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Immunology

(B.Sc.- Zoology Sem. - V)

Contact us:

8252299990

8404884433

AISECT University, Hazaribag

Matwari Chowk, in front of Gandhi Maidan, Hazaribag (JHARKHAND)-825301

www.aisectuniversityjharkhand.ac.in info@aisectuniversityjharkhand.ac.in

UNIT I

Immunology

Immunology is the branch of microbiology which deals with the study of our protection from foreign macromolecules or invading organisms and our responses to them. These invaders include viruses, bacteria, protozoa, or even larger parasites. In addition, we develop immune responses against our own proteins (and other molecules) in autoimmunity and against our own aberrant cells in tumour immunity.

Our first line of defence against foreign organisms are barrier tissues such as the skin that stop the entry of organism into our bodies. If, however, these barrier layers are penetrated, the body contains cells that respond rapidly to the presence of the invader. These cells include macrophages and neutrophils that engulf foreign organisms and kill them without the need for antibodies. Immediate challenge also comes from soluble molecules that deprive the invading organism of essential nutrients (such as iron) and from certain molecules that are found on the surfaces of epithelia, in secretions (such as tears and saliva) and in the blood stream. This form of immunity is the innate or non-specific immune system that is continually ready to respond to invasion.

A second line of defence is the specific or adaptive immune system which may take days to respond to a primary invasion (that is infection by an organism that has not hitherto been seen). In the specific immune system, we see the production of antibodies (soluble proteins that bind to foreign antigens) and cell-mediated responses in which specific cells recognize foreign pathogens and destroy them. In the case of viruses or tumours, this response is also vital to the recognition and destruction of virally infected or tumorigenic cells. The response to a second round of infection is often more rapid than to the primary infection because of the activation of memory B and T cells. We shall see how cells of the immune system interact with one another by a variety of signal molecules so that a coordinated response may be mounted. These signals may be proteins such as lymphokines

which are produced by cells of the lymphoid system, cytokines and chemokines that are produced by other cells in an immune response, and which stimulate cells of the immune system.

Definition:

Immunity is the ability of the body to protect against all types of foreign bodies like bacteria, virus, toxic substances, etc. which enter the body.

Immunity is also called disease resistance. The lack of immunity is known as susceptibility.

The science dealing with the various phenomena of immunity, induced sensitivity and allergy is called immunology.

Types of Immunity:

There are two major types of immunity: innate or natural or nonspecific and acquired or adaptive.

(A) Innate or Natural or Nonspecific Immunity (L. *innatus* = inborn):

Innate immunity is inherited by the organism from the parents and protects it from birth throughout life. For example, humans have innate immunity against distemper, a fatal disease of dogs.

As its name nonspecific suggests that it lacks specific responses to specific invaders. Innate immunity or nonspecific immunity is well done by providing different barriers to the entry of the foreign agents into our body. Innate immunity consists of four types of barriers— physical, physiological, cellular and cytokine barriers.

1. Physical Barriers:

They are mechanical barriers to many microbial pathogens. These are of two types. Skin and mucous membrane.

(a) Skin:

The skin is physical barrier of body. Its outer tough layer, the stratum corneum prevents the entry of bacteria and viruses.

(b) Mucous Membranes:

Mucus secreted by mucous membrane traps the microorganisms and immobilises them. Microorganisms and dust particles can enter the respiratory tract with air during breathing which are trapped in the mucus. The cilia sweep the mucus loaded with microorganisms and dust particles into the pharynx (throat). From the pharynx it is thrown out or swallowed for elimination with the faeces.

2. Physiological Barriers:

The skin and mucous membranes secrete certain chemicals which dispose off the pathogens from the body. Body temperature, pH of the body fluids and various body secretions prevent growth of many disease-causing microorganisms. Some of the important examples of physiological barriers are as follows:

(a) Acid of the stomach kills most ingested microorganisms,

(b) Bile does not allow growth of microorganisms,

(c) Cerumen (ear wax) traps dust particles, kills bacteria and repels insects,

(d) Lysozyme is present in tissue fluids and in almost all secretions except in cerebrospinal fluid, sweat and urine. Lysozyme is in good quantity in tears from eyes. Lysozyme attacks bacteria and dissolves their cell walls. Lyso-enzyme is also found in saliva,

(e) Nasal Hair. They filter out microbes and dust in nose,

(f) Urine. It washes microbes from urethra,

(g) Vaginal Secretions. It is slightly acidic which discourages bacterial growth and flush microbes out of vagina,

(h) Sebum (sweat). It forms a protective acid film over the skin surface that inhibits growth of many microbes.

3. Cellular Barriers:

These are certain white blood corpuscles (leucocytes), macrophages, natural killer cells, complement system, inflammation, fever, antimicrobial substances, etc.

(i) Certain Leucocytes:

Neutrophils and monocytes are major phagocytic leucocytes.

(a) Polymorpho-nuclear Leucocytes (PMNL- neutrophils):

As they have multilobed nucleus they are normally called polymorphonuclear leucocytes (PMNL-neu- trophils). Neutrophils are short lived and are highly motile phagocytic killers. Neutrophils are formed from stem cells in the bone marrow. Neutrophils are the most numerous of all leucocytes. They die after a few days and must therefore, be constantly replaced. Neutrophils constitute about 40% to 75% of the blood leucocytes in humans.

(b) Monocytes:

They are the largest of all types of leucocytes and somewhat amoeboid in shape. They have clear cytoplasm (without cytoplasmic granules). The nucleus is bean shaped. Monocytes constitute about 2-10% of the blood leucocytes. They are motile and phagocytic in nature and engulf bacteria and cellular debris. Their life span is about 10 to 20 hours. Generally, they change into macrophages after entering tissue spaces.

(ii) Macrophages:

Monocytes circulate in the bloodstream for about 8 hours, during which time they enlarge and then migrate into the tissues and differentiate into specific tissue macrophages. Macrophages are long lived and are highly motile phagocytic.

Macrophages contain more cell organelles especially lysosomes. Macrophages are of two types, (a) Some take up residence tissues becoming fixed macrophage- ages and (b) whereas other remain motile and are called wandering macrophages. Wandering macrophages move by amoeboid movement throughout the tissues. Fixed macrophages serve different functions in different tissues and are named to reflect their tissue location. Some examples are given below:

i. Pulmonary alveolar macrophages in the lung

ii. Histiocytes in connective tissues

iii. Kupffer cells in the liver

iv. Glomerular Mesangial cells in the kidney

v. Microglial cells in the brain

vi. Osteoclasts in bone

(iii) Natural Killer Cells (NK Cells):

Besides the phagocytes, there are natural killer cells in the body which are a type of lymphocytes and are present in the spleen, lymph nodes and red bone marrow. NK cells do not have antigen receptors like T cells and B cells. NK cells cause cellular destruction in at least two ways:

(a) NK cells produce perforins which are chemicals that when inserted into the plasma membrane of a microbe make so weak that cytolysis (breakdown of cells particularly their outer membrane) occurs and creates pores in the plasma membrane of the target cells. These pores allow entry of water into the target cells, which then swell and burst. Cellular remains are eaten by phagocytes.

(b) Another function of NK cells is apoptosis which means natural cell death. It occurs naturally as part of the normal development, maintenance and renewal of cells, tissues, and organs.

Thus, functions of NK cells are to destroy target cells by cytolysis and apoptosis. NK cells constitute 5%-10% of the peripheral blood lymphocytes in humans.

(iv) Complement:

Complement is a group of 20 proteins, many of which are enzyme precursors and are produced by the liver. These proteins are present in the serum of the blood (the fluid portion of the blood excluding cells and clotting factors) and on plasma membranes. They are found circulating in the blood plasma and within tissues throughout the body. They were named complement by Ehrlich because they complement the actions of other components of the immune system (e.g., action of antibody on antigen) in the fight against infection. Jules Bordet is the discoverer of complement. Complement proteins create pores in the plasma membrane of the microbes. Water enters the microbes. The latter burst and die. The proteins of complement system destroy microbes by (i) cytolysis (ii) inflammation and (iii) phagocytosis. These proteins also prevent excessive damage of the host tissues.

(v) Inflammation:

Inflammation is a defensive response of the body to tissue damage. The conditions that may produce inflammation are pathogens, abrasions (scraping off) chemical irritations, distortion or disturbances of cells, and extreme temperatures. The signs and symptoms of inflammation are redness, pain, heat and swelling.

Inflammation can also cause the loss of function in the injured area, depending on the site and extent of the injury. Inflammation is an attempt to dispose of microbes, toxins, or foreign material at the site of injury to prevent their spread to other tissues, and to prepare the site for tissue repair. Thus, it helps restore tissue homeostasis.

Broken mast cells release histamine. Histamine causes dilation of capillaries and small blood vessels. As a result, more blood flows to that area making it red and warm and fluid (plasma) takes out into the tissue spaces causing its swelling. This reaction of the body is called inflammatory response.

(vi) Fever:

Fever may be brought about by toxins produced by pathogens and a protein called endogenous pyrogen (fever producing substance), released by macrophages. When enough pyrogens reach the brain, the body's thermostat is reset to a higher temperature, allowing the temperature of the entire body to rise.

Mild fever strengthens the defence mechanism by activating the phagocytes and by inhibiting the growth of microbes. A very high temperature may prove dangerous. It must be quickly brought down by giving antipyretics.

4. Cytokine Barriers:

Cytokines (Chemical messengers of immune cells) are low molecular weight proteins that stimulate or inhibit the differentiation, proliferation or function of immune cells. They are involved in the cell to cell communication. Kinds of cytokines include interleukins produced by leucocytes, lymphocytes produced by lymphocytes, tumour necrosis factor and interferon's (IFNs). Interferon's protect against viral infection of cells.

(B) Acquired Immunity (= Adaptive or Specific Immunity):

The immunity that an individual acquires after the birth is called acquired or adaptive or specific immunity. It is specific and mediated by antibodies or lymphocytes or both which make the antigen harmless.

It not only relieves the victim of the infectious disease but also prevents its further attack in future. The memory cells formed by B cells and T cells are the basis of acquired immunity. Thus, acquired immunity consists of specialized B and T lymphocytes and Antibodies.

Characteristics of Acquired Immunity:

(i) Specificity:

It is the ability to differentiate between various foreign molecules (foreign antigens).

