BT401 :- IMMUNOLOGY

unit - I :-

Immune system, organs and cells of immune system. Immunity, Innate immune mechanism. Acquired immune mechanism, Antigen, Antigenecity (factors affecting antigenecity) Humoral immunity, main pathways of complement system.

unit - II :-

Antibody structure and classes, Antibody diversity genes of antibodies, theories of formation of antibodies.

unit - III :-

Cell mediated Immunity: Pc mediated immunity, NK cells mediated immunity, ADCC, delayed type hypersensitivity, cytokines and brief idea of MHC.

unit - IV :-

Hyper sensitivity and vaccination: General features of hypersensitivity, various types of hypersensitivity, vaccination: Discovery, principles, significance, concept of Autoimmunity.

unit - V :-

Immunological Techniques: Antigen - Antibody reactions: Perception, agglutination, Complement fixation, immuno diffusion, ELISA.

Hybridoma Technology:- monoclonal antibodies and their applications in immunodiagnosis.

Practical Syllabus

Immunology and Bio-physical techniques:-

1. Antigen-Antibody reaction- determination of blood group.
2. Pregnancy test
3. Widal test
4. ouchterloney immunodiffusion
5. Radial immunodiffusion.
6. ELISA
7. Isolation of casein by iso-electric precipitation.
8. Production of antibodies and their titration.

UNIT-1

Immunology:-

The study of cells and molecules of immune system and their use to protect the body against foreign invaders are called immunology.

→ Immunology is a branch of biology that covers the study of immune system in all organisms.

→ The Russian biologist ERIIYAIIYICH mechnikob; made advanced studies on immunology and received nobel prize for his work in 1908. He pinned small thorns into starfish larvae and noticed unusual cells surrounding the thorns. This was the active response of the body trying to maintain its integrity. This phenomenon of phagocytosis in wa which the body depends itself against a foreign body and then coined the term immunology.

Immune system:-

The immune system is a host defense system comprising many biological structures and process within an organism that protects against disease. To function properly an immune system must detect a wild variety of agents, known as pathogens, from viruses to parasitic worms and distinguish them from the organisms own healthy tissue.

→ The immune is made up of a network of cells, tissues and organs that work together to protect the body.

Part - A :- Organs of immune system

Lymphoid organ :-

The organs concerned with immune reactions are called lymphoid organs. There are a number of organs that have various functions in the development of immune system response based on their functions. They are two types of organs. They are :- ① primary lymphoid organs
② Secondary lymphoid organs

① Primary lymphoid organs (or) central lymphoid organs :-

It is also called as central lymphoid organs. The primary lymphoid organs are considered with production and maturation of immune cells. The primary lymphoid organs are :- (a) Bone marrow
(b) Thymus
(c) Bursa of fabricus

(a) Bone marrow :-

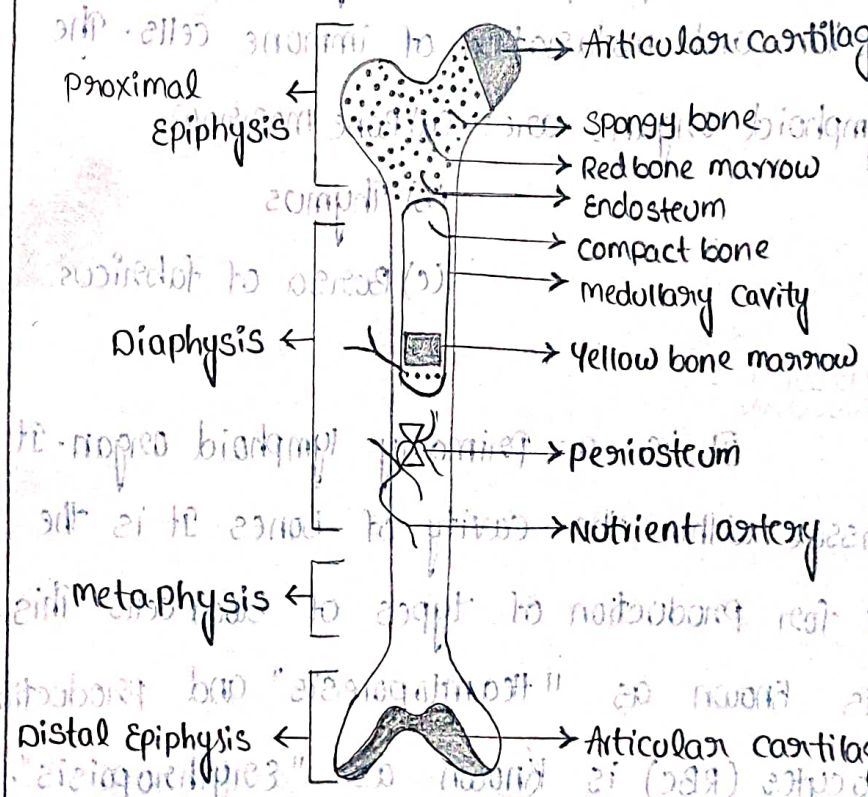
It is a primary lymphoid organ. It is a soft tissue within the cavity of bones. It is the major site for production of types of blood cells. This process is known as "Heamtopoiesis" and production of Erythrocytes (RBC) is known as "Erythropoisis".

Bone marrow is the site of origin and development of B-lymphocytes (or) B cells in mammals. Particularly in humans and mice after birth. Before

birth the yolk sac and total bone marrow are the major sites of the B-lymphocytes maturation.

Bone marrow is present in the cavity of most of the bones in the body like skull bones, fumour bones, sternum and spinal bones. In bone marrow different types of blood cells are produced due to the presence of stem cells. It is also the site for maturation of B-lymphocytes.

Both B-lymphocytes and T-lymphocytes are produced in bone marrow. The precursor of these lymphocytes is called lymphoid progenator.



(b) Thymus:

Thymus is a primary lymphoid organ and it is a critical organ of immune system because it is a site for maturation of T-lymphocytes is named as thymus derived lymphocyte (or) T-lymphocytes (or) T-cells that means soon after the production of T-cells in the bone marrow, they migrated to the thymus and mature into T-lymphocytes so the thymus is the most aquisite for lymphocytes proliferation.

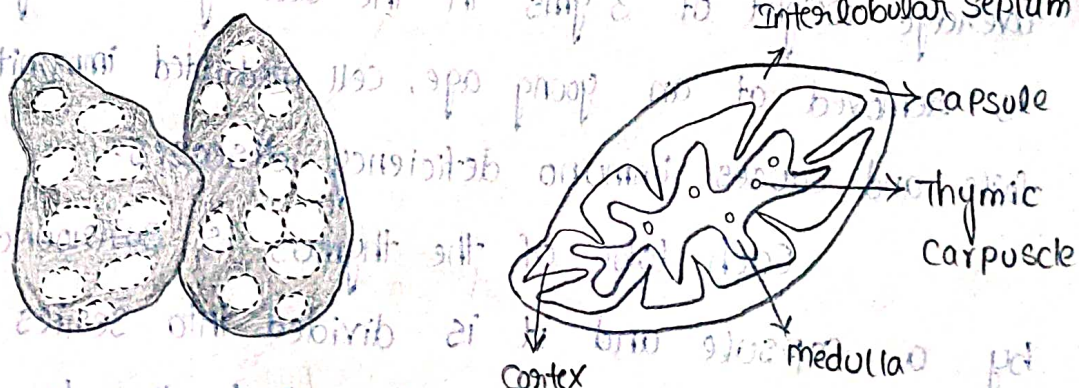
Thymus is bulged, flat and greyish coloured organ situated just above the heart and extending into the neck on the front side of trachea the thymus show peak activity during childhood it is larger size at younger age. there after it gradually becomes smaller and externally small at old age the average weight of thymus is 10 gms. infants and its age dependent involution leaves the thymus with an average weight of 3 gms in the old age. If thymus is removed at an young age, cell mediated immunity fails and causes immuno deficiency diseases.

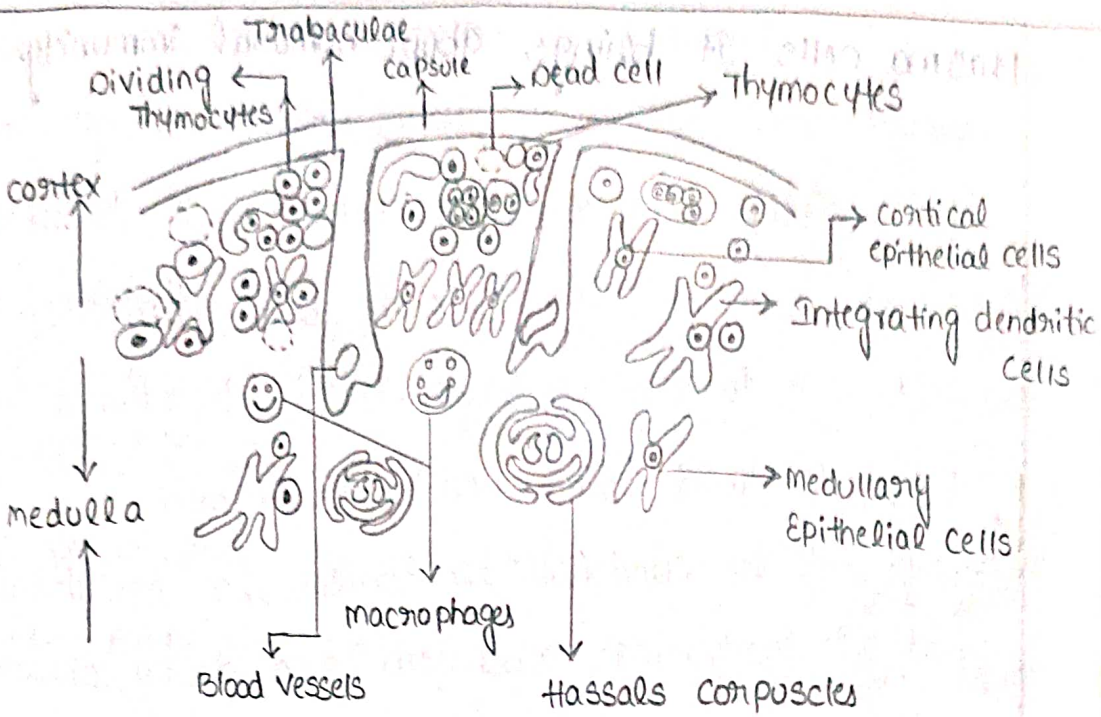
Each lobe of the thymus is surrounded by a capsule and it is divided into series of lobules, which are separated from each other by strands of connective tissue is called "trabeculae".

Each lobule is organised into two compartments outer and inner compartments. The outer compartment is called cortex where as the inner compartment is called as medulla. The cortex is density packed with thymocytes, where as the medulla is sparsely populated with thymocytes. Thymocytes developed from prothymocytes. Then T-lymphocytes are produced in bone marrow migrated through blood stream and enters into the cortex of the thymus that acts as thymocytes. Thymocytes divide rapidly in cortex and give rise to T-lymphocytes. Some of epithelial cells of the outer cortex possess long membrane extention that surrounds as many as 50 thymocytes. The cells are called nurse cells.

Functions:-

It produces T-lymphocytes and bring about cell mediated immunity and graft rejection.





(c) Bursa of fabricus :-

Bursa of fabricus is a primary lymphoid organ in birds where stem cells from yolk sac and bone marrow mature proliferate and differentiate into bursa derived lymphocyte called B-lymphocyte (or) B-cells. Bursa of fabricos arises as a pouch from the dorsal part of cloaca (fluid gut) in birds. Bursa of fabricus is sensitive to harmones, administration of testosterone at the early Embroy stage completely prevents its formation (Harmonal bursectomy) surgical removal of Bursa (bursectomy) from newly hatched chickens destroys their subsequent ability to produce antibodies. They have unique Ag cells called B-Ag.

Functions :-

It produces B-lymphocytes, macrophages and

plasma cells. It brings about humoral immunity.

② Secondary lymphoid organ (or) Peripheral lymphoid organ :-

As stated Earlier, the lymphocytes mature proliferate and differentiate in the primary (or) central lymphoid organs. These lymphocytes originate ~~their form~~ through circulation to the secondary (or) peripheral lymphoid organs. These organs are concerned with immune and they are storage organs of different immune cells.

The secondary lymphoid organs across the Ag and provides site for mature lymphocytes to interact with Ag. So in the secondary lymphocyte organs lymphocytes are made functional so these

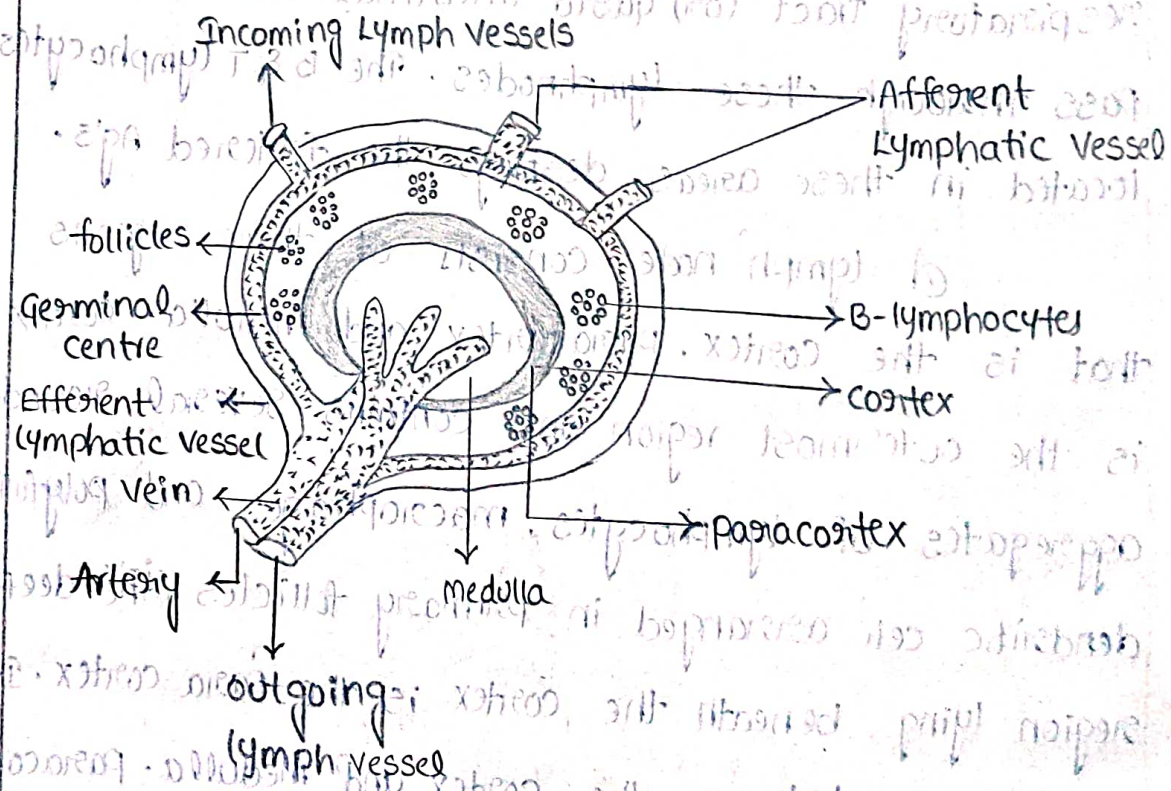
organs are small and poorly developed at but these are grow progressively with age. Thus organs includes lymphnodes, lymph vessels, spleen, malt, payers patch and tonsils.

(a) Lymphnodes and lymphatic vessels:-

Lymph nodes are small bean shaped structures clustered at junctions of the lymphatic vessels which are distribute throughout the body. Any foreign particals that enters the body through the respiratory tract (or) gasto intestinal tract mamust pass through these lymphnodes. The B & T lymphocytes located in these areas destroys the filtered Ag's.

A lymph node consists of three regions that is the cortex, paracortex and medulla. cortex is the outermost region and contains several rounded aggregates of lymphocytes, macrophages and ~~poly~~follicular dendritic cell arranged in primary follicles. The deeper region lying beneath the cortex is the para cortex. It is the zone between the cortex and medulla. paracortex possess large number of T-lymphocytes and also contain dendritic cells thought to have migration from tissues to the lymph node medulla the inner most region of lymphnode is more sparsely populated with lymphoid lineage cells.

Each lymphnode has a number of lymph vessels called afferent lymphatic vessels the lymph now paraculates inwards through the cortex, paracortex and medulla allowing phagocytic cells and dendritic cell to trap pathogens Ag's carried by the lymph. The lymph then is drained into a single large lymph vessel called Efferent lymphatic vessel that carries lymph through thoracic duct, which empties into a large vein in the neck.



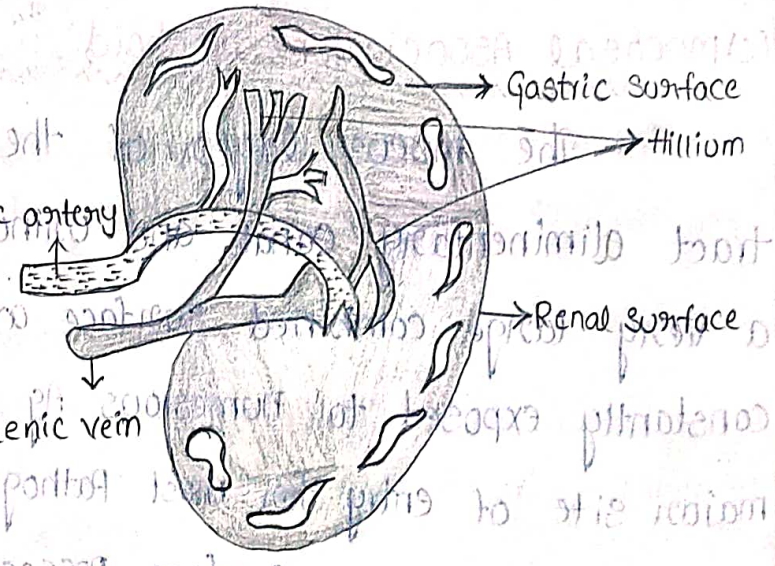
(b) Spleen:-

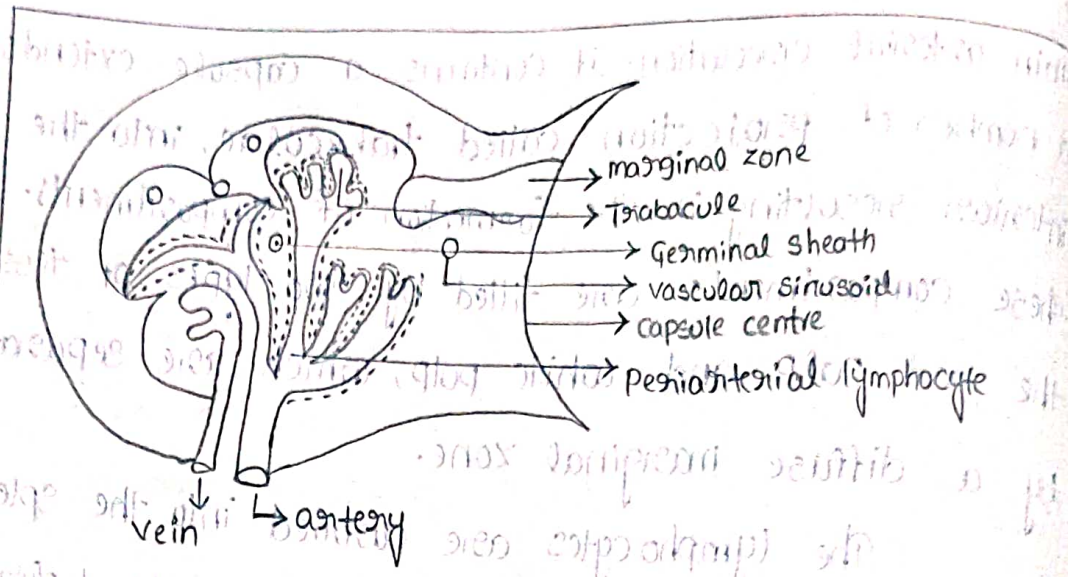
The spleen is a large secondary lymphoid organ located in the upper part of the abdominal cavity behind the stomach. The spleen is five (5) inches long and 200gms weight in adults. It is deep red in colour and has direct communication with the

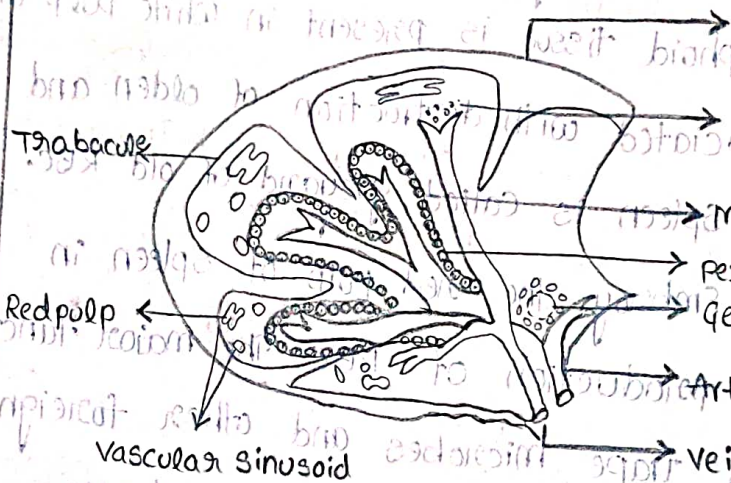
main arterial circulation. It contains a capsule extends a number of projection called trabeculae, into the interior resulting in the formation of compartments. these compartments are filled by two types of tissues the red pulp and white pulp, which are separated by a diffuse marginal zone.

The lymphocytes are carried into the spleen through the splenic artery. It filters the blood for microbes. The lymphoid tissue is present in white pulp and red pulp is associated with distruction of olden and dead RBC. Hence spleen is called greyard of old RBC.

In the Embroy the red pulp of spleen in involved in the production of RBC. The major function of spleen is to trape microbes and other foreign particals that passess through the blood stream.







T.S of Spleen

(c) mucosal Association lymphoid Tissue (MALT):-

The mucus layer of the respiratory tract aliminentary canal and urinogenital tract have a very large combined surface area, which is constantly exposed to numerous Ag and it is the major site of entry for most Pathogens. These vulnerable membrane surface possess a group of organized lymphoid tissue which is defined it from Pathogens and Ag's. The group of organised lymphoid

in colour and

Tonsils are collections of lymphoid tissue phasing into the aerodigestive tract. The set of lymphatic tissues known as waldeyers tonsillar ring includes the adenoid tonsil (pharangeyal tonsils), two tubal tonsils, two palatine tonsils and the lingual tonsil. Tonsils in human include from anterior (front), superior (top), posterior (back) and inferior (bottom)

Type	Epithelium	capsule	Crypts	Location
① Adenoid (pharangeal tonsils)	ciliated Pseudostr-atified columnal (Respiratory Epithelium)	Incompleted Encapsulated	No crypts but small folds	Roof of pharynx
② Tubal tonsils	ciliated Pseudostr-atified columnal (Respiratory epithelium)	completely capsulated	crypts are present	Roof of Pharynx
③ Palatine Tonsils	Non Kera-tinized Stratified Squamousa	Incompletely encapsulated	long-branched	Sides of oropharynx
④ Lingual Tonsils	Non Kera-tinized Stratified Squamousa	Incompletely encapsulated	long branched	Behind terminal Sulcus (Tounge)

→ Normally each tonsil measures upto 2.5 cm in length, 2.0 cm in width and 1.2cm in thickness.

Development:-

Tonsils tend to reach their largest size near tuberty and they gradually undergo

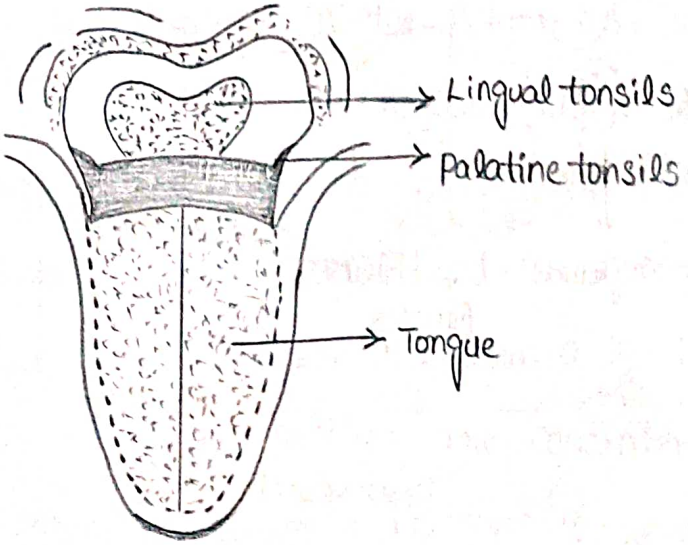
Functions:-

Tonsils are the major sites for production of T-lymphocytes also known as T-cells.

tissues is known collectively as MALT. These tissues do not posses a capsule they are several types of MALT that is (i) Tonsils (ii) payers patch.

(i) Tonsils :-

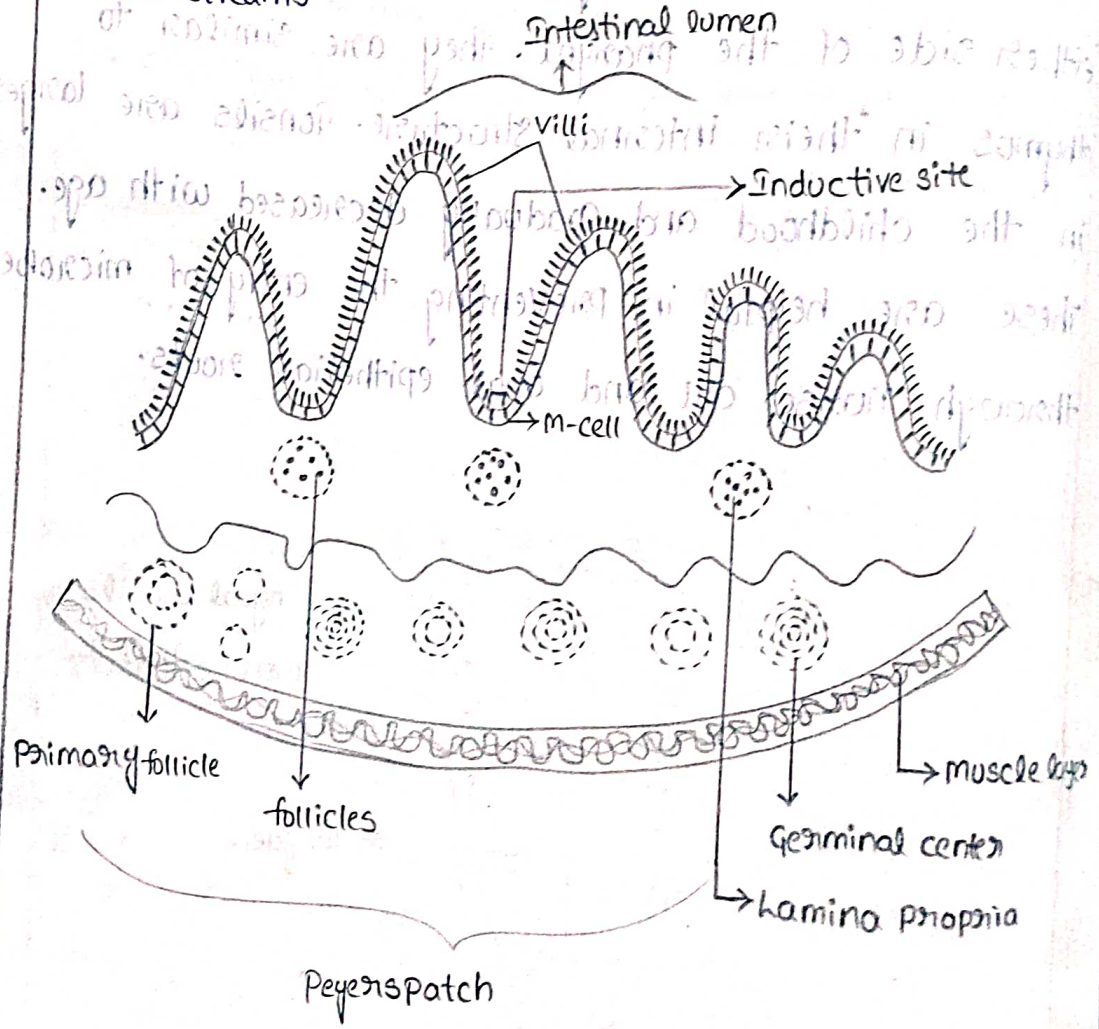
Tonsils are lymphoid tissue present on either side of the pharynx. They are similar to thymus in their internal structure. Tonsils are larger in the childhood and gradually decreased with age. These are helpful in preventing the entry of microbes through nurse cell and oral epithelial roots.



(ii) Payer's patch :-

These are the secondary lymphoid organ present in the mucos of small intestine each payers patch is a nodula of 30-40 lymphoid particles peyerspatch can developed into 20 follicles with germinal centres

T and B lymphocytes present in the surface of payerpatch that contain folds. In the folds of these mucosal layer certain Ab's like Ig A are present. These immono globin avoid the entry of virus (or) bacteria and any other toxic compounds into the blood streams.



Peyers patch:-

Peyers patch are organised lymphoid follicles named after the swis anatomist Johann conrad peyer. They are an important part of gut associated lymphoid tissue. usually found in humans in the lowest portion of the small intestine mainly in the distal jejunum and the ileum but also could be detected in the deodenum.

Structure:-

Peyers patch are appeared as a elongated thickenings of the intestinal epithelium measured a few cms in length about 100 are found in humans. It appears as a oval (or) round lymphoid follicles (similar to lymphnodes) located in the submucosa layer of the ileum and extends into the mucous layer. The number of peyers patches peaks at the age of 15 to 25 and then declines during adulthood. In the distal ileum there are numerous and form a lymphoid ring. Atleast 46% of peyeris patches are concentrated in the distal (25 cm) of ileum in humans. There are large variations in size, shape and distribution of peyers patches from one individual to another one. In adults B-lymphocytes are seen to be dominant at the follicles germinal centers. T-lymphocytes are found in zones between follicles.

Functions:-

Peyers patch that contains macrophages, dendritic cells, B-lymphocytes and T-lymphocytes acts on gastro intestinal system much as the tonsils acts on the respiratory system, both of them trap the foreign particles surveilling them and destroying them.

→ maturation of the B-lymphocytes takes place in peyers patch.

part - B :- Cells of Immune System

The cells that participate in immune response iginate from bone marrow by a process called as Hematopoiesis" through which various blood cells re produced.

The different cells that participate in ne immune system response include WBC, T and B- ymphoids all these cells are produced from single ype of stem are called Haematopoitic stem cell (HSC) resent in bone marrow.

