monomer joined at their carboxy terminus of Fc region by "J" chain and also with a secretary component (Sc).
- "J" chain is synthesized by the same plasma cells.
- Secretary component is a glycoprotein (71 KDa), synthesized in the epithelial cells of the mucous membrane (not by plasma cells) and present as coil about the double $F_C$cylinders.
- Synthesis of Sc is independent of the production of IgA.

- The dimer IgA (SIgA) is much longer (11S with molecular weight of 360KDa).
- The Sc protects the SIgA from proteolysis by the gastrointestinal enzymes.
- Two IgA sub classes have been identified IgA1 and IgA2.
- The sub class IgA2 lacks disulphide bonds between the heavy and light chains. It is a minor component in serum but more present in secretions.
- The sub classes IgA1 and IgA2 were recorded in humans, cat, mouse, sheep and pigs. IgA posses about 7% carbohydrate.

Functions

- Provide local immunity to mucosal surface of respiratory and intestinal tract.
- It does not fix complement but can activate alternative complement pathway.
- It helps in phagocytosis and intracellular killing of microorganisms
- It is a minor component in systemic humoral immunity but plays a major role in mucosal immunity. IgA antibodies found in gut contents or feces are known as *copro antibodies*.

IMMUNOGLOBULIN E (Ig E)

- It is synthesized by plasma cells located beneath body surfaces. It has two ε heavy chains and two light chains (either κ or λ).
- Constant region of heavy chain composed of four domains (C_H 1 to C_H4).
- It has sedimentation coefficient of 8S (molecule weight 190 KDa). IgE has about 12 % carbohydrate.
- The serum concentration is very low with shortest half-life.

Functions

- *Reaginic antibody:* Provide protection against microbes by degranulation of mast cells .IgE adhere to cell surface through C_H3 and C_H4 domains and release of inflammatory mediators (vesoreactive substances).
- It mediates immediate hypersensitivity reaction (Type-1) e.g. anaphylactic shock, hay fever, asthma etc.
- Provide immunity against parasitic worms.
- It does not fix complement components.

IMMUNOGLOBULIN D (Ig D)

- It is secreted by plasma cells in spleen and lymph nodes. It has two δ heavy chains and two light chains (either κ or λ) with molecular weight of 180 KDa (7S).
- Mouse IgD lacks C_H2 domain and hinge region separates this two domains. But cattle, sheep, pig and humans have 3 constant domains C_H1 to C_H3.
- It has no interchain disulphide bonds. It is susceptible to proteolytic enzymes and thus could not be detected in serum but found in low concentration in plasma.

Functions

- It is not found in all animal species
- Function is not well established

CHARACTERISTICS OF IMMUNOGLOBULINS

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	IgG	IgM	IgA	IgE	IgD
Molecular weight	180,000	900,000	360, 000	190,000	180,000
Sub units	1	5	2	1	1
Heavy chain	γ	μ	α	ε	δ
Sedimentation coefficient	7S	19 S	7 S to 11S	8S	7S
Electrophoretic	γ	β	β- γ	β- γ	γ

mobility					
Carbohydrate percentage	3	12	7	12	12
Mainly synthesized in	Spleen and lymph nodes	Spleen, bone marrow and lymph nodes	Intestinal and respiratory tracts	Intestinal and respiratory tracts	Spleen and lymph nodes
Biological property	Major Ig in serum. Provides the majority of antibody-based immunity against invading pathogens. Moderate complement fixer (IgG3) can cross placenta.	First response antibody. Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated immunity before there is sufficient IgG.	Most produced Ig. Found in mucosal areas, such as the gut, respiratory and urogenital tract, and prevents their colonization by pathogens. Resistant to digestion and is secreted in milk.	Binds to allergens and triggers histamine release from mast cells and is involved in allergy. Also protects against parasitic worms.	Function unclear. Works with IgM in B-cell development; mostly B cell bound

IMMUNOGLOBULINS IN BIRDS

- Predominant serum immunoglobulin in birds is lgY similar to mammalian IgG,
- Heavy chain is Epsilon.
- Chicken possesses the full sized 180 KDa isoform of lgY. Ducks and geese possess full sized and truncated (120KDa)isoforms.
- A monomeric IgM can be defected in the amniotic fluid of eggs and in one day old chicks.
- IgA is present in chicken secretions and polymers are common.
- An avian homolog of IgD has been identified.

ALLOTYPES AND IDIOTYPES

Allotypes

- Immunoglobulins of one individual may be structurally different from another individual of the same species. This variation is called allotypes and it is determined genetically.

- This may occur due to single amino acid variation as heritable polymorphism.
- These have been identified within IgG, IgA and on κ chains.

Idiotypes

- When structural difference is due to variation in amino acid sequence in the variable domain of heavy and light chains,these variants are called Idiotypes.

ABNORMAL IMMUNOGLOBULINS

- Bence Jones protein in multiple myeloma etc.,
- Bence Jones proteins – Excess immunoglobulin light chains found in the urine of myeloma patients.
- They precipitate out when the urine is warmed and re-dissolve at higher temperatures.