Immunology

- From Latin word "Immunis" → to exempt (external factor);
  is the source of English word 'immunity' → a state of protection from infectious disease.

Immunity:- overall ability of a living body to fight against diseases.

- **Innate Immunity**
  - born with
  - non-specific
  - less efficient
- **Adaptive Immunity**
  - acquired
  - specific
  - memory formation

Innate Immunity

refers to an immediate or early antigen-non specific defense mechanisms that are present in almost since birth without being induced and are designed to react and/or eliminate any antigen.

① Physical Barriers: (Mechanical)

(a) Skin: First Barrier – (outermost – stratum corneum)
(b) Mucous and cilia

② Physiological Barriers:-

(a) Saliva – lyzozyme
(b) Acid in stomach – Gastric acid
(c) Tears (lyzozyme) – (from Lacrimal gland of eye)
(d) Cerumen (Ear wax) – Ceruminous glands
(modified sweat gland)
aporine.

(e) vaginal secretions :- acidic (maintained by Bartholin gland)
↳ prevent growth of bacteria, fungus, etc.

(f) Sebum (oil) from skin :- Secretion depends on the blood secretions.

③ Cellular barriers :

(i) Leucocytes — Neutrophils and monocytes both are Phagocytosis
↓
Polymorphonuclear Leucocytes.
* largest of all WBCs
* Differentiate into macrophages.

(ii) Macrophages :-

(a) Alveolar Macrophage → "BALT" Bronchus Associated Lymphoid tissue

(b) Connective tissue → Histiocyte

(c) Liver → Kupffer cells.

(d) Glomerular

(e) Mesangial cells — kidney

(f) Brain — Microglial

(h) Bone — osteoblasts

(iii) Natural killer cells (NK) :-

(a) Secrete "Perforin"

(b) Induction of Apoptosis (cell death)

(iv) Complement System:- 30 proteins in Inactive state.

Serum and Plasma membrane of cells.

Once activated they induce → (i) Cytolysis
(ii) Inflammation
(iii) Phagocytosis

(v) Inflammation:- Redness, swelling, heat, Pain → causes

↓

Response generated by body due to Secretion of Pro-inflamentory substances. → Histamines
→ Prostaglandin
→ ~~Fact~~ IL-6, IL-B
} Vasodilation

(vi) Fever - Secretion of Pyrogens

(4) Cytokine Barrier :-

Low molecular weight proteins that stimulate/inhibit the proliferation and differentiation of immune cells.

eg. Interleukins → Leucocytes.
Tumor Necrosis factor - TNF
Interferons - IFNγ

Adaptive Immunity

It refers to antigen-specific induced defense mechanisms that take several days to develop and are designed to react / eliminate a specific antigen.

1. Specificity
2. Diversity
3. Self and Non-self recognisation
4. Memory formation recognisation.

Adaptive immune responses involves:-

- Antigen-presenting cells (APCs) such as macrophages and dendritic cells.
- The activation and proliferation of antigen-specific B-lymphocytes
- The activation and proliferation of antigen-specific T-lymphocytes.
- The production of antibody molecules, cytotoxic T-lymphocytes (CTLs) and cytokines.

Types of Acquired Immunity (based on mode of development)

Active

Generation of antibody on antigen exposure

- Natural
- Artificial (vaccines)

Passive

Generated antibody provide defence against Ag (Antigen

- Natural (Colostrum)
- Artificial (Anti venam, Tetanus toxoid)

Adaptive Immunity

★ Lymphocytes and antigen-presenting cells (APC) cooperate in Adaptive Immunity.

∴ An effective immune response involves two major groups of cell:- (i) Lymphocytes
(ii) Antigen-presenting cells (APC)

(i) Lymphocytes:-

Lymphocytes are one of many types of white blood cells (WBCs) produced in bone marrow by the process of hematopoiesis.

Lymphocytes leave the bone marrow, circulate in the blood and lymphatic systems and reside in various lymphoid organs. Because they produce and display antigen binding cell surface receptors, lymphocytes mediate the defining immunologic attributes of specificity, diversity, memory and self-nonself recognition.

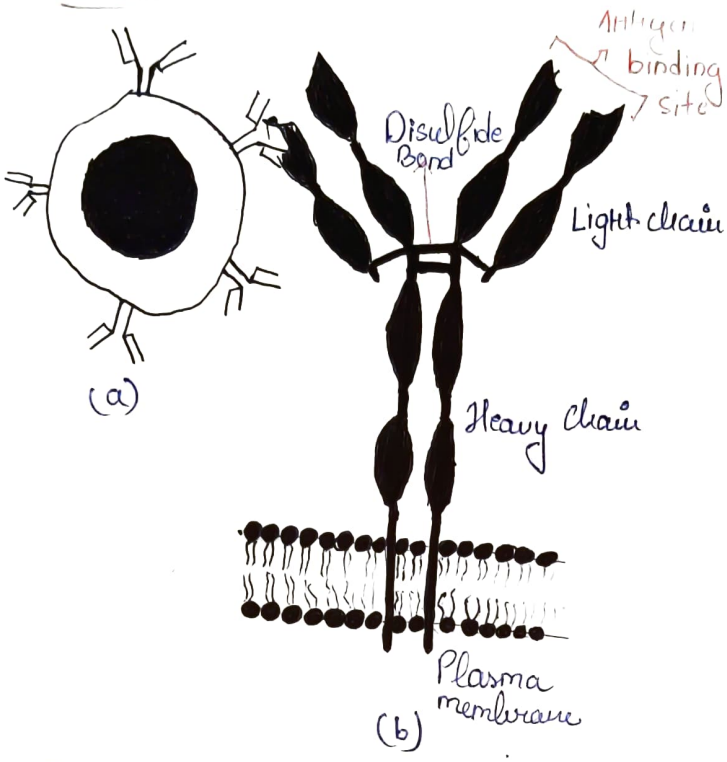
- B lymphocytes (B cells)
- T lymphocytes (T cells)

∴ B lymphocytes:-

B lymphocytes mature in bone marrow, and release, each expresses a unique antigen-binding receptor on its membrane. This antigen-binding or B-cell receptor is a membrane-bound antigen molecule.

Antibodies are glycoproteins that consist of two shorter, identical polypeptides called **heavy chains** and two shorter, identical polypeptides called **light chains**.

B cell



(a) The surface of B cells host about 10^5 molecules of membrane bound antibody per cell.

(b) Membrane bound and soluble (secreted) antibody showing the heavy chains (blue) and light chains (red).

∴ Each heavy chain is joined to a light chain by disulfide bonds, and the heavy/light chain pairs are linked together by additional disulfide bonds.

∴ The amino-terminal ends of the pairs of heavy and light chains form a site to which antigen binds.

∴ When a naive B cell (one that has not previously encountered antigen) first encounters the antigen that matches its membrane-bound antibody, the binding of the antigen to the antibody causes the cell to divide rapidly.

Its progeny differentiate into memory B cells and effector B cells called plasma cells. Memory B cells have a longer life span than naive cells, and they express the same membrane bound antibody as their parent B cell.

Plasma cells produce the antibody in a form that can be secreted and have a little or no membrane-bound antibody. Secreted antibodies are the major effector molecules of humoral immunity.

T lymphocytes :—

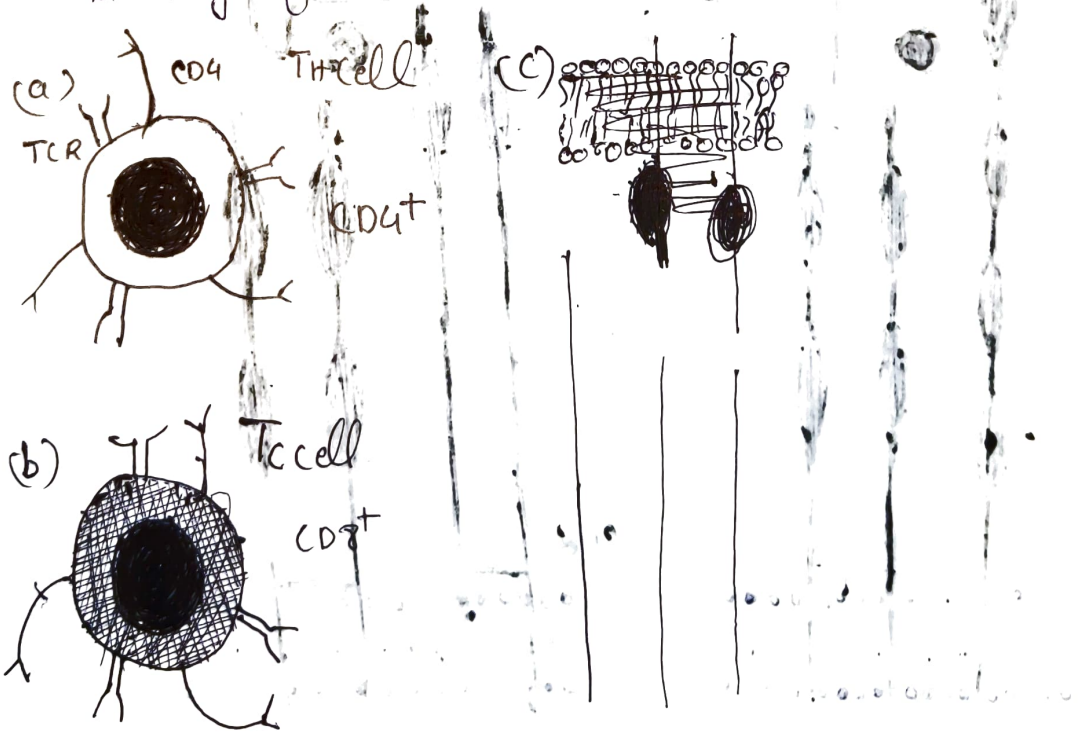
∴ T lymphocytes also arise in the bone marrow. Unlike B cells which mature in the marrow, T cells migrate to the Thymus to mature.

Maturing T cells express a unique antigen-binding molecule, the T-cell receptor (TCR), on their membranes.

- There are two well-defined subpopulations of T cells:-
  - ① T helper (T_H) cells
  - ② T cytotoxic (T_C) cells

* The T helper and T cytotoxic cells distinguished from one another by the presence of either $CD4^+$ or $CD8^+$ membrane glycoproteins on their surfaces.