These are also called as Pluripotent stem ells that means they have capacity to differentiate nto different types of blood cells.

The HSC in the bone marrow proliferates and differentiates into two main lineage.

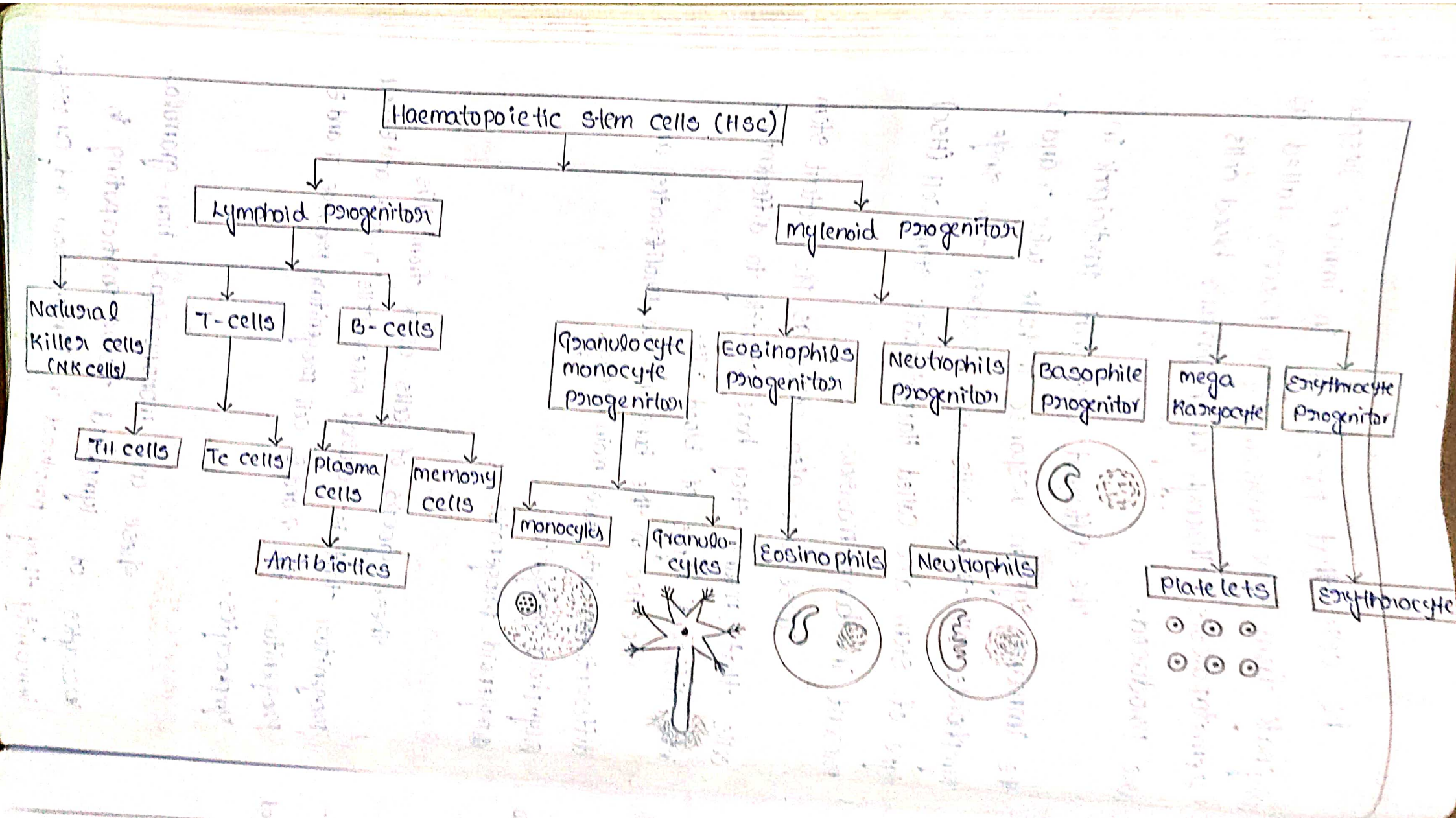
1) Lymphoid progenitor
2) mylenoid progenator

1) Lymphoid progenitor:-

These groups of cells arise from this progenitor that is natural killer cells T-cells and B-cell progenitor. The T and B cell progenitor produce T and B lymphocytes.

Lymphocytes:-

These are mononuclear, non-granular kocytes of lymphoid tissue participating in immunity they produced in bone marrow and constitute

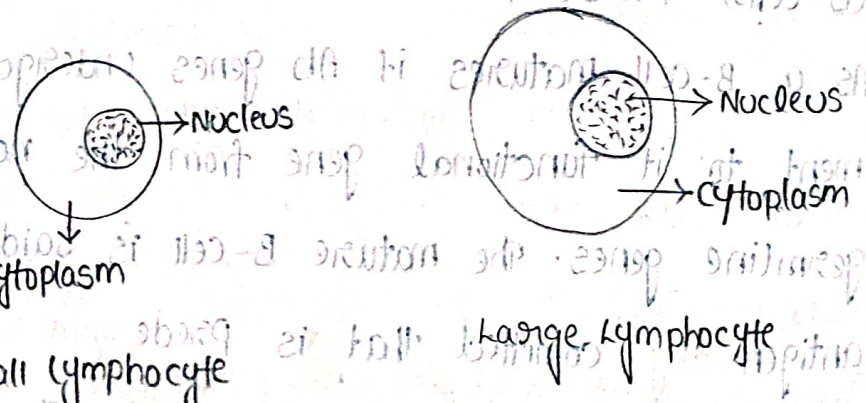

Haematopoietic stem cells (HSC)
Lymphoid progenitor
Myleroid progenitor
Natural Killer cells (NK cells)
T-cells
B-cells
Granulocyte monocyte progenitor
Eosinophils progenitor
Neutrophils progenitor
Basophile progenitor
mega Karyocyte
Erythrocyte Progenitor
TH cells
Tc cells
Plasma cells
memory cells
monocytes
Granulo-cytes
Eosinophils
Neutrophils
Platelets
Erythrocyte
Antibiotics

20-40% of WBC. They have unique structural and functional ability that is required and responsed to the Ag's. The primitive cell that do not undergo any interaction are called naive cells.

During the coarse of development they enlarge size they are called lymphoblasts which proliferate and differentiate into affector cells and memory cells.

The affector cells interms differentiate into either T-cells (or) B-cells depending upon the environment created the T-cells (or) thymocytes differentiate into cytotoxic T-cells (T_c cells) and helper T-cells (T_h cells) and suppressor are T-cells (T_s cells)

The B-cells can also differentiate into plasma cells and memory cells. The plasma cells are capable of producing Ab's specific to a particular Ag and the memory cells are helpful in the remember the identifying Ag-Ab complex.



B-lymphocytes :-

The B-lymphocytes which are matur in the bursa of fabricus of birds and in bone marrow of mammals is called B-lymphocytes. Afte production and maturation in bone marrow, they migrated to various secondary lymphoid tissues the cells are mononuclei agranular cells.

mature B-cells can be distinguished from other lymphocytes by their ability to synthesized and this play in membrane bound immunoglobin molecules and serve as receptors for Ag's. They give humoral immunity. B-lymphocytes proliferates and differentiate into ① plasma cells

② memory cells

multipotent stem cells present in bone marro give birth to lymphoid progenator cell that differentiate further to mature into B-lymphocytes (B-cells). First pro B-cells and then pre B-cells. As a B-cell matures it Ab genes undergo rearrangement to it functional gene from the non-functional germline genes. the mature B-cell is said to be antigenically comitted that is predermines in its ability to recognise a specific Ag. the mature

G_0 phase, produces Ab molecules that remains bound to its surface and doesnot secrete Ab's. It will secretes Ab's only when it differentiates into plasma cells following its interaction with specific Ag.

Plasma cells:-

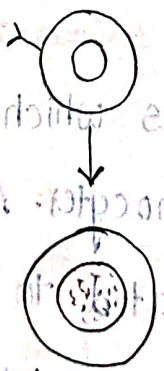
Plasma cells lack membrane bound Ab's where synthesis and secretes one of the five classes of the Ab's. All the clonal progene from a single B-cell secretes Ab's with the same Ag binding specificity. These cells die within one (or) two weeks.

Memory cells:-

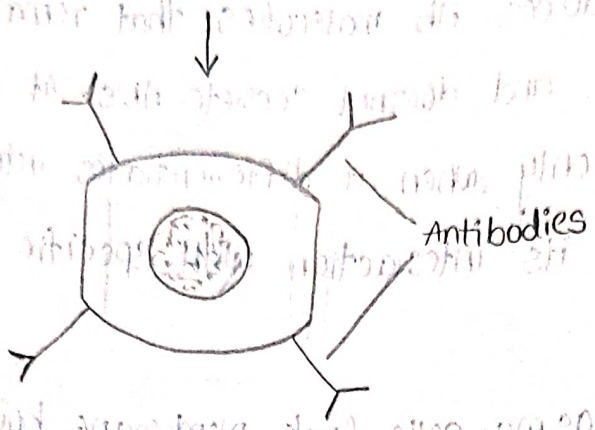
memory recognize and store information about the Ag they survive for a longer time. These cells respond quickly and efficiently during the subsequent similar Ag stimulation these are involved in secondary immone response.

Differentiation of B-cells in bonemarrow & Ab formation

Stem cell in bone marrow

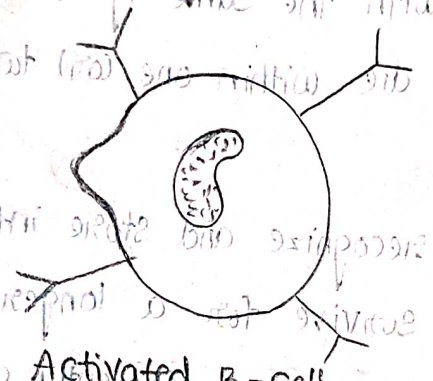


Lymphoid progenitor cell (pre-B-cell)

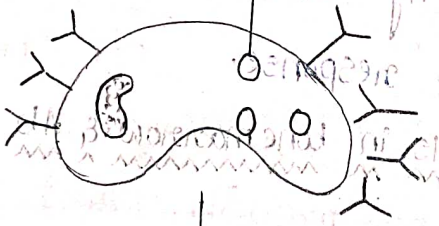


Mature B-cell (Naive-B-cell)

→ Ag-stimulation



Activated B-cell



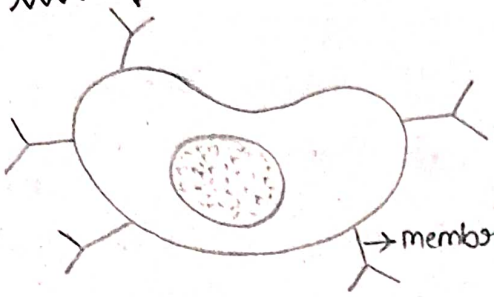
continue next diagram

Plasmacell that secret Ab's of various isotypes.

T-lymphocytes :-

The lymphocytes which mature in thymus are called T-lymphocytes. After production in bone marrow they will migrated to thymus for their maturation. The T-cells possess receptors on their surface are called T-cell receptors. These receptors recognizes the Ag that is bound to particular glass of

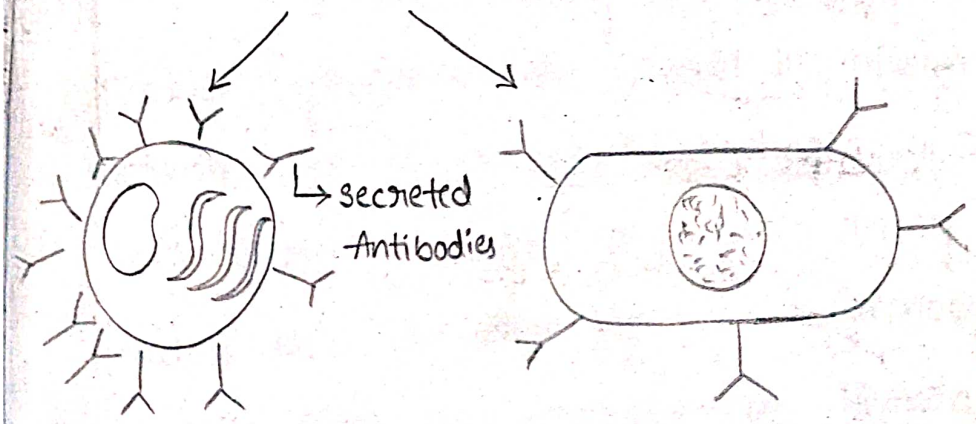
Differentiation of memory cells into plasma cells and memory cell :-



Mature B-cell (Naive B-cell)

Ag stimulation →

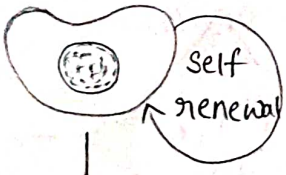
Activated B-cells

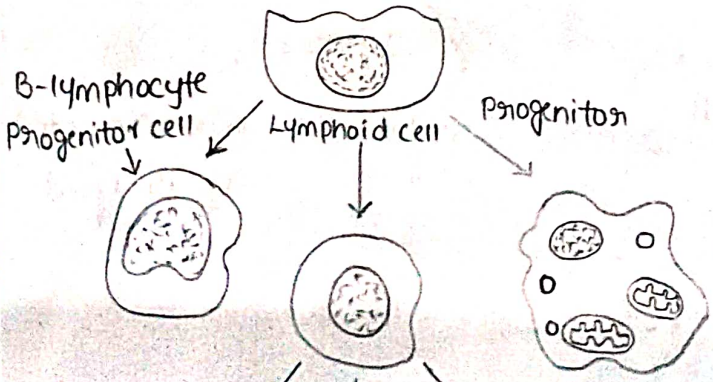


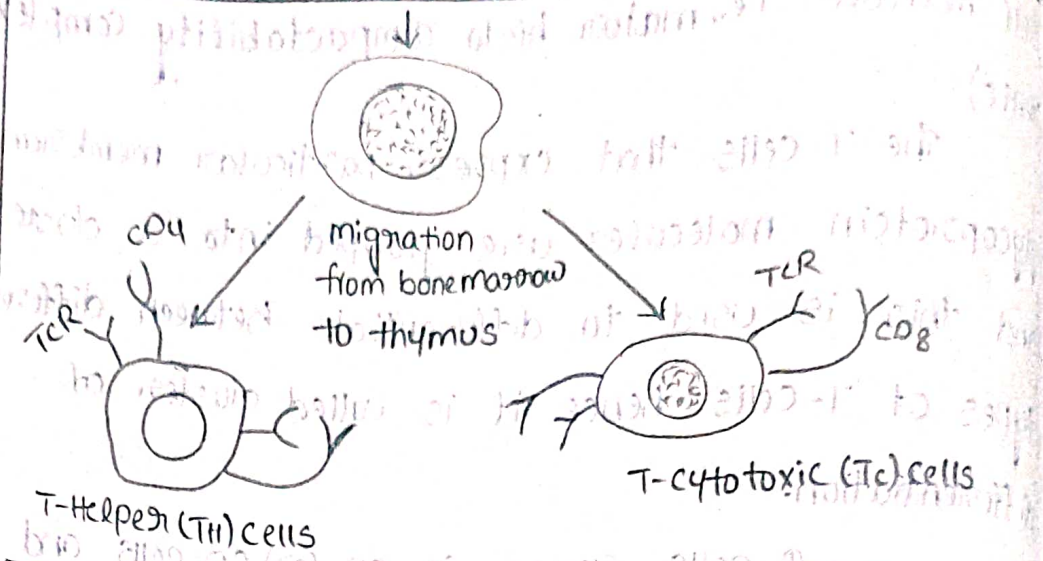
Plasma cells

Memory cells

Stem cells in bonemarrow



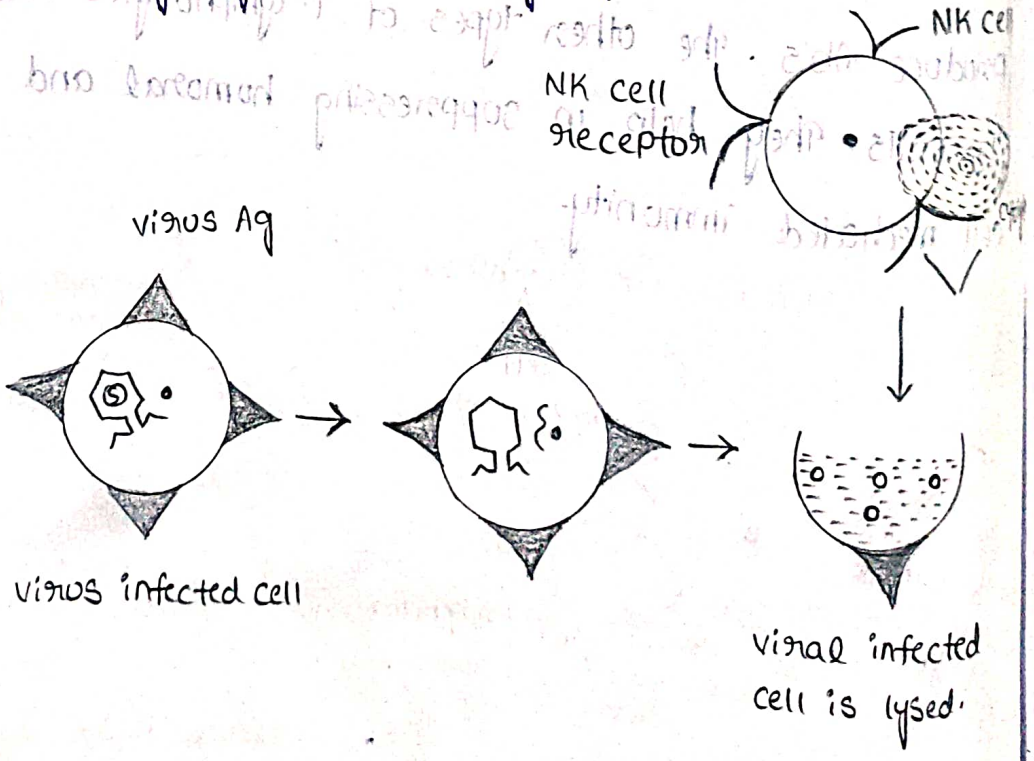




Differentiation of T-lymphoid into TH & Tc cells

Natural Killer cells:-

These are also called as Null cells. These cells do not express membrane molecu and receptors like T & B-cell lineages. They constitute 5-10% of the total lymphocytes. These cells play cytotoxic activity against humoral cells.



The NK cells attached themselves to the Ab's at the Fc region and destroy the target cells. This type of immunity by the NK cells is called Ab dependent cell mediated cytotoxicity (ADCC). A special type of NK cells (or) NK⊥T cells. These cells have receptors on their surface the characteristics of both NK & T-cells. These cells have receptors on their surface CD_{16} hence these behave like a T-cells and produces cytokinins that make it behave like a NK cell.

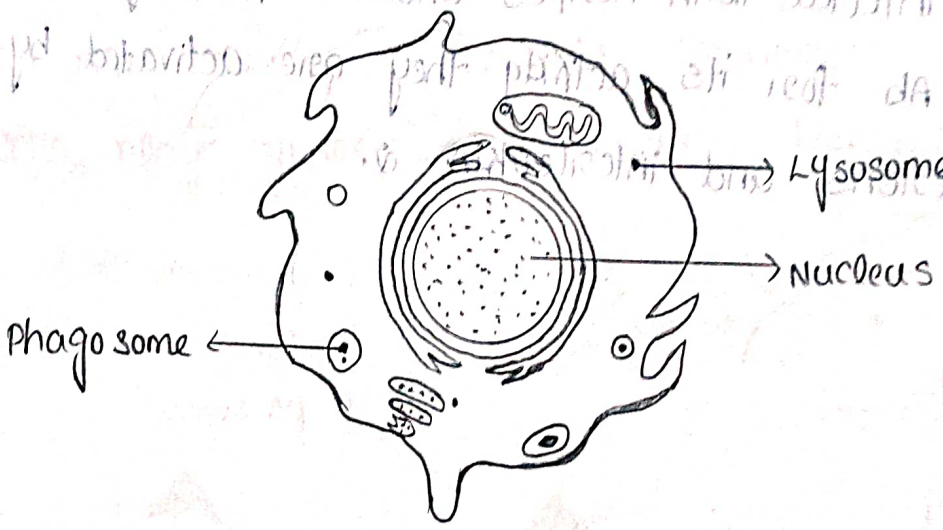
These cells destroys the cancer cells and cells infected with herpes and mumps. They do not need Ab for its activity they are activated by interferons and interleukin-2.

⑧ Mylenoid progenation:-

Mononuclear cell:-

Mononuclear cells are, monocytes.

→ Monocytes are mononuclear phagocytic leukocytes, possessing an oval (or) kidney shapes nucleus. The granules in the cytoplasm that is stained with grey (or) blue monocytes cells are produced in bone marrow. These monocytes are circulating in blood for about 1 & 8 hrs, enlarge in size and they migrated into the tissue where they differentiate into macrophage.



Macrophages :-

Macrophages are differentiate from monocyte into the tissues of the body. Some of the macrophages are freely circulated and some of tissue may be fixed in the tissue.

① Alveolar macrophage - lungs

② Histeocyte - connective tissue

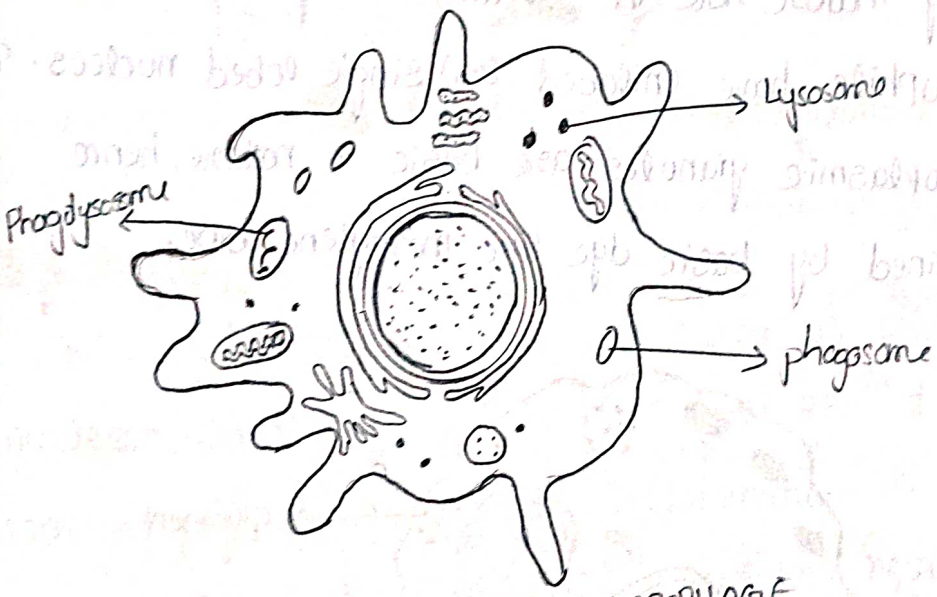
③ Kupffer cells - Liver

④ mesangil cells - Kidney

⑤ microgleal cells - Brain

⑥ osteocytes - Bones.

The cytokinis secreted by T-cells and interferons activates the macrophages. these activated macrophages produced high levels of class-III MHC molecules for TH cells to function efficiently.



MACROPHAGE

Granulocytes:-

The WBC are leucocytes that contain granules in the cytoplasm are called granulocytes. there are three types of granulocytes are recognized in the body. They are :- ① Basophills

② Eosinophills

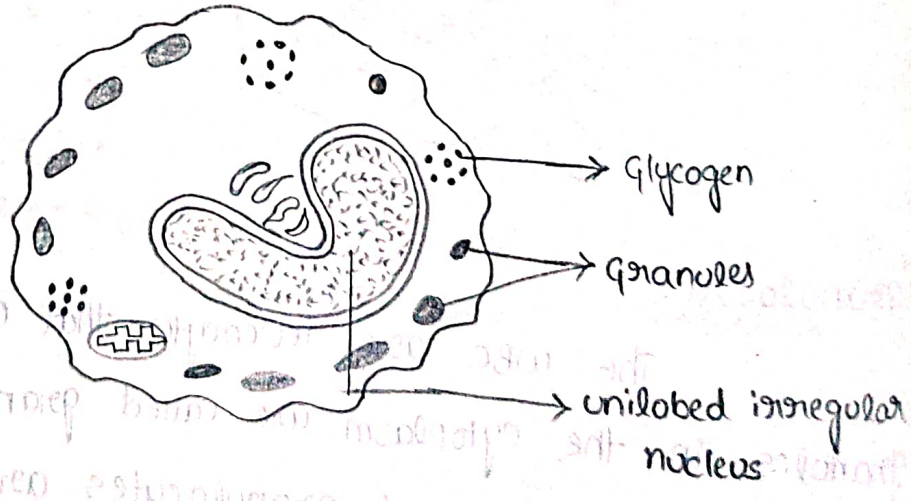
③ Neutrophills

① Basophils :-

They constitute less than 1% of the total circulating WBC. Since they are very less in number, they are not involved in phagocytosis. However they provide the defense mechanism against allergies.

→ Basophils possess high affinity receptor for IgE and their by become coated with these Ab's. once coated, Ag trigers the basophils to secret vaso active mediator which are inflammatory and play major role in certain allergic response.

→ Basophils have unilobed (or) single lobed nucleus. The cytoplasmic granules are basic in nature, hence stained by basic dye like methylene blue.

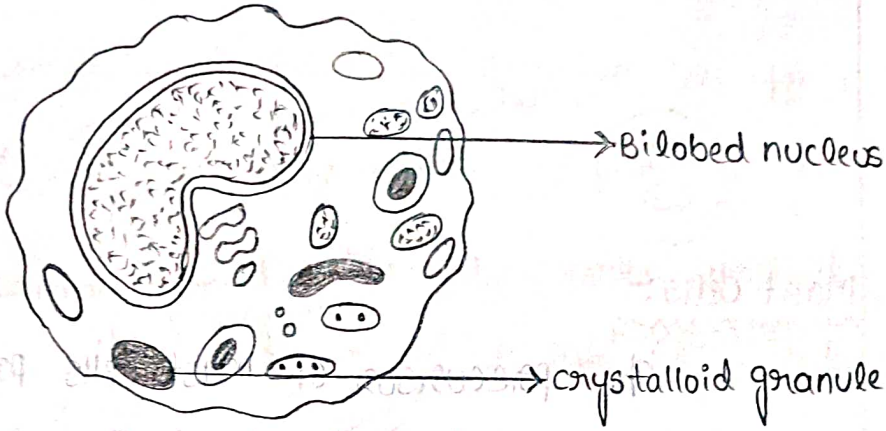


② Eosinophils :-

They constitute 1 to 3% of the total circulating WBC. Eosinophils, that is like neutrophils. they are motile cells that migrated from blood stream

into tissues phases. These granulocytes are consider to play a role in that defense against parasitic organism by phagocytosis.

Eosinophils possess a bilobed nucleus with acidophillic granules. Hence their cytoplasm is stained by acidic dye that is Eosine red.



③ Neutrophils:-

Neutrophils are also called as polymorpho-nuclear cell. They constitute 50-70% of RBC. This arrived 1st at the site of inflammation. As a result the increase in the number of circulating neutrophils is called leukocytosis. The movement of this neutrophils into nucleus is called extravasiation. These neutrophils circulating in blood stream for 7-10 hours before they migrate into the tissue where the enzyme a life span of only a few days.

The neutrophils are considered to be aquiphagocytic cell similar to macrophages. These nucleus

is multilobed and its cytoplasm is stained by both acidic and basic dye.

Mast cells:-

The precursor of mast cells produced in bone marrow released into blood. These precursor developed into mast cells only, when they enter into the tissues. The cytoplasm of these cell consist of histamines and other pharmacologically active substances.

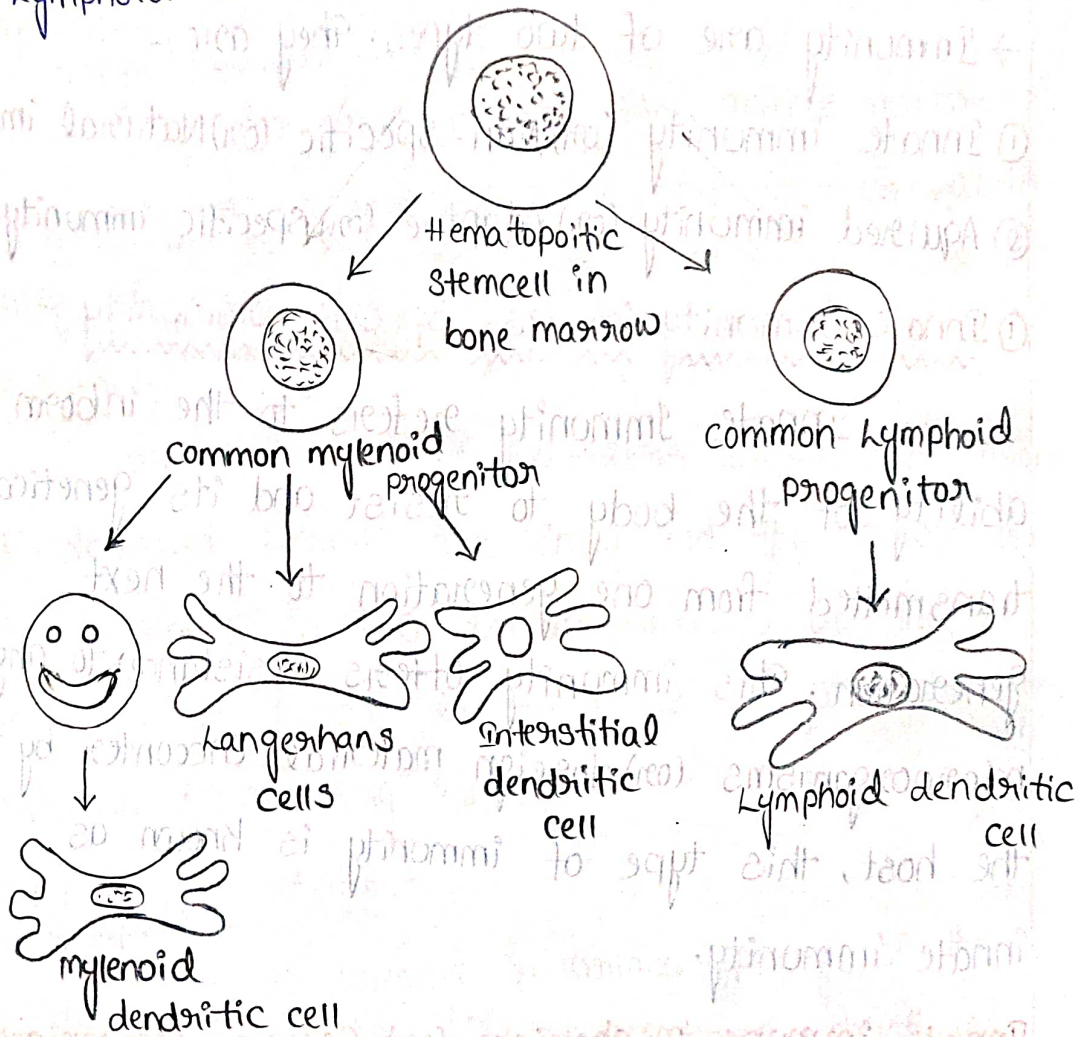
Mast cells together with basophils play an important role in defense mechanism against allergy.

