Indira Gandhi
National Open University
School of Sciences

BZYET-141
IMMUNOLOGY

Block

2

ANTIGEN-ANTIBODY

Course Design Committee

Prof. M.S. Nathawat
Former Director
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Prof. Pratima Ray
Department of Biotechnology
Jamia Hamdard University, Delhi-110062

Prof. Sarita Sachdeva
Department of Biotechnology
Manav Rachna International Institute of Research and Studies, Faridabad
Haryana-121004

Dr. Indrakant K. Singh
Molecular Biology Research Laboratory Department of Zoology, Deshbandhu College University of Delhi, Delhi-110019

Dr. Moses Rinchui N.
Department of Zoology, Deshbandhu College
University of Delhi, Kalkaji, New Delhi-110019

Prof. Neera Kapoor
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Dr. Bano Saidullah (Retd.)
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Dr. Abdul Kareem
School of Sciences, IGNOU, Maidan Garhi
New Delhi-110068

Dr. Arvind Kr. Shakya

School of Sciences, IGNOU, Maidan Garhi
New Delhi-110068

Dr. Swati Omanwar
Former, Consultant, School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Block Preparation Team

Dr. Sarita Nanda
Department of Biochemistry, Daulat Ram College
University of Delhi, Delhi-110007 (Unit 6)

Prof. Sarita Sachdeva
Head, Department of Biotechnology, Manav Rachna International Institute, of Research and Studies
Faridabad, Haryana-121004 (Unit 7)

and

Dr. Sarita Nanda
Department of Biochemistry, Daulat Ram College
University of Delhi, Delhi-110007 (Unit 7)

Dr. Nikhat Manzoor
Department of Biosciences, Jamia Millia Islamia,
New Delhi-110025 (Unit 8)

School of Sciences

Prof. Neera Kapoor (Units 6 to 8)

Course Coordinator	**:**	**Prof. Neera Kapoor**
Course Editor	**:**	**Dr. Indrakant K. Singh** **Department of Zoology, Deshbandhu College** **University of Delhi, Delhi-110019**
Associate Editor	**:**	**Dr. Moses Rinchui N.** **Department of Zoology, Deshbandhu College** **University of Delhi, Kalkaji, New Delhi-110019**

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Mr. Hemant Kumar, SO (P), MPDD, IGNOU

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BLOCK 2: ANTIGEN-ANTIBODY

Human body can remain intact and unharmed in the midst of the entire universe, where there are other life forms and inanimate objects that might otherwise threaten survival. Since the evolution of multi-cellularity, it had become necessary for each organism to contain cells and molecules capable of defending it. These cells and molecules together comprise what is called as **immune system.** So, the immune system is actually a series of cells, some are organized as discrete components within tissue and others are molecules which work together to allow immune response to occur. All the important components of the immune system are in place but in a resting state or non responsive form until the person or the host is actually exposed to something that they must be defended against. Hence, we can define that the most important function of immune response is to provide protection against anything that would be harmful to the host. Also, what is crucial is that not everything is potentially harmful to the host, so the immune system should have the ability to take a decision, as to when to respond. The decision to respond or not to respond is without doubt one of the most critical functions of the immune system and the state of non responsiveness is known as immunological tolerance that is actually opposite of response.

Block 2 consists of 3 units in all. Unit 6 “Antigens” defines antigens as substances that can be recognized by B cells and T cells when combined with MHC and which may be self or non-self. Antigens that can induce specific immune reaction in the body are known as **immunogens**. However, there are antigens that can react with antibody but lack the ability to induce immune reaction. They are known as **haptens**. Therefore, all immunogens are antigens but not all antigens are immunogens. Based on this concept it is important to note that a substance that has the property of immunogenicity also possesses the property of antigenicity but not vice versa. Immunogenicity is the ability to induce an immune response either humoral or cellular mediated or both and antigenicity is the ability of the antigen to bind with antibodies or cell receptors.

The antibodies can be classified into five classes (IgM, IgG, IgA, IgD, and IgE) based on their structural, physicochemical and immunological properties. IgG stands for another term for antibody which makes up to 80% of all circulation. The heavy chain that consists of one variable domain and three identical constant is IgG. IgA and IgD have four constant domains per heavy chain. The variable domain determines the binding specificity of the antibody. The constant domain of heavy chain determines the immunological mechanism of action of corresponding antibody class after the adaptive defense is into action against a particular pathogen.

Unit 8 “Antigen Antibody Interactions” tells that Antigen-Antibody (Ag-Ab) reactions are highly specific interactions between antigens and antibodies. These are weak non-covalent interactions involving electrostatic bonds, H-bonds, hydrophobic interactions, and Van der Waal bonds. This forms the basis for some very significant immunologic assays designed for the detection of both antigens and/or antibodies. They can therefore, be used in the diagnosis of diseases, identification of some clinically relevant biomolecules and estimation of the strength of an immune response.

Objectives

After studying this block you will be able to:

- define an antigen,
- list the structure and properties of antigens,

- differentiate between alloantigen and auto antigen,
- differentiate between T and B epitopes,
- differentiate between hapten and antigens,
- explain how small molecules can become immunogenic,
- define Adjuvants and discuss their functions,
- visualize the concept of antibodies,
- describe structure and functions of antigen and antibody,
- explain the various mechanisms of antigen-antibody interaction,
- appreciate antigen-antibody binding,
- comprehend antigenic determinants,
- explain the immunological variants of antibodies present in mammalian body,
- distinguish among isotype, idiotype and allotype antibodies,
- differentiate between polyclonal and monoclonal antibodies, and
- discuss the principles and applications of important immunological tools and techniques.

UNIT 6

ANTIGENS

Structure

6.1 INTRODUCTION

In this unit you will study about the molecules which can produce antibodies in our body. Our body is surrounded by several kinds of inorganic and organic foreign molecules. Some of them can evoke immune response in our body. It is necessary to understand which kind of molecules can produce these immune responses and whether the intensity of response is the same with different kinds of molecules. You can also understand how the physical and chemical structure of these molecules affect their immunogenicity. You will also come to know whether molecules of our own body can also trigger an immune response. You will understand how the immune response evokes varied signals and response in the immune cells. You will also get an idea of how small molecules, which normally do not produce immune response, can be made to be immunogenic.

Objectives

After reading this unit you will be able to:

- define an antigen,
- list the structure and properties of antigens,
- differentiate between alloantigen and auto antigen,
- differentiate between T and B epitopes,
- differentiate between hapten and antigens,
- explain how small molecules can become immunogenic,
- define adjuvants, and
- discuss their functions.

6.2 BASIC PROPERTIES OF ANTIGENS

6.2.1 What are Antigens?

The dictionary meaning of antigen says that word antigen is a noun which means "substance that causes production of an antibody". It originates from the word Antigen in German and antigène in French. **Antigen is a part of a molecule which can induce the living body to form specific antibodies or produce T cells which can recognise their presence in the body**. They are made up of complex molecules like proteins, peptides, and polysaccharides. Sometimes nucleic acids and lipids along with carrier proteins and polysaccharides can evoke immune response. Antigens are also called immunogens or allergens. Allegens or immunogens are present on the surface of viruses, fungi, bacteria and cells. They can be part of their coats, capsules, cell walls, flagella, fimbriae and toxins (Fig 6.1).

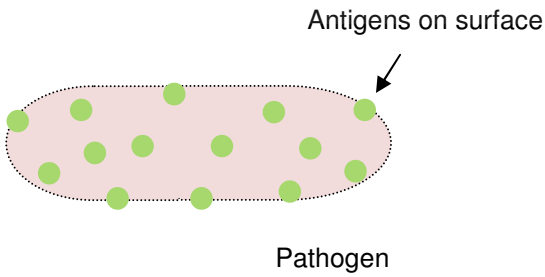


Fig 6.1: Antigens are shown on the surface of the bacteria.

6.2.2 Properties of Antigen which Influence its Immunogenicity

Foreignness

An antigen should be quite different from the inherent molecules of the body in which they produce the immune response. Thus, **only foreign molecules can evoke immune response.** For example, when a pathogen infects the

human body then antibodies which recognize the antigen of the pathogen begin to rise in the blood. The difference in the ability of foreign molecules to stimulate an immune response is called **immunogenicity.**

Molecular Size

Molecules should have minimal molecular weight of 8-10 KDa to act as antigen. Those molecules which can evoke strong immune response fall in the range 14KDa- 600 KDa. Molecules smaller than 8 KDa do not or evoke much weaker response on their own. Serum albumin from mammals (69 KDa) is a fairly good antigen. Hormone angiotensin (1031 Da) is a poor antigen.

Chemical Nature and Composition

The **molecules with complex physical structure are more immunogenic** than the simple ones. Polymers of proteins, polysaccharide, lipids and nucleotide can act as antigens. It is presumed that chemicals which have aromatic radicals can impart greater immunogenicity to the biomolecule. Complex bacterial lipopolysaccharides (LPS) are good antigens. Complex proteins are better antigens than large repeating polymers such as lipids, carbohydrates and nucleic acids.

Physical Form

It has been observed that **soluble antigens are less immunogenic than the particulate antigens**. Interestingly, molecules which have lost their 3D structure i.e., those molecules which are denatured become more immunogenic than their native structures.

Antigen Specificity

Antigens have unique sites which impart them the uniqueness or antigen specificity. These are called the **Antigenic Determinants or Epitopes.** These are the sites that form bonds with the antibody.

Species Specificity

Each species has tissues that bear unique kind of antigens which are called the species-specific antigens. These **species-specific antigens** can produce unique antibodies when introduced in another species. Human blood proteins can be differentiated from animal proteins by specific antigen antibody reaction.

Organ Specificity

Some antigens are unique to a particular organ. These antigens can be similarly found on the organs of different species. Thus, antigens are said to be **organ specific**.

Auto Specificity

The antigens present in the self-tissues normally do not elicit immune response. But in a disease condition they may evoke immune response and produce auto-antibodies. Such antigens are called as **autoantigens.**

Autoantigens ⟶ Autoimmune responses ⟶ Autoantibodies.

Genetic Factors

Every species does not respond similarly to antigens. Some species may elicit an immune response against the antigen but others may not. This is due to the **difference in their genetic makeup to respond to the immunogenicity of the molecule.** The species or individuals may lack or have altered genes that code for the receptors for antigens on B and T cells.

Age

The ability to generate immune response is **dependent on the age**. The children below twelve and elders above sixty have lesser immunogenicity power than those between thirteen to sixty years of age.

Degradability

Those antigens that are easily phagocytosed can elicit better immune response. Thus, molecules which have **better biodegradability are active antigens**. This is because for most antigens the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by antigen presenting cell (APC). In fact, inert molecules like dust which cannot be degraded in the body produce an allergic response rather than the immunogenic response.

Dose of the Antigen

The amount or the dose of antigen exposed to the body determines its immunogenic response. Exceptionally low a dose or extremely high dose may not produce an optimal response. Only appropriate dose can trigger an optimal response.

Route of Administration

Antigens can be administered by various routes to elicit the immune response. It can be introduced through **Intravenous/subcutaneous/Intradermal routes**. The antigens introduced through subcutaneous/intradermal route give better response than the intravenous route.Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes.

Adjuvants

Some molecules which are not immunogenic but when injected with additional substances enhance immune response are called **adjuvants.** Aluminum hydroxide is one of the examples of adjuvant. Adjuvants are commonly used to improve the effectiveness of a vaccine They are injected alongside an antigen to help the immune system generate antibodies that fight the antigen. You will study about adjuvants in detail in Section 6.6 of this Unit.

6.3 GENERAL STRUCTURE OF ANTIGENS

6.3.1 Chemical Nature and Composition

Antigenic molecules are complex in nature. These molecules should have stable internal bonding which gives them a unique specific three-dimensional

structure. It should not have long stretches of long repeating units. They should neither be too big nor too small. **Ideally, molecules whose molecular weight ranges from 8-10K Da are strong immunogens**. Exceedingly small molecules can be made immunogens by conjugating to larger molecules. Antigens belong to proteins, polysaccharides, lipids, or nucleic acids family of biomolecules. The presence of aromatic radicals is presumed to promote antigenicity of the molecule. Peptide antigens can elicit strong immune response if they contain high number of hydrophilic and charged amino acids like lysine, glutamine, glutamic acid, asparagine, and aspartic acid. They should be structurally dissimilar with the host biomolecules. They should be able to interact easily with the immune cells to elicit an immune response.

6.3.2 Physical Form

The physical form of the molecule can change its immunogenicity. It has been observed that **particulate matters** can evoke greater immune response than its **soluble form.** Interestingly, the **denatured form** of a biomolecule can trigger greater immune response than its normal structure**. Inert molecules** like dust which cannot be degraded by the immune cell **exerts an allergic response** rather than antibody formation.

6.4 TYPES OF ANTIGENS

There are different types of antigens depending on the antigen specificity. Antigen specificity, is based on the part of the immunogen which elicits the immune response, called the antigenic determinant or epitope. These regions specifically bind with the antibody molecules. An antigen can have at least one to several epitopes on it. Based on these epitopes the antigens can be classified as **Alloantigens** and **Autoantigens.** They can also be classified based on origin and its function.

Between 1901-1920, **Landsteiner** demonstrated the ABO blood group system. In the 1920s, **Michael Heidelberger** and **Os wald Avery** observed that antigens could be precipitated by antibodies and went on to show that antibodies were made of protein.

6.4.1 Classification of Antigens based on the Origin (Fig 6.2)

Based on the origin, antigens are classified as exogenous, endogenous, autoantigens and alloantigens.

Exogenous Antigens

Exogenous antigens are those antigens that enter the body of an organism from outside. They can enter the body by **ingestion, inhalation** or **injection.** They are non-self-antigens. They occur in body fluids and extracellular space. **They are present along with MHC Class II molecules.** Examples include bacteria, viruses, allergens like pollen or toxic food. Some antigens start out as exogenontigens but later become endogenous (e.g. intracellular viruses).

Endogenous Antigens

Endogenous antigens are those antigens that originate from within the cell, as a part of normal cell metabolism or infected by products of the cellular metabolism. **They are self or non self-antigens present on the cell membrane which are presented along with the MHC class I molecules.** The endogenous antigens are processed by the macrophages which are later accepted by the cytotoxic T-cells. Examples: by products of regular cell metabolism or molecular components of pathogens inside the infected cells, blood group antigens. Exogenous antigens after metabolism may become endogenous antigens.

Differences in Exogenous and Endogenous antigens are listed in Table 6.1.

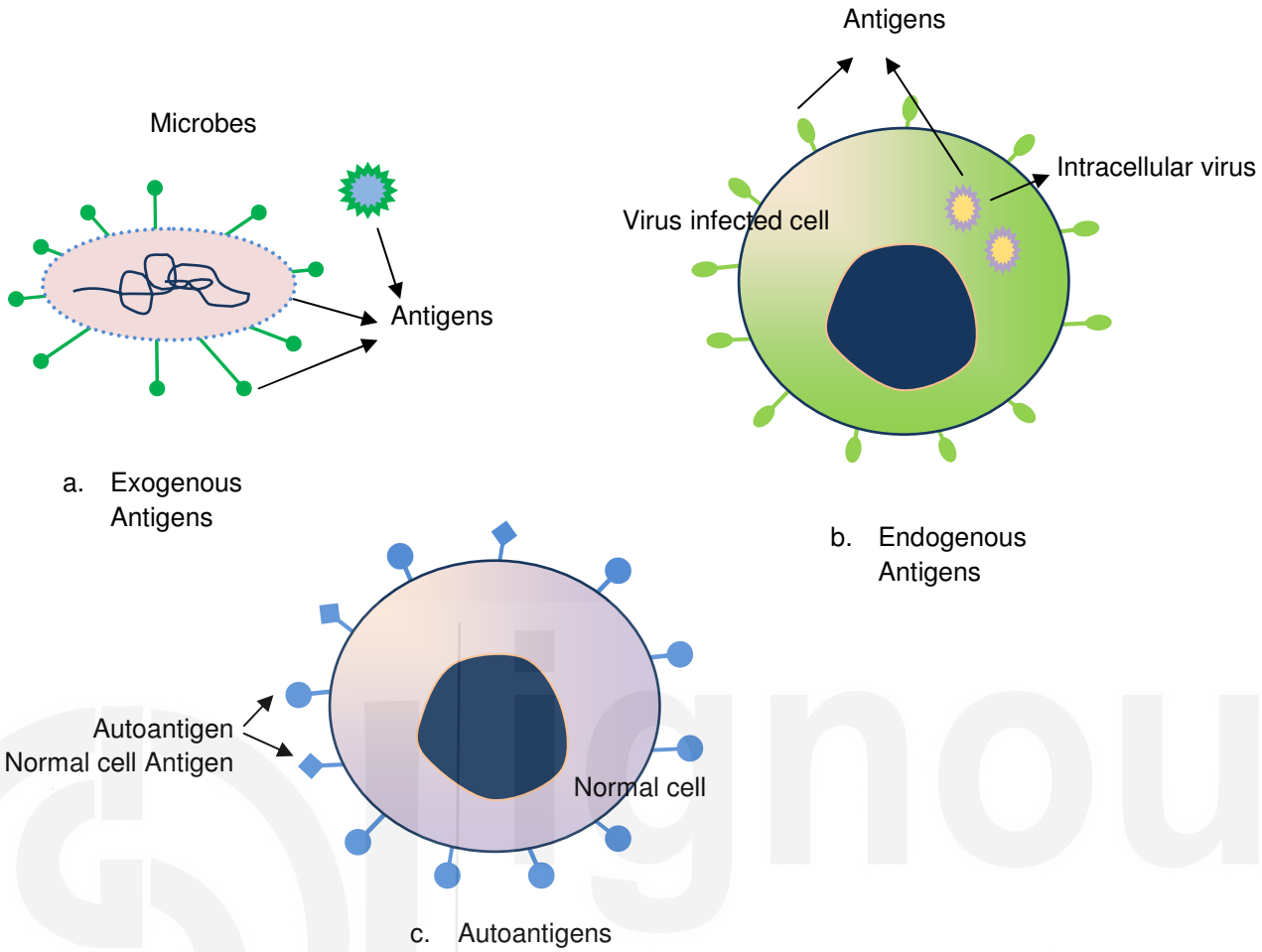


Fig. 6.2: Types of Antigens (a) Exogenous Antigens present on the surface of pathogens (b) Endogenous Antigens are nonself antigens present inside or outside the cell (c) Autoantigens are normal self antigens on the cell.

The Antigens present on the RBC surface are made up of glycoprotein/sphingolipids/glycolipid

Allo-Antigens (Fig 6.3)

Allo-antigens are antigens that are found only in some members of the same species e.g., Antigens A and B present in blood. They are determined genetically and they can be present in some members of the species, the other members of the species who lack it are capable of producing antibodies against it. They are also known as **isoantigens.** They produce an immune response to non-self antigens from members of the same species. **Blood group antigens and histocompatibility antigens are two major types of alloantigens.**

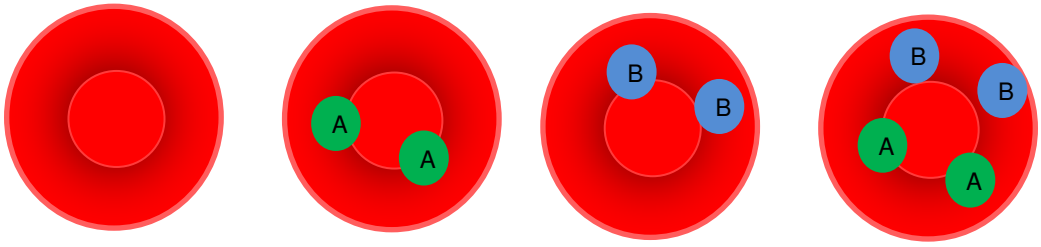


Fig. 6.3: Alloantigens are present on the surface of human RBCs and they determine the blood group of an individual. If blood of Group A is injected into blood Group B it evokes immune response.

Autoantigens

Every species has a unique set of antigen which is recognized as self by the immune system. The body can recognize between self from non-self-antigens and induce immune response against non-self. But sometimes in an autoimmune disorder antibody can be produced against self antigens. Such antigens are called autoantigens. These antigens under normal conditions should not be the target of the immune system but due to genetic and environmental factors, the normal immunological tolerance has been lost in these patients. Examples: Nucleic acids, Nucleoproteins etc. For example, in Hashimoto's thyroiditis, antibodies are produced against antigens present on the thyroid gland. This leads to gradual destruction of thyroid gland.

Hyshimoto's thyroiditis: When the immune system attacks the thyroid, a butterfly shaped gland in the neck. Initially inflammation of the thyroid causes hyperthyroidism. Over time, the inflammation causes hypothyroidism. Symptoms include fatigue and weight gain.

Superantigens

Superantigens are class of antigens that are capable of evoking non-specific T cells response bypassing normal antigen processing. These antigens are usually proteins produced by virus and bacteria to combat against immune system. They are more potent than normal antigen. **Binding of super antigens to T cell receptors results in T cell proliferation and massive release of cytokines.** When the immune system encounters a conventional T dependent antigen, only a small fraction of T cell population is able to recognize the antigen and becomes activated (monoclonal/oligoclonal response). But some antigens polyclonally activate a large fraction of T cells. These are called superantigens. Bacterial superantigens are the best studied as compared to other microbes. Staphylococcal enterotoxins (food poisoning) and streptococcal pyrogenic exotoxins (shock) are examples of super antigens.