(ii) Diversity:

It can recognise a vast variety of foreign molecules (foreign antigens).

(iii) Discrimination between Self and Non-self:

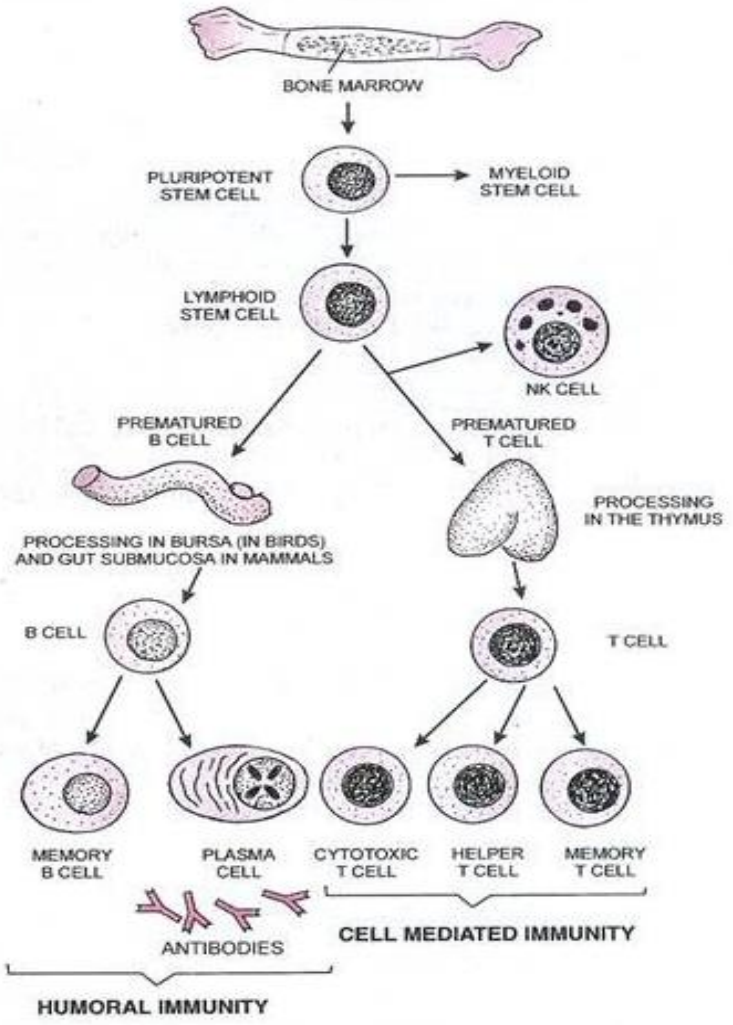
It can recognise and respond to foreign molecules (non-self) and can avoid response to those molecules that are present within the body (self) of the animal.

(iv) Memory:

When the immune system encounters a specific foreign agent, (e.g., a microbe) for the first time, it generates immune response and eliminates the invader. This is called first encounter. The immune system retains the memory of the first encounter. As a result, a second encounter occurs more quickly and abundantly than the first encounter.

The cells of the immune system are derived from the pluripotent stem cells in the bone marrow. Pluripotent means a cell that can differentiate into many different types of tissue cells. The pluripotent stem cells can form either myeloid stem cells or lymphoid stem cells.

Myeloid stem cells give rise to monocytes, macrophages, and granulocytes (neutrophils eosinophil's, and basophils). RBCs and blood platelets (lymphoid stem cells) form B lymphocytes (B cells), T lymphocytes (T-cells) and natural killer (NK) cells.



Development of B and T lymphocytes. Both arise from bone marrow precursors. Natural killer (NC) cells are a third population of lymphocytes that are distinct from T cells and B cells.

Components of Acquired Immunity:

Acquired immunity has two components: humeral immunity or Antibody mediated immune system (AMIS) and cellular immunity or cell mediated immune system (CMIS).

I. Antibody Mediated Immune System (AMIS) or Humoral Immunity:

It consists of antibodies (specialised proteins produced in the body in response to antigen) that circulate in the body fluids like blood plasma and lymph. The word 'humour' pertains to fluid. B lymphocytes (B cells) produce antibodies that regulate humoral immunity. The T-lymphocytes themselves do not secrete anti-bodies but help B lymphocytes produce them.

Certain cells of the bone marrow produce B lymphocytes and mature there. Since B lymphocytes produce antibodies, therefore, this immunity is called antibody mediated or humoral immunity. Humoral immunity or antibody-mediated immune system (AMIS) provides defence against most extracellular bacterial pathogens and viruses that infect through the respiratory and intestinal tract.

Formation of Plasma B cells and Memory B cells:

When antibodies on B cell's surface bind antigens (any substances that cause antibodies formation) the B cell is activated and divides, producing a clone (descendants of a single cell) of daughter B cells. These clones give rise to plasma B cells and memory B cells. This phenomenon is called clonal selection.

(a) Plasma B Cells (Effector B cells):

Some of the activated B cells enlarge, divide and differentiate into a clone of plasma cells. Although plasma cells live for only a few days, they secrete enormous amounts of antibody during this period.

(b) Memory B Cells:

Some activated B cells do not differentiate into plasma cells but rather remain as memory cells (Primed cells). They have a longer life span. The memory cells remain dormant until activated once again by a new quantity of the same antigen.

Role of AMIS:

The AMIS protects the body from (i) viruses (ii) some bacteria and (iii) toxins that enter the body fluids like blood and lymph.

II. Cell-Mediated Immune System (CMIS) or T-Cell Immunity:

A healthy person has about a trillion lymphocytes. Lymphocytes are of two types: T lymphocytes or T cells and B lymphocytes or B cells. As we know both types of lymphocytes and other cells of the immune system are produced in the bone marrow. The process of production of cells of immune system in the bone marrow is called haematopoiesis.

Because T lymphocytes (T cells) mature in the thymus, this immunity is also called T- cell immunity.

The T-cells play two important functions—effector and regulatory.

The effector function includes cytolysis (destruction of cells by immune processes) of cells infected with microbes and tumour cells and lymphokine production. The regulatory functions are either to increase or to suppress other lymphocytes and accessory cells.

Types of T-cells and their Functions:

1. Helper T cells (T_H):

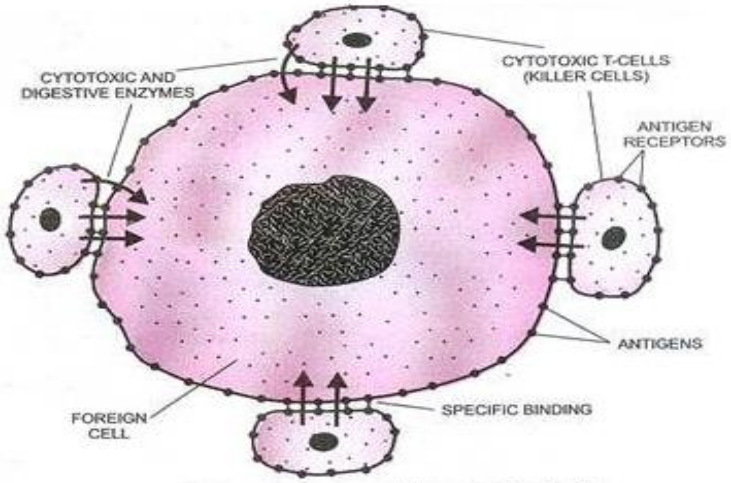
T_H cells are most numerous of the T cells. They help in the functions of immune system. They produce a growth factor that stimulates B-cell proliferation and differentiation and stimulates antibody production by plasma cells; enhance activity of cytotoxic T cells.

2. Cytotoxic T cells (T_c) or Killer cells:

These cells can kill microorganisms and even some of the body's own cells directly hence they are called killer cells. The antigen receptors on the surfaces of the cytotoxic cells cause specific binding with antigens present on the surface of foreign cell.

Cell after binding, the cytotoxic T cell secretes hole-forming proteins, called perforins, that punch large round holes in the membrane of the foreign cell. Then fluid flows quickly into the cell from the interstitial space. In addition, the cytotoxic T cell releases cytotoxic substances directly into the foreign cell. Almost immediately, the foreign cell becomes greatly swollen and it usually dissolves shortly thereafter.

Thus, they destroy body cells infected by viruses and attack and kill bacteria, fungi, parasites, and cancer cells.



Killing of a foreign cell by cytotoxic T-cells.

3. Memory T Cells (Primed Cells):

These cells are also formed by T-lymphocytes because of exposure to antigen and remain in the lymphatic tissue (e.g., spleen, lymph nodes). They recognize original invading antigens even years after the first encounter.

These cells keep ready to attack as soon as the same pathogens infect the body again. They proliferate and differentiate into cytotoxic T cells, helper T cells, suppressor T cells, and additional memory cells.

4. Suppressor Cells (Regulatory T cells (T_R)):

These cells can suppress the functions of cytotoxic and helper T cells. They also inhibit the immune system from attacking the body's own cells. It is believed that suppressor cells regulate the activities of the other cells. For this reason, the suppressor cells are classified as regulatory T cells.

Natural Killer (NK) Cells:

NK cells attack and destroy target cells, participate in antibody dependent cell mediated cytotoxicity. They can also attack parasites which are much larger than bacteria.

Types of Acquired Immunity:

Acquired (= Adaptive) Immunity is of two types: active immunity and passive immunity.

1. Active Immunity:

In this immunity person's own cells produce antibodies in response to infection or vaccination. It is slow and takes time in the formation of antibodies. It is long lasting and is harmless. Active immunity may be natural or artificial.

(a) A person who has recovered from an attack of smallpox or measles or mumps develops natural active immunity.

(b) Artificial active immunity is the resistance induced by vaccines. Examples of vaccines are as follows: Bacterial vaccines, (a) Live- BCG vaccine for tuberculosis, (b) Killed vaccines- TAB vaccine for enteric fever. Viral vaccines, (a) Live – sabin vaccine for poliomyelitis, MMR vaccine for measles, mumps, rubella, (b) Killed vaccines- Salk vaccine for poliomyelitis, neural and non-neural vaccines for rabies. Bacterial products. Toxoids for Diphtheria and Tetanus.

2. Passive Immunity:

When ready-made antibodies are directly injected into a person to protect the body against foreign agents, it is called passive immunity. It provides immediate relief. It is not long lasting. It may create problems. Passive immunity may be natural or artificial.

