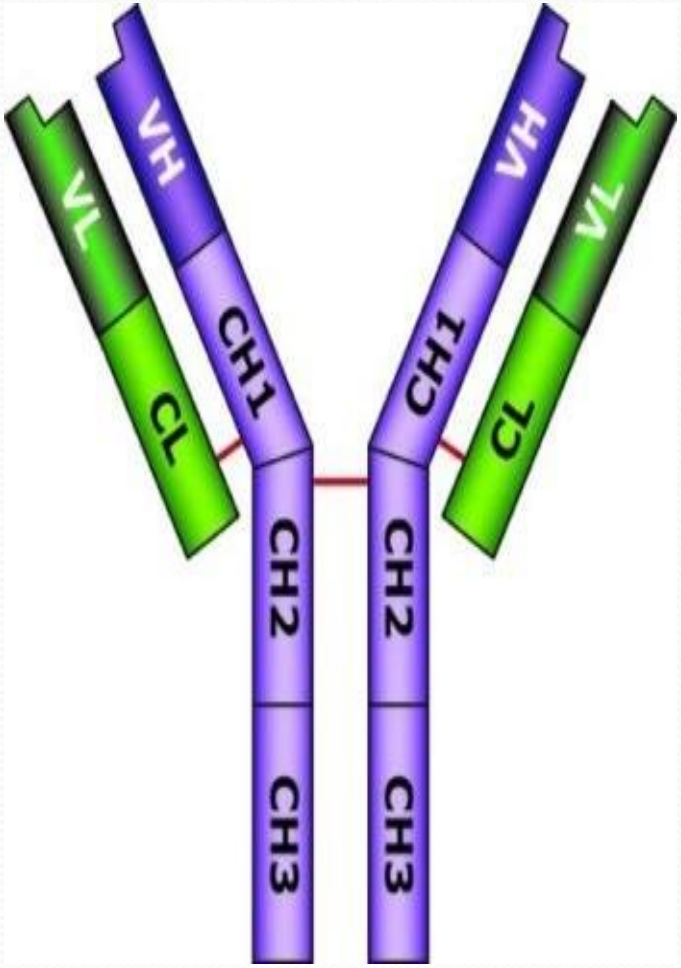
Antibody



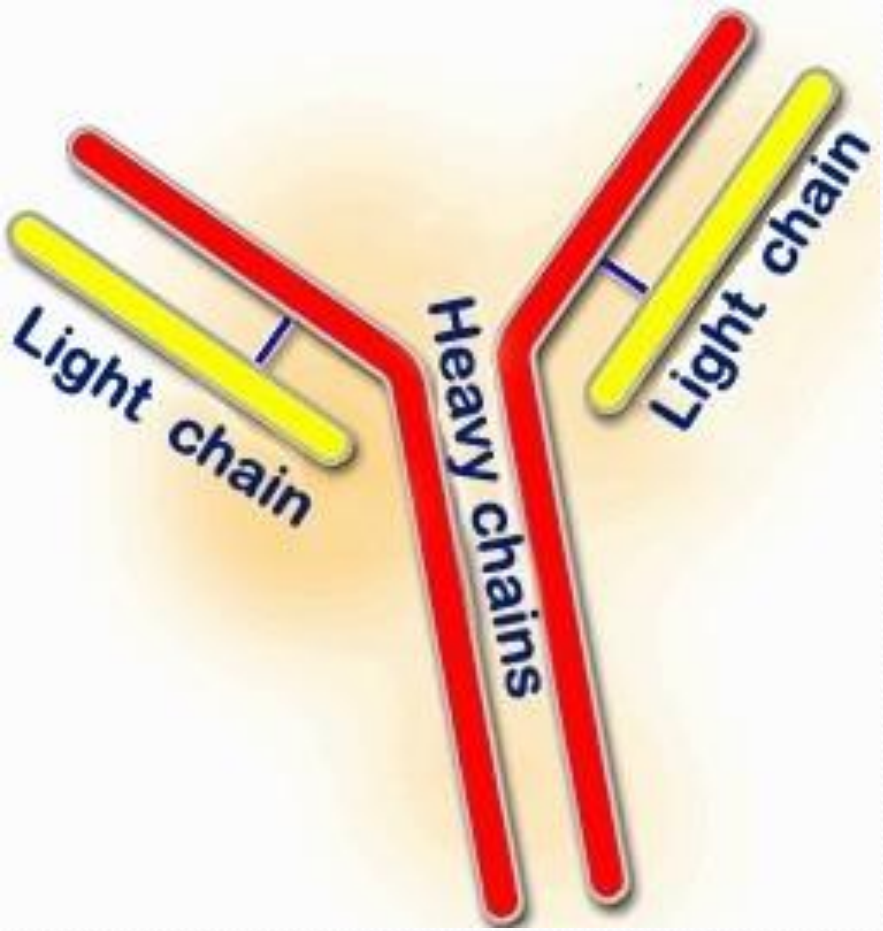
Introduction

- Also called immunoglobulin (Ig)
- Immunoglobulin is a glycoprotein that is made in response to an antigen and can recognize and bind to the antigen that caused its production.
- Protects us from microbial infection.

- Are gamma globulins
- Synthesized by plasma cells
- Constitute 25-30 % of total serum proteins
- Antibodies are present in serum, tissue fluids and mucosal surfaces and on surface of B-cells where they acts as antigen receptor.

Basic structure

- Composed of 4 polypeptide chains.
- 2 identical ligh chains (25kDA each) and 2 identical heavy chains (50-73 kDA each)
- Linked by disulphide bonds
- Light chains similar in all immunoglobulins
- Light chains occur in 2varieties :-kappa(k) and lambda(λ)
- Kappa chains are more frequently found.



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- Heavy chains:- gamma , alpha,mu,delta and epsilon.

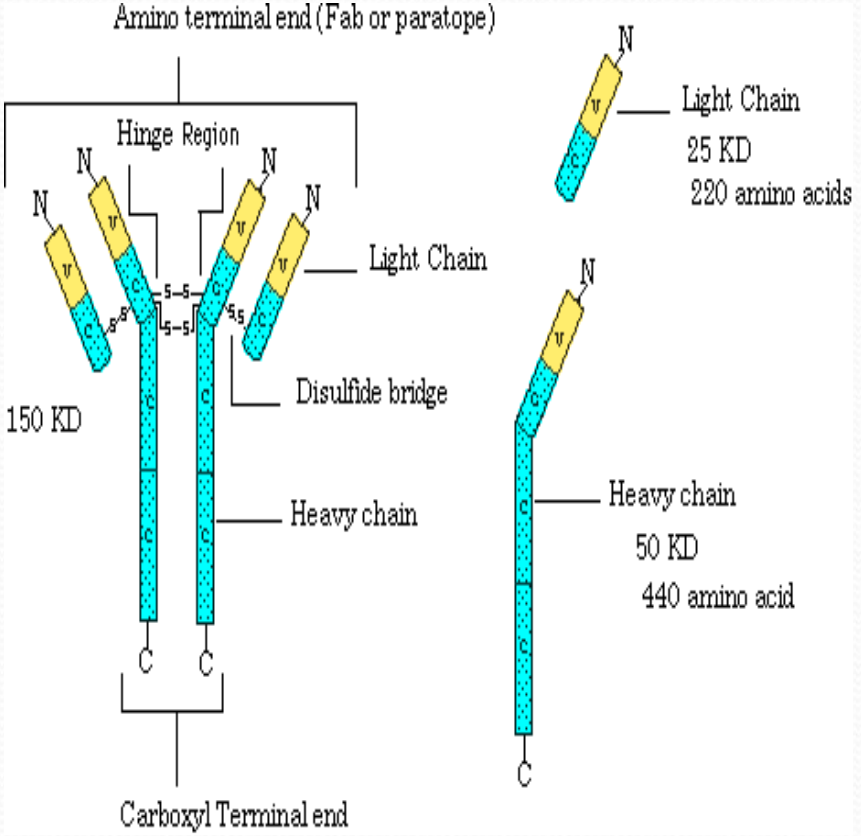
- One Ig contain one type of light chain and one type of heavy chain..(each 2/2)
- Composition :-