Table 6.1: Difference between Exogenous and Endogenous Antigen.

Exogenous Antigens	***Endogenous Antigens***
Those antigens that enter the body from extracellular source	*Those antigens generated from within the cell or intracellular in nature*
Route of entry to the body through ingestion, inhalation or injection	*By products of the cellular metabolism*
Non self antigens	*Either self or non self*
Occur in body fluids and extracellular space	*Presented on the cell membrane*
Present along with MHC Class II molecules	*Presented along with the MHC class I molecules*
Examples: bacteria, viruses, allergens like pollen or toxic food	*Examples: by products of regular cell metabolism or molecular components of pathogens inside the infected cells*

SAQ 1

Mark the sources as alloantigens or auto antigens.

S.No.	Source	Alloantigen/autoantigen
A)	Bacteria	
B)	Drugs	
C)	Self molecules	
D)	Mutated proteins in the body	

6.4.2 Classification of Antigens based on their Function

Antigens can be functionally described as two types, **Complete and Incomplete**. Complete antigens can elicit immune response on their own but incomplete antigens cannot evoke an immune response till they are complexed with proteins. Complete antigens are called **immunogens** while incomplete antigens are called **Haptens** and the protein to which they are complexed are called **Carriers**. Complete antigens are generally of high molecular weight and may be proteins or polysaccharides. You will study about Haptens in detail later in this Unit.

T Lymphocytes and B lymphocytes are cells of the immune system which help to provide long term immunity against antigens

6.5 B AND T CELL EPITOPES

Epitopes are the sites on the surface of the antigens where antibody binds to recognize it as a foreign body (Fig 6.4). They are also known as antigen determinants and are immunologically active regions which bind to the antigen-specific membrane receptors on the cells of the immune system or secreted antibodies. (Fig 6.5)The epitope can be in primary, secondary, tertiary or even in quaternary structure. The B-cells and T-cells can identify the epitopes on the antigen, but the properties of epitopes are different for both the cases. The epitopes thus have universally accessible sites on the exposed surface of immunogen. T-cells do not recognize any random antigen. They interact with the part of the antigen which is presented by a special molecule called MHC (Major Histocompatibility Complex) on antigen presenting cells or nucleated cells.

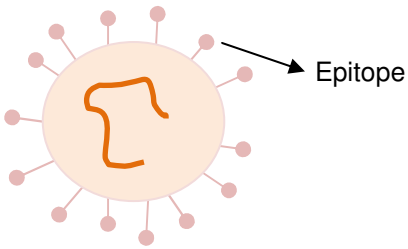


Fig. 6.4: An antigen has antigen determinant sites called epitopes. Antibodies formed are specific to these sites.

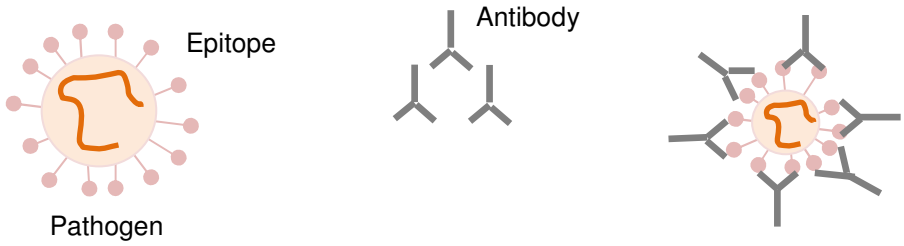


Fig. 6.5: Antibodies formed in response to the antigen are specific to the epitopes.

6.5.1 Properties of B-cell Epitopes

- The **epitope** must have a shape that is complementary to the antigen binding site on an antibody.
- The size of the epitope should not be larger than the receptor.
- Epitopes mostly comprise of hydrophilic amino acids. Hydrophobic amino acids are buried inside and thus cannot be an epitope for B-cell receptors or antibodies.
- The amino acid sequence on the epitopes of B-cells can be a sequential or non-sequential one. In sequential epitopes, the amino acids are in contiguous fashion along the polypeptide while non-sequential has residues from different segments of polypeptide brought from nearby folded conformation.
- Epitopes display mobility of site. They are generally present in the flexible region.
- The antigen city of B cell epitopes is determined by the presence of group of amino acids in sequence of the polypeptide chain of the protein surface in display.

6.5.2 Properties of T-cell Epitopes

- T cell epitopes are processed and presented on antigen presenting cells through class I or class II MHC molecules.
- The receptors on T cells cannot bind to the free peptides or antigens independently.
- The antigenic peptide which is processed by the APC or nucleated cells interact with the T-cell receptor and form a trimolecular complex of MHC – peptide-T cell receptor.
- In contrast to B cell epitopes, T-cell epitopes can have **internal epitopes.** The internal epitopes contain hydrophobic amino acid sequence.
- T cell epitopes presented by MHC I molecules are typically linear peptides with length that ranges from 8-11 amino acids.

6.5.3 Ligand Cell Interaction and Signal Transduction

The B cell receptor complex which contains immunoglobulin binds to B epitopes on the antigen. This binding alone cannot signal any message. The adjoining protein activates the immunoreceptor present on these proteins when B cell receptor binds to B antigen. This signaling is enhanced by the co-receptor CD4 on the B cell.

The T cell receptor (TCR) recognises the T epitope presented by APC on MHC molecules and binds to it. They are associated with other proteins of CD3 which get activated and stimulate the immunoreceptors. This signaling is enhanced by the co-receptor CD8 on the T cell. The responses from the immunoreceptors can be modulated by the inhibitory signals around. Thus, it helps to regulate immune responses as per the surrounding conditions. (Fig 6.6)

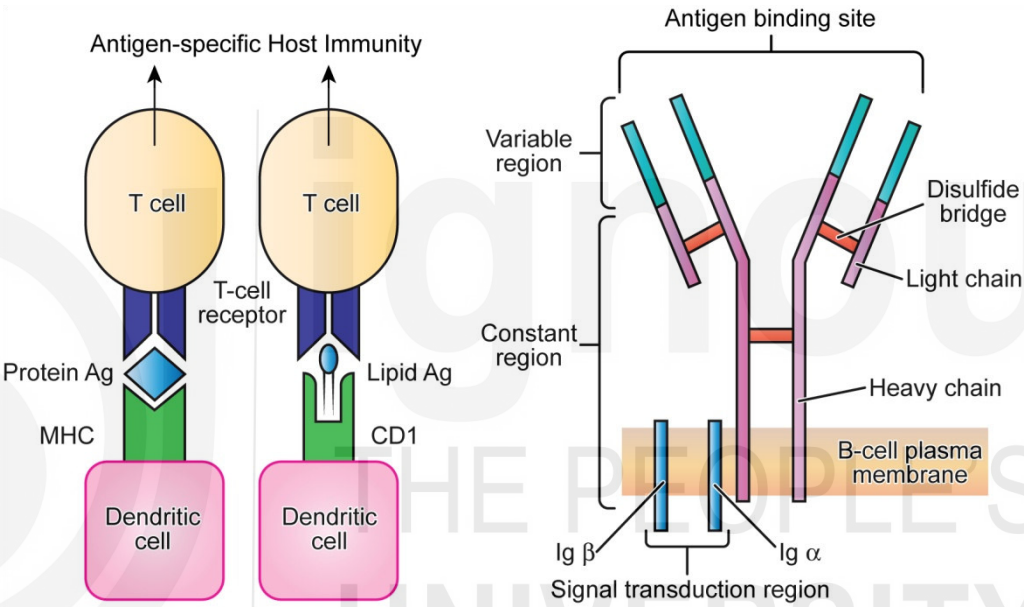


Fig. 6.6: T cell binds to the T cell epitopes bound to the Antigen presenting/ Dendritic cells. The B cells have immunoglobulin bound to its cell surface. The B cell epitopes of the soluble antigens bind to these immunoglobulins. The adjoining proteins of the B and T cell with the MHC of the cell help in signal transduction

SAQ 2

Fill in the blanks:

i) An antigen soluble in the blood can activate …… cells.

ii) The antigen attached to the macrophage will activate …… cells.

SAQ 3

Fill in the Blanks:

…… are produced when …… is injected with …… in the host animal.

6.6 HAPTENS AND ADJUVANTS

6.6.1 Haptens

Molecules less than 1 KDa cannot elicit an immune response. However, when these molecules are linked to larger molecules like proteins, they evoke immune response. **Such small molecules are referred to as Haptens and the linking big molecules are called Carrier.** The term hapten is derived from the Greek word haptein, meaning "to fasten." The carrier proteins which are popularly used are albumins. Table 6.2 presents differences between Antigen and Hapten.

Table 6.2: Differences between Antigen and Hapten.

Antigen	***Hapten***
An organic molecule which can evoke an immune response on its own	*A small molecule which cannot evoke an immune response*
It can evoke immune response without being bound to any other molecule	*It has to be bound to an organic molecule called carrier to evoke immune reponse*
Its molecular weight can be as low as 8KDa	*Its moklecular weight can be as low as 1 Kda*
It interacts with major histocompatibility complex	*It does not interact with major histocompatability complex but its carrier can*
Examples are proteins, peptides, nucleic acid and polysaccharides	*Examples are biotin, dinitrophenol, p- aminobenzoic acids, antibiotics like pencillin, anaesthetics like Halothane*

6.6.2 Adjuvants

The term Adjuvant is derived from the word "adjuvare" in latin meaning "to help". An adjuvant is a pharmacological or immunological agent that improves the immune response to an antigen..These can be used with antigen like vaccine or hapten to improve its ability to produce the immune response. The commonly used adjuvants are:

- Inorganic compounds: aluminum hydroxide, alum
- Mineral oil: Paraffin oil
- Killed bacteria

Fig. 6.7 depicts interaction of haptens, carrier protein and adjuvant for development of antibodies.

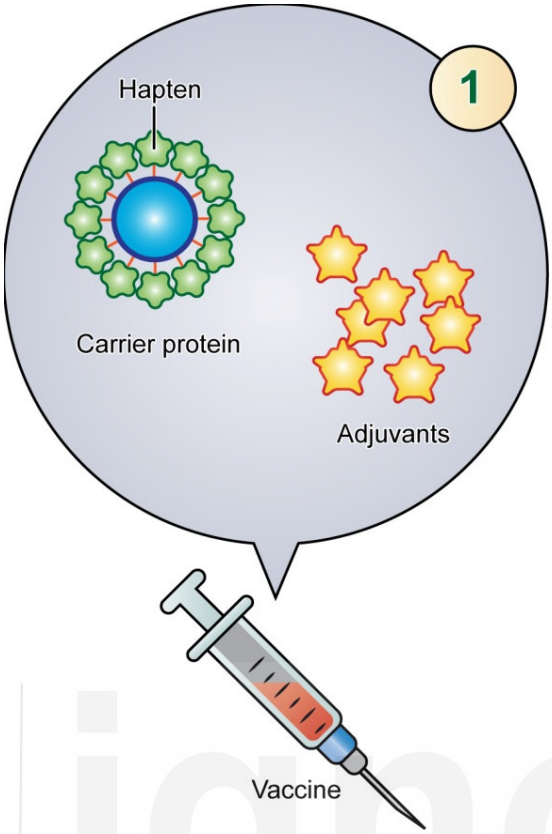


Fig. 6.7: Antibodies can be experimentally developed by injecting small molecules, called hapten, conjugated to carrier proteins along with adjuvant.

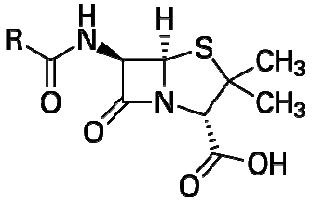
Functions of Adjuvants

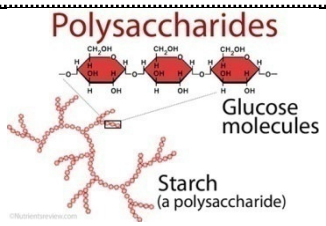
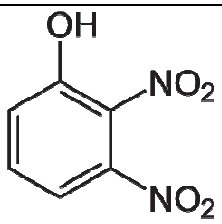
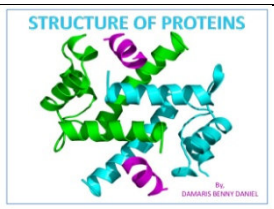
Antibodies are experimentally produced by injecting antigens with adjuvant in the host animals like mice, rats, rabbits, and many other animals. Since adjuvant enhances immunogenicity, the dose required by antigen to elicit the immune response is decreased. It is hypothesized that the adjuvant can enhance the immune response by some of the mechanisms mentioned below:

- ❖ It causes slow release of antigens into the bloodstream.
- ❖ It enhances the immune response at the site of injection.
- ❖ They activate T cells by the phagosomes formed or by activating the antigen presenting cells. The activated T cells release cytokines which stimulate antibody formation by the cells.

SAQ 4

Mark the following as Antigens/Hapten.

S. No.	Molecule	Mark it as Antigen/Hapten
A)		

B)		
C)		
D)		

6.7 SUMMARY

Let us summarise what you learnt in this Unit.

- All molecules cannot evoke immune response by producing antibodies.
- Only antigens (non self, alloantigens) which are complex organic molecules (like proteins, peptides, polysaccharide, lipids, nucleic acids) with molecular weight greater than 8 K Da and are foreign molecules to the body can evoke this response.
- But sometimes our own biomolecules (self antigens, auto antigens) can act as foreign molecules due to structural changes (in autoimmune diseases) and trigger the body to produce antibodies against themselves.
- Antigens can be classified as exogenous, endogenous, autoantigens and alloantigens based on origin.
- Based on their function, they can be classified as complete and haptens.
- Only a small portion of the molecule, which is called the epitope, binds to the B and T cell. The mechanism of binding to the T and B cells are different and the signals produced are also not the same.The response produced from the antigenic binding to the immune cells intensifies the immune response leading to antibody formation.
- Sometimes small molecules called haptens, added to the body fluid as drugs, analgesics or by injection (along with solutions called adjuvants) can trigger antibody formation after they bind to protein in the blood.
- Adjuvants help the haptens to become immunogenic by several mechanisms like slowing their rate of release and enhancing local immune response.

6.8 TERMINAL QUESTIONS

1. Why is the O negative blood group a universal donor?
2. Why do the doctors match the donors before the organ transplant?
3. How does the protein structure influence the capacity of antigens to form antibodies?
4. Write down the differences between alloantigen and autoantigen.

6.9 ANSWERS

Self Assessment Questions

1. A) Alloantigen, B) Alloantigen, C) Autoantigen, D) Autoantigen.
2. i) B cell, ii) T cell.
3. Antibodies, Antigens, Adjuvant.
4. A) Hapten, B) Antigen, C) Hapten, D) Antigen.

Terminal Questions

1. The O negative blood donor does not have any antigenic group on their RBC therefore they do not evoke immune response in the recipient blood.
2. Every organ has organ specific antigens and so if antigens of donor and recipient do not match then these can evoke immune response. Therefore doctors match the antigens before transplantation.
3. Proteins have several antigenic determinant sites which are called epitopes. These are either linear group of amino acids or have 3D structure. The antibodies formed are specific to the epitopes.
4. Alloantigens are antigens of particular species which may not be present in all members of the species. Those members who do not have it can produce antibodies against it eg blood group antigens. Autoantigens are self antigens which are recognized by self and they do not evoke immune response.

UNIT 7

ANTIBODIES

Structure

7.1 INTRODUCTION

The immune system which protects our body possibly works by recognizing and responding to antigens which are present on the surface of harmful viruses or bacteria. Non- living substances like chemicals, drugs and toxins can also be categorized as antigens. Hence, the immune system recognizes and destroys or actually tries to destroy the substances which are antigens. Also, it is important to mention that our body cells also have proteins which include a group of antigens called HLA antigens and immune system usually does not react against them. When we are born, we already have a basic defense system and this innate immunity protects as against all antigens and acts as a barrier forming the first line of defense.

The most basic type of defense is actually provided by our body in the form of physical and chemical barriers. These barriers regulate the initial interactions between the host and the non self and so exist at the interface between the

individual and the environment. At the external interface, the skin, the tears and sweat are effective physical and chemical barriers to non self. The tears and sweat contain antibacterial substances; most popularly known as the enzyme lysozyme in tears and normal bacterial flora of the skin which help to protect against the external harmful organism. Where internal environment is concerned there is a range of defense ie: the acid pH of the stomach neutralizes the ingested bacteria, normal bacterial flora of the intestine inhibits the growth of pathogenic bacteria and microbial invasion into tissue, and cilia which line the gastro-intestinal, respiratory and genitor-urinary tract help to remove bacteria from the body system.

In this unit you will learn that there are several antibody variants present in our own and other members of the same species. You will understand how antibodies are classified based on their uniform presence, variation of the same within one's body and variation observed among different members of the same species. You will also acquire knowledge about the structure, function and formation of antibodies that have multiple specific interactions or single specific interaction with the antigen. Antibodies bind with high specificity to antigens used to challenge the immune system, but they may also show cross reactivity by binding to other antigens that share chemical properties with the original antigen.

Objectives

After studying this unit, you will be able to:

- visualize the concept of antibodies,
- explain the basic structure of antibodies,
- analyze the different features of antibodies,
- appreciate antigen-antibody binding,
- comprehend antigenic determinants,
- recognize the various functions of antibody,
- explain the immunological variants of antibodies present in mammalian body,
- distinguish between isotype, idiotype and allotype antibodies, and
- differentiate between polyclonal and monoclonal antibodies.

7.2 WHAT ARE ANTIBODIES

The pharmablast cells of lymphoid tissue produce large Y shaped molecules called antibodies. The circulating antibodies provide **humoral immunity** which does not last longer like cellular immunity and also does not act on small quantities of antigens. These proteins are involved in immunological reaction and are therefore called **immuno-globulins** ("Antibodies" and "Immunoglobulins" are used interchangeably). They are classified into five major categories based on their physicochemical and biological properties. You will study about them in detail in this Unit.

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.

BOX 7.1: 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. They were awarded the Nobel prize for this in 1972. In 1973 the actual structure was shown by atomic resolution. In 1975 the invention of monoclonal antibodies was done by George Kohler and Cesar Milstein and this opened up the field of further discovery in this field.

7.2.1 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).

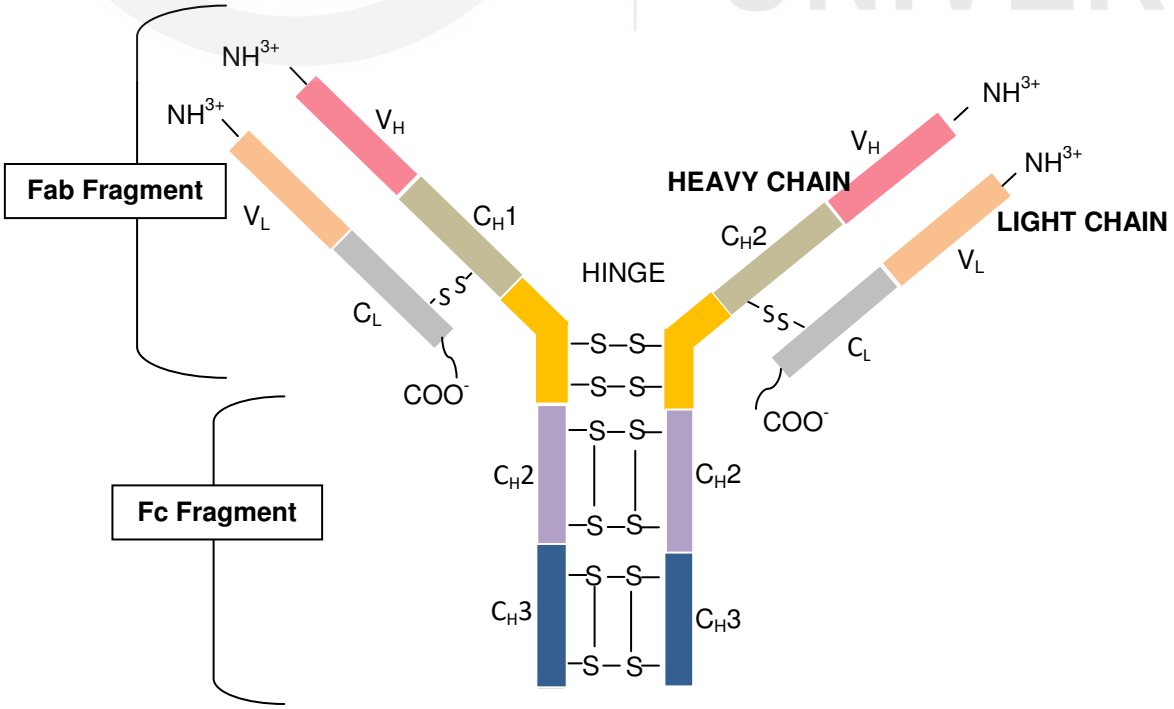


Fig. 7.1: Structure of Immunoglobulin.

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.

SAQ 1

Describe the structure of an antibody.