(a) Natural passive immunity is the resistance passively transferred from the mother to the foetus through placenta. IgG antibodies can cross placental barrier to reach the foetus. After birth, immunoglobulins are passed to the new-born through the breast milk. Human colostrum (mother's first milk) is rich in IgA antibodies. Mother's milk contains antibodies which protect the infant properly by the age of three months.

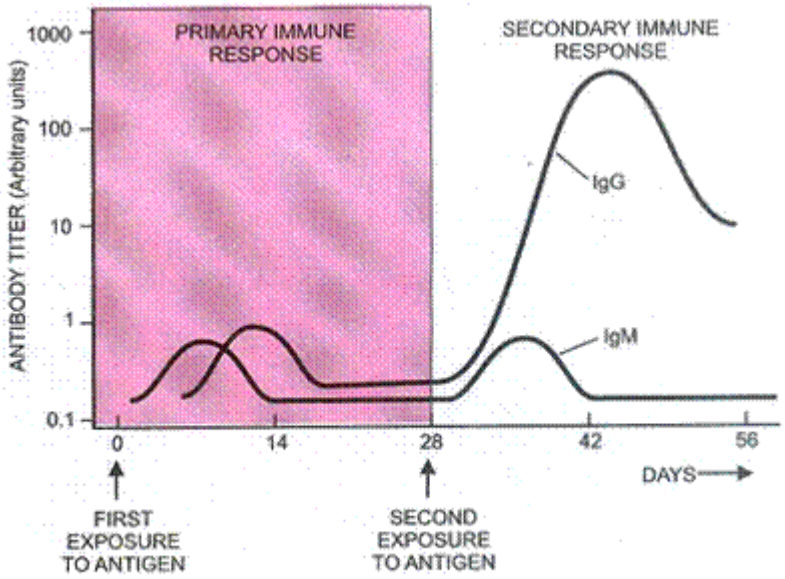
(b) Artificial passive immunity is the resistance passively transferred to a recipient by administration of antibodies. This is done by administration of hyper-immune sera of man or animals. Serum (pi. sera) contains antibodies. For example, anti-tetanus serum (ATS) is prepared in horses by active immunisation of horses with tetanus toxoid, bleeding them and separating the serum. ATS is used for passive immunisation against tetanus. Similarly, anti-diphtheric serum (ADS) and anti-gas gangrene serum (AGS) are also prepared.

Immune Response:

The immune response involves primary immune response and secondary immune response.

(a) The primary immune response:

After an initial contact with an antigen, no antibodies are present for a period of several days. Then, a slow rise in the antibody titre o (arbitrary units) occurs, first IgM and then IgG followed by a gradual decline in antibody titre. This is called the primary immune response.



Production of antibodies in the primary and secondary responses to a given antigen.

(b) The secondary immune response:

Memory cells may remain in the body for decades. Every new encounter with the same antigen results in a rapid proliferation of memory cells. This is also called "booster response". The antibody titre after subsequent encounters is far greater than during a primary response and consists mainly of IgG antibodies. This accelerated, more intense response is called the secondary immune response. Antibodies produced during a secondary response have an even higher affinity for the antigen.

A person who had been suffering from diseases like measles, smallpox or chicken pox becomes immune to subsequent attacks of these diseases. It includes spleen, lymph nodes, tonsils, Peyer's patches of small intestine and appendix.

The increased power and duration of the secondary immune response explain why immunization (method of providing immunity artificially, it is called vaccination) is usually accomplished by injecting antigen in multiple doses.

UNIT II

Cells of immune system: Lymphocytes, phagocytic cell, granulocytes, and dendritic cells

- WBCs are the principle cells of immune system formed hematopoietic stem cell by the process of hematopoiesis. Hematopoiesis occurs in yolk sac during 1st week of gestation. After 3rd month of gestation, hematopoiesis occurs in liver and spleen of fetus and after birth, it occurs in bone marrow.

The cells of immune system are:

1. Lymphocytes-
   - T-lymphocytes
   - B- lymphocytes
   - NK cell
2. Phagocytic cells
   - Monocytes
   - Macrophages
3. Granulocytic cells
   - Neutrophils
   - Basophils
   - Eosinophils
4. Dendritic cells

I. Lymphocytes:

- Lymphocytes are small, round cells found in peripheral blood, lymph, lymph nodes, lymphoid organs and in tissues.
- Lymphocytes represent 20-45% of total cells in peripheral blood and 99% of total cells in lymph and lymph node.

- According to side lymphocytes are divided into small (5-8µm), medium (8-12µm) and large (12-15µm).
- Depending on life span lymphocytes are classified into short lived (2 weeks) and long lived (3 years or more or even lifelong).

Broadly lymphocytes are divided into three sub-populations, based on function and cell membrane components.

1. **T-lymphocytes**
2. **B-lymphocytes**
3. **Natural killer cell**

II. Phagocytic cells:

- Monocytes and macrophages are mononuclear phagocytic cells.
- Granulocyte-monocyte progenitor cell differentiates into promonocytes and neutrophil.
- Promonocytes leaves the bone marrow and enter blood stream where they differentiate into mature monocytes.
- Monocytes circulates in blood for about 8 hours, during which they enlarge and then enter tissues and differentiates into specific macrophages and dendritic cells.

1. Monocytes:

- Blood monocytes measure 12-15 µm with a single lobed kidney shaped nucleus.
- It accounts for (2-8%) of blood leucocytes.

Immunological Functions of monocytes:

- Helps in antigen processing and presentation
- Releases cytokines
- Specialized function in tissues
- Cytotoxicity

2. Macrophages:

- Monocyte migrates to tissue and differentiates into macrophages.

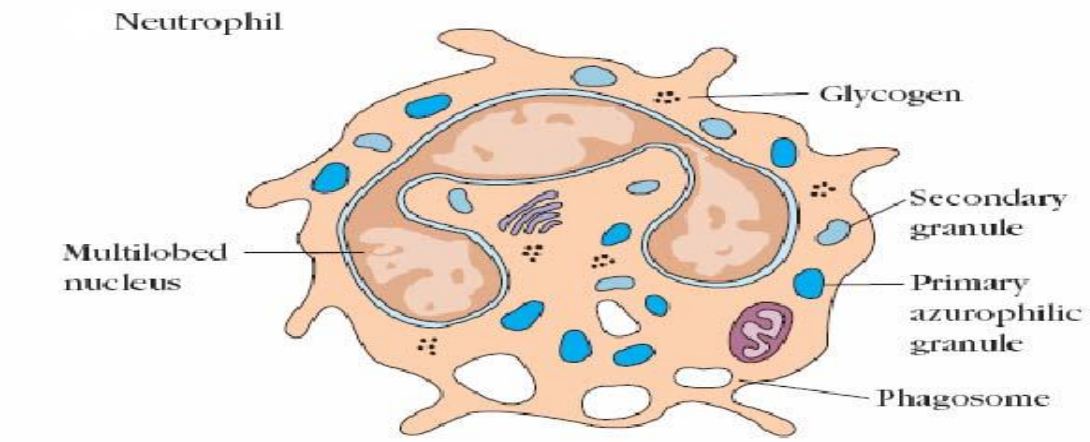
- Differentiation of monocytes into macrophages involves following changes:
- Cell enlarges 5-10 folds
- Intracellular granules increase in number and complexity
- Increase phagocytic ability
- Produces higher level of hydrolytic enzymes and cytokines
- Macrophages serve different functions in different tissues.
- Alveolar macrophages: in lungs
- Histiocyte: connective tissue
- Kuffer cell: liver
- Mesangial cell: kidney
- Microglial cell: brain
- Osteoclast: bone

Immunological functions of macrophages:

- Phagocytosis
- Antigen presentation to T-cell
- Secretion of lymphokines IL-1, IL-6. IL-12. TNF-α etc. to activates inflammatory response
- Secretion of granulocyte monocyte colony (GMC) stimulating factors.

II. Granulocytic cells:

1. Neutrophil:

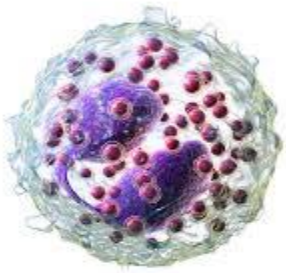


- Neutrophils are (11-14µm) in diameter with multilobed nucleus with granules in cytoplasm.
- It constitutes 50-70 % of total circulating WBC and remains for 7-8 hours in blood and then migrates to tissues
- Life span is 3-4 days.
- Also known as polymorphonuclear (PMN) leucocyte.
- Neutrophils is stained by both acidic and basic dye.

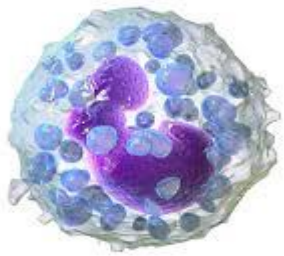
Immunological functions of Neutrophil:

- Phagocytic role in acute inflammatory response.
- It is the first immune cell to responds in inflammation.

2. Eosinophils:



Eosinophil



Basophil

- Eosinophils are (11-15µm) in diameter, heavily granulated with bilobed nucleus
- It is stained by acidic dye i.e. Eosin
- They are phagocytic and motile

Immunological functions of Eosinophil:

- Granules contain various hydrolytic enzymes that kill parasites which are too large to be phagocytosed by neutrophils.
- Provide allergic inflammation

3. Basophils:

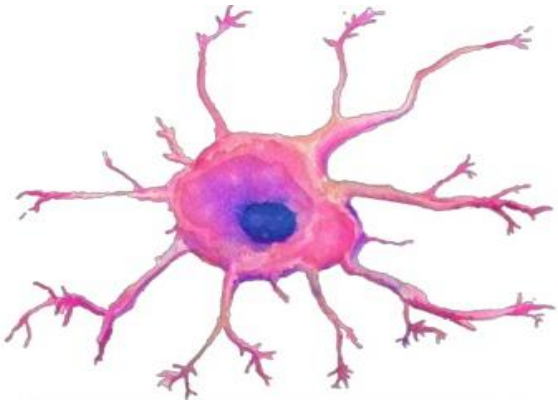
- Basophils are non-phagocytic cell found in small number in blood and tissue

- Cytoplasm contains large number of prominent basophilic granules containing histamine, heparin, serotonin, and other hydrolytic enzymes
- Stained by basic dyes

Immunological functions:

- Provide anaphylactic and atopic allergic reaction

IV. Dendritic cell:



- Dendritic cells have long cytoplasmic extensions that resembles to dendrites of nerve cell.
- They have highly pleomorphic with a small central body and many long needle-like processes.
- Dendritic cells are antigen presenting cell (APC) because they possess MHC class.