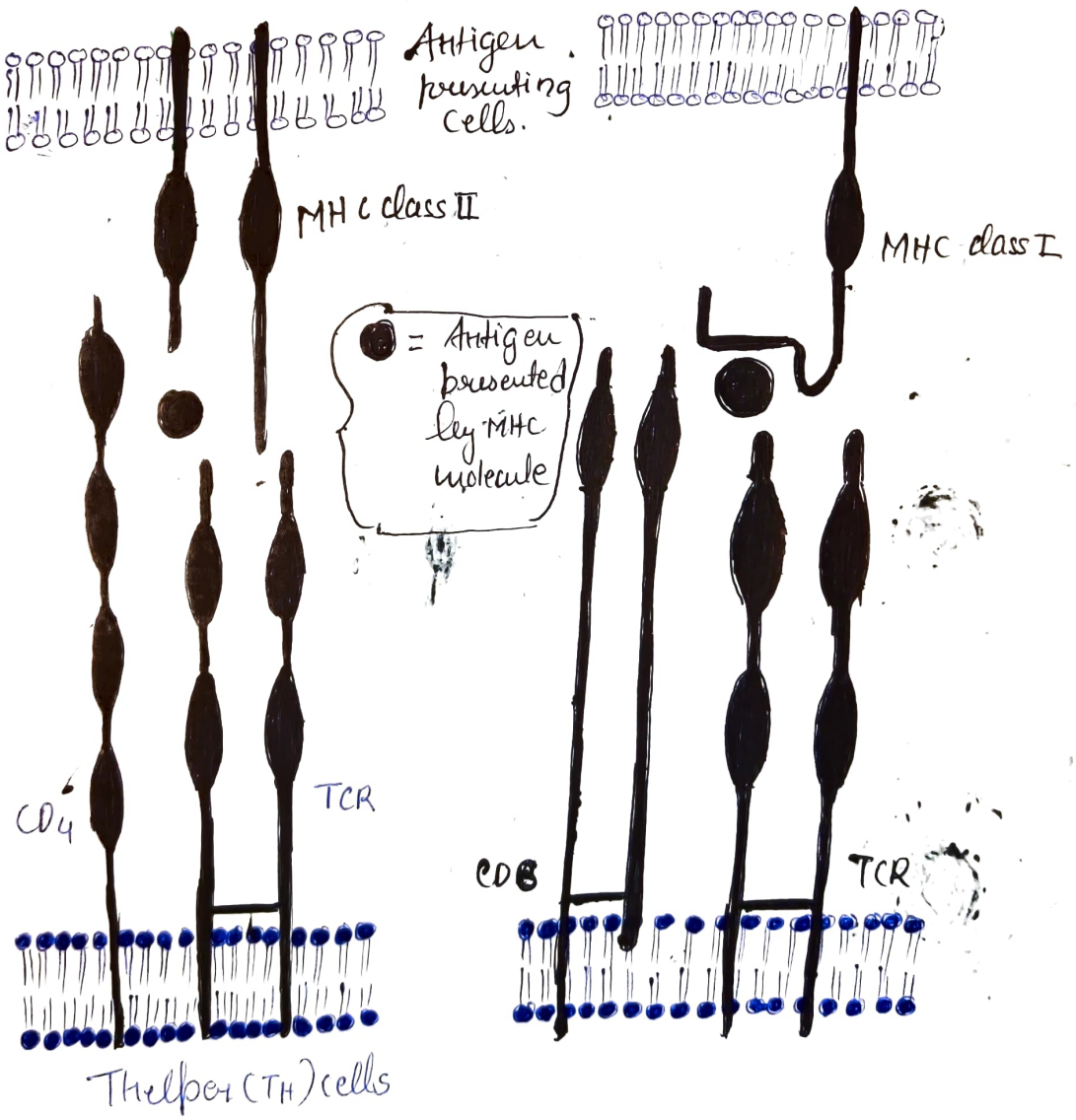
- T cell displaying $CD4^+$ generally function as T_H cells, whereas those displaying $CD8^+$ generally function as T_C cells.
- A recently characterized third type of T cell, called a T regulatory (T_{reg}) cell carries $CD4^+$ on its surface markers associated with its stage of activation.



cellular membrane - bound antibodies on B cells

cells recognise bare antigen most T-cell receptors can recognize only antigen that is bound to cell membrane proteins called major histocompatibility complex (MHC) molecules.

- MHC molecules are polymorphic (genetically diverse) glycoproteins found on cell membranes.
- MHC are of two types, MHC I and MHC II. MHC I are expressed by nearly all nucleated cells of species and MHC II molecules which are expressed only by antigen-presenting cells (APCs).



Antigen-presenting cells interact with T cells:-

Activation of both the humoral and cell-mediated branches of immune system requires cytokines produced by T_H cells. It is essential that activation of T_H cells themselves be carefully regulated, because a T-cell response directed against self components can have fatal autoimmune consequences.

- One safeguard against unregulated activation of T_H cells is that the antigen receptors of T_H cells can recognize only antigen that is displayed together with class II MHC molecules on the surface of antigen-presenting cells.

These specialized cells which includes macrophages, B lymphocytes and dendritic cells are distinguished by two properties:-

(i) they express class II MHC molecules on their membranes

(ii) they can produce cytokines that cause T_H cells to become activated.

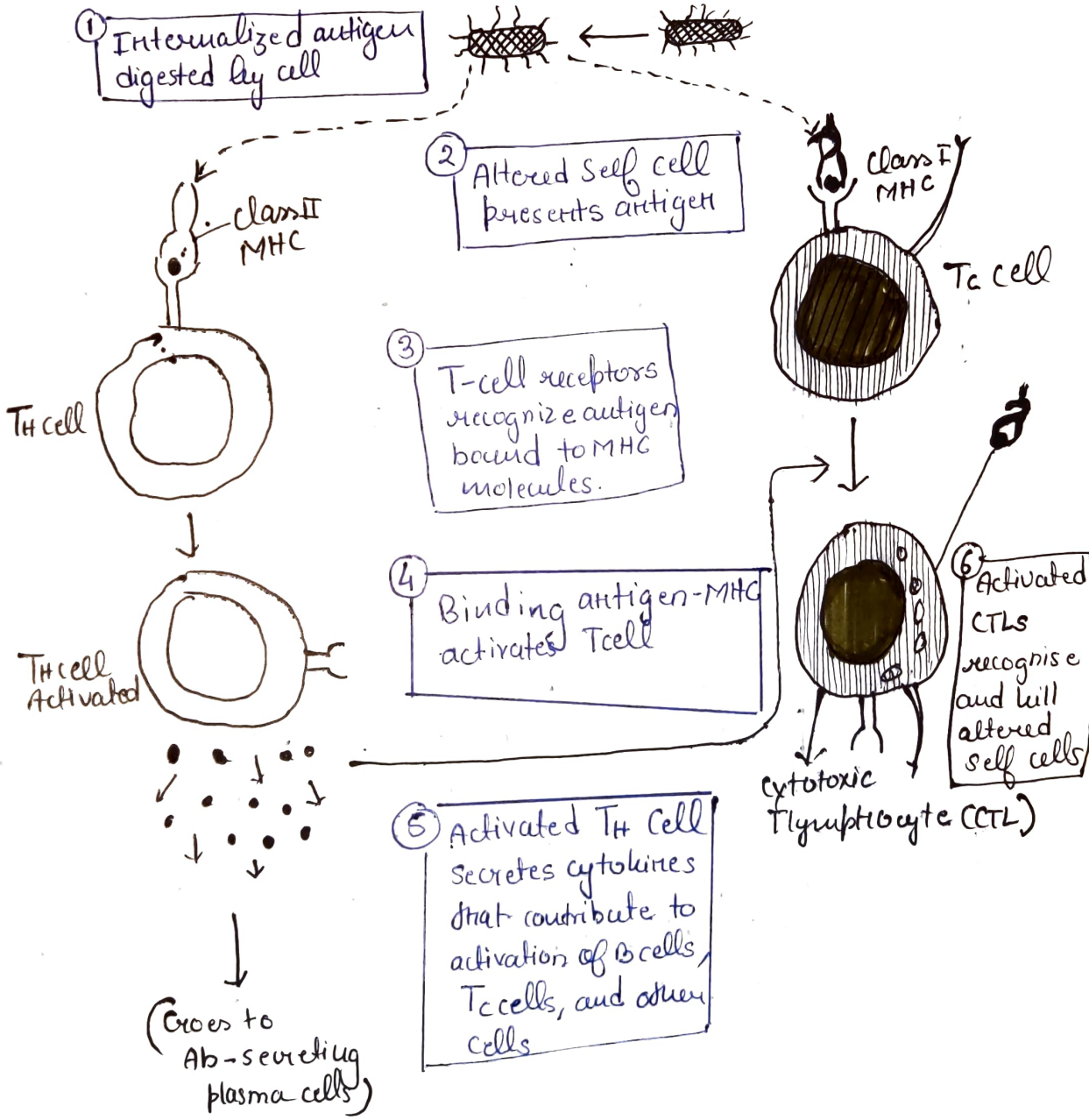
Antigen presenting cells (APCs) first internalize antigen by either phagocytosis or endocytosis, and then display a part of that antigen on their membrane bound to a class II MHC molecule. The T_H cell interacts with the antigen-class II MHC molecule complex on the membrane of the antigen-presenting cell (APC). APC then produces an additional signal leading to activation of the T_H cell.

The antigen receptors of B and T lymphocytes are diverse :-

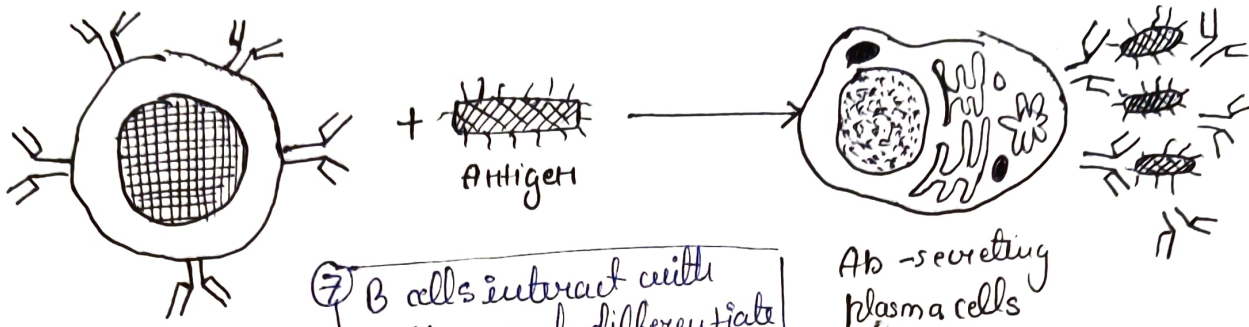
The antigenic specificity of each B cell is determined by the membrane-bound antigen-binding receptor (i.e., antibody) expressed by the cell.