7.2.2 Types of Immunoglobulins

The pharmablast cells of lymphoid tissue produce large Y shaped molecules called **antibodies**. 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.

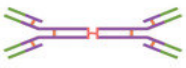
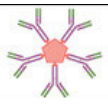
Immunoglobulin E (γ-Globulin E or IgE)

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.

Table 7.1: 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.	

7.3 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.

7.4 THE GENERATION OF DIVERSITY IN IMMUNOGLOBULINS

An antibody response can be generated against any substance which can act as a target. Also, the response even to a simple antigen wearing a single antigenic determinant is diverse.It comprises of many different antibody molecules, each with a different specificity for that antigen and with a unique affinity or binding strength. Total number of antibody specificity available in an individual is called as **antibody repertoire** or **immunoglobulin repertoire** and in humans is at least 10^{11}. The number of antibody specificities present at any one time is however, limited, by total number of B – cells which are present in an individual as well as by any individual that encounters with antigens.

There are two main hypotheses for origin of this diversity. The **Germline Theory** holds that there is a separate gene for each different immunoglobulin chain and that antibody repertoire is largely inherited. The **Somatic Diversification Theory** proposes that the observed repertoire is generated from a limited number of inherited V region sequences that undergo alteration within B-cells during the individuals lifetime. Also, the cloning of immunoglobulin genes revealed that elements of both theories were correct and that DNA sequence encoding each V region is generated by rearrangements of a relatively small group of inherited gene segments. The process of somatic hyper-mutation in mature activated B cells, further enhances the diversity of immunoglobulins.

7.5 ANTIBODIES BIND TO CONFORMATIONAL SHAPES ON ANTIGEN SURFACE

The biological function of antibodies is to bind to the pathogens and their products. This facilitates their removal from the body by recognizing only a small region in the large surface of the molecule, eg: a protein or a polysaccharide. The structure which is recognized by the antibody is called **epitope or intergenic determinant**. Many of the important pathogens have polysaccharide coats and antibodies that recognise this epitope, are essential in providing immune protection from such pathogens as they are formed by sugar subunits of these molecule. At the same time, the antigens that provoke an immune response are actually proteins eg: antibodies that work against viruses recognized viral coat protein .The structures which are recognized by the antibodies are located on the surface of the protein. These sites are composed of amino acids from different parts of the polypeptide chain and have been brought together by protein folding and are called **conformational or continuous epitope.** This is because structures recognized are made up of segments of protein that are discontinuous in amino acid sequences of the antigen but are brought together in 3D structure. Also an epitope composed of single segment of polypeptide chain is called **continuous or linear epitope**.

Most of the antibodies raised against intact fully folded proteins recognize discontinuous epitopes but some will bind to peptide fragments of the protein. Antibodies which are raised against peptides of a protein or against synthetic peptide corresponding to the part of its sequence are occasionally found to bind to the natural folded protein. This makes it possible to use synthetic peptides in vaccines that aim at raising antibodies against a pathogen protein. Antigens can bind in pockets or groves or on extended surfaces in the binding site of antibodies. Antigen binding to antibodies usually bind in the cleft between the V regions of the heavy and light chain where they make special specific contacts with some but not necessarily in all, hyper-variable loops is also the usual mode of binding for carbohydrate antigen and small molecules; such as hapten antigens can bind in pockets or groups on extended surfaces in the binding sites of antibodies.

7.6 INTERACTION FORCES INVOLVED IN ANTIGEN-ANTIBODY BINDING

The interaction between antibody and its antigen can be disrupted by high salt concentration; by extremes of pH; by detergents and sometimes by competition with high concentration of pure epitope itself**. Hence, it can be concluded that binding between antigen and antibody is therefore a reversible non covalent interaction.** Further, electrostatic interactions which occur between charged amino acid side chains as in salt bridges have been also studied. Interactions also occur between electric dipoles as in hydrogen bonds or can involve short-range van der Waals forces. Extremes of pH or

high salt concentrations actually disrupt the antigen antibody binding by weakening electrostatic interactions or hydrogen bond. This feature has been used in purification of antigens with the use of affinity columns of immobilized antibodies and vice versa. In some cases, water molecules are trapped in pockets, in the interface between antigen and antibody. The ones specially between polar amino acid residue can contribute to binding and hence to the specificity of the antibody. The strength of the hydrophobic interaction is proportional to the surface area that is hidden from water. Further, the contribution of each of these forces towards an interaction between antigen and antibody, totally depends on a particular antigen and antibody involved.

A striking difference has been observed in antibody interaction with protein antigens. Antibodies that possess many aromatic amino acids in their antigen binding site, participate in van der Waals and hydrophobic interaction and sometimes in hydrogen bonds. These forces operate over very short ranges and serve to put together two surfaces that are complementary in shape. In contrast, electrostatic interactions between charged side chain and hydrogen bonds binding oxygen or nitrogen atoms are committed specific features and contribute to strengthening the overall in interaction of antigen and antibody .Overall surface complementarities, has an important role in antigen antibody interaction in most antibodies that have been studied in detail and it has been found that only few residues make a major contribution to the binding energy and hence to the final specificity of the antibody. Genetic engineering can facilitate with site directed mutagenesis for the antigen and antibody binding to its complementary epitope.

7.7 AFFINITY, AVIDITY AND CROSS REACTIVITY

It has been noticed that all antibodies do not bind with the same strength, **specificity** and **stability**. In fact they exhibit different affinity depending on the molecular complementarily between the antigen and antibody. The binding affinity of a protein – protein interaction can be described as the strength of the interaction between a receptor and its respective ligand. An example of this is an antibody's epitope and antigen.

Avidity describes the measure of overall or accumulated strength of a protein, protein complex i.e. the total strength of all the non-covalent interaction between an antibody binding to its ligands. It is determined by binding efficiency of complex, valency of proteins and structural arrangement of proteins in the complex. Cross reactivity measures the extent to which different antigens appear similar to the immune system. The molecular determinants of specificity and cross reactivity define to nature of antigenic variation and the selective process that shape the distribution of variants (Fig. 7.2). Antibodies raised against pathogenic molecular components that resemble self molecules mark host cells for destruction and cause damages but later cross react with self antigens, this phenomenon is called **molecular mimicry**.

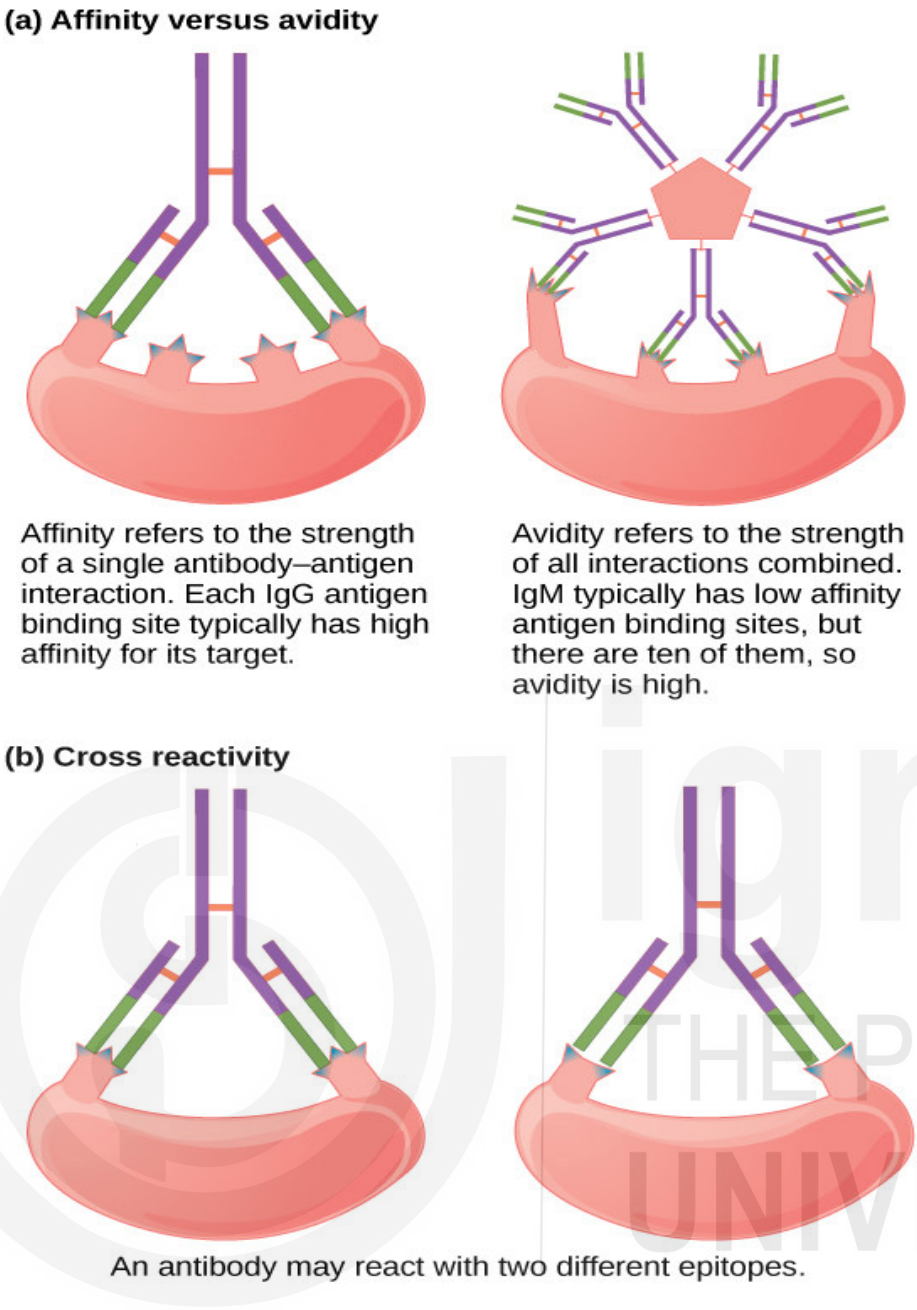


Fig. 7.2: Affinity, avidity and cross reactivity.

7.8 ISOTYPES, ALLOTYPES, IDIOTYPES

Depending on the structural variations the antibodies are classified as Isotypes, Allotypes and Idiotypes.

7.8.1 Isotypes

The name Isotypes originates from the Greek word **iso** which means equal or same. Thus Isotype antibodies refers to same group of antibodies present uniformly in different members of the same species. For example all the members of the mammalian species contain same group of antibodies. This group contains five different types of antibodies namely IgG, IgM, IgA, IgD, IgE. The protein type, α (alpha) forms IgA, δ (delta) forms IgD, ε (epsilon) forms IgE, γ(gamma) forms IgG, and μ (miu) forms IgM. This imparts them varied biochemical characteristics which help them to perform, different

functions. Antibodies are formed by plasma cells by switching from producing IgM to other types. During class switching there is no alteration in L chain or in the variable portion of the H chain which form the antigen binding site. But they show class switching by acquiring different H chain in the constant regions. They are found in all members of the species. For example all individuals of a species who are normal will exhibit all five types of antibodies in their serum. The different species do not have same type of constant region. Thus they may possess different isotypes. When these antibodies are taken from one species and are injected into the other species it will form antibody against these antibodies. They are used to measure the antibody levels and to check the immunodeficiency. They are also used to detect the B cell tumors (Fig. 7.3).

Different classes of isotypes present in the mammalian system are tabulated in Table 7.2.

Table 7.2: Properties of different isotypes.

Properties	**IgM**	**IgG**	**Secretory IgA**	**IgE**	**IgD**
Exists as	Pentamer	Monomer	Dimer	Monomer	Monomer
Nature of HC	μ (mu)	γ (gamma)	α (alpha)	ε (epsilon)	δ (delta)
Weight (Daltons)	900,000	150,000	385,000	200,000	180,000
% of total antibody in serum	6%	60%	13%	0.02%	1%
Diffuses across Placenta	No	Yes	No	No	No
Complement Fixation	yes	Yes	No	No	No
Function	Main antibody of primary responses. It serves as B cell receptor	Main blood antibody of secondary responses, helps to kill the pathogen	It is secreted into secretions like mucus, tears, saliva and colostrum	It is secreted in response to allergens or in presence of parasitic infection	It acts as B cell receptor

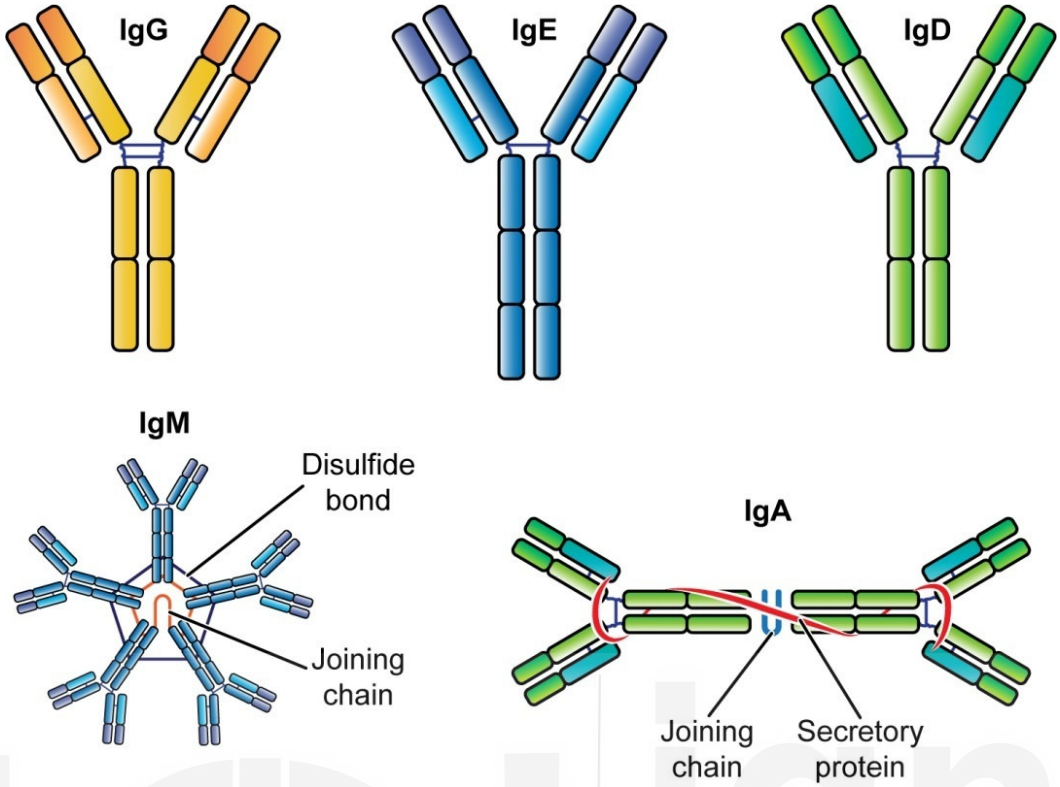


Fig. 7.3: Isotypes of antibodies, IgM, IgG, IgA, IgE and IgD, found in the mammalian blood.

Functions of different Isotypes

1. IgG can persist for many months and years following infection/ or injection , thus they give long term protection.
2. IgG helps in clearance of pathogens and toxins by initiating phagocytosis.
3. IgA is the antibody which is found in secretions like saliva, tears and mucous. Thus they can be transported out of the body easily. They also act as the first defense system in the mucosal tissues of the intestine, nose and lungs.
4. IgM acts as receptor on the B cell which enhances phagocytosis.
5. IgE is the predominant antibody which participates in allergic and antiparasitic actions.
6. IgD acts as B cell receptor which helps in initiating induction of antibody production.

7.8.2 Allotypes

The term Allotype originates from the Greek word **Allo** which means 'different'. It has been observed that each individual member of a species inherits different alleles of the genes.. When these are injected from one member to another member of the species it will produce antibodies against allotypic determinant. Thus the antibodies formed by these alleles vary in their amino acid sequence of the constant and variable regions of the proteins present.

For example IgG (IgG1) for individual X will be slightly different from individual Y (IgG1). So, if IgG1 of individual X1 is injected into individual Y then it will produce antibodies. The most important types are identified by the markers of three types. They are **Gm** (G1m4, G1m 17) which are variants of gamma 1 heavy chain), **Am** (Alpha chain variants) and **Km** (Km1 and Km2 variants of Kappa light chain). The allotypes are used for paternity testing and monitoring bone marrow graft. They are observed during blood transfusion and pregnancy (Fig. 7.4).

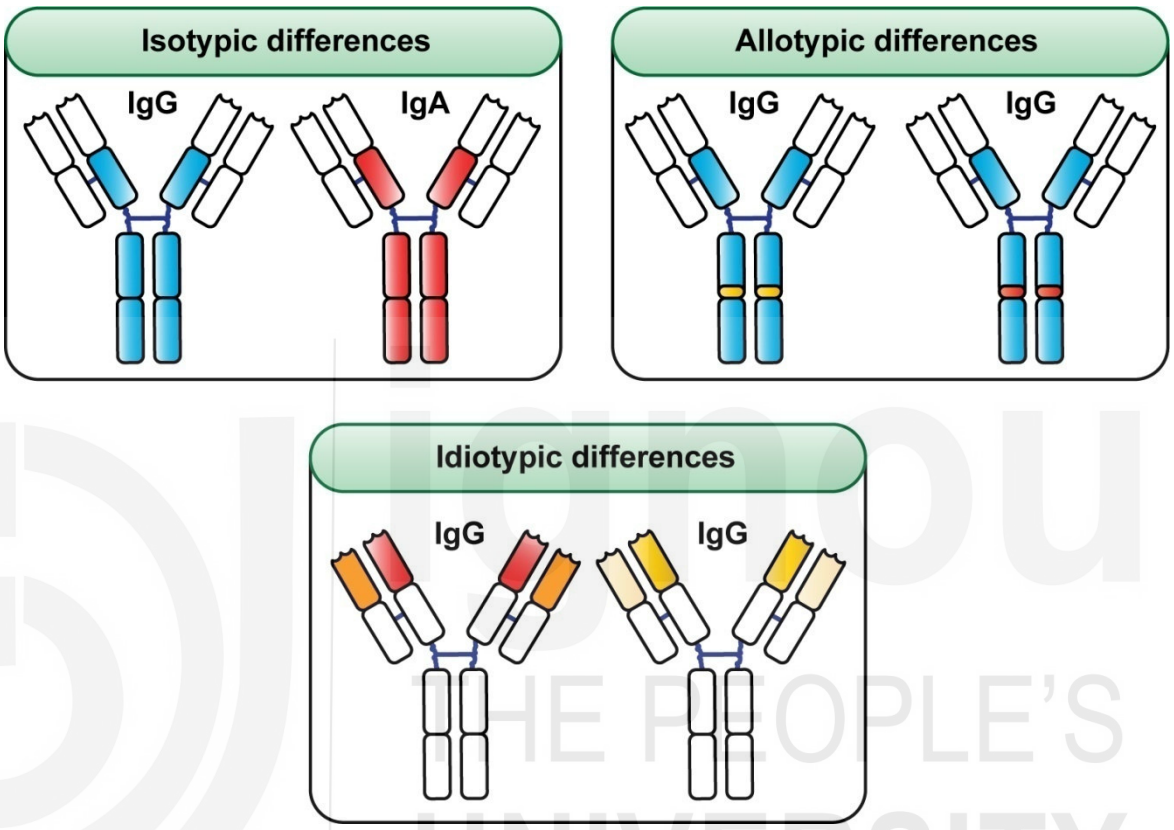


Fig. 7.4: The areas in dark show the difference in various chains of Isotype, allotype and Idiotype antibodies.

7.7.3 Idiotypes

The **term idio**- comes from Greek word idios **meaning** 'own, distinct', and it usually **means** personal, own, peculiar, or distinct depending on the context. It means distinct antibodies present in our body that recognize different antigens. For example, IgG1 may have entities which are specific to antigen 1, antigen 2, and antigen 3 and so on. **These antibodies of one type, present in the same body and are specific to different antigens are called the Idiotypes**. They have variability in the amino acid sequence in three to four CDRs surrounded by relatively invariant residues. These CDRs or Complementarity determinant regions are the areas which contact the epitope of the antigen and are called idiotypes. When we inject these idiotypic antibodies into genetically identical twin, idiotypic antibodies will be produced. In twins who are genetically identical, the isotype and allotype antibodies are same but idiotypes can be different. Idiotypes are used in the treatment of B cell tumors and as vaccines.

SAQ 2

Fill in the Blanks:

i) The isotypes have similar …… regions of …… and …… chains but different …… region of …… chains.

ii) The allotypes may have different amino acids in CDR regions of the …… chain or the …… chains.

iii) The idiotype provides …… antibodies to interact with different …… in one's own body.

iv) The fetus acquires immunity from the mother as …… can cross …… .