Variable and constant region

- Light and Heavy chains are subdivided into variable and constant region.
- Each heavy and light chain contains amino terminal in variable region carboxy terminal in constant region

- **Variable region** extends from N-terminal about 100-11-a.acids. and amino acid sequence in these region is highly variable.

- **Constant region** extends from end of variable region to C-terminal and amino acid sequence is relatively constant



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- Heavy chains are structurally and antigenically distinct for each class.

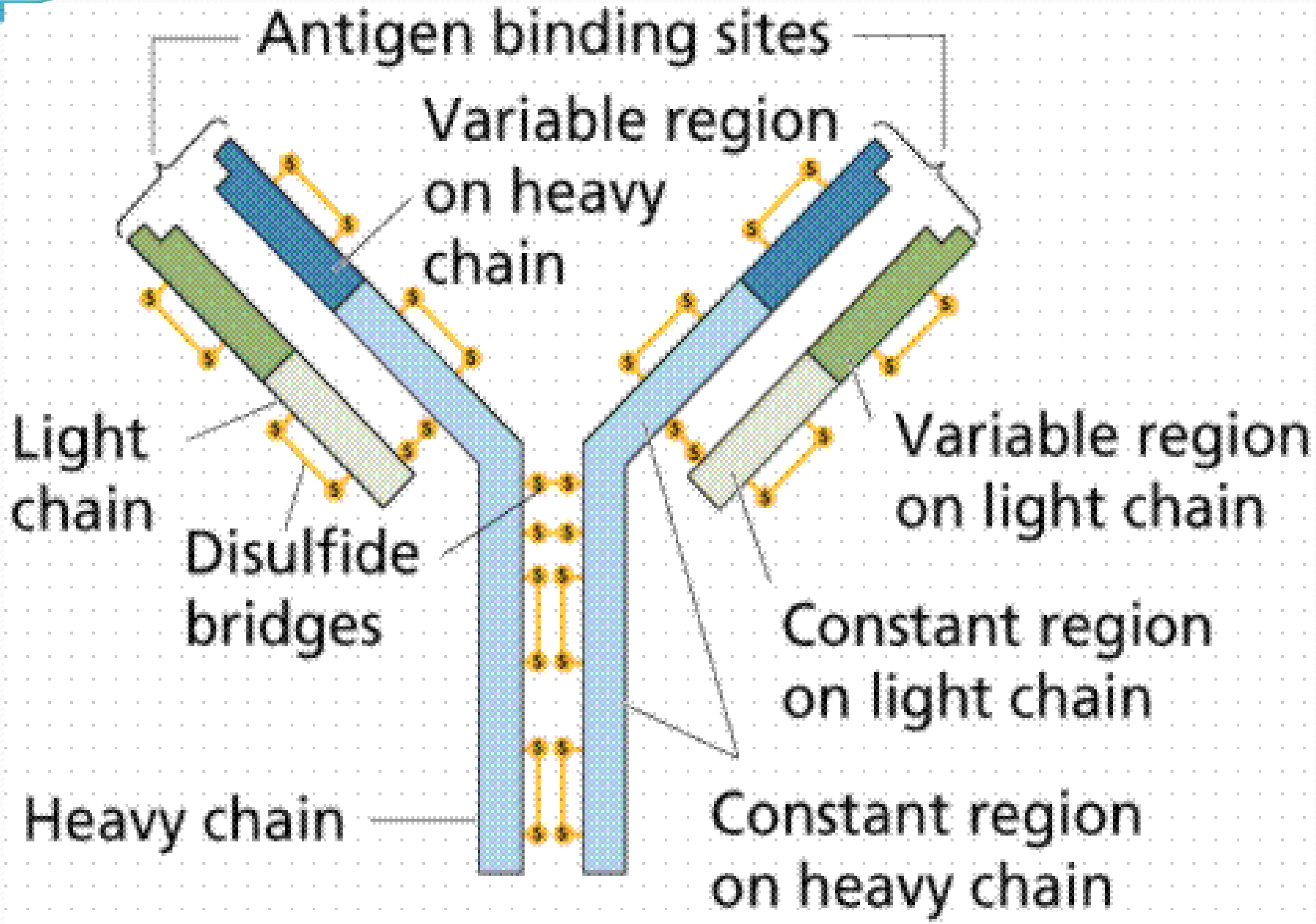
- L and H chains are linked together by both inter and intra chain S-S bonds.

H and L chain domains:-

- Each H and L chains are made up of several small but similar regions called domains.

- L- chain:- two domain (V_L and C_L)

- H-chain :- 4 domain in IgA, IgD ,IgG (V_H,C_H1,C_H2 and C_H3) while 5 domains in IgM, IgE (V_L,C_H1,C_H2,C_H3 and C_H4).