- The Humoral and Cell-Mediated Branches of Immune System.

✯ Cell Mediated response



★ Humoral response :-



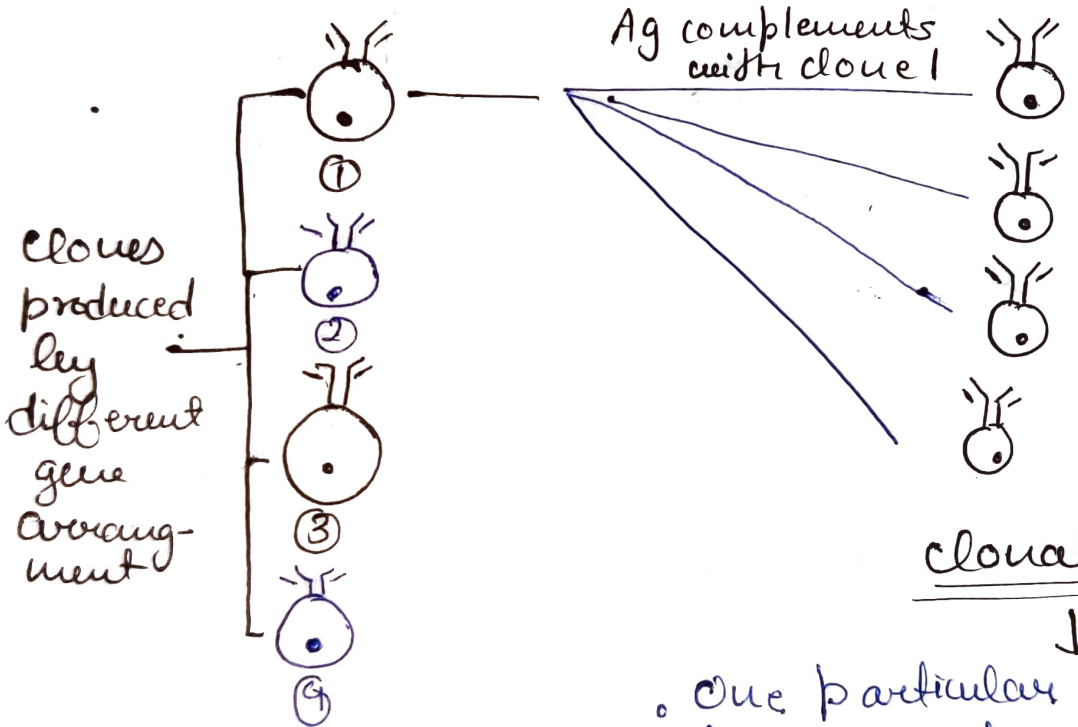
(7) B cells interact with antigen and differentiate into antibody-secreting plasma cells.

(8) Antibody binds antigen and facilitates its clearance from the body.

- In Humoral response, B cells interact with antigen and then differentiate into antibody-secreting plasma cells. The secreted antibody binds to the antigen and facilitates its clearance from the body.
- In cell mediated response, various sub-populations of T cells recognise antigen presented on self cells. T_H cells respond to antigen by producing cytokines.
  Tc cells respond to antigen by developing into cytotoxic T lymphocytes (CTLs), which mediate killing of altered self cells (e.g. virus-infected cells)

- As B cells matures in bone marrow, its specificity is created by random rearrangements of a series of gene segments that encode the antibody molecule – At maturity, each B cell possesses a single functional gene encoding the antibody heavy chain and a single functional gene encoding the antibody light chain. All antibody molecules on a given B lymphocyte have identical specificity; giving each B lymphocyte, the clone of daughter cells to which it gives rise, a distinct specificity for single antigen. The mature B lymphocyte is therefore said to be Antigenically committed.

- The attributes of specificity and diversity also characterize the antigen-binding T-cell receptor on T cells. As in B-cell maturation, the process of T-cell maturation includes random rearrangements of a series of gene segments that encode the cells antigen-binding receptor.



★ structure of variable region of Ab is different

clonal selection

↓

- One particular clone of B-cell is selected upon specific exposure to an Ag complementory for the Ab on that clone.
- Prdiferation and maturation of only that clone bellow giving rise to Plasma B-cell and memory cells

- B cells - Antigenitically committed.

Lymphocytes Synthesis

- **Primary lymphoid Organs:-**
  - lymphoid organs provide appropriate microenvironments for the development and maturation of lymphocytes. (Bone marrow, Thymoxine)
- **Secondary lymphoid organs:-**
  - Trap antigen, generally from nearby tissues or vascular spaces and are sites where mature lymphocytes can interact effectively with antigen (Spleen, Payer's Patch).

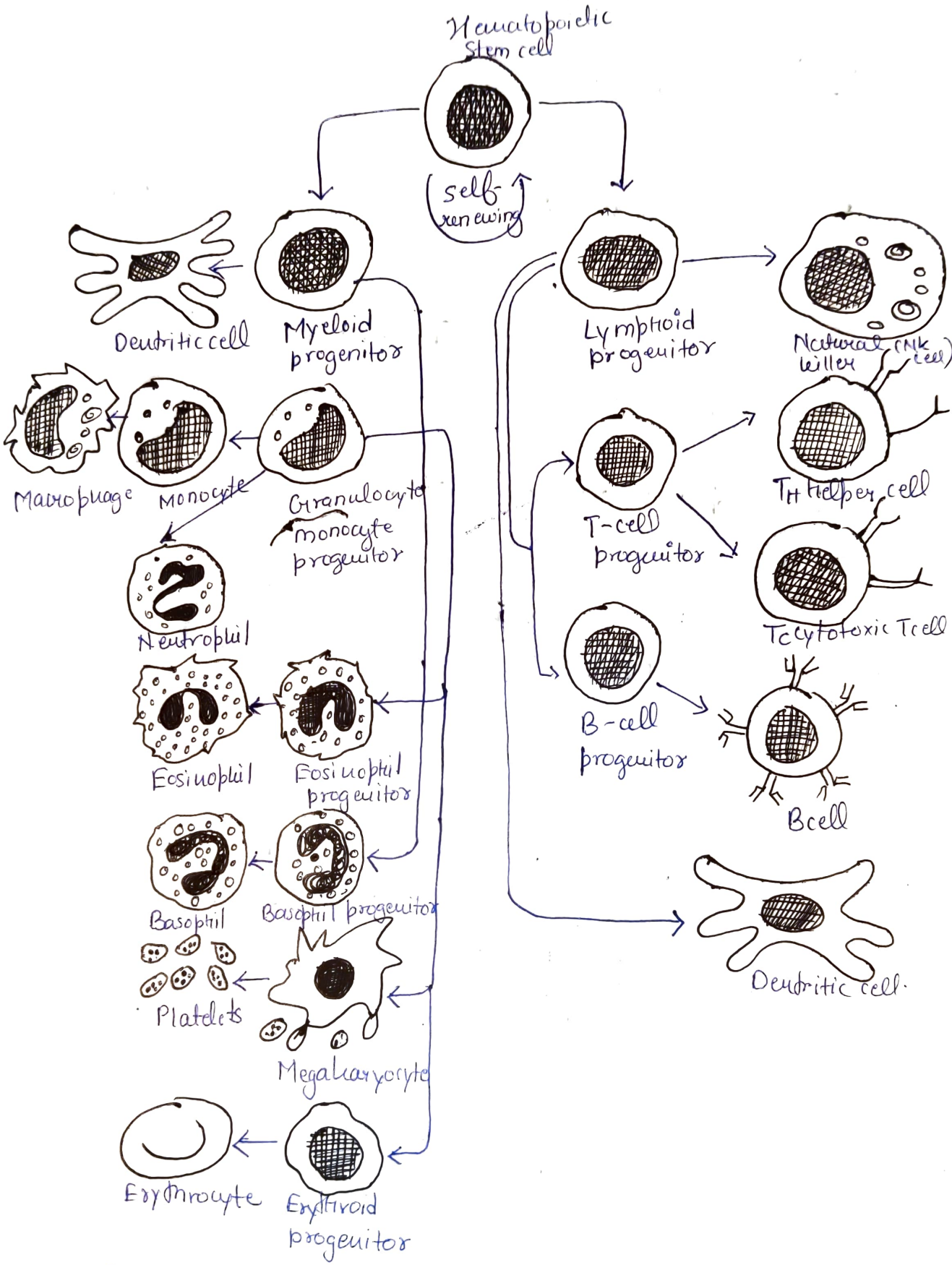
★ Hematopoiesis:-

- All blood cells arise from a type of cell called the Hematopoietic stem cell (HSC).
- Stem cells are cells that can differentiate into other cell types.

In humans, hematopoiesis, the formation and development of red and white blood cells, begins in the embryonic yolk sac during the first weeks of development. Yolk sac stem cells differentiate into primitive erythroid cells that contain embryonic hemoglobin.

- By third month of gestation, hematopoietic stem cells have migrated from the yolk sac to the fetal liver and subsequently colonize the spleen; these two organs have major roles in hematopoiesis from the third to seventh months of gestation.

Hematopoiesis



★ Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. All lymphoid cells descend from lymphoid progenitor cells and all cells of the myeloid lineage arise from myeloid progenitors.

Natural killer Cells:-

↓

Lymphatic Origin (Component of Innate Immune System)

- Natural killer cells are part of innate immune system and most do not have T-cell receptors or immunoglobulin incorporated in their plasma membranes; in other words, they do not express the membrane molecules and receptors that distinguish T- and B-cell lineages.

- NK cells recognize potential target cells in two different ways. A NK cell employ NK-cell receptors to distinguish abnormalities, such as a reduction in the display of class I MHC molecules or unusual profile of surface antigens displayed by some tumor cells and cells infected by some viruses.

Natural killer (NK) cell.