7.9 MONOCLONAL ANTIBODIES (mAbs)

Monoclonal antobodies are identical immunoglobulins generated from a single stimulated B cell clone. **These antibodies are specific to only one epitope of a single antigen**. They are generally produced artificially. *In vitro*, production of monoclonal antibodies is done by introducing an antigen of interest to a model organism, say a mouse. And the resulting B cells are then fused with myeloma cells. The product of fusion of the two different cells is called hybridoma. The hybridoma cells are then cultured which continue to produce antibodies against the specific antigen. Hybridoma cells have growth property of cancerous cell and antibody producing property of normal B cell. Monoclonal antibodies are used as therapeutic agents in chemotherapy and other immune diseases (Fig. 7.5). The advantage of producing monoclonal antibodies is its specificity and affinity. mAbs are currently used to treat cancer, but their exorbitant cost has prevented them from being used more widely to treat infectious diseases, still their potential for laboratory and clinical use is deriving the development of new cost-effective solutions like plantibodies.

Kohler and Milstein were awarded Noble Prize in Physiology and Medicine in 1975 for their discovery of the technique for generating **monoclonal antibodies** of the desired specificity

A plantibody is an antibody that is produced by plants that have been genetically engineered with animal DNA encoding a specific human antibody known to neutralize a particular pathogen.

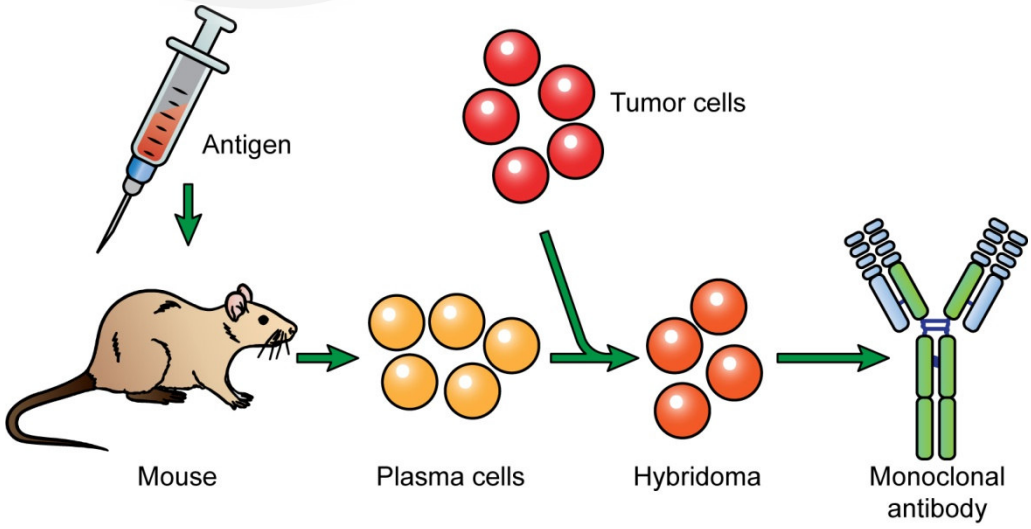


Fig. 7.5: The mouse is injected with antigens as is done for production of polyclonal antibody. The animal is sacrificed and the plasma cells of such animal are extracted. These are fused with the myeloma cells which form the hybridoma. These hybridoma cells in culture produce the monoclonal antibodies.

7.10 POLYCLONAL ANTIBODIES (pAbs)

Polyclonal antibodies are antibodies which are specific to more than one epitope of an antigen. They are secreted by B cells in response to specific antigens. Therefore, they can be defined as **collection of antibodies that bind to different epitopes of the specific antigen**. These antibodies are produced when a person is infected by a pathogen or when antigen is injected by subcutaneous, intradermal, intramuscular or the peritoneal mode. Different antibodies are produced that react with various epitopes of antigen. They are used as vaccines for prevention of several diseases. The injections are repeated after an interval to boost the reaction. Polyclonal antibodies are useful for techniques like agglutination or immunoprecipitation as these are based on macromolecular antigen-antibody complex formation. The serum (the fluid after removing cellular components and clotting factors from blood) isolated from such blood contains the polyclonal antibodies. Polyclonal antisera are useful for some types of laboratory assays, but other assays require more specificity. Diagenistic tests that are polyclonal antisera are typically only used for screening because of the possibility of false-positive and false-negative results. The monoclonal and polyclonal antibodies can be differentiated experimentally by Ouchterlony double diffusion and affinity chromatography (Fig. 7.6 and Fig. 7.7). You will study about these techniques in detail in next unit.

Table 7.3 shows differences between polyclonal and monoclonal antibodies.

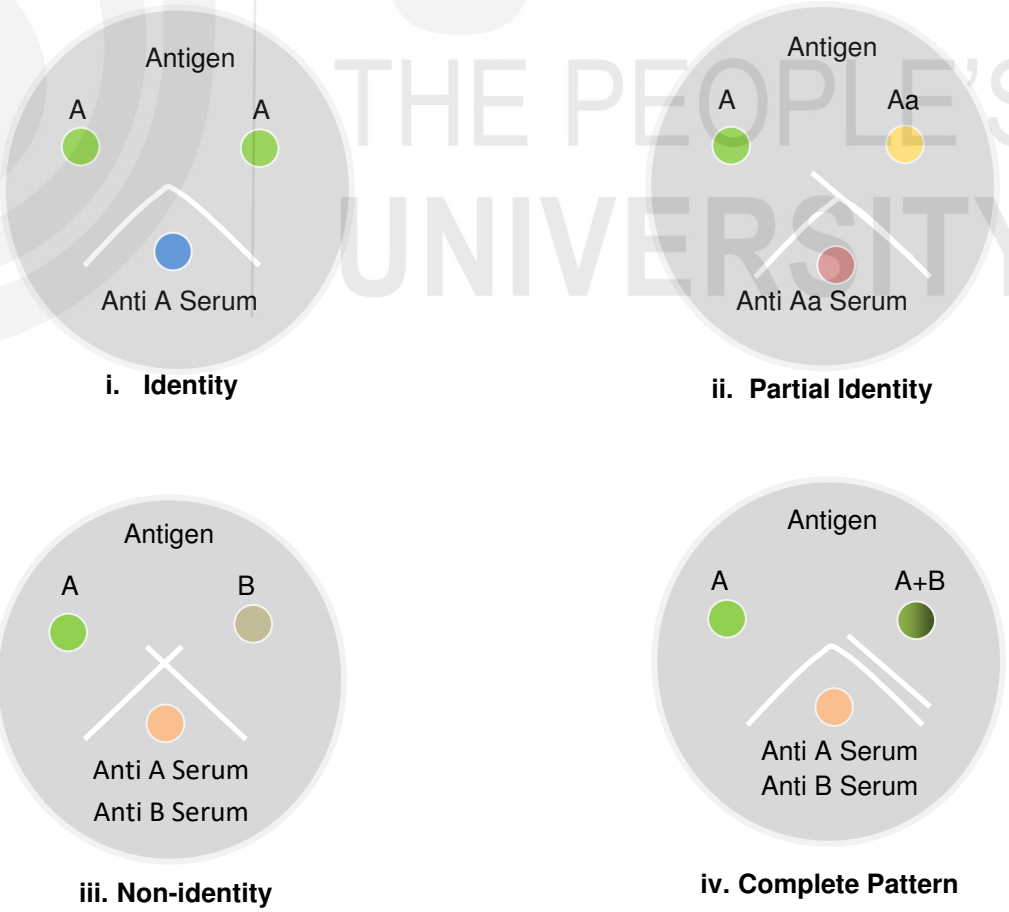


Fig. 7.6: The technique Ouchterlony double diffusion shows the difference in specificity to the different antigens. They can show: Complete pattern identity, Partial identity, Non identity.

Table 7.3: Differences between Polyclonal and Monoclonal antibodies.

Polyclonal Antibodies	Monoclonal Antibodies
They are heterogenous population of antibodies that are secreted against specific antigen.	They are homogeneous population of antibodies produced by a single clone of B cells.
They are produced by different clones of plasma B cells.	They are produced by one single clone of plasma B cells.
There is no requirement for the production of hybridoma cell lines.	Production of monoclonal antibodies requires hybridoma cell lines.
They interact with different epitopes on the same antigen.	They interact with a specific epitope on the antigen.

Highlights of Monoclonal and Polyclonal Antibodies

Polyclonal antibodies can show cross reactivity with molecules which are similar to original antigen. Thus these polyclonal antibodies can show partial, complete, or no reactivity with the epitopes of antigens. The monoclonal antibodies show specific reactivity to only one epitope of the antigen and these antibodies have specific reactivity with high affinity. Polyclonal antibodies are naturally produced by antigens or produced by injecting it in the animal. The monoclonal antibodies are produced by culture of spleen cells (hybridomas) of polyclonal antibody producing animal. Their production is limited and costly but they have been widely used in screening and therapies of some of the serious diseases like cancer.

SAQ 3

i) What are the differences in functions of members of the isotype antibodies?

ii) How many CDRs are present in monoclonal and polyclonal antibodies respectively?

iii) If we inject isotype antibodies of member1 to member 2 what will be the reaction?

iv) If we inject an antigen into the body of mouse then what kind of antibodies will be produced?

7.11 FUNCTIONS OF ANTIBODIES

Humoral response differentiated plasma cells play a crucial role. The antibodies they secrete are particularly significant against extracellular toxins

and pathogens. Once they are secreted into the bloodstream, these antibodies can circulate freely and can act independently of plasma cells. It has also been noticed that these antibodies can be transferred from one individual to another. It has also been noticed that an individual who has immune response against the disease, can donate to another non individual and hence confer temporary immunity through antibodies present in his/her blood stream. This phenomenon is called **passive immunity** which helps in combating the disease. Antibodies are known to coat extracellular pathogens and neutralized key sites on the pathogen in the host cells. Antibody **neutralization** can prevent pathogens from entering host cells. The neutralized antibody coated pathogen can then be filtered out by the screen and eliminated in urine or feces.

Another important role of antibodies is that they also mark pathogens for destruction by phagocytic cells such as macrophages or neutrophils. They are highly attracted to macromolecules complexed with antibodies. This process is called **opsonization**. In **complement fixation** IgM and IgG in the serum bind to antigen providing docking sites on to which sequential complement proteins can bind (Fig. 7.7). The combination of antibodies and complement and hence oponization even further promote rapid clearing of the pathogens.

The antibody has a range of functions and uses, both in biological defense and as a clinical tool.

1. Host Defence

i) Defend against microorganisms (bacteria, viruses).

ii) The first antibody, which appears, is IgM and followed by IgG.

iii) In fetal life, IgM appears at 6 months of intrauterine life.

iv) Mother (milk) is a rich source of IgA and IgG.

v) Recruits another component like complement which destroys the antigen.

vi) Causes neutralization of toxins.

vii) Leads to the removal of foreign antigen from the circulation.

2. Clinical Medicines

i) Used in the diagnosis of diseases and monitoring of the progress of diseases.

ii) It can be used as passive therapy by giving pooled antibodies.

3. Diagnosis and Research

These can be used for diagnostic and research purposes.

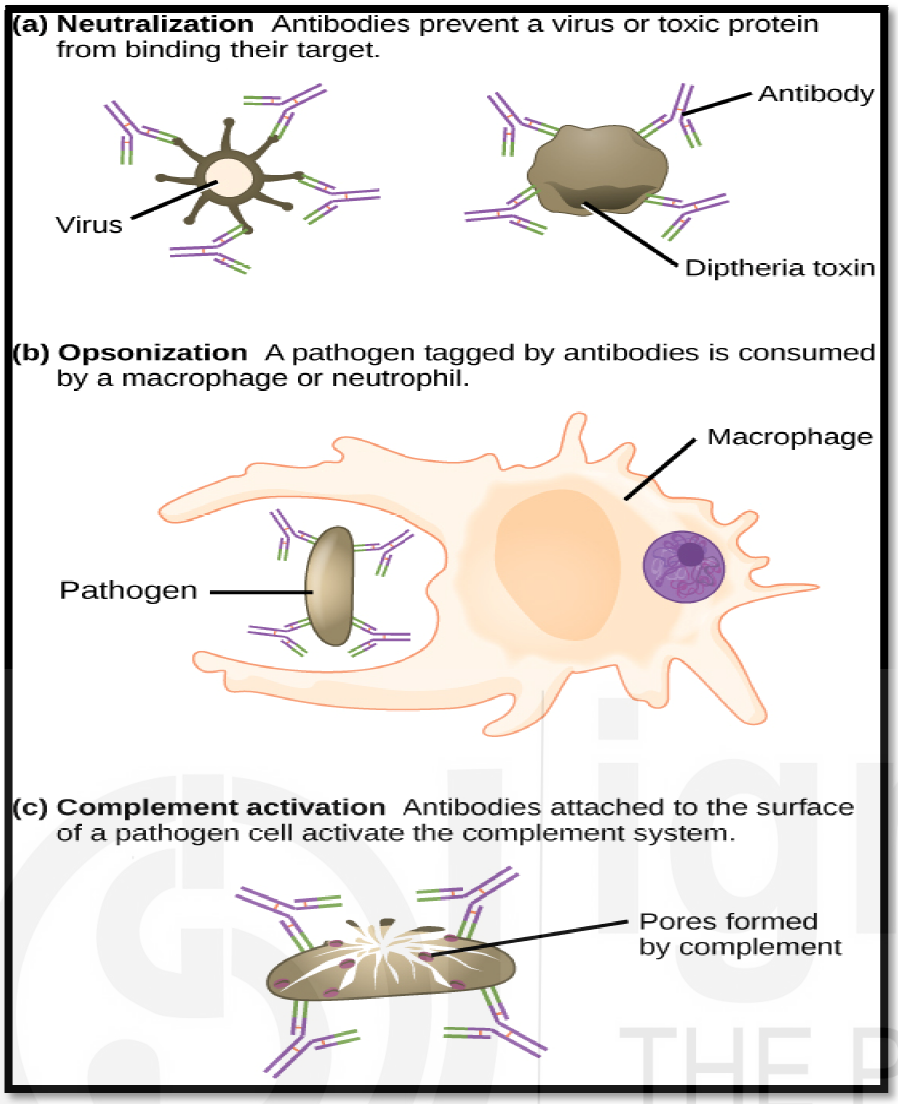


Fig. 7.7: Neutralization, Opsonization, Complement activation: Important functions of antibodies.

7.12 SUMMARY

Let us summarise what you have learnt in this unit:

- Antibodies or Immunoglobulins have a basic four peptide structure of two identical heavy and two identical light chains joined by interchain disulfide links.Papain splits the molecule at the exposed flexible hinge region to generate two identical univalent antigen binding fragments-Fab and a fragment Fc. Pepsin proteolysis gives a divalent antigen binding fragment $F(ab)_2$ lacking the Fc segment.
- There are 10^8 or more different IgG molecules in normal serum . Analysis of myeloma protein which are homogenous Ig produced by single clones of malignant plasma cells, has shown the N-terminal region of heavy and light chains to have variable amino acid structure and the remainder to be relatively constant in structure.
- Isotypes of Ig variants are based on different heavy chain constant structures, all of which are present in each individual. Examples are the Ig classes IgG,IgA etc. Allotypes are heavy chain variants encoded by

alternative genes or allelic genes, at single loci and are therefore genetically disturbed, example GM groups. An idiotype is the collection of antigenic determinants on an antibody, usually associated with the hypervariable regions, recognized by other antigen specific receptors, either antibody or T cell receptors.

- The Isotype antibodies can show variety within an individual as they are developed specific to different antigens and they form the Idiotype (Ab1, Ab2, Ab3) of one individual.
- Such idiotypic antibodies can be specific to several epitopes of antigens which are also called polyclonal antibodies, or be specific to only one epitope of the antigen which are called the monoclonal antibody.
- The polyclonal antibodies are formed in response to invading pathogens or intoxicants whereas monoclonal antibodies are engineered in the laboratory.
- The variable region domains bind antigen and three hypervariable loops on the heavy chain and three on the light change from the antigen binding site. The constant region domain of the heavy chain carries out a secondary biological function after the binding of antigens i.e., complement fixation and macrophage binding.
- 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 e.g., sites on bacterial cell wall polysaccharides .This flexibility also helps the antibodies to interact with antibody binding proteins that mediate immune effector mechanism.
- Antigens can bind in pockets or groves or on extended surfaces in the binding site of antibodies. Antigen binding to antibodies usually takes place in the cleft between the V regions of the heavy and light chain where they make special specific contacts. Binding between antigen and antibody is a reversible non covalent interaction.
- Overall surface complementarities, has an important role in antigen antibody interaction in most antibodies that have been studied in detail and it has been found that only few residues make a major contribution to the binding energy and hence to the final specificity of the antibody.
- Genetic engineering can felicitate with site directed mutagenesis for the antigen and antibody binding to its complementary epitope. The antibodies are particularly significant against extracellular toxins and pathogens. Once they are secreted into the bloodstream these antibodies can circulate freely and can act independently of plasma cells.
- Antibodies are known to coat extracellular pathogens and neutralized key sites on the pathogen in the host cells. Antibody neutralization can prevent pathogens from entering host cells. The neutralized antibody coated pathogen can then be filtered out by the screen and eliminated in urine or feces.

7.13 TERMINAL QUESTIONS

1. Define antibodies. What is the significance of antibodies?
2. What are the different classes of antibodies? Enumerate their significance.
3. Is antibody molecule flexible? How is it able to demonstrate diversity?
4. How does an antibody bind with antigen? What forces interplay in this binding mechanism?
5. Explain the terms: affinity, avidity and cross reactivity.
6. What are the prime functions of antibodies in human beings?

7.14 ANSWERS

Self Assessment Questions

1. Antibodies or Immunoglobulins have a basic four peptide structure of two identical heavy and two identical light chains joined by interchain disulfide links. Papain splits the molecule at the exposed flexible hinge region to generate two identical univalent antigen binding fragments- Fab and a fragment Fc. Pepsin proteolysis gives a divalent antigen binding fragment $F(ab)_2$ lacking the Fc segment.
2. i) Variable , light, heavy, constant, heavy
   ii) Light, heavy
   iii) Specific , antibodies
   iv) IgG , placenta
3. i) IgM: Main Antibody of primary response, initiates immune reaction, helps to fix complement

   IgG: Main antibody of secondary response, helps in removing the pathogen

   IgA: main antibody which is secreted in mucus, tears, saliva and colostrum

   IgE: Antibody participating in allergy and antiparasitic activity

   IgD: Acts as B cell receptor which induces antibody production.

   ii) Monoclonal has one and Polyclonal has many CDRs.

   iii) If we inject isotype antibodies from member 1 to member 2 it will produce antibodies to antibodies as they are allotypes i.e., antiantibody.

   iv) If we inject an antigen into the body of a mouse then it will cause production of polyclonal antibodies. Spleen of such an animal is used to produce monoclonal antibodies in the laboratory.

Terminal Questions

1. Refer to Section 7.2.
2. Refer to Section 7.2.2.
3. Refer to Section 7.4.
4. Refer to Section 7.5 and 7.6.
5. Refer to Section 7.7.
6. Refer to Section 7.11.

UNIT 8

ANTIGEN ANTIBODY INTERACTIONS

Structure

8.1 INTRODUCTION

Antigens are foreign invaders (viruses, bacteria, fungi, parasites), that induce an immune response in the body. Antibodies are specialized Y-shaped proteins having specific binding sites (paratopes) that interact with the epitopesor antigenic determinants (Fig. 8.1). Antigen-Antibody (Ag-Ab) reactions are highly specific interactions between antigens and antibodies. These are weak non-covalent interactions involving electrostatic bonds, H-bonds, hydrophobic interactions, and Van der Waal bonds.

Like enzyme-substrate reactions, Ag-Ab reactions take place at optimum conditions (temperatures, pH, ionic strength etc.) that vary depending on the epitopes, paratopes and the type of bonds involved. Optimum pH is in the range of 6.5-8.4, outside which the Ag-Ab reactions would be strongly

inhibited. In fact, antibodies being immunoglobulins undergo conformational changes at extreme temperatures and pH values. Although non-covalent and reversible, the interactionsare strong enough to form an Ag-Ab complex. This forms the basis for some very significant immunologic assays designed for the detection of both antigens and/or antibodies. They can therefore, be used in the diagnosis of diseases, identification of some clinically relevant biomolecules and estimation of the strength of an immune response.

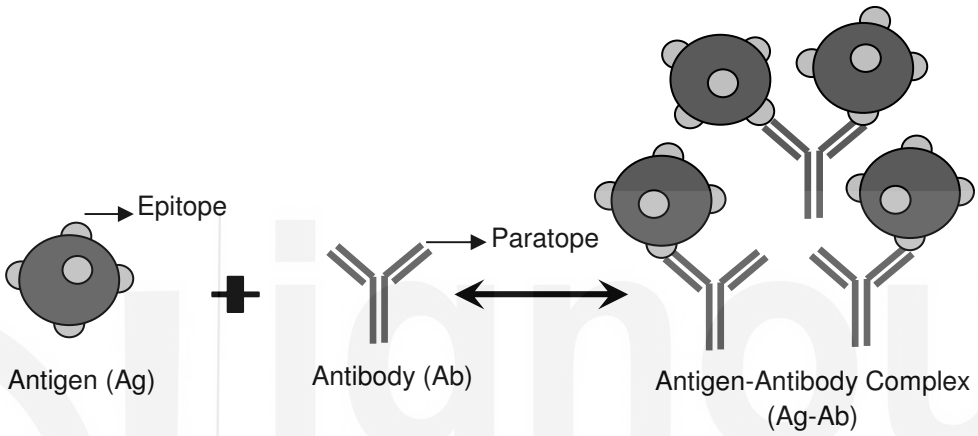


Fig. 8.1: Antigen Antibody Reactions.

