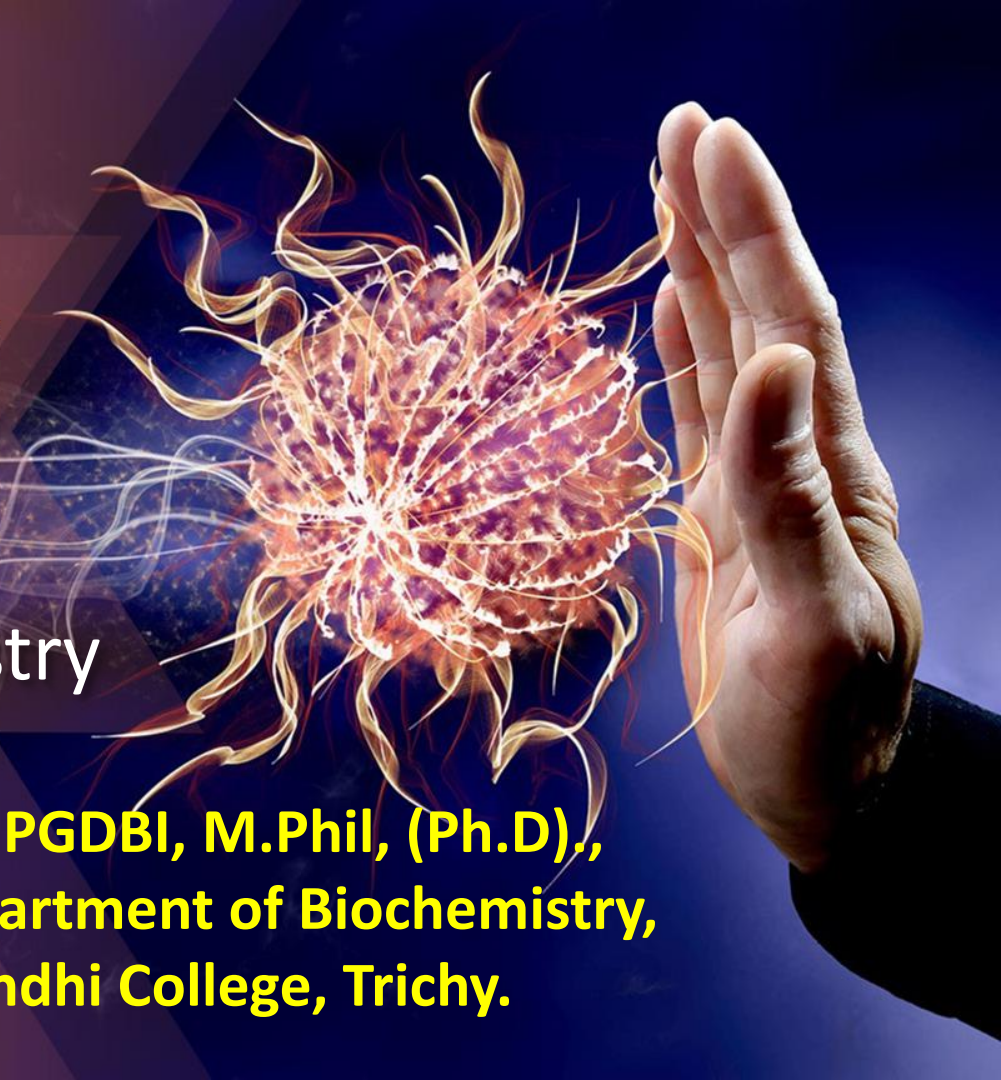
Core Course : VIII
Course code: 16SCCBC8
Course title: Immunology
Class: III B.Sc., Biochemistry
Unit: III



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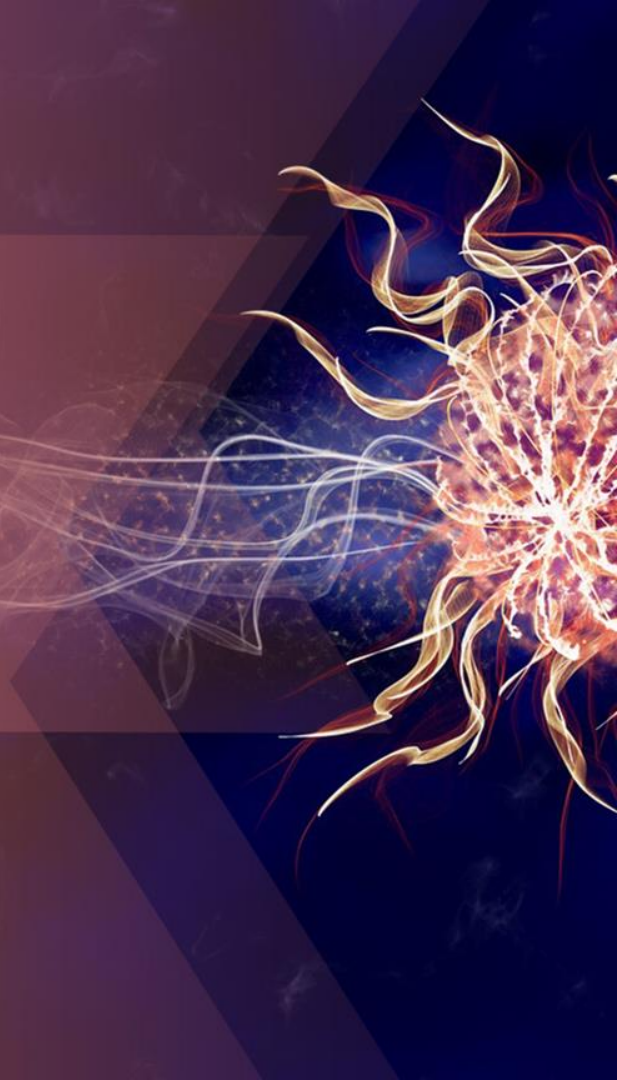
Synopsis

- Immunoglobulin Structure, Types and Function
- Factors Determining Antigenicity
- Antigen – Antibody Interactions
  - Precipitation
  - Agglutination
  - Cytolysis
  - Complement fixation
  - Flocculation
  - Opsonisation
  - Immunofluorescence
- Antitoxins

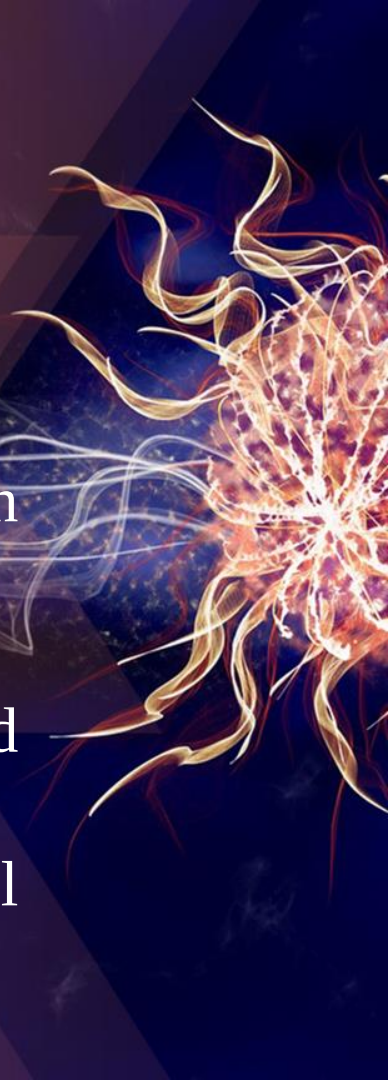


Immunoglobulin Structure, Types and Function

Immunoglobulin

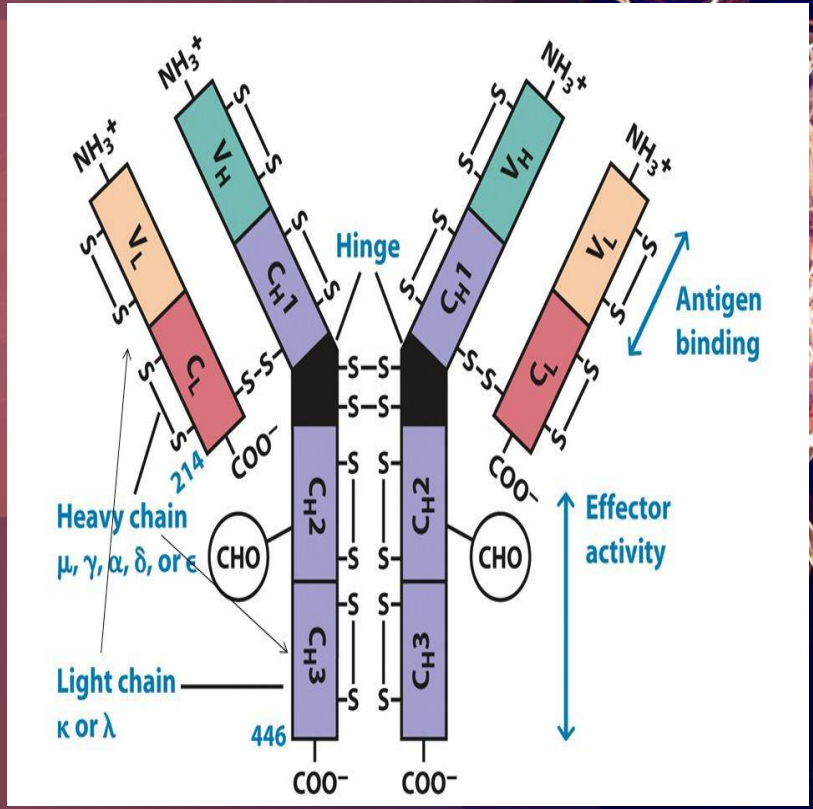
Immunoglobulin are:

- Glycoproteins molecules
- Functions as antibodies
- Produced by plasma cells in response to an immunogen
- Constitute 25-30 % of total serum proteins
- Antibodies are present in serum, tissue fluids and mucosal surfaces.
- All antibodies are immunoglobulins, but all immunoglobulins may not be antibodies



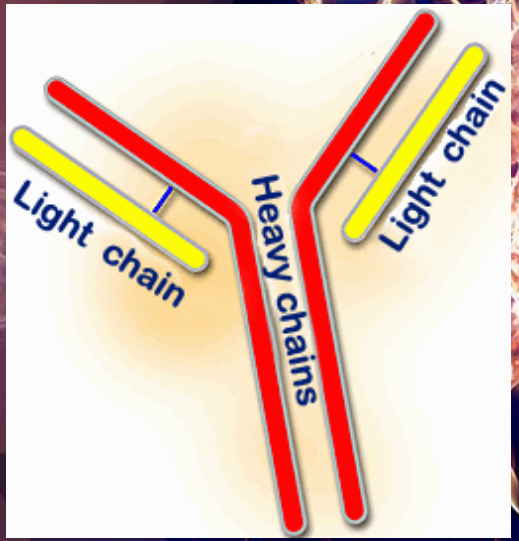
Structure of Immunoglobulin

- Immunoglobulin Composed of 4 polypeptide chains.
- It has 2 identical light (23kD) and 2 identical heavy chains (50 – 70 kD).
- Linked by disulphide bonds
- Light chains similar in all immunoglobulins
- Light chains occur in 2 varieties kappa and lambda
- 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



A. Heavy and Light Chains

- Each immunoglobulin peptide chain has intra chain disulphide bonds- form loops
- Each loop is compactly folded to form a globular structure or domain
- Heavy chains are structurally and antigenically distinct for each class
- Light chain contains a single variable domain (VL) and a single constant domain (CL)
- Heavy chain contains one variable domain (VH) and 3 constant domains (CH1, CH2, CH3)
- Hinge region is the segment in heavy chain - between CH1, CH2

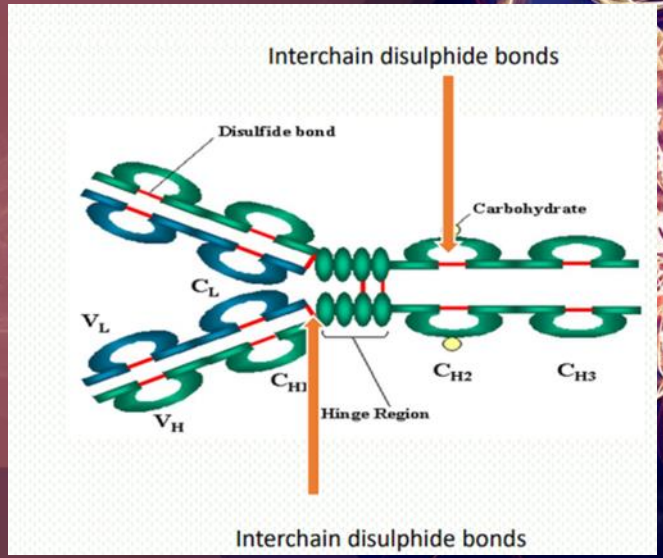


B. Disulfide bonds

- Inter-chain disulfide bonds - The heavy and light chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions.
- The number of inter-chain disulfide bonds varies among different immunoglobulin molecules.
- Intra-chain disulfide bond binds - Within each of the polypeptide chains there are also intra-chain disulphide bonds.

C. Constant and Variable region

- Both the heavy and light chain can 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) and Heavy Chain - VH (110 amino acids) and CH (330-440 amino acids)



D. Hinge region

- This is the region at which the arms of the antibody molecule form a Y.
- It is called the hinge region because there is some flexibility in the molecule at this point.