- In addition, some tumor cells and cells infected by certain viruses display antigens against which the immune system has raised an antibody response so that antitumor or antiviral antibodies are bound to their surfaces. Because NK cells express a membrane receptor (CD16) for a specific region of the antibody molecule, they can attach to these antibodies and subsequently destroy the targeted cells. This is an example of a process known as Antibody-dependent cell-mediated cytotoxicity (ADCC).

ANTIGENS AND ANTIBODIES

- The Hallmark Molecules Of The Adaptive Immune system are the antibody and the T-cell receptor.

The antibody and T-cell receptor molecules display a higher degree of specificity, recognizing specific antigenic determinants or epitopes.

Epitopes are the immunologically active regions of an immunogen that bind to antigen-specific membrane receptors on lymphocytes or to secreted antibodes.

- Secreted antibodies circulate in the blood, where they serve as the effectors of humoral immunity by searching out antigens and marking them for elimination.

The T-cell receptor expressed on the surface membrane of the T cell recognizes only processed antigen fragments that are complexed with MHC molecules.

- In general, the population of antibodies produced in response to a particular antigenic stimulus is heterogeneous. Most antigens are structurally complex, containing many different epitopes, and the immune system usually responds by producing antibodies to several of the epitopes on the antigen.

In other words, several different B-cell clones are stimulated and proliferate. The output of the plasma cells of a single B-cell clone is a monoclonal antibody that specifically binds a single antigenic determinant. Together the secreted products of all stimulated B-cell clones, the group of monoclonal antibody, make up the **polyclonal** and heterogenous serum antibody response to an immunizing antigen.

Members of the antibody protein family have common structural features and are known collectively as immunoglobulins (Igs).

∴ Immunogenicity Versus Antigenicity :-

Immunogenicity is the ability to induce a humoral and/or cell mediated immune response :-

B cells + antigen → effector B cells + memory B cells
↓
Plasma cell → Secretes antibody

T cells + antigen → effector T cells + memory T cells
↓
CTLs, T_H, etc → Secretes cytokines and cytotoxic factors

Although a substance that induces a specific immune response is usually called an antigen, it is more appropriately called an immunogen.

Antigenicity is the ability to combine specifically with the final products of the above responses (i.e., Secreted antibody / or surface receptors on T cells). Although all molecules that have the property of immunogenicity also have the property of antigenicity, the reverse is not true. Some small molecules, called **haptens**, are antigenic but incapable by themselves of inducing a specific immune response. In other words, they lack immunogenicity.

HAPTENS

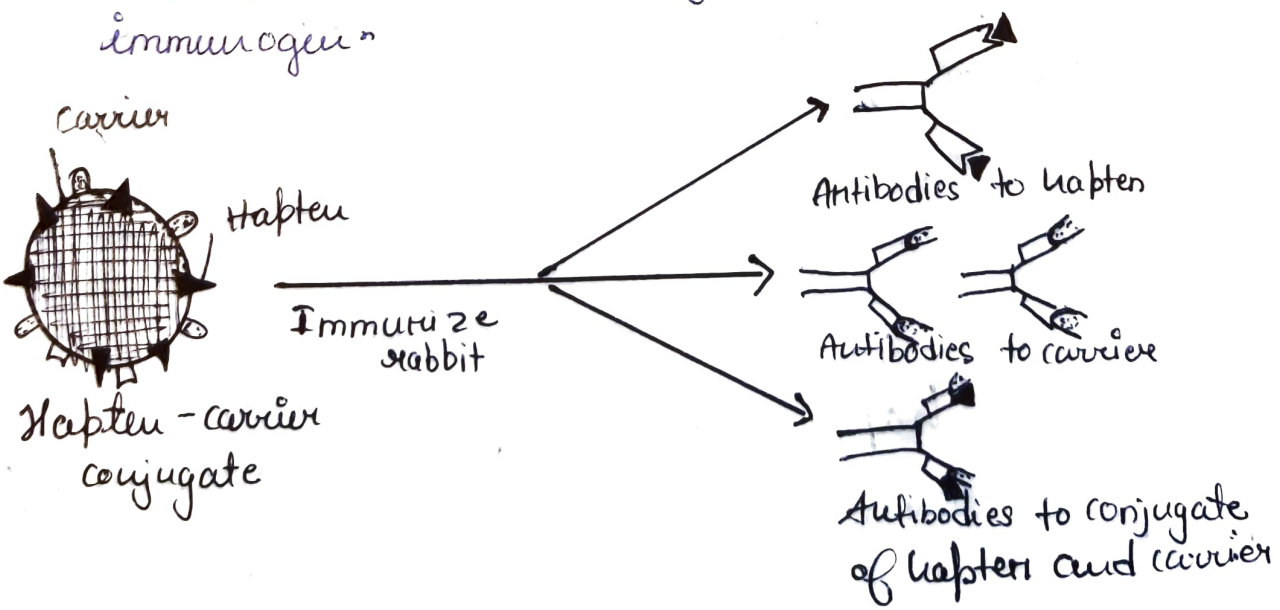
- Haptens are antigenic but incapable of inducing a specific immune response. In other words, they lack immunogenicity.

The chemical coupling of a hapten to a large immunogenic protein called a carrier, yields an immunogenic hapten-carrier conjugate.

Animals immunized with such a conjugate produce antibodies specific for three types of antigenic determinant:-

(1) the hapten determinant
(2) unaltered epitopes on the carrier protein
(3) new epitopes formed by regions of both the hapten and the carrier molecule in combination.

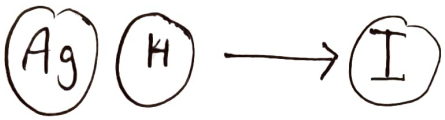
∴ By itself, a hapten cannot function as an immunogenic epitope. But when multiple molecules of a single hapten are coupled to a carrier protein, the hapten becomes accessible to the immune system and can function as an immunogen.



Adjuvants :-

⊙ Adjuvants (from the Latin **adjuvare**, to help) are substances that, when mixed with an antigen and injected with it, enhance the immunogenicity of that antigen.

- Adjuvants are often used in research and clinical settings to boost the immune response when an antigen has low immunogenicity or when only small amounts of an antigen has low immunogenicity or when only small amounts of an antigen are available.



eg: Keyhose Limpet Hemocyanin (KLH) "protein".
Bovine Serum Albumin (BSA)

- **Water-in-oil adjuvants :-**

They prolong the persistence of antigen. A preparation known as **Freund's incomplete adjuvant** contains antigen in aqueous solution, mineral oil, and an emulsifying agent such as **mannide monooleate**, which disperses the oil into small droplets surrounding the antigen; the antigen is then released very slowly from the site of injection.

- **Freund's complete adjuvant:-**

The first deliberately formulated highly effective adjuvant, developed by **Jules Freund** many years ago and containing **heat-killed Mycobacteria** as an additional ingredient. **Muramyl dipeptide**, a component of the mycobacterial cell wall, activates dendritic cells and macrophages, making Freund's complete adjuvant far more potent than the incomplete form.

∴ Epitopes :-

Lymphocytes recognize discrete sites on the macromolecule called epitopes, or antigenic determinants. Studies with small antigens have revealed that B and T cells recognize different epitopes on the same antigenic molecule.

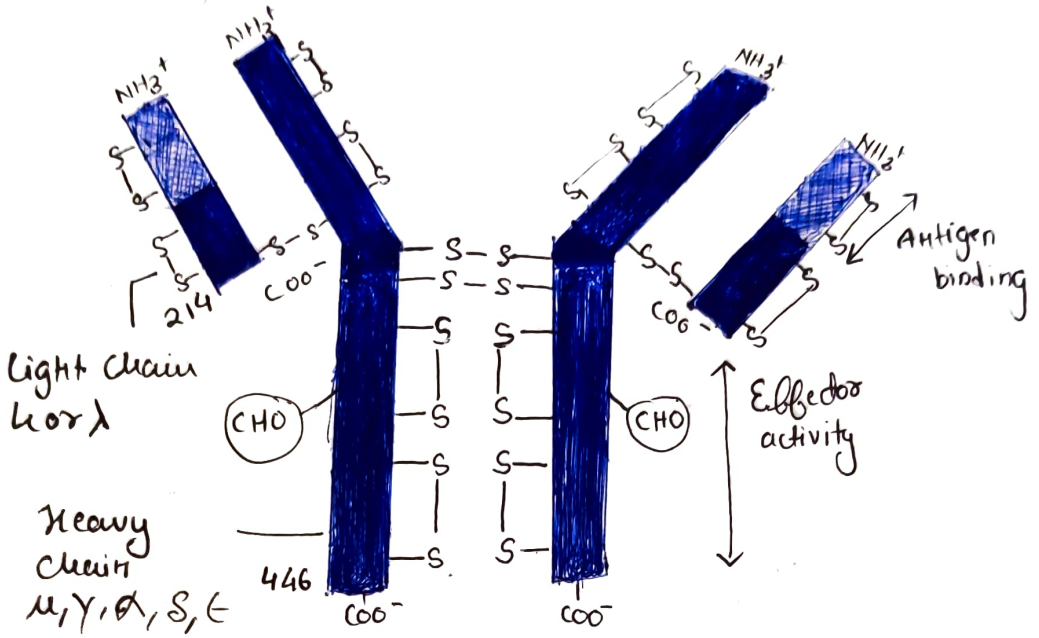
For example, when mice were immunized with glucagon, a small human hormone of 29 amino acids, antibody was elicited to epitopes in amino-terminal portion, whereas T cells responded only to epitopes in the carboxyl-terminal portion.

- B cell recognize soluble antigen when it binds to antibody molecules in the B-cell membrane. Because B cells bind antigen that is free in solution, the epitopes they recognize tend to be highly accessible sites on the exposed surface of the immunogen.
- T-cells epitopes from proteins differ in that they are ~~are~~ peptides usually derived from enzymatic digestion of pathogen proteins and are recognized by the T-cell receptor only when in complex with an MHC antigen. Thus there is no requirement for solution accessibility such as with the B-cell epitope.

★ Comparison of antigen recognition by T cells and B cells.

Characteristic	B cells	T cells
Interaction with antigen	Involves binary complete of membrane Ig and Ag	Involves ternary complex of T-cell receptor, Ag, MHC
Binding of soluble antigen	Yes	NO
Involvement of MHC molecules	None required	Required to display processed antigen
Chemical nature of antigens	Protein, polysaccharide, lipid	Mostly proteins, but some lipids and glycolipids presented on MHC like molecule.
Epitope properties	Accessible, hydrophillic, mobile peptides containing sequential or nonsequential amino acids	Internal linear peptides produced by processing of antigen and bound to MHC molecules.