Objectives

After studying this unit you will be able to:

- describe structure and function of antigen and antibody,
- explain the various mechanisms of antigen-antibody interaction, and
- discuss the principles and applications of important immunological tools and techniques.

8.2 AFFINITY AND AVIDITY

Ag–Ab binding is a very specific phenomenon wherein both entities should have complementary binding sites that fit very closely. The binding strength between the two is described by the terms ‘Affinity’ and ‘Avidity’. Antibodies having low affinity for antigens will bind weakly and hence dissociate easily while those that bind tightly will remain bound for a longer period. Since antibodies are bivalent or multivalent having at least two Ag-binding sites, the overall strength of the interaction will consider all the binding affinities of the Ag-sites. **This accumulated strength including multiple affinities is called Avidity. While, the strength of interaction between a single antigen binding site on an antibody with a single antigenic determinant is called Affinity** (Fig. 8.2).

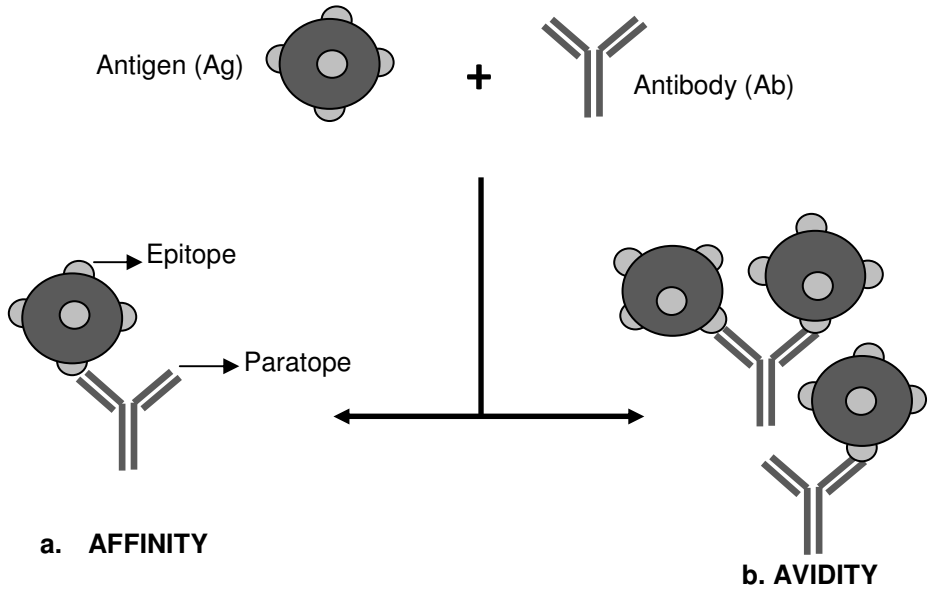


Fig. 8.2: Antigen–Antibody interaction showing Affinity and Avidity.

This interaction between a monovalent antigen and a single Ag-binding site on the antibody can be expressed as follows:

$$Ag + Ab \rightleftharpoons Ag\text{-}Ab$$

The ratio of the forward and backward rate constants of the above reversible reaction gives a measure of the affinity and is the Association constant or Equilibrium constant (Ka).

$$Ka = [Ag\text{-}Ab]/[Ab][Ag]$$

where, [Ag-Ab] is the molar concentration of Ag-Ab complex (bound form)

[Ag] is the concentration of unbound antigen

[Ab] is the concentration of unbound antibody

The value of association constant Ka depends on both the forward and backward reactions, and also varies for different Ag-Ab complexes. It can be estimated by ***Equilibrium Dialysis*** and other similar methods.

Surface plasmon resonance (SPR) is the resonant oscillation of conduction electrons that are in resonance with the oscillating electric field of incident light, which will produce energetic plasmonic electrons through excitation.

Equilibrium Dialysis

Ag-Ab binding affinity can be conveniently determined by equilibrium dialysis (Fig. 8.3). This method is economical and illustrates comprehensively the Ag-Ab interactions but it has now been replaced by other more advanced techniques like surface plasmon resonance.

Two compartments of a chamber are separated by a semipermeable membrane which allows a particular ligand to pass through. In case I, suppose there is no antibody in compartment (A) while compartment (B) contains a radioactively labelled ligand.

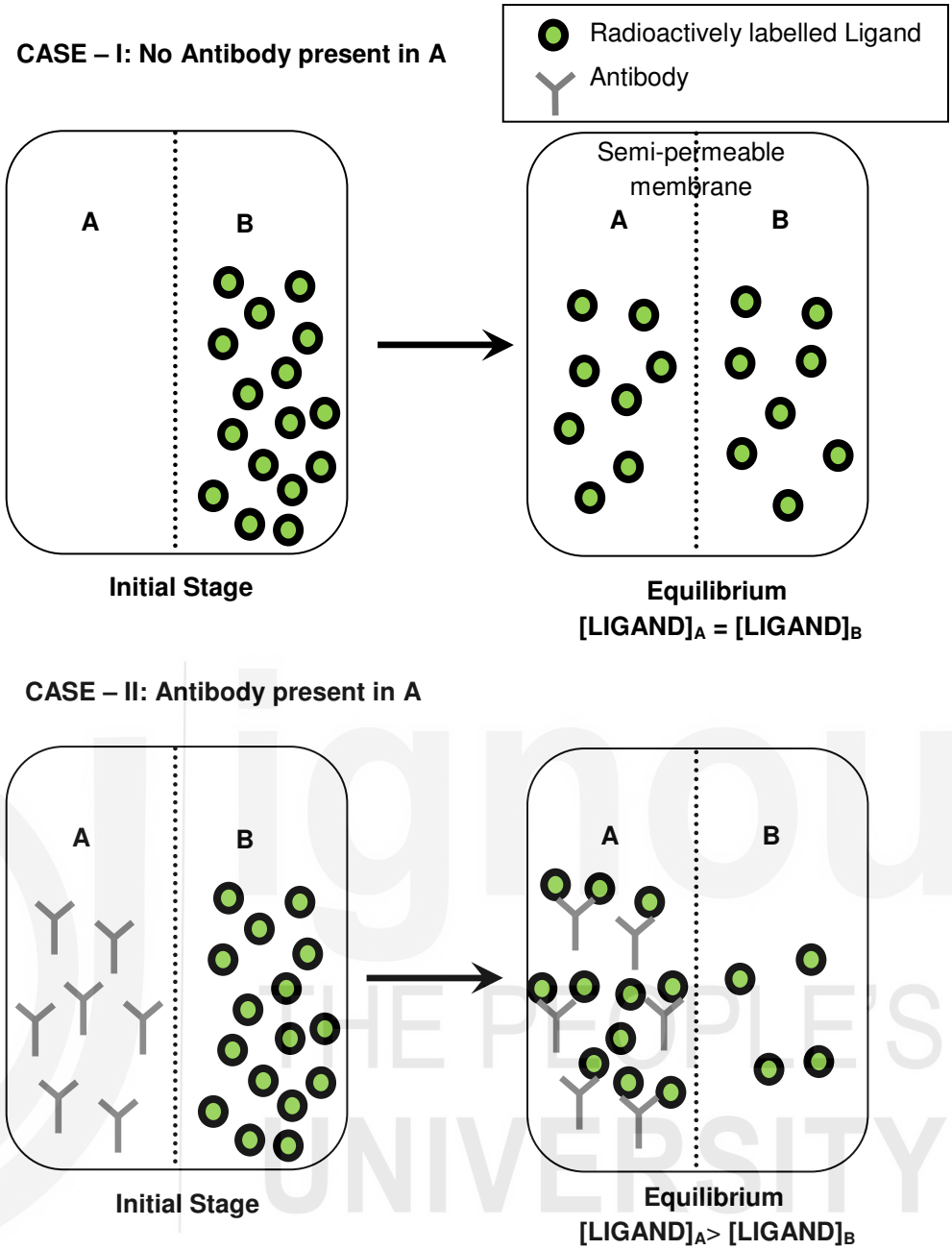


Fig. 8.3: Equilibrium Dialysis using radioactively labelled antigen molecules.

Then after a certain time period, the ligand will diffuse and equilibrate on either side of the membrane. In the second case, when an antibody is introduced into compartment (A), the labelled ligand will diffuse across the membrane to bind with the antibodies (Abs). Hence, at equilibrium, some amount of ligand will be bound with the Abs forcing the unbound ligand to equally get distributed in compartment A and B. As a result, the total ligand concentration will be much higher in the compartment containing the Ab. Greater the concentration of bound ligand, higher would be the affinity of the Ab.

$$[\text{Ag-Ab complex}] = [\text{bound ligand}] = [\text{ligand}]_A - [\text{ligand}]_B$$

Where

[Ag-Ab complex] = Concentration of Ag-Ab complex

[bound ligand]= Concentration of ligand bound to Ab

$[\text{ligand}]_A - [\text{ligand}]_B$= Difference in the ligand concentration in the two compartments

The concentration of antibody introduced in compartment A is a known value, the concentration of bound antigen and free antigen can be estimated from the amount of radioactivity in compartment A (with and without Abs), the equilibrium constant and affinity can be calculated.

8.3 MECHANISMS OF ANTIGEN ANTIBODY INTERACTIONS

You will study about various mechanisms of antigen antibody Interactions in this section.

8.3.1 Immunoprecipitation

i) Precipitation reaction in solution

This phenomenon is based on the ability of antibodies to bind to more than one epitopes on a single antigen. Ag-Ab complex so formed is large and bulky - precipitate, separating out of the solution. The precipitate can be centrifuged and both the antigen and antibody can be separated with the help of biochemical techniques. This method can be applied in the purification of specific antigens from a mixture of heterogeneous soluble molecules. **It is to be noted that precipitation phenomenon can take place only when the concentration of both the antigen and antibodies are equal.** If either of the two (antigen or antibody) are in excess, then monovalent binding will preferably take place not resulting in precipitation of the complex.

ii) Precipitation reaction in solid gels

Immunoprecipitation in solid agar media also occurs only when both antigens and antibodies are present in equal concentrations (as in solutions). As the reaction proceeds, the Ag-Ab complex can be seen as visible lines or zones of precipitation (precipitin lines/zones)(Fig 8.4).There are several variations of this technique, the most frequently used method is the **Radial Immunodiffusion** and the **Ouchterlony** method of immunoprecipitation which are discussed in the next section.

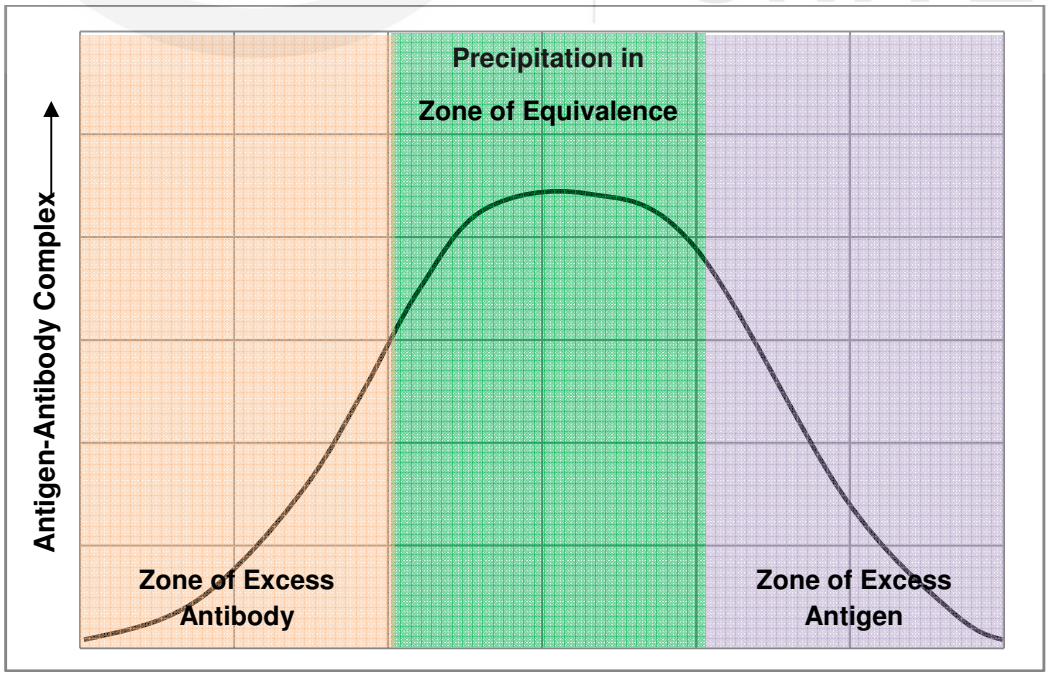


Fig. 8.4: Precipitation of Ag-Ab complex.

Co-immunoprecipitation

Co-immunoprecipitaion is a modification of immunoprecipitation technique which is frequently used for isolating protein antigens from cell and tissue samples using antibodies specific to the particular protein. The procedure involves the following steps (Fig 8.5):-

1. A mixture of lysed cell extract and antibodies raised against the protein of interest is used to form an Ag-Ab complex.
2. A secondary antibody is added pre-attached to a synthetic bead.
3. The secondary antibody binds specifically to the tail region (Fc) of the primary antibody that increases the bulk of the precipitate.
4. The Ab-Ag-Bead complexes are collected as a pellet by centrifugation.
5. The protein of interest is then separated from the precipitating antibodies by SDS-gel electrophoresis.
6. Secondary antibodies can also be attached to magnetic beads. This aids in separation by passing the solution over a magnetic column, thereby purifying the protein of interest.

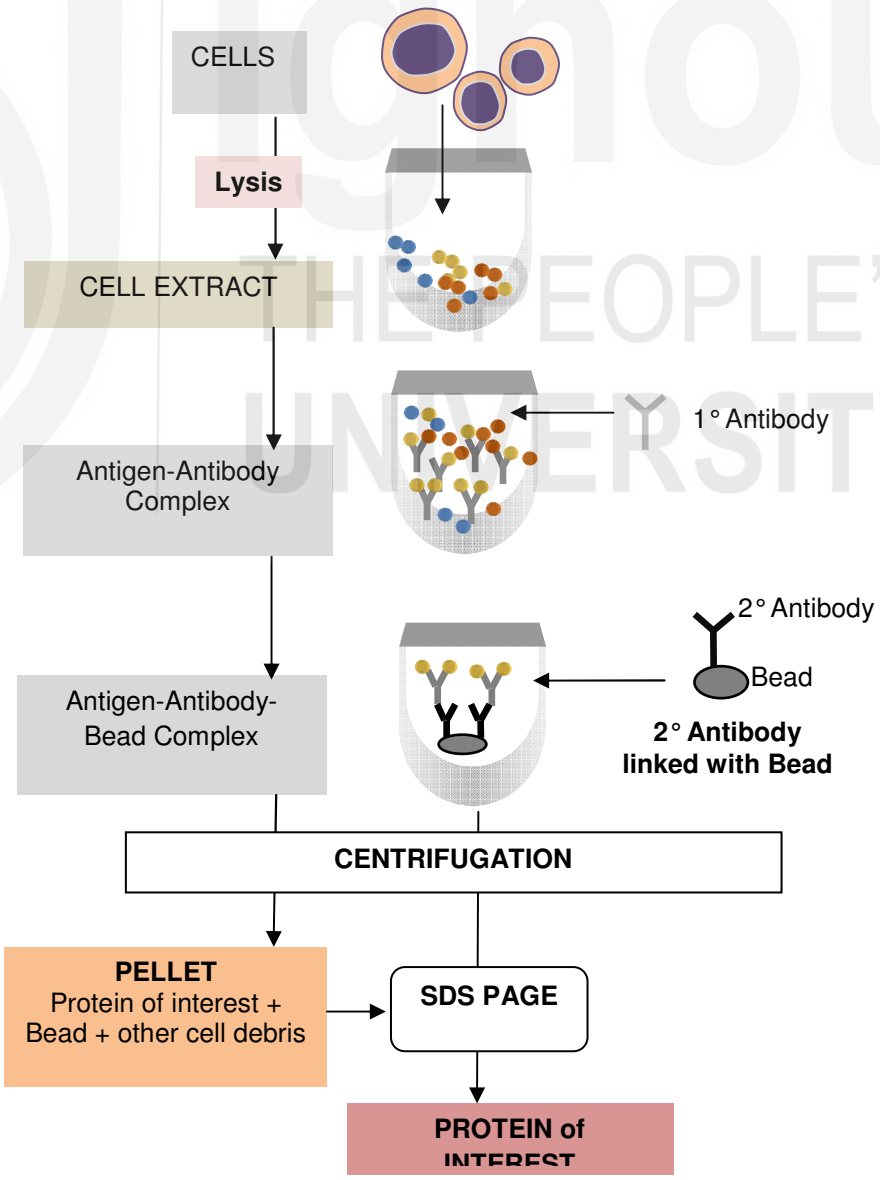


Fig. 8.5: Steps involved in Co-immunoprecipitation.

7. The amount of bound protein and other associated protein that may have co-precipitated along with the target protein can also be determined in test samples. The quantity of immunoprecipitate can be estimated using western blotting.

8.3.2 Agglutination Reactions

Antibodies are generally divalent or multivalent. As a result single antibody can bind to at least two epitopes or antigenic determinants. Besides, bacteria and other pathogens generally have several epitopes on their surface. Interaction between several antigenic determinants and antibody paratopes that lead to cross-linking and visible clumping of Ag-Ab complex formed are called 'agglutination reactions'. The antibodies that produce such reactions are called 'agglutinins'. The basic principle behind these reactions are similar to precipitation reactions except that the antigens here are not soluble components but conjugated to large sized particulate matter like bacterial cells, RBCs, charcoal or latex. As a result, the visibility of the cross-linked product is greater. It is important to note that the epitopes present on the antigenic surfaces should be exposed so that they are available for antibodies to attach (Fig. 8.6).

The conjugated antigens are mixed with serum samples containing antibodies of interest. At the end of the reaction an Ag-Ab complex is observed in the form of visible clumps, the quality and quantity of which will depend upon the incubation period, the affinity and the quantity of conjugated antigen and reaction conditions like pH, temperature etc. Agglutination reactions have immense diagnostic applications. One very useful application of this phenomenon is '**Hemagglutination**' where antibodies are made to bind the antigen molecules present on the surface of red blood cells (RBCs). Other applications include latex agglutination, flocculation and bacterial agglutination. In general, serial dilutions of the serum (antibodies) are made to which the particulate antigen carrier is added at a fixed concentration and incubated at 37°C until visual clumping is observed. This may take several hours.

Bacterial agglutination reactions can detect antibodies generated during a bacterial infection. The antibodies are specific for the antigenic determinants present on the bacterial cell surfaces. The end product is a visible clump formed due to the antibacterial antibodies cross-linking with bacterial cells. Quantitative analysis of the reaction product and the agglutinin titre (patient serum) can be used in the diagnosis of bacterial infections. When serial dilution of the patient sera gives visible agglutination in a well, then that particular well gives the agglutinin titre of the patient.

Agglutination assays can be performed and analysed easily. They are extremely sensitive and sometimes prove to be the most sensitive immune assays available at present. Their applications are varied and include clinical diagnosis of both infectious and non-infectious diseases such as, serotyping of *Vibrio cholerae* (cholera), *Salmonella typhi* (typhoid), RPR and FTA-ABS test for syphilis, and Antistreptolysin O titre test for *Streptococcus* infection. They can easily detect both antigens and antibodies in serum and other body fluids.

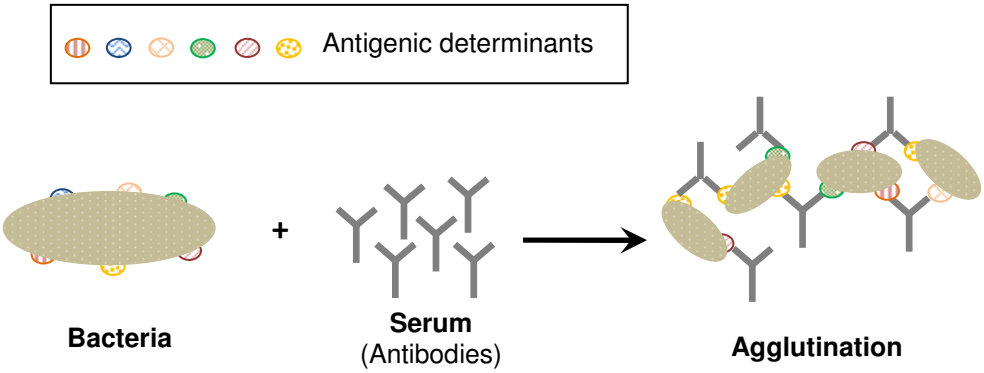


Fig. 8.6: Agglutination Reactions.

8.3.3 Neutralization Reactions

Activated B cells generate antibodies that provide protection against pathogens in many ways:

(a) **Neutralization:** where antibodies bind to the pathogen and block receptors used to gain entry into the host cell.

(b) **Opsonization:** where they make the pathogen more susceptible to phagocytosis.