Immunological functions:

- Involved in antigen presentation to T-cells during primary immune response.
- Very little role in phagocytosis.

Primary Lymphoid Organs:

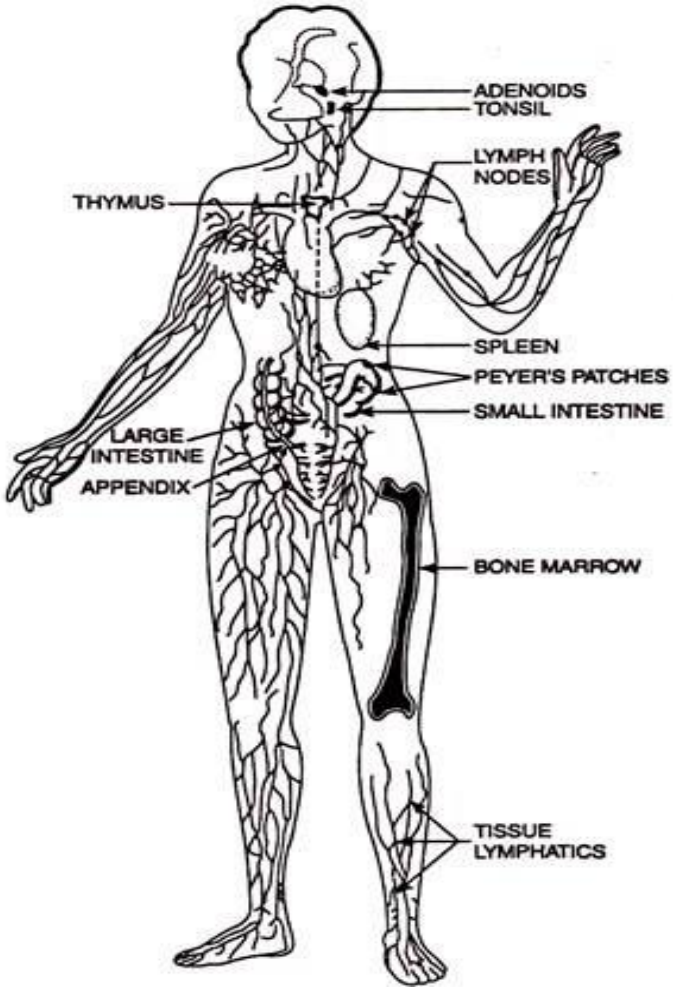
Immature lymphocytes generated in hematopoiesis, the process of formation and development of blood cells, mature and become committed to a particular antigenic specificity within the primary lymphoid organs, namely, thymus, bursa of Fabricius (in birds) and bone marrow (in mammals). A lymphocyte becomes immuno-competent, i.e., capable of mounting an immune response only after it matures within a primary lymphoid organ.

1. Thymus:

Thymus is a greyish, flat, bilobed lymphoid organ situated above the heart and extending into the neck on the front and sites of trachea. It develops from the epithelium of third and fourth pharyngeal pouches and, on maturity, acts as the site of development and maturation of lymphocytes named thymus-derived lymphocytes or T-lymphocytes or T-cells.

The thymus reaches peak activity in childhood and attains its largest size at puberty. Thereafter, the thymus begins to atrophy without any apparent effect on T-lymphocyte function and is extremely small in old age.

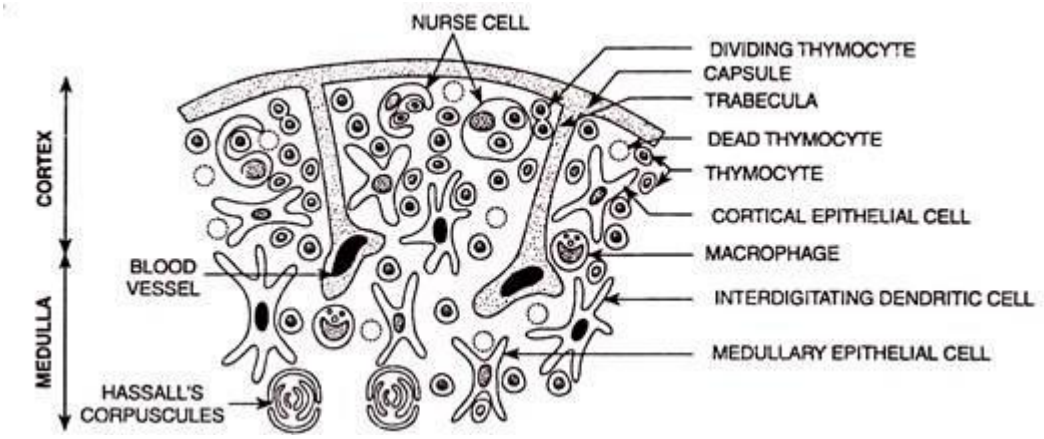
For convenience, the average weight of the thymus is 70 g in infants and its age-dependent involution leaves the thymus with an average weight of 3 g at the old age. This is probably due to the fact that T-lymphocytes are very long-lived and can circulate in the resting state for long periods of time.



Each lobe of thymus is surrounded by a capsule and is divided into a series of lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments-outer and inner. The outer component is called cortex, whereas the inner component is called medulla.

The cortex is densely packed with thymocytes, whereas the medulla is sparsely populated with thymocytes. Thymocytes develop from prothymocytes. The latter are produced in bone marrow, migrate through blood stream, enter the cortex of the thymus, and act as thymocytes. Thymocytes divide rapidly in the cortex and give rise to T-lymphocytes.

Of the T-lymphocytes produced in thymus only 5% leave the thymus as viable cells. Though the reason for this apparent wasteful process is not known, some believe that it is the elimination of T-lymphocyte clones that react against self.



Both the cortex and the medulla of the thymus are criss-crossed by a three-dimensional network consisting of epithelial cells, dendritic cells, and macrophages, which make up the framework of the organ and contribute to the growth and maturation, of thymocytes.

Some epithelial cells of the outer cortex possess long membrane extensions that surround as many as 50 thymocytes. These cells are called nurse cells. Other epithelial cells of the cortex have long interconnecting cytoplasmic extensions that form a network and have been found to interact with many of the thymocytes when they traverse the cortex.

The function of the thymus is to generate T-lymphocytes and to confer immunological competence on to them during their stay in the organ. T-lymphocytes so educated in the thymus become capable of mounting cell-mediated immune response against appropriate antigen.

This is affected under the influence of the thymic microenvironment and several hormones such as thymosin and thymopoietin produced by the epithelial cells of the thymus. The competent T-lymphocytes immediately move from thymus to the secondary or peripheral lymphoid organs.

2. Bursa of Fabricius:

Bursa of Fabricius is a primary lymphoid organ in birds where stem cells from yolk sac, foetal lever, and bone marrow mature, proliferate, and differentiate into bursa-derived lymphocytes called B-lymphocytes or B-cells.

Bursa of Fabricius arises as a pouch from the dorsal part of cloaca (fluid gut) in birds, Bursa of Fabricius is sensitive to hormones: administration of testosterone at the early embryo stage completely prevents its formation (hormonal bursectomy).

Surgical removal of bursa (bursectomy) from newly hatched chickens destroys their subsequent ability to produce antibodies. The B-cells mature, proliferate, and differentiate into bursa and then migrate from it and reach outer or superficial cortex of the germinal follicles and medullary cords of peripheral lymph nodes and lymphoid follicles of spleen where, following appropriate antigenic stimulation, transform into plasma cells and secrete antibodies. Like thymus, the bursal of Fabricius starts to shrink or atrophy at puberty.

3. Bone Marrow:

Bone marrow is the site of origin and development of B-lymphocytes or B-cells (bone marrow derived lymphocytes) in mammals particularly in humans and mice after birth. Before birth, the yolk sac, foetal lever, and total bone marrow are the major sites of B-lymphocyte maturation. Bone marrow, therefore, is the mammalian equivalent of the bursa of Fabricius in birds.

Development of B-lymphocytes (B-cells) begins with the differentiation of lymphoid stem cells into the earliest distinctive progenitor B cells (pro-B cell), which proliferate within the bone marrow filling the extravascular spaces between large sinusoids in the shaft of a bone.

Proliferation and differentiation of pro-B cells into precursor B cells (pre-B cells) requires the microenvironment provided by the bone marrow stromal cells.

The stromal cells within the bone marrow:

(1) Interact directly with the pro-B and pre-B cells and

(2) Secrete various cytokines that are required for development.

Bone marrow is not the site of origin and development of B-lymphocytes (B-cells) in all mammals. In cattle and sheep, the foetal spleen is the primary lymphoid tissue wherein the maturation, proliferation, and diversification of B-cells take place during early gestation.

During later gestation this function is performed by ideal Peyer's patch, a patch of tissue embedded in the wall of the intestine. In rabbit, gut-associated tissues (e.g. appendix) act as primary lymphoid tissue for maturation, proliferation, and diversification of B-cells.

Secondary Lymphoid Organs:

As stated earlier, the lymphocytes mature, proliferate, and differentiate in the primary or central lymphoid organs. These lymphocytes migrate therefrom via circulation to the secondary or peripheral lymphoid organs. Here they bind appropriate antigens and undergo further antigen-dependent differentiation.

Once in the secondary lymphoid organs, the lymphocytes do not remain there but move from one lymphoid organ to another through the blood and lymphatics. The passage of lymphocytes facilitates the induction of an immune response. Lymph nodes and the spleen are the most highly organized secondary or peripheral lymphoid organs, whereas mucosa-associated lymphoid tissue (MALT) is the less organized lymphoid tissue.

1. Lymph Nodes:

Lymph nodes are small, encapsulated, bean-shaped structures clustered at junctions of the lymphatic vessels which are distributed throughout the body. Lymph nodes contain a reticular network packed with lymphocytes, macrophages and dendritic cells, and filter out pathogenic microorganisms and antigens from the lymph.

As the lymph percolates through a lymph node, any pathogen or antigen that is brought in with the lymph is trapped by the phagocytic cells and dendritic cells.