ANTIBODY STRUCTURE



- Antibody molecules have a common structure of four peptide chains. This structure consists of two identical **light (L) chains**, polypeptide of about 22,000 Da, and two identical **heavy (H) chains**. Each light chain is bound to a heavy chain by a disulfide bond and by monocovalent interactions such as salt linkages, hydrogen bonds, and hydrophobic interactions to form a heterodimer (H-L).

- The first 110 or amino acids of the amino-terminal region of a light or heavy chain varies greatly among antibodies of different antigen specificity.

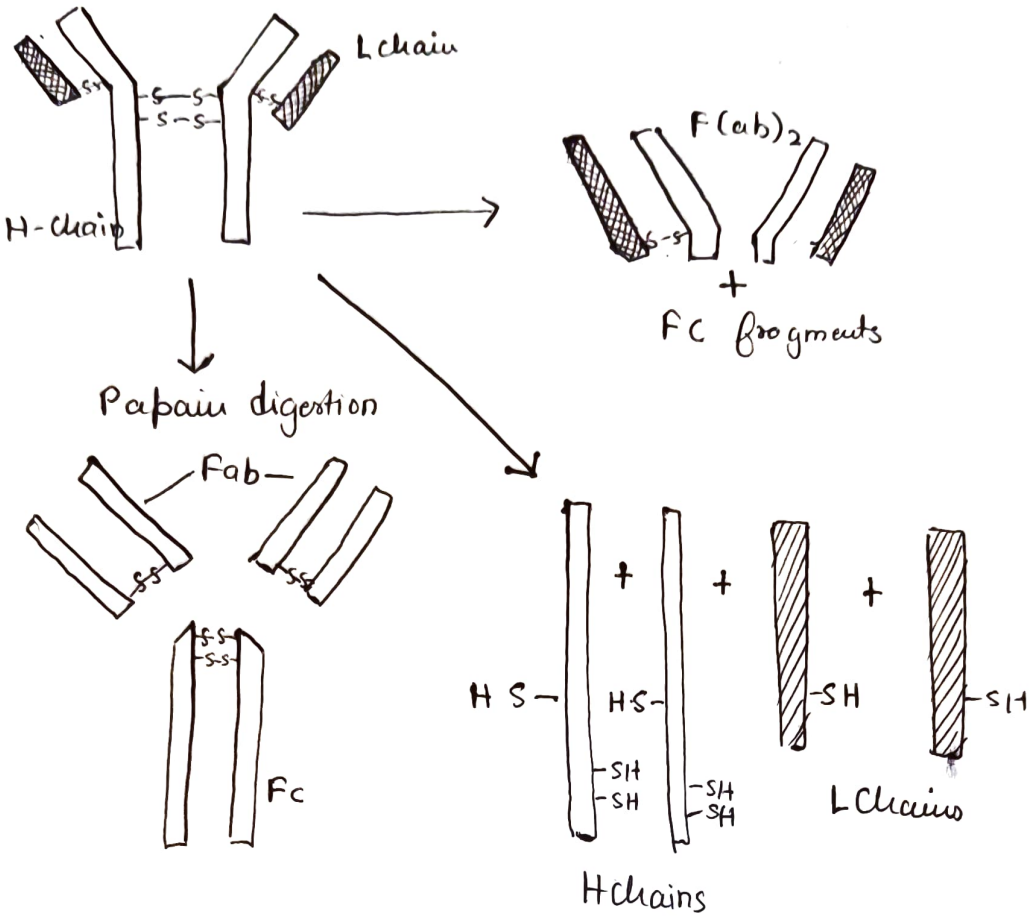
- These segments of highly variable sequence called **V regions**: **V_L in light chains and V_H in heavy**. All of the difference in specificity displayed by different antibodies can be traced to differences in the amino acid sequences of V regions.

In fact, most of the differences among antibodies fall within areas of the V regions called **complementarity-determining regions (CDRs)**.

- It is these CDRs, on both light and heavy chains, that constitute the antigen binding site of the antibody molecule.
  By contrast, within each particular class of antibody far fewer differences are seen when one compares sequences throughout the rest of the molecule.
- The regions of relatively constant sequence beyond the variable regions have been dubbed C regions, C_L in light chains and C_H in heavy. Antibodies are glycoproteins, with few exceptions, the site of attachment for carbohydrates are restricted to the constant region.
  - It probably increases the solubility of the molecule.

Chemical Digestion

- In a key experiment, brief digestion of IgG with proteolytic enzyme papain produced three fragments, two of which were identical, with a third that was quite different.
- The two identical fragments (45,000 Da each) had antigen-binding activity and were called Fab fragments ("fragment, antigen binding")
  The other fragment (50,000 Da) had no antigen-binding activity at all.
  Because it was found to crystallise during cold storage, it was called the Fc fragment ("fragment, crystallizable").
- Digestion of IgG with a different proteolytic enzyme, pepsin, demonstrated again that the antigen-binding properties of an antibody can be separated from the rest of the molecule. Pepsin digestion generated a single 100,000 Da fragment, composed of two Fab-like subunits designed the F(ab)$_2$ fragment, which binds antigen. The Fc fragment was not recovered from pepsin treatment because it had been digested into numerous small peptides.



- The antibody to the Fab fragment could react with both the H and the L chains, whereas the antibody to the Fc fragment reacted only with the H chain.

These observations led to the conclusion that the Fab fragment consists of a portion of a heavy plus an intact light chain and the Fc contains only heavy-chain components.

★ Light-chain sequences revealed constant and variable regions :-

The amino-terminal half of the chain, consisting of 100 to 110 amino acids, was found to vary among different Bence-Jones proteins.

This region was called the variable (V) region.

- The carboxyl-terminal halves of the molecules, called the constant (C) region, had one of two amino acid sequences.

This led to the recognition that there were two light chain types, kappa(κ) and lambda (λ).

- In humans, 60% of light chains are kappa and 40% are lambda, whereas in mice, 95% of light chains are kappa and only 5% are λ.

★ A normal antibody molecule contains only one light-chain type, either κ or λ never both.

- The amino acid sequences of λ light-chain constant regions show minor differences that are used to classify λ light chains into subtypes.

In mice and in humans, there are four subtypes: λ1, λ2, λ3 and λ4. Amino acid substitutions at only a few positions are responsible for the subtype differences.

★ <u>There are five major classes of heavy chains</u>

- For heavy-chain sequence analysis, myeloma proteins were reduced with mercaptoethanol and alkylated and the heavy chains were separated by gel filtration in a denaturing solvent.

- The remaining part of the protein revealed five basic sequence patterns, corresponding to five different heavy-chain constant (C) regions: μ, δ, γ, ε and α

- Each of these five different heavy chains is called an isotype. The lengths of constant regions is approx 330 amino acids for δ, γ and α and 440 amino acids for μ and ε.

- The heavy chains of a given antibody molecule determine the class of that antibody: - IgM(μ), IgG(γ), IgA(α), IgD(δ) or IgE(ϵ). H chains of each class may pair with either κ or λ light chains. A single antibody molecule has two identical heavy chains and two identical light-chains, H_2L_2 or $(H_2L_2)_n$ of this basic four-chain structure.

Class	Heavy Chain	Subclasses	Light Chain	Molecular formula.
IgG	γ	$\gamma^1, \gamma^2, \gamma^3$ γ^4	κ or λ	$\gamma_2\kappa_2$ $\gamma_2\lambda_2$
IgM	μ	None	κ or λ	$(\mu_2\kappa_2)_n$ $(\mu_2\lambda_2)_n$ $n=1$ or 5
IgA	α	$\alpha 1 \alpha 2$	κ or λ	$(\alpha_2\kappa_2)_n$ $(\alpha_2\lambda_2)_n$ $n=1,2,3,4$
IgE	ϵ	None	κ or λ	$\epsilon_2\kappa_2$ $\epsilon_2\lambda_2$
IgD	δ	None	κ or λ	$\delta_2\kappa_2$ $\delta_2\lambda_2$

Types of Immunoglobulin

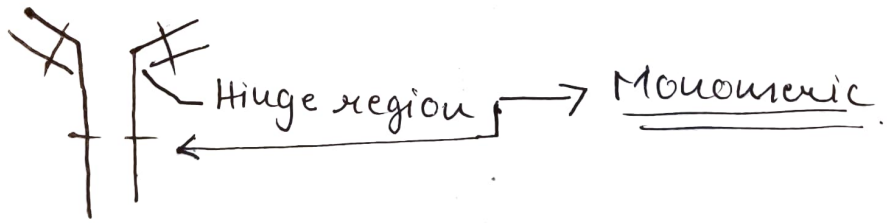
① IgG – Immunoglobulin G.

- IgG, the most abundant class in serum, constitutes about 80% of the total serum immunoglobulin.
- The IgG molecule consists of two γ heavy chain and two κ or two λ light chains.

There are four human IgG subclasses, distinguished by differences in γ-chain constant-region sequences and numbered according to their decreasing average serum concentrations: IgG1, IgG2, IgG3, and IgG4

IgG1
IgG2
IgG3 } Isotypes
IgG4 → cannot activate complement system.

- 150 kDa; — only antibody that can cross placenta. Provides Natural Passive Immunity for the foetus.



Function: –

- It neutralise toxin
- IgG3 and IgG1 activates complement system (example of synchrony of working of innate and acquired.)
- Binds to Fc receptors or Phagocytic cells culminating in a process called as Opsonisation.