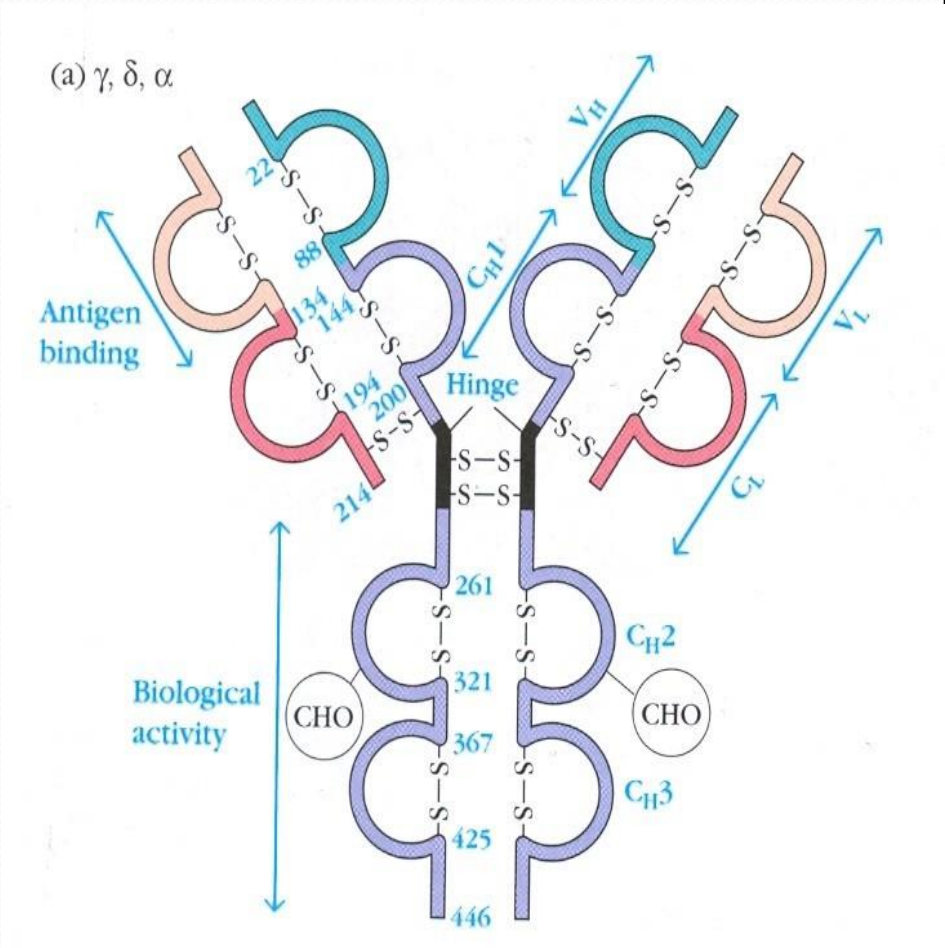

Antigen binding sites
Variable region on heavy chain
Light chain
Disulfide bridges
Variable region on light chain
Constant region on light chain
Heavy chain
Constant region on heavy chain

Immunoglobulin fold:-

Folded loop like structure (B-pleted sheet)

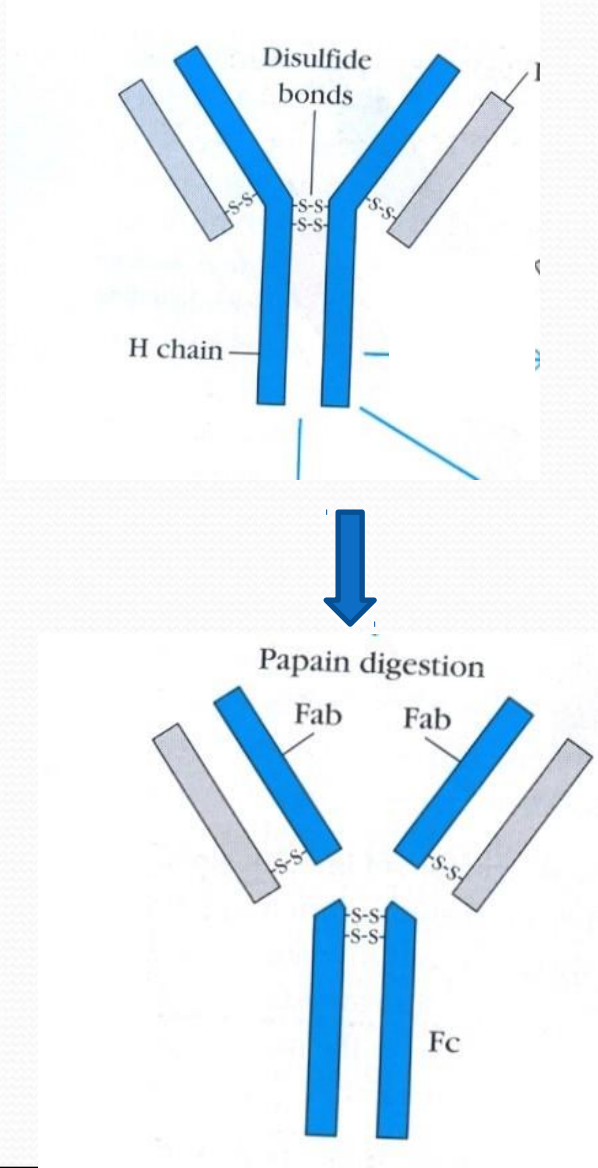
Hinge region:-

- H-chain of arms extends into hinge region.
- Rich in proline and cystine.
- Disulphide bond.