E. Domains

- Three dimensional images of the immunoglobulin molecule show that it is not a straight molecule rather, it is folded into globular regions each of which contains an intra-chain disulfide bond .
- These regions are called domains. 1. Light Chain Domains - VL and CL 2. Heavy Chain Domains - VH , CH1,CH2CH3 (or CH4)

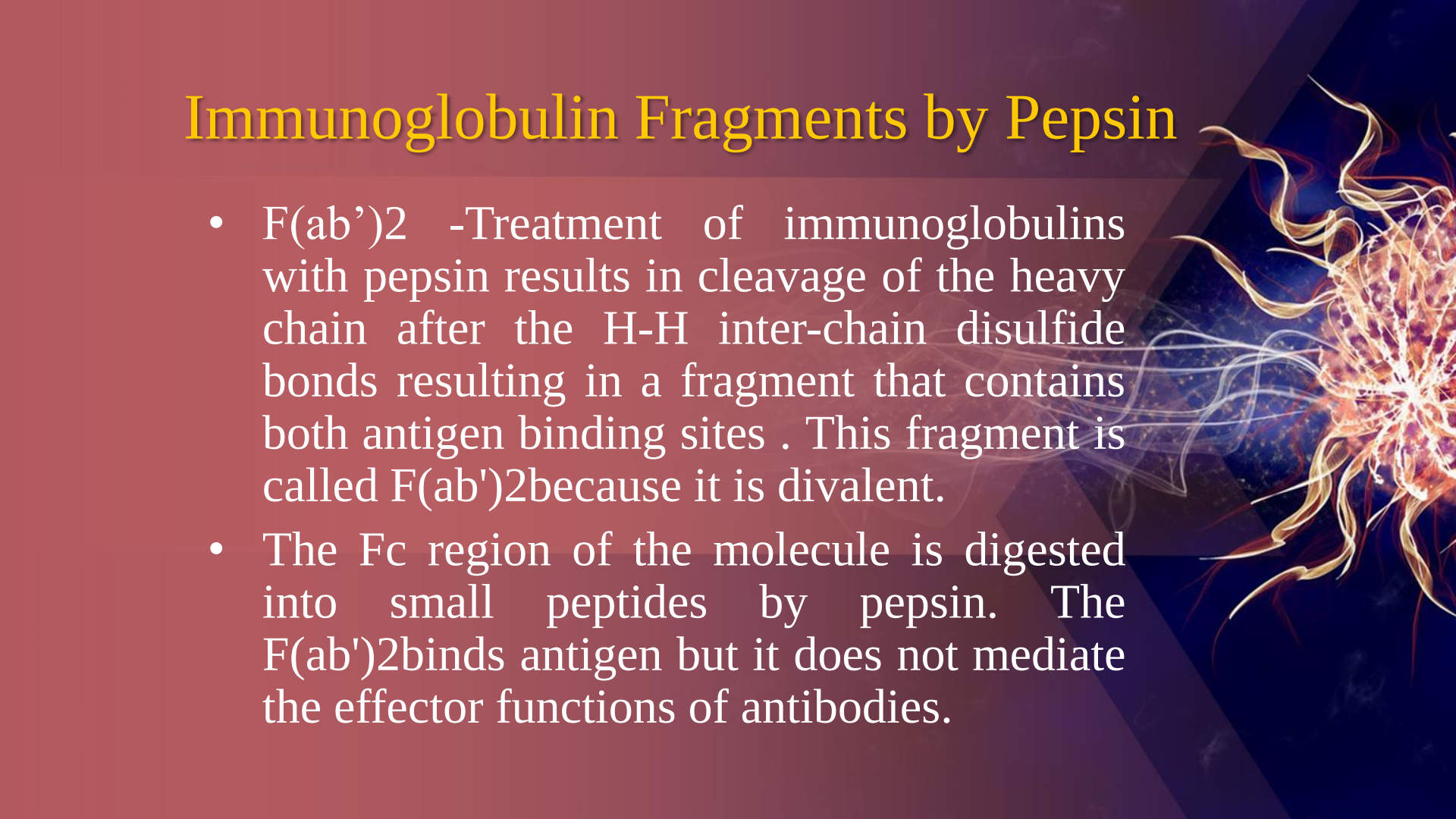
Immunoglobulin Fragments by Papain

- A. Fab Digestion with papain breaks the immunoglobulin molecule in the hinge region before the H-H inter-chain disulfide bond.
- Fab -These fragments are called the Fab fragments because they contain the antigen binding sites for the antibody.
- Each Fab fragment is monovalent whereas the original molecule was divalent.
- The combining site of the antibody is created by both VH and VL

• B. Fc Digestion with papain also produces a fragment that contains the remainder of the two heavy chains each containing a CH2 and CH3 domain. • This fragment was called Fc because it was easily crystallized.

Immunoglobulin Fragments by Pepsin

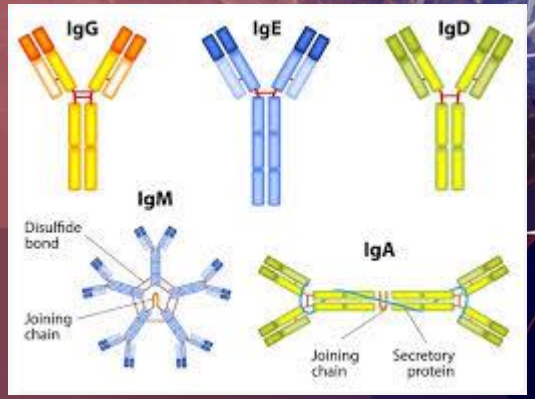
- F(ab’)2 -Treatment of immunoglobulins with pepsin results in cleavage of the heavy chain after the H-H inter-chain disulfide bonds resulting in a fragment that contains both antigen binding sites . This fragment is called F(ab')2because it is divalent.
- The Fc region of the molecule is digested into small peptides by pepsin. The F(ab')2binds antigen but it does not mediate the effector functions of antibodies.



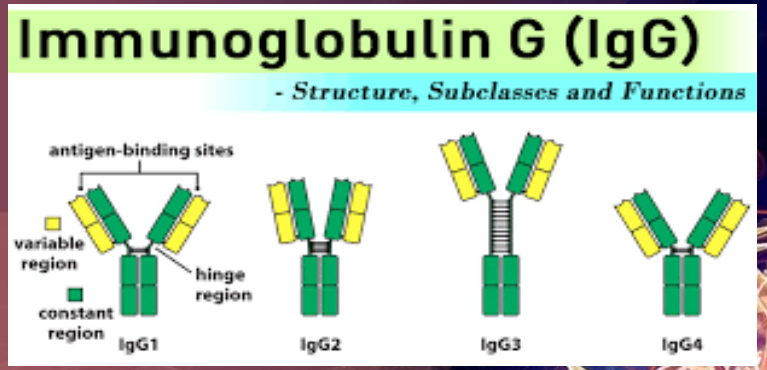
Types of Immunoglobulin

- The immunoglobulins can be divided into five different types, based on differences in the amino acid sequences in the constant region of the heavy chains.

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



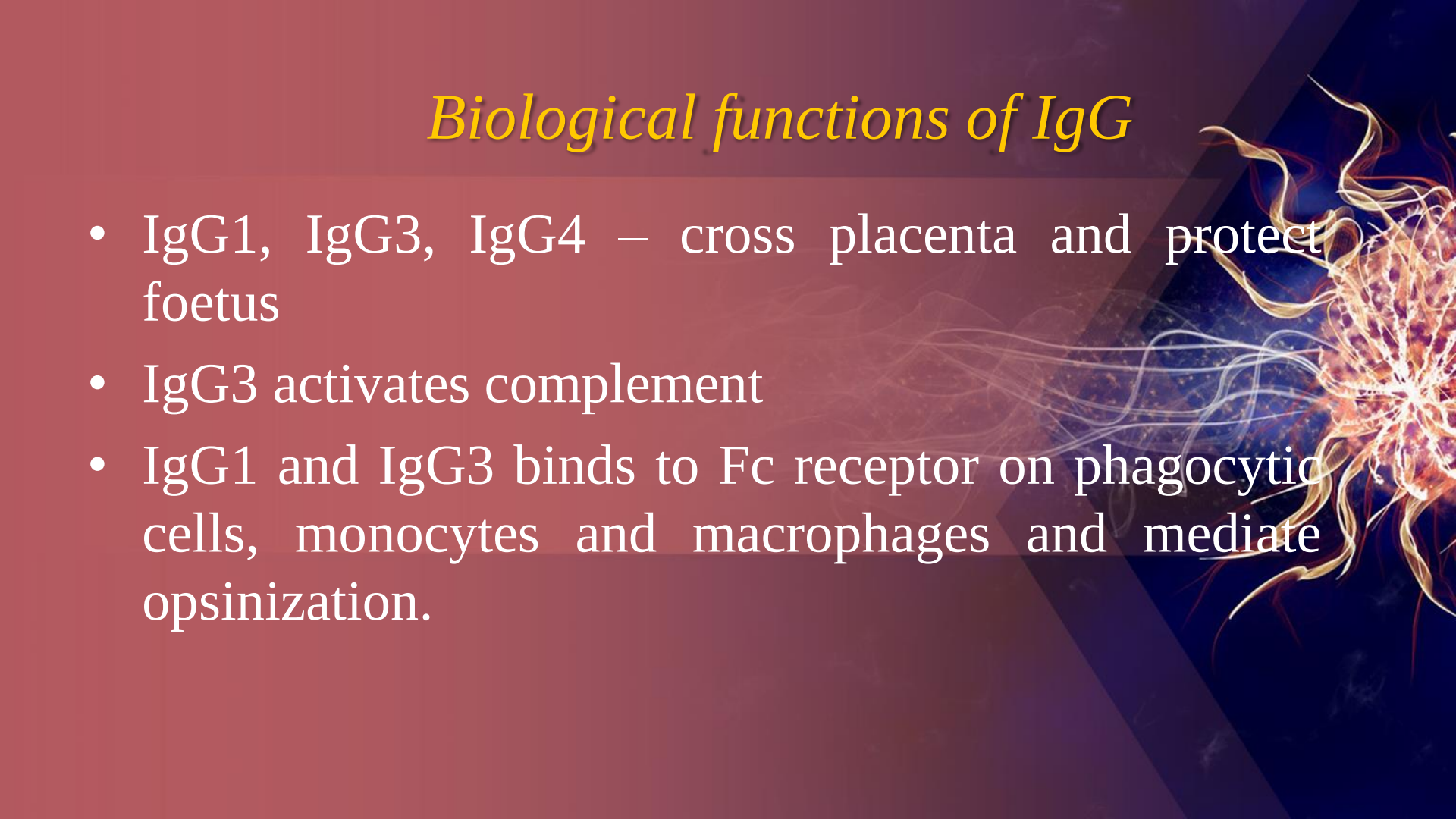
Immunoglobulin G (Ig G)



- Most abundant class 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 neonates at birth.
- Acts against bacteria and viruses by opsonizing
- Neutralize toxin
- Activate complement by classical pathway
- Catabolism of IgG is unique in that it varies with its serum concentration
- Sub classes: Ig G1, Ig G2, Ig G3, Ig G4.

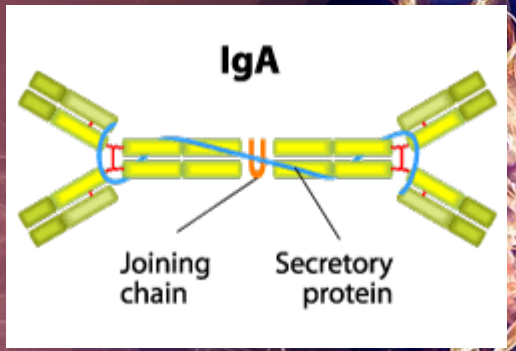
Biological functions of IgG

- IgG1, IgG3, IgG4 – cross placenta and protect foetus
- IgG3 activates complement
- IgG1 and IgG3 binds to Fc receptor on phagocytic cells, monocytes and macrophages and mediate opsinization.



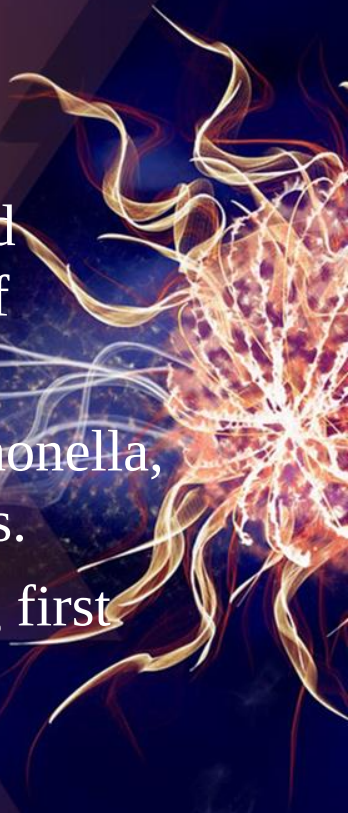
Immunoglobulin A (Ig A)

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



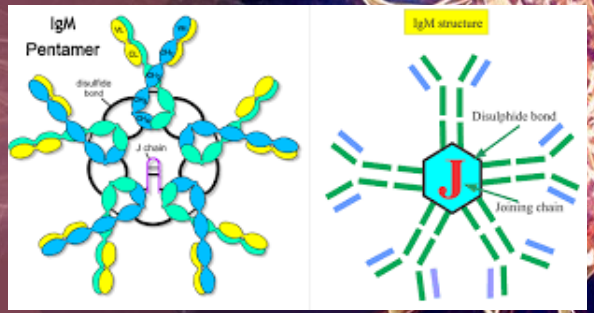
Biological functions of IgA

- 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 defence against salmonella, Vibrio cholera, N. gonorrhoeae, influenza virus and poliovirus.
- Secretory IgA present in breast milk protects new born during first months of life.
- Activates complement by the alternative pathway
- Promotes phagocytosis and intracellular killing of microorganisms



Immunoglobulin M (Ig M)

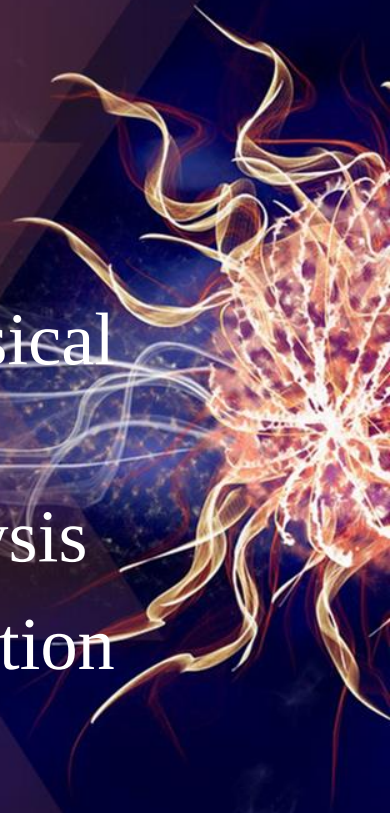
- Accounts for 5-10% of total serum proteins
- Polymer of five monomeric units (pentamer)
- Held together by disulfide bonds and ‘J’ chain
- Mol. Wt. of 900,000- 10,00,000 (millionaire molecule)
- Half life: 5 days; Most of IgM (80%) present intravascularly
- Present in low concentration in intercellular tissue fluids
- Cannot cross placenta



- Presence of IgM antibody in serum of new born 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