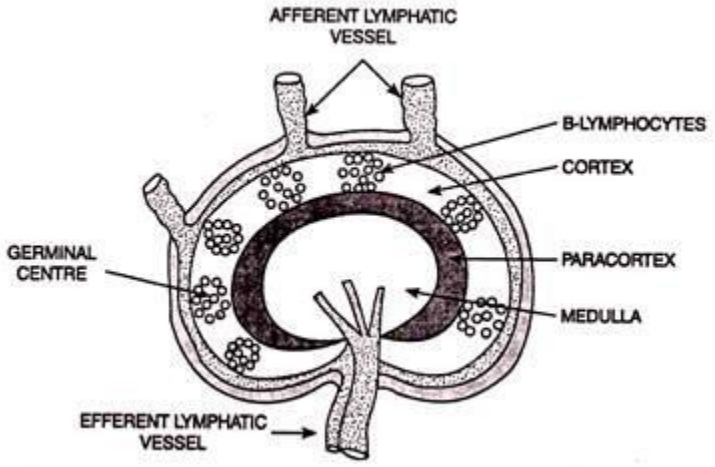
A lymph node consists of three regions: the cortex, the paracortex, and the medulla. Cortex is the outermost region and contains several rounded aggregates of lymphocytes (mostly B-lymphocytes), macrophages, and follicular dendritic cells arranged in primary follicles. Each follicle has a pale-staining germinal centre surrounded by small dark-staining lymphocytes.

The deeper region lying beneath the cortex is the paracortex. It is the zone between the cortex and the medulla. Paracortex possesses large number of T-lymphocytes and contains inter-digitating dendritic cells thought to have migrated from tissues to the lymph node.

Because of the presence of large number of T-lymphocytes in it. the Para-cortex is also referred to as a thymus-dependent area in contrast to the cortex which is a thymus-independent area. Medulla, the inner most region of lymph node, is more sparsely populated with lymphoid-lineage cells. Of the lymphoid-lineage cells present, many are plasma cells actively secreting antibody molecules.

Each lymph node has a number of lymph vessels called afferent lymphatic vessels, which pierce the capsule of a lymph node at numerous sites and empty lymph into the sub-capsular sinus. The lymph now percolates slowly inward through the cortex, paracortex, and medulla, allowing phagocytic cells and dendritic cells to trap pathogens and antigens carried by the lymph.

The lymph then is drained into a single large lymph vessel called efferent lymphatic vessel that carries the lymph to the thoracic duct, which empties into a large vein in the neck.



2. Spleen:

The spleen, which is about 5 inches long and 200 g in weight in adults, is an ovoid encapsulated, and the largest secondary or peripheral lymphoid organ. Spleen is specialized for trapping blood-borne antigens and is present high in the left abdominal cavity and being encapsulated, its capsule extends a number of projections, 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 (Fig. 42.4). The red pulp consists of a network of sinusoids populated by large number of erythrocytes (red blood cells) and macrophages and few lymphocytes.

In fact, red pulp is the region where old and defective erythrocytes are destroyed and eliminated. The white pulp consists of the branches of the splenic artery that make a periarteriolar lymphoid sheath (PALS) populated heavily by T-lymphocytes.

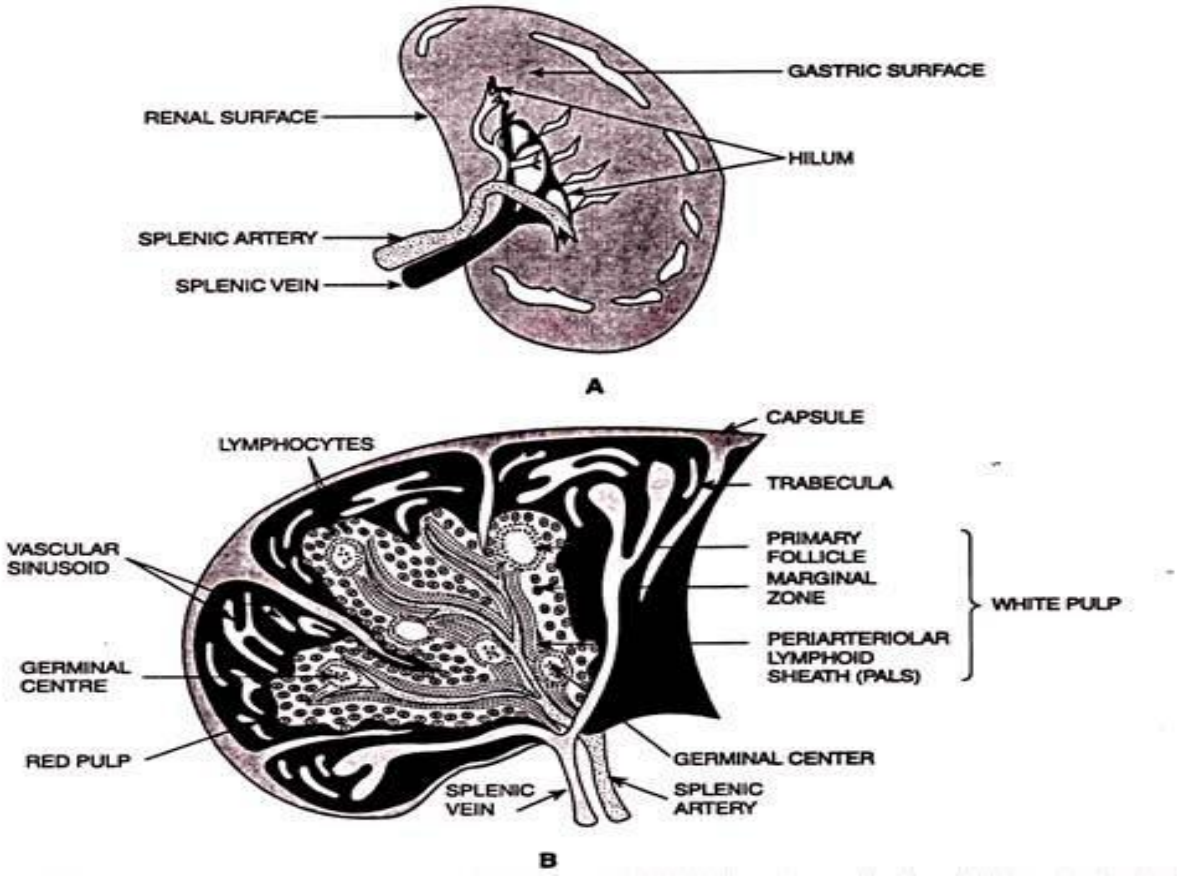
Periarteriolar lymphoid sheath (PALS) is attached with primary lymphoid follicles that are rich in B-lymphocytes. The marginal zone separating the red pulp from white pulp is populated by lymphocytes and macrophages.

When the blood-borne antigens enter the spleen the B- and T-lymphocytes present in periarteriolar lymphoid sheath (PALS) are initially activated. Here interdigitating dendritic cells capture antigen and present it combined with class II MHC molecules

(major histocompatibility molecules) to T_H cells (T helper cells). Once activated, these T_H cells can then activate B- lymphocytes (B-cells).

The activated B-lymphocytes, together with some T_H cells then migrate to primary follicles in the marginal zone. When the primary follicles are challenged by antigen, they differentiate into characteristic secondary follicles.

The latter contain germinal centres (similar to those occurring in lymph nodes) where rapidly dividing B-lymphocytes and plasma cells are surrounded by dense clusters of concentrically arranged lymphocytes.



3. Mucosal-Associated Lymphoid Tissue (MALT):

The mucous membranes lining the alimentary, respiratory, and genitourinary systems have a very large combined surface area (about 400 m^2; nearly the size of a basketball court), which is constantly exposed to numerous antigens and is the major site of entry for most pathogens.

These vulnerable membrane surfaces possess a group of organized lymphoid tissues which defend it from pathogens and antigens. The group of organized lymphoid tissues is known collectively as mucosal-associated lymphoid tissue (MALT).

There are several types of MALT; the most studied one is the gut-associated lymphoid tissue (GALT) which includes tonsils, Peyer's patch, appendix, and loosely organised clusters of lymphoid cells in the lamina propria of intestinal villi.

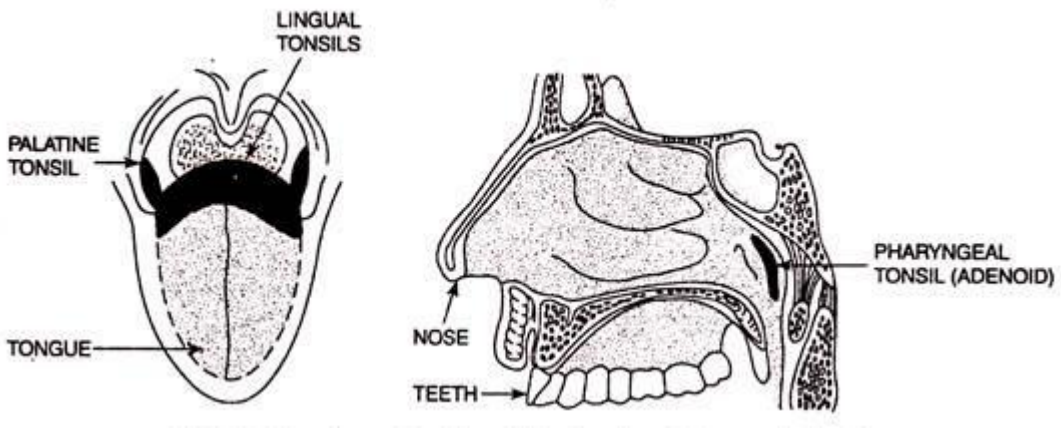
Mucosal-associated lymphoid, tissue (MALT) is functionally very significant in immune system of the body because of the presence of large number of antibody-producing plasma cells in it. The number of plasma cells in MALT for exceeds that of the total of the number of plasma cells present in spleen, lymph nodes, and bone marrow.

(i) Tonsils:

There are three groups of tonsil present at three different locations: palatine, lingual, and pharyngeal (adenoids). Palatine group of tonsils occur at the sides of the back of the mouth; lingual in the basal region of the tongue; and pharyngeal (adenoids) in the roof of the nasopharynx.