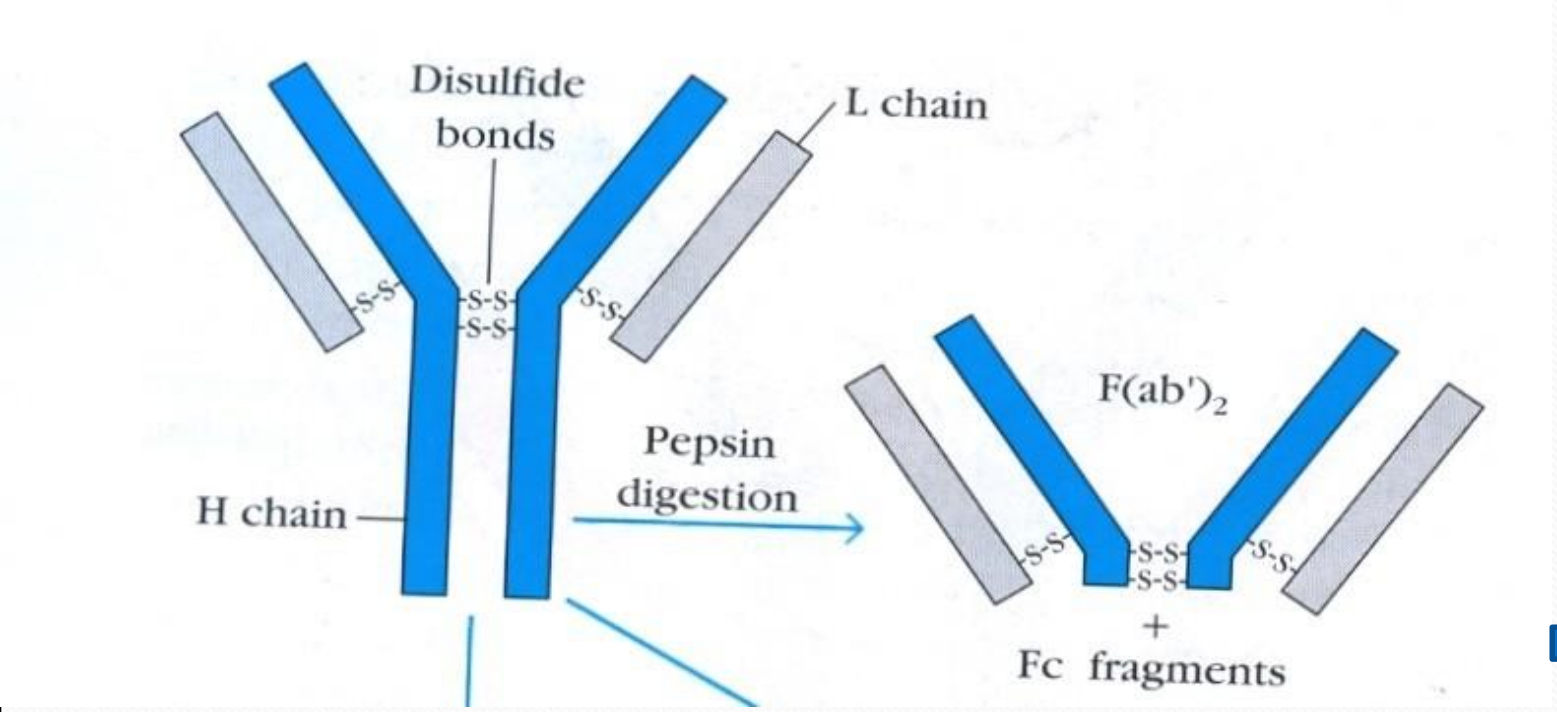
enzymes

- **Papain** cleavage occurs above the S-S bond of hinge region.
- Produces 3 fragments
- 2 identical fragments called Fab fragments – antigen binding activity.
- Other fragment called Fc fragment (Fraction crystallizable)



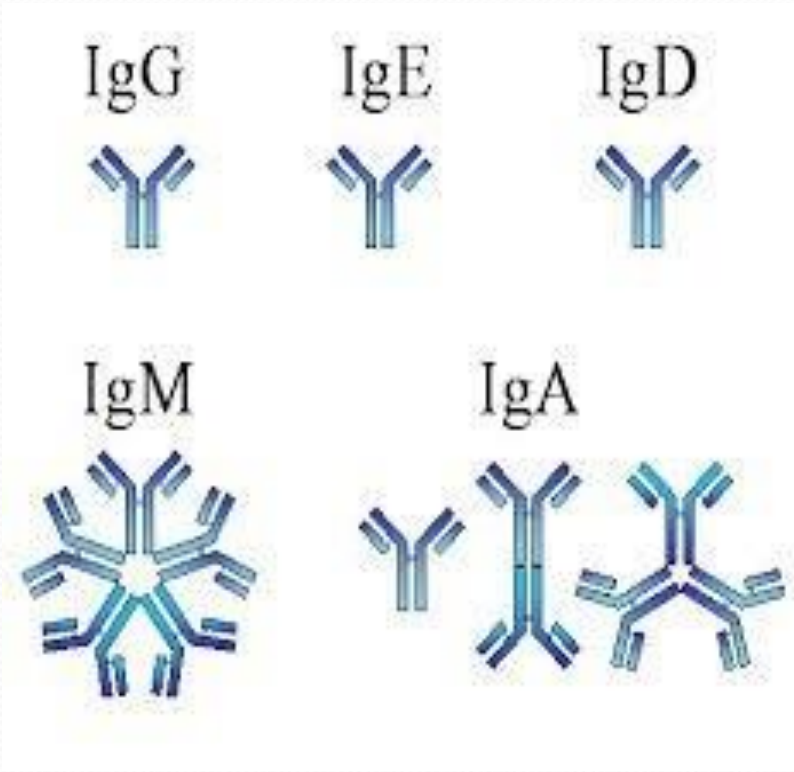
Pepsin digestion

- Pepsin cleavage occurs below the S-S bond of hinge region.
- Produce a single fragment composed of two Fab like subunits F(ab)2 binds antigen
- Fc fragment is not recovered- digested to small numerous peptides.



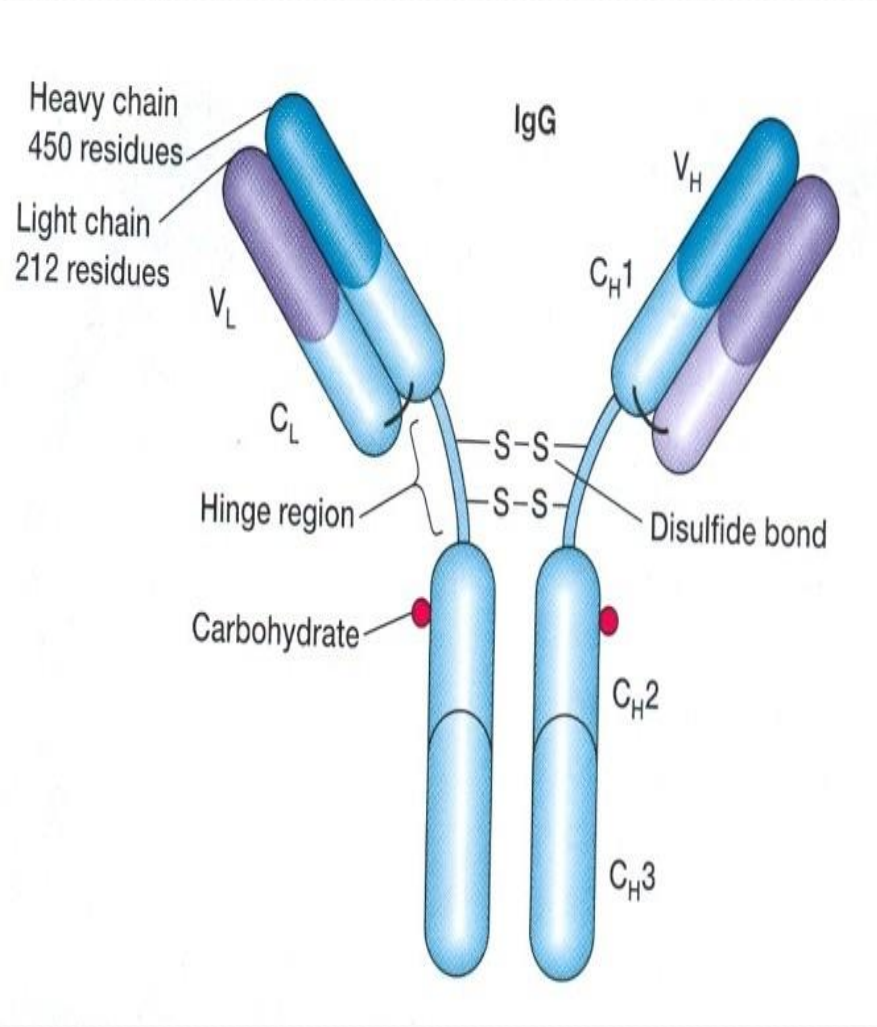
Classification of Igs:-

- Based on structure and antigenic nature of H chain the immunoglobulins are classified into 5 classes.
- Ig G- (gamma)
- Ig A- *(alpha)*
- *Ig M- (mu)*
- *Ig D- (delta)*
- *Ig E- (epsilon)*