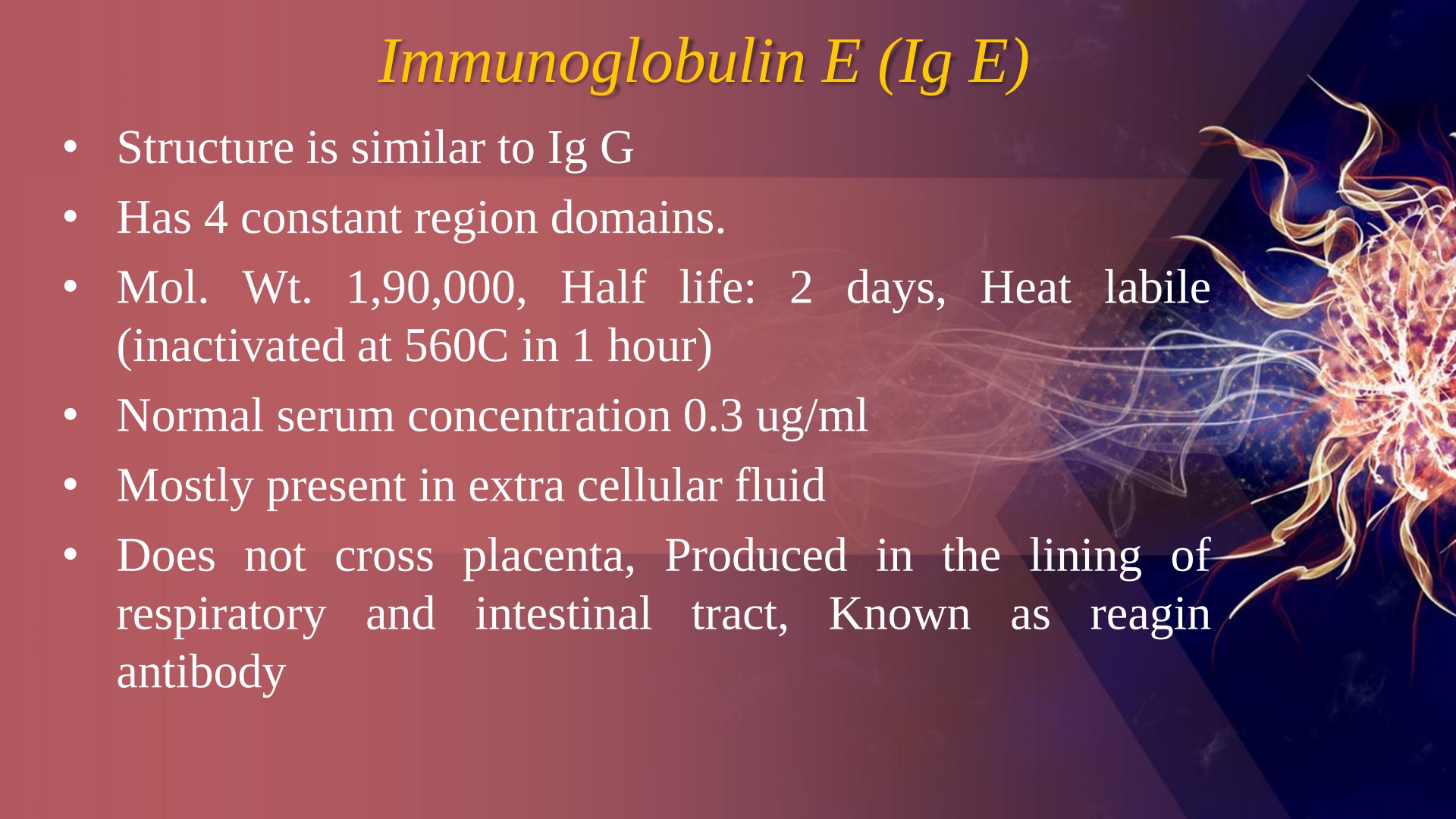
Biological functions of IgM

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

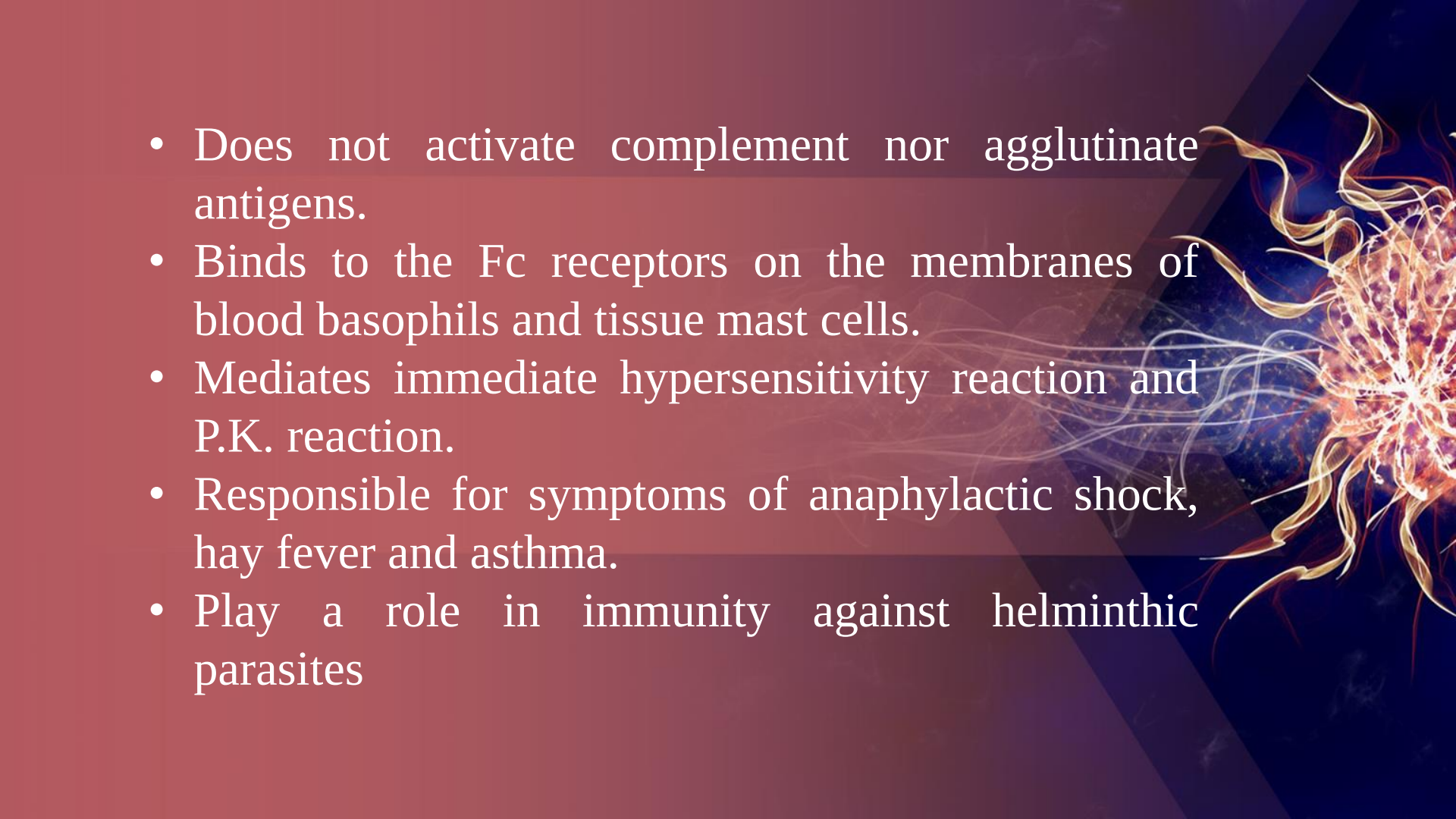


Immunoglobulin E (Ig E)

- Structure is similar to Ig G
- Has 4 constant region domains.
- Mol. Wt. 1,90,000, Half life: 2 days, Heat labile (inactivated at 560C in 1 hour)
- Normal serum concentration 0.3 ug/ml
- Mostly present in extra cellular fluid
- Does not cross placenta, Produced in the lining of respiratory and intestinal tract, Known as reagin antibody

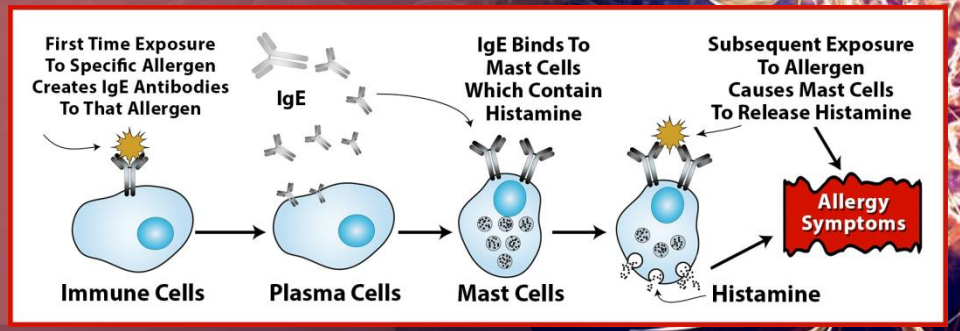


- Does not activate complement nor agglutinate antigens.
- Binds to the Fc receptors on the membranes of blood basophils and tissue mast cells.
- Mediates immediate hypersensitivity reaction and P.K. reaction.
- Responsible for symptoms of anaphylactic shock, hay fever and asthma.
- Play a role in immunity against helminthic parasites



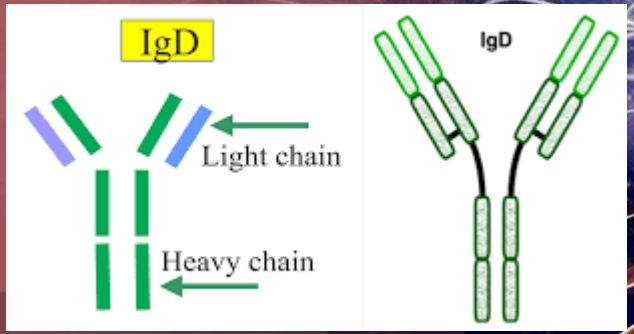
Biological functions of IgE

- Cross-linking of IgE molecules on the surface of a mast cell or basophil causes the release of histamine; the synthesis of prostaglandins, leukotrienes and other chemokines; the production of various cytokines.
- IgE plays a major role in combating parasitic infections
- IgE plays a role in combating pulmonary fungal infections



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.



Biological functions of IgD

- On B cell surface it initiate immune response

Short information on functions of different Ig:

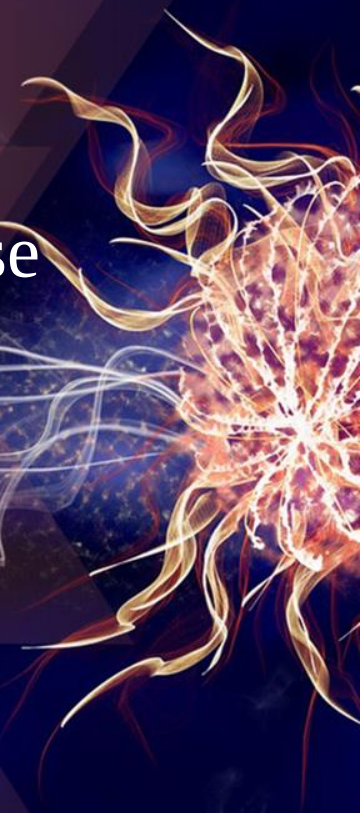
IgG: Protects the body fluids

IgA: Protects the body surfaces

IgM: Protects the blood stream

IgE: Mediates type I hypersensitivity

IgD: Initiation of B cell activation



Factors determining antigenicity

Antigen and properties

- **Antigen** is a substances usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody or specially sensitized T cells or both.
- Molecules that stimulate immune responses are called I**mmunogens.**

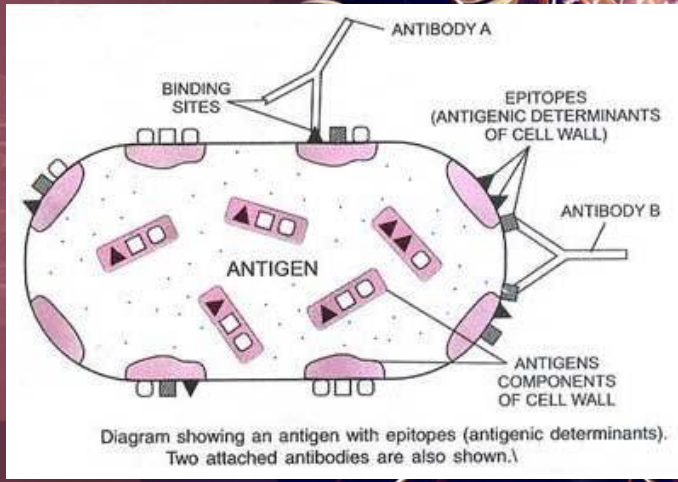
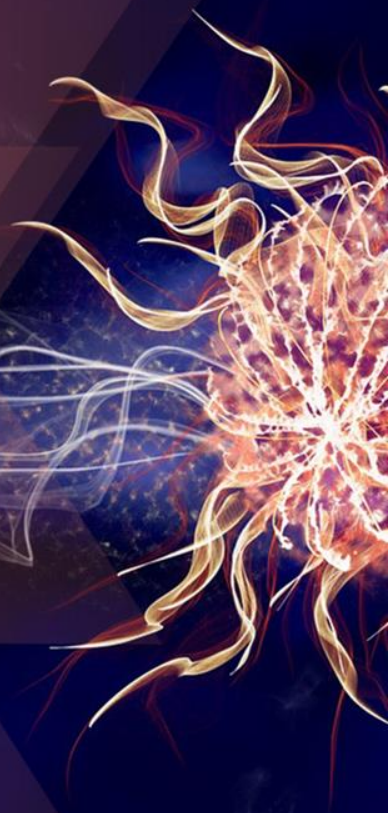
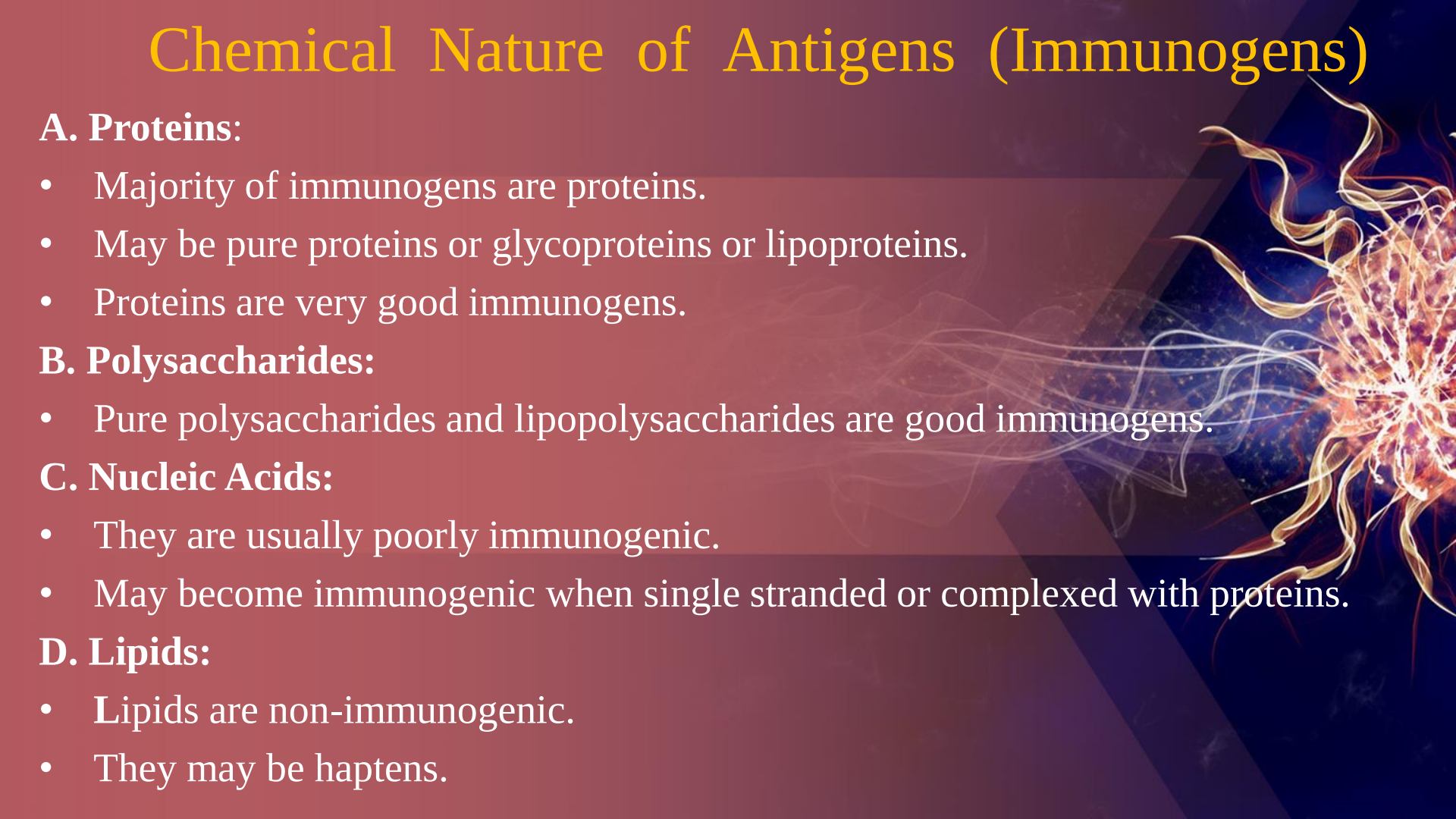


Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.\

- **Epitope** is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants.**
- **Adjuvants** are substances that are non-immunogenic when alone but enhance the immunogenicity of any added immunogen.



Chemical Nature of Antigens (Immunogens)



A. Proteins:

- Majority of immunogens are proteins.
- May be pure proteins or glycoproteins or lipoproteins.
- Proteins are very good immunogens.

B. Polysaccharides:

- Pure polysaccharides and lipopolysaccharides are good immunogens.

C. Nucleic Acids:

- They are usually poorly immunogenic.
- May become immunogenic when single stranded or complexed with proteins.

D. Lipids:

- **L**ipids are non-immunogenic.
- They may be haptens.

Property of antigens/ Factors Influencing Immunogenicity

1. Foreignness

An antigen must be a foreign substances to the animal to elicit an immune response.

2. Molecular Size

The most active immunogens tend to have a molecular mass of 14,000 to 6,00,000 Da. Examples: tetanus toxoid, egg albumin, thyroglobulin are highly antigenic. Insulin (5700) are either non-antigenic or weakly antigenic.

3. Chemical Nature and Composition

In general, the more complex the substance is chemically the more immunogenic it will be. Antigens are mainly proteins and some are polysaccharides. It is presumed that the presence of an aromatic radical is essential for rigidity and antigenicity of a substance.

4. Physical Form

Particulate antigens are more immunogenic than soluble ones. Denatured antigens are more immunogenic than the native form.

5. Antigen Specificity

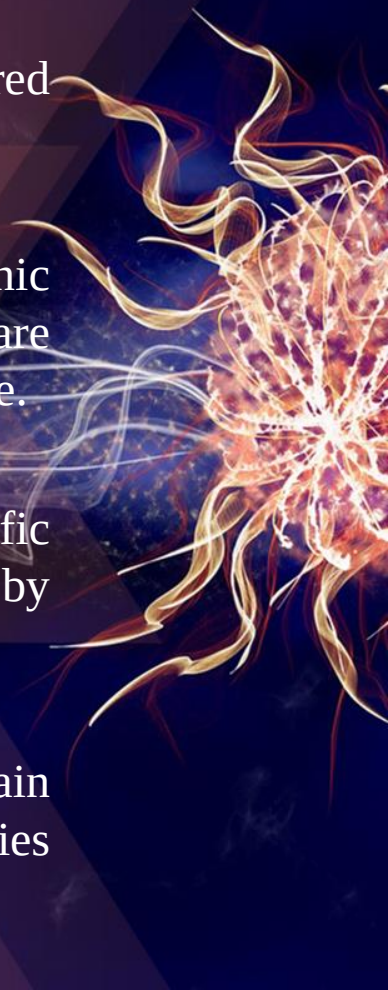
Antigen Specificity depends on the specific active site on the antigenic molecules (Antigenic determinants). Antigenic determinants or epitopes are the regions of antigen which specifically binds with the antibody molecule.

6. Species Specificity

Tissues of all individuals in a particular species possess, species specific antigen. Human Blood proteins can be differentiated from animal protein by specific antigen-antibody reaction.

7. Organ Specificity

Organ specific antigens are confined to particular organ or tissue. Certain proteins of brain, kidney, thyroglobulin and lens protein of one species share specificity with that of another species.



8. Auto-specificity

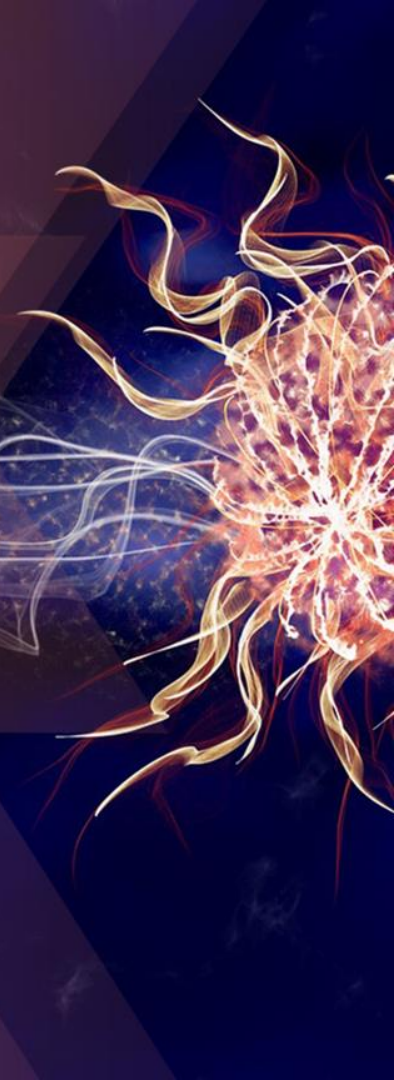
The autologous or self antigens are ordinarily not immunogenic, but under certain circumstances lens protein, thyroglobulin and others may act as ***autoantigens.***

9. Genetic Factors

Some substances are immunogenic in one species but not in another (i.e. responders and non-responders). The species or individuals may have altered genes that code for the receptors for antigen on B cells and T cells. They may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

10. Age

Age can also influence immunogenicity. Usually the very young and the very old have a diminished ability to elicit and immune response in response to an immunogen.



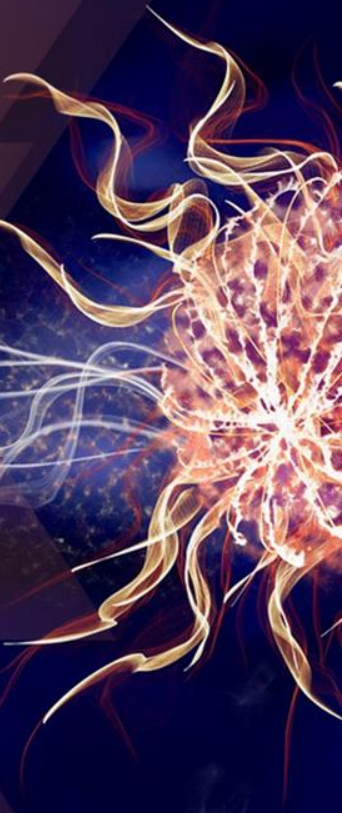
Antigen and antibody interactions

Introduction

- The antigens and the antibodies combine specifically with each other. This interaction between them is called Antigen-Antibody reaction.
- It may be abbreviated as Ag – Ab reaction.

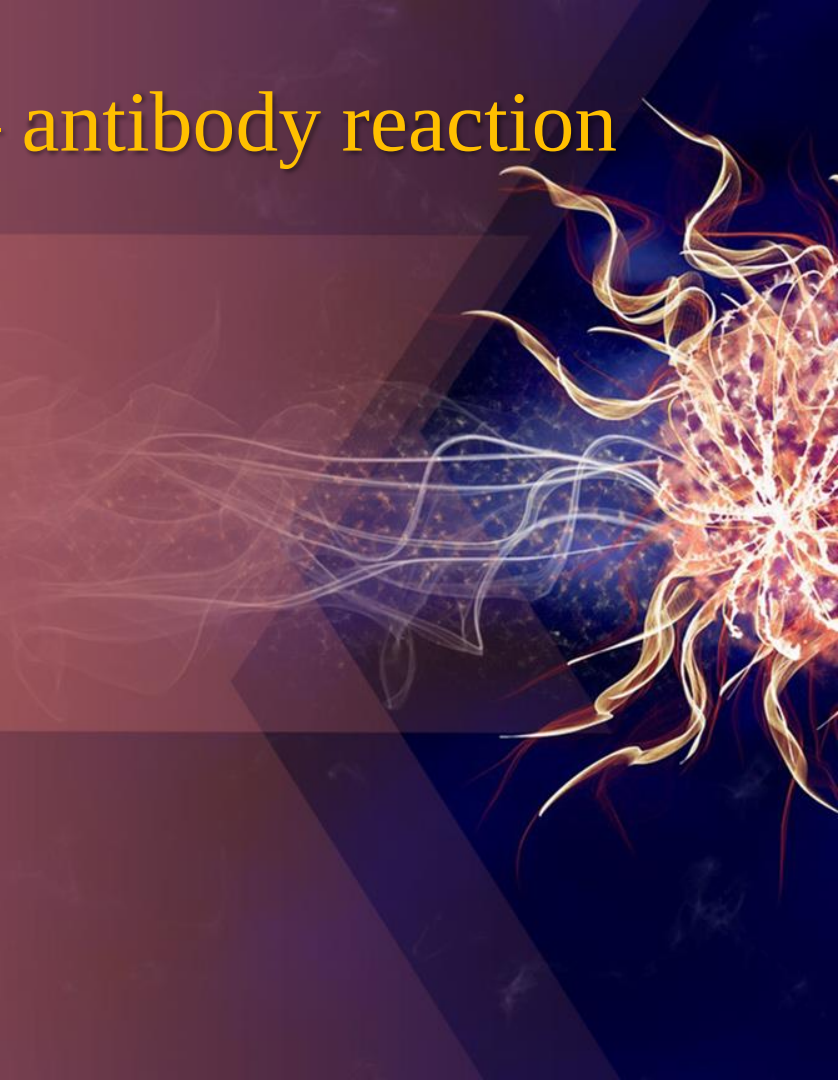
- The reactions between Ag and Ab occur in three stages.

1. First stage: The reaction involves the formation of Ag-Ab complex.
2. Second stage: Leads to the visible events like precipitation, agglutination etc.
3. Third stage: Includes the destruction of Ag or its neutralization



Salient features of antigen – antibody reaction

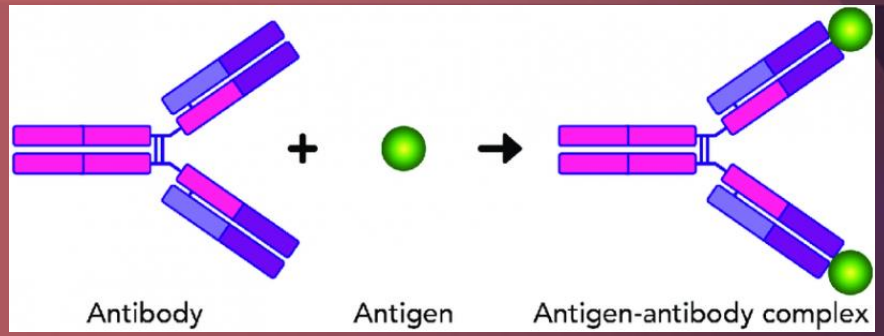
- Immune complex
- Specificity of Ag-Ab reaction
- Binding sites of Ag-Ab
- Binding forces of Ag-Ab
- Avidity
- Bonus effect
- Cross reaction



1. Immune complex

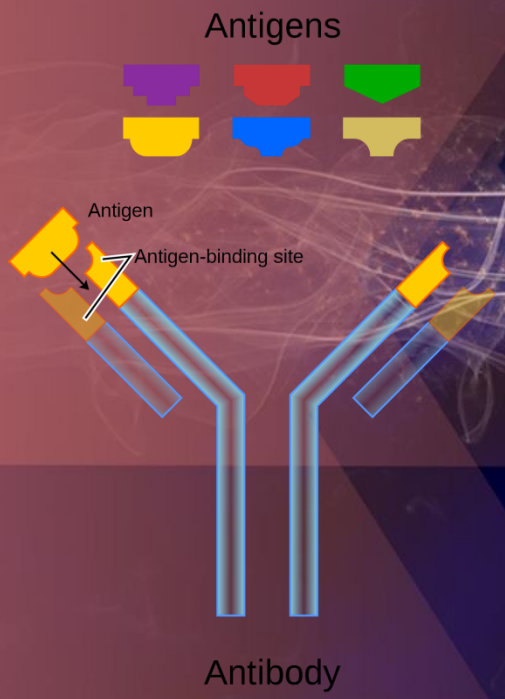
- When ag and ab are brought together, the ab binds with the ag to form complex molecule called immune complex or ag-ab complex.