Dendritic cells:-

Dendritic cell constitute only 0.2% of WBC in the blood and are present in even small numbers in skin and mucous membranes of the nose, lungs and intestine. They derive their name due to long membrane extensions resembling the dendrites nerve cells. Stem cells originated dendritic cells are

of four types. They are:-

① Langerhans cells
② Interstinal dendritic cells
③ myelenoid dendritic cells
④ Lymphoid dendritic cells.



part :- c :- Immunity and its types

Immunity :-

Immunity broadly involves the resistance show and protection offered by the host organism against the infectious diseases.

→ Immunity are of two types. They are :-

① Innate immunity (or) Non-specific (or) Natural immunity

② Aquired immunity (or) Adaptive (or) specific immunity.

① Innate Immunity (or) less specific Immunity :-

Innate Immunity refers to the inborn ability of the body to resist and its genetically transmitted from one generation to the next generation. This immunity offers resistance to any microorganisms (or) foreign material encounter by the host, this type of immunity is known as innate immunity.

Innate immune mechanism (or) Defence mechanism involved in innate immunity :-

→ All the living organisms are naturally gifted the resistance to certain infection from birth. The component of a immunity are present before on set of infection provides immunity against many infections.

→ It is also not specific for any particular Ag.

→ There are four factors that are responsible for this immunity. They are :- ① physical barriers
② Biochemical barriers
③ cellular factors
④ Genetic factors

① physical barriers :-

These barriers include skin, mucous membrane, cilia, coughing and sneezing. physical barriers avoid entry of mo's (microorganisms) into the body.

(i) Skin :-

Skin is a major barriers for infectious agent. It doesnot permit the Entry and the growth of the microbes on it's surface. This is due the secretion of sweat glands. In case of skin damages, infection occurs very rapidly.

(ii) mucous membrane :-

It is present in various openings of the human body like respiratory tract, digestive tract and urinogenitical tract. In all these the surface is lined by mucous membrane, the epithelial cells that are present in these tract, which secret mucous. The mucous trap the microbes which try to enter into the body through these opening. The mucous also act as a protective barrier to prevent the adherence of microbes to the Epithelial cells.

(iii) Cilium :-

The Epithelial cells of the respiratory tract are lined with cilia which have short hair like projections, the microbes are trapped in the mucous of the respiratory tract are removed by constant movement of cilia.

(iv) Coughing and Sneezing :-

The mechanical actions of coughing and sneezing prevents the entry of foreign particals into the body particularly through the digestive tract and respiratory tracts.

② Biochemical barriers :-

They are different biochemical barriers that fight against infectious agents and they include.

(i) Secretion of skin :-

A high concentration of salt in drying sweats contains bacteriosidal activity. The acidity of pH 5.5, which is microbisidal. However certain areas of body particalarly the soles ~~of~~ that contains defficient in sweat glands, so these areas are easily attacked by the microbes.

(ii) Secretion of digestive tract :-

The high acidity in the stomach has microbisidal. This ~~activity~~ acidity is due to the

presence of HCl. HCl is secreted by oxyntic cells in the stomach.

(iii) Lysozymes :-

Lysozymes is an enzyme that have ability to lysis the many gram negative bacteria. This enzyme was discovered by flamming. It is present in saliva, tears, nasal secretions, human milk and in the most other tissue fluids except cerebrospinal fluid, sweat and urine.

(iv) Interferance :-

It is a group of soluble, non toxic, glycoproteins. These are antiviral agents that can inhibits intracellular viral replication in the viros infected cells that can also inhibits proliferation in humans, so interferons can be administration in the treatment of cancer (chemotheraphy).

③ Cellular factors :-

Different types of cells that participate in providing the innate immunity. Some of them are phagocytes, NK cells and polymorphonuclear cells. Phagocytes are discovered by metchnikoff in cellular immunity. They are directly involved in attacking the microbes and this process is known as phagocytosis.

④ Genetic factor:-

Natural immunity is also due to genetic factors. So immunity refers at the level of species, races and art the level of individuals. Genetic factors at species level refers to species immunity. It means the members of one species (or) resistance to a disease by the members of others are suspectable for same disease.

Ex:- Rats are resistance to diptheria where as guiniapigs are suspectable to the same diseases.

Types of innate immunity:-

Innate immunity can be divided into Species, ratial and individual immunity.

(i) Specious immunity:-

Specious resistance (specious immunity) is that in which a disease affecting one speciousus doesnot affect the other specious. For convinience, humans do not contract cattle plague, chicken, cholera and hog cholera, infectious horse anaemia etc. while animals are not effected by many human diseases such as enteric fever, Sparlet fever, syphilis, Gonorrhoea, measels etc.

(ii) Ratial immunity:-

Ratial resistance (Ratial Immunity) is that in which various races (breads) show

marked differences in their resistances to certain infectious disease. Lakh Africans affected by sickle-cell aneamia, a genetic disease, are resistance to malaria, while malaria effects other human races.

(iii) Individual immunity:-

Having the same natial background and opportunity for explosal. Some individual of the race experience fewer (or) less severe infections than other individual of the same race. For convience, children are more suspectable to disease such as measels, chicken pox while aged individuals are suspectable to other diseases like pneumonia.

② Aquired immunity (or) Specific immunity (or) Adaptive immunity (or) more Specific immunity:-

The resistance developed by man during his life is known as acquired immunity. It is a more specific type of immunity. It is also called as specific immunity (or) Adaptive Immunity. During the life span of an individual develops acquired immunity against a particular disease. It means if an individual is exposed to this disease and recovers from that diseases, so he develops resistance against that particular diseases. This is because acquired immunity is capable of recognizing and selectively eliminating Specific Ag. It

Contains four characteristic features.

① Antigen Specificity:-

Immune Specificity can distinguish subs differences among Ag's.

(ii) Diversity :-

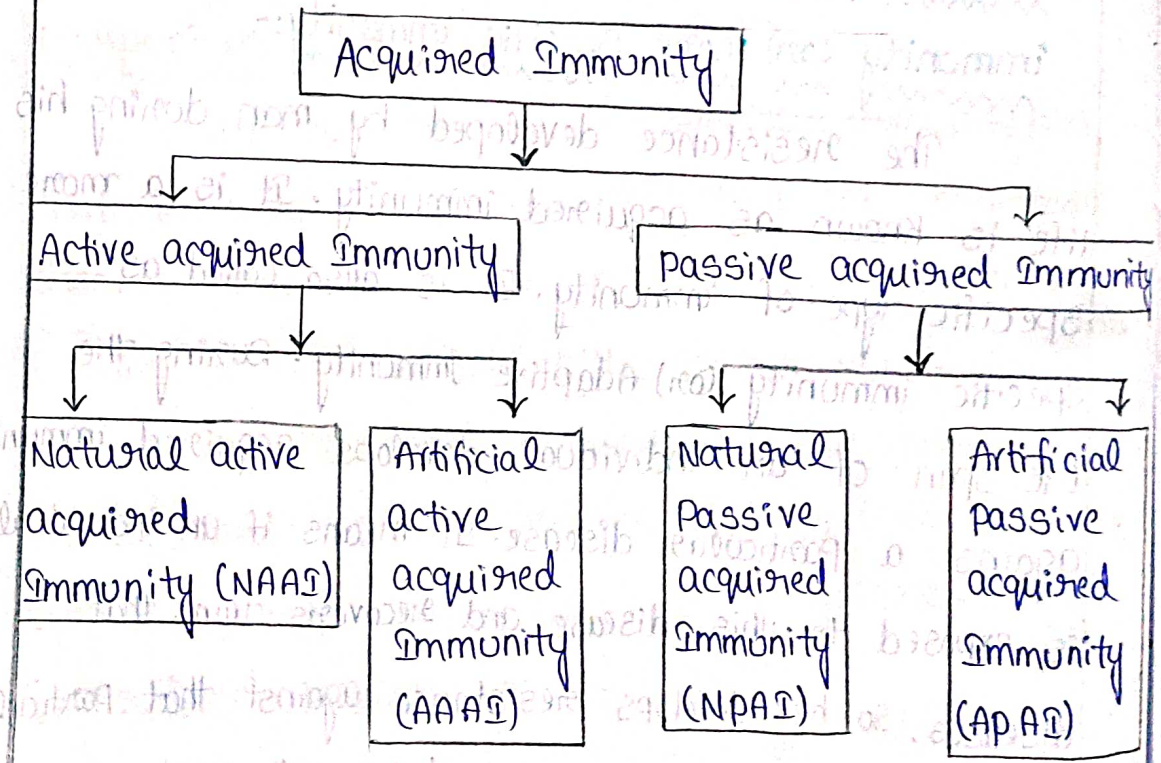
The immune system generates a variety of molecules to recognise the Ag.

(iii) Immunological memory:-

Once immune system has recognise and responded to an Ag. It exhibits immunological.

(iv) Self and Non-Self Recognization:-

Immune System is capable of distinguish self from non-self molecules.



There are two types of acquired immunity.

① Active acquired immunity

② Passive acquired immunity

① Active Acquired immunity:-

In this type of immunity protection is acquired through the generation of immune products by the infected individuals against specific Pathogen. It can be acheived by 2 ways.

(i) Natural Active acquired immunity.

(ii) Artificial Active acquired immunity.

(i) Natural Active Acquired immunity:-

The individual acquired this immunity by natural way. If a person is infected by a pathogen and recovers from the disease naturally then he may be immune against that particular pathogens.

(ii) Artificial active acquired immunity:-

The ~~artificial~~ Individual is artificially immunized by means of vaccination that is when an inactive form of Ag is administrated into the body artificially. Then the person immune system get stimulated against the inactive Pathogen. When there is second encounter of the same Ag then the body becomes resistant against that Ag.

Eg:- vaccines.

② Passive Acquired immunity:-

In this type of immunity, the person get

immunized in passive way that is the person immune cells are immune system doesnot Participate actively in destroying the pathogens. It is of two types,

(i) Natural Passive acquired immunity.

(ii) Artificial Passive acquired immunity.

(i) Natural Passive acquired immunity:-

The person gets immunized passively in a natural way.

Ex:- The transfer of Ab from mother to foetus across the pleasant a transfer of Ab from mother to child.

(ii) Artificial Passive acquired immunity:-

In this type of immunity the immunity gets immune products artificially from an external source. It means the immune cells (or) Absorbe prepared in another organism, isolated and artificially injected into the individual.

Humoral Immunity (or) Ab mediated Immunity:-

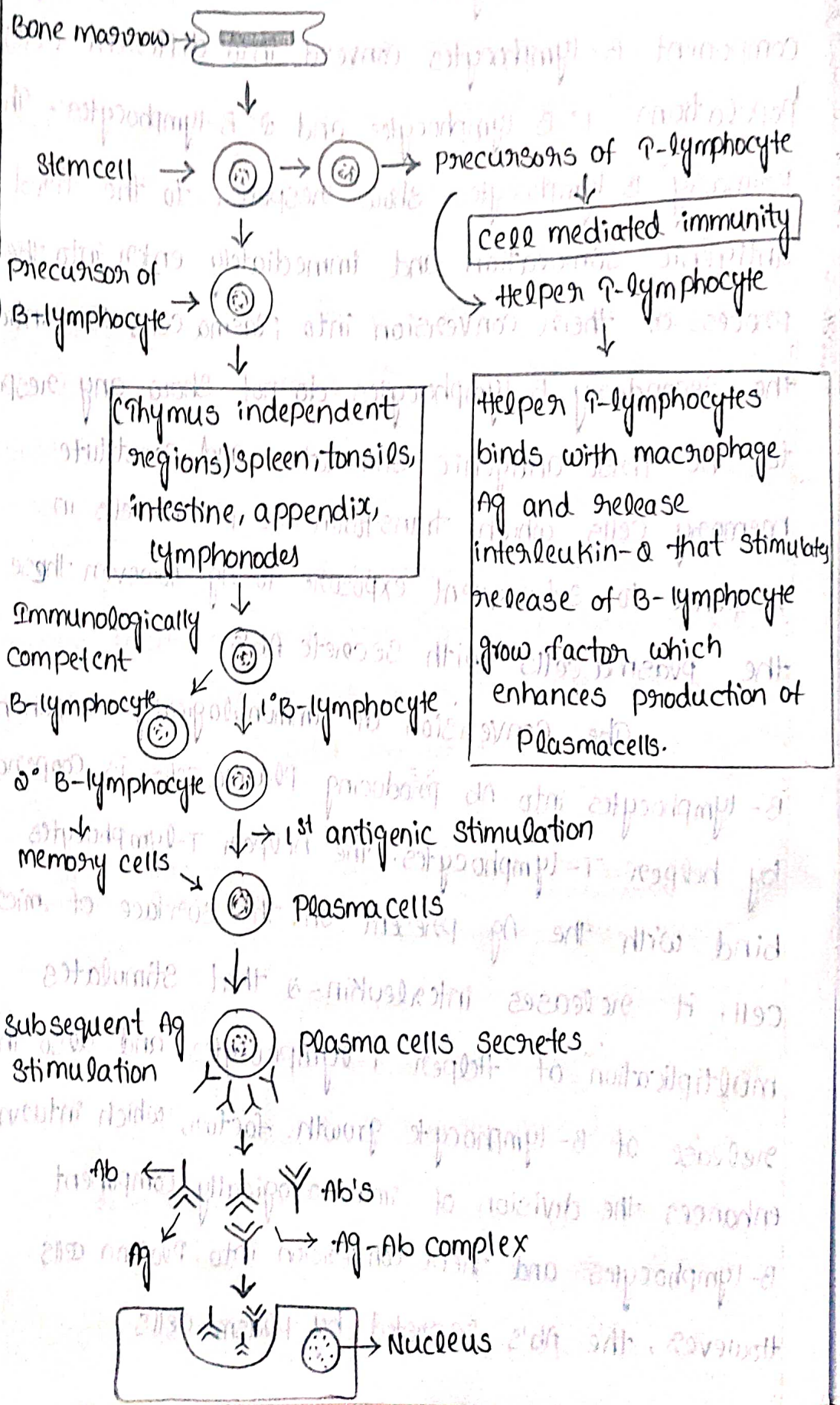
The Ab mediated immunity is that where the B-lymphocytes synthesize Ab's. It responds to the detection of Ag's and these Ab's counteract with those Ag's. Ab mediated immunity is often referred to as humoral immunity.

the precursors of B-lymphocytes which originate from the stem cells of bone marrow are

processed within thymus independent tissues of the lymphatic system (the spleen, tonsils, intestine, apendix and lymph nodes) and become immunological complement. It response to antigenic stimulation the immunologically component B-lymphocytes convert into different cells populations. 1° B-lymphocytes and 2° B-lymphocytes. The primary B-lymphocytes show response to the first antigenic stimulation and immediately enter into the process of their conversion into plasma cells. In contrast the secondary B-lymphocytes do not show any response to the first antigenic stimulation and constitute memory cells which transform into plasma cells in response to subsequent exposure to Ag. However these are the plasma cells with secrete Ab's.

The conversion of immunologically component B-lymphocytes into Ab producing plasma cells is cooperated by helper T-lymphocytes. The helper T-lymphocytes bind with the Ag present on the surface of microphage cell, it releases interleukin-2 that stimulates multiplication of Helper T-lymphocytes and also the release of B-lymphocyte growth factor, which inturn enhances the division of immunologically component B-lymphocytes and their conversion into plasma cells. However, the Ab's secreted by plasma cells clumped

together (agglutinate) with Ag's present in body's circulatory system forming Ab-Ag complexes which are up taken by scavenger WBC.



complement system:-

→ It is a part of immune system

→ It enhances the ability of the antibody and phagocytic cells to clear microbes and damages the organism, promotes inflammation and attacks the pathogenic cell membrane.

→ Complement system consists of a number of small proteins found in the blood.

→ Synthesised by the liver and normally circulating as inactive precurssor.

① classical pathway:-

→ classical pathway is simple stepwise reaction brought about by the activation of complement system.

→ The classical pathway is activated by a Ag-Ab complex, gram negative bacteria and animal viruses.

→ The complement ~~compound~~ components involved in the classical pathway are 11 components namely C_1 to C_9. The component C_1 has three subunits namely C_{1q}, C_{1r}, C_{1s}.

→ The 11 components of classical pathway react in the following sequence:

C_{1q}, C_{1r}, C_{1s}, C_4, C_2, C_3, C_5, C_6, C_7, C_8, C_9.

→ The activating agent such as Ag-Ab complex interact with the 1st component of the complement C_1.

→ The C1q of the complement C1 actually recognizes the Ag-Ab complex and binds with the Fc portion of the Ab. The process of binding complement to the Ab is called complement fixation; Ca^{2+} are essential for effective C1q binding.

→ Attachment of C1 to the immune complex activates the C1r.

→ Activated C1r functions as serine, histadine, esterase and it activates C1s.

→ C1s activates complements C_4 and C_2 in the presence of Mg^{2+} ions.

→ The complement C_4 and activation splits into a small fragments C_4a and large fragment called C_4b. C_4b becomes activated and this activated C_4b becomes attached either to the $Ab-C_1$ complex (or) to the surface of microbes.

→ The activated C_2 is also split into a small fragment called C_2a and large fragment called C_2b. C_2b reacts with C_4b to form C_4b2b complex.

→ The C_4b2b complex functions as C_3 convertage enzyme and it activates C_3 components.

→ The activated C_3 component is cleaved into a small fragments C_3a and large fragment C_3b. The C_3b bind with C_4b2b frames C_4b2b3b complex.

→ The C_3b has binding sites for macrophages and

phagocytes and hence the C_4b_2b complex is adhered to macrophages (or) phagocytes along with microbes.

→ The C_4b_2b complex activates the next complement C_5. The activated C_5 splits into a small fragment called C_5a and large fragment called C_5b. C_5b binds with C_6 and C_7 to form a trimolecule called C_5b6_7 complex.

→ C_5b6_7 complex binds with C_8 to form a tetramolecular complex called C_5b_678.

→ The C_5b67_8 complex has a binding site for C_9. It binds with C_9 to form a C_5b6_789 complex.

→ This complex causes a distruption of the lipid bilayer of the membrane of microbes and this result in lysis of cell. when the lysis cell is bacterium, the lysis is called bacteriolysis. when the lysis cell in RBC, the lysis is called Heamolysis.

Ag - Ab complex

↓

C_1q

↓

C_1r

↓

C_1s

↓ C_2 → C_2a ; C_4 → C_4a

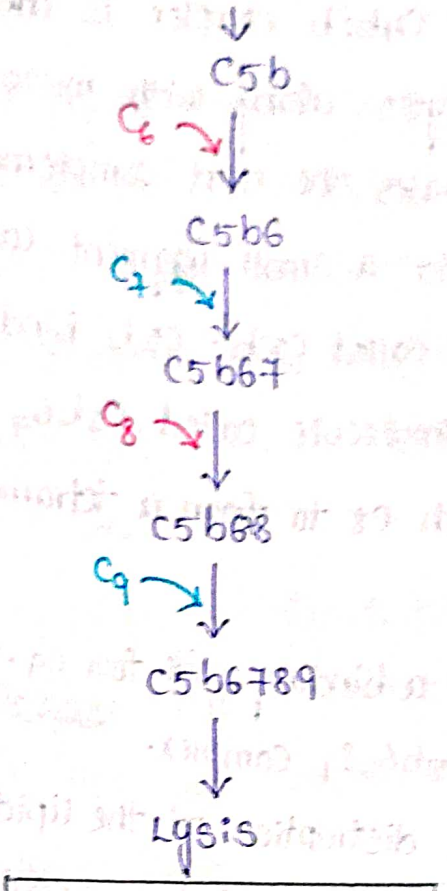
C_4b_2b

↓ C_3 → C_3a

C_4b_2b3b

↓ C_5 → C_5a

C_4b_2b3b → Phagocytosis



Classical Pathway

Significance of Classical Pathway:-

When a microbe enter the body, the body response producing Ab. The Ab binding with the microbe to form an Ag-Ab complex (or) immune complex. When the immune complex is formed the compliment system is activated to enhance the removal of the microbe. This is done in the following ways.

→ The complement system helps to adhere the microbe to the neutrophils and macrophages.

→ It facilitates the ingestion of microbes by neutrophils and macrophages through Phagocytosis.

→ It brings about the lysis of microbes.

⑧ Alternative pathway (or) properdin Pathway:-

→ The activation of complement C_3 without the prior participation of C1, C4 and C2 is called alternative pathway.

→ The alternative pathway was discovered by pillemer in 1954.

→ Alternative pathway is a stepwise reaction of the complement system to bring about the distruction of microbes in the body.

→ The alternative pathway is activated by aggregated Ab's IgG (or) IgA lipopolysachaside, bacterial endotoxin and yeast.

→ The alternative pathway is the independent of the Fc fragments of Ab's.

→ About 6 complement components are involved in the alternative pathway. They are :- C_3, B, D, P, H and I.

→ In this pathway C_3 is starting component and it is activated without the activation of C1, C4 and C2.

In alternative pathway C3 is activated by Pz complex. Pz complex is formed by the reaction of properdin, a normal serum protein with zymosyn in the presence of mg^{2+}.

The Pz complex functions as a protinase enzyme, it cleaves C_3 into a small fragment C3a and large fragment C3b.

→ The C3b is coated on the surface of microbe.
→ Then factor B binds with C3b to form a complex called C3bB complex.
→ The C3b B complex is weak bridge and is stabilized by factor P called properdin.
→ Activated factor D activates and cleaves the C3bB complex releasing C3 cleavage enzymes C3bBb called C3 convertase.
→ The activated C3bBb splits C3 into C3a and C3b.
→ C3b is deposited near by microbes and interaction with factors B & D.

once C3b is formed a cycle of activation occors which maintain C3b formation without the original activation agent.

C3b may activate the C5 and enter into the classical pathways and produce C5b6789 complex in sequence resulting in lysis of the microbes.

Aggregated Ab (or) Pz complex

↓

C3

↓ → C3a

C3b

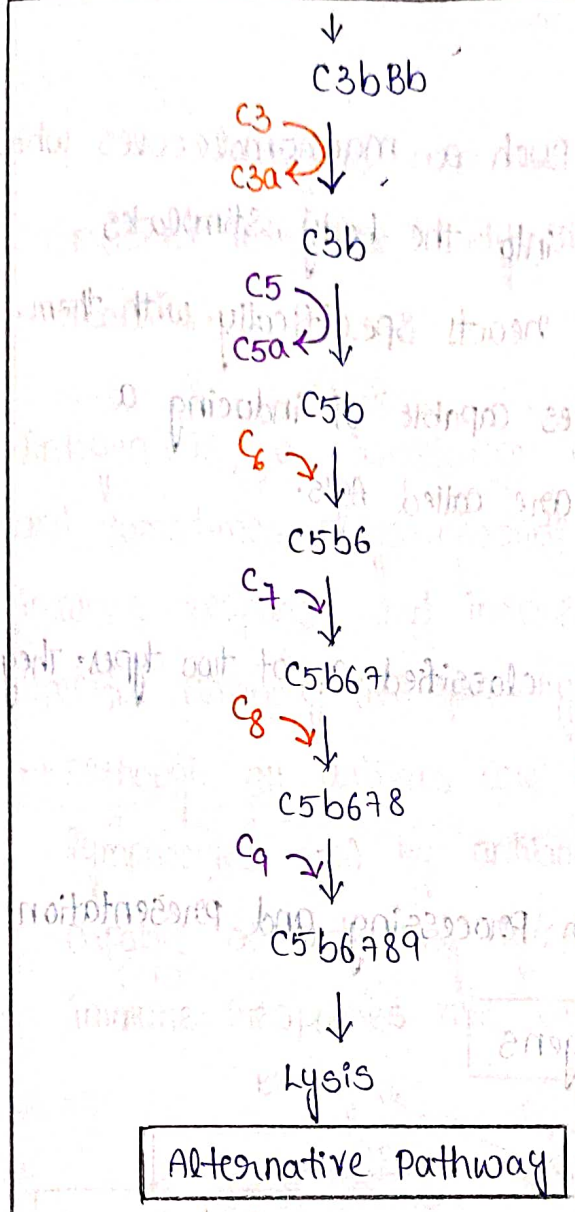
B ↓

C3bB

P ↓

C3bB

D ↓



Alternative Pathway

Part - D :- ANTIGEN

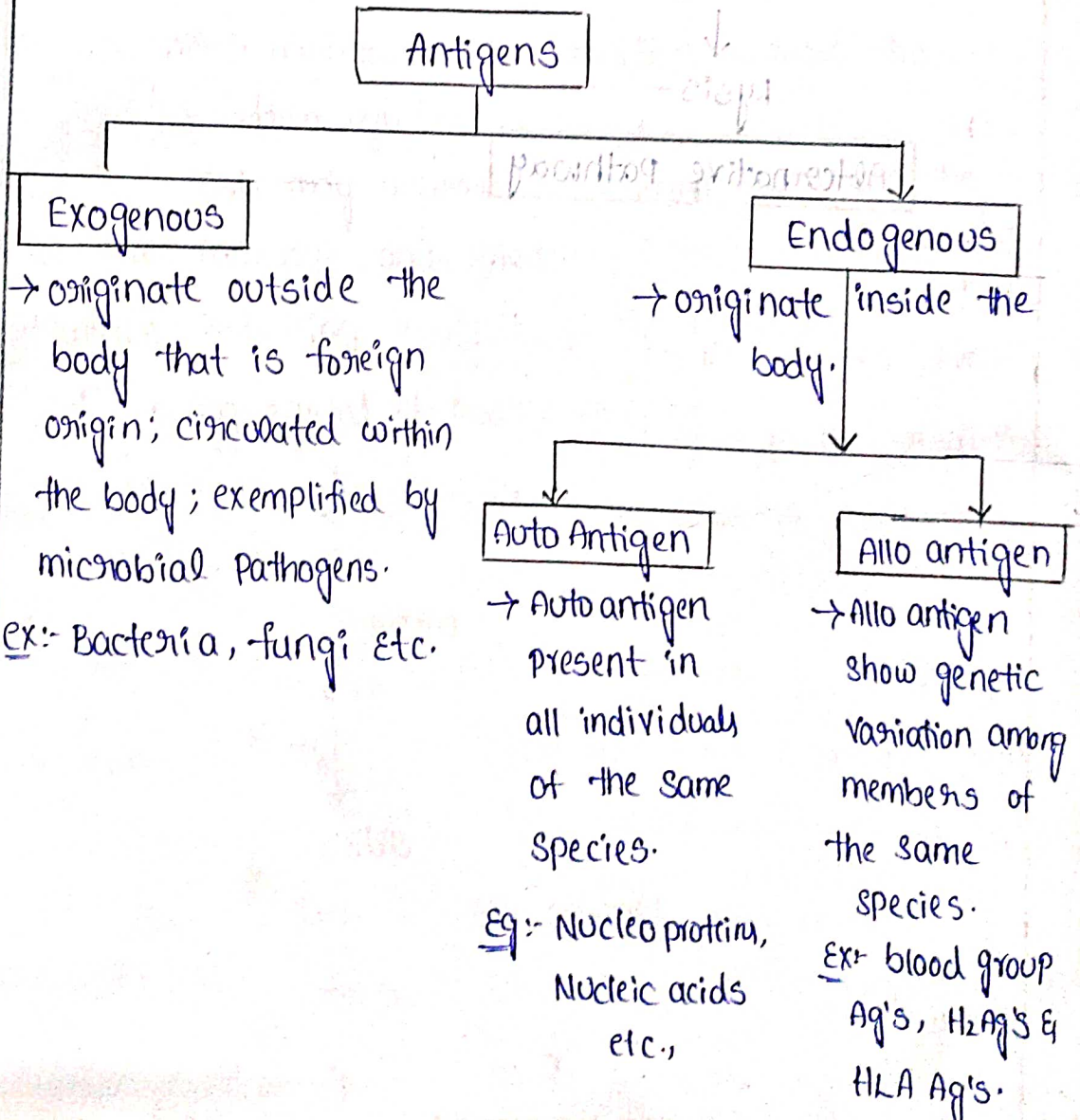
Antigen :-

An antigen is such a macromolecules which depends upon introduction into the body, stimulates production of Ab's and reacts specifically with them. In simple terms substances capable of inducing a specific immune response are called Ag's.

Types of Ag's :-

Ag's can be broadly classified as of two types, they are :- ① exogenous Ag's.
② endogenous Ag's

→ on the basis of their processing and presentation.



Part-D:- Antigen

Definition:-

Antigens are substances which, when introduced into the body, stimulate the production of antibodies.

(or)

Antigen is a substance usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody (or) specially sensitized T-cells (or) both.

→ Although all antigens are recognised by specific lymphocytes (or) by antibodies, only some antigens are capable of activating lymphocytes - molecules that stimulate immune responses are called immunogens.

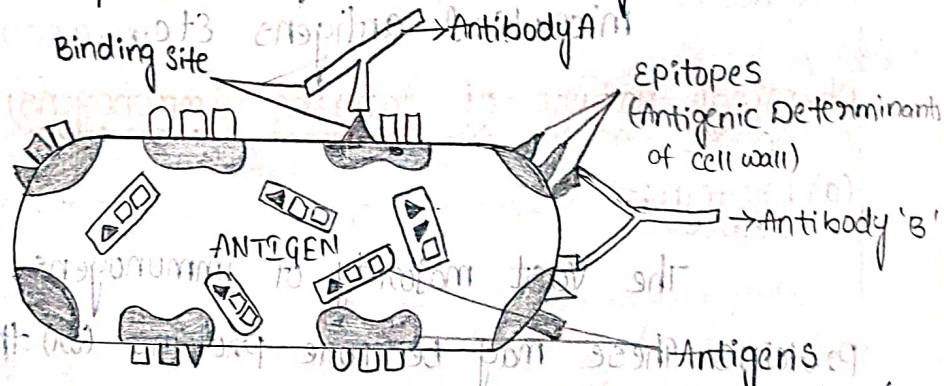


Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.

→ Adjuvants are substance that are non-immunogenic alone but enhance the immunogenicity of any added immunogen.