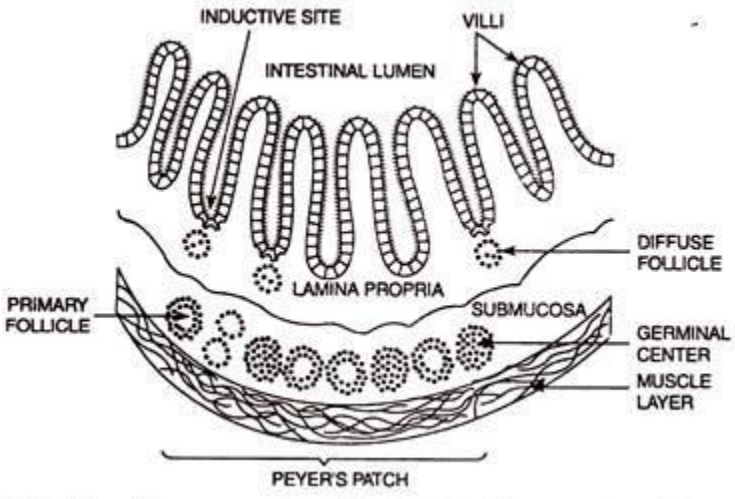
All the aforesaid tonsil groups are nodule-like and consist of a meshwork of reticular cells and fibres interspersed with lymphocytes, macrophages, granulocytes, and mast cells.

The B-lymphocytes are organised into follicles and germinal centres. The germinal centres are surrounded by regions showing T-lymphocyte activity. However, the tonsils protect against antigens that enter through the nasal and oral epithelial routes.



(ii) Peyer's Patch:

Peyer's patches occur in the sub-mucosal layer present beneath the lamina propria lying under the epithelial layer of intestinal villi. Each Peyer's patch is a nodule of 30-40 lymphoid follicles. Like lymphoid follicles in other sites, those that compose Peyer's patches can develop into secondary follicles with germinal centres.



(iii) Lamina Propria:

Lamina propria occurs under the epithelial layer of intestinal villi. It is populated with large number of plasma cells, macrophages, activated T helper cells (activated T_H cells) in loose clusters. More than 15,000 lymphoid follicles have beer, reported within the lamina propria of a healthy child.

UNIT III

Antibody-Mediated (Humoral) Immunity

The antibody-mediated or humoral immunity is that where the B-lymphocytes synthesize antibodies response to the detection of antigens and these antibodies counteract with those antigens. Antibody-mediated immunity is often referred to as humoral immunity because the antibody molecules flow extracellularly through the blood and other vital body fluids which are called humors in Greek.

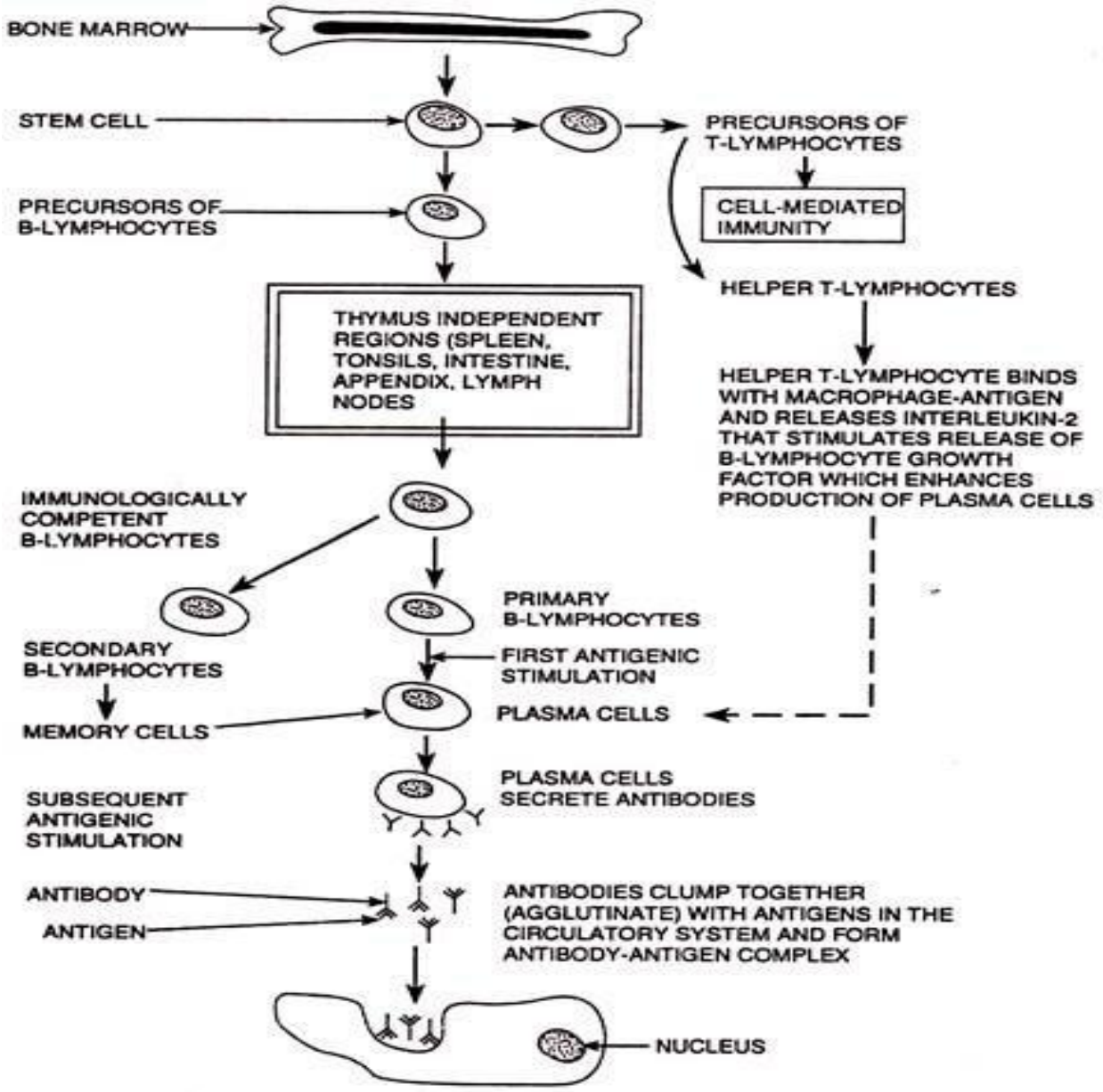
The precursors of B-lymphocytes, which originate from the stem cells of bone marrow, processed within thymus-independent tissues of the lymphatic system (the spleen, tonsils, intestine, appendix, and lymph nodes) and become immunologically competent. In response to antigenic stimulation, the immunologically competent B-lymphocytes convert into two different cell populations primary B-lymphocytes and secondary 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 antigens, perhaps years later. However, these are the plasma cells which secrete antibodies.

Once thought to be totally independent system, the conversion of immunologically competent B- lymphocytes into antibody producing plasma cells is cooperated by helper T-lymphocytes (helper T-cells). An antigen with at-least two different antigenic determinants first attaches on the surface of a macrophage cell which then migrates to lymphatic tissues and interact with helper T-lymphocyte.

Once the helper T-lymphocyte binds with the antigen 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 in turn enhances the division of immunologically competent B-lymphocytes and their conversion into plasma cells.

However, the antibodies secreted by plasma cells clump together (agglutinate) with antigens present in body's circulatory system forming antibody-antigen-complexes which are up-taken by scavenger white blood cells. The diagrammatic representation of the process of antibody-mediated (humoral) immunity is given below-



Antigens

Definition:

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

Chemical Nature:

The antigens are mostly the conjugated proteins like lipoproteins, glycoproteins and nucleoproteins.

Structure:

Antigenic determinants or epitopes (Gk. epi – upon, topos- place) are components of antigen. Each antigen carries many epitopes. Each Y-shaped antibody molecule has at least two binding sites that can attach to a specific epitope on an antigen. An antibody can also bind to identical epitopes of two different cells at the same time which can cause neighbouring cells to aggregate. Antigens combine with the antibody. The combination is very much like the lock and key analogy.

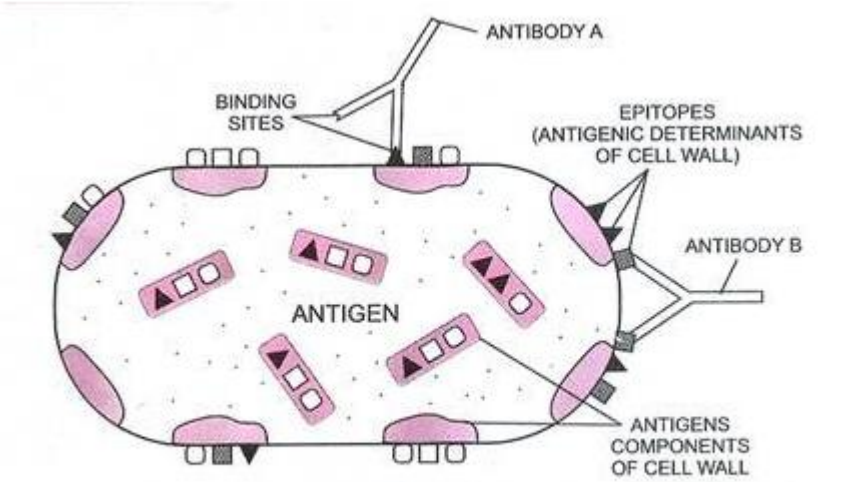


Diagram showing an antigen with epitopes (antigenic determinants). Two attached antibodies are also shown.\

Types:

Based upon the ability of antigens to carry out their functions, antigens are of two types: complete antigens and incomplete antigens (haptens). A complete antigen is able to induce antibody formation and produce a specific and observable reaction with the antibody so produced.

Haptens (Gr. hapten to grasp; partial antigens) are substances which are incapable of inducing antibody formation by themselves but can be capable of inducing antibodies on combining with larger molecules (normally proteins) which serve as carriers.

Antigens which are present on the body's own cells are called the autoantigens or self-antigens. The antigens on the non-self-cells are known as foreign antigens or non-self-antigens.

H antigen:

Red blood corpuscles of all ABO blood groups possess a common antigen, the H antigen, which is a precursor for the formation of A and B antigens. Due to universal distribution, H antigen is not ordinarily important in grouping or blood transfusion. However, Bhende et al (1952) from Mumbai reported a very rare example in which A and B antigens and H antigens were absent from the red blood corpuscles. This is known as Bombay or Oh blood group. Such individuals will have anti A, anti B and anti H antibodies. Therefore, they can accept the blood only from their own group.