Ag + Ab ⟶ Ag-Ab complex

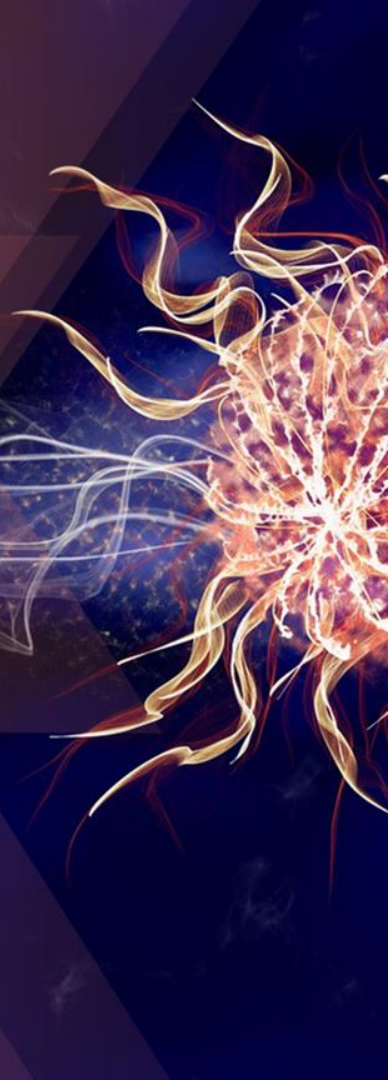


2. Specificity of Ag-Ab reaction

- Specificity refers to the ability of an individual antibody to react with only one antigenic determinant or the ability of a population of antibody to react with only one antigen.

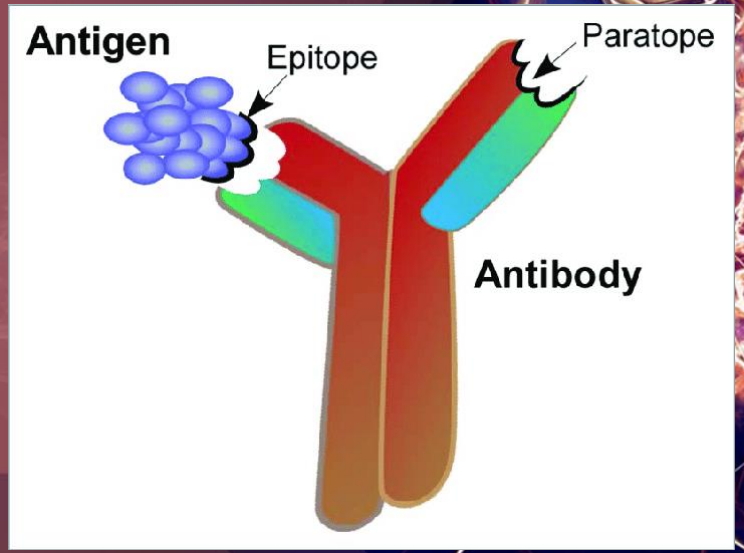


- For example, the antibody produced against lens antigen will react only with lens-antigen.
- Similarly, the antibody produced against kidney antigen will react with only kidney- antigen.
- A standard lock can be opened by its own key only as one antibody can react with its own antigen.



3. Binding site of Ag-Ab reaction

- In antigen - antibody reaction, the antibody attaches with the antigen.
- The part of antigen which combines with antibody is called Epitope.
- An epitope, also known as antigenic determinant, is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells, or T cells.
- The part of an antibody that recognizes the epitope is called a paratope.



4. Binding force of Ag-Ab reaction

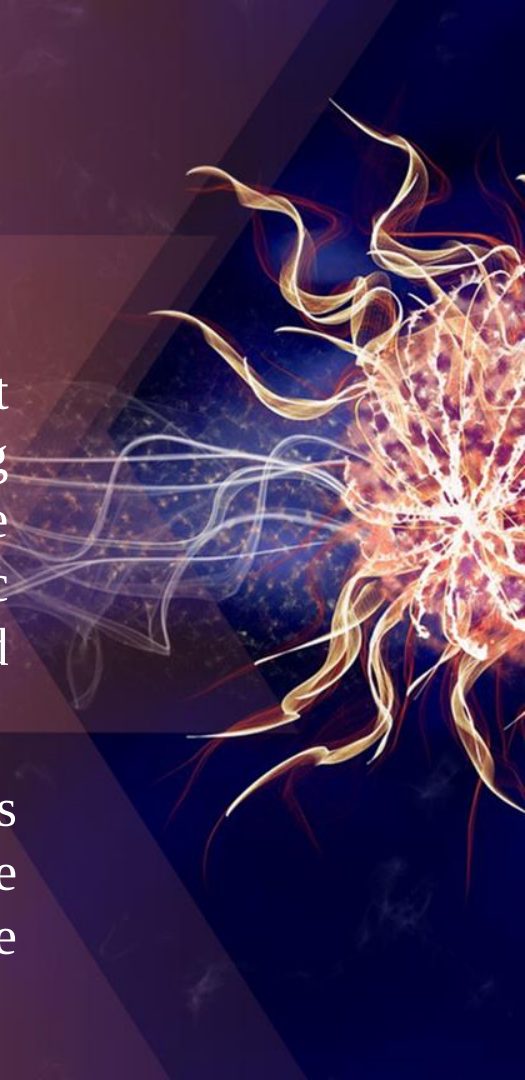
The binding between antigen and antibody in Ag – Ab reaction is due to three factors namely:

- Closeness between antigen and antibody.
- Non – covalent bonds or Intermolecular forces .
- Affinity of antibody.

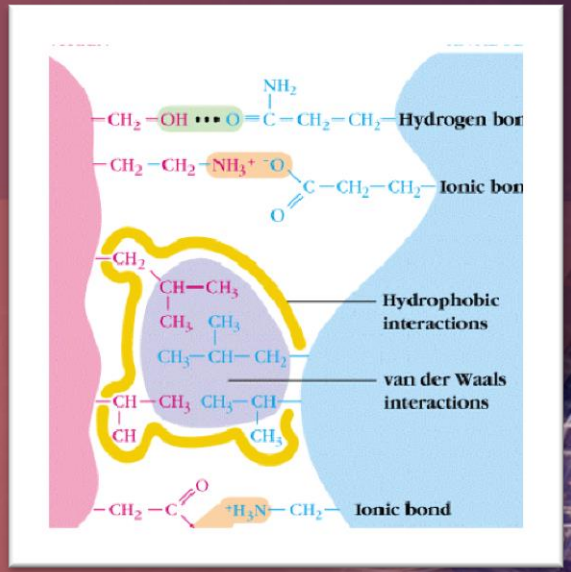
Closeness between antigen and antibody: When antigen and antibody are closely fit, the strength of binding is great. When they are apart binding strength low.

Non – Covalent Bonds: The bonds that hold the antigen to the antibody combining site are all noncovalent in nature. These include hydrogen bonds, electrostatic bonds, Van der Waals forces and hydrophobic bonds.

Affinity of antibody: Antibody affinity is the strength of the reaction between a single antigenic determinant and a single combining site on the antibody.



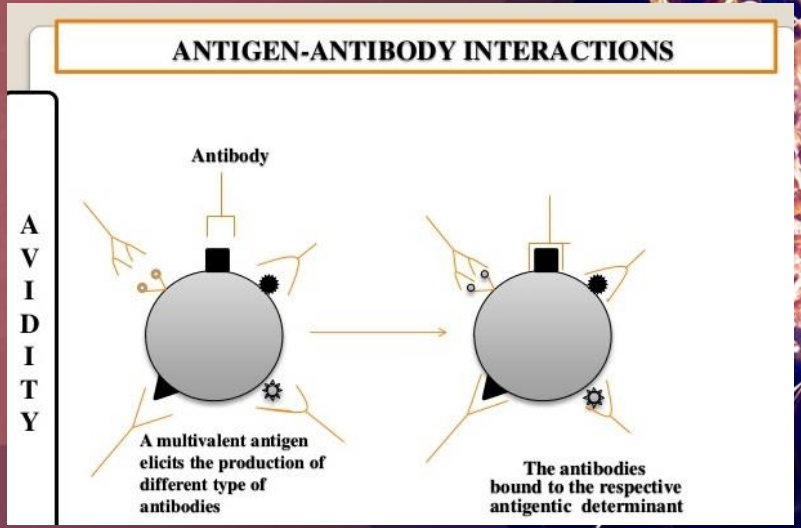
- The non – covalent interaction that form the basis of antigen – antibody binding include hydrogen bond, ionic bond, hydrophobic interaction and Van der Waals interaction.



A strong antigen – antibody interaction depends on a very close fit between the antigen and antibody which requires high degree of specificity.

5. Avidity

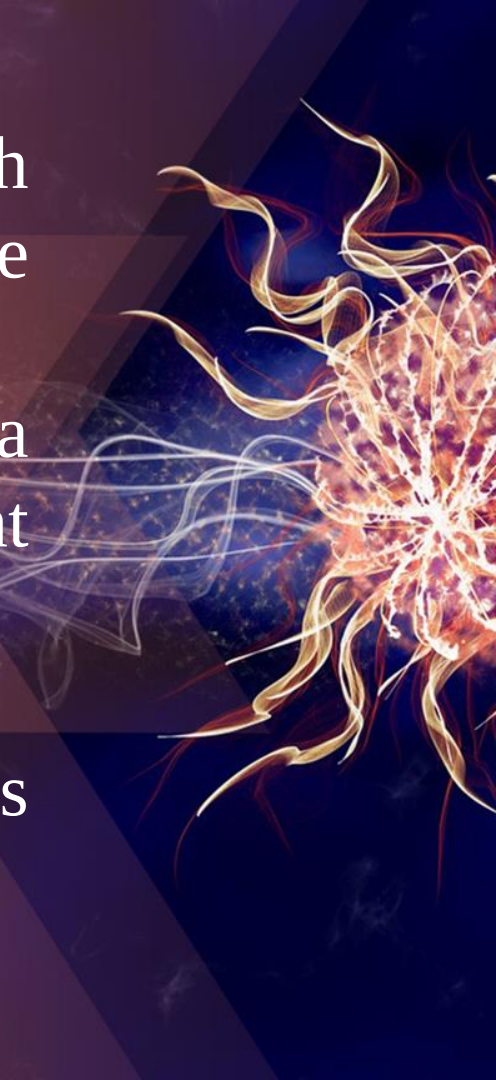
- It is the strength of the bond after the formation of Ag-Ab complexes.
- It is used to denote the overall capacity of antibodies to combine with the multivalent antigen.
- A multivalent Ag has many types of antigenic determinants.



- When injected into the blood, each antigenic determinant stimulates the production of a particular antibody.
- The various antibodies produced by a single Ag combine with the different antigenic determinants of the Ag.

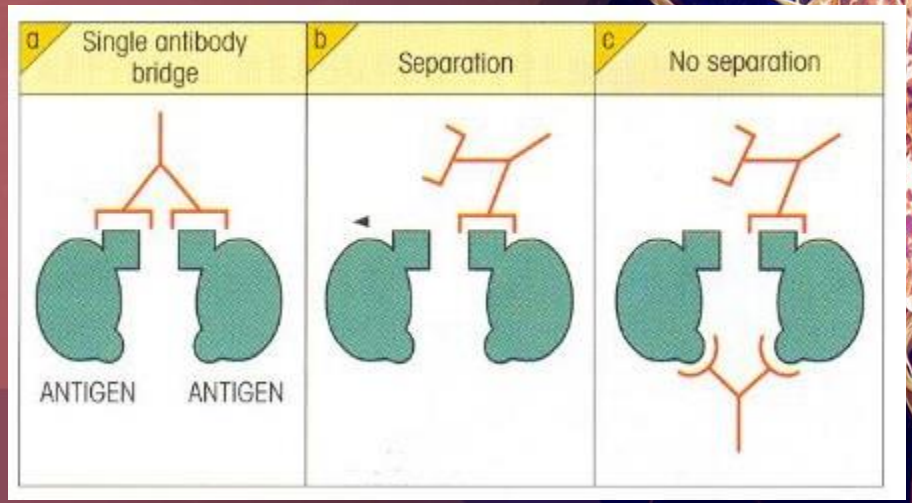
  $$nAb + mAg \leftrightarrow AbnAgm$$

- Where nAb is No. of Ab’s and mAg is No. of Antigenic determinants.



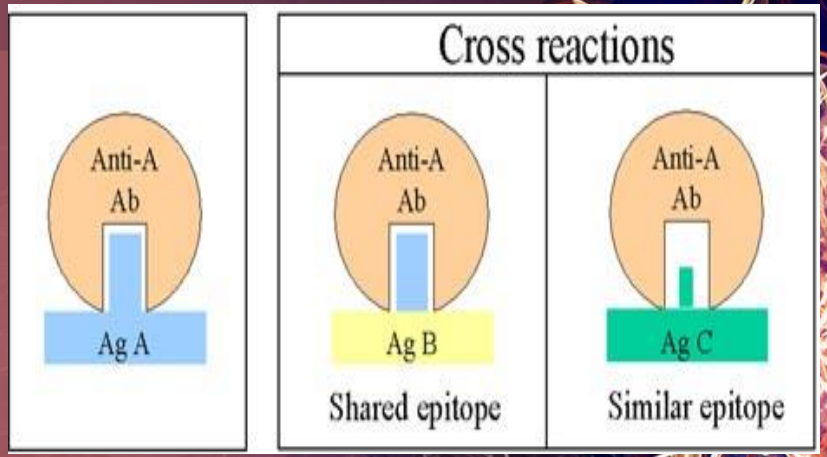
6. Bonus effect

- The phenomenon of giving extra strength to the Ag – Ag complex by the binding of two Ab to two Ag molecules is called bonus effect.
- It is highly possible because the Ag are multivalent and there are many types of Ab.
- Bonus effect increases the binding strength of Ag and Ab molecules

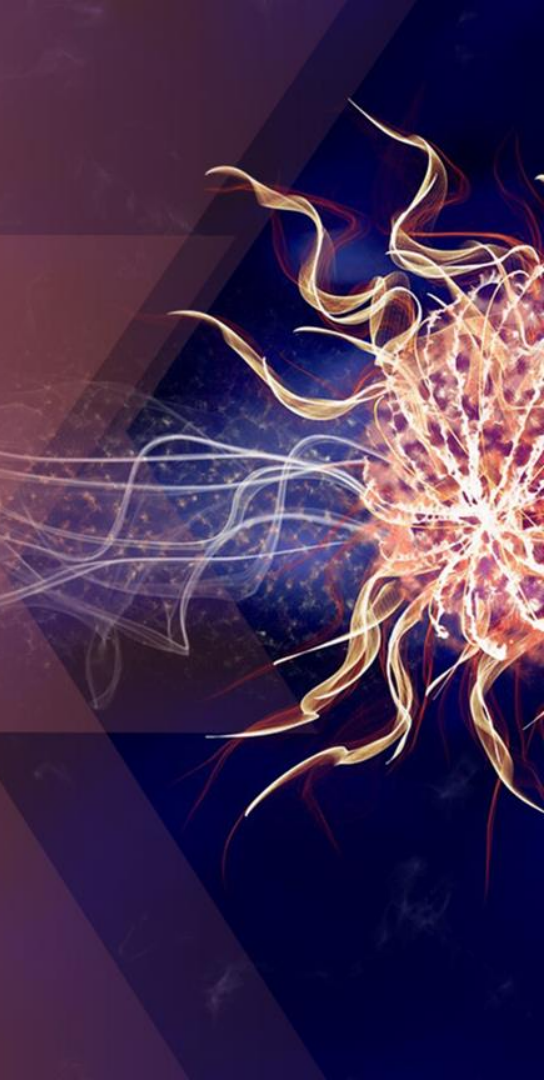


7. Cross Reaction

- An antiserum raised against an Ag, can also react with a similar Ag of another type. This is called cross reaction and the Ag which produces the cross reaction is called Cross reactive Ag. But the strength of Ab raised against its own Ag is strong.
- The bonds involved in cross reactions are weak.