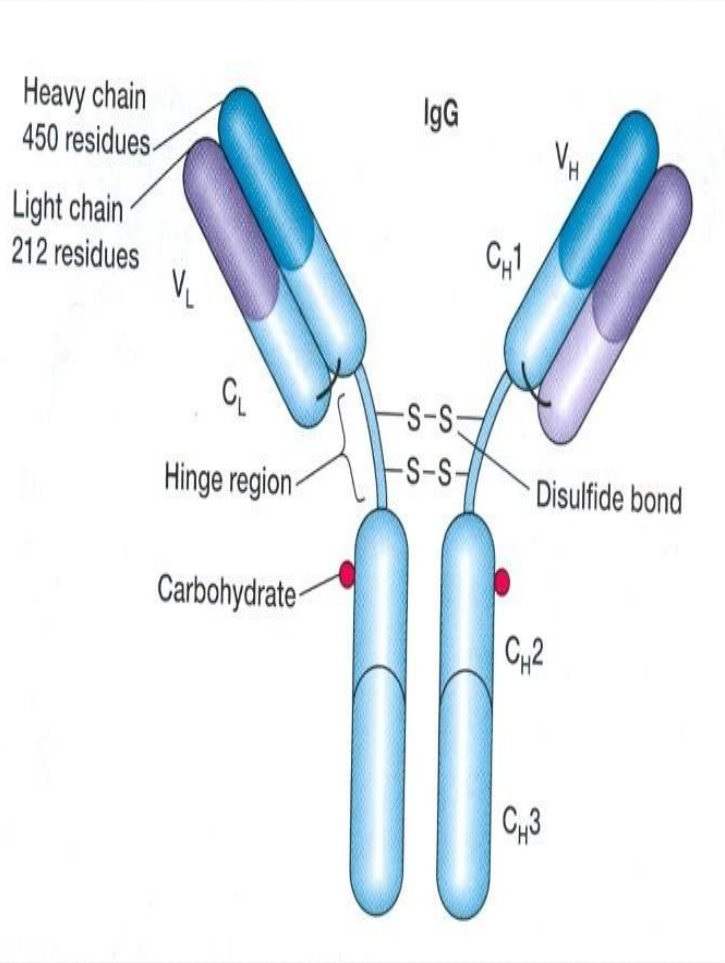


Immunoglobulin G (Ig G)

- Most abundant class of Ig in serum
- Constitutes 80% total immunoglobulin
- Present in blood, plasma and tissue fluids
- Contains less carbohydrate than other immunoglobulins
- It has a half life of 23 days: the longest of all of the immunoglobulin isotypes

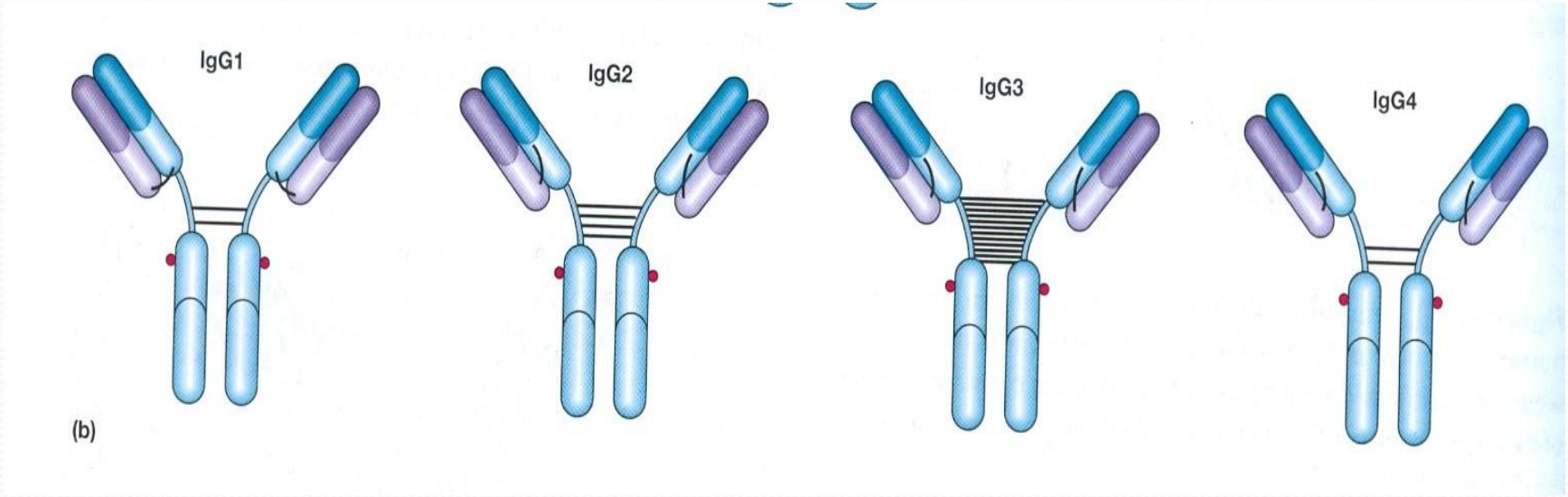


- Crosses placenta and provide natural immunity to foetus and neonate at birth
- Acts against bacteria and viruses by opsonizing
- Neutralize toxin
- Activate complement by classical pathway



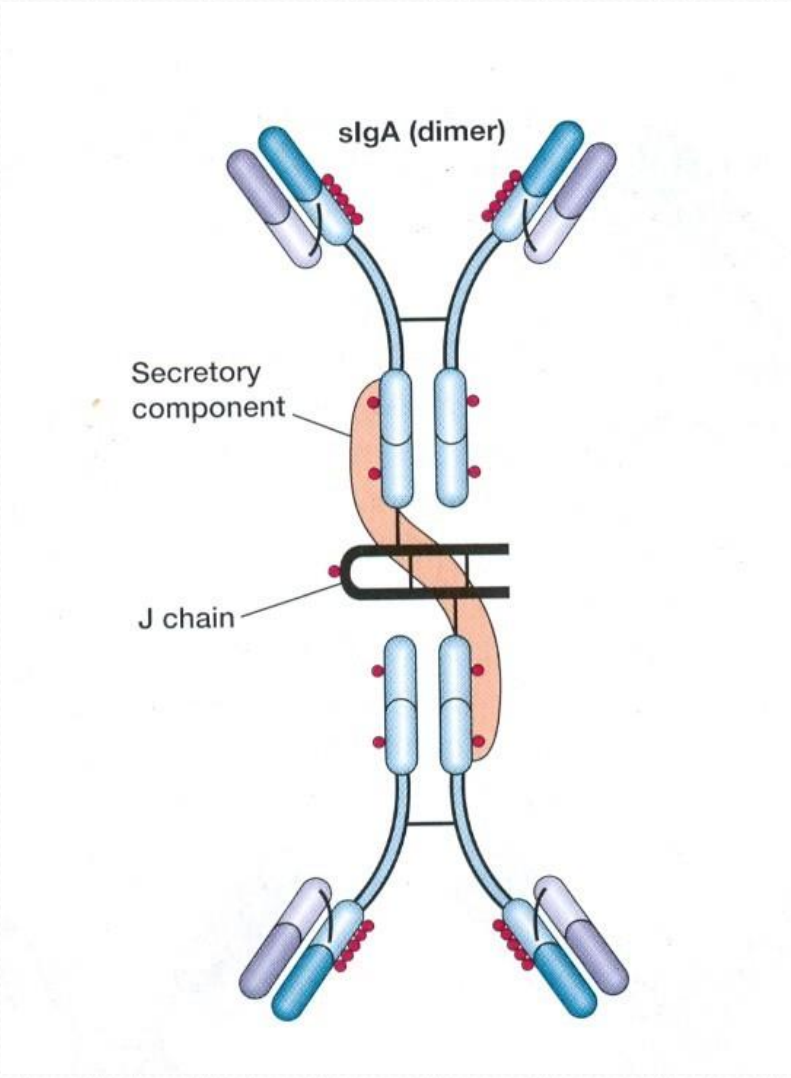
Sub classes of Ig G

- Ig G1, Ig G2, Ig G3, Ig G4.