(c) **Complement fixation:** where they bind to pathogens and the resulting complex initiates a complement cascade causing cell lysis.

(d) **Antibody-dependent cell-mediated cytotoxicity (ADCC):** where they assist immune cells in recognizing and killing, antibody coated target pathogens, through cytotoxic T cells and natural killer cells. This phenomenon has immense therapeutic potential.

The antibodies initiate either of these responses depending on the antigen specificity and the immunoglobulin isotype involved. These mechanisms eliminate the pathogen by inducing phagocytosis causing destruction of the pathogen-antibody complexes and disintegrating the pathogen cell membranes.

Neutralization is a very effective means of protection against infection. These are Ag-Ab reactions that reduce the damaging effects of pathogens with the help of specific antibodies (also called antitoxin) that prevent pathogens from binding to target receptors (Fig. 8.7). Majority of the viruses and several bacteria bind to one or more cell-surface proteins to get entry into the host cells via the process of endocytosis. For instance, hemagglutinin of the influenza virus binds to surface proteins of epithelial cells. Antibodies that can bind to such proteins will block the entry of the pathogen and hence prevent the initiation of an infection. Such antibodies, called ‘neutralizing antibodies’, when bound to the pathogen make them vulnerable to phagocytosis by macrophages and also block the entry of toxins into cells. Neutralizing

antibodies have been used for the treatment of snake bites as they can neutralize venom toxins. The tetanus vaccine, which is an inactive form of tetanus toxoid that stimulates production of anti-tetanus antibodies binds to receptors on neurons preventing the entry of toxin.

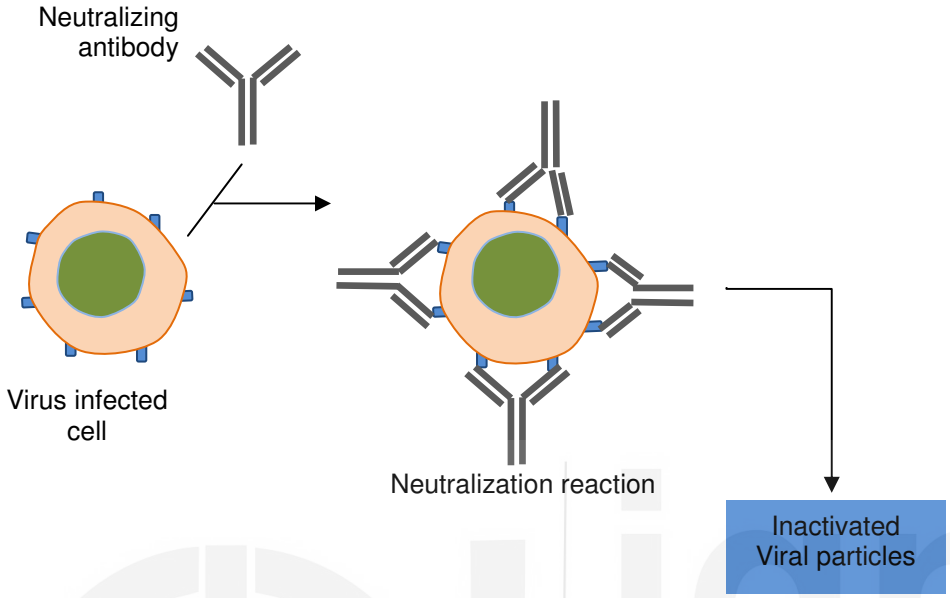


Fig. 8.7: Neutralization of viral particles by serum antibodies.

8.3.4 Complement Fixation

Antigen and antibody reaction product (Ag-Ab complexes) may trigger a '**complement cascade'. The compliment cascade is a sequence of events that involves complement components (specific serum proteins) as part of the innate immune system**. Specific antibodies bind on the surface of pathogens, which then combine with the complement. After which, cascade of reactions are initiated resulting in the generation of a complex that attacks the pathogen membranes making them porous. As the cell constituents leak out, the pathogen gets killed (Fig. 8.8). This ability of specific antibodies (e.g. IgGs and IgM) to initiate membrane damage in association with the complement is called **'complement fixation'**. This phenomenon can occur only when antibodies are bound to antigens.

8.3.5 Opsonization

Opsonization is a process that prepares a pathogen for destruction with the help of specific proteins called 'opsonins'. These proteins form coating on pathogens that are recognized by phagocytes (macrophages and neutrophils). Opsonization can be:

(a) **Antibody-independent:** where the initial components of the complement cascade are responsible, mainly C3b protein. Phagocytes have C3b receptors and readily reach out for pathogens that are coated with C3b proteins.

(b) **Antibody-dependent or ADCC:** where antibodies bind to the antigenic determinants on pathogens. The Fc component of the antibody is free to bind Fc receptors present on the phagocytes. Pathogens coated with complement or antibody bind to phagocytic cells resulting in complement-receptor mediated or Fc-receptor mediated phagocytosis of pathogens, respectively. The protein coating around the pathogens (especially viruses) annihilates the virulence of the pathogen and prevents its binding to host cell receptors. Complement receptors present on the surface of RBCs also bind immune complexes, which are then transported to the liver for phagocytosis by macrophages where they undergo intracellular digestion (Fig. 8.9).

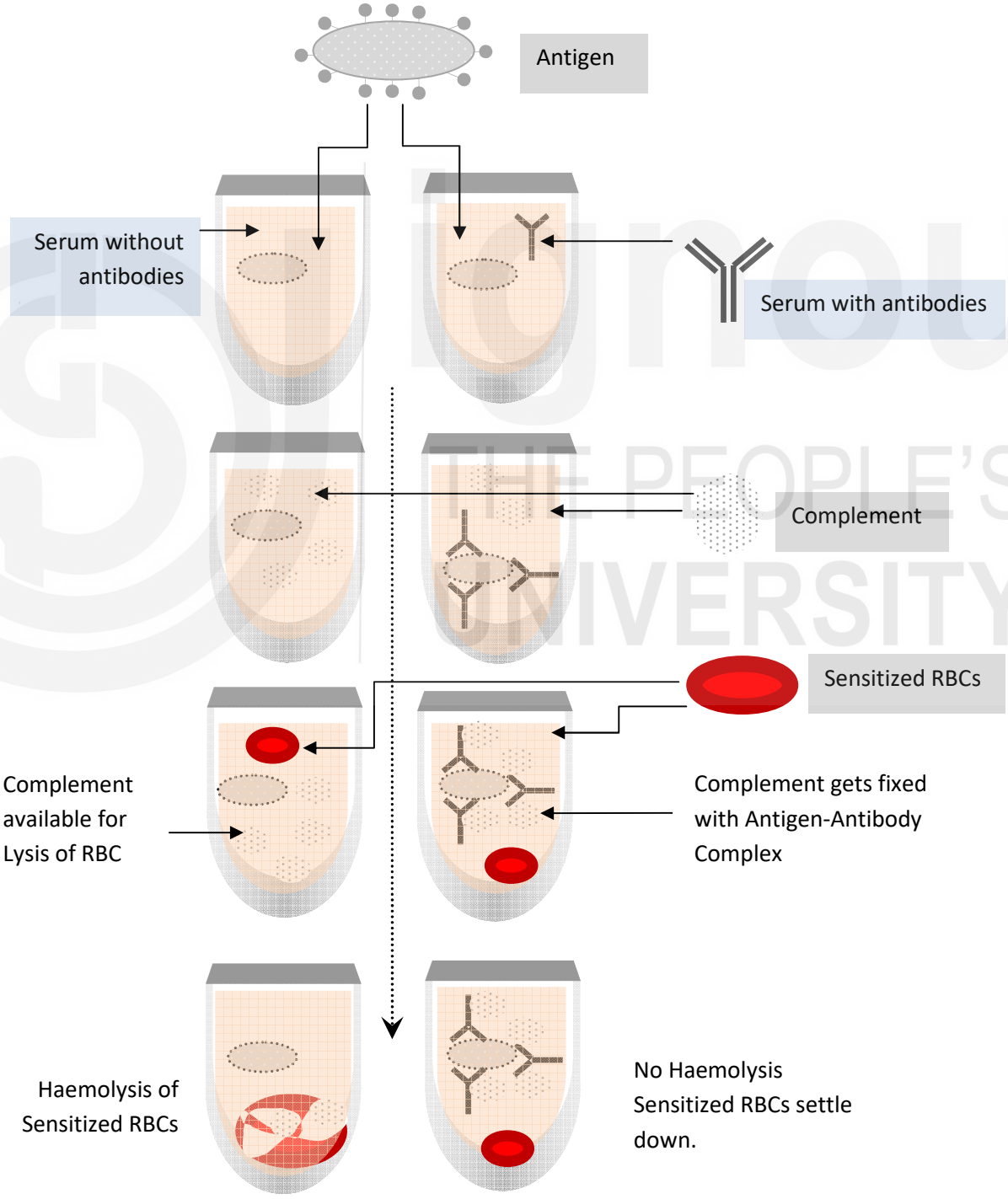


Fig. 8.8: Complement fixation.

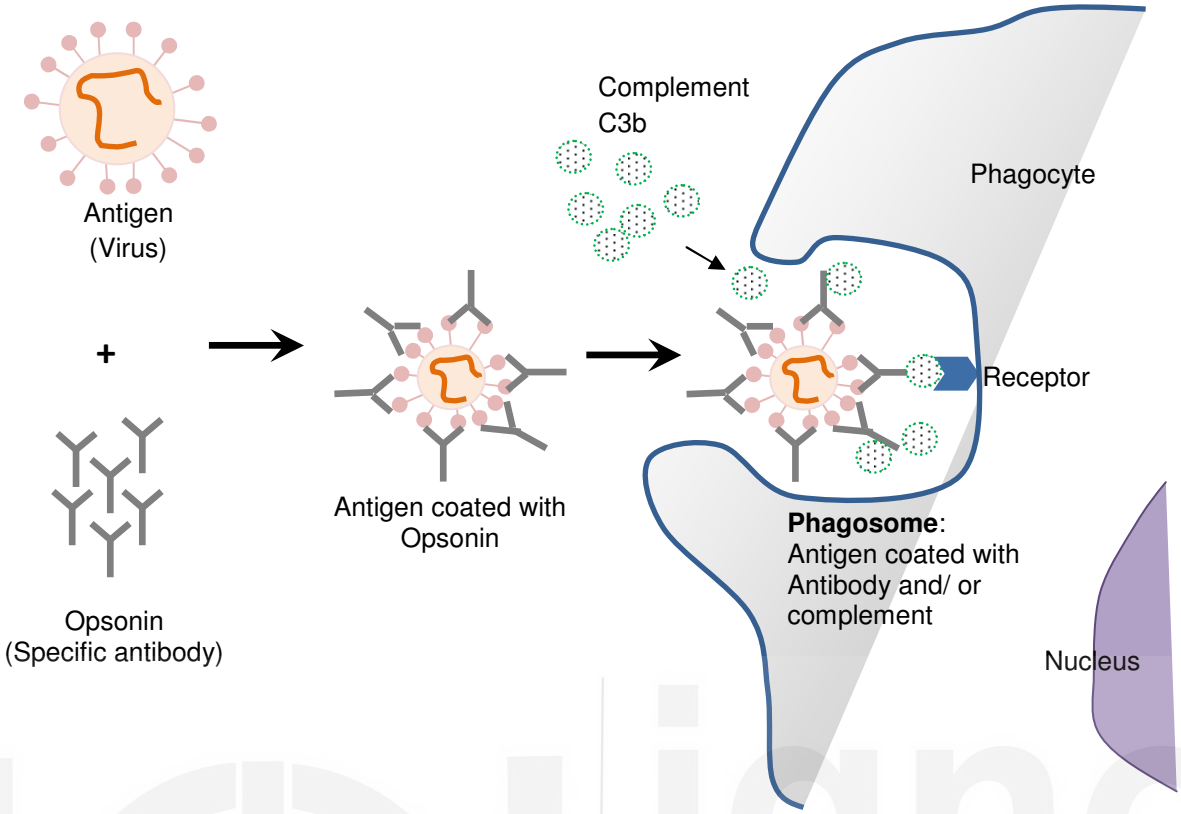


Fig. 8.9: Opsonisation of pathogens.

SAQ 1

State whether the following statement are 'True' or 'False'.

i) Antigen and antibody reaction product can cause a 'complement cascade'.

ii) Neutralization is a very effective means of protection against infection.

iii) Agglutination assays cannot be performed and analysed easily.

iv) Co-immunoprecipitaion is a modification of immunoprecipitation technique.

8.4 BASIC TOOLS AND TECHNIQUES

8.4.1 Radial immunodiffusion or Mancini immunodiffusion

This method is based on the phenomenon of formation of precipitin when antigens and antibodies interact with each other to form visible precipitate at optimum equivalent concentrations. Either the antigen or antibody is suspended in agarose gel while the other is placed in the well and allowed to migrate through the gel to form precipitation zones. This is a simple technique to detect formation of Ag-Ab complexes. The relative concentrations of antigens can be estimated as well as the conditions under which the precipitate is formed. Agar or agarose gels, prepared on glass plates or petridishes, are impregnated with specific antibodies (antisera). The samples

containing the antigens are placed in different wells cut out in the gel surface. Antigens specific to the antibody will diffuse across the gel uniformly and in all directions away from the well. On reaction with the antibody there would be formation of precipitation at the a point where their concentrations become equivalent. Formation of ring shaped precipitation bands around the well indicates that a reaction has taken place. The size of the zone diameter will indicate the intensity of the reaction and hence correspond to the amount of antigenin the solution. Absence of precipitation bands indicate that no reaction has taken place (hence absence of the antigen of interest). For quantitative estimation, a calibration curve is prepared using one or more standards. The zone diameters are measured and plotted against their respective concentrations. The concentration of unknown sample can be estimated from the standard curve. A control is always taken each time for comparison.

8.4.2 Ouchterlony Double Diffusion

This is another commonly used technique for detection and estimation of antigens and antibodies by using their property to precipitate in gels. In this method of immunoprecipitation, the antigen (sample of interest) and antibody (usually known sera) are placed in separate wells. After a period of time they leave the wells and diffuse radially towards each other forming a concentration gradient. Once they reach the optimum equivalent concentration, the reaction product (Ag-Ab complex) precipitates forming visible lines of precipitation called the 'precipitin lines'. Here, the antigen (pathogen) is placed in the centre while the serum samples are placed in the surrounding wells. The given diagram (Fig.8.10 A) indicates that the serum samples of only two individuals (A and C)are positive for the particular pathogen showing precipitin lines between the serum and the central pathogen containing well. The other remaining serum samples are negative for the pathogen. Besides being rapid and easy to perform, the procedure gives accurate results.

The resulting gels give information regarding the identification of the antigen of interest. The pattern of precipitin lines also gives information regarding the interactive properties of the precipitation complex formed (Fig 8.10B). These immunodiffusion assays help in qualitative and quantitative estimation of antigen and antibodies in clinical samples like; assessment of the immunoglobulin class and purity of a particular antigen present in the serum sample, diagnosis of diseases and conducting serological surveys to determine prevalence of a disease in a particular population. Table 8.1 lists advantages and limitations of Immunodiffusion.

Table 8.1: Advantages and Limitations of Immuno diffusion techniques.

Advantages	Limitations
Results are more specific and sensitive in comparison to other available methods.	Reaction time is long (upto 48 hours)
Precipitation areas can be made more visible with staining methods.	Results may get affected by presence of metal ions bound proteins in the test samples.

The gel plates can be preserved for longer period.	Radial immunodiffusion is considered comparatively uneconomical in comparison to other methods.
Separate precipitation lines obtained for each Ag-Ab reaction help in distinguishing various antigens and cross reaction if any.	Recently replaced by more sensitive and automated methods, such as ELISA.

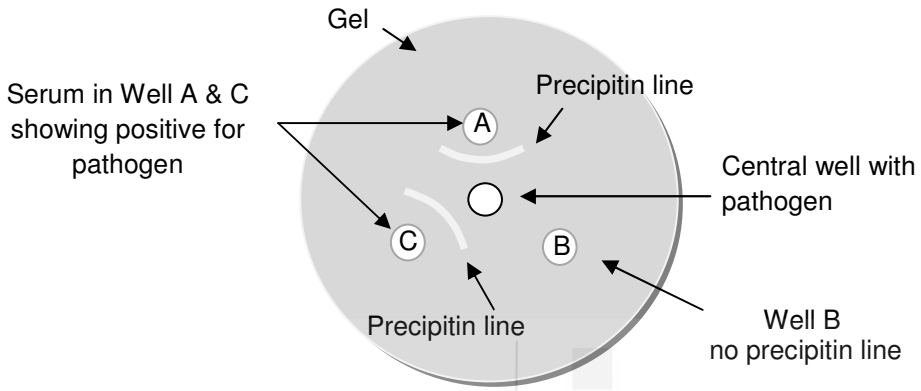
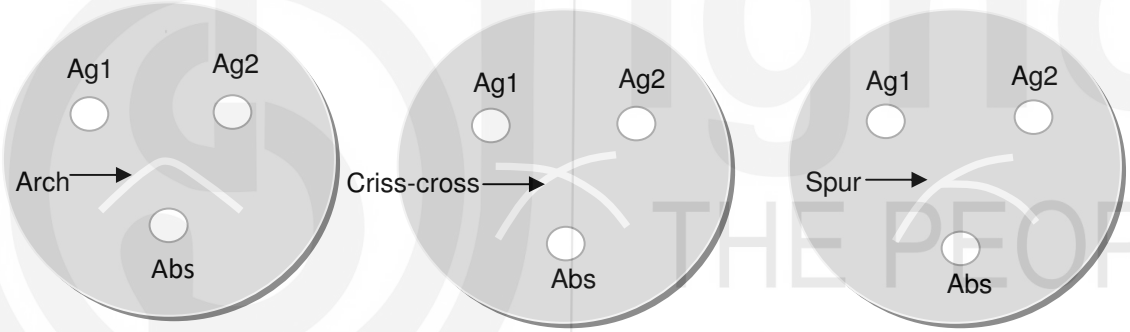


Fig. 8.10: A) Precipitation zone formed after Radial Immunodiffusion.



i. Identity **ii. Non-Identity** **iii. Partial Identity**

Fig. 8.10: B) Precipitin lines formed after Ouchterlony Double Diffusion.

8.4.3 Haemagglutination

Haemagglutination tests are the most frequently used immunoassays world over. They are routinely used for blood typing wherein human RBCs are mixed with antisera to blood group antigens (Type A or B). The presence of either antigen on the cell surface will cause agglutination or visible clumping. These assays can also be modified to estimate antibodies raised against any antigen which can be attached to the surface of RBCs. The test is highly sensitive and can be easily performed. Another variation of this technique, called **Haemagglutination inhibition reaction**, is used for the detection of viruses and antiviral antibodies in patients. The multivalent protein molecules on the viral surface (e.g. hemagglutinin glycoprotein on influenza virus) interact with macromolecules on the RBC surface (sialic acid) and induce agglutination of the RBCs.

Haemagglutination assay is performed in a microtitre plate. Antiserum (antibodies to RBCs) is added to the first well and then serially diluted down to the last well. In other words the concentration of antibodies in well 2 is half of

that in well 1, and so on till the last well. A buffer solution (control) is added to well number 9. Next, same amount of RBCs are added to each well and the plate is incubated. At the end of the incubation period, agglutination is observed in the first three wells only. In the remaining wells, RBCs settle down at the bottom of the well as a tight red button indicating a negative reaction. Since well number 9 contained no agglutinating antibodies (buffer + RBCs only), no positive reaction is observed (Fig 8.11).The first three wells contain antisera concentration high enough to allow haemagglutination (positive reaction) where large clumps are formed on agglutination that float in the well and do not settle down. From the fourth well onwards, the diluted antiserum does not allow the antibodies to establish cross-linking and hence the RBCs settle down to the bottom of the well.

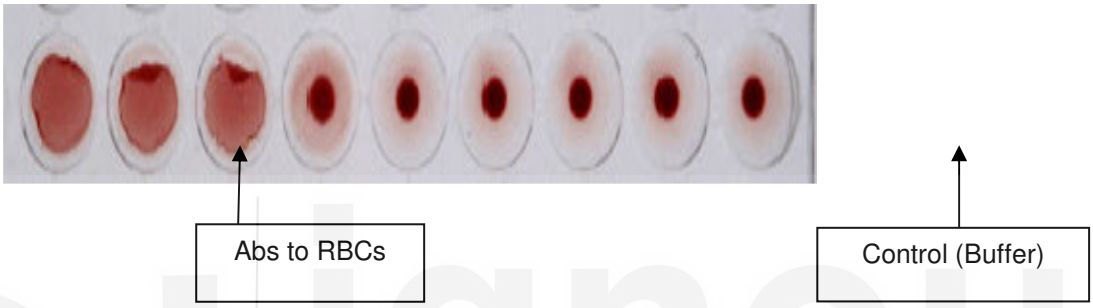


Fig. 8.11: Haemagglutination assay using antibodies against RBCs

8.4.4 Western Blotting (Fig 8.12)

Western Blotting is an analytical technique widely used for the detection, identification, and quantification of specific proteins in a complex mixture. This technique is also called 'Immunoblotting' since antibodies are used as probes to detect specific antigens after they have been separated. The technique mainly involves the following major steps:

1. **Protein separation using SDS-PAGE (Sodium dodecyl sulphate–polyacrylamide gel electrophoresis):** In the presence of an applied electric field, the protein molecules present in serum sample get separated as their mobility depends on mass and charge. Low molecular molecules move faster followed by larger ones. The molecules that are positively charged would migrate towards anode and the negatively charged molecules towards cathode. The role of SDS (Sodium dodecyl sulphate, a detergent) is to unfold the protein and impart negative charges.