- **Example:** The serum raised against albumin of hen’s egg can cross react with albumin obtained from duck’s egg.
- The antiserum raised against human insulin will react with the insulin of Pig, Sheep, whale etc.
- The antiserum raised against *Pneumococcal* polysaccharides will react with *E.coli*, blood group A and collagen Ag’s.

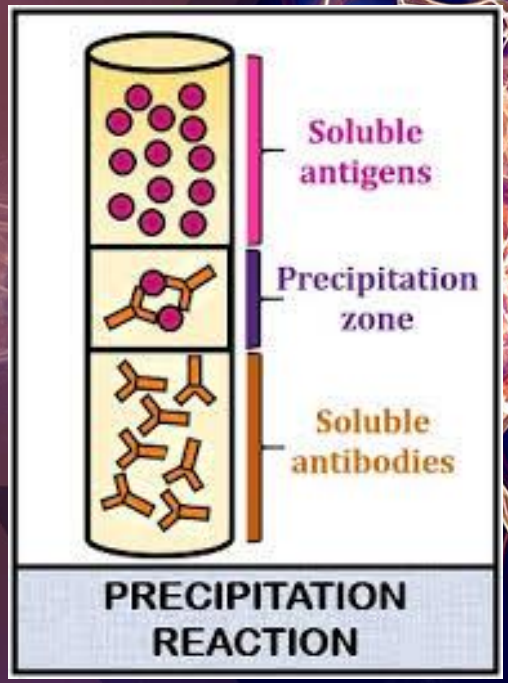


Types of antigen and antibody reaction

- Precipitation
- Agglutination
- Cytolysis
- Complement fixation
- Flocculation
- Opsonisation
- Immunofluorescence

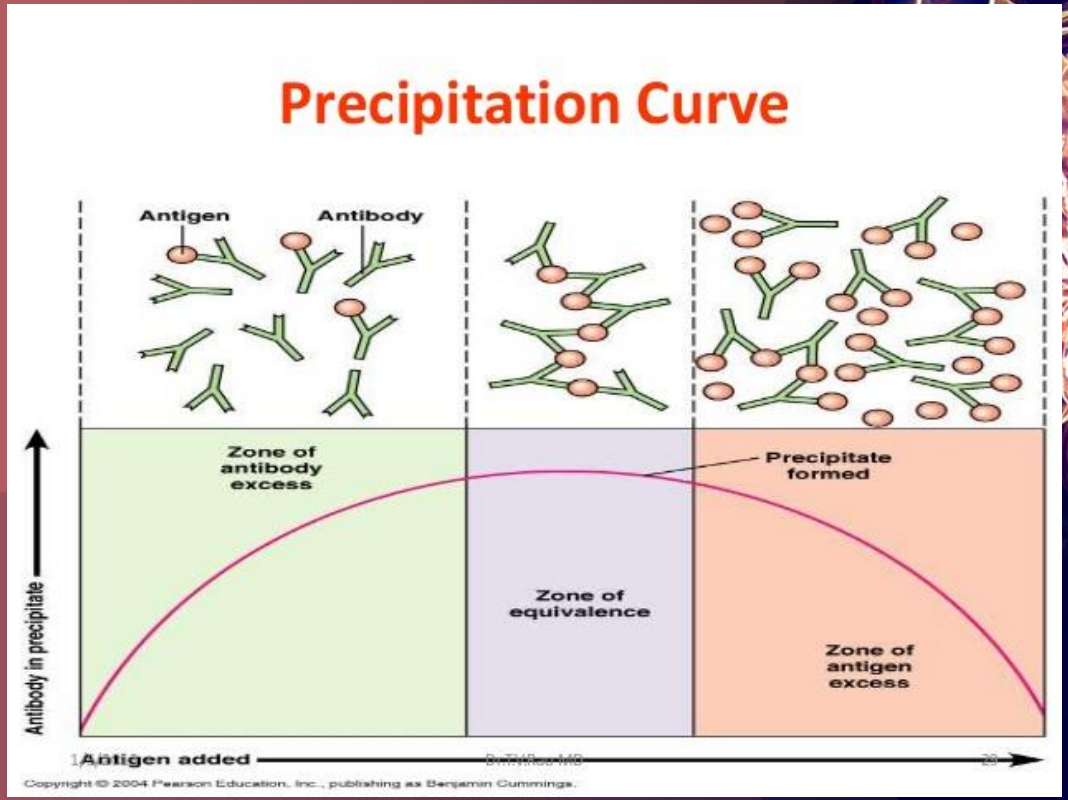
1. Precipitation

- Reaction refers to an ag – ab reaction between a soluble ag and its ab resulting in the formation of insoluble precipitate.
- The ab causing precipitation is called precipitin
- When the amount of precipitin formed in different tubes is plotted on a graph paper and curve is obtained which is called precipitin curve.



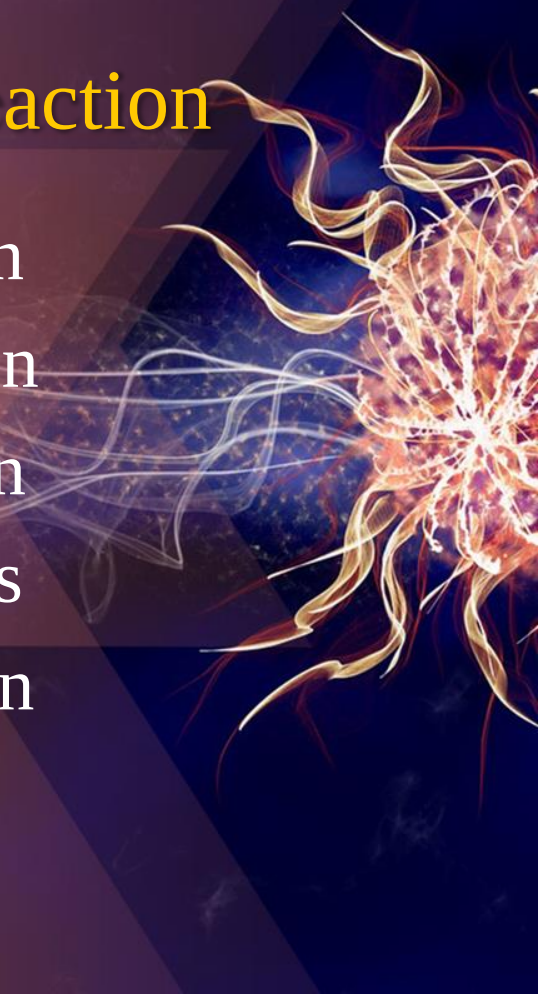
Three zone of precipitin curve

- Zone of antibody excess
- Zone of equivalence
- Zone of antigen excess



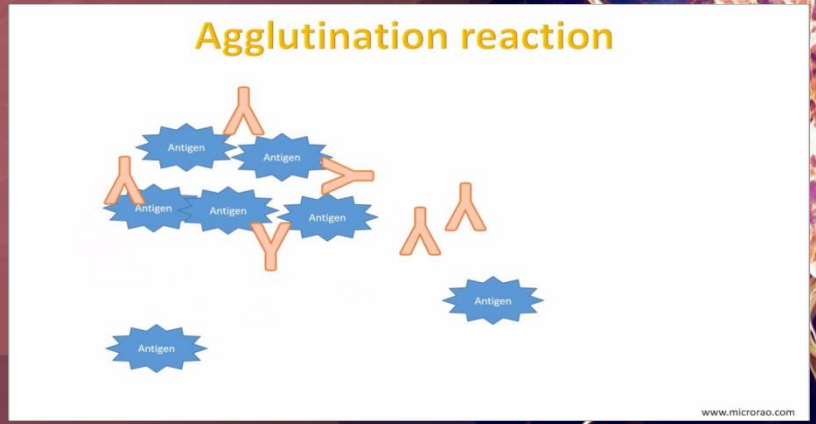
Application of Precipitin Reaction

- Single immunodiffusion
- Double immunodiffusion
- Radial immunodiffusion
- Immuno electrophoresis
- Rocket immunodiffusion

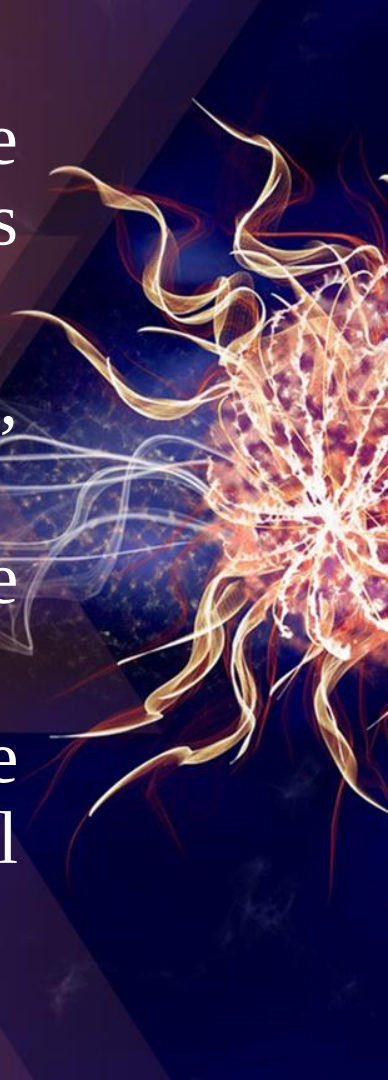


2. Agglutination

- Agglutination is an Antigen – Antibody reaction where the Ab of serum causes the cellular Ag to adhere to one another to form clumps. The process is called clumping.

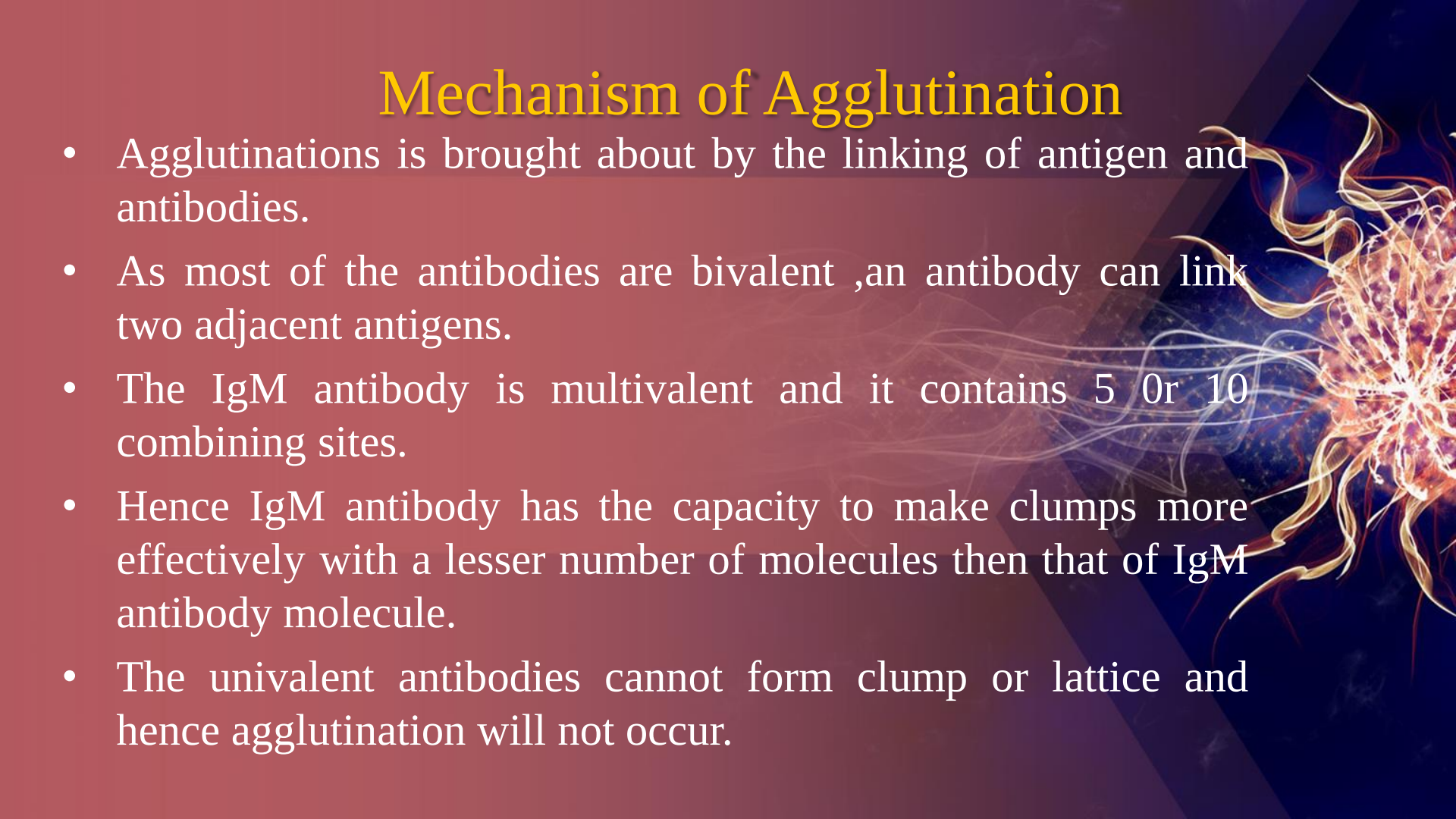


- The antibody that cause agglutination are called agglutinins and particulate antigens aggregated are called agglutinogens.
- The particulate antigen include bacterial, viruses, RBC, platelets lymphocytes, etc.
- When red blood cells are agglutinated, the reaction is called Heamagglutination .
- When bacterial cells are agglutinated ,the agglutination is called Bacterial Agglutination.