→ Epitope is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors (or) lymphocytes (or) to

secreted antibodies. It is also called antigenic determinants.

Physical nature of Antigens :-

Autoantigens :-

For example, are a person's own self antigens.

Ex:- Thyroglobulin, DNA, corneal tissue, etc.,

Alloantigens are antigens found in different members of the same species (the red blood cell antigens A and B are examples).

→ Heterophile antigens are identical antigens found in the cells of different species.

Examples:- Forrssman antigen, cross-reacting microbial antigens etc.,

Chemical nature of Antigens (Immunogens):-

(a) protein:-

The vast majority of immunogens are proteins. These may be pure proteins (or) they may be glycoproteins (or) lipoproteins. In general, proteins are usually very good immunogens.

(b) polysaccharides:-

Pure polysaccharides and lipopolysaccharides are good immunogens.

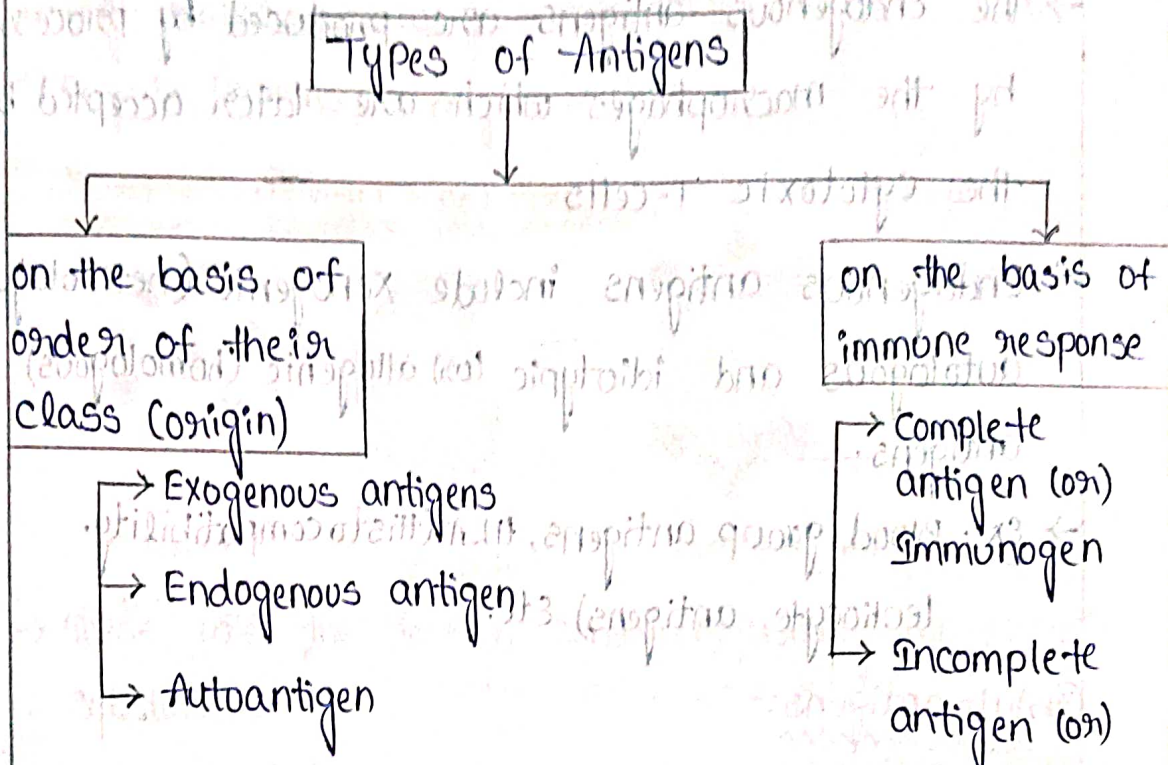
(c) Nucleic acids:-

Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded (or) when complexed with proteins.

(d) Lipids :-

In general lipids are non-immunogenic, although they may be haplens.

Types of antigens :-



on the basis of order of their class (origin) :-

① Exogenous Antigen :-

→ These antigens Enter the body (or) system and start circulating in the body fluids and trapped by the Apcs (Antigen processing cell such as macrophages, dendritic cells Etc).

→ The uptake of these exogenous antigen by Apcs are mainly mediated by the phagocytosis.

Examples :- Bacteria, Viruses, fungi, Etc.

→ Some antigens start out as exogenous antigens, and later become Endogenous (for example, intracellular Viroses).

② Endogenous Antigen:-

→ These are body's own cells. (or) sub fragments (or) Compounds (or) the antigenic products that are Produced.

→ The Endogenous antigens are ~~produced by~~ processed by the macrophages which are later accepted by the cytotoxic T-cells.

→ Endogenous antigens include xenogenic (heteriologus), autologous and idiotypic (or) allogenic (homologous) antigens.

→ Ex:- Blood group antigens, HLA (Histo compatibility leukocyte antigens) etc.

③ Auto antigens:-

→ An autoantigens is usually a normal protein (or) complex of proteins (and sometimes DNA (or) RNA) that is recognized by the immune system of patients suffering from a specific autoimmune disease.

→ These antigens should not be under normal conditions, the target of the immune system, but due mainly to genetic and Environmental factors, the normal immunological tolerance for such an antigen has been lost in these patients.

Ex:- Nucleoproteins, Nucleic acids etc.,

on the basis of immune response:-

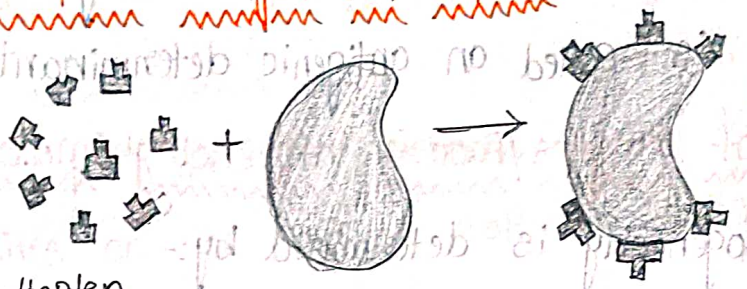
① Complete Antigen (or) Immunogen:-

→ Posses antigenic properties denovo i.e, these are able to generate an immune response by themselves.

→ High molecular weight (more than 10,000).

→ may be proteins (or) polysaccharides.

② Incomplete Antigen (or) Hapten:-



→ These are the foreign substance, usually non-protein substances.

→ unable to induce an immune response by itself, they require carrier molecule to act as a complete antigen.

→ The carrier molecule is an non-antigenic component and helps in provoking the immune response.

Ex:- Serum protein such as Albumin (or) Globulin.

→ low molecular weight (less than 10,000).

→ Haptens can react specifically with its corresponding antibody.

Ex:- capsular polysaccharide of pneumococcus, polysaccharide "c" of beta haemolytic streptococci, cardiolipin antigens etc.

Determinants of Antigenicity:-

The whole antigen does not evoke immune response and only a small part of it includes B and T-cell response.

→ The small area of chemical grouping on the antigen molecule that determines specific immune response and reacts specifically with antibody is called an antigenic determinants.

Property of antigens / Factors influencing immunogenicity

Immunogenecity is determined by:-

① Foreignness:-

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

② Molecular size:-

→ The most active immunogens tend to have a molecular mass of 14,000 to 6,00,000 Daltons.

Ex:- Tetanus toxoid, Egg albumin, thyroglobulin are highly antegenic.

→ Insulin (5700) are either non-antigenic (or) weakly antigenic.

③ Chemical Nature and Composition:-

→ In general, the more complex the substance is chemically the more immunogenic it will be.

→ Antigens are mainly proteins and some are polysaccharides.

→ It is presumed that presence of an aromatic

radical is essential for rigidity and antigenicity of a substance.

④ physical form:-

→ In general particulate antigens are more immunogenic than soluble ones.

→ Denatured antigens are more immunogenic than the native form.

⑤ Antigen Specificity:-

→ Antigen Specificity depends on the specific active sites on the antigenic molecules (Antigenic determinants)

→ Antigens determinants (or) Epitopes are the regions of antigen which specifically binds with the antibody molecule.

⑥ Species Specificity:-

→ Tissues of all individuals in a particular species possess, species specific antigen.

→ Human blood proteins can be differentiated from animal protein by specific antigens- antibody reaction.

⑦ organ Specificity:-

→ organ specific antigens are confined to particular organ (or) tissue.

→ Certain proteins of brain, kidney, thyroglobulin and lens proteins of one species share specificity with that of another species.

⑧ Auto-Specificity:-

→ The autologous (or) self antigens are ordinarily not immunogenic, but under certain circumstance lens protein, thyroglobulin and others may act as autoantigens.

⑨ Genetic factor:-

→ Some substances are immunogenic in one species but not in another.

→ Similarly, some substances are immunogenic in one individual but not in others (i.e, responders and non-responders).

→ The species (or) individual may lack (or) have altered genes that code for the receptors for antigens on B-cells and T-cells.

→ They may not have the appropriate genes needed for the Apc to present antigen to the helper T-cells.

⑩ Age:-

→ Age can also influence immunogenicity.

→ usually the very young and the very old have a diminished ability to elicit and immune response in responses to an immunogen.

⑪ Degradability:-

→ Antigens that are easily phagocytosed are generally more immunogenic.

→ This is because for most antigens (T- dependant antigens) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T-cells by an antigen presenting cell (Apc).

⑫ Dose of the antigen:-

→ The dose of administration of an immunogen can influence its immunogenicity.

→ There is a dose of antigen above (or) below which the immune response will not be optimal.

⑬ Route of Administration:-

→ Generally the subcutaneous route is better than the intravenous (or) intragastric routes.

→ The route of antigen administration can also alter the nature of the response.

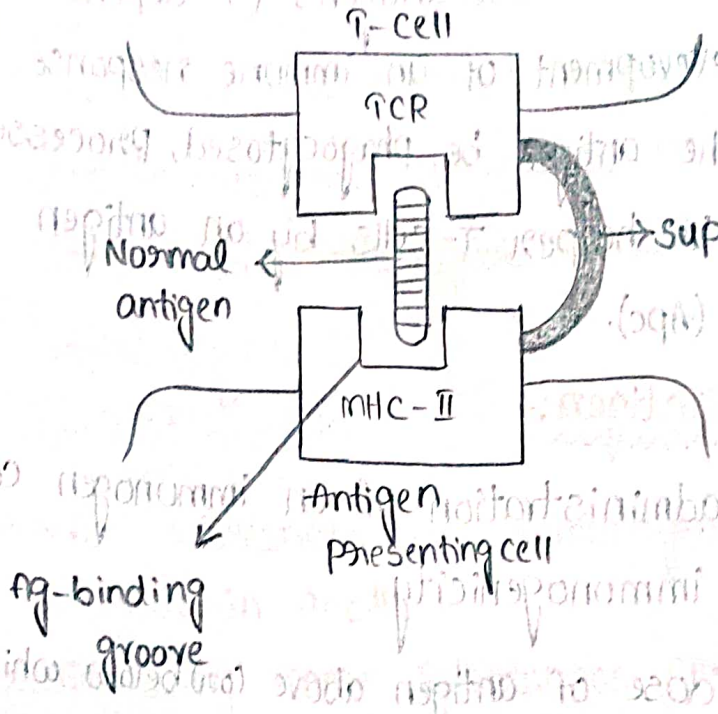
→ Antigen administered intravenously is carried first to the spleen, where as antigen administered subcutaneously moves first to local lymphnodes.

⑭ Adjuvants:-

→ Substances that can enhance the immune response to an immunogen are called adjuvants.

→ The use of adjuvants, however, is often hampered by undesirable side effects such as fever and inflammation.

Eg:- Aluminum hydroxide


T- cell
TCR
Normal antigen
Super antigen
MHC - II
Antigen presenting cell
Ag-binding groove

UNIT-II

ANTIBODIES [IMMUNOGLOBULINS]

Structure of Antibodies (or) immunoglobulins :-

Antibodies (or) Immunoglobulins (Ig) are glycoproteins formed by plasmacells in response to antigenic stimulation and counteract with antigen's with great specificity.

→ They were first discovered by Pilselius and Kobot in 1939.

→ Immunoglobulins are represented as Ig. These are also called as antibodies (Ab's).

→ Ig molecules are found in blood serum, body fluids and tissues. These are produced only by vertebrates.

→ They are synthesized by B-lymphocytes. In 1962, Rodney porter and GM. Edelman proposed the basic structure of Ig.

→ The normal structure of any Ig molecule is in the shape of 'Y'.

→ Ig molecules consists of four polypeptide chains of these four chains, two chains are heavy chains and the other two are light chains.

→ The molecular weight of light chain is 25,000 daltons and the molecular weight of heavy chain is 50,000 daltons.

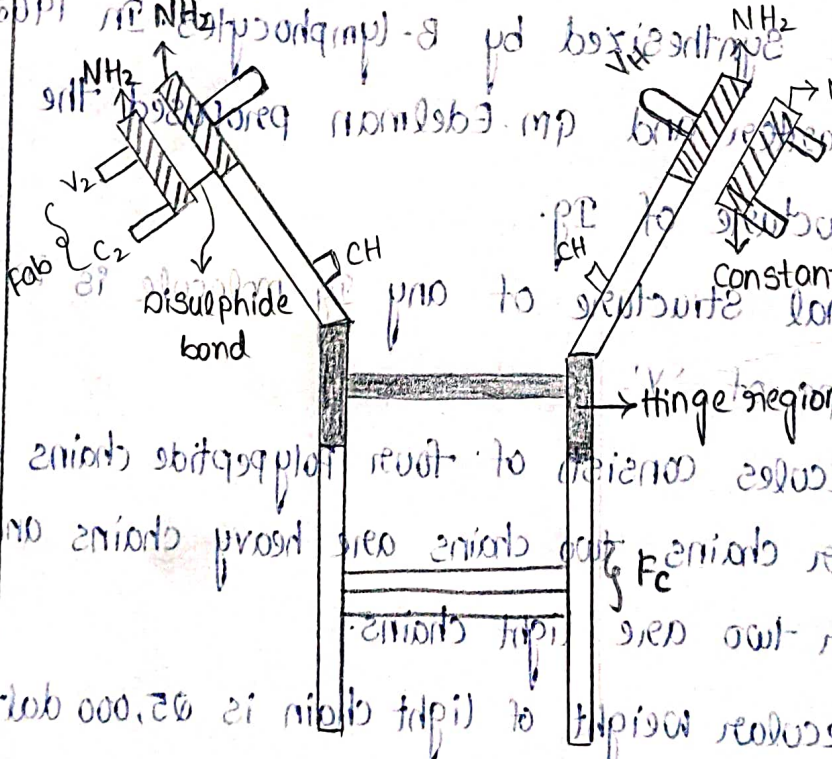
→ The heavy chain consists of $\alpha, \delta, \varepsilon, \gamma, \mu$ Subunits

and light chain consists of Kappa (or) Lambda.

→ The light chain and heavy chains are connected by disulfide bonds.

→ The two heavy chains are connected by each other by disulfide bonds. There is a hinge region between the two heavy chains. It provides flexibility to the Ig within the two heavy chains and the two light chains intrachain disulphide bonds are present.

→ The immunoglobulin molecule contains two regions. they are:- ① variable region.
② constant region.



→ In the variable region the aminoacid sequence should variability in different Ig's. This region is also called hyper variable region (or) complementary determine region the variable region is located at the End of limb of Y and it is m-termines of the Ig molecule.

→ The constant region is located at the base of stalk of the Y and it is c-termines.

→ The variable region and the constant region are formed by both heavy and light eka chains.

→ Based on the functional aspect Ig molecule has two regions. They are:- ① Fab region
② Fc region

→ Fab region is called fragment of Ag binding region it is located at the ends of the two limbs of Y (or) Ig reacts with Ag epitopes.

→ Fc region is also called crystalligible fragment. It is located at the state of Y (or) Ig.

→ Fc region is involved in the attachment of the immune cells and also is complement fixation.

Classes of immunoglobulins (or) Types of immunoglobulins (Ab's):-

These are five types of immunoglobulins molecules in numbers. They are:-

① Immunoglobulin G [IgG] - γ heavy chain
② Immunoglobulin A [IgA] - α heavy chain

③ Immunoglobulin M [IgM] - μ heavy chain

④ Immunoglobulin D [IgD] - δ heavy chain

⑤ Immunoglobulin E [IgE] - ε heavy chain

① Immunoglobulin G [IgG]:-

IgG is the most abundantly occuring immunoglobulin in human serum according for 80% of the total Ig's this Ab is termed as (maternal Ab) because it is only class of Ab's has crosses the placenta and convert immunity upon foetus that lossed for the first month after birth.

It's molecular weight is 160 - 170 Kilodaltons. It Exist as a monomer. Each heavy chain consist of above 440 amino acids while each light chain have 220 amino acids.

A functional IgG Ab consists of two Ag binding unit. Each unit consisting of one heavy chain one light chain therefore IgG are bivalent and can bind two identical epitope (or) Ag.

There are four subclasses of IgG is humans namely :- (i) IgG_1

(ii) IgG_2

(iii) IgG_3

(iv) IgG_4

These subclasses mainly differ from each other in possessing different chemical composition in heavy

and the numbers and arrangement of interchain disulphide bonds. 65% of IgG Ab's in serum are IgG_1 Ab's, where are 23% are IgG_2 Ab's. These subclasses Ab's performs different biological functions. they are:-

→ If IgG_1 and IgG_3 are Ab's are anti RH Ab's and upon recognization of their specific Ag bind to receptor expressed on monocytes and macrophages and make them better phagocytes.

→ If IgG_1 and IgG_3 are Ab's are

→ If IgG_2 Ab's are opsonic and develop in response to antitoxic

→ If IgG_4 Ab's however functions as skin sensitizing immunoglobulins.

→ IgG half life is 23 days.

③ Immunoglobulin A [IgA]:-

→ IgA constitute 50% of Ig in the serum. It is found in body serum such as saliva, tears, breast milk and mucosal secretions from the gastro intestinal respiratory and urinogenital tracts.

→ All of this mucosal surfaces are associated with 'MALT' that produce IgA.

→ It's molecular weight is 385 kilodaltons. It exists as a dimer the secretory IgA dimer consists of two monomer; A, Secretory component 'J' chain. The joining chain is J-chain that specifiliates the polymerisation of IgA.

→ Everyday human secretes 5 to 15 gms of secretary IgA in the mucous secretions.
→ Secretary IgA protects the surface tissue against microbial pathogens by forming immunobarriers.
→ Breast milk contains several types mucocytes and secretory IgA and many other molecules that help to protect the new born baby against the infections during the first month of life.
→ It's half life is 6 days.
→ Secretory IgA present in intestine bind to protozone parasite (Entameoba histolitica) bacteria and virus and thus their adherence of mucosal surfaces and invasion of host tissues by them this phenomenon is refers to as immune exclusion.
→ The secretory component helps in transport IgA dimer across the cell membrane.

③ Immunoglobulin m [Ig m]:-

→ Ig m is the largest Ab and constituents total 5 to 10% of the total Ig present in the human serum.
→ It's molecular weight is 900 kilo daltons (KD).
→ Ig m is usually a polymer of 5 monomeric units (pentamer) each possessing to two heavy chains and two light chains.
→ The monomers are arranged in a pinwheel array help together by disulphide bonds at a special

J-chain (joining chain).

→ The five monomeric units are arranged in such away that fc region (or) at the centre of pentamers and the Ag binding site are at the periphery.

→ Ig m is the first immunoglobulin and is expressed as membrane bound Ab and B-lymphocytes. Igm is secreted into serum during primary Ab response following the introduction of Ag into the body since this Ab is so large it doesnot cross the placenta.

→ Ig m are generally have low affinity but high availability (strength of binding to Ag)

→ It's half life is 5 days.

→ Ig m molecule agglutinate the gram-ve bacteria and activates the complement by the classical pathway.

→ It Enhance the insaction of Pathogens by the process of phagocytosis.

④ Immunoglobulin D [Ig D] :-

→ Ig D occurs in low concentration that is 0.2% of the total Ab's present in the blood serum.

→ It's molecular weight is 180 Kilodaltons.

→ These Ab's are monomeric and their monomeric structures are similar to IgG.

→ Ig D molecules are short lived be particularly suspectable to proteolytic Enzymes and to heat.

→ These Ab's donot fix complement and fail to cross placenta

→ They abundantly occur in combination with IgM on the surface of B-lymphocytes that bind to Ag and signal the B-lymphocyte to initiate the formation.

→ It has been found that IgD is especially abundant on memory cells differentiated from B-lymphocytes and most probably play an important role in 2° Ab response.

⑤ Immunoglobulin E [IgE]:-

→ IgE occurs in blood serum in extremely small amounts that is 0.002% of total Ab's and it exist as a monomer.

→ These Ab's despites low concentration are classic skin sensitizing and an _____ Ab because immediate hyper sensitivity (allergies) are mediated by them. Therefore they are popularly called allergic antibodies.

→ IgE molecules are mostly present on the surface of basophils and mast cells.

→ IgE molecules bind to allergies and stimulates basophills which cause degranulation of cells releasing pharmalogically active molecules.

→ It's molecular weight is 160-200 kilodaltons. It is rich in carbohydrates. It cannot cross through placenta.

→ It's half life is 2.5 days.

Antibody functions / functions of immunoglobulins:-

Antibody molecule shows a different role played by each of it's two regions the Ag binding fragments (Fab) and the crystalized fragments (Fc) its Ag binding fragment region functions to bind Ag where as the crystalligible fragment region mediates binding to tissues of the host various cells of the immune system, certain phagocytic cells (or) the 1st component of the complement system.

When Ab molecule binds Ag this normally doesn't cause distruction of the Ag rather Ab serves to mark and identify the Ag for immunological attack and to activate immune responses that destroy the Ag. However the binding of Ab to Ag that is Ag-Ab interaction takes place in different ways and is the central damage of Ab mediated immunity (humoral) inside the body of the host.

The important functions attributed to Ab's by widely of Ag-Ab interactions in the host body they are:-

① Neutralization
② Immune complex formation
③ opsonization
④ Complement fixation
⑤ Ab dependent cell mediated cytotoxicity.

In addition Ab's also contribute in the causation of hypersensitivity, auto immunity and immune diffusion by way of Ag-Ab interaction.

	monomer	Pentamer	2° IgA dimer	monomer	monomer
Properities	Ig G	Ig M	Ig A	Ig D	Ig E
Structure	monomeric	monomeric & Pentameric	Dimeric & monomeric	monomeric	monomeric
occurance	Blood (Transfered from mother to foetus)	surface of B-cells and blood	milk; saliva; tears, Nasal, respiratory & intestinal secretion	cell surface of B-cells, serum (0.2%)	surface of mast cells basophiles.
Percentage of total Ab's in the serum	80%	6%	13%	less than 1%	less than 1%
molecular weight (daltons)	150,000	900,000	385,000	180,000	200,000
Light chain	Kappa (or) Lambda	Kappa (or) Lambda	Kappa (or) Lambda	Kappa (or) Lambda	Kappa (or) Lambda
Heavy chain	γ (gamma)	μ (mu)	α (Alpha)	δ (delta)	ε (Epsilon)

	monomer	Pentamer	2° dimer	monomer	monomer
Properties	IgG	IgM	IgA	IgD	IgE
Number of Ag binding site	2	10	4	2	2
Sub-units	H_2L_2	$(H_2L_2)_5$	$(H_2L_2)_2$	H_2L_2	H_2L_2
Fixes complement	It fixes with complement	It fixes with Complement	It doesnot fixes with complement unless aggregated	It doesnot bind Complement	It doesnot fixes with Complement.
Half-life	23 days	5 days	6 days	3 days	2.5 days
Sub-classes	IgG (1-4)	-	α_1, α_2	-	-
characteristics/ properties	main form of antibodies in circulation increased after immunization, secreted during Secondary response.	Functions as antigen receptors on surface prior to immunization, secretes during primary response.	main antibody type in external Secretion saliva and mother's milk also called as breast milk	Functions as antigen receptors on surface prior to immunization; other functions are unknown	Responsible for allergic symptoms in immediate hypersensitivity reactions.

Properties	monomer Ig G	pentamer Ig M	2° dimer Ig A	monomer Ig D	monomer Ig E
specific	* Also called maternal antibody. * Heavy chain contains 440 A.A * Light chain contains 220 A.A * Bivalent can bind to two identical epitopes * 4 subclasses 65% ← IgG1 22% ← IgG2 IgG3 IgG4 } Differ in chemical composition in heavy chain & number arrangement of disolphide bonds.	* Arranged in pinwheel array disulphide bonds J chain. * Has low affinity but highly available	* Two monomeric unit are joined by J chain.	* Short lived, * Succeptable to proteolytic enzyme and heating	

	monomer	Pentamer	"0" dimer	monomer	monomer
Properties	IgG	IgM	IgA	IgD	IgE
Functions	IgG_1, IgG_3- Recognises specific antigen-bind to receptor IgG_2- opsonic response to antigen toxicity IgG_4- skin sensitizing immunoglobulins	It agglutinates gram-ve bacteria and activates with the classical path.	IgA associates with malt. It protects the surface tissue against microbial pathogens (immuno barriers) present in intestine, bind to protosomal parasite, bacteria, virus, Adherence of mucosal surfaces and invasion of host (immune exclusion)	play an important role in secondary immune response	Bind to allergins and stimulate basophills degranulation Pharmacological activity.

① Neutralization (NAb):-

→ Ab that ~~define~~ defined a cell from an Antigen/ infection body by neutralizing any effect it has biologically.

Ex:- NAb is diptheric antitoxin, which can neutralize the biological effect of diptheric toxin.

② Opsonization:-

Refers to an immune process where Particles such as bacteria are targeted for destruction by an immune cell known as phagocyte. The process of opsonization is a mean of identifying the invading Particle to the phagocyte.

Ex:- Ab molecule binds to IgG & IgE complement Proteins C_3b, C_4b. Fab region of the Ab reacts with epitopes of the Ag.

③ Immune complex formation:-

An immune complex formation some times called Ag-Ab complex, is a molecule formed from the integral binding of antibody to a soluble Ag. The bound Ag and Ab out as a unitary objective Effectively on Ag of its own with a specific Epitope.

Ex:- Immune complex is a prominent features of auto immune diseases including systemic lupus, Erythematosus, Rheumatoid arthritis.

UNIT-III

CELL MEDIATED IMMUNITY

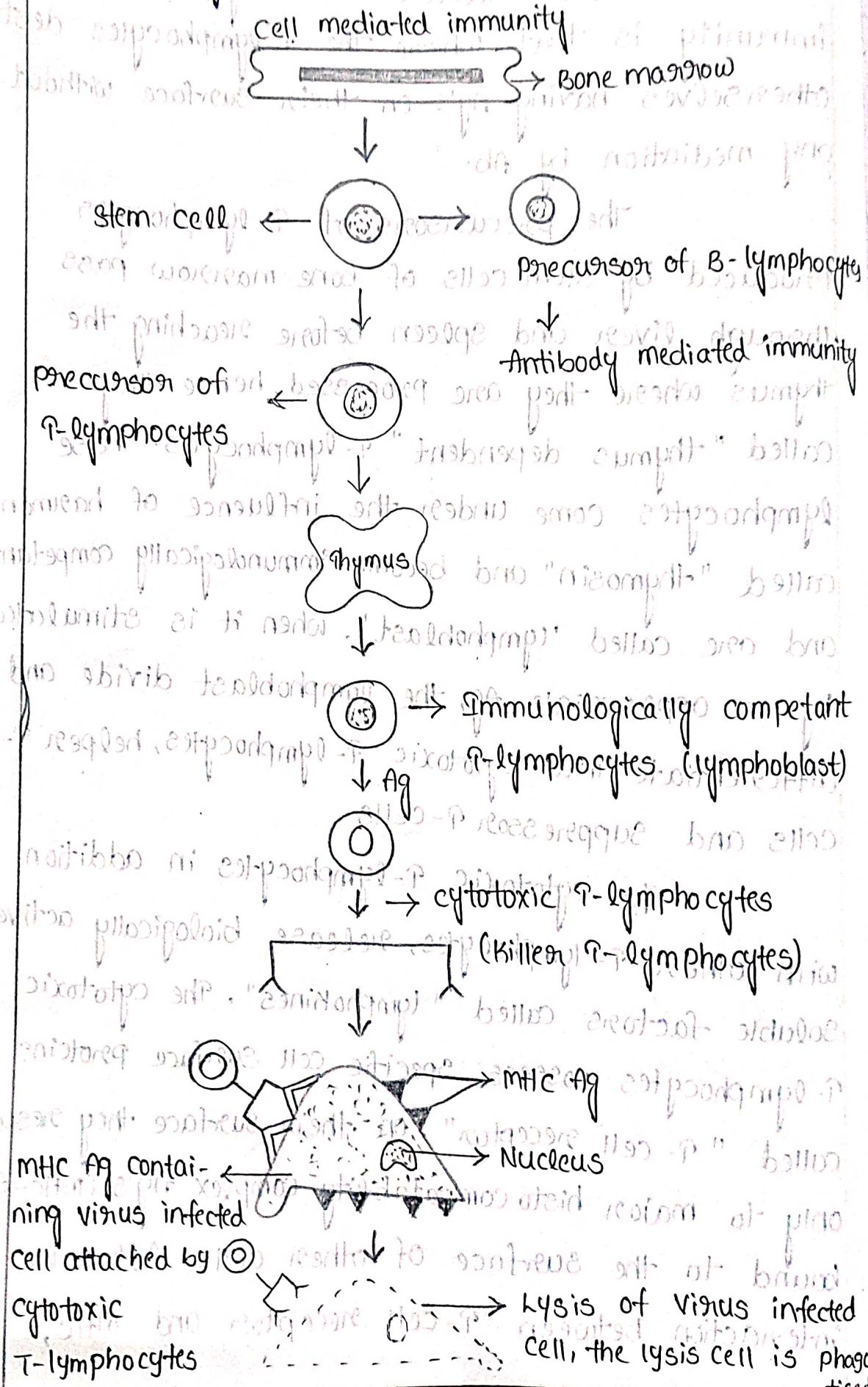
Cell mediated immunity (or) Cellular immunity:-

The cell mediated immunity (or) cellular immunity is that where the T-lymphocytes destroy otherselves having Ag's on their surface without any mediation by Ab.

The precursors of T-lymphocytes produced by stem cells of bone marrow pass through liver and spleen before reaching the thymus where they are processed, hence they called "thymus dependent" T-lymphocytes. These lymphocytes come under the influence of hormone called "thymosin" and become immunologically competant and are called 'lymphoblast'. when it is stimulated by an appropriate Ag, the lymphoblast divide and differentiate into cytotoxic T-lymphocytes, helper T-cells and suppressor T-cells.