2. **Protein transfer from the gel to membrane:** The gel containing separated protein bands are removed and transferred to protein binding membrane (nitrocellulose or polyvinylidene fluoride)for further detection and analysis. The transfer takes place in the presence of an applied electric field which is made possible as the proteins in the gel are all negatively charged (electrophoretic transfer).

3. **Prevention of non-specific binding:** Since antibodies are also proteins, they can bind anywhere on the membrane. This can disrupt the specificity and sensitivity of the analysis. Non-specific binding of antibodies can be avoided by adding a blocking agent like Bovine Serum Albumin (BSA) so that all spaces are occupied excluding the protein bands.

4. **Antibody probing:** Membrane is incubated with primary antibodies that will target the specific antibodies located in the membrane bands. Excess primary antibodies are washed off before adding labelled secondary antibodies. The secondary antibody binds to the already bound primary antibodies. Besides helping in the detection of antibody of interest, secondary antibodies also enhance the sensitivity of the assay by amplifying the detection signal. Excess secondary antibodies are also washed off.

5. **Detection of proteins on membrane (Blotting):** Detection is done on the basis of the type of label present on the secondary antibodies. If the label is an enzyme, then enzyme-substrate reaction will give a coloured insoluble product at the site of relevant antibody visualized using ELISA (Enzyme Linked Immuno Sorbent Assay). Chemiluminescent or radiolabeled secondary antibodies can also be used for detection with appropriate instrumentation.

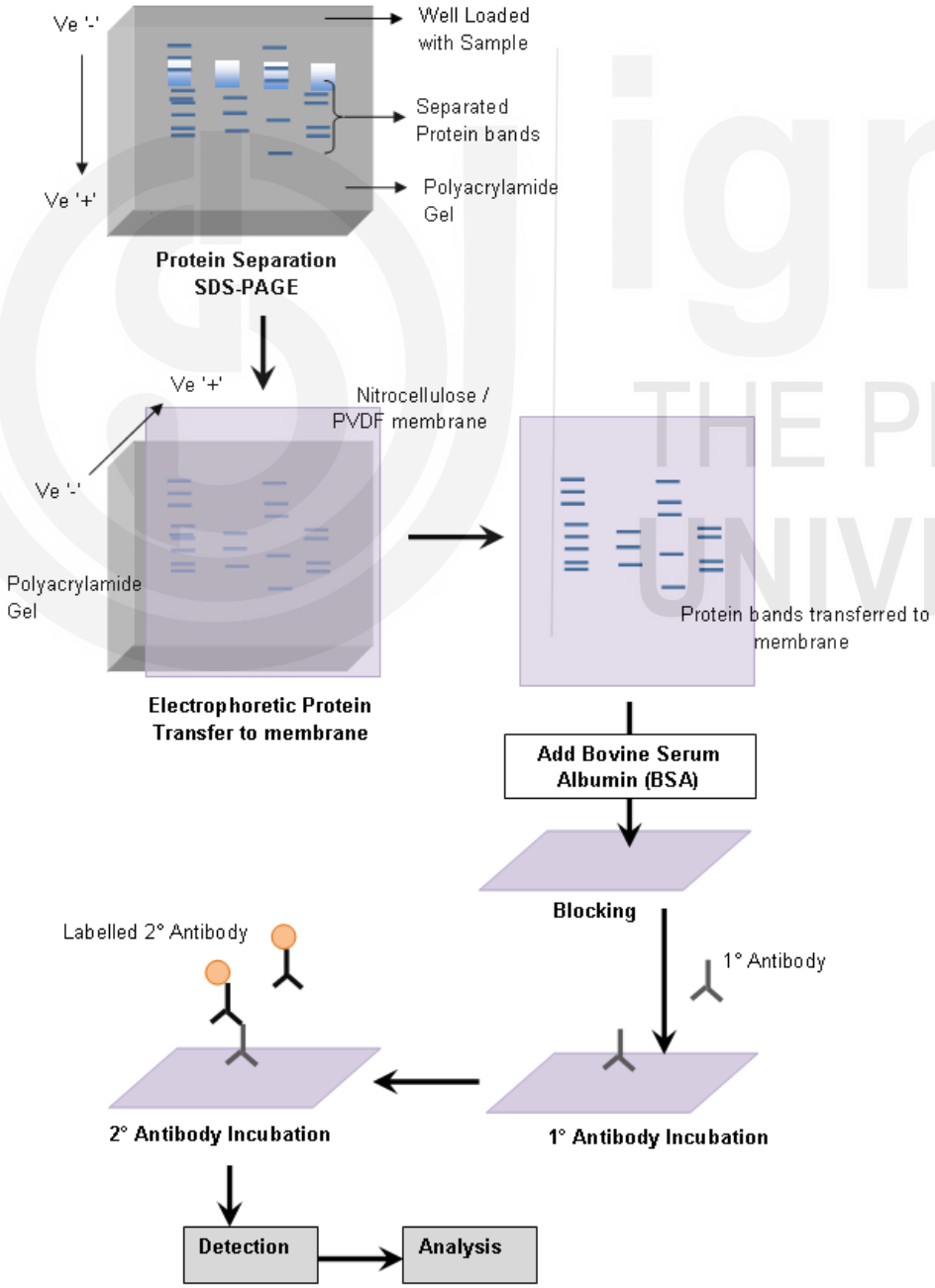


Fig. 8.12: Western blotting technique.

8.4.5 Enzyme Linked Immuno Sorbent Assay (ELISA) (Fig 8.13)

ELISA uses antigens or antibodies that are covalently bound to enzymes. These enzymes catalyze the conversion of suitable chromogenic substrate into a coloured reaction product. High sensitivity, safety and low cost makes ELISA a useful technique in the estimation and detection of antigens and antibodies. The technique has several variations that are used on a regular basis. In each case, a standard curve is plotted using known concentrations of antigens and antibodies.

(a) Indirect ELISA

This assay is used for the identification of antibodies and determination of their concentration. Wells in a microtitre plate are coated with the antigen of interest. Serum sample (containing primary antibody) is added to the well and allowed to react with the attached antigen. Excess unbound primary antibody is washed off and the residual bound antigen is detected by adding an enzyme-linked secondary antibody that binds to the primary antibody. The well is washed again to remove excess secondary antibody. Substrate to the enzyme is then added so that a product is formed, the quantity of which can be measured using an ELISA plate reader and a standard curve of known primary antibody concentrations. **Indirect ELISA is regularly used for the diagnosis of AIDS by detecting antibodies to HIV in the patient serum.**

(b) Sandwich ELISA

This variation estimates the antigen present in a sample. The mictrotitre wells in this case are coated with the antibody (not the antigen). The sample (containing antigen) is added to the well coated with antibody and washed. A secondary antibody (enzyme linked) is added which binds to the antigen but on a different epitope or binding site. After another washing that removes excess unbound antibody; the substrate is added to obtain a coloured product that can be measured easily. It is important to note that since the two antibodies used here bind to different epitopes on the antigen, they have to be monoclonal and specific for different binding sites.

(c) Competitive ELISA

In this case, the antibody is first mixed with the serum sample that may contain the antigen of interest. An Ag-Ab complex will be formed if specific antibodies are present in the sample. An inhibitor antigen conjugated with chromophore is then introduced. The conjugate antigen competes with the native antigen for the binding site of the antibody. Hence, in competitive ELISA, the intensity of the signal emit would be inverse to the concentration of the antigen of interset.

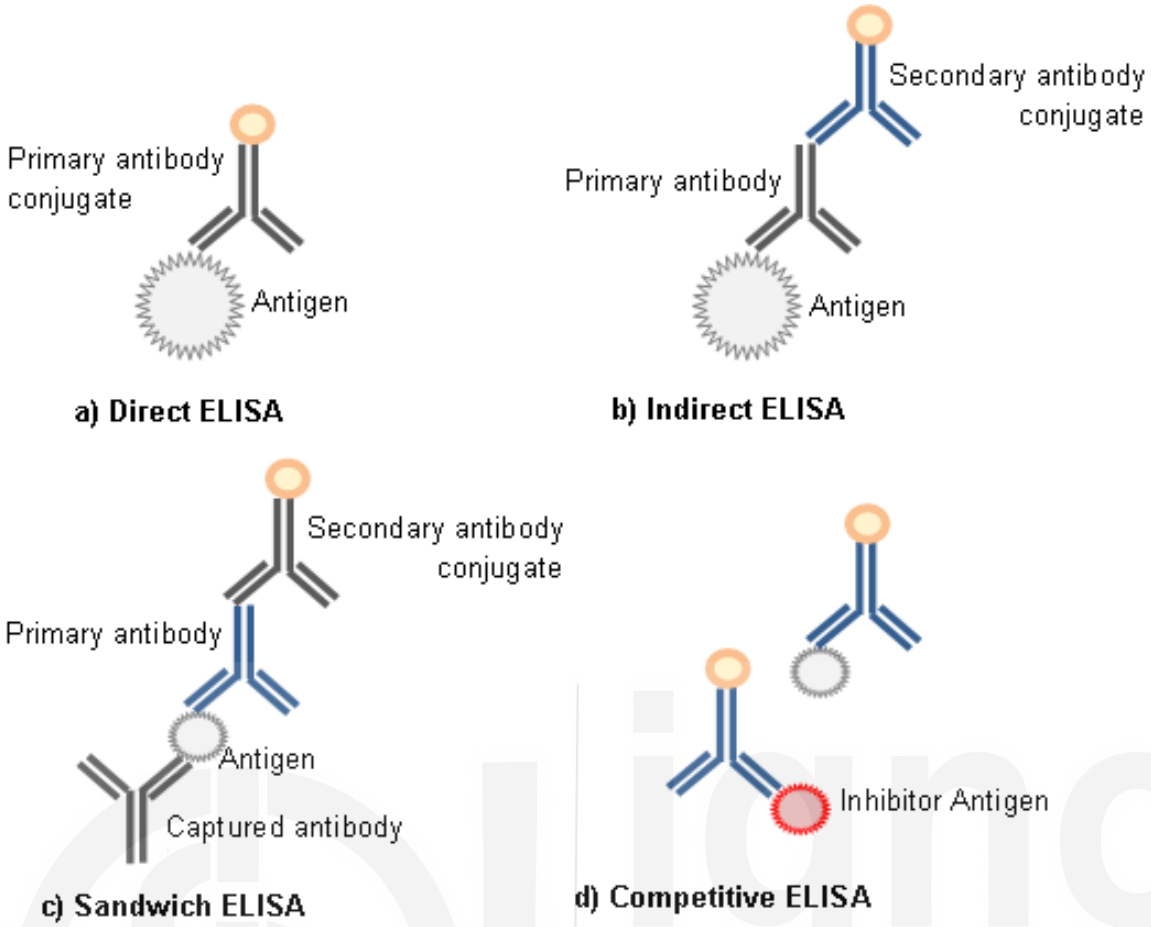


Fig. 8.13: Different types of ELISA.

8.4.6 Immunofluoresence (Fig 8.14)

This is a visualization technique widely used for the analysis of cells, tissues and cellular components for the presence of specific molecules and their distribution. Fluorescent antibodies are prepared by attaching fluorescent stains or 'Fluorochromes' to monoclonal antibodies. The antibody part of this conjugate binds to the molecule of interest while the fluorochrome attachment emits light of a particular wavelength (or colour) when excited. This mechanism is called 'Fluorescence'. Diverse fluorochromes that can be attached to different monoclonal antibodies are available which can be used either alone or in combination for visualization of different molecules. There are two types of Immunofluorescence assays:

(a) **Direct Immunofluorescence:** where fluorescent antibodies bind directly to the target molecule or antigen and

(b) **Indirect Immunofluorescence:** where a primary antibody (without a fluorochrome) is added which binds to the target molecule and then a secondary antibody (raised against the primary antibody and fluorochrome linked) is added. The slides are then observed under a fluorescence microscope. Since, fluorescent antibodies bind to the target molecule indirectly via the primary antibodies, the second type of assay is called Indirect Immunofluorescence. The indirect assay is more sensitive than direct assay since more than one labelled antibody enhance the signal to give a better image.

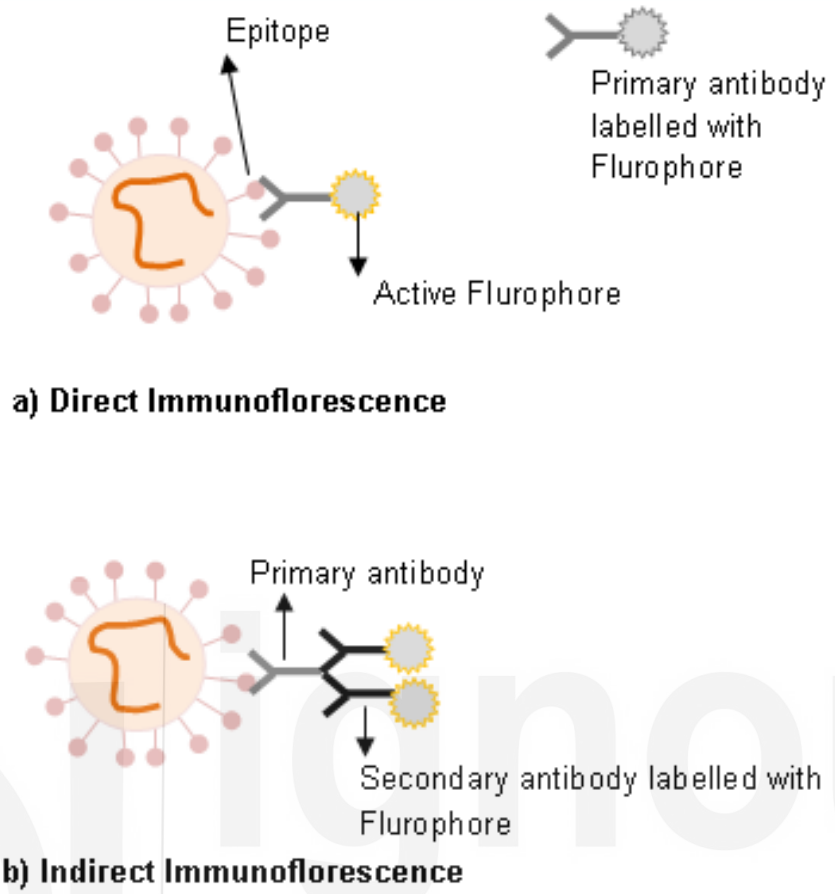


Fig. 8.14: Immunofluorescence assays.

8.4.7 Immunoelectrophoresis (Fig 8.15)

Immunoelectrophoresis is a combination of two techniques: (a) electrophoresis which separates an antigen mixture into its different components and (b) double Immunodiffusion that helps in the identification of separated antigens.

In this technique, the antigen mixture is placed in a well cut out in a gel(agarose) coated slide and subjected to electrophoresis. Application of an electric field separates the antigen mixture into its individual constituents based on the size of the molecule and the nature of its charge. The molecules that are positively charged migrate towards the anode while negatively charged molecules migrate towards cathode. Similarly, smaller molecules will move faster leaving behind larger molecules. After the electrophoresis, a trough is created in the gel, parallel to the direction of electrophoretic migration and antibodies are loaded in it. Both antigens and antibodies diffuse towards each other across the gel and elliptical precipitate 'precipitin lines' are formed after a particular time period when equivalent concentrations are reached. The shape, intensity and location of precipitin lines would determine the nature of the antigens. Absence of precipitin lines indicates no Ag-Ab reaction. Besides having high resolving power, this technique can identify different antigens present in the serum and also indicates if there is any relationship between them (identical, partially identical or non-identical).

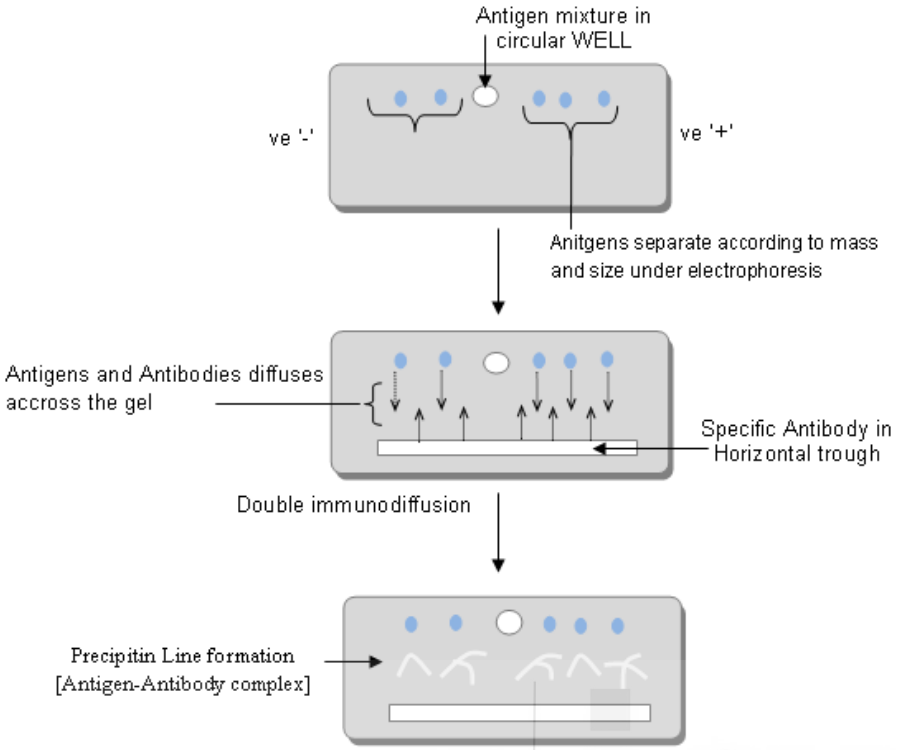


Fig. 8.15: Immunoelectrophoresis.

SAQ 2

Fill in the blanks:

i) ……. vaccine stimulates production of anti-tetanus antibodies that prevents the entry of toxin in neurons.

ii) Affinity can be defined as the strength of non-covalent interaction between …… binding site with a single antigenic determinant

iii) The ability of specific antibodies to initiate membrane damage of the pathogens in association with the complement is called …… .

iv) ……. are routinely used for blood typing where human RBCs are mixed with antisera to blood group antigens (Type A or B).

8.4.8 Flow Cytometry (Fig 8.16)

Flow cytometry is an automated technique that uses diverse fluorescent antibodies for staining those cells that are to be separated and analyzed. Flow cytometry is a modern technique getting more useful with advancements in technology. The assay can detect up to 12 fluorescences and light-scattering molecules routinely. In this assay, cells can be stained with antibody conjugated with one or more than one fluorochromes (fluorescein, phycoerythrin, or PE) that make detection easier. For intracellular staining, cells are permeabilized with a detergent which allows entry of antibodies into cell interior and bind intracellular components. Based on the binding of fluorophores, FAC (Fluorescence Activated Cell Sorter) of a flow cytometer sorts heterogeneous mixture of cells on the basis of light scattering and fluorescent signal from individual cells. Flow Cytometer uses laser beam and a series of detectors or photomultiplier tubes to identify the scattered light or

fluorescent signals of particular wavelengths emitted from single intact cells flowing in a single stream. When a cell crosses the path of the laser beam, the laser signal is interrupted and the scattered light is recorded. The light scattered is detected by a photodiode (detector) in the forward direction; the intensity of which can be used to determine the size of cells in the stream. Hence, greater the forward light scattering, larger would be the cell size. The light scattered from the side is detected by another detector which is placed perpendicular to the path of the laser beam. The amount/ intensity of side scattered light determines the complexity of the scattering cell. Increasing amounts of side scattering indicates that the cell has a higher number of intracellular membranous structures (endoplasmic reticulum and mitochondria).Therefore, this technique is used for analyzing cytokine synthesis, cell cycle and enzyme activity. In addition, Flow cytometers are routinely used for the detection and diagnosis of different blood cancers and AIDS.