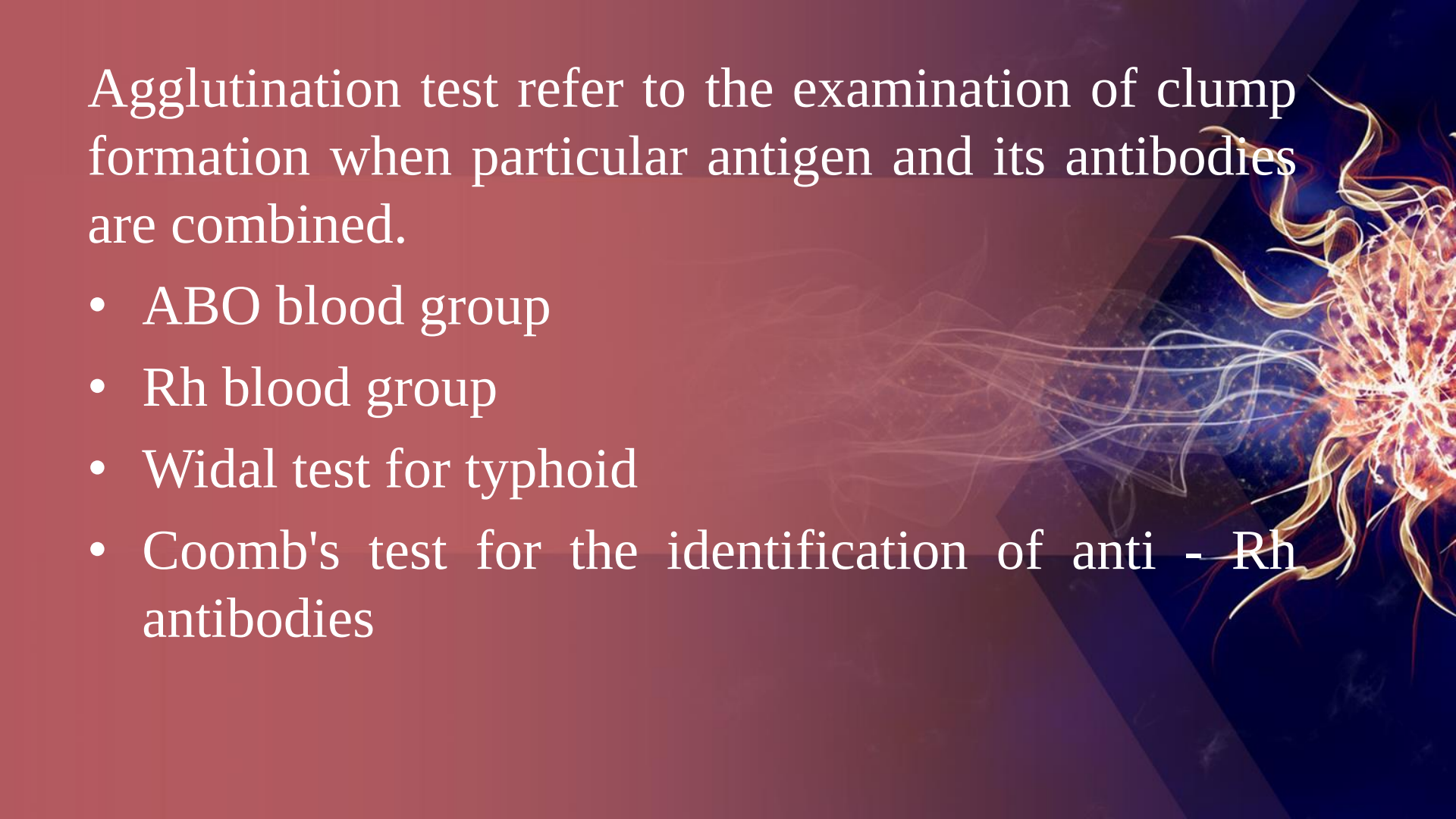
Mechanism of Agglutination

- Agglutinations is brought about by the linking of antigen and antibodies.
- As most of the antibodies are bivalent ,an antibody can link two adjacent antigens.
- The IgM antibody is multivalent and it contains 5 0r 10 combining sites.
- Hence IgM antibody has the capacity to make clumps more effectively with a lesser number of molecules then that of IgM antibody molecule.
- The univalent antibodies cannot form clump or lattice and hence agglutination will not occur.



Agglutination test refer to the examination of clump formation when particular antigen and its antibodies are combined.

- ABO blood group
- Rh blood group
- Widal test for typhoid
- Coomb's test for the identification of anti - Rh antibodies



3. Cytolysis

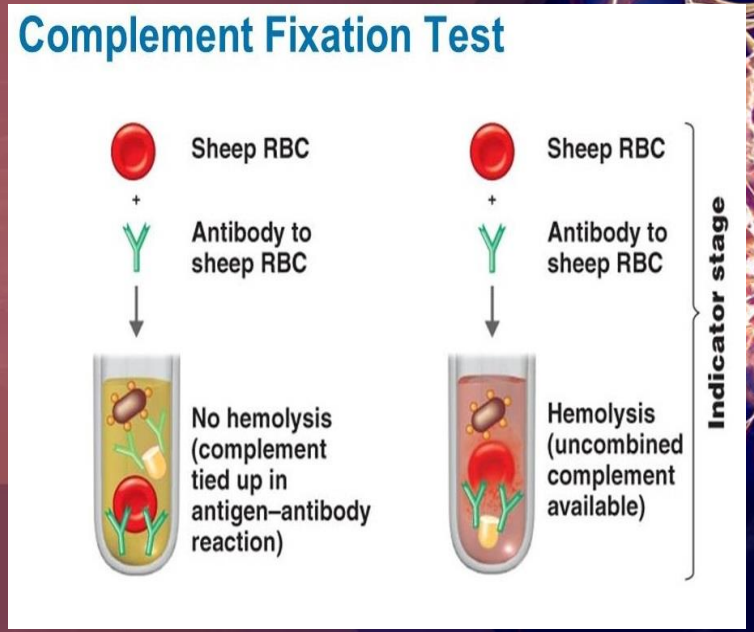
- Definition: Is the dissolution of a cell.
- Example: RBC is lysed – haemolysis and Bacterial cell – bacteriolysis.

Mechanism of Cytolysis:

- The antigen-antibody complex activates the complement.
- Complement binds to the surface antigen of microbe or cell.
- The compliment fixed on the surface of the cell causes the disruption of the lipid bilayer of the membrane of the microbe.
- As a result, a hole is made on the microbe.
- Through this hole the content of the cell are released and the cell is lysed.

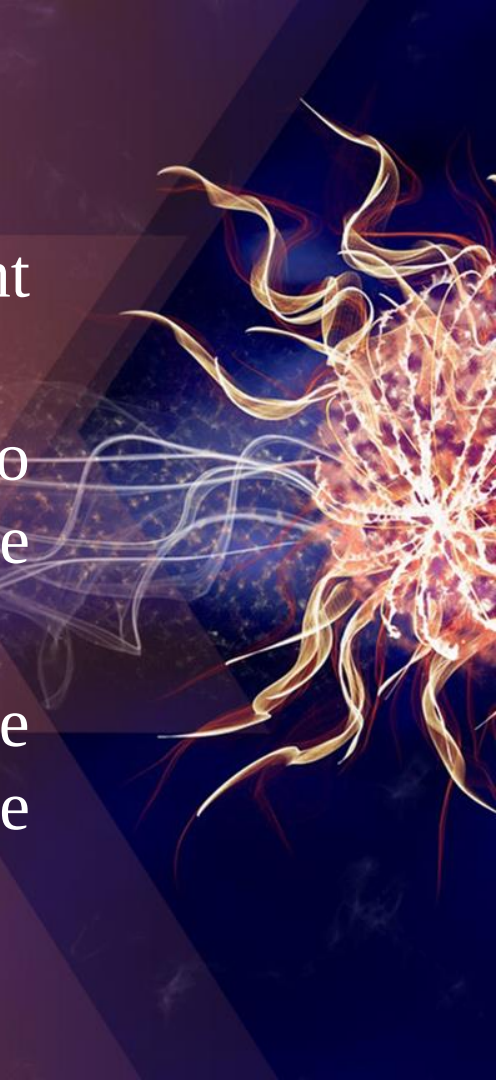
4. Complement fixation

- The binding of complement to antigen – antibody complex is called complement fixation.
- When complement is added to a serum containing an antigen and its antibody, the complement is activated and immediately it binds to the Antigen-antibody complex and the complement is said to be fixed.



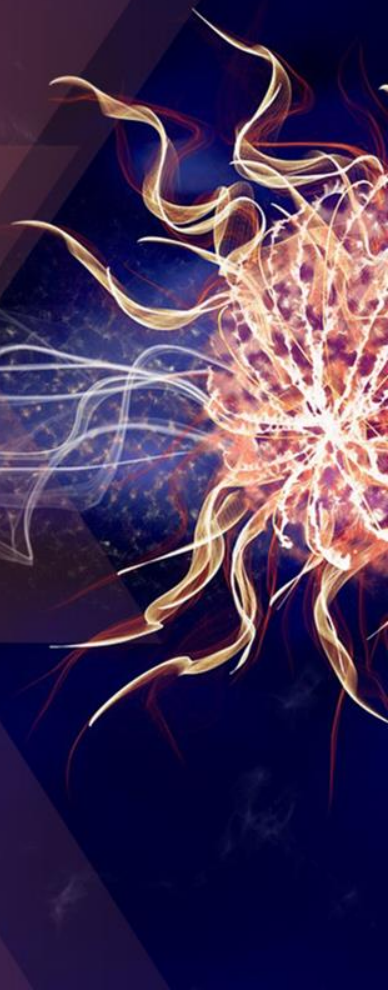
5. Flocculation

- Antigen- Antibody reaction brought about by exotoxin and antitoxin.
- Reaction produces flocculates which do not sediment but remain dispersed in the medium.
- Flocculation is somewhat like precipitation, but in precipitation the precipitate will not sediment.

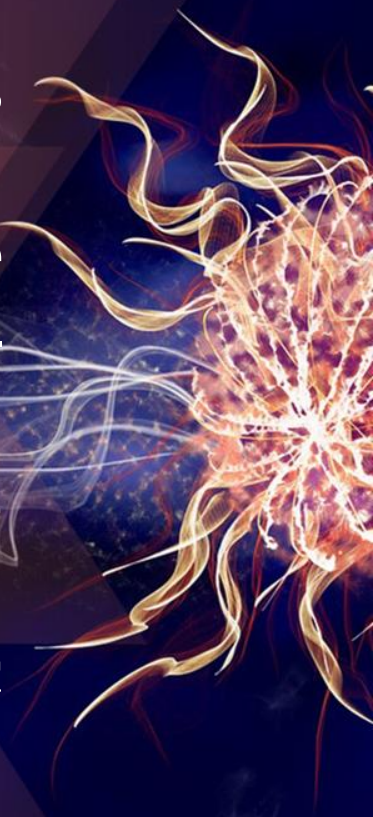


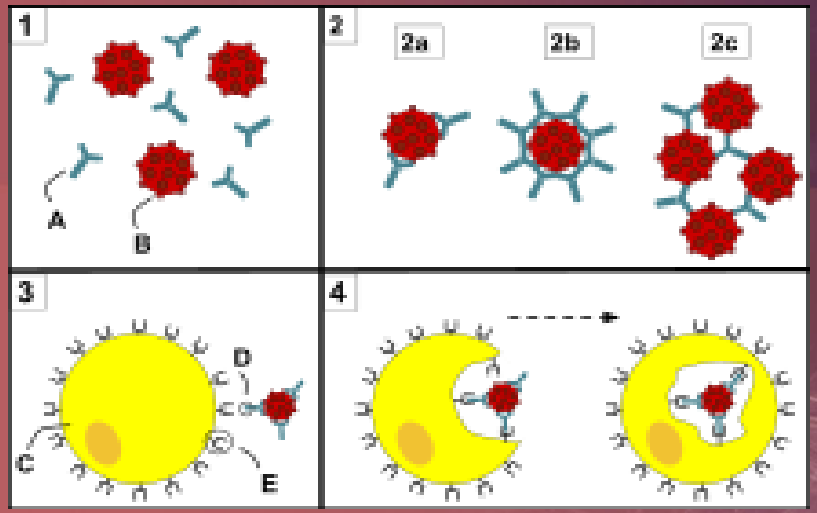
6. Opsonisation

- Process by which a particular antigen becomes more susceptible to phagocytosis by combination with an opsonin.
- Opsonin is an antibody
- when combines with a particulate antigen, increases the susceptibility of the antigen to phagocytosis.
- In Opsonisation - the antibody combines with the surface antigen of bacteria.



- This antigen-antibody complex activates complement system.
- The activated complement is attached to the antigen- antibody complex to form an antigen-antibody complement complex.
- The antigen-antibody-complement is adhered to the phagocytic cells.
- The microbe (antigen) fixed on the phagocytic cell is killed by phagocytosis or lysis.





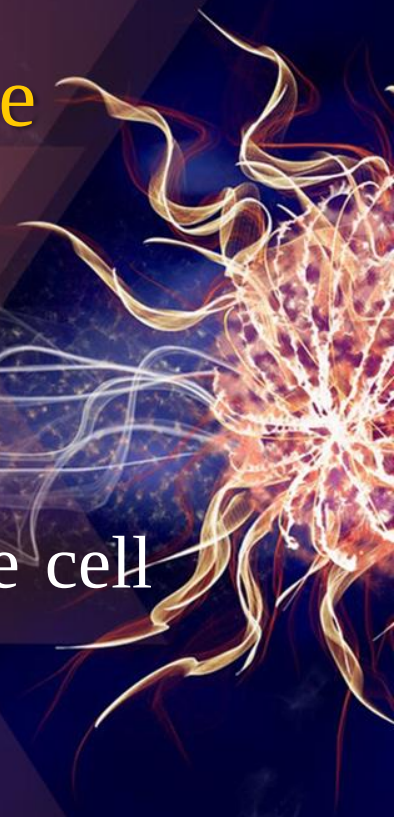
1) Antibodies (A) and pathogens (B) free roam in the blood.
2) The antibodies bind to pathogens, and can do so in different formations such as: opsonisation (2a), neutralisation (2b), and agglutination (2c).
3) A phagocyte (C) approaches the pathogen, and Fc region (D) of the antibody binds to one of the Fc receptors (E) on the phagocyte.
4) Phagocytosis occurs as the pathogen is ingested.