The cytotoxic T-lymphocytes in addition with other T-lymphocytes, release biologically active soluble factors called "lymphokines". The cytotoxic T-lymphocytes possess specific cell surface proteins called "T-cell receptor". on their surface they respond only to major histo compatability complex Ag's (MHC-Ag's) bound to the surface of other cells. After the interaction between T-cell receptor and MHC,

Ag is established and the cytotoxic T-lymphocyte cells binds the MHC Ag containing cell. They later undergoes lysis and is phagocytised.



The cell mediated immunity is important in controlling those infections where the pathogens are intracellular and reproduce within the infected cells.

Ex:- viral and some protozoan like trypanosomes etc.

In such infections the Ab's proved to be an ineffective because the Ab's are unable to penetrate and attack intracellular pathogens multiplying within the host cell. In addition, the cellular immunity is consider to play an important role in monitoring and regulating the proliferation of abnormal types of cells (humoral cells) and thus inhibit the tumour development.

Ab dependent cell mediated cytotoxicity:-

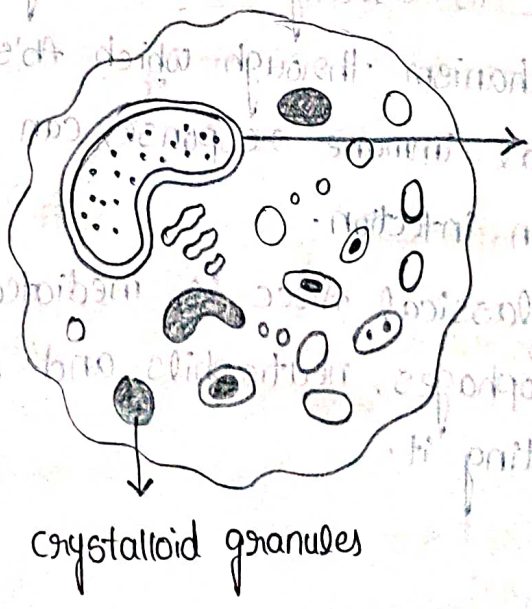
The ADCC is a mechanism of cell mediated immune defense where by an effector cell of immune system actively lysis the target cell whose membrane surface cell have been bound by specific type Ab's. In human ADCC is usually mediated by IgG. It is one of the mechanism through which Ab's, as a part of humoral immune response, can act to limit and contain infection.

Classical ADCC is mediated by NK cells but macrophages, neutrophils and Eosinophils can also mediating it.

Eosinophils :-

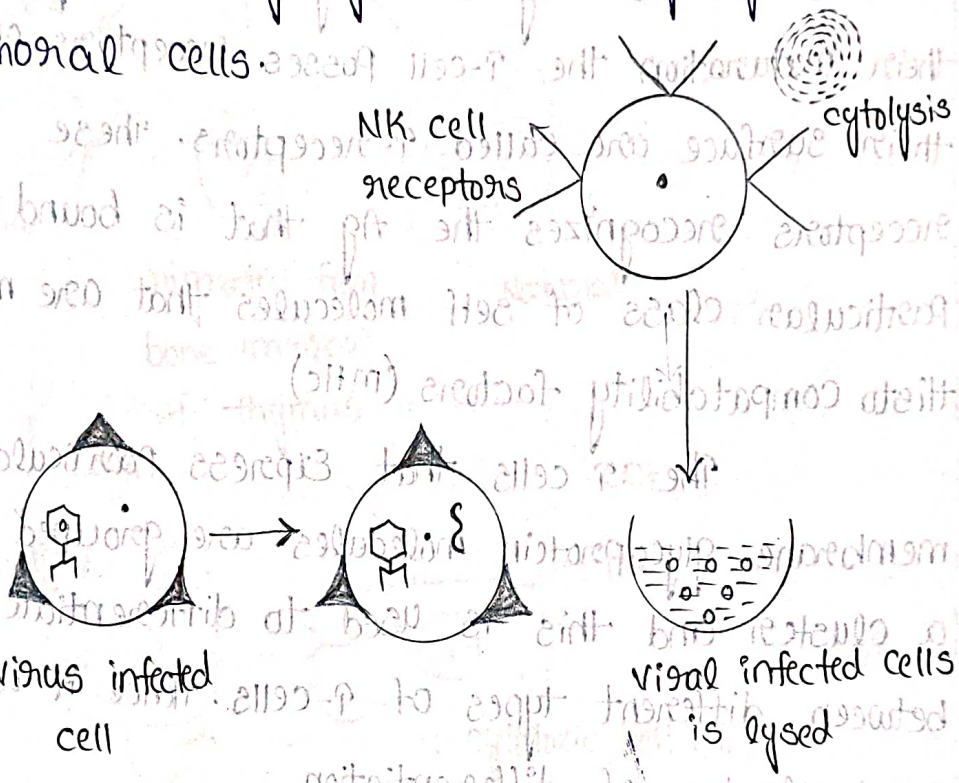
Large parasites like helminths are too big to be engulfed and killed by phagocytosis. They also have an external structure (or) integument that is resistant to attack by substance released neutrophils and macrophages. After IgE coat these parasites, the Fc receptors of an eosinophils will then recognise IgE. Subsequently interaction between Fc receptor and the Fc portion of helminthus bound to IgE signals the eosinophils to degranulate.

They constitute 1 to 3% of the total circulatory WBC. Eosinophils that is like neutrophils. they are motile cells that migrated from blood stream into tissue phases. These granulocytes are consider to play a role in that defense against paralytic organisms by phagocytosis.



Natural Killer cells:-

These are also called as Null cells. These cells do not express membrane bound molecules and receptors. The T & B-cell lineages, they constitute 5-10% of the total lymphocytes. These cells play cytotoxic activity against humoral cells.



The NK cells attached themselves to the Ab's at the Fc region and destroy the target cells. This type of immunity by the NK cell is called "Ab dependent cell mediated cytotoxicity (ADCC)" is a special type of NK cells (or) NKT cells. These cells have the characteristics of both NK & T-cells. These cells have receptors on their surface CD_{16}, hence they behave like a T-cells and produces cytokinins that make it behave like a NK cell.

These cells destroy the cancer cells and cells infected with herpes and mumps. They do not

need Ab's for its activity. They are activated by interferons and interleukins-2.

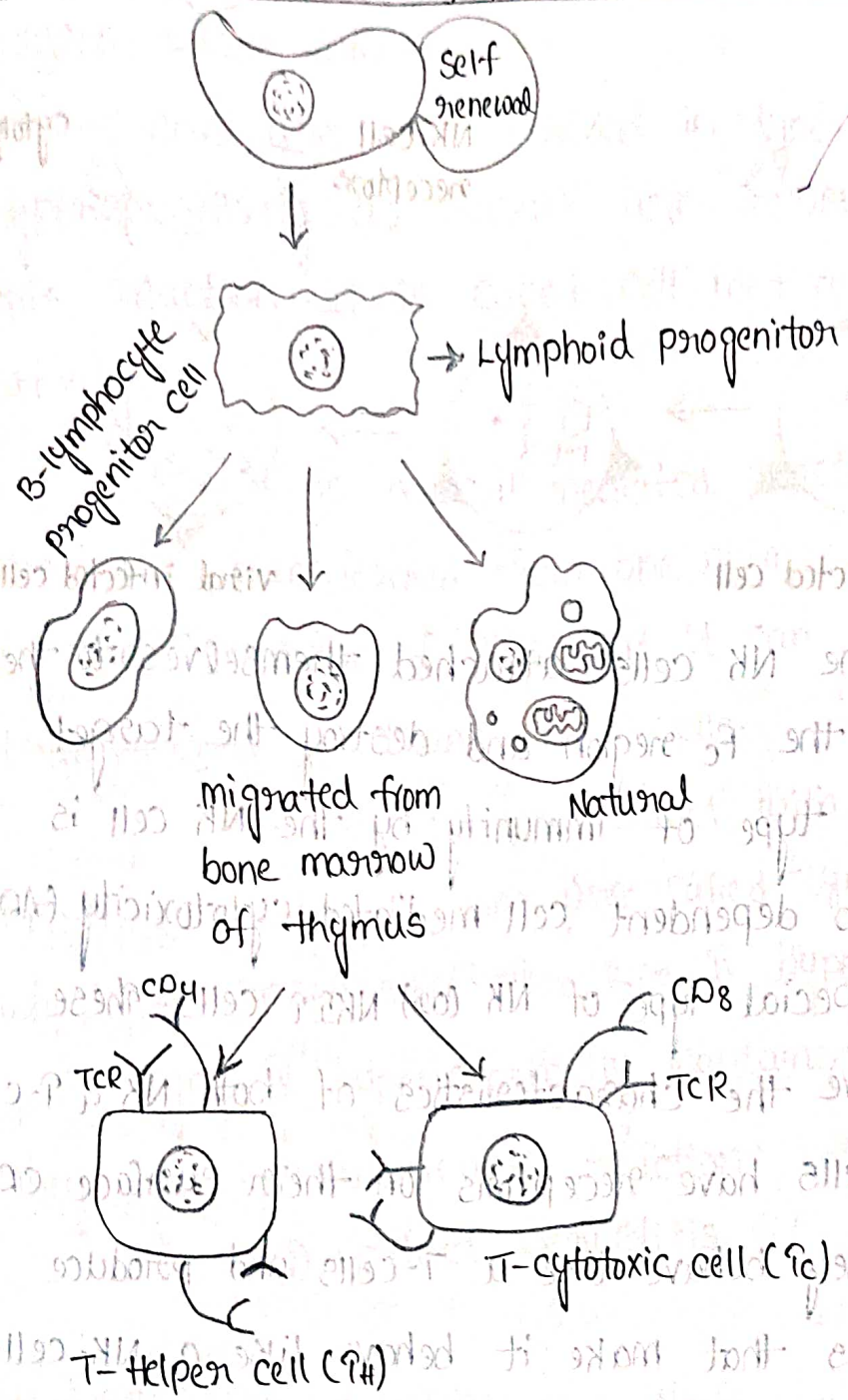
T-cell mediated immunity:-

The lymphocytes which mature in thymus are called T-lymphocytes. After production in bone marrow they will migrated to thymus for their maturation the T-cell posses receptors on their surface are called T-receptors. These receptors recognizes the Ag that is bound to particular class of self molecules that are major histo compatability factors (MHC)

The T-cells that express particular membrane glycoprotein molecules are grouped into a cluster and this is used to differentiate between different types of T-cells. Hence it is called cluster of differentiation.

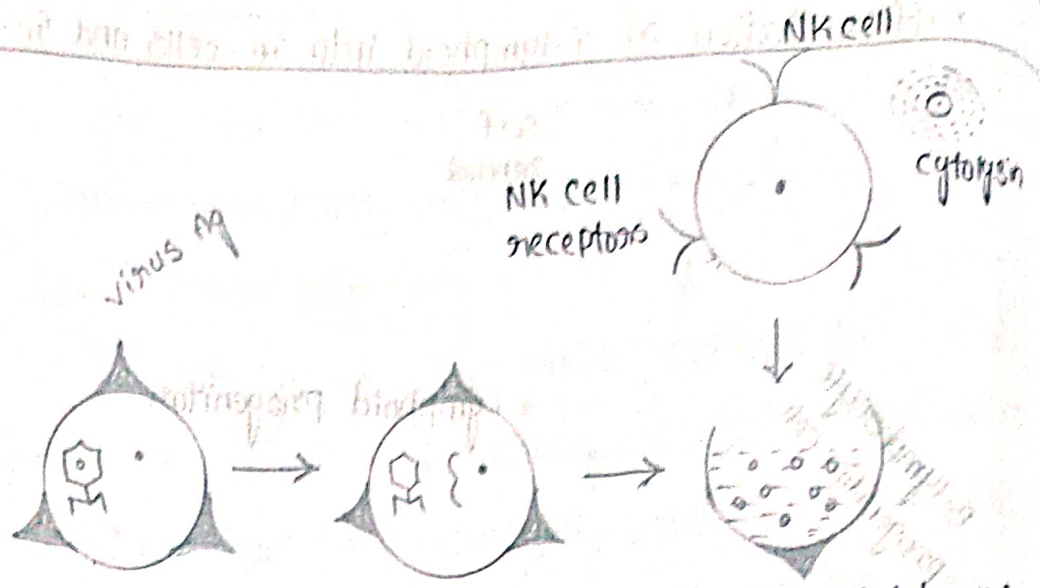
T-cells during first response (T_1) produces cytokinins that results in inflammation and activities of T-cells and macrophages. T_H cells during their second response (TH_2) activates mainly B-cells and produce Ab's the other types of T-lymphocytes are T_S cells. They help in supressing humoral and cell mediated immunity.

Differentiation of T-lymphoid into T_H-cells and T_C-cells



NK cell mediated immunity:-

These are also called as Null cells. These do not express membrane bound molecules and receptors like T and B lineages. They constitute 5-10% of the total lymphocytes. These cells play cytotoxic activity against humoral cells.



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These cells destroys the cancer cells and cells infected with herpes and mumps. They do not need Ab for its activity they are activated by interferons and interleukins.

Delayed type hypersensitivity:-

Type-IV hypersensitivity is caused by the interaction between the Ag's sensified T-cells. This leads to inflammatory reaction and

causes tissue damages.

Ab's are not involved in type-IV hypersensitivity as T-cells are involved in this reaction. It is called cell mediated hypersensitivity.

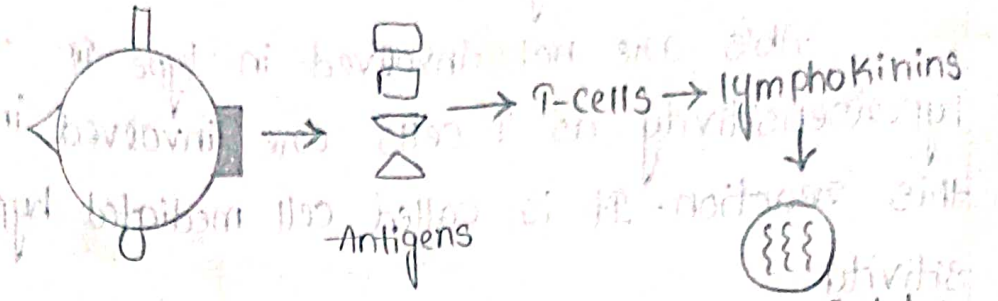
As it is a cell mediated reaction, it can be passively transferred from one animal to another by the transfer of Ab's. But it can be transferred by trans of T-cells.

The T-cells on contact with the Ag, produce soluble proteins are called lymphokinins which is responsible for type-IV hypersensitivity.

Type-IV hypersensitivity contains two types. They are:- ① Tuberculin reaction
② contact dermitis

mechanism:-

When T-cells primed to an Ag (viral (or) bacteria) come in contact with same Ag for the second time the cells releases the soluble proteins called lymphokinins. these lymphokinins activates macrophages to kill intracellular bacteria like tuberculae bacilli and leads to the formation of inflammatory cells like giant cells and epithelial cells.



Tuberculin reaction:-

When a small dose of tuberculin injected intradermally in an individual. these sensitized to tuberculoprotein than can manifest the reaction within 48-72 hrs time. In unsensified individuals this tuberculin injection promotes the sensitized person shows swelling and redness at the injection times within 48-72 hours.

Tuberculin like hypersensitivity reaction is developed in many infections with bacteria like mycobacterium leprea, fungus, viruses and parasites. A similar reaction is developed in allograph reaction, many auto immune diseases like systemic lupus erythrumotosis, Rhematoid arthraitis, good patcher syndrome etc.

Contact dermitis type reaction:-

Delayed type hypersensitivity some time result from skin contact with a variety of

chemicals which are nickel, chromium, hair dye, formaldehyde, cosmetic poision oak, poision ivy, picryl chloride dinitro chloro benzene, drugs such as penicillin etc. sensitization particularly labels and lisions are appear at contact area. these substances are involved are not antigenic in nature but may occur antigenicity in combination with skin proteins. this type of hypersensitivity reactions are detected by patches test. sensitivity is indicated by itching and local reaction which may vary with in 24-48 hours

major Histo compactability

The concept that the rejection of foreign tissue in the result of an immune response to cell surface molecules now called histo compactability Ag's originated from the work of peter gorer in mid 1930's. Gorer was using in breed strains of mice to identify blood group Ag. The coarse of these studies 4 types of genes designated I to IV that blood work carried out in 1940's and 1950's by gorer George Sell established Ag's ~~endoded~~ encoded by the genes in the group designated to II part in the rejection of transplant a tumours and other tissues.

The MHC complex is collection of genes arranged within along continues stretch of DNA on chromosome 6 in humans and on chromosome-17 in mice. The MHC is referred to as MHC

complex in human and has H_2 complex in mice. Although the arrangement of genes in some what different in both cases the MHC genes are organised into regions encoding and classes of molecules.

① MHC class-I :-

Genes encode glycoproteins expressed on the surface of nearly all nucleated cells the major function of the class-I cell products is presentations of peptide Ag's to Tc cells.

② MHC class-II genes encode glycoproteins expressed primarily on Ag presenting cells (macrophages, dendritic cells and B cells) where they present processed antigenic peptides to T_H cells.

③ MHC class-III genes encode in addition to other products various secreted protein that have immune functions including components of complement system and molecules involved in inflammation.

MHC class-I :-

MHC class-I molecule is found on almost on every nucleated cell of the body. MHC class is a heterodimer composed of two polypeptide chain and a long α-chain that is encoded in MHC and short β-chain β_2 microglobulin which is not encoded in MHC. The α-chain has

4 regions.

(i) cytoplasmic region containing sites for phospholation and binding to cytoskeleton elements.

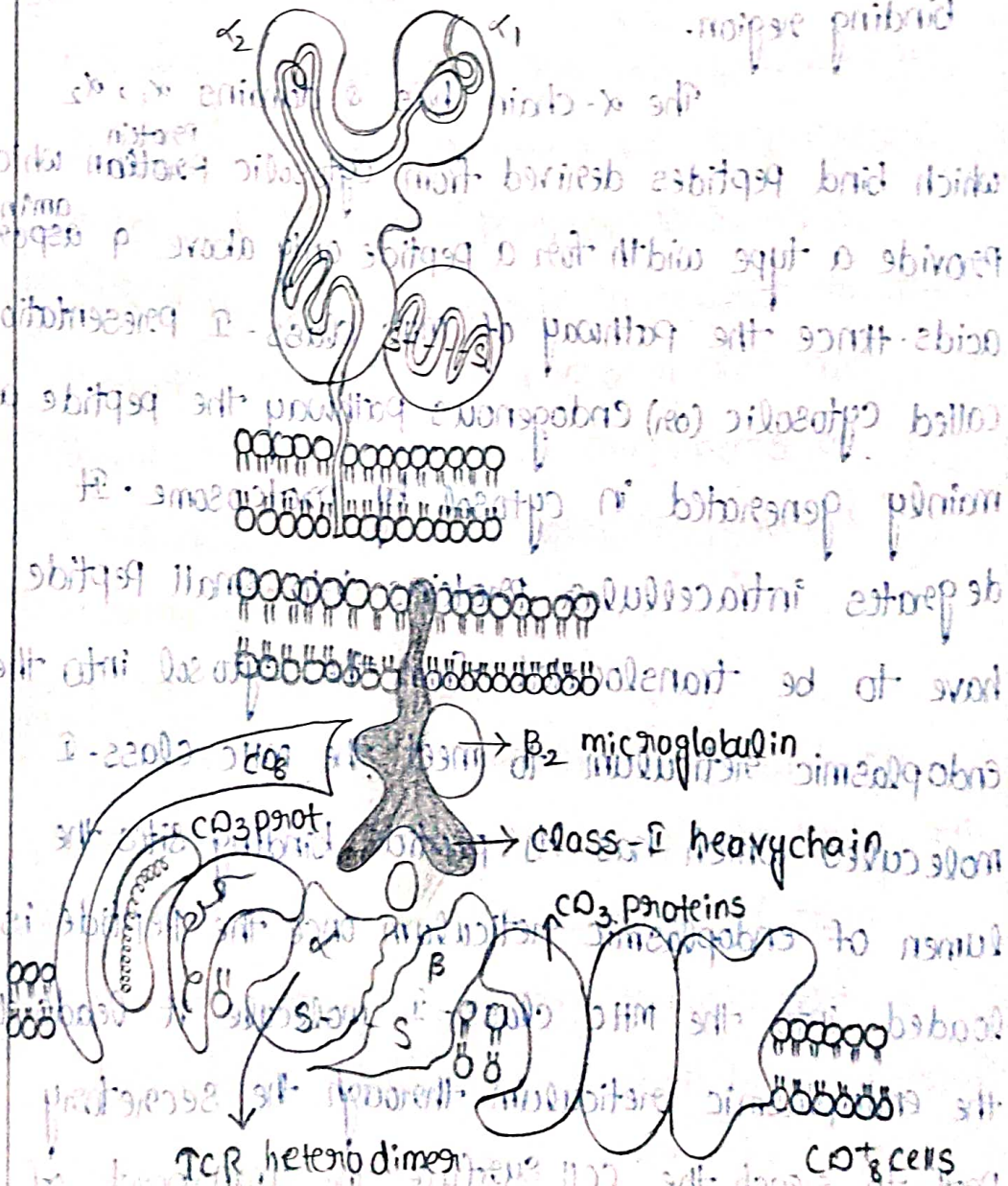
(ii) A transmembrane region contains hydrophilic amino acids by which the molecule is anchored in the cell membrane.

(iii) A highly polymorphic peptide binding is formed by α_1 & α_2 domain, the B_2 microglobulin associates with the α-chain and helps to maintain the proper confirmation of molecules. The variability is most common in α_1 & α_2 molecules which comprises peptide binding region.

The α-chain has 2 domains α_1, α_2 which bind peptides derived from cytosolic ~~protein~~ protein which provide a type width for a peptide only above 9 ~~aspesic~~ amino acid acids. Hence the pathway of MHC class-I presentation called cytosolic (or) endogenous pathway the peptide are mainly generated in cytosol by proteosome. It degrates intracellular proteins into small peptide have to be translocated from the cytosol into the endoplasmic reticulum to meet the MHC class-I molecules which has its peptide binding sites the lumen of endoplasmic reticulum once the peptide is loaded into the MHC class-I molecule it leads to the endoplasmic reticulum through the secretory path to reach the cell surface the transport of

the MHC class-I molecule through the secreted path to reach the cell surface and also involves the several post transition modification of MHC molecules to T cells CD_8.

As virus infect a cell by entering it's cytoplasm, this cytosolic MHC class-I dependent pathway of Ag presentation is the primary way of a virus infected cell to signal T-cell-MHC class T-cells (CTL's). The fate of the virus infected cell is almost always apotdosis initiated by the CTL.



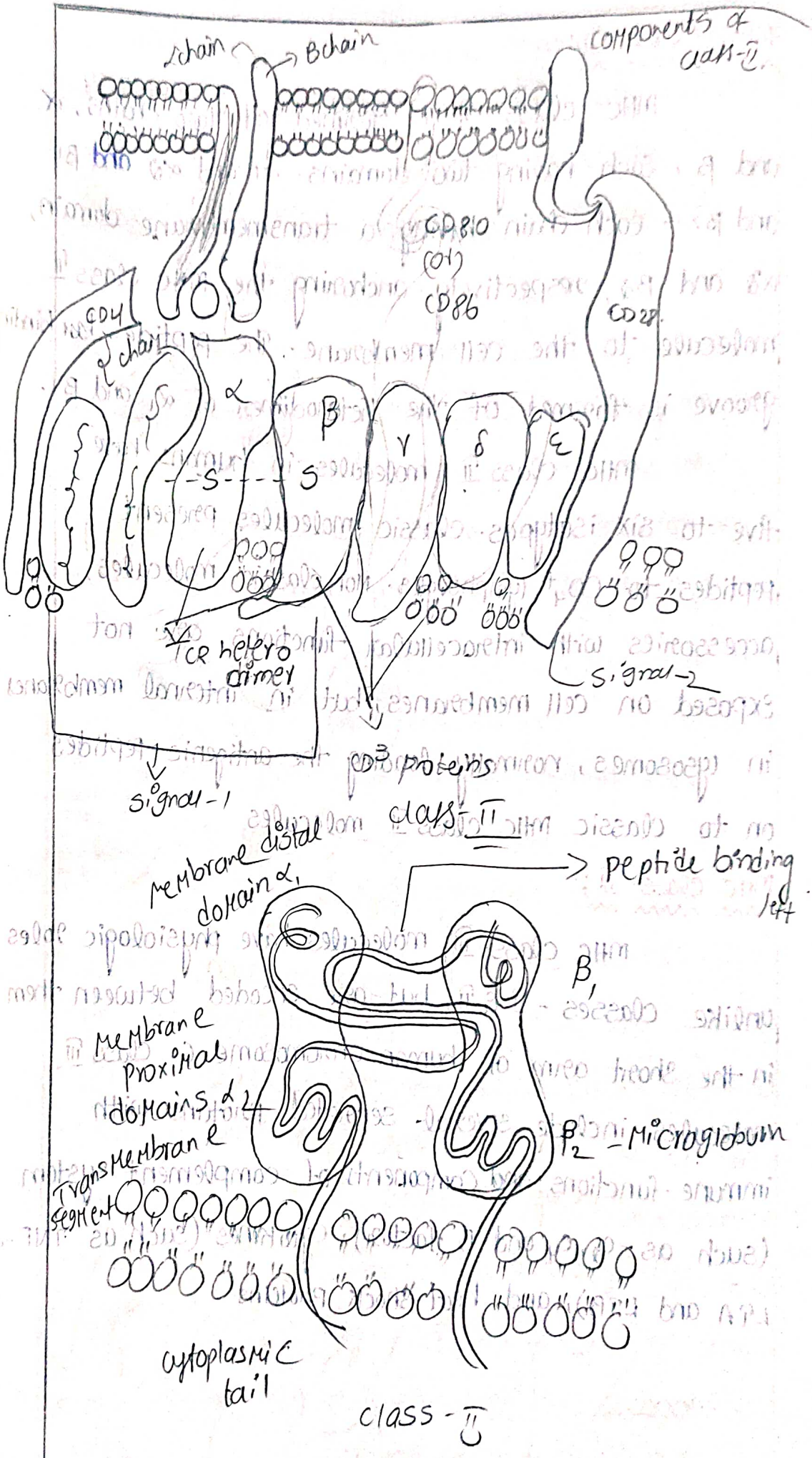
MHC class - II :-

MHC class - II is formed of two chains, α and β, each having two domains - $\alpha 1$ and $\alpha 2$ and $\beta 1$ and $\beta 2$ - each chain having a transmembrane domain, $\alpha 2$ and $\beta 2$, respectively, anchoring the MHC class II molecule to the cell membrane. The peptide - binding groove is formed of the heterodimer of α_1 and $\beta 1$.

MHC class II molecules in humans have five to six isotypes. classic molecules present peptides to CD_4^+ lymphocytes. Non classic molecules, accessories with intracellular functions, are not exposed on cell membranes, but in internal membranes in lysosomes, normally loading the antigenic peptides on to classic MHC class II molecules

MHC class - III :-

MHC class - III molecules have physiologic roles unlike classes - I & II, but are encoded between them in the short arm of human chromosome 6. Class III molecules include several secreted proteins with immune functions: and components of complement system (such as C_2, C_4 and B factor), cytokines (such as TNF-α, LTA and LTB), and heat shock proteins.


components of class-I
α chain
β chain
CD8
(α)
CD86
CD4
CD28
α chain
α
β
γ
δ
ε
S
S
TCR hetero dimer
signal-2
signal-1
CD3 proteins
class-II
membrane distal domain α1
peptide binding left
β1
membrane proximal domain α2
β2-Microglobum
transmembrane segment
cytoplasmic tail
class-II

cytokines:-

Cytokines that are important in cell signalling. These cytokines are involved in autocrime signalling, paracrime signalling and endocrine signelling as immuno mediating agents.

Cytokines includes hemokines, interferons, interleukins, lymphokines and tumour necrosis factors. Cytokines are produced by a broad range of cells including immune cells like macrophage, B-lymphocytes, T-lymphocytes and mast cell as well as endothelial cells and cytokines modulate the balance between humoral and cell mediated immune responses and they regulated the maturation growth and responsiveness of particular cell populations.

Structure of cytokines:-

Cytokines can be classified into four types. They are four α-helix bundle family it contain 30 structures with four bundles of α-helix. This family can be sub-divided into 3 types.

(a) The interleukin-2 subfamily.

(b) The interferon - subfamily.

(c) The interleukin - 10 subfamily.

→ The interleukin-1 family which primarily includes

interleukin-1 and interleukin-18

→ The interleukin-1 family it contains a specific effect in promoting proliferation of T-cells that cause cytotoxic effect.

→ The 16 K not cytokinins include members of the transforming growth factor β-superfamily including $TGF\beta_1$, $TGF\beta_2$, $TGF\beta_3$.

UNIT-V

IMMUNOLOGICAL TECHNIQUES

Ag-Ab reactions (or) Ag-Ab interactions:-

The interactions between Ag and Ab is called Ag-Ab reaction.

→ Ag-Ab reaction is the basis of HI and antibody mediator immune response.

→ Ag-Ab reaction is characterized by following salient features.

① **Immune complex:-**

When Ag and Ab are brought together. The Ab binds with Ag to form a complex molecule called immune complex (or) Ag-Ab complex.

$$Ag + Ab \rightarrow Ag\text{-}Ab \text{ complex.}$$

② **Affinity:-**

Affinity refers to the strength of binding between a particular molecule of Ab and a single antigenic determinant. Ab's which bind strongly to the Ag is used to denote the binding capacity of an Ab with a "univalent Ag".

③ **Avidity:-**

Avidity refers to the capacity of an antiserum containing various antibodies to combine with the whole Ag that stimulate the production of Ab's. Avidity is used to denote the overall "capacity of Ab's to combine with multivalent antigen".

④ Detection of Ag-Ab reaction (or) Types of Ag-Ab reaction:-

Ag-Ab reaction is brought about by the contact of Ag and Ab. This process of contact cannot be seen by naked eye. But once the contact is made the Ag-Ab reaction leads to visible manifestation such as precipitation, agglutination etc. The Ag-Ab reaction can be detected by the following techniques.

① precipitation
⑤ Agglutination
③ Complement fixation
④ Immuno diffusion
⑤ ELISA.

① Precipitation:-

ppt is referred to as Ag-Ab reaction between soluble Ag and its Ab resulting in the formation of insoluble ppt. The Ab causing ppt is called precipitating.

Mechanism:-

ppt is due to the formation of Ag-Ab complex. The Ag is multivalent and the Ab is bivalent. Each Ab in bivalent molecule, it can bridge into two multivalent Ag molecule. This bridging leads to formation of a lattice, which forms the ppt.

when Ag and Ab are in optimum concentration the ppt is complete and a large lattice is formed.