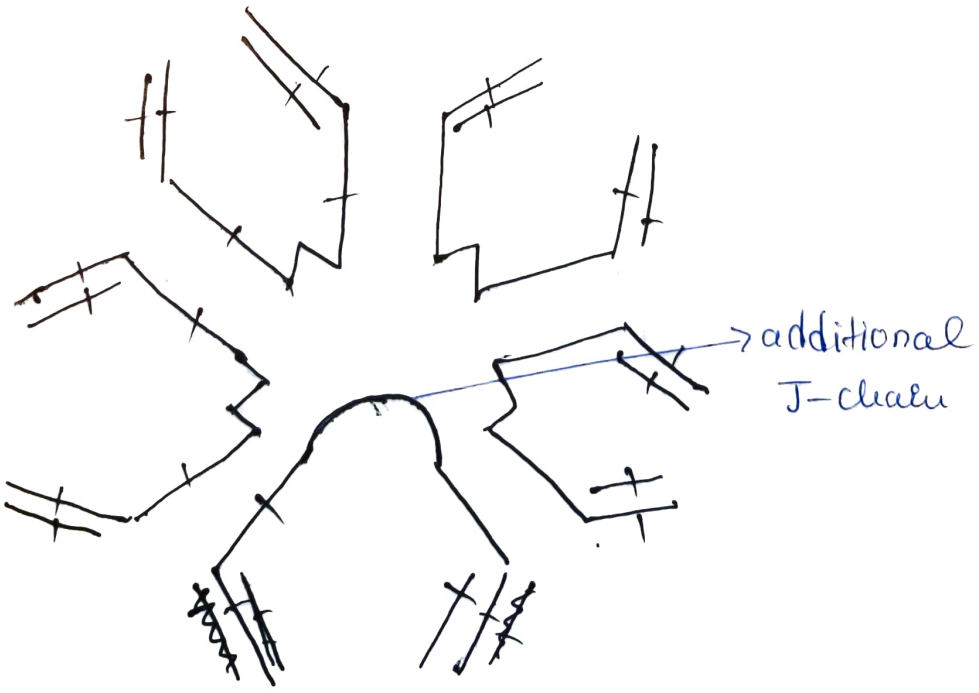
② Immunoglobulin M (IgM):-

IgM accounts for 5% to 10% of total serum immunoglobin, with an average serum concentration of 1.5 mg/ml.

Monomeric IgM (180,000 Da) is expressed as membrane-bound antibody on B cells.

- IgM is secreted by plasma cells as a pentamer in which five monomeric units are held together by disulfide bonds that link their carboxyl-terminal heavy-chain domains.

- The monomeric subunits are arranged with their Fc region the center of the pentame and 10 antigen binding sites on the periphery of the molecule. Each penta contains an additional Fc-linked polypeptide called joining chain, which is disulfide-bonded to boxyl-terminal cysteine residue of two of the 10μ chain. The J chain appears to be required for polymerization the monomers to form pentameric IgM; it is added before secretion of the pentamer.



- Found in lymph and earliest antibody to secreted by bacteria.

Function:-

1. Perform Agglutination Reaction
2. More efficient in Complement Activities → innate
3. Act as a secretory Ig.

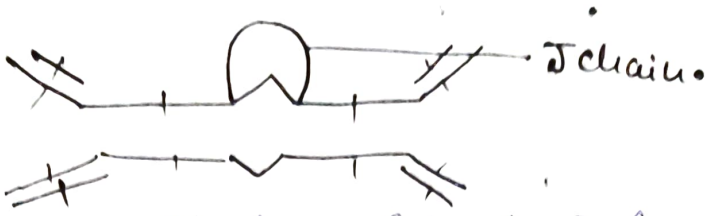
3. Immunoglobulin A (IgA)

2nd most abundant. Although IgA constitutes only 10% to 15% of total immunoglobulin in serum, it is the predominant immunoglobulin class in external secretions such as breast milk, saliva, tears and mucus of bronchial, genitourinary and digestive tracts.

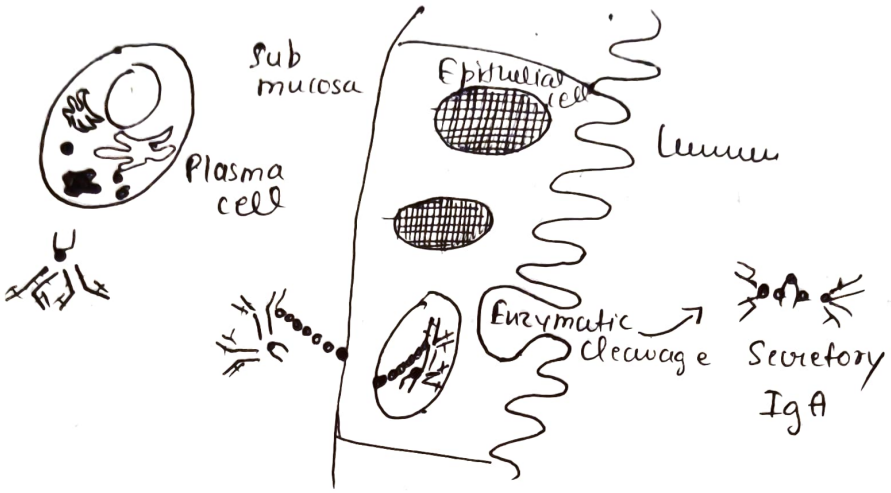
The IgA of external secretions, called Secretory IgA, consists of a dimer or tetramer, a J chain polypeptide, and a polypeptide chain called secretory component.

- Secretory component is derived from the receptor that is responsible for transporting polymeric IgA across cell membranes. The J-chain polypeptide in IgA is identical to that of IgM.

- IgA has been shown to provide an important line of defense against bacteria such as Salmonella, Vibrio Cholerae and Neisseria gonorrhoeae and viruses such as influenza, Polio. V STD



• Structure of Secretory IgA



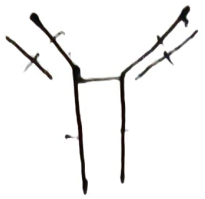
∴ Secretory ~~component~~ contains IgA is formed during transport through mucous membrane epithelial cells.

(4) Immunoglobulin D (IgD)

IgD was first discovered when a patient developed a multiple myeloma whose myeloma protein failed to react with anti-isotype antisera against the then-known isotypes: IgA, IgM, and IgG.

When rabbits were immunized with this myeloma protein, the resulting antisera were used to identify the same class of antibody at low levels in normal human serum.

- The new class called IgD, has a serum concentration of 30 µg/ml and constitutes about 0.2% of the total immunoglobulin in serum. IgD, together with IgM, is the major membrane-bound immunoglobulin expressed by mature B cells, and its role in the physiology of B cells is under investigation. No biological effector function has been identified for IgD.



150 kDa

- Surface of activated B cells.
- Functions as typical Ag-receptor.

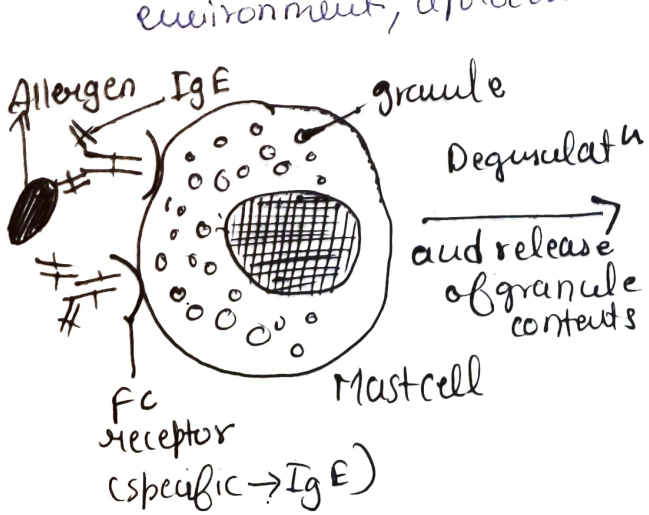
(5) Immunoglobulin E (IgE) :-

- The potent biological activity of IgE allowed it to be identified in serum despite its extremely low average serum concentration (0.3 μg/ml)
- IgE antibodies mediate the immediate hypersensitivity reactions that are responsible for allergic reactions was first demonstrated in 1921 by K. Prausnitz and H. Kustner, who injected serum from an allergic person intradermally into a non allergic individual.
  - a wheal, flare reaction developed there.

- This reaction is called P-K reaction.

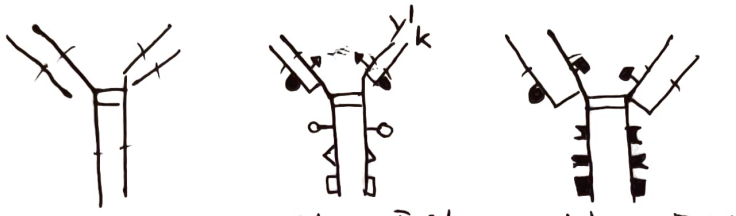
IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells.

Cross-linkage of receptor bound IgE molecules by antigen (allergen) induces basophils and mast cells to translocate their granules to the plasma membrane and release their contents to the extracellular environment, a process known as degranulation.



Isotype :-

- Isotypic determinants are constant region determinant that completely define each heavy chain class or subclass within a species.
- It also includes the light chain type and subtype all members of a species carry the same collection of constant region genes, which also include multiple alleles.



Mouse IgG1 Mouse IgM

Allotype :-

Although all members of a species inherit the same set of isotypic genes, there can be multiple allels for a gene. These allels differ minutely in the amino acid sequences and the sum of the such allotypic determinants, determines its allotype.