7. Immunofluorescence

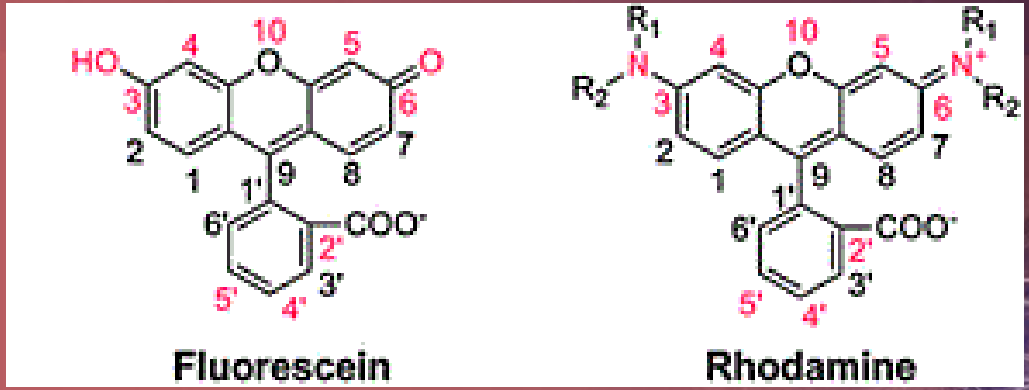
- Immunofluorescence is a powerful technique that utilizes fluorescent-labelled antibodies to detect specific target antigens.
- Fluorescein is a dye which emits greenish fluorescence under UV light. It can be tagged to immunoglobulin molecules.
- This technique is sometimes used to make viral plaques more readily visible to the human eye. Immunofluorescent labelled tissue sections are studied using a fluorescence microscope.

Applications of immunofluorescence

- Used to locate and identify Ag in tissues
- Used to identify pathogenic bacteria
- Ab directed to detect the ag present in the cell or tissue



Examples for fluorescence dye

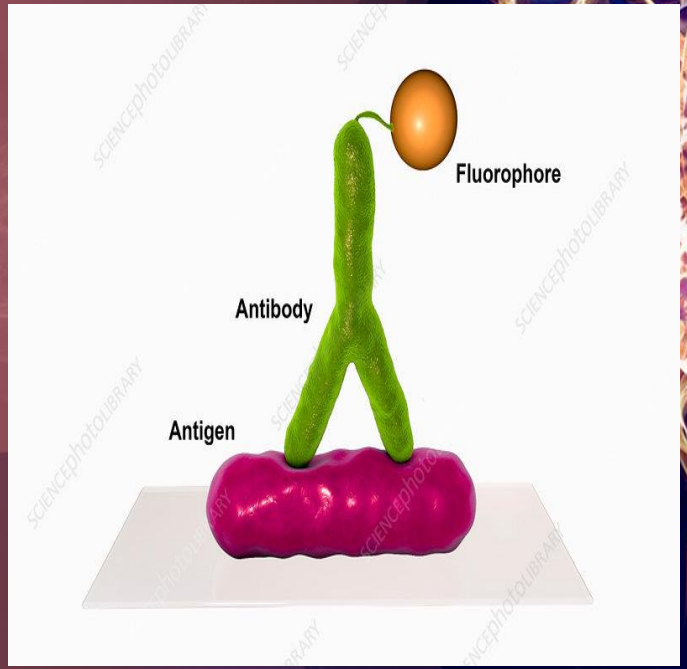


Different methods of Immunofluorescence

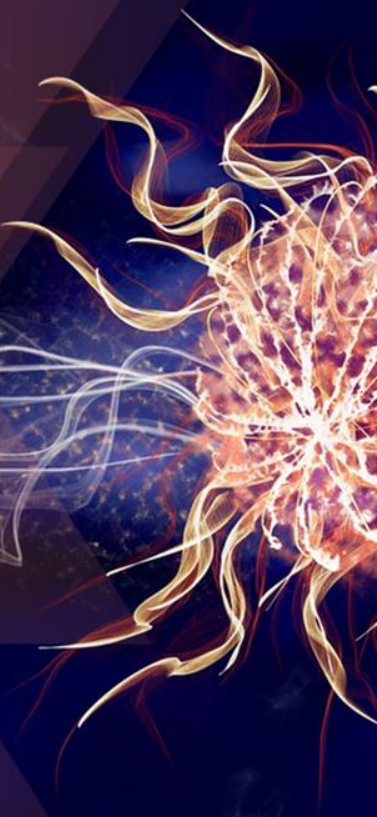
- Direct method
- Indirect method
- Sandwich method

Direct method

- It is a simple and common procedure.
- Ag is fixed on the slide
- Fluorescein labelled Ab’s are layered over it
- Slide is washed to remove unattached Ab’s

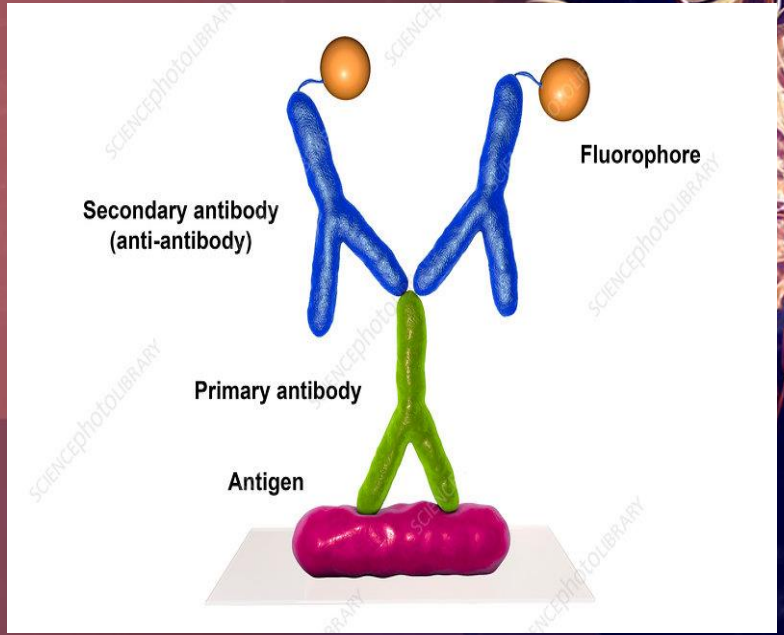


- Examined under UV light in an fluorescent microscope
- The site where the Ab attaches to its specific Ag will show apple green fluorescence
- Use: Direct detection of Pathogens or their Ag’s in tissues or in pathological samples.



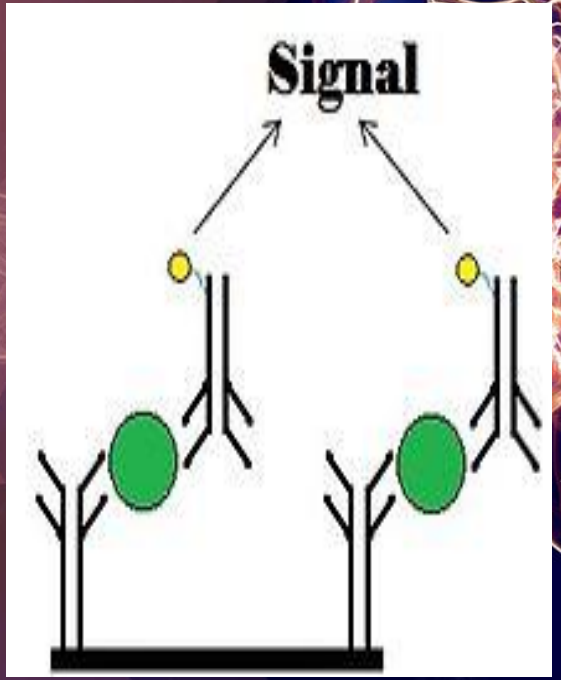
Indirect method

- Indirect test is a double-layer technique
- The unlabelled antibody is applied directly to the tissue substrate
- Treated with a fluorochrome-conjugated anti-immunoglobulin serum.

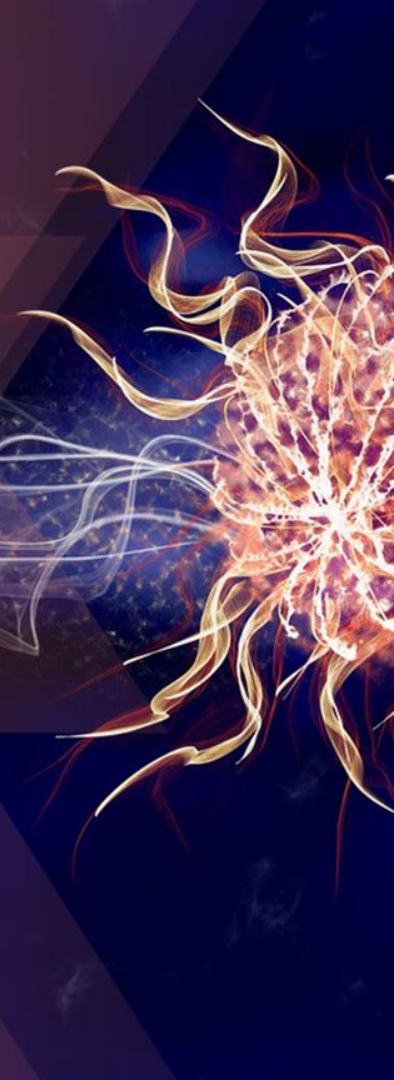


Sandwich method

- Used to test number of cells producing Ab for specific Ag.
- For example: lymphocytes are fixed in ethanol.
- The fixed cells are treated with polysaccharide Ag of *Pneumococcus*.
- This Ag will combine with lymphocytes to produce Ab against *pneumococcus* polysaccharide Ag.



- Fluorescent labelled Ag was added.
- This will bind with Ag which is linked to Ab producing lymphocyte
- Hence, Ag is sandwich between lymphocyte and fluorescent labelled Ab.



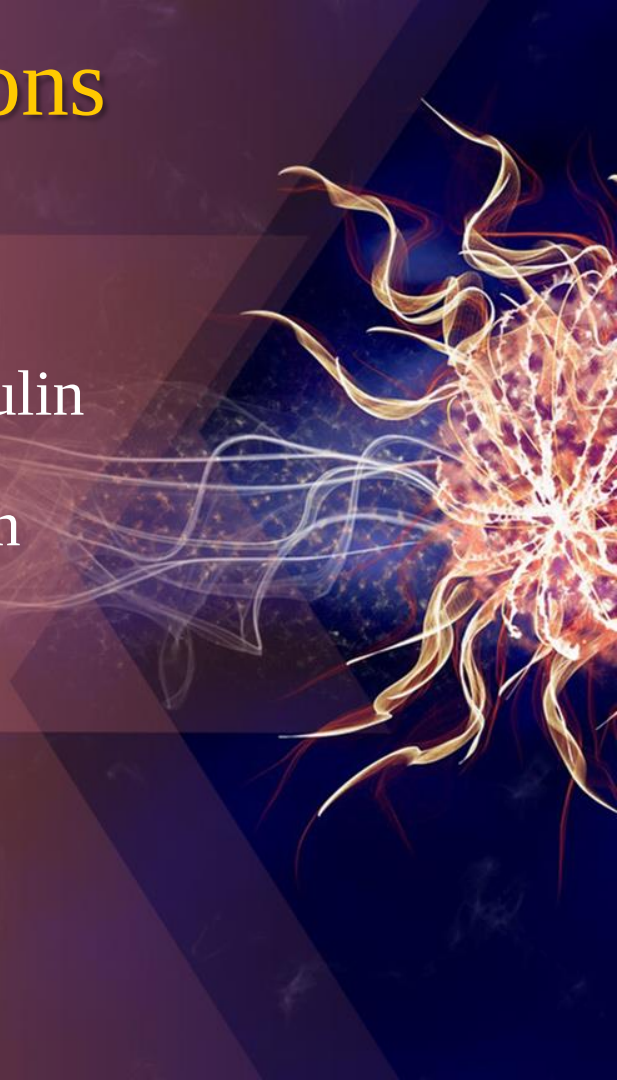
Antitoxins

- An **antitoxin** is an antibody with the ability to neutralize a specific toxin. Antitoxins are produced by certain animals, plants, and bacteria in response to toxin exposure.
- Although they are most effective in neutralizing toxins, they can also kill bacteria and other microorganisms.
- Antitoxins are made within organisms, and can be injected into other organisms, including humans, to treat an infectious disease.
- This procedure involves injecting an animal with a safe amount of a particular toxin.

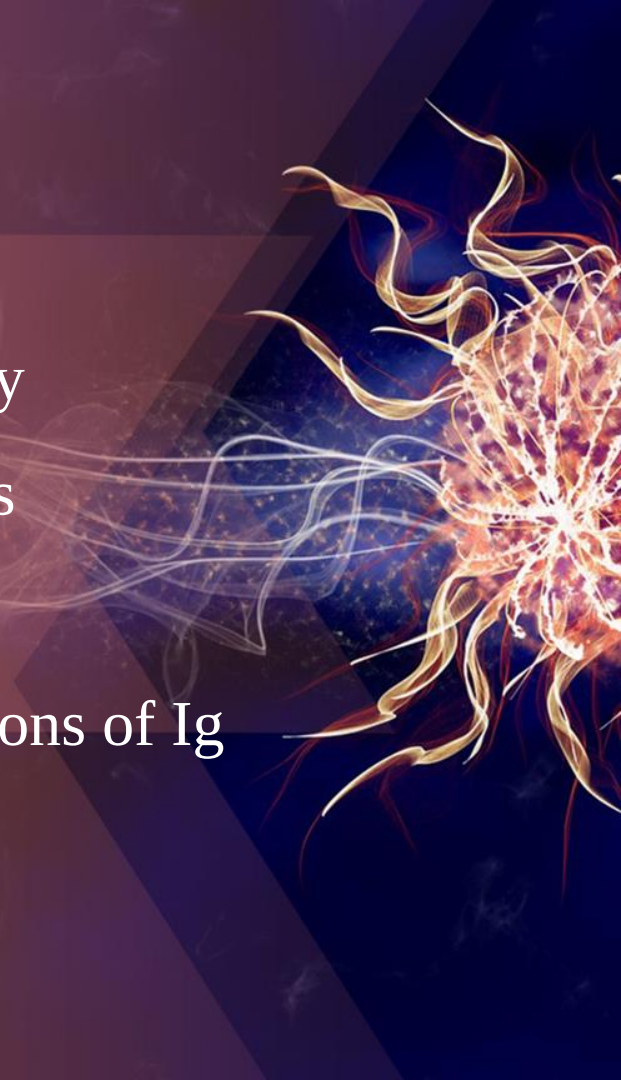
- The animal's body then makes the antitoxin needed to neutralize the toxin.
- Later, blood is withdrawn from the animal. When the antitoxin is obtained from the blood, it is purified and injected into a human or other animal, inducing temporary passive immunity.
- To prevent serum sickness, it is often best to use an antitoxin obtained from the same species (e.g. use human antitoxin to treat humans).
- Example: Botulism antitoxin and Diphtheria antitoxin

Important Questions

1. What is an antibody? Give an Example
2. State the general structure of immunoglobulin
3. Write a short note on agglutination reaction
4. Explain bacteriolysis
5. What is an antigen? Explain its types and characteristics



6. Write a note on epitopes
7. Write down the features of antigenicity
8. Describe antigen-antibody interactions
9. Name any two antitoxin
10. Explain the structure, types and functions of Ig

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