Precipitating test:-

Precipitating test is a test of Ag-Ab reaction. It can be carried out by a classical experiments.

→ A set of five (or) more reaction tubes are arranged serially and are marked a constant volume of anti-serum is added to each tube. The Ag is added in high volume from tube A to E.

→ Ag and Ab react together resulting in ppt. The amount of ppt formed is determined by the proportion of Ag and Ab.

→ maximum amount of ppt is formed when the Ag and Ab are in optimal proportion. This occurs in the central tube. When the Ab is in excess (or) the Ag is in excess. The amount of precipitate formed will be less. This occurs in side tubes.

→ When the amount of ppt formed in different tubes is plotted on a graph paper. A curve is obtained. This curve is called "precipitation curve". The curve shows a peak where maximum ppt is formed. This occurs when the proportion of Ag-Ab is optimum. The amount of ppt formed on a side tubes is low and hence the curve depends on the sides. The precipitating curve shows three zones namely ① zone of Ab excess

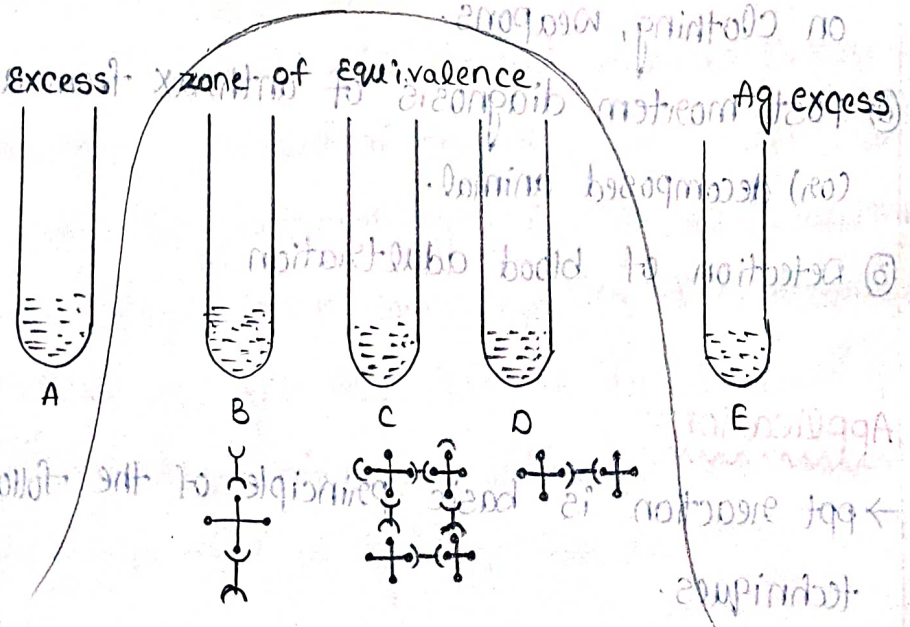
② zone of Equivalent

③ zone of Ag excess

→ The zone of equivalence lies in the pack of the curve (tube c) here the proportion of Ag and Ab is optimum. Here all the Ag and Ab are completely precipitated into a large lattice which is insoluble.

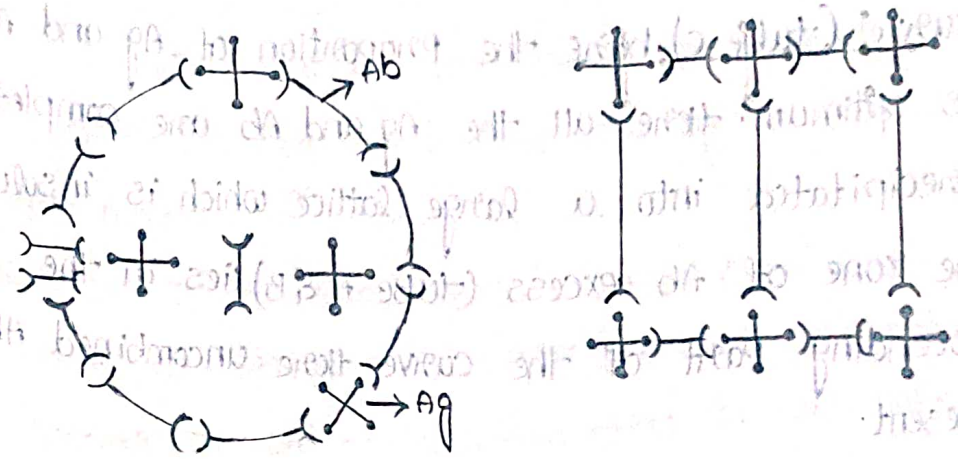
→ The zone of Ab excess (tube A & B) lies in the ascending part of the curve. Here uncombined Ab is present.

→ All the available antigenic determinants of Ag are occupied by the binding sites of Ab. There is no Ag antigenic determinant left out. Hence the Ag-Ab complex is insoluble.



→ The zone of Ag excess (tubes D & E) lies in the descending part of the curve here uncombined Ag determinants are present. All the Ab's are combined. In Ag excess the binding between Ag and Ab is weak and hence the Ag-Ab complexes are soluble because large lattice formation is inhibited.

→ The precipitation test is used to find out the amount of Ab present in the serum of an immunized animal.



mechanism of precipitation

Advantages (or) uses:-

① Identification of blood / seminal fluid in stains on clothing, weapons.

② Post mortem diagnosis of anthrax from a dead (or) decomposed animal.

③ Detection of blood adultration &

. . . .

Application:-

→ ppt reaction is basic principle of the following techniques.

① Single immunodiffusion

② Double immunodiffusion

③ Radial immunodiffusion

④ Immuno Electrophoresis

⑤ Rocket immunodiffusion

⑥ Agglutination:-

Agglutination is an Ag-Ab reaction where the Ab of serum causes the cellular Ag to

adhere to one another to form clumps. It is the clumping of a particular Ag and it's Ab. The Ab's that cause agglutination are called agglutins and the particular Ag's aggregated are called agglutinogens.

The particular Ag's include bacteria, virus, RBC, platelets, lymphocytes etc.

The red blood cells are agglutinated the reaction is called haemagglutination. when bacterial cells are agglutinated, agglutination is called bacterial agglutination.

Mechanism of agglutination:-

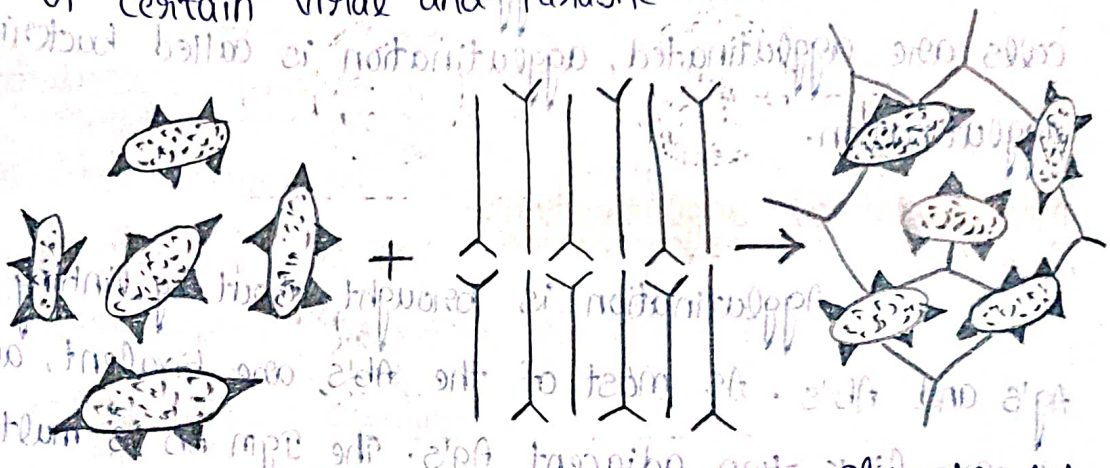
Agglutination is brought about by linking of Ag's and Ab's. As most of the Ab's are bivalent, an Ab can link two adjacent Ag's. The IgM Ab is multivalent and it contain 5 (or) 10 combining sites. Hence it can link more number of Ag's. Hence IgM Ab has the capacity to make clumps more effectively with a lesser number of molecules than that of IgG Ab molecule.

Agglutination test:-

Agglutination test refers to the examination of clump formation when particulate Ag and it's Ab's are combined.

Agglutination test has a wide application in the clinical field. It is used to test blood groups and infectious diseases. The following are the applications of agglutination test.

① ABO blood grouping
② Rh blood typing
③ Widal test for typhoid
④ Coomb's test for the identification of anti Rh antibody.
⑤ Brucella agglutination test for Brucellosis.
⑥ Cold agglutination test for pneumonia and malaria.
⑦ Heamagglutination inhibition test for the diagnosis of certain viral and parasite disease.



cells (or) particles with Ag on their surface

Homologous Ab's

clumping (or) agglutination of cells (or) particals.

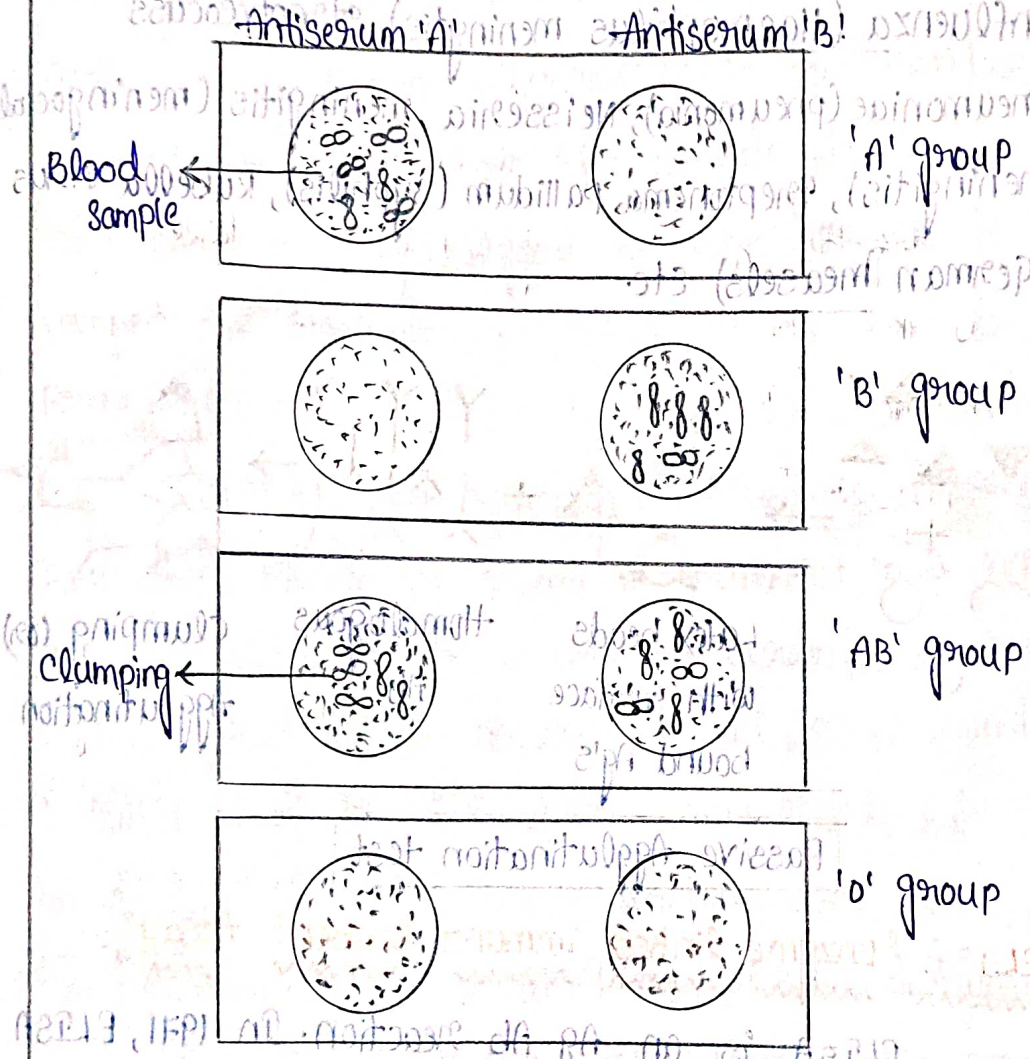
Agglutination test

ABO blood grouping:-

The typing of blood for ABO group (or) Rh group involves the agglutination reaction. For typing blood a drop of blood sample is mixed with drop of antiserum A and another drop of blood sample is mixed with a drop of antiserum B on a glass slide.

If the blood sample is clumped with

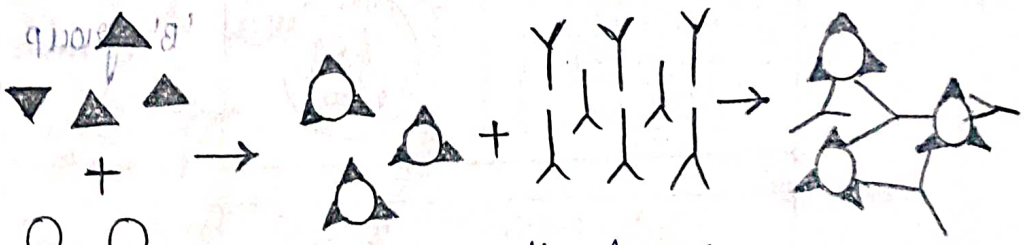
antiserum A, the sample belongs to group A. If the sample is clumped with antiserum B, the sample belongs to B. If the sample is clumped with both antiserum A and antiserum B, the blood sample belongs to A and B. If there is no agglutination the blood sample belongs to group 'o'.



Passive agglutination test:-

The passive agglutination test, the soluble Ag's are taken out, pasre 1st attached to the one of the surface of one of the carriers like latex beads, polysterene partical, RBC and then mixed with patient serum. Homologous Ab's present in

serum attach with Ag's present on the surface of carriers forming Ag-Ab complexes that agglutinate. The excellent example of passive agglutination test using latex beads has carrying is one of the modern pregnancy test - other example are the detection of pathogens haemophilus influenza (Haemophilus meningtis), streptococuss Pneumoniae (pneumonia), Neisseria meningitis (meningocal meningitis), Treptonema pallidum (syphilis), Rubella virus (German measels) etc.



Passive Agglutination test

ELISA (Enzyme linked immuno sorbent Assay):-

ELISA is an Ag-Ab reaction. In 1971, ELISA was introduced by Peter perlmann & Eva Engvall at stockholm university in sweden. It is a common laboratory technique which is usually used to measure the concentration of Ab's (or) Ag's in blood.

ELISA is a plate based assay technique which is used for detecting and quantifying

substances such as peptides, proteins, antibodies and hormones. An Enzyme conjugated with an Ab reacts with colourless substrate to generate a coloured product such substrate is called chromogenic substrate. A number of Enzymes have been used for ELISA such as alkaline phosphatase, horse radish peroxidase and beta galactosidase. Specific substrate such as ortho-phenyl diamine dihydrochloride (for peroxidase), Paranitrophenyl phosphate (for alkaline phosphatase) are used which are hydrolysed by above enzymes to give colored end product.

Principle :-

ELISA's are typically performed in 96-well polystyrene plates. The serum is incubated in a well and each well contains a different serum. A positive control serum and a negative control serum would be included among the 96 samples being tested. Antibodies (or) Antigens present in serum are captured by corresponding Ag (or) Ab coated on to solid surface. After sometime, the plate is washed to remove serum and unbound Ab's (or) Ag's with a series of wash buffer. To detect the bound Ab's (or) Ag's, a secondary antibodies that are attached to an enzyme such as peroxidase (or) alkaline phosphatase are added to each well. After an incubation period the unbound secondary Ab's are washed off. When a suitable

substrate is added, the enzyme reacts with it to produce a colour. This colour produced is measurable as a function (or) quantity of Ag's (or) Ab's present in the given sample. The intensity of colour / OD is measured at 450 nm. The intensity of the colour gives an indication of the amount of Ab (or) Ag.

Types of ELISA:-

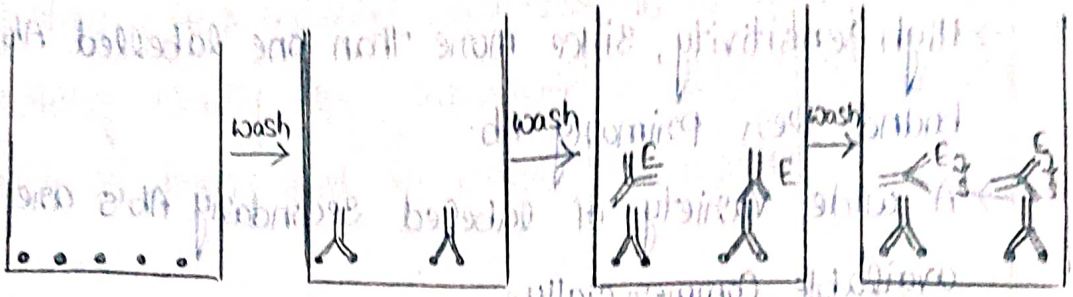
→ Frequently there are three types of ELISA on the basis of binding structure between the Ab and Ag.

① Indirect ELISA
② Sandwich ELISA
③ Direct ELISA

① Indirect ELISA:-

Ab can be detected (or) quantitatively determined by indirect ELISA. In this technique, Ag is coated on the microtiter well. serum (or) some other sample containing 1° Ab is added to the microtiter well and allowed to react with the coated Ag. Any free primary Ab is washed away and the bound Ab to the Ag is detected by adding an enzyme conjugated 2° Ab that binds to the 1° Ab. Unbound secondary Ab is then washed away and a specific substrate for the enzyme is added. Enzyme hydrolyzes the substrate to form coloured products. The amount of coloured

end product is measured by spectrophotometric plate readers that can measure the absorbance of all the wells of 96-well plate.



Ag-coated well | Specific Ab binds to Ag | Enzyme linked Ab binds to specific Ab | Substrate is added and converted by enzyme into coloured product the rate of colour formation is proportion to amount of specific Ab.

Procedure of Indirect ELISA:-

① Coat the micro-titer plate wells with Ag.

② Block all unbound sites to prevent false +ve results.

③ Add sample containing Ab (Eg:- rabbit monoclonal Ab) to the wells and incubate the plate at 37°C.

④ Wash the plate, so that unbound Ab is removed.

⑤ Add 2° Ab conjugated to an enzyme (Eg:- anti-mouse IgG).

⑥ Wash the plate, so that unbound enzyme-linked Ab's are removed.

⑦ Add substrate which is converted by the enzyme to produce a coloured product.

(5) Reaction of a substrate with the enzyme to produce a coloured product.

Advantages:-

→ High sensitivity, since more than one labelled Ab is bound per primary Ab.
→ A wide variety of labeled secondary Ab's are available commercially.
→ Maximum immunoreactivity of the primary Ab is retained because it is not labelled.
→ Versatile because many primary Ab's can be made in one species and the same labelled secondary Ab can be used for detection.
→ Flexibility, since different primary detection Ab's can be used with a single labelled secondary Ab.
→ Cost savings, since fewer labeled Ab's are required.
→ Different visualization markers can be used with the same primary Ab.

Disadvantages:-

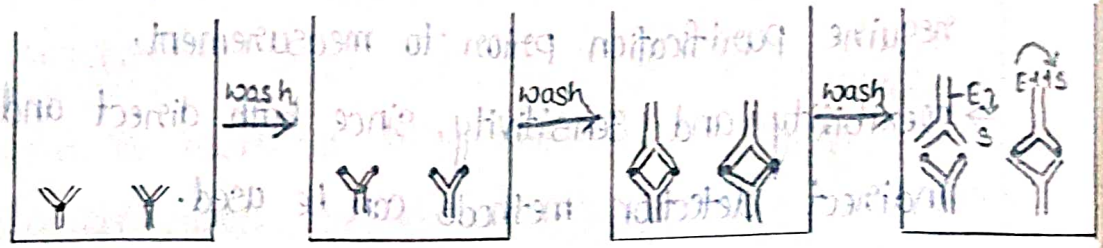
→ Cross-reactivity might occur with the secondary Ab, resulting in non-specific signal.
→ An extra incubation step is required, in the procedure.

(3) Sandwich ELISA:-

Ag can be detected by Sandwich ELISA.

In this technique, Ab is coated on the microtiter well. A sample containing Ag is added to the well and allowed to react with the Ab attached to the well,

forming Ag-Ab complex. After the well is washed, a second enzyme-linked Ab specific for a different epitope on the Ag is added and allowed to react with the bound Ag. Then after unbound secondary Ab is removed by washing. Finally the substrate is added to the plate which is hydrolyzed by enzyme to form colored products.



Monoclonal Ab-coated well

Ag binds to Ab

A second mAb, linked to enzyme, binds to immobilized Ag.

Substrate is added & converted by enzyme into colored product the rate of colour formation is proportional to amount of Ag.

Procedure of Sandwich ELISA:-

① Prepare a surface to which a known quantity of Ab is bound.

② Add the Ag-containing sample to the plate and incubate the plate at 37°c.

③ Wash the plate, so that unbound Ag is removed.

④ Add the enzyme-linked Ab's which are also specific to the Ag and then incubate at 37°c.

⑤ Wash the plate, so that unbound enzyme-linked Ab's are removed.

⑥ Add substrate which is converted by the enzyme to produce a colored product.

④ Reaction of a substrate with enzyme to produce a coloured product.

Advantages:-

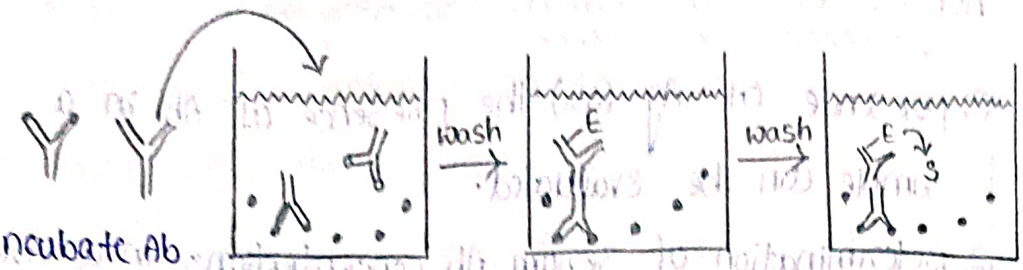
→ High specificity, since two Ab's are used the Ag is specifically captured and detected.

→ Suitable for complex samples, since the Ag does not require purification prior to measurement.

→ Flexibility and sensitivity, since both direct and indirect detection methods can be used.

③ Competitive ELISA:-

This test is used to measure the concentration of an Ag in a sample. In this test, Ab is first incubated in solution with a sample containing Ag. The Ag-Ab mixture is then added to the microtitre well which is coated with Ag. The more the Ag present in the sample, the less free Ab will be available to bind to the Ag coated well. After the well is washed, enzyme conjugated secondary Ab specific for isotype of the primary Ab is added to determine the amount of primary Ab bound to well. The higher the concentration of Ag in the sample, the lower the absorbance.



Incubate Ab with Ag to be measured

Add Ag-Ab mixture to Ag-coated well

Add enzyme conjugated secondary Ab

Add substrate and measure colour.

Procedure :-

① Ab is incubated with sample containing Ag.

② Ag-Ab complex are added to the microtitre well which are pre-coated with Ag.

③ Wash the plate to remove unbound Ab.

④ Enzyme linked secondary Ab which is specific to the primary Ab is added.

⑤ Wash the plate, so that unbound enzyme-linked Ab's are removed.

⑥ Add substrate which is converted by the enzyme into a fluroscent signal.

Advantages :-

① High specificity, since two antibodies are used.

② High sensitivity, since both direct and indirect detection methods can be used.

③ Suitable for complex samples, since the Ag does not require purification prior to measurement.

Applications of ELISA:-

① presence of Ag (or) the presence of Ab in a sample can be evaluated.

② Determination of serum Ab concentrations in a virus test.

③ used in food industry when detecting potential food allergens.

④ Applied in disease outbreaks - tracking the spread of disease.

Eg:- HIV, bird flu, common colds, cholera, STD etc.

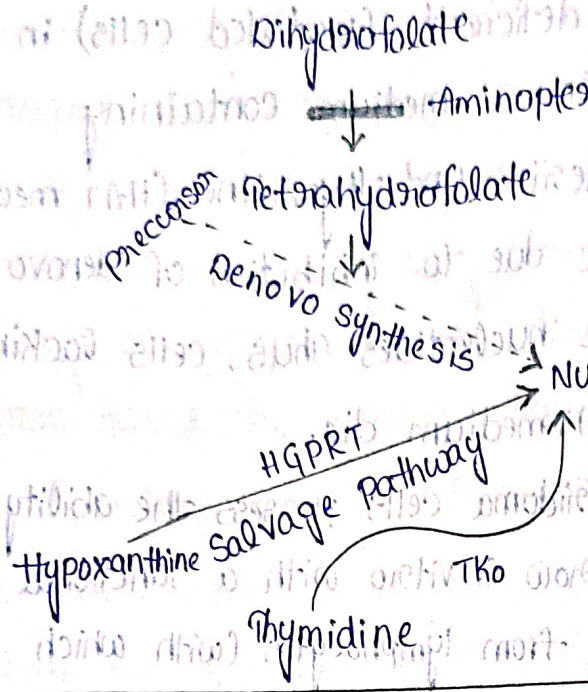
Monoclonal Antibodies (MAb's)

monoclonal Antibodies (MAb) is a single type of Ab that is directed against a specific antigenic determinant (epitope)

→ The production of MAb's by the hybrid cells is referred to as hybridoma technology.

Principle for creation of hybridoma cells:-

The myeloma cells used in hybridoma technology must not be capable of synthesizing their own antibodies. The selection of hybridoma cells is based on inhibiting the nucleotide (consequently the DNA) synthesizing machinery. The mammalian cells can synthesize nucleotides by two pathways - denovo synthesis and salvage pathway.



Pathway for synthesis of nucleotides

The denovo synthesis of nucleotides requires tetrahydrofolate which is formed from dihydrofolate. The formation of tetrahydrofolate (and therefore nucleotides) can be blocked by the inhibitor aminopterin. The salvage pathway involves the direct conversion of purines and pyramidines into the corresponding nucleotides. Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is a key enzyme in the salvage pathway of purines.

It converts hypoxanthine and guanine respectively to ionise monophosphate and guanosine monophosphate. Thymidine kinase (TK), involved in the salvage pathway of pyramidine's converts thymidine to thymidine monophosphate (TMP). Any mutation in either one of the enzymes (HGPRT (or) TK) blocks the salvage pathway.

When cells deficient (mutated cells) in HGPRT are grown in a medium containing hypoxanthine aminopterin and thymidine (HAT medium), they cannot survive due to inhibition of denovo synthesis of purine nucleotides. Thus, cells lacking HGPRT grow in HAT medium die.

The hybridoma cells possess the ability of myeloma cells to grow invitro with a functional HGPRT gene obtained from lymphocytes (with which myeloma cells are fused). Thus, only the hybridoma cells can proliferate in HAT medium and this procedure is successfully used for their selection.

Production of monoclonal Antibodies:-

The establishment of hybridomas and production of MABs involves the following steps.

① Immunization
② cell fusion
③ Selection of hybridomas
④ screening the products
⑤ cloning and propagation
⑥ characterization and storage.

① Immunization:-

The very first step in hybridoma technology is to immunize an animal (usually a mouse), with appropriate Ag. the Ag, along with an adjuvant like freund's complete (or) incomplete adjuvant is injected

subcutaneously. The injections at multiple sites are repeated several times.

This Enables increased stimulation of B-lymphocytes where are responding to Ag. Three days prior to killing of animal, a final dose of Ag is intravenously administered. The immune-stimulated cells for synthesis of Ab's have grown so maximally by this approach. The concentration of desired Ab's is assayed in the serum of the animal at frequent intervals during the course of immunization. When the serum concentration of the Ab's is optimal, the animal is sacrificed. The spleen is aseptically removed and disrupted by mechanical (or) enzymatic methods to release the cells. The lymphocytes of the spleen are separated from the rest of the cells by density gradient centrifugation.

② Cell fusion:-

The thoroughly washed lymphocytes are mixed with HGPRT defective myeloma cells. The mixture of cells is exposed to polyethylene glycol (PEG) for a short period (few minutes), since it is toxic. PEG is removed by washing and the cells are kept in a fresh medium. These cells are composed of a mixture of hybridomas (fused cells), free myeloma cells and free lymphocytes.

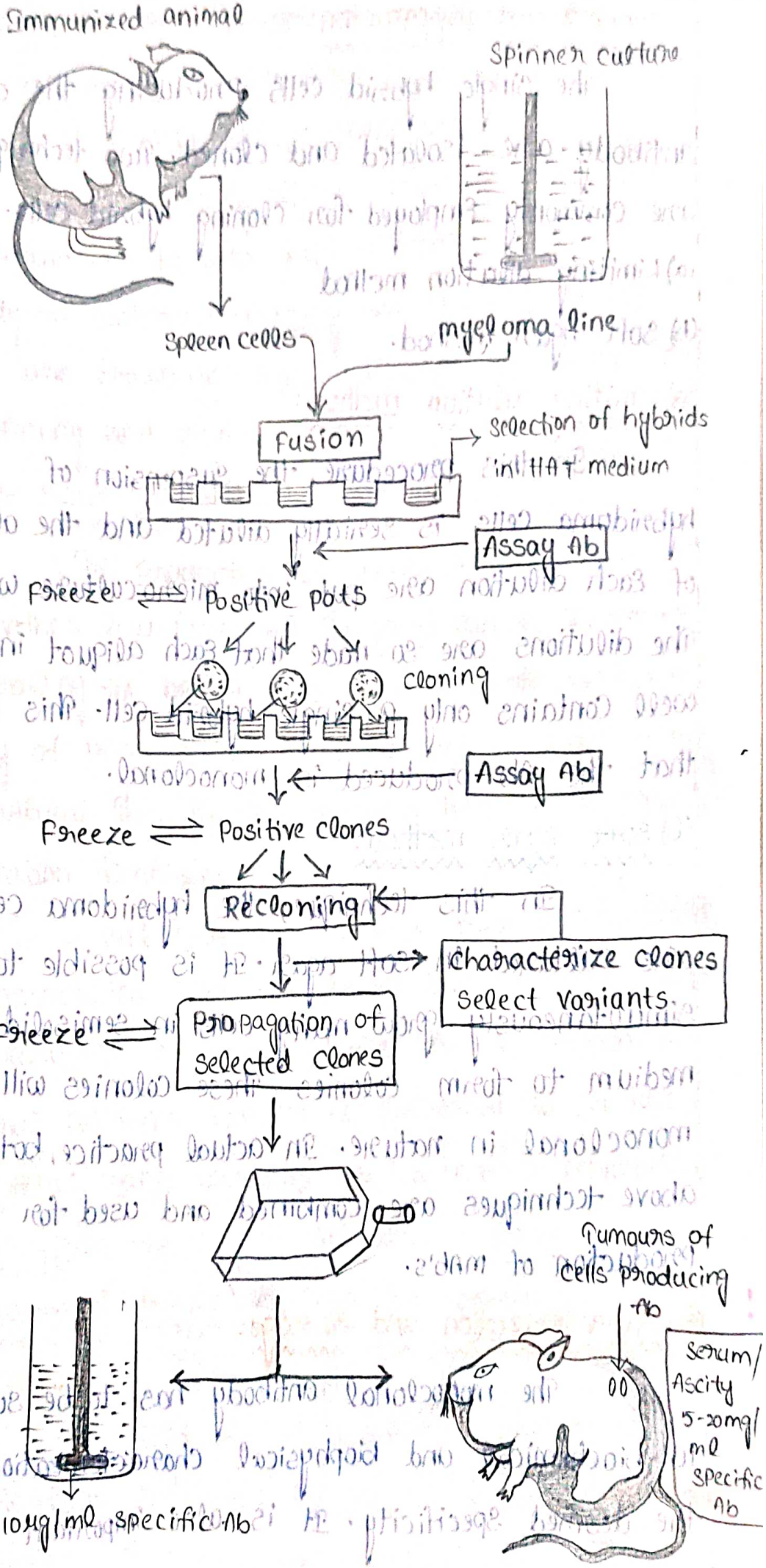
③ Selection of hybridomas:-

When the cells are cultured in HAT medium only the hybridoma cells grow, while the rest will slowly disappear. This happen in 7 to 10 days of culture. Selection of a single Ab producing hybrid cells is very important. This is possible if the hybridomas are isolated and grown individually. The suspension of hybridoma cells is so diluted that the individual aliquots contain an average one cell each. These cells, when grown in a regular culture medium, produce the desired Ab.