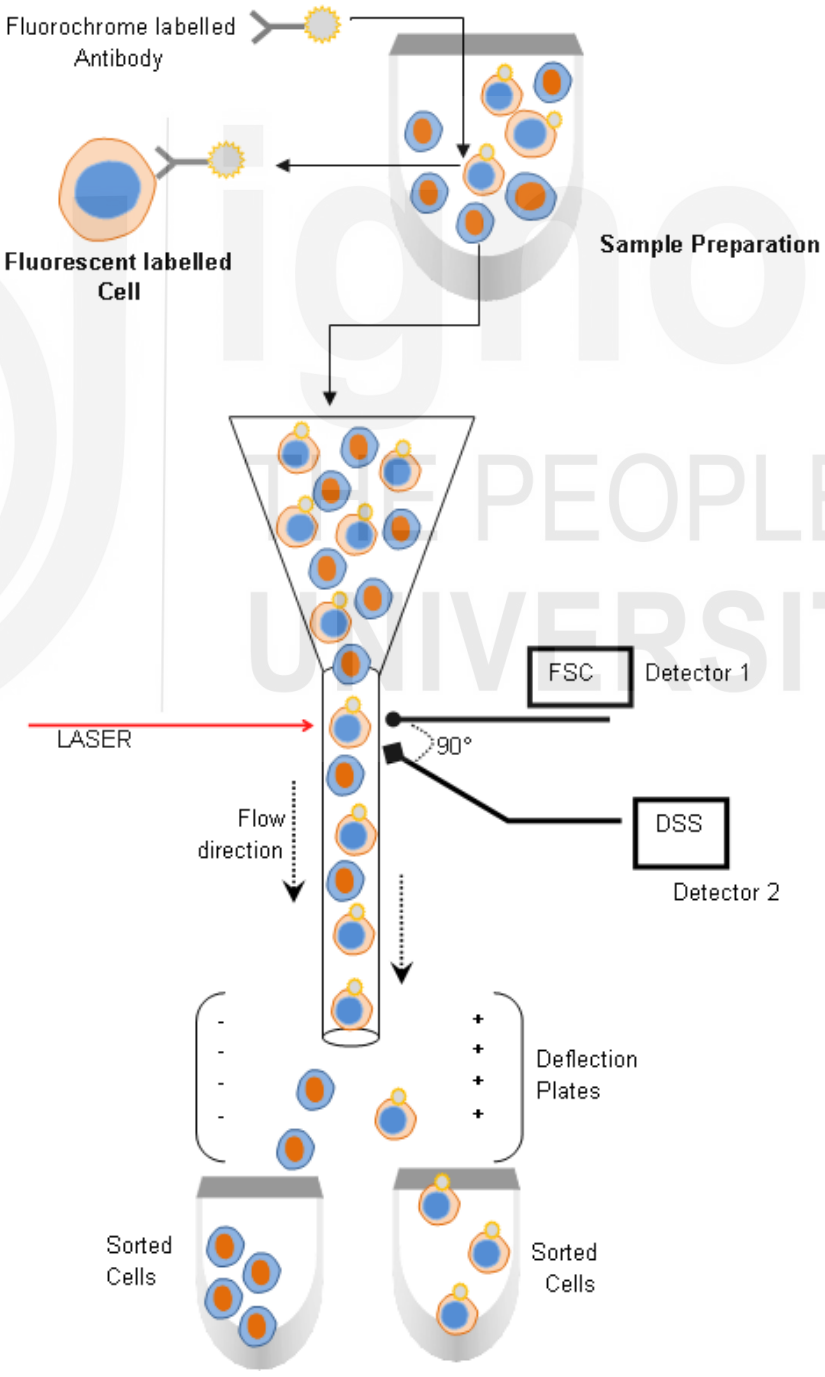


Fig. 8.16: Flow Cytometric Analysis.

8.5 SUMMARY

Let us summarise what you have learnt in this Unit:

- Antibodies react with antigens at specific binding sites (paratopes vs epitopes) forming reversible non-covalent bonds at optimum conditions. The resulting antigen-antibody (Ag-Ab) complex is the basis for immunologic assays designed for their detection.
- The binding strength is defined in terms of affinity (single binding) or avidity (multiple accumulated binding) and can be estimated by Equilibrium dialysis
- When the concentration of the antigen and antibodies and equal, Ag-Ab complex precipitates from which specific antigens/proteins can be purified from a mixture of heterogeneous soluble molecules. In radial immunodiffusion, either antigen or antibody is suspended in the gel and the other is placed in the well and allowed to migrate through the gel to form precipitation zones while in Ouchterlony double diffusion both are placed in separate wells and migrate towards each other forming visible lines of precipitation.
- Ag-Ab interactions between several antigenic determinants and antibody paratopes that lead to cross-linking and visible clumping of complex formed are called agglutination reactions. The antibodies that cause these reactions are called agglutinins. Agglutination reactions have immense diagnostic applications
- Hemagglutination assay estimates antibodies to antigens present on the surface of RBC. The antibodies bound to specific bacterial strains are measured through bacterial agglutination reactions. However, in virus especially influenza, the presence of antibody against it is measured by Hemagglutination inhibition reaction.
- Antibodies can bind to pathogens, blocking host cell receptors (Neutralization reaction) or making them more susceptible to phagocytosis (Opsonization). The Ag-Ab complex can initiate a complement cascade causing cell lysis (Complement fixation). Antibody coated target pathogens can be destroyed using cytotoxic T cells and natural killer cells.
- Western Blotting is an analytical technique that uses antibodies as probes for detection, identification, and quantification of specific proteins in a complex mixture. Protein separation is done using SDS PAGE and transferred to nitrocellulose membrane before detection.
- Enzyme-linked immunosorbent assays (ELISA) use antigens or antibodies covalently bound to enzymes that convert substrate into a coloured reaction product.
- Immunofluorescence uses monoclonal antibodies attached to fluorochromes (fluorescent antibodies) to visualize cells, tissues and cellular components for the presence of specific molecules and their distribution.
- Immunoelectrophoresis is a combination of electrophoresis which separates an antigen mixture into its different components and double Immunodiffusion that helps in the identification of separated antigens.

8.6 TERMINAL QUESTIONS

1. Why do antigens and antibodies precipitate?
2. Briefly discuss Immunoprecipitation in solid gels.
3. How are agglutination reactions different from precipitation reactions?
4. How can antibodies provide protection against pathogens?
5. What causes haemagglutination and what is it used for?
6. Why is the technique of Western blotting also called Immunoblotting?
7. Describe the technique of Western Blotting.
8. Discuss the differences between indirect ELISA and Sandwich ELISA.
9. How do fluorescent antibodies work?
10. What is the principle of flow cytometry?

8.7 ANSWERS

Self Assessment Questions

1. i) True, ii) True, iii) False, iv) True.
2. i) Tetanus, ii) Single antigen binding, iii) Complement fixation, iv) Haemagglutination immunoassays.

Terminal Questions

1. Immunoprecipitation is based on the ability of multivalent antibodies to bind to more than one epitopes on a single antigen. Ag-Ab complex thus formed becomes so large and bulky that it precipitates, separating out of the solution. The precipitate can be centrifuged to obtain the complex as pellet from which the antigen and antibody can be separated with the help of biochemical techniques. Precipitation take place only when the concentration of both the antigen and antibodies are equal. If either of the two (antigen or antibody) are in excess, then monovalent binding will be preferred not resulting in precipitation of the complex.
2. Immunoprecipitation in solid agar media occurs when both antigens and antibodies are present in equal concentrations. The resulting Ag-Ab complex is seen as visible lines or zones of precipitation (precipitin lines/zones). There are several variations of this technique but the most frequently used on gels is Radial Immunodiffusion and the Ouchterlony method of immunoprecipitation.
3. Both are antigen antibody reactions. While precipitation reactions lead to formation of a complex precipitate, the agglutination reactions form a cross-linked clump in the form of Ag-Ab complex. The antigens here are not soluble components but conjugated to large sized particulate matter like bacterial cells, RBCs, charcoal or latex. As a result, the visibility of the cross-linked product is greater.

4. Antibodies provide protection against pathogens in several ways mainly (a) As neutralizing antibodies that bind to the pathogen and block receptors used to gain entry into the host cell; (b) Opsonizing or making the pathogen more susceptible to phagocytosis; (c) Complement fixation where they bind to pathogens and the resulting complex initiates a complement cascade causing cell lysis; (d) Antibody-dependent cell-mediated cytotoxicity (ADCC) where they assist immune cells by recognizing and killing antibody coated target pathogens, using cytotoxic T cells and natural killer cells.

5. The presence of either antigen on the cell surface will cause agglutination or visible clumping. These assays can also be used to estimate antibodies raised against any antigen which can be attached to the surface of RBCs. Haemagglutination inhibition assay can be used for the detection of viruses and antiviral antibodies in patients. The multivalent protein molecules on the viral surface such as hemagglutinin glycoprotein interact with sialic acid on the RBC surface and induce agglutination of the RBCs.

6. It is also called 'Immunoblotting' since antibodies are used as probes to detect specific antigens after they have been separated.

7. This technique is widely used for the detection, identification, and quantification of specific proteins in a complex mixture. It mainly involves the following major steps:

   i) Proteins present in serum sample are separated in the presence of an applied electric field using SDS-PAGE on the basis of charge and mass.

   ii) The separated protein bands are electrophoretically transferred from the gel to a protein binding membrane. Nitrocellulose and Polyvinylidene fluoride membranes are commonly used.

   iii) Addition of bovine serum albumin prevents non-specific binding of antibodies and all the spaces are occupied excluding the protein bands of interest.

   iv) Membrane is incubated with primary antibodies that will target specific antibodies in membrane bands. Excess primary antibodies are washed off before adding labelled secondary antibodies which bind to the primary antibodies.

   v) Proteins are detected on the basis of the type of label present on the secondary antibodies which can be an enzyme, a chemiluminescent or radiolabeled molecule.

8. (a) Indirect ELISA is used for the qualitative and quantitative estimation of antibodies and determination of their concentration. Wells in a microtitre plate are coated with the antigen of interest. Then the test serum sample is added to the well coated with antigen and allowed to react. The unbound excess primary antibody is washed off while the bound antigen is detected by adding an enzyme-linked secondary antibody that binds to the

primary antibody. The excess antibody is removed from the well. Substrate to the enzyme is then added so that a product is formed. The amount of product can be measured using an ELISA plate reader and a standard curve of known primary antibody concentrations.

(b) Sandwich ELISA estimates the presence of antigen in a sample which is added to the mictrotitre wells coated with the antibody. A secondary antibody (enzyme linked) is added which binds to the antigen but on a different epitope or binding site. After another washing that removes excess unbound antibody; the substrate is added to obtain a coloured product that can be measured easily.

9. Fluorescent antibodies are prepared by attaching fluorescent stains or 'Fluorochromes' to monoclonal antibodies. The antibody part of this conjugate binds to the molecule of interest while the fluorochrome attachment emits light of a particular wavelength (or colour) when excited. The mechanism is called 'Fluorescence'. Diverse fluorochromes are available that can be connected to different monoclonal antibodies and used either alone or in combination for visualization of different molecules.

10. Flow cytometry uses diverse fluorescent antibodies for staining cells that are to be separated and analyzed. Fluorescence Activated Cell Sorter (FACS) is a specialized adaptation of a flow cytometer that sorts a heterogeneous mixture of cells on the basis of light scattering and fluorescent signal from individual cells. It quantifies the number of cells that bind to specific fluorescent antibodies. It consists of laser beam, series of detectors or photomultiplier tubes that identify particular wavelength of the scattered light and fluorescent signals emitted from intact cells flowing in a single stream. Whenever a cell crosses the path of the laser beam, the laser signal is interrupted and the scattered light is recorded. A photodiode (detector) detects the intensity of the scattered light, which can be used to determine the size of cells in the forward stream. Hence, greater the forward light scattering, larger would be the cell size. Another detector that measures the amount of side scattered light is placed perpendicular to the path of the laser beam. This detector determines the complexity of the scattering cell. Increased intensity of side scattering indicates that the cell has a higher number of intracellular membranous structures (endoplasmic reticulum and mitochondria).

Therefore, flow cytometers are routinely used for the detection and diagnosis of different blood cancers and AIDS, for analyzing cytokine synthesis, cell cycle and enzyme activity.

GLOSSARY

ABO blood-group antigen : Antigenic determinants of the blood-group system defined by the agglutination of red blood cells exposed to anti-A and anti-B antibodies.

Adjuvants : Factors that are added to a vaccine mixture to enhance the immune response to antigen by activating innate immune cells. Dead mycobacterium were among the original adjuvants, but more refined preparation include alum, cytokines, and/or lipids.

Affinity : The strength with which a monovalent ligand interacts with a binding site. It is represented quantitatively by the affinity constant K_a.

Agglutination : The aggregation or clumping of particles (e.g., latex beads) or cells (e.g., red blood cells).

Agglutinin : A substance capable of mediating the clumping of cells or particles; in particular, a hemagglutinin (HA) causes clumping of red blood cells.

Allotypes : A set of **allotypic determinants** characteristic of some but not all members of a species.

Antagonist, antagonize : A molecule that inhibits the effect of another molecule.

Antigen : Any substance (usually foreign) that binds specifically to an antibody or a T-cell receptor; often is used as a synonym for **immunogen.**

Antigen processing : Degradation of antigens by one of the pathways yielding antigenic peptides that are displayed bound to MHC molecules on the surface of antigen-presenting cells or altered self cells.

Antigenic determinant : The site on an antigen that is recognized and bound by a particular antibody, TCR/MHC-peptide complex, or TCR-ligand-CDI complex; also called **eptiope**.

Antigenicity : The capacity to combine specifically with antibodies or T-cell receptor/MHC.

Antiserum : Serum from animals immunized with antigen that contains antibodies to that antigen.

Apoptosis : A process, often referred to as **programmed cell death,** where cells initiate a signaling pathway that results in their own demise. Apoptosis requires ATP and is typically dependent on the activation of internal caspases. In contrast in **necrosis,** it does not result in damage to surrounding cells.

Autoimmunity : An abnormal immune response against self antigens.

Avidity : The strength of antigen-antibody binding when multiple epitopes on an antigen interact with multiple binding sites of an antibody.

B lymphocytes (B cells) : Lymphocytes that mature in the bone marrow and express membrane-bound antibodies. After interacting with antigen, they differentiate into antibody-secreting plasma cells and memory cells.

B-cell receptor (BCR) : Complex comprising a membrane-bound immunoglobulin molecule and two associated signal-transducing Igα/Igβ molecules.

Cancer stem cells : A subset of cells within a tumor that has the stem-cell-like ability to give rise to all cells within that tumor and the ability to self-renew indefinitely. They are thought to be responsible for tumor growth.

Carrier effect : A **secondary immune response** to a hapten depends on use of both the **hapten** and the **carrier** used in the initial immunization.

Cascade induction : The property of cytokines that pertains to their ability to induce one cell to release cytokines that then act upon another to induce the release of other cytokines and growth factors.

CD4 : A glycoprotein that serves as a co-receptor on class II MHC-restricted T cells. Most helper T cells are $CD4^4$.

Cell line : A population of cultured tumor cells or normal cells that have been subjected to chemical or viral **transformation.** Cell lines can be propagated indefinitely in culture.

Cell-mediated immune response : Host defense that are mediated by antigen-specific cells. It protects against intracellular bacteria, viruses, and cancer and is responsible for graft rejection. Transfer of primed T cells confers this type of immunity on the recipient.

Chemokines : Any of several secreted low-molecular-weight cytokines that mediate **chemotaxis** in particular leukocytes via receptor engagement and that can regulate the expression and/or adhesiveness of leukocyte **integrins**.

Clone : Cells arising from a single progenitor cell.

Complement : A group of serum and cell membrane proteins that interact with one another and with other molecules of innate and adaptive immunity to carry out key effector functions leading to pathogen recognition and elimination.

Endogenous pathway : Intracellular route taken by antigen that is processed for presentation by MHC class I, typically associated with proteins generated in the cytosol.

Epitope : The portion of an antigen that is recognized and bound by an antibody or TCR-MHC combination; also called **antigenic determinant.**

Exogenous pathway : Intracellular route taken by antigen that is processed for presentation by MHC class II, typically associate with proteins that are endocytosed.

Flow cytometer : An instrument that uses lasers along with sophisticated optics to measure multiple fluorescent and light scattering parameters from thousands of cells as flow rapidly, one-by-one in front of the laser beam.

Gene therapy : General term for any measure aimed at correction of a genetic defect by introduction of a normal gene or genes.

Hapten : A low-molecular-weight molecule that can be made immunogenic by conjugation to a suitable carrier.

Hemagglutinin (HA) : Any substance that causes red blood cells to clump, or agglutinate. Most commonly the virally-derived glycoprotein found on the surface of influenza virus that binds to sialic acid residues on host cells causing them to agglutinate. See also **agglutinin.**

Hematopoiesis : The formation and differentiation of blood cells.

Hematopoietic stem cell (HSC) : The cell type from which all lineages of blood cells arise.

Herd immunity : When the majority of the population is immune to an infectious agent, thus significantly reducing the pathogen reservoir due to the low chance of a susceptible individual contacting an infected individual.

Humoral immune response : Host defenses that are mediated by antibody present in the plasma, lymph, and tissue fluids. It protects against extracellular bacteria and foreign macromolecules. Transfer of antibodies confers this types of immunity on the recipient.

Hybridoma : A **clone** of hybrid cells formed by fusion of normal lymphocytes with **myeloma cells;** it retains the properties of the normal cell to produce antibodies or T-cell receptors but exhibits the immortal growth characteristic of myeloma cells. Hybridomas are used to produce **monoclonal antibody.**

Hypersensitivity : Exaggerated immune response that causes damage to the individual. **Immediate hypersensitivity** (types I, II and III) is mediated by antibody or immune complexes, and delayed-type hypersensitivity (type IV) is mediated by T_H cells.

IgD : Immunoglobulin D. An antibody class that serves importantly as a receptor on naïve B cells.

IgM : Immunoglobulin M. An antibody class that serves as a receptor on the course of an immune response Secreted IgM exists primarily in pentameric form.

Interferons (IFNs) : Several glycoprotein **cytokines** produced and secreted by certain cells that induce an antiviral state in other cells and also help to regulate the immune response.

Interleukins (ILs) : A group of **cytokines** secreted by leukocytes that primarily affect the growth and differentiation of various hematopoietic and immune system cells.

Interstitial fluid : Fluid found in the spaces between cells of an organ or tissue.

Lymph : Interstitial fluid derived from blood plasma that contains a variety of small and large molecules, lymphocytes, and some other cells. It circulates through the lymphatic vessels.

Memory B cell : An antigen-committed, persistent B cell. B-cell differentiation results in formation of **plasma cells,** which secrete antibody, and **memory cells,** which are involved in the **secondary responses.**

Memory T cells : T cells generated during a primary immune response that become long lived and more easily stimulated by the antigen to which they are specific. They include two main subsets, central and effector memory T cells, and are among the first participants in the faster more robust secondary immune response to antigen.

MHC (major histocompatibility complex) : A group of genes encoding cell-surface molecules that are required for antigen presentation to T cells and for rapid graft rejection. It is called the H-2 complex in the mouse and the HLA complex in humans.

Monoclonal : Deriving from a single clone of dividing cells.

Natural killer (NK) cells : A class of large, granular, cytotoxic lymphocytes that do not have T- or B-Cell receptors. They are antibody-independent killers of tumor cells and also can participate in **antibody-dependent cell-mediated cytotoxicity.**

Pleiotropic : Having more than one effect. For example, a cytokine that induces both proliferation and differentiation.

Polyclonal antibody : A mixture of antibodies produced by a variety of B-cell clones that have recognized that same antigen. Although all of the antibodies react with the immunizing antigen, they differ from each other in amino acid sequence.

Polygenic : The presence of multiple genes within the genome that encode proteins with the same function but slightly different structures.

Sarcoma : Tumor of supporting or connective tissue.

Secondary lymphoid organs (SLOs) : Organs and tissues in which nature, immunocompetent lymphocytes encounter trapped antigens and are activated into **effector cells.** In mammals, the **lymph nodes, spleen,** and **mucosal-associated lymphoid tissue (MALT)** constitute the secondary lymphoid organs.

Serum : Fluid portion of the blood, which is free of cells and clotting factors.

Stem cell : A cell from which differentiate cells derive. Stem cells are classified as totipotent, pluripotent, multipotent, or unipotent depending on the range of cell types that they can generate.

T helper (T_H) cells : T cells that are stimulated by antigen to provide signals that promote immune responses.

T lymphocyte : A lymphocyte that matures in the thymus and expresses a T-cell receptor, CD3, and CD4 or CD8.

Vaccination : International administration of a harmless or less harmful form of a pathogen in order to induce a specific adaptive immune response that protects the individual against later exposure to the pathogen.

Vaccine : A preparation of immunogenic material used to induce immunity against pathogenic organisms.

SUGGESTED READINGS

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Acknowledgement of Figures

1. Fig. 6.1 to Fig. 6.5: are drawn by Dr. Indrakant K. Singh and Dr. Moses Rinchui N., Department of Zoology, Deshbandhu College University of Delhi, Delhi-110019.

2. Table 7.1: https://courses.lumenlearning.com/boundless-biology/chapter /antibodies/

   Fig. 7.1 and Fig. 7.6: are drawn by Dr. Shivani G. Varmani, Department of Biomedical Science, Bhaskaracharya College of Applied Sciences University of Delhi, Delhi-110075.

   Fig. 7.2: https://vivaopen.oercommons.org/courseware/lesson/652/ overview

   Fig. 7.7: https://courses.lumenlearning.com/boundless-biology/chapter/ antibodies/

3. All figures of Unit 8 are drawn by Dr. Indrakant K. Singh and Dr. Moses Rinchui N., Department of Zoology, Deshbandhu College University of Delhi, Delhi-110019.