Immunoglobulin A (Ig A)

- Constitutes 10-15% of total immunoglobulins
- Present in milk, saliva, tears, mucous of respiratory tract, digestive tract and genitourinary tract.
- In serum exist as monomer
- In external secretions exist as dimer called secretory Immunoglobulin.
- Has 'J' chain and secretory piece.
- Half life: 6-8 days

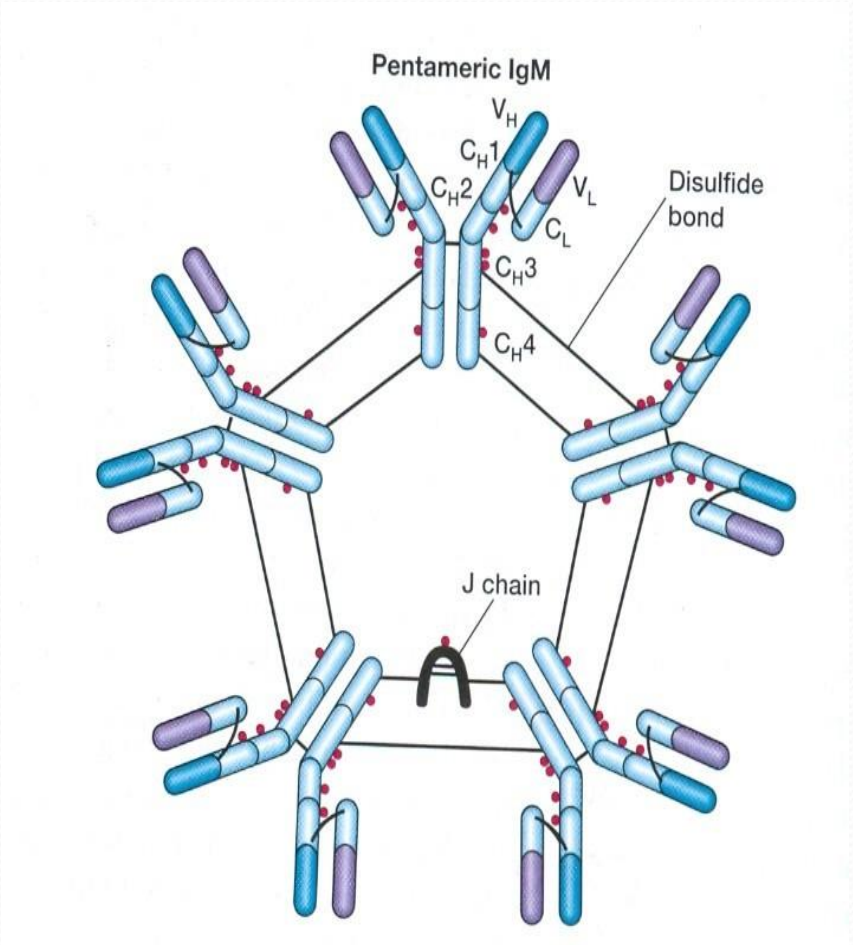


Functions:-

- Provides local immunity.
- Secretory Ig A binds to surface antigens of microorganism and prevent its attachment and invasion of the mucosal surfaces of respiratory and digestive tract- immune elimination.
- Secretory IgA provides important line of defense against *Salmonella, Vibrio cholerae, N. gonorrhoeae*, influenza virus and poliovirus.
- Secretory IgA present in breast milk protects newborn during first months of life.
- Activates complement by the alternative pathway
- Promotes phagocytosis and intracellular killing of microorganisms

Immunoglobulin M (Ig M)

- Accounts for 5-10% of total serum proteins
- Polymer of five monomeric units (pentamer)
- Held together by disulfide bonds and ‘J’ chain
- Mol. Wt. of 900,000-10,00,000 (millionaire molecule)
- Half life: 5 days



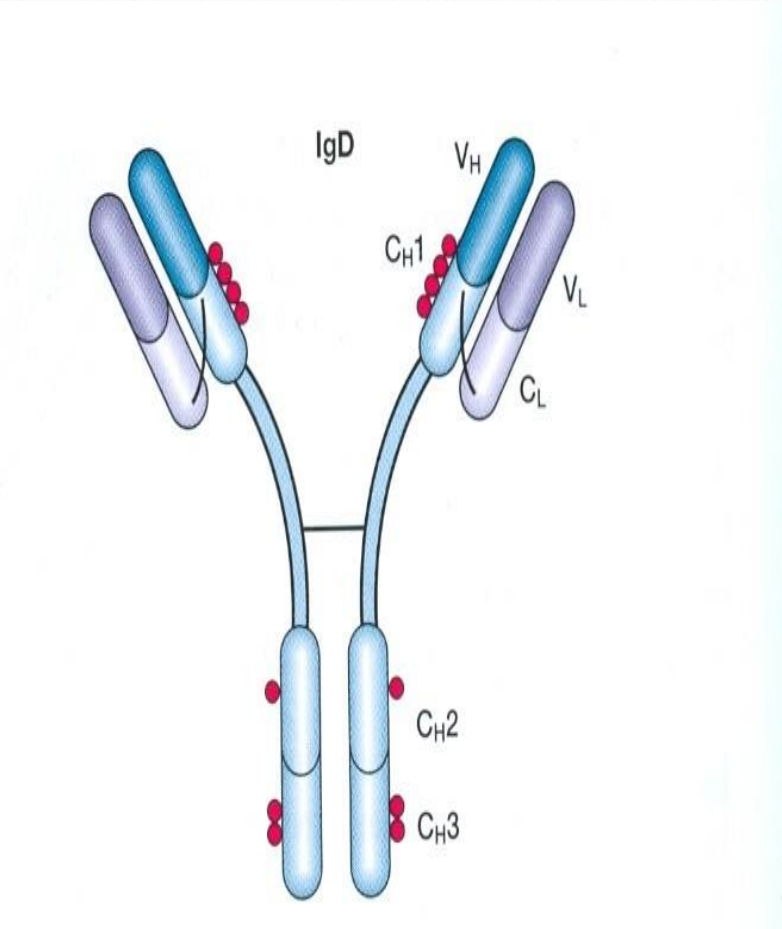
- Most of IgM (80%) present intravascularly
- Present in low concentration in intercellular tissue fluids
- Cannot cross placenta
- Presence of IgM antibody in serum of newborn indicate congenital infection.
- Earliest immunoglobulin to be synthesized by foetus (20 weeks)
- First immunoglobulin to be produced in primary response to antigen
- Relatively short-lived hence it's demonstration in the serum indicates recent infection
- Monomeric IgM appears on the surface of unstimulated B lymphocytes and act as receptors for antigens