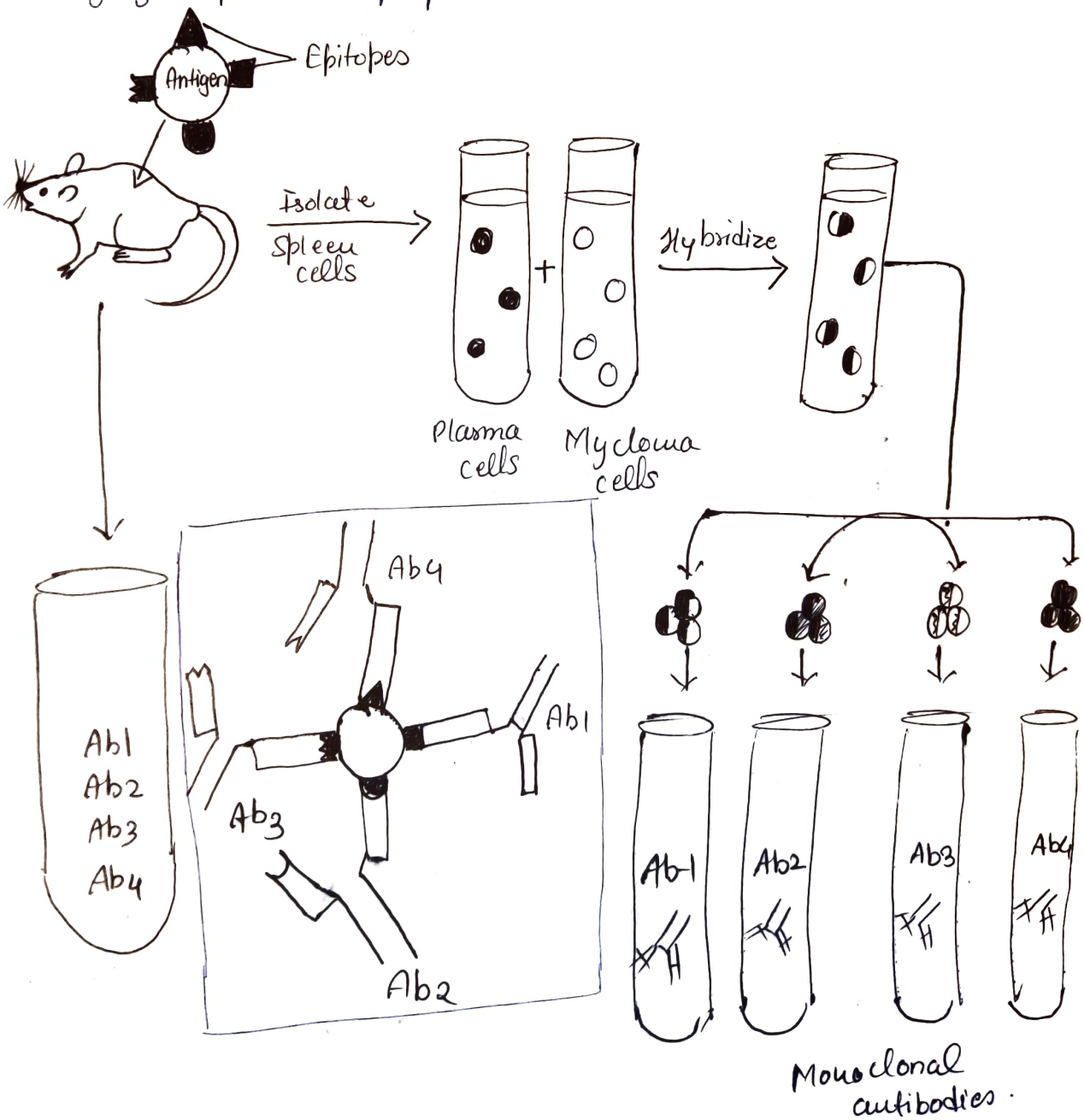
Mouse IgG1 (strain A) Mouse IgG1 (strain B)

Idiotype :-

Unique sequence of the V_H and V_L region of the antibody can also act as antigenic determinants. Each individual antigenic determinant is called an Idiotype, the sum of all Idiotope found in an organism is called Idiotype.

Monoclonal Antibodies

As noted earlier, most antigens offer multiple epitopes and therefore induce proliferation and differentiation of a variety of B-cell clones, each derived from a B cell that recognizes a particular epitope.



- The resulting serum antibodies are heterogeneous, comprising a mixture of antibodies, each specific for one epitope. Such a polyclonal antibody response facilitates the localization, phagocytosis and complement-mediated lysis of antigen; it thus has clear advantages for the organism in vivo.

- Unfortunately, the antibody heterogenity that increases immune protection in vivo often reduces the efficacy of an antiserum for various in vitro uses. For most research, diagnostic and therapeutic purposes, monoclonal antibody, derived from single clone and thus specific for a single epitope are preferable.

  - Direct biochemical purification of a monoclonal antibody from a polyclonal antibody preparation is not feasible.

- In 1975, Georges Köhler, and Cesar Milstein devised a method for preparing monoclonal antibody, which quickly became one of immunology's key technologies.

  - By fusing a normal activated, antibody-producing B cell with a myeloma cell (a cancerous plasma cell), they were able to generate a hybrid cell, called **hybridoma**, that possessed the immortal-growth properties of the myeloma cell and secreated the antibody by the B cell. The resulting clones of hybridoma cells which secrete large quantities of monoclonal antibody can be cultured indefinitely.

How to Measure Ag-Ab Interaction?

∴ **Antibody affinity** – quantitative measure of binding strength

The combined strength of noncovalent interactions between a single antigen-binding site on an antibody and a single epitope is the affinity of the antibody for that epitope.

– Low-affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high-affinity antibodies bind antigen more tightly and remain bound longer.

The association b/w a binding site on an antibody (Ab) with a monovalent antigen (Ag) can be described by the equation.

$$Ag + Ab \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ag\text{-}Ab$$

Where k_1 is the forward (association) rate constant
k_{-1} is reverse (dissociation) rate constant.

Ag-Ab complex to the molar concentration of bound antigen and antibody at equilibrium is as follows: –

$$K_a = \frac{[Ag\text{-}Ab]}{[Ab][Ag]}$$

Low-affinity Ag-Ab complexes have K_a values b/w 10^4 10^{11} L/mol; high-affinity complexes can have K_a values as 10^{11} L/mole.

For some purpose, the dissociation of antigen-antibody complex

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

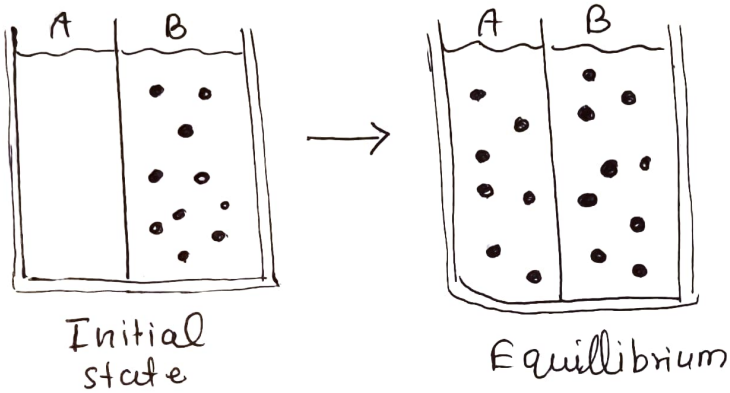
The equilibrium constant for this dissociation reaction the dissociation constant, is equal to the reciprocal of ka

$$K_d = \frac{[Ab][Ag]}{[Ab\text{-}Ag]} = 1/ka$$

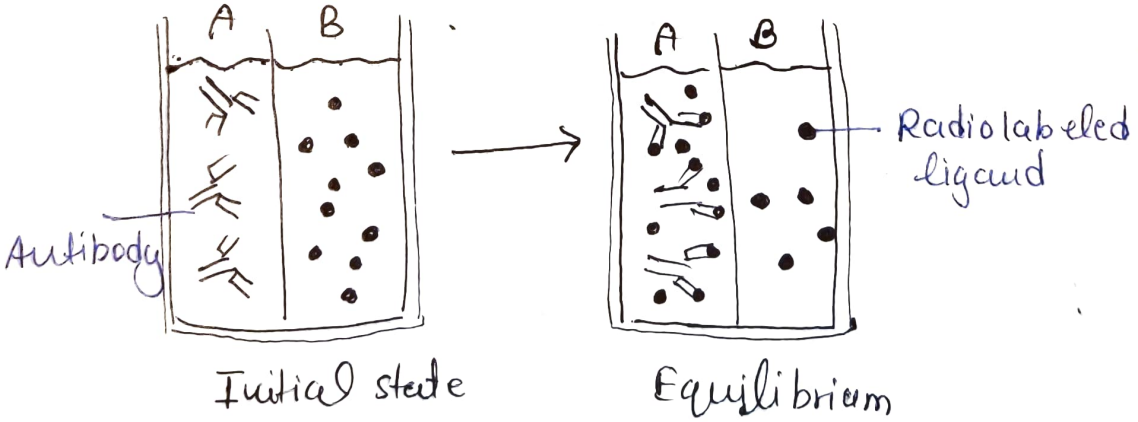
Techniques to Measure Ag-Ab Interaction

① Equilibrium Dialysis

control No antibody present



Experimental: Antibody in A.

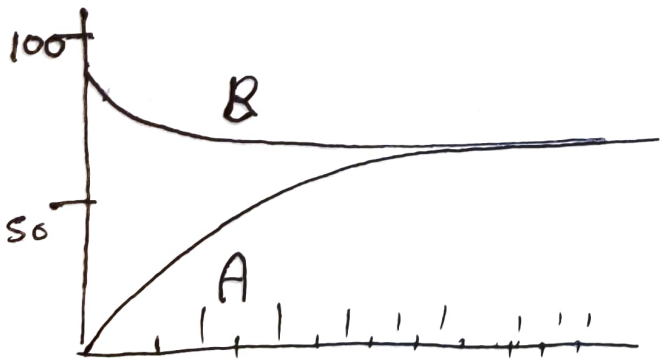


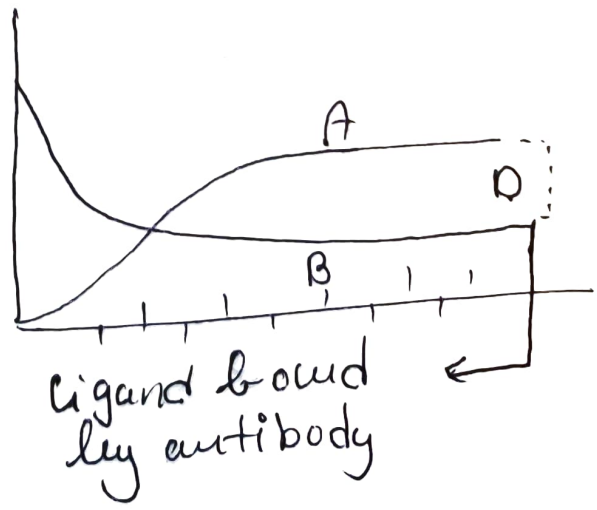
The affinity constant, ka, formerly determined by **equilibrium dialysis**, is now derived by newer methods, especially surface plasmon resonance (SPR)

Because equilibrium dialysis illustrates important principles and remains for some immunologists the standard against which other methods are evaluated.

- This procedure uses a dialysis chamber containing two equal compartments separated by a semipermeable membrane.
- Antibody is placed in one compartment, and a radioactively labeled ligand small enough to pass through the semipermeable membrane is placed in other compartment.
- Suitable ligands include Haptens, oligosaccharides and oligopeptides. In the absence of antibody, ligand added to compartment B will equilibrate on both sides of the membrane.
- In the presence of antibody, part of labelled ligand will be equally distributed in both compartments. Thus the total concentration in the two compartments represents the concentration of ligand bound to the antibody.