④ Screening the product:-

The hybridomas must be screened for the secretion of the Ab of desired ~~specifically~~ specificity. The culture medium from each hybridoma culture is periodically tested for the desired Ab specificity. The two techniques namely ELISA and RIA are commonly used for this ~~process~~ purpose.

In both the assays, the Ab binds to the specific Ag (usually coated to plastic plates) and the unbound Ab and other components of the medium can be washed off. Thus, the hybridoma cells producing the desired Ab can be identified by screening. The Ab secreted by the hybrid cells is referred to as monoclonal Antibody (mAb).


Immunized animal
Spinner culture
Spleen cells
myeloma line
Fusion
Selection of hybrids in HAT medium
Assay Ab
Freeze
Positive pots
cloning
Assay Ab
Freeze
Positive clones
Recloning
Characterize clones
Select variants
Freeze
Propagation of selected clones
Tumours of cells producing Ab
Serum/ Ascity 5-20mg/ ml specific Ab
~10ug/ml specific Ab

⑤ Cloning and propagation:-

The single hybrid cells producing the desired antibody are isolated and cloned. Two techniques are commonly employed for cloning hybrid cells.

(a) Limiting dilution method

(b) Soft Agar method.

(a) Limiting dilution method:-

In this procedure, the suspension of hybridoma cells is serially diluted and the aliquots of each dilution are put into microculture wells. The dilutions are so made that each aliquot in a well contains only a single hybrid cell. This ensure that the Ab produced is monoclonal.

(b) Soft Agar method:-

In this technique, the hybridoma cells are cultured in soft agar. It is possible to simultaneously grow many cells in semisolid medium to form colonies. These colonies will be monoclonal in nature. In actual practice, both the above techniques are combined and used for maximal production of MAb's.

⑥ Characterization and storage:-

The monoclonal antibody has to be subjected to biochemical and biophysical characterization for the desired specificity. It is also important to

Elucidate the MAb for the immunoglobulin class (or) sub-class, the epitope for which it is specific and the number of binding sites it possesses.

The stability of the cell lines and the MAb's are important. The cells must be characterized for their ability to withstand freezing and thawing. The desired cell lines are frozen in liquid nitrogen at several stages of cloning and culture.

Large scale production of MAb's:-

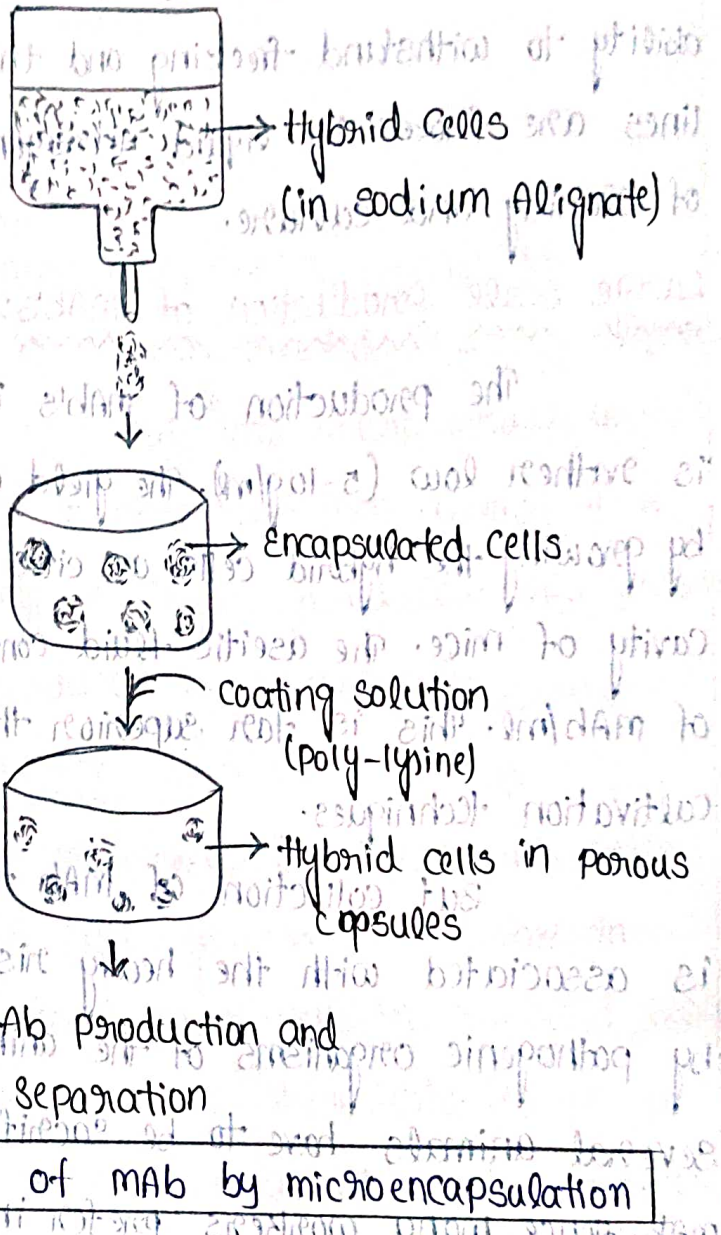
The production of MAb's in the culture bottles is rather low (5-10 g/ml). The yield can be increased by growing the hybrid cells as cites in the peritoneal cavity of mice. The aseitic fluid contains about 5-20mg of MAb/ml. This is far superior than the invitro cultivation techniques.

But collection of MAb from ascitic fluid is associated with the heavy risk of contamination by pathogenic organisms of the animal. In addition, several animals have to be sacrificed to produce MAb. Hence, many workers prefer invitro techniques rather than the use of animals.

Encapsulated hybridoma cells for commercial production of MAb's:-

The yield of MAb production can be substantially increased by increasing the hybridoma cell density in suspension culture. This can be

done by encapsulating the hybridomas in alignate gels and using a coating solution containing poly-lysine. These gels allow the nutrients to enter in and antibodies to come out.



Production of MAb by microencapsulation

By this approach, a much higher concentration of MAb production (10-100 µg/ml) can be achieved. Damon biotech company and Cell-Tech use encapsulated hybridoma cells for large-scale production of MAbs. They employ 100-liter fermenters to yield about 100g of MAbs in about 2 weeks period.

Applications of monoclonal Antibodies:-

The four types of applications are:

① Diagnosis Applications

② Therapeutic Applications

③ protein purification

④ miscellaneous applications.

① Diagnostic Applications:-

mAb's have revolutionized the laboratory diagnosis of various diseases. For this purpose, mAb's may be Employed as diagnostic reagents for biochemical analysis (or) as tools for diagnostic imaging of diseases.

(i) mAbs in Biochemical Analysis:-

Diagnostic tests based on the use of mAbs as reagents are routinely used in radioimmunoassay's (RIA) and Enzyme-linked immunosorbent assays (ELISA) in the laboratory. These assays measure the circulating concentrations of hormones (insulin, human chorionic gonodotropin, growth harmone, progesterone, thyroxine, gastrin, renin) and several other tissue and cell products (blood group Ag's, blood clotting factors, interferons, tumor markers).

A number of diagnostic kits using mAbs have become commercially available for instance it is now possible to do the early diagnosis of the condition/diseases.

(a) Pregnancy:-

By detecting of urinary levels of human Chorionic gonadotropin.

(b) Cancers:-

Estimation of plasma carcino-embryonic Ag in colorectal cancer and prostrate specific Ag for prostrate cancer. Besides diagnosis, estimation of tumor markers is also useful for the prognosis of cancers.

(c) Harmonal disorders:-

Analysis of thyroxine, triiodothyronine and thyroid stimulating harmone for thyroid disorders.

(d) Infectious diseases:-

By detecting the circulatory levels of Ag's specific to the infectious agent.

Eg:- Ag's of Neisseria gonorrhoeae and herpes simplex virus. for the diagnosis of sexually transmitted diseases (STD's).

(ii) MAbs in diagnostic imaging:-

Radiolabeled-MAbs are used in the diagnostic imaging of disease and the technique is referred to as immunoscintinography. The radio isotopes commonly used for labeling MAb are iodine-131 and technetium-99.

MAbs are success-fully used in the diagnostic imaging of cardiovascular diseases,

cancers and sites of bacterial infections cardiovascular diseases.

(a) myocardial infraction (MI):-

The cardiac protein myosin gets exposed whenever myocardial necrosis (death of cardiac cells) occurs. Antimyosin MAb labeled with radioisotopes indium chloride (^{111}In) is used for detecting myosin and thus the site of myocardial infraction. Imaging of radiolabeled MAb, is usually done after 24-48 hrs of intravenous administration. This is carried out either by planner gamma camera (or) single photon Emission computed tomography (SPECT). It is possible to detect the location and the degree of damage to the heart by using radiolabeled antimyosin MAb. Thus, this technique is useful for the diagnosis of heart attacks.

(b) Deep vein thrombosis (DVT):-

DVT refers to the formation of blood clots (thrombus) within the blood veins, primarily in the lower extremities. For the detection of DVT, radioisotope labeled MAb directed against fibrin (or) platelets can be used. The imaging is usually done after 4 hrs of injection. Fibrin specific MAbs are successfully used for the detection of clots in thigh, pelvis, calf and knee regions.

(c) Atherosclerosis :-

Thickening and loss of Elasticity of arterial

walls is referred to as atherosclerosis. Atherosclerotic plaques cause disease of coronary and peripheral arteries. Atherosclerosis has been implicated in the development of heart diseases. MAb tagged with a radiolabel directed against activated platelets can be used to localize the atherosclerotic lesions by imaging technique.

⑤ Therapeutic Applications:-

MAbs have a wide range of therapeutic applications. MAbs are used in the treatment of cancer, transplantation of bone marrow and organs, autoimmune diseases; cardiovascular diseases and infectious diseases. The therapeutic applications of MAbs are broadly grouped into 2 types.

(i) Direct use of MAbs as therapeutic agents

(ii) MAbs as targeting agents.

(i) Direct use of MAbs as therapeutic agents:-

MAbs can be directly used for enhancing the immune function of the host. Direct use of MAbs causes minimal toxicity to the target tissues (or) the host.

(a) In the treatment of AIDS:-

Immunosuppression is the hall mark of AIDS. This is caused by reduction in CD_4 (cluster determinant Ag 4) cells of T-lymphocytes. The HIV binds to specific receptors on CD_4 cells by using surface membrane

glycoprotein (gp 120). Genetic Engineers have been successful to attach Fc Portion of mouse mAb to human CD_4 molecule. This complex has high affinity to bind to membrane glycoprotein gp 120 of virus infected cells. The Fc fragment induces cell-mediated destruction of HIV infected cells.

(b) In the treatment of autoimmune diseases:-

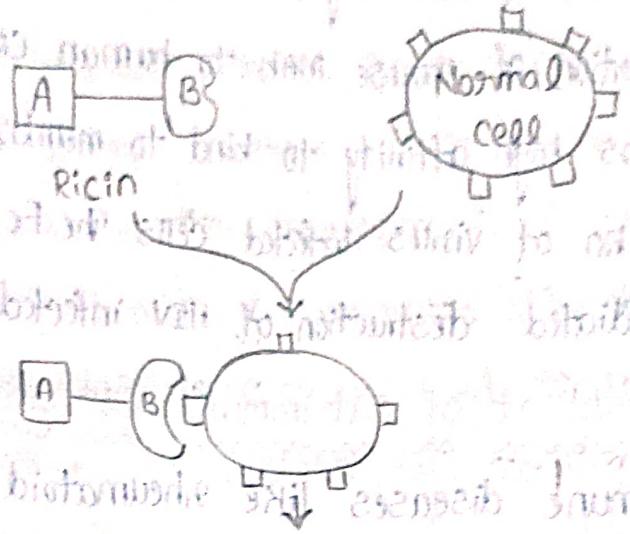
Autoimmune diseases like rheumatoid arthritis and multiple sclerosis are of great concern. Some success has been reported in the clinical trails of rheumatoid arthritis patients by using mAbs directed against T-lymphocytes and B-lymphocytes.

(ii) mAbs as targeting agents in therapy:-

Toxins, drugs, radioisotopes etc., can be attached (or) conjugated to the tissue specific mAbs are carried to target tissues for efficient action. This allows higher concentration of drugs to reach the desired site with minimal toxicity. In this way mAbs are used for the appropriate delivery of drugs (or) isotopes.

(a) mAbs in drug delivery:-

In general, the drugs are less effective invivo when compared to invitro. This is mainly due to the fact that sufficient quantity of the drug does not reach the target tissue. This problem can be solved by using tissue-specific mAbs. The drugs can be coupled with mAb and specifically targeted to reach the site of action.



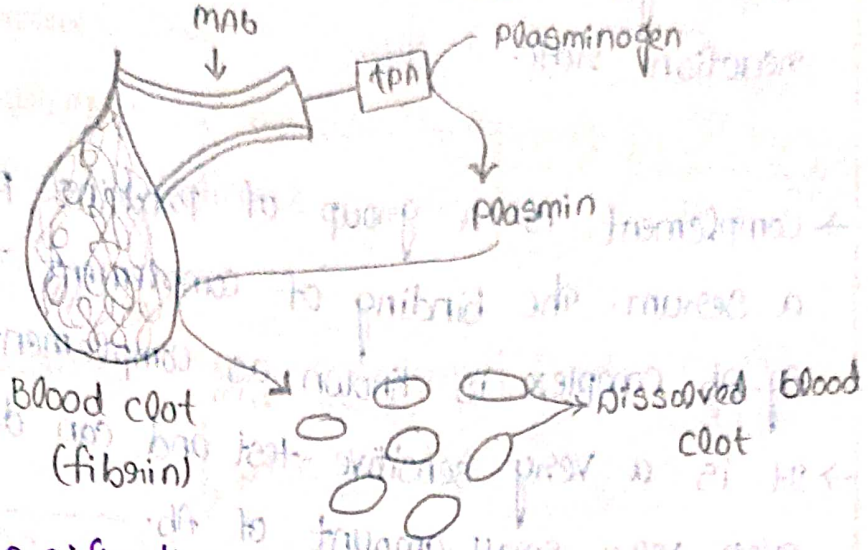
Normal cell death

(b) MAb in dissolution of blood clots

A great majority of natural deaths are due to a blockage in coronary (or) cerebral artery by a blood clot (thrombus). Fibrin is the major constituent of blood clot which gets dissolved by plasmin. plasmin turn is formed by the activation of plasminogen by plasminogen activator. The blockage of arteries occurs due to inadequate dissolution of blood clots. Tissue plasminogen activator (TPA) can be used as a therapeutic agent to remove the blood clots.

A MAb directed against fibrin can be coupled to TPA and used for degradation of blood clots. MAb-TPA complex due to a high affinity gets attached to fibrin. Due to the concentration of TPA at the target spots, there is more efficient conversion of plasminogen to plasmin

which in turn dissolves blood clot (fibrin). Good success of clot lysis has been reported by using mAb-TPA complex in experimental animals.



③ Protein purification :-

mAbs can be produced for any protein. And the so produced mAb can be conveniently used for the purification of the protein against which it was raised. mAbs columns can be prepared by coupling them to cyanogen bromide activated sepharose (chromatographic matrix). The immobilized mAbs in this manner are very useful for the purification of proteins by immuno affinity method.

④ Miscellaneous Applications :-

Abzymes :-

① Abzyme is an Ab that expressess catalytic activity.

② An Abzyme also called catmab (catalytic monoclonal Ab) and most often called catalytic Ab, is a mAb with catalytic activity.

③ These abzymes are capable of catalyzing the

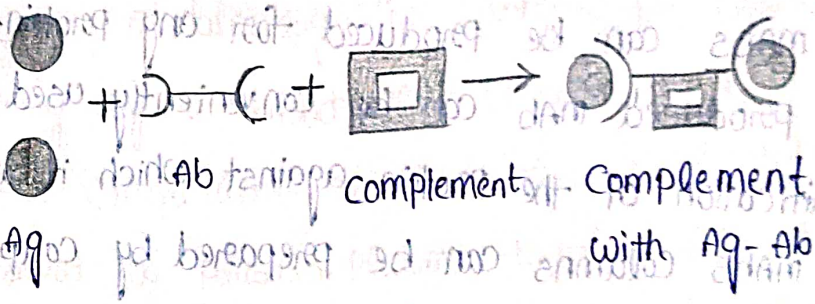
destruction of thousands of target molecules.

④ They bind storage to the transition state with high association constant, enhances the reaction rate.

Complement Fixation:-

→ Complement is a group of proteins present in a serum. the binding of complement to an Ag-Ab complex is known as complement fixation.

→ It is a very sensitive test and can detect even very small amount of Ab.



Complement fixation test:-

→ The patient serum is heated at 56°C for 30 minutes to inactivate the complement present in it.

→ The serum is serially diluted and added to known amounts of specific Ag and guineapig animal and then it is incubated at 37°C for ~~30 minutes~~ 1 hour.

→ To this mixture, add sheep RBC'S with the Ab and it is incubated at 37°c for 30 minute's.

→ If Ab's are present in the patient serum, they react with Ag's and form Ag-Ab complex that fixes the complement, no free complement is

available to lyse the sheep RBC.

→ So a positive test indicated by no lysis of sheep RBC.

→ If the patient serum contains no Ab's, Ag-Ab complex is not formed and complement is not fixed.

→ free complement is available to lyse sheep RBC.

→ A -ve test is indicated by the lysis of sheep RBC.

Positive test:-

Ag + patients serum + complement (containing Ab)

↓ 37°C for 1 hr

[Ag + Ab + Complement] + sheep RBC and Ab to sheep RBC.

↓ 37°C for 1 hr

[Ag + Ab + Complement] + Free Complement not available. No lysis of RBC.

Negative test:-

Ag + patient's serum + complement

(No Ab's)

↓ 37°C for 1 hr

Ag + free complement + sheep RBC and Ab to sheep RBC

↓ 37°C for 30 min

Free Ag + [sheep RBC & Ab to sheep RBC + Complement]

lysis of RBC

Immunodiffusion:-

Immunodiffusion is a technique for detecting (or) measuring Ab's and Ag's by their precipitation, when diffused together through a gel (or) medium is known as immunodiffusion.

Single immunodiffusion in one dimension method:-

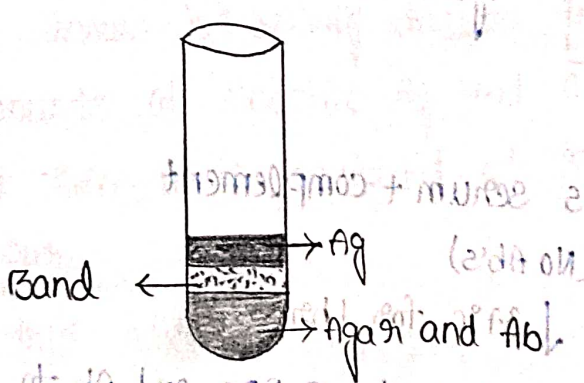
→ In this reaction one of the reactants is stationary while the other moves by diffusion.

→ Ab is incorporated in Agar gel at 56°C in a test tube and allowed to solidify.

→ Ag solution is layered overlying.

→ Ag diffuses through the gel, reacts with Ab and forms precipitation band.

→ Since the number of bands indicate the number of Ag's present, the test is used for the detection of the number of Ag's present in the mixture.



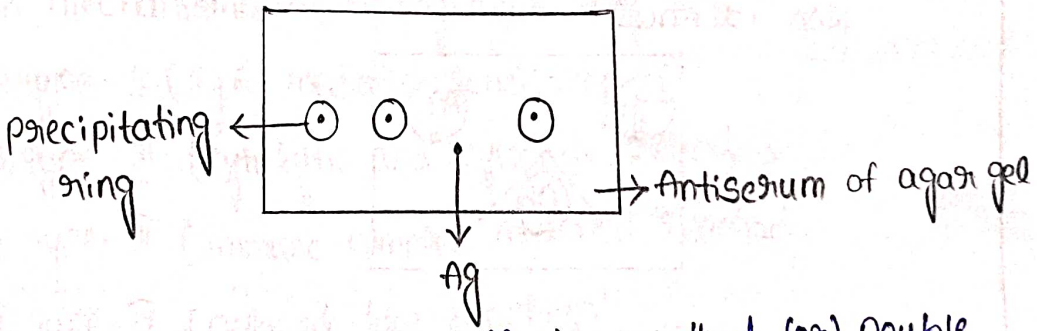
Radial immunodiffusion/ Mancini method/ single immuno diffusion:-

→ Antiserum is incorporated into a agar gel at 56°C into a petriplate.

→ The gel is allowed to set. wells are cut on surface of it.
→ then Ag is added to wells.
→ It diffuses radially from the well and forms ring shaped bands of ppt around the well.
→ Diameter of the ring gives an estimate of concentration of Ag.

uses :-

→ It is used to estimate the immunoglobulins (Ab's) and soluble Ag's.
→ It is used for the quantification of Ab's and protein in body fluids like csf (cerebro spinal fluid), urine, milk.



→ ouchterylony immunodiffusion method (or) Double immunodiffusion method.
→ This method is also known as agar gel diffusion.
→ Agar gel is poured on a slide (or) into a petriplate and allowed to solidify.
→ wells of 3 to 4 nm diameter are cut on gel surface using a template.
→ Antiserum is placed in the central well and Ag is present in surroundings.

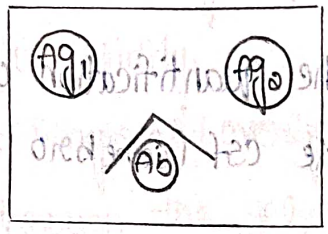
→ If two adjacent Ag's are identical, the lines of ppt will fuses to form a single arc.

→ If they are not identical the lines will cross each other.

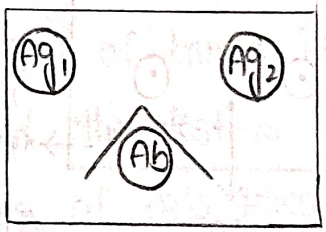
→ It is used to detect and compare different Ag's and Ab's.

→ It is used for the diagnosis of bacterial, viral and fungal infection.

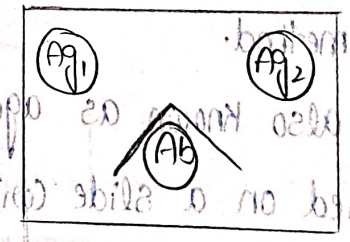
Identical



Non-identical



Partially identical



ouchtercony immunodiffusion

UNIT - IV

HYPERSENSITIVITY

Hypersensitivity:-

The hypersensitivity refers to the injurious consequences in the sensitized host due to contact with specific allergins. The term allergy was used by Von Pirquet.

→ The initial contact sensitizes immune system and it is known as sensitizing dose (or) primary dose. Subsequent contact with same allergins causes manifestation of actual allergic reaction. This is called shocking dose.

classification:-

In 1963 "Robert Coombs and peter Gell classified hypersensitive reactions" into 4 types based on differences in mechanisms of pathogenisis. they are:-

① Type - I (IgE mediated sensitivity)
② Type - II (cytolytic and cytotoxic reaction)
③ Type - III (Immune complex mediated reaction)
④ Type - IV (Delayed type reactions)

① Type - I hypersensitivity:-

Type - I hypersensitivity is a IgE dependent reactions. It contains two categories. They are:- (i) Anaphylaxis (ii) Atopy

(i) Anaphylaxis:-

This is classical immediate hypersensitive reaction. The term was coined by Richard. Anaphylaxis reactions are clinical manifestation which include smooth muscle contraction and increased vascular

permeability.

mechanism:-

When an allergin enters into host during primary dose, the IgE Ab's are bounded to surface receptors of mast cells (or) Basophils because these cells are carrying a large number of receptors called Fc Endoplasmic reticulum receptors. IgE molecules are attached to these receptors by Fc end. At 2° shocking dose the IgE molecules attach to this cells bind to Ag molecules and results in crosslinking. This cross-linking high permeability of cell to Ca^{2+} ions and leads to degranulation. The released granules are of two types. They are :-

(a) Primary granules

(b) Secondary granules

(a) primary granules:-

→ The primary granules are preformed granules in mast cells and basophils. They include histamine, serotonins, chaemotatic factors and Heparins.

→ Histamines are produced by decarboxylation of histidine. It is most important vasoactive amine in human anaphylaxis, when it is released, this stimulates a burning (or) itching sensation. It is called flare reaction. This release of histamine causes edema by increasing capillary permeability. This is called wheal reaction. Hence the release of histamine leads to wheal and flare reaction.

→ serotonin is derived from decarboxylation of tryptophan found in intestinal mucosa, brain tissue and platelets.

→ Heparin is a acidic mucopolysaccharide but the reactions contributed by heparin is observed in dogs but not in humans.

(b) secondary granules:-

→ The secondary granules are newly formed granules upon stimulation by mast cells, basophils and other type of immune cells. These secondary granules include prostaglandins and leukotrienes, platelets activating factor etc.

→ prostaglandins and leukotrienes:-

These are derived from a precursor called arachodonic acid by two pathways. prostaglandins are derived from ~~lipoxygenase pathway~~ cyclooxygenase pathway (cox). The leukotrienes are derived from lipoxygenase pathway. The granules causes branched contrinction, increase in vascular permeability and excess mucous production.

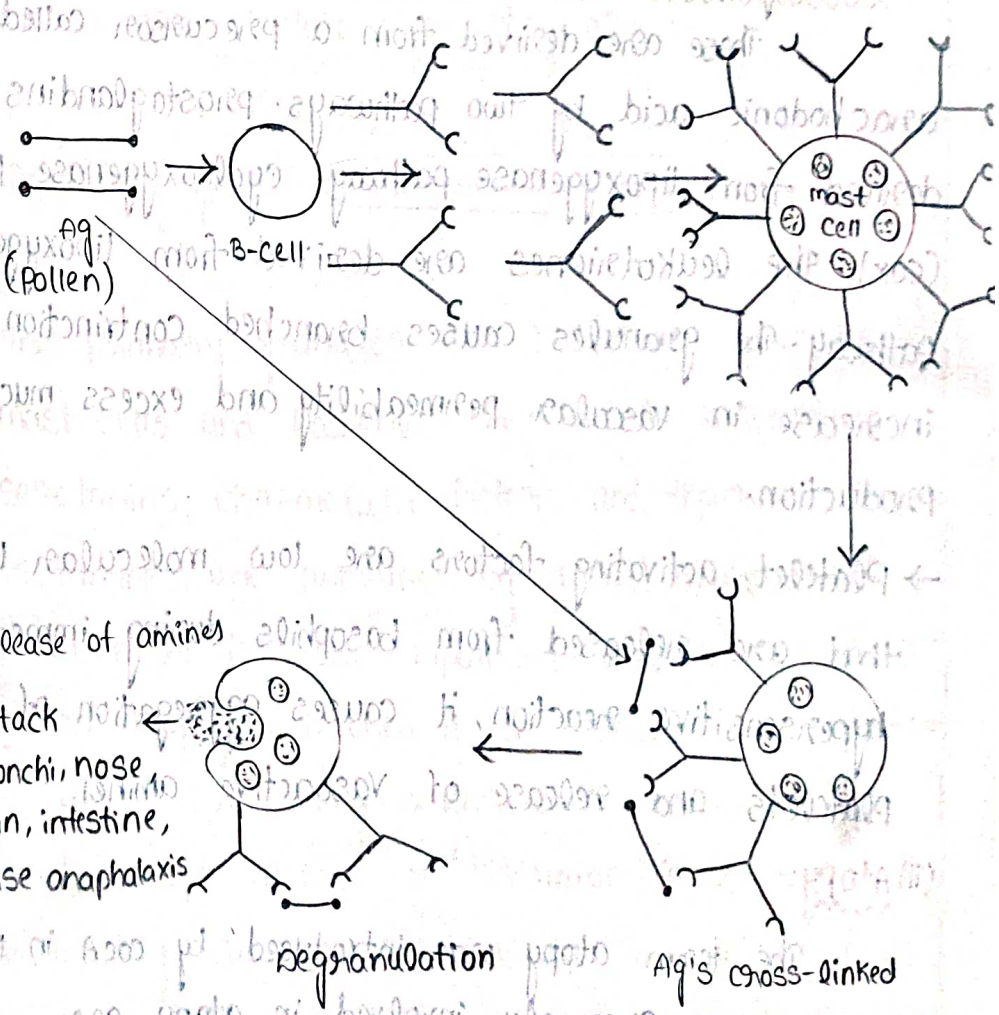
→ Platelet activating factors are low molecular lipid that are released from basophils during immediate hypersensitive reaction, it causes aggregation of platelets and release of vasoactive amines.

(ii) Atopy:-

The term atopy was introduced by coca in 1903. The allergins commonly involved in atopy are characteristically the inhalents such as pollen, cow dust,

foods like egg, milk etc. Atopy is genetically determined one. Atopy sensitization is developed spontaneously following natural contact with the ap atopents. About 10% of persons have tendency to over produce IgE. The biological consequences of atopic reactions includes allergic rhinities (HAY fever), Asthama, atopic determitisis (Eczema) and food allergies.