Antigen Presenting Cells (APCs):

The cells that can engulf antigen and present fragments to T cells are called antigen presenting cells (APCs).

There are three types of antigen presenting cells in the body: macrophages, dendritic cells and B cells.

1. Macrophages:

Macrophages are usually found in a resting state. Their phagocytic capabilities are greatly increased when they are stimulated to become activated macrophages. The macrophages are present along with lymphocytes in almost all the lymphoid tissues, e.g., monocytes as blood macrophages and histocytes as tissue macrophages.

2. Dendritic Cells:

These cells are characterized by long cytoplasmic processes. Their primary role is to function as highly effective antigen-trapping and antigen presenting cells. These cells are nonphagocytic in nature. They are found in lymph nodes, spleen, thymus and skin. The different types of dendritic cells are:

(i) Langerhans's dendritic cells in epidermis of skin which trap the organisms coming in contact with body surface.

(ii) Dendritic cells in spleen, which trap the antigen in blood.

(iii) Follicular dendritic cells in lymph nodes which trap the antigen in the lymph.

Thus, macrophages and dendritic cells play an important role in the trapping and presentation of antigens to T and B cells to initiate the immune response.

Steinman was awarded Nobel Prize (2011) for his discovery of the dendritic cell and its role in adaptive immunity.

3. B-cells:

B-cells express on their surface intra-membrane immunoglobulin (Ig) molecules that function as B cell antigen receptors. Since all the receptors on a single B cell are identical, each B cell can bind only one antigen. This makes them much more efficient antigen-presenting cells than macrophages, which must ingest any foreign material that comes their way.

Immunoglobulins

Antibodies or Immunoglobulins are **globular proteins** present in the serum and tissue fluids. They are produced by the **plasma cells** (B-cells) and are used in the **immune system** of the body to neutralize pathogenic microbes or other toxic foreign components.

Antibodies play a very crucial role in the immune system of an organism. Antibodies bind to definite molecules of microbes called antigens with high affinity and specificity. This enables our immune system to detect foreign organisms such as invading pathogens, of its products and initiate the mechanism to eliminate these foreign particles. The production of antibodies by the plasma cells is also stimulated by the antigens.

Classification of immunoglobulins

The immunoglobulins constitute about 20 – 25% of the total serum proteins. Based on the Physiochemical and Antigenic differences, the immunoglobulins are classified into **FIVE** categories. These immunoglobulins variants are called as **Isotypes**. The five isotypes or classes of the immunoglobulins are given below.

(1). *Immunoglobulin-G (IgG)*

(2). *Immunoglobulin-M (IgM)*

(3). *Immunoglobulin-A (IgA)*

(4). *Immunoglobulin-E (IgE)*

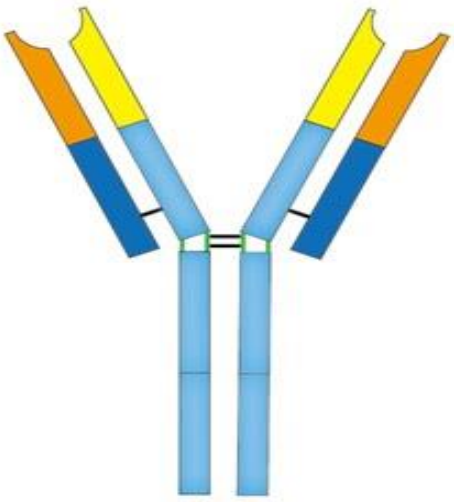
(5). *Immunoglobulin D (IgD)*

Structure of Immunoglobulins

The basic unit of a single immunoglobulin consists of **four linear** polypeptide chains.

These peptide chains are named as two identical **Heavy Chains** and two identical **Light Chains**.

The heavy chains are long and heavy with a molecular weight of 50 – 70 kDa.



'Y' Shaped of an Antibody

The light chains are smaller and lighter in weight with a molecular weight of 25 kDa.

The heavy chains are designated as '**H**' and the light chains are designated as '**L**'

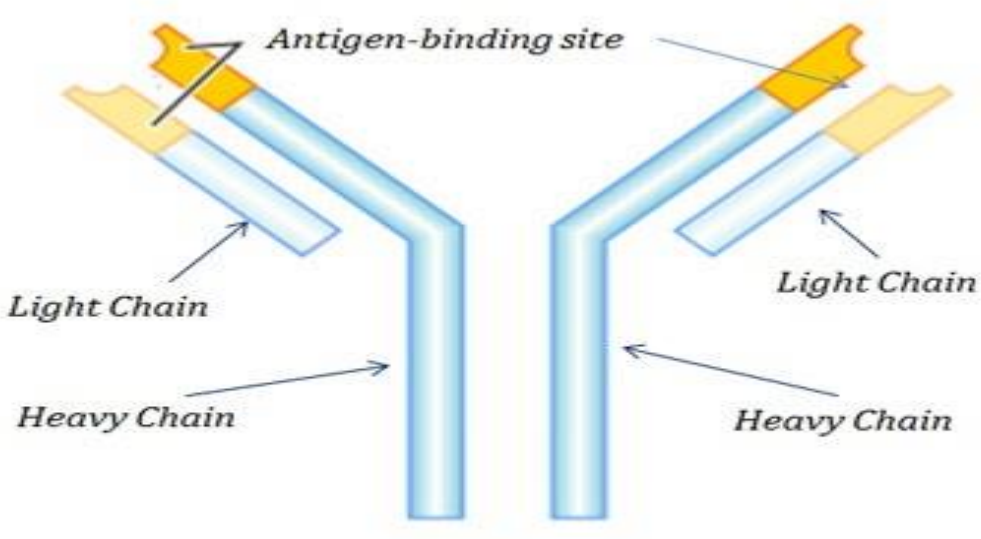
Since an immunoglobulin contain two heavy (H) chains and two light (L) chains, they are together represented as H_2L_2.

H_2L_2 is the basic structural unit of any class (isotypes) of immunoglobulins.

Both H chains and L chains are connected through disulphide

Some antibodies are very complex as in Immunoglobulin M (IgM) which is a pentamer. In such case, the basic structural units will be H_2L_2 and they are multiplied in 'n' times $(H_2L_2)_n$.

The H chain and L chains are inter-connected and oriented in such ways that the individual immunoglobulin unit (H2L2) appears in the shape of '**Y**' or '**T**'.



Heavy (H) and Light (L) Chains in an Antibody

Heavy Chain of Immunoglobulins

As we mentioned above, each immunoglobulin is with two Heavy (H) chains.

Each heavy chain is 420 – 440 amino acids long with free '**N**'and '**C**' terminals.

The two heavy chains are covalently connected to each other through 1 to 5 disulphides (S-S) bonds.

Each heavy chain is bound to a light (L) chain with a single disulphide bond and many non-covalent interactions such as salt bridges, hydrogen bonds and hydrophobic interactions.

The binding of heavy chains with the light chain creates a heterodimer (HL).

The interaction between two such heterodimers through disulphide bonds, hydrogen bonds and hydrophobic interaction create a tetramer (HL)2 or H2L2.

The H2L2 is thus the basic structure of an immunoglobulin.

The heavy chains are structurally distinct for each class (isotypes) of immunoglobulins.

They differ in their size, amino acid sequence, antigenicity, and the carbohydrate content.

For example, the heavy chains of Immunoglobulin M (IgM) contains mu (μ), IgG contains gamma (γ), IgA contains alpha (α), IgE contains epsilon (ε) and IgD contains delta (δ) chains.

Light Chain of Immunoglobulins

As the name suggests, the light (L) chains are lighter than the heavy chains in their molecular weight.

Each light chain consists of 220 to 240 amino acids with free 'N' and 'C' terminals.

Each light chain is attached to the heavy chain by a single disulphide bond and many non-covalent interactions.

The light chains are structurally and chemically similar in all classes of immunoglobulins.

There are two types of light chains, named as *kappa* (κ) and *lambda* (λ) chains.

The kappa and lambda chain differ in their amino acid sequence in their constant regions (see below for constant and variable region of the immunoglobulin).

Each immunoglobulin is with either two kappa chains or with two lambda chains, never both.

In humans, 60% of the light chains are kappa and 40% is lambda.

Variable and Constant regions of Heavy and Light Chains

Each polypeptide chain (H and L chain) of an immunoglobulin possesses two terminal (end) regions designated as N-terminal (amino terminal) and C-terminal (carboxy terminal).

In each chain, the amino terminal region is called Variable region (V region) and the carboxy terminal is called Constant region (C region).

Both the 'H' chain and 'L' chains contain V and C regions.

The V and C region consists of repeating units of structural units called **Domains**.

The variable region of the heavy chain is called '**VH**' region and the constant region of heavy chain is called '**CH**' region

Similarly, the variable and constant regions of light chains are designated as '**VL**' and '**CL**' respectively.

The sequencing studies of the variable region of the heavy chains revealed the existence of five different categories of immunoglobulins designated as μ, δ, γ, ε and α.