Functions

- It agglutinates bacteria
- Activates complement by classical pathway
- Causes opsonization and immune heamolysis
- Believed to be responsible for protection against blood invasion by microorganisms

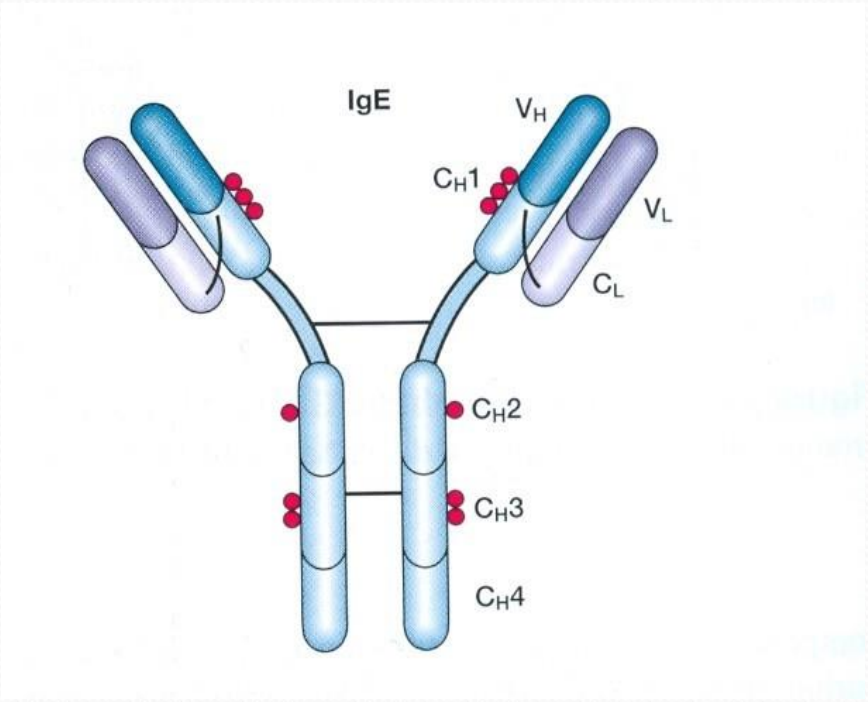
Immunoglobulin D (Ig D)

- Structure is similar to IgG
- Serum concentration 30 micrograms per ml
- Constitutes 0.2% of total immunoglobulins
- Half life: 3 days
- IgD together with IgM is major membrane bound immunoglobulin on unstimulated B lymphocytes-acts as recognition receptors for antigens



Immunoglobulin E (Ig E)

- Structure is similar to Ig G
- Has 4 constant region domains.
- Mol. Wt. 1,90,000
- Half life: 2 days
- Heat labile (inactivated at 56^0C in 1hour)
- Normal serum concentration 0.3 ug/ml
- Mostly present extra cellularly
- Does not cross placenta



- Produced in the lining of respiratory and intestinal tract
- Does not activate complement nor agglutinate antigens
- Binds to the Fc receptors on the membranes of blood basophils and tissue mast cells
- Mediates immediate hypersensitivity reaction
- Play a role in immunity against helminthic parasites

Properties and biological activities of Immunoglobulins

	Ig G	Ig A	Ig M	Ig D	Ig E
1. Structure	Monomer	Monomer in serum/ Dimer in secretion	Pentamer / monomer	Monomer	Monomer
2. Heavy chain CH domain	Gamma Three	Alfa Three	Mu *Four*	Delta Three	Epsilon Four
3. Mol. Wt.	1,50,000	1,60,000	9,00,000	1,80,000	1,90,000
4. Serum concentration (mg/ml)	12	2	1.2	0.03	0.00004
5. Present on membrane of mature B cell	–	–	+	+	–
5. Intravascular Distribution (%)	45	42	80	75	50
6. Crosses placenta	+	-	-	-	-
7. Present in milk	+	+	-	-	-
8. Selective secretion by seromucous glands	-	+	-	-	-
9. Activation of complement Classical Alternate	 + -	 - +	 + -	 - -	 - -
10 Binds to FC receptor of phagocytes	+	-	-	-	-
11 Induces mast cell degranulation	-	-	-	-	+

Overall Functions:-

- Based on antigen recognition and binding:-

1. mAb as B-cell receptor
2. sAb as antigen neutralizing agent

- Based on effector response:-

1. Complement activation
2. Opsonozation
3. ADCC

- Based on Ig class:-

1. Neonatal immunty
2. Mucosal immunity
3. IgE mediated hypersensitivity reaction.

Antigenic determinant of Antibodies

- Antibodies are complex glycoproteins and they themselves can act as immunogens and induce antibody formation against them called **anti Ig antibodies.**

- Whole Ig molecule is not immunogenic to the host system which produces antibodies to them, rather small sites or regions on Ig molecule acts as immunogen.

- These sites or regions are called antigenic determinant.

Contd…

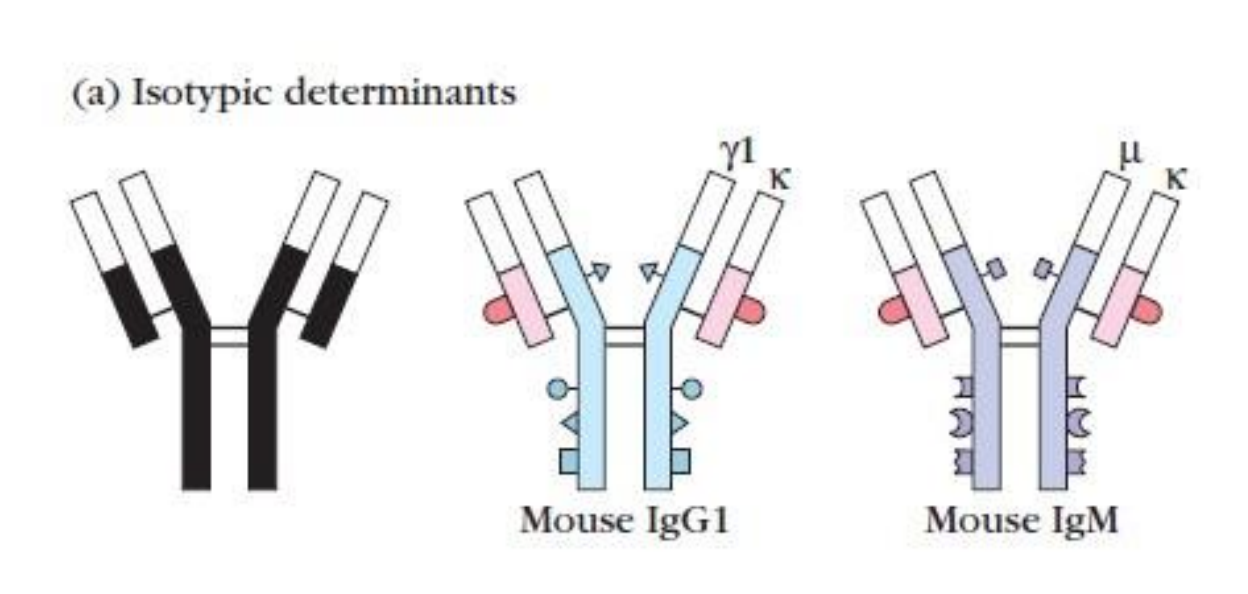
- Based on their location on Ig , they are classified into three types:-**isotypes,allotypes and idiotypes.**

Isotypes:-formed by unique sequence of aminoacids located in the constant region of H and L chain.