*For example: HAY fever is observed in population of us the inhalation of pollen grains leads to sensitization of mast cells in nasal mucosa and conjuctiva. This induces release of pharmacological active mediators that increase vascular permeability.



② Type-II hypersensitivity (or) cytolytic / cytotoxic reaction:-

The type-II hypersensitivity is also called cytolytic and cytotoxic reactions. These are also comes under immediate type of hypersensitivity and it is a Ab mediated destruction of cells. This type of reaction is best exemplified while blood transfusion reaction in which host Ab's reacts with foreign Ag's are incompactable transfused blood cells and that mediates the destruction of these cells. Ab mediated cell destruction by activating complement system to create pores on membrane of foreign cells. Ab's can also mediate cell destruction by ADCC method i.e., Ab dependent cell mediated cytotoxicity.

mechanism:-

In this process, cytotoxic cells carriers Fc cell receptors to bind to Fc region of Ab on target cell and promotes killing of cells.

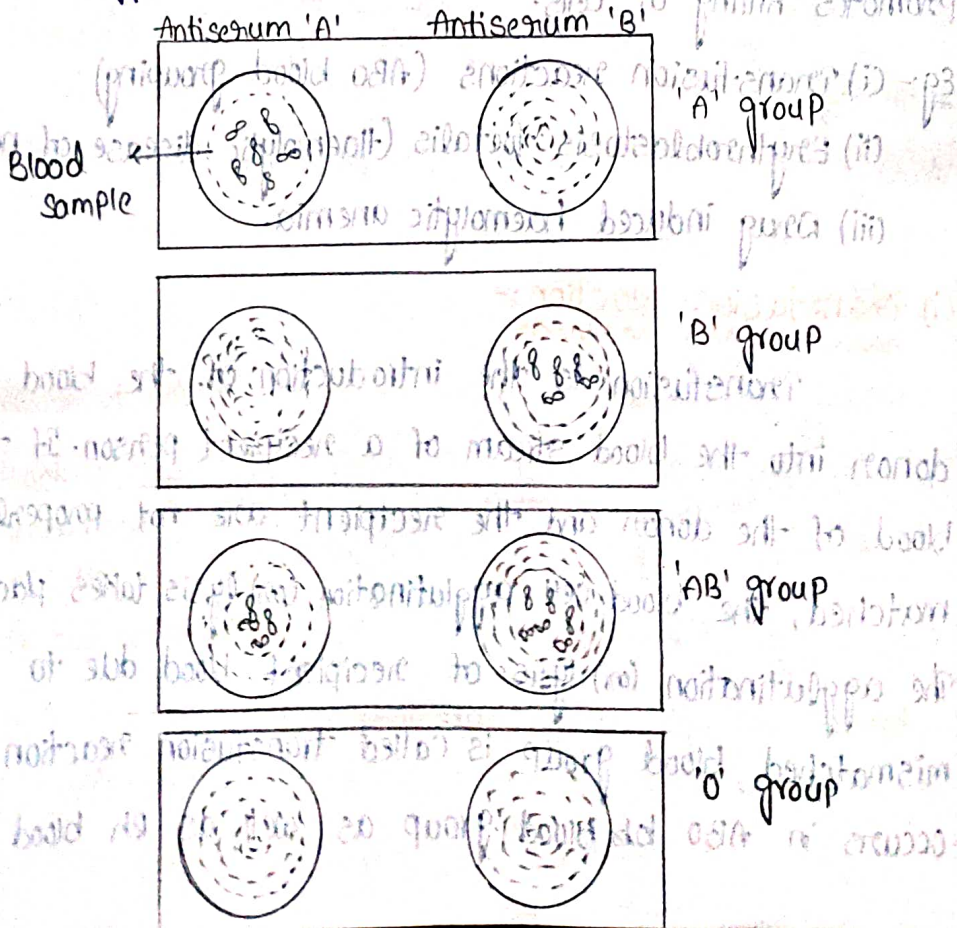
Eg:- (i) Transfusion reactions (ABO blood grouping)
(ii) Erythroblastosis foetalis (Haemolytic disease of new born).
(iii) Drug induced haemolytic anemia.

(i) Transfusion reaction:-

Transfusion is the introduction of the blood of donor into the blood stream of a recipient person. If the blood of the donor and the recipient are not properly matched, the blood cell agglutination (or) lysis takes place. The agglutination (or) lysis of recipient blood due to mismatched blood group is called transfusion reaction. It occurs in ABO bb blood group as well as Rh blood group.

The ABO blood group system has four groups in human beings namely Group A, Group B, Group AB, Group O. This grouping is based on presence of ABO blood group Ag's and Ab's.

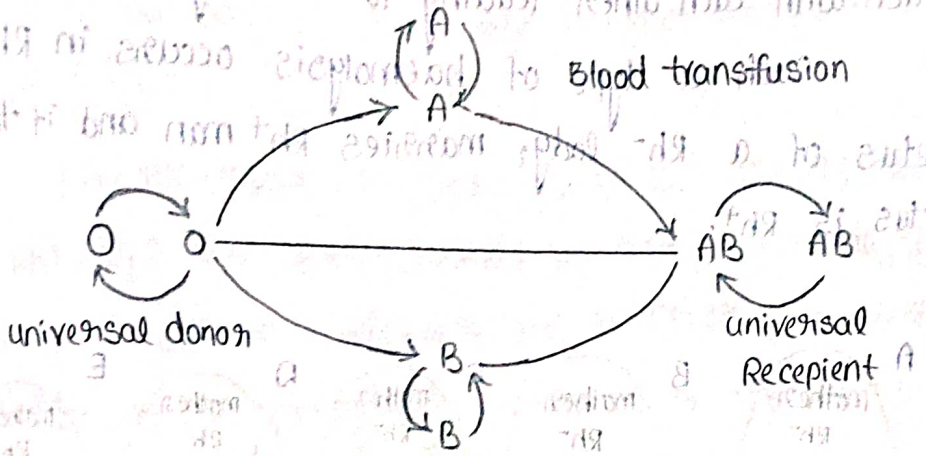
There are two types of ABO blood group Ag's namely Ag A, Ag B. They occur in surface of RBC. The typing of blood for ABO group (or) Rh group involves the agglutination reaction. When a drop of blood sample is mixed with antiserum A and another drop of blood sample is mixed with antiserum B on a glass slide, If the blood sample is clumped with antiserum A, the sample belongs to group A. If the sample is clumped with antiserum B, the sample belongs to group B. If the sample is clumped with both antiserum A and antiserum B, the sample belongs to group AB. If there is no agglutination, the sample belongs to 'O'.



AB blood group person has no Ab's in his blood. so he can receive blood from any group person. so AB group person is called universal receipient.

Similarly 'o' group person has no Ag's in his blood. so, he can donate blood to any other group. so 'o' group person is called universal donar.

The Ab's involved in transfusion reaction belong to a class of Ig M. these are called isohaemo-agglutins and the reaction is an isoimmune reaction.



(ii) Erythroblastosis foetalis:-

Erythroblastosis foetalis is a haemolytic disease caused by the reaction of Rh Ag and Rh Ab. It occurs in Rh+ baby developing in an Rh- mother. the Rh Ab involved in this reaction belongs to IgG type. It is an iso-immune reaction.

→ Landsteiner (1940) found an Ag in Rhesus monkey and it is called Rh Ag (or) Ag D. some human beings possess this Ag on their RBC and others do not contain it. the person having Ag D on their RBC are called Rh+ve/Rh+. the person who donot contain Ag D are called Rh-ve/Rh-.

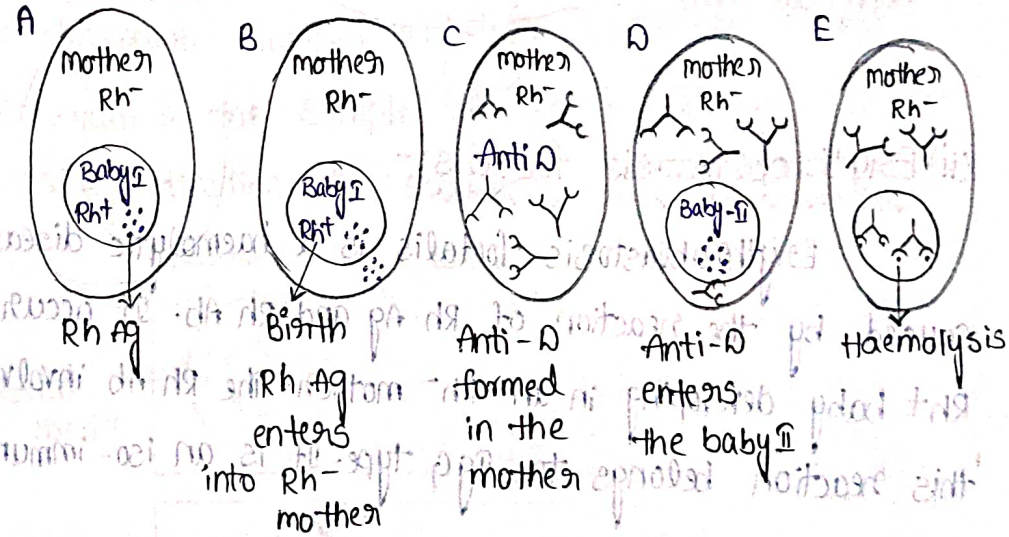
→ In India, 93.5% are Rh+ and 7.5% are Rh-

In China, 99.5% are Rh+ and 0.5% are Rh-.

The Rh blood group system has no natural Ab but when a Rh^- person receives blood either one ml (or) more from a Rh^+ person the blood of Rh^- person respond and produce Rh Ab called anti-D.

The anti-D remains in the blood and it doesnot do any harm to its possessor. Now, the person is sensitized to Ag-D. when he receives Rh^+ blood for the second time, Ag-D and Anti-D cross react with each other leading to haemolysis.

Such a type of haemolysis occurs in Rh foetus of a Rh^- lady; marries Rh^+ man and if their foetus is Rh^+.



The Rh^+ baby developed in the uterus of mother the Rh^-, the foetal blood cells may pass through ruptured placenta at birth into the Rh^- maternal blood. The mother's immune system recognizes the Rh Ag's and gets sensitized. The sensitized immune system produces Rh Ab's. The Rh Ab's are of the IgG type which are small in size. So, they can pass

through the placental barrier and enter the foetal blood circulation. Generally the first child of this 'genetically incompatible marriage' is unaffected because foetus is delivered by the time the mother gets sensitized and produces anti D Ab's.

During second pregnancy, if the second child is Rh^+, these Ab's crosses the placental border and enter the foetal blood circulation. The blood cells of the Rh^+ foetus are destroyed, causing erythroblastosis foetalis.

Haemolytic anemia and jaundice are symptoms.

prevention:-

If the mother is found to be Rh^- and the foetus is Rh^+, 1g of anti-D Ab's should be administered to the mother at 28th week (7th month) and 34th week (8½ month) of gestation as a prophylactic measure. If the Rh^- mother delivers Rh^+ baby, then anti-D Ab's should be administered to the mother soon after delivery. This develops passive immunity and prevents the formation of anti-D Ab's in the mother's blood, by destroying the Rh^+ foetal erythrocytes, before the mother's immune system is sensitized. This has to be done every time the lady is pregnant.

(iii) Drug induced haemolytic anemia:-

certain antibiotics like insulin, cephalosporins, streptomycin can absorb non-specifically to the proteins on RBC members forming a complexes that is similar to a Hallen carrier conjugate. This induced the formation of Ab's which can bind to adsorbed

drug on RBC inducing complement mediated lysis and thus a progressive anemia. when drug is withdrawn from patient, haemolytic anemia disappears.

③ Type - III Hypersensitivity :-

Type - III hypersensitivity is called immune complex mediated reaction. Generally this complexing of Ag with Ab facilitates clearance of Ag by phagocytic cells, in some cases large amounts of immune complexes leads to tissue damaging hypersensitive reaction. The magnitude of reaction depends on quantity of immune complexes and their distribution within body. The two important reactions characterized (a) Arthus reaction (b) serum sickness

(a) Arthus reaction :-

The arthus reaction was also called localized hypersensitivity reaction. Arthus reaction was observed by arthus in 1903, he injected normal horse serum subcutaneously into rabbit. The initial injection have no local effect but at shocking dose with 4-8 hours intense local reactions like edema and haemorrage necrosis occurs. This is called Arthus reaction. Here tissue damage is due to formation of Ag-Ab precipitates that are deposited on walls of blood vessels. This leads to increase in vascular permeability and infilteration of the site with neutrophils.

Some of the agents that cause this type

of hypersensitivity are bacterial spores, fungal spores, artificial proteins etc.

eg:- pigeon fanciers dorset disease occurs due to inhalation of dritoecal proteins of pigeons.

(b) serum sickness:-

This reaction appears following a single injection of high concentration of foreign serum such as diptheria antitoxin etc. This type of hypersensitive reactions are called generatized.

mechanism:-

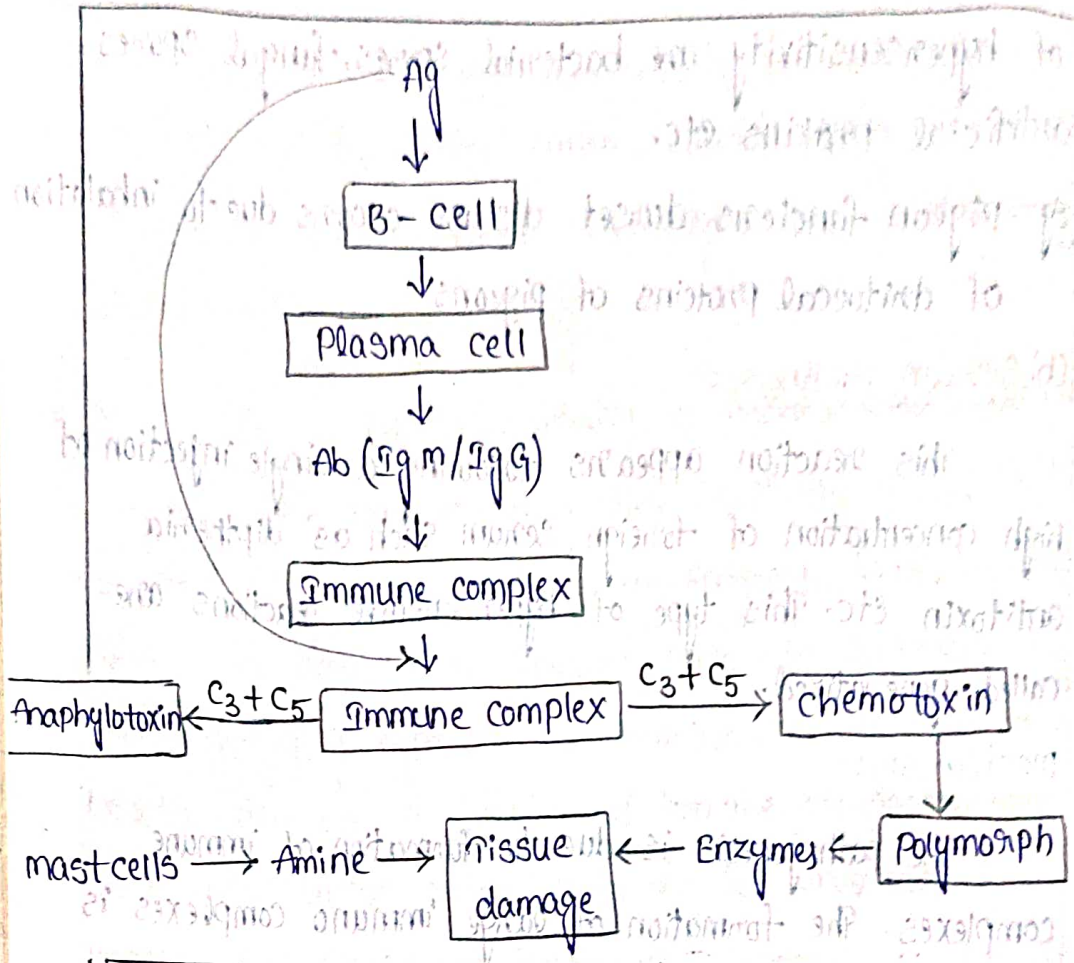
The pathogenesis is due to formation of immune complexes. The formation of large immuno complexes is not easily cleared by phagocytosis. This immune complexes deposited on Endothelial line of blood vessels in various parts of body causing inflamma infilteration.

The plasma concentration of complement reduces due to massive complement activation and fixation by Ag-Ab complexes. Formation of circulating immune complex contributes to pathogenisis of a number of conditions other than serum sickness as follows.

Autoimmune diseases like systemic new erythema, Goots pasters syndrome, Rheumatoid arthritis.

Drug allergic reaction like pencilin, sulphanamides, drug allergies etc.

Infectious diseases like hepatitis, malaria, trypanosomiasis, post streptococal glomerulonephritis.



Mechanism of immune complex mediated hypersensitivity

④ Type-IV hypersensitivity (or) Delayed type:-

Type-IV hypersensitivity is caused by the interaction between the Ag's sensitized T-cells. This reaction leads to inflammatory reactions and causes tissue damages.

Ab's are not involved in Type-IV hypersensitivity. As T-cells are involved in this reaction. It is called cell mediated hypersensitivity. As it is a cell mediated reaction, it can be passively transformed from one animal to another by the transfer of Ab's. But it can be transferred by trans of T-cells.

The T-cells on contact with Ag produce soluble proteins called lymphokinins which is

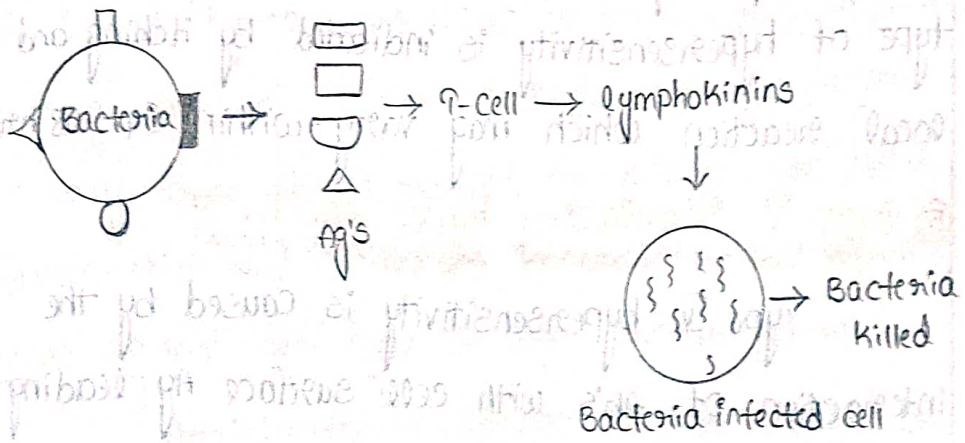
responsible for type-IV hypersensitivity.

Type-IV hypersensitivity contains two types of reaction. ① Tuberculin reaction

② contact Dermititis

mechanism:-

When T-cells primed to an Ag (viral (or) bacterial) come in contact with same Ag for second time the cells releases Soluble proteins called lymphokinins. These lymphokinins activates macrophages to kill intracellular bacteria like tuberculae bacilli and leads to the formation of inflammatory cells like giant cells and epithelial cells.



① Tuberculin reaction:-

When a small dose of tuberculin injected intradermally in an individual, these sensitized to tuberculoprotein that can manifest the reaction within 48 to 72 hrs time. In unsensitized individuals, this tuberculin injection promotes the sensitized person shows swelling and redness at the injection time within 48 to 72 hrs.

Tuberculin like hypersensitivity reaction is developed in many infections with bacteria like mycobacterium leprae, fungus, viruses and parasites. A similar reaction is developed in allograph reactions,

many autoimmune disease like systemic lupus erythromotosis, Rhematoid arthritis, Good patcher syndrome etc.

④ Contact dermititis type reaction:-

Delay type hypersensitivity sometime result from skin contact with a variety of chemicals which are Ni, Cr, hair dye, formaldehyde, cosmetics, poison oak, poison IBy, picryl chloride, dinitro chloro benzene, drugs such as penicillin etc. sensitization particularly label and lisions are appear at contact area. The substances are involved are not antigenic in nature but may occur antigenicity in combination with skin proteins. This type of hypersensitivity is indicated by itching and local reaction which may vary within 04 - 48 hrs.

⑤ Type-V Stimulatory hypersensitivity:-

Type V hypersensitivity is caused by the interaction of Ab's with cell surface Ag leading to stimulation of cells. In type-II hypersensitivity the interaction between cell surface occurs but here instead of stimulation destruction of cell occurs.

This phenomenon of stimulation occurs in graves disease (THYROTOICOSIS). Stimulation of thyroid cells by thyroid stimulating harmone is another example of type-V hypersensitivity.

Paratoxicosis (graves disease):-

Thyrotoxicosis is a disease condition owing to

the over activity of thyroid gland.

It is caused by an Ab called long acting thyroid stimulator (LATS). It is an IgG type. It acts on the thyroid cell surface Ag which is basically a receptor for thyroid stimulating harmone produced by the pituary gland. This causes the release of thyroxine in higher dose from the thyroid cell. This causes the graves disease.

AUTOIMMUNE DISEASES

Ag's present in one's own cells are called autoantigens (or) self-antigens. However when these Ag's are altered by the action of bacteria, virus, chemicals (or) drugs, they elicit the production of Ab's in the own body. These Ab's produced by auto Ag's are called Auto Ab's. This kind of immune response where Ab's are formed against self Ag's is called autoimmunity.

Autoimmunity is defined as humoral (or) cell mediated immune response against the constituents of body's of own tissues.

Autoimmune disease was observed by Donoth and Landsteiner in 1904.

Classification of autoimmune diseases:-

The autoimmune disease are broadly classified into three types. They are:-

① Haemolytic autoimmune diseases
② Localized autoimmune diseases.
③ systemic autoimmune diseases.

① Haemolytic autoimmune diseases:-

Haemolytic autoimmune disease is a clinical disorder resulting in the destruction of components of blood. Here auto Ab's are formed against one's own RBC.

Eg:- Haemolytic Anemia, Leucopania.

Haemolytic Anemia:-

→ Haemolytic Anemia is a clinical disorder where there is reduction below normal number of RBCs (or) quantity of haemoglobin.

→ The reduction in RBC number is caused by the destruction (or) lysis of RBC's and this process is called Haemolysis.

→ The lysis of RBC's is due to the production of auto Ab's against Ag's present on the Ab.

→ It is an Ab mediated autoimmune disease.

→ The Auto Ab's to red cells associated with haemolytic diseases are of two classes namely cold Ab and warm Ab. The cold Ab is activated at 4°C but not at 37°C and it belong to the class of IgM Ab. The warm Ab is acted at 37°C but not at 4°C. It belongs to the class of IgG Ab.

→ It is type II hypersensitivity reaction which damages the membranes of RBC occurs.

→ Autoimmune haemolytic anemia is also caused by drug therapy, diseases of the lymphoid tissues, secondary to infections.

⑧ Localized autoimmune disease:-

In localized autoimmune disease, a particular organ is effected due to auto Ab's, hence it is also called as organ specific autoimmune disease.

Eg:- Thyrotoxicosis, addisons disease, mysthenia gravis.

Thyrotoxicosis (or) Gravis disease :-

Thyrotoxicosis is an autoimmune disease. The primary cause for this disease stimulation of thyroid gland to secrete more thyroid hormones, here the stimulation is done by an auto Ab. this Ab directed against the receptors for thyroid stimulatory hormone. this Ab is called long acting stimulatory hormone. this Ab is called long acting stimulator (LATS) (or) thyroid stimulating Ab [TSH]. The LATS is IgG Ab, the LATS is a stimulating molecule it is shown to activate the present in the thyroid cell membrane. Thus, it is consider that LATS has a complementary site similar to thyroid stimulating hormone, it is type V hypersensitivity reaction.

Thyrotoxicosis is a condition of hyper thyroidism. over secretion of thyrosine result in Exothalmos and goitre.

Myasthenia Gravis :-

myasthenia Gravis is a disease of skeletal muscle characterized by gradually increasing weakness of muscles that may one easily fatigue.

This disease is caused by auto Ab against muscle Ag and acetyl choline receptor Ag.

The neuromuscular junction is severally affected in these patients. when auto Ab's against acetyl choline receptors are produced, acetyl choline cannot be produced. Hence the nerve impulse cannot be transferred from nerves to muscle.

③ Systematic Autoimmune disease:-

Systematic autoimmune disease affects the whole body (or) many organs. Hence it is also called as non-organ specific autoimmune disease.

Eg:- Systematic Lupus Erythematosis
Rheumatoid Arthritis

Systematic Lupus Erythematosis:-

→ Lupus Erythematosis is a autoimmune disease.
→ It is a skin disease affectly mostly women than men.
→ It is characterized by appearance of red spots over the bridge of nose and cheeks and it takes the shape of butterfly, it is immune complex mediated autoimmune disease.
→ The primary cause for this disease is the formation of autoimmune antibody to the nuclear complements. These Ab's are called antinuclear complex/factor (ANF) (or) antinuclear autonuclear body.
→ The antinuclear factor reacts with the break down products of nuclei in the normal wear and tear of

cells and form immune complexes.

→ these immune complex is the main cause for the tissue damage occuring in this diseases.

→ In the patients a distinct cell called lupus Erythemato-sis cells appear in the blood and bone marrow. these cells are actually a mature neutrophils that has phagocytes that break down nuclear material in the presence of antinuclear Auto Ab's.

Rheumatoid Arthritis:-

It is chronic systematic disease of the joints marked by inflammatory changes in the synovial membrane and articular and bind atropy of bones.

These disease caused by auto Ab's of the IgM and hence they are called Rheumatoid factors. The synovial fluid of these patients contain increased number of T-cells and macrophages.

Vaccination

Vaccine:-

Vaccine (vacca = cow) is a preparation/suspension (or) extract of dead/attenuated (weakened) germs of a disease which on inoculation (injection) into a healthy person provides temporary/permanent active/passive immunity by inducing Ab's formation.

→ Thus Ab provoking agents are called vaccines.

→ The principle of immunisation (or) vaccination is based on the property of 'memory' of the immune systems. Vaccines also generate memory-B and T cells that

recognise the pathogen quickly.

→ In snake bites the injection which is given to the patients contains preformed Ab's against snake venom. This type of immunisation is called passive imunisation.

→ The process of introduction of vaccine into an individual to provide protection against a disease is called vaccination. In vaccination, a preparation of antigenic proteins of pathogens (or) inactivated / weakened pathogens (vaccine), is introduced into the body.

These Ag's generate the primary immune response and the memory B and T cells. When the vaccinated person is attacked by the same pathogen, the existing memory T (or) B cells recognise the Ag quickly and attack the invaders with a massive production of lymphocytes and Ab's.

→ Vaccination is the administration of a vaccine (or) toxoid.

Toxoid:-

Toxoid is a modified bacterial toxin that has been made non toxic but retains the capacity to stimulate the formation of antitoxin.

Types of vaccines:-

There are several basic types of vaccines.

(a) Attennuated whole-agent vaccines.

(b) Inactivated whole-agent vaccines.

(c) Toxoids

(d) Subunit vaccines.

(e) Conjugated Vaccines

(f) Nucleic acid Vaccines (or) DNA Vaccines.

(a) Attenuated whole-agent Vaccine :-

It uses living but attenuated (weakened) microbes.

Eg:- ① Sabin Polio vaccine

② Vaccines against mumps, measles, rubella (MMR), tuberculosis bacillus and orally administrated typhoid vaccines.

(b) Inactivated whole-agent Vaccines :-

It uses microbes that have been killed.

Eg:- ① Inactivated Virus vaccines (rabies, influenza, polio)

② Inactivated bacterial vaccines (pneumococcal pneumonia, Cholera, thyphoid, pertussis (whooping cough))

(c) Toxoids :-

Toxoids are inactivated toxins, are vaccines directed at the toxins produced by a pathogen.

Eg:- Vaccines against tetanus and diptheria.

(d) Subunit vaccines :-

→ It use only those antigenic fragments of a micro-organism that best stimulate an immune response.

→ These are produced by genetic modification techniques, others microbes are programmed to produce the desired antigenic fraction, are called recombinant Vaccines.

Eg:- Vaccine against hepatitis B virus consists of a portion of viral protein coat that is produced by genetically modified yeast.

(e) Conjugated vaccines:-

It deals with poor immune response of children. The polysaccharides are combined with proteins such as diptheria toxoid.

Eg:- Haemophilic influenza type b.

(f) Nucleic acid vaccine (or) DNA vaccine:-

Vaccines are among the newest and most promising vaccines.

Eg:- Plasmid of naked DNA injected into muscle results in the production of protein encoded in the DNA.

Vaccines classification:-

① First generation vaccines
② Second generation vaccines
③ Third generation vaccines
④ Vaccines under study.

① First generation vaccines:-

These are produced by conventional methods.

Eg:- Small pox vaccine, Salk's Polio vaccine.

② Second generation vaccines:-

They are prepared with the help of genetic engineering technique.

Eg:- Hepatitis B and Herpes virus.

③ Third generation vaccines:-

These are synthetic vaccines which are under trial.

④ Vaccines under study:-

Vaccines against malaria, Herpes, Hepatitis C, AIDS, Leprosy etc are under study.

Recombinant Antigen Vaccines:-

In this method, gene Encoding any immunogenic Protein can be isolated and cloned in bacterial, yeast (or) mammalian cells using r-DNA technology. A number of genes Encoding surface Ag's from viral, bacteria and protozoan pathogens have been successfully cloned bacterial, yeast, insects and mammalian cell system and the expressed Ag's used for vaccine development. The first such recombinent Ag vaccines approved for human use was the Hepatitis B vaccine.

This vaccine was developed by cloning the gene for the major surface Ag of Hepatitis B virus (HBV's Ag) in yeast cells. The recombinent yeast cells are grown in large fermentors and HB's Ag accumulates intracellularly in the cells. The yeast cells are harvested and distrupted by high pressure, releasing the Hepatitis B Ag which is then purified by conventional biochemical techniques. The recombinent Hepatitis B Vaccine has been shown to induce the production of Protective Ab's.

Advantages:-

→ Toxoid vaccines are prepared in large quantities by using this method.

→ Animals immersed with these recombinent vaccines having sub cases mounted a protect immune response to a subsequent challenge

with the live pathogen.

Limitations :-

→ They primarly induces humoral immunity they donot tend to induce much activation of Class-I MHC restricted T_c-cells.

other recombinent vaccines that are evaluated in animal models include the B-subunit of cholera toxin, the Enterotoxin of E-coli, the circumsporozite of the malarial parasite and glycoprotein membrane Ag from Epstein barr virus.