The more higher the affinity of the antibody, the more ligand is bound.





② Surface Plasmon Resonance (SPR)

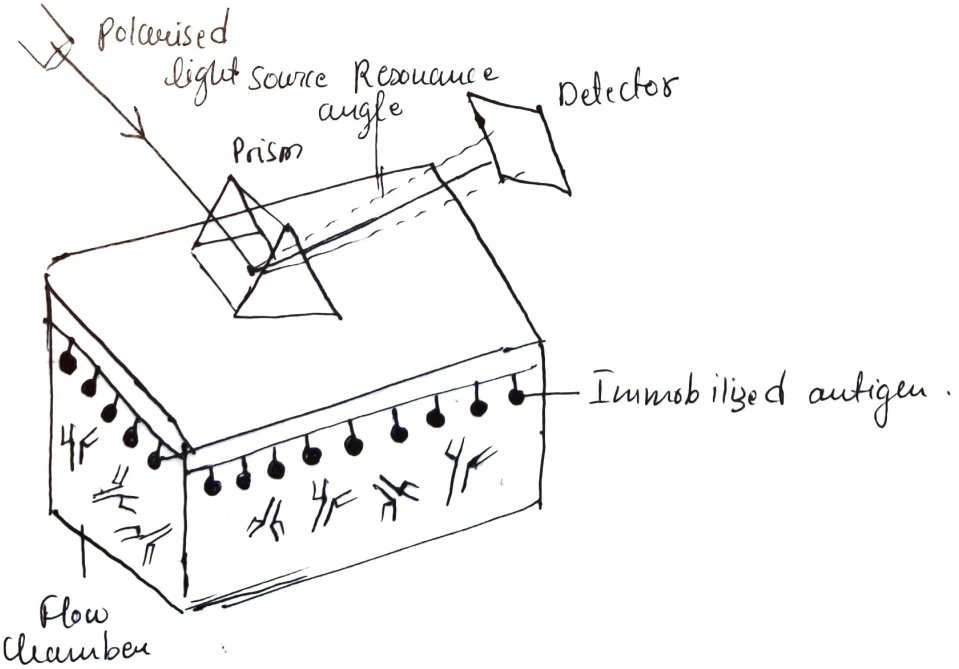
- A beam of polarized light is directed through a prism onto a thin gold film (coated with antigen on the opposite side) and reflected off the gold film, toward a light-collecting sensor.

  However at a unique angle, some incident light is absorbed by the gold layer, its energy transformed into charge waves called surface plasmons.

- A sharp dip in the reflected light intensity can be measured at that angle, called **resonant angle**. The angle depends on the colour of the light, the thickness and conductivity of the metal film, and the optical properties of the material close to the gold layer's surfaces.

— The SPR method takes advantages of the last of these factors: the binding of antibodies to the antigen attached to the chip has a strong enough effect to produce a detectable change in the resonant angle.

  — The change has been found to be proportional to the number of bound antibodies.



Antigen - Antibody Interaction

- The interactions between antigens and antibodies are known as **antigen - antibody reactions.**

- The reactions are highly specific, and an antigen reacts only with antibodies produced by itself or with closely related antigens.

  Antibodies recognize molecular shapes (epitopes) on antigens.

The affinity of the antibody for the antigen is one of the most important factors in determining antibody efficacy in vivo.

- The antigen - antibody interaction is bimolecular irreversible association b/w antigen and antibody.
  The association b/w antigen and antibody includes various non-covalent interactions b/w epitope and variable region (V_H/V_L) domain of antibody.

★ Chemical bonds Responsible for Ag-Ab Reaction:-

(1) Electrostatic bonds:-

This result from the attraction b/w oppositely charged ionic groups of two protein side chains; for example, an ionized amino group (NH_4^+) on a lysine in the Ab and an ionized carboxyl grp (COO^-) on an aspartate residue in the Ag

② Hydrogen bonding :-

When the Ag and Ab are in very close proximity, relatively weak hydrogen bonds can be formed b/w hydrophillic groups (e.g., OH and C=O, NH and C=O, and NH and OH groups.

③ Hydrophobic interactions :-

Hydrophobic groups, such as the side chains of valine, leucine, and phenylalanine, tend to associate due to Van der Waals bonding and coalesce in an aqueous environment, excluding water molecules from their surroundings.

— As a consequence the distance b/w them decreases, enhancing the energies of attraction involved.

④ Van der Waals bonds :-

These forces depend upon interactions b/w the "electron clouds" that surround the Ag and Ab molecules. The interaction has been compared to that which might exist b/w alternating, dipoles in two molecules.

✱ Strength of Ag-Ab interaction :-

① Affinity :-

Affinity measures the strength of interaction b/w an epitope and an antibody's antigen binding site. It is defined by same basic thermodynamic principles that govern any reversible biomolecular interact

$$K_a = \frac{[Ab\text{-}Ag]}{[Ab][Ag]}$$

affinity.

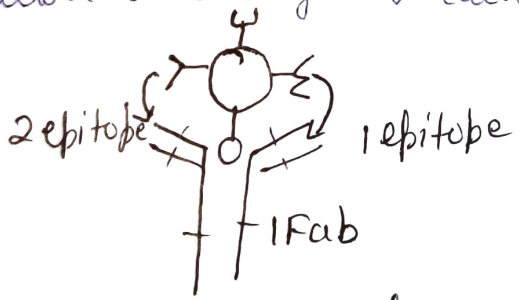
- Combined strength of total non-covalent interactions b/w single Ag-binding site of Ab and single epitope is affinity of Ab for that epitope.

- Low affinity Ab : Bind Ag weakly and dissociates readily.
- High affinity Ab : Bind Ag tightly and remain bound longer.

② Avidity :-

When complex antigens containing multiple epitopes are mixed with antibody containing multiple binding sites, the interaction of an antibody molecule with an antigen molecule at a single epitope will increase the probability of reaction b/w these two molecules at a second site.

Such multiple interaction b/w a multivalent antibody with an antigen is called avidity.



multiple epitope.

③ Cross Reactivity:-

Ag-Ab reactions are very very specific but in some cases the antibody elicited by one antigen (Ag) can cross react with another unrelated antigen.

This is possible only if the two antigens share a very very common or similar epitope. However the affinity of the antibody for the original epitope is much higher than the epitope for which it shows the cross reactivity.

e.g; Cross reactivity observed for the polysaccaride Antigen in ABO blood grouping.

Types of Interactions:-

① Precipitation:-

It is a type of antigen-antibody reaction, in which the antigen occurs in a soluble form. When a soluble antigen reacts with its specific antibody, at an optimum temperature and pH in the presence of electrolyte antigen-antibody complex forms insoluble precipitate. called **precipitins**.

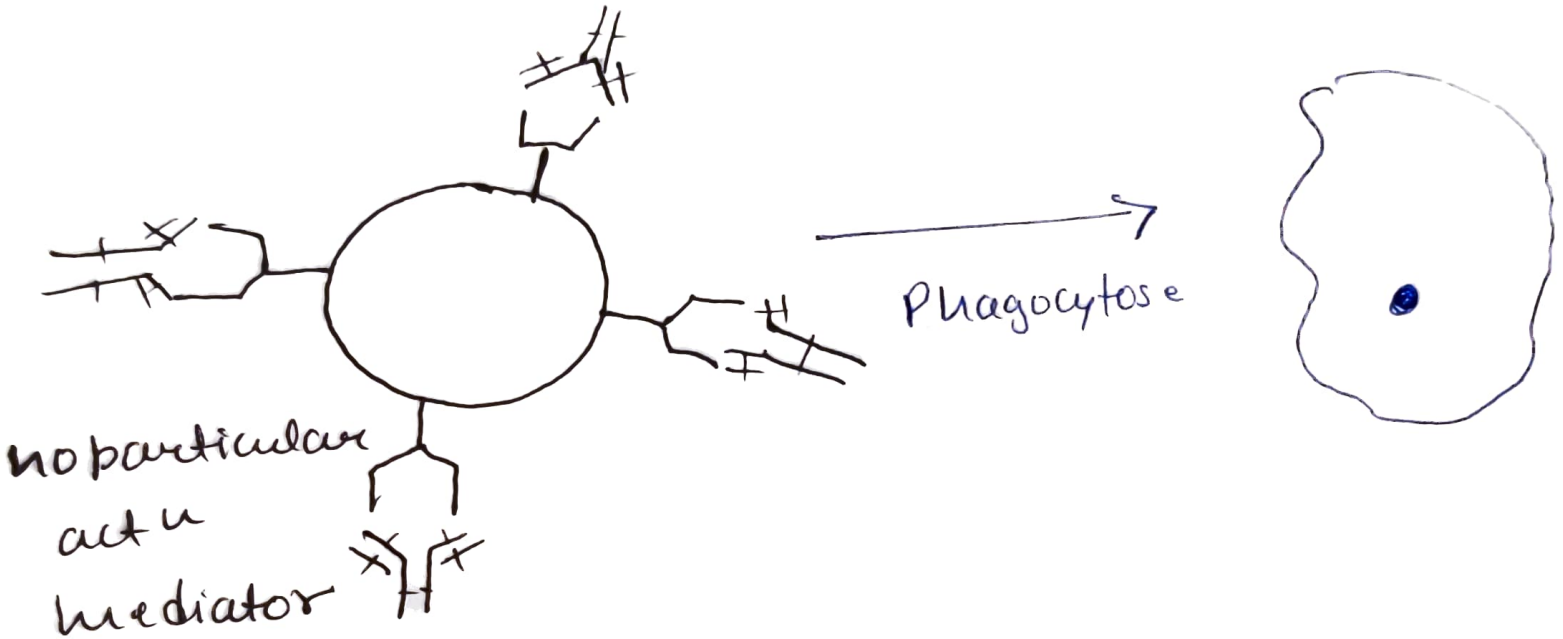
② Agglutination:-

The interaction b/w antibody and a particular antigen results in visible clumping called Agglutination.

Antibodies that produce such reactions are called **agglutinins**

eg: IgM, IgG (bad)

(3) Opsonization:-



- Opsonization, promotion of phagocytosis of antigens by macrophages and neutrophils.