- Hence different isotypes differ from each other in their constant regions.
- Classes and subclasses of Igs are isotypes of one another.
- Isotypes are present in all the members of same species and they are same in all.

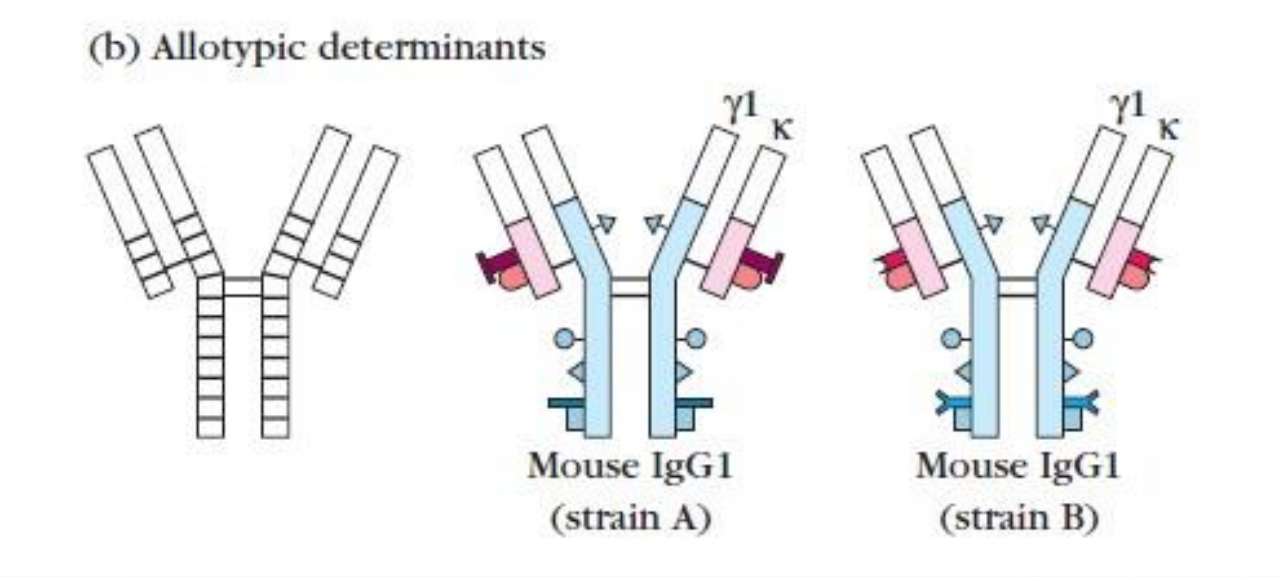
- Isotypes are different in different species.
- Eg…IgG of mouse is different than IgG of rabbit.

Simplest way of producing isotype antibodies is to inject antibodies from one species to another.



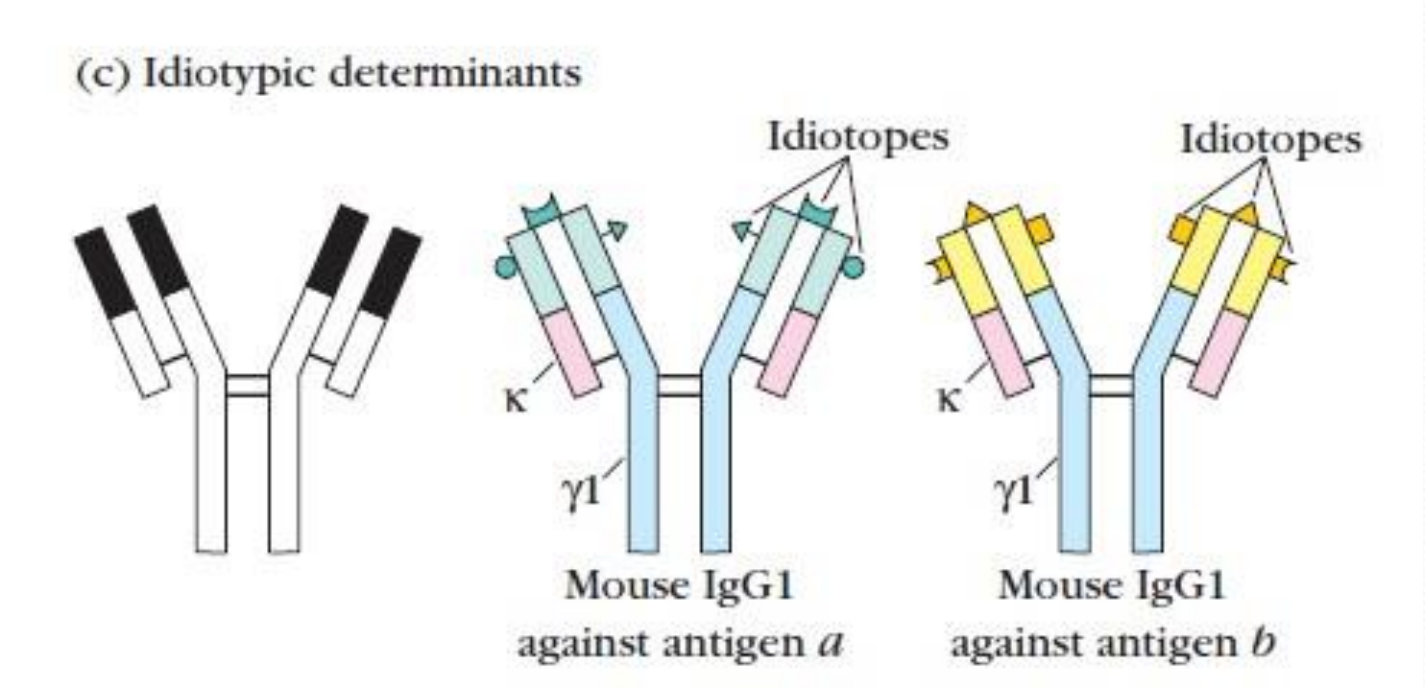
Allotypes

- The antigenic determinant are present in the constant region of H and L chains and are encoded by polymorphic alleles, are called allotypes.
- Since some members of a species carry alleles not all, they are present in some members of species.
- Allotypes differ in sequences of one to four aminoacids from one another.



Idiotypes

- Are located in the hypervariable region of the V_H and V_L domains and one member of a species acts antigenic determinant to other member of the same species.



Thank You