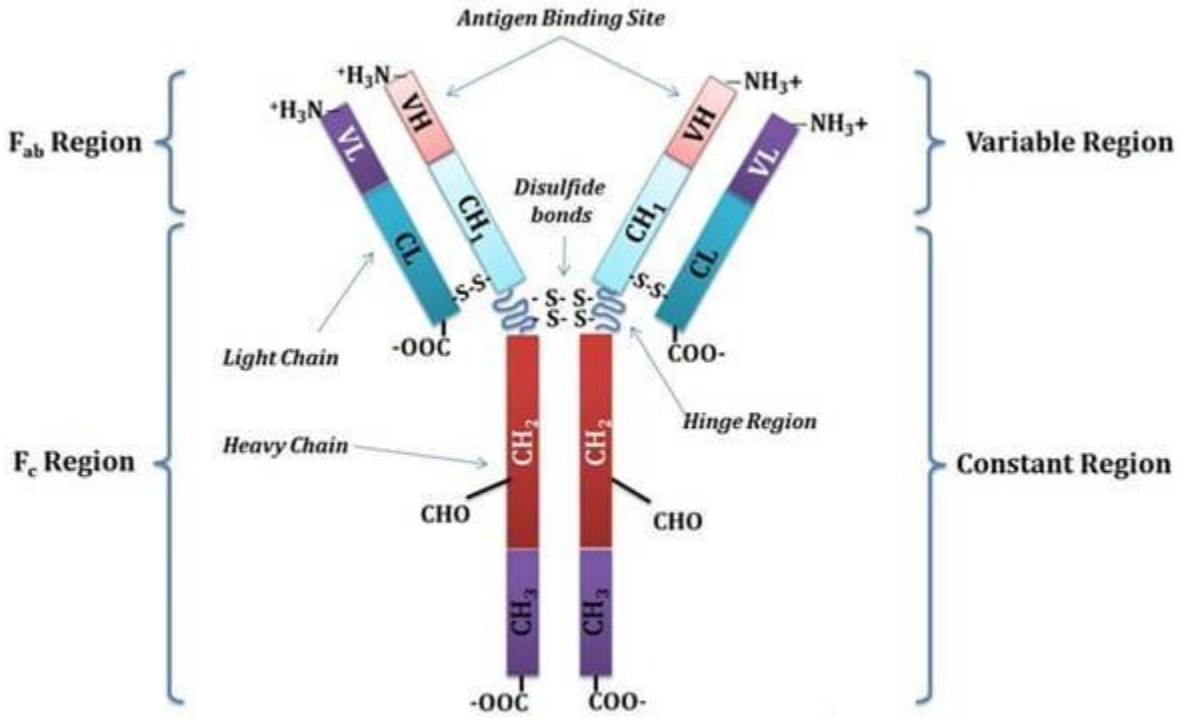
Each of these five different heavy chains is called an isotype.

The sequence of the heavy chain molecule determines the class of that antibody – IgG (γ), IgM (μ), IgA (α), IgE (ε) and IgD (δ).

Each heavy chain contains one variable region (VH) and three or four constant (CH) regions.

IgG and IgA have three constant regions in the heavy chains, designated as CH1, CH2 and CH3.

The heavy chains IgM and IgE have four constant regions designated as CH1, CH2, CH3 and CH4.



Variable (V) and Constant (C) Regions of an Antibody

Each light chain consists of one variable (VL) and one constant (CL) region.

The variable region is different in each class of immunoglobulins.

The variable region of both H chain and L chains consist of 100 – 110 amino acids.

The variable regions of both heavy and light chains consist of three highly variable regions called **Hyper Variable Regions**.

The antigen binding site of immunoglobulin (called ***Fab***) is located in this hyper-variable region.

The 'Fab' region consists of a short stretch of only 5 – 10 amino acids.

The hyper variable region is responsible for the high specificity of immunoglobulins towards specific antigens.

All immunoglobulins are glycoproteins (proteins conjugated with oligosaccharide units).

The site of attachment of **carbohydrates** in antibodies is restricted to the constant regions.

The exact role of carbohydrate regions in the immunoglobulins is not fully known.

They may be helping in increasing the solubility of antibodies in the body fluids.

The constant (C) region consists of two basic amino acid sequences.

The '**Fc**' fragment is located in the constant region of the heavy chain.

The Fc region crystallizes under low ionic conditions.

The constant regions of the heavy chains have much biological significance such as activation of complement, binding of cell surface receptors and placental transfer.

The constant region of the light chain has no biological functions.

Hinge Region of Immunoglobulin

The hinge region is a highly flexible amino acid stretch present in between the CH1 and CH2 region of heavy chains of some immunoglobulin isotypes (IgG, IgA and IgD).

The hinge region is absent in IgM and IgE.

The heavy chains of IgM and IgE possess additional **hinge-like** domain with approximately 110 amino acids.

The hinge region connects the two heavy chains with many disulphide bonds.

The hinge region is rich in cysteine and proline amino acids.

The number of disulphide bonds in the hinge region varies with different immunoglobulin isotypes.

UNIT IV

Cell-Mediated Immune System (CMIS) or T-Cell Immunity:

A healthy person has about a trillion lymphocytes. Lymphocytes are of two types: T lymphocytes or T cells and B lymphocytes or B cells. As we know both types of lymphocytes and other cells of the immune system are produced in the bone marrow. The process of production of cells of immune system in the bone marrow is called haematopoiesis.

Because T lymphocytes (T cells) mature in the thymus, this immunity is also called T- cell immunity.

The T-cells play two important functions—effector and regulatory.

The effector function includes cytolysis (destruction of cells by immune processes) of cells infected with microbes and tumour cells and lymphokine production. The regulatory functions are either to increase or to suppress other lymphocytes and accessory cells.

Structural organization of MHC complex

Definition

The term histo-compatible refers to the individuals who the same tissues i.e. identical twins. This term is used to determine how identical two unrelated individuals are. In case of two histo-compatible individuals, a tissue or organ from a donor (the person giving the organ or tissue) that will not be rejected by the recipient (the patient in whom the tissue or organ is transplanted).

Thus, histocompatibility is the property of having the same or mostly the same alleles of a set of genes called the ‘major histocompatibility complex’. These genes are expressed in most tissues as antigens to which the immune system makes antibodies.

Major histocompatibility complex (MHC) is a tightly linked cluster of genes present on chromosome 6 in humans (and chromosome 17 in mice) which encodes the MHC proteins. The MHC proteins are present on plasma membrane of almost all human tissue/cells. The MHC proteins participate in intercellular recognition and antigen presentation to T lymphocytes.

Generally, a group of linked MHC genes is inherited as a unit from parents. These linked groups are called haplotypes. MHC genes are polymorphic {i.e. there are a large number of alleles for each gene). Also, they are polygenic (i.e. there are a number of different MHC genes). Human MHC molecules are also called human leucocyte antigens (HLA).

In the mid-1930s Peter Gorer (England) established the concept of rejection of foreign tissue due to an immune response to cell surface molecules. This gave the birth to the study of histocompatibility antigens. He identified four types of genes (I to IV) which encode blood cell antigens.

During 1950 George Snell (U.S.A.) pioneered the concept that antigens encoded by the genes took part in the rejection of transplanted tumours. He called these genes as histocompatibility genes. For this work Snell was awarded the Nobel Prize in 1980.

Classes of MHC Molecules:

The MHC genes are organized into three classes I, II and III which express three classes of molecules Classes I, II and III, respectively table. Classes I MHC genes consists of A, B and C gene loci. They secrete glycoproteins which are referred to as Class I MHC molecule. Glycoproteins are expressed on the surface of about all nucleated cells. Class I MHC molecules present the peptide antigens to T_C cells.

Class	Class II			Class III			Class I	
Regions	DP	DQ	DR	C_4, C_2 and BDF		B	C	A
Gene Product	DP αβ	DQ αβ	DR αβ	C Proteins	TNF-α TNF-β	HLA-B	HLA-C	HLA-C

The human Class I MHC gene spans about 2,000 kb (about 20 genes) at the telomeric end of the HLA complex, whereas the Class II MHC genes (about 1,000 kb) are located at the centromeric end of HLA. Class III genes (flanked by about 10,000 kb long) located between the two genes.

The DP, DQ and DR region of Class II MHC genes in humans encode the Class II MHC molecules called glycoproteins. They are expressed on antigen presenting cells such as macrophages, dendric cells and B cells, and present the processed antigenic peptides to T_H cells. Class II molecules have specialised function in the immune response.

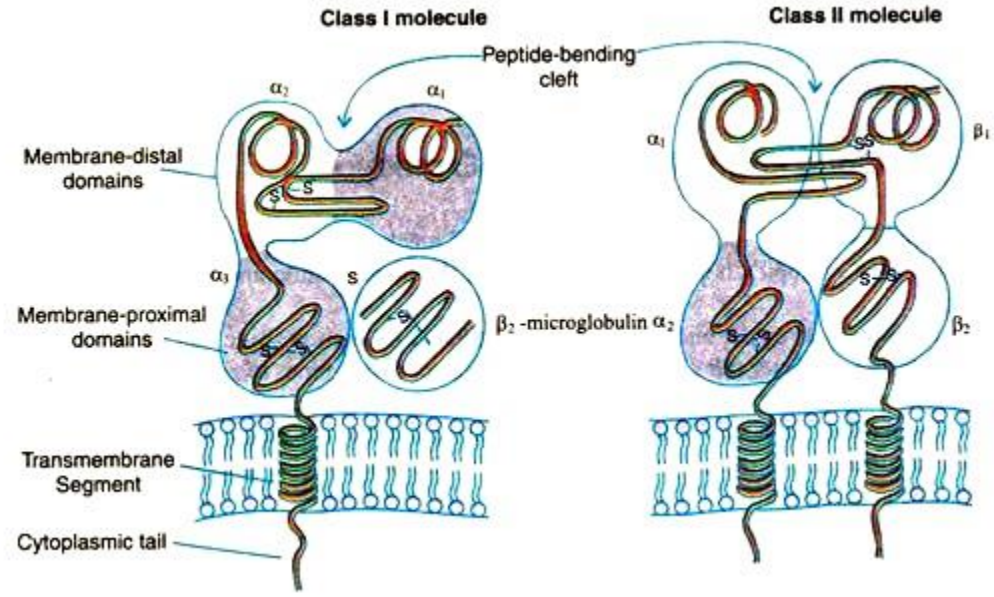
Both Class I and Class II molecules have common structural features. They have role in antigen processing. In addition, the Class III MHC gene is flanked by Class I and Class II regions and encodes molecules critical to immune function. Class III MHC molecules consist of complement components C4, C2, BF, inflammatory cytokines, including tumour necrosis factor (TNF) and heat shock proteins.

Structure of MHC Molecules:

The Class I molecule is a trans-membrane glycoprotein consisting of two chains: a-chain or heavy chain (of 42 KD molecular weight) non-covalently associated with a light chain called β_2-micro-globulin (molecular weight 12 KD).

The α-chain is organized into three extracellular domains (α_1, α_2, α_3) and a 'trans-membrane segment' (hydrophobic) followed by a short stretch of hydrophilic 'cytoplasmic tail' (Fig. 22.20A). These are encoded by A, B and C regions of HLA complex and expressed on the surface of plasma membrane of almost all cells except erythrocytes.

B_2-micro-globulin molecule is expressed by different chromosomes. Association of the α-chain with B_2-micro-globulin is must for expression of Class I molecules on cell membrane. The α_1 and α_2 form the antigenic-binding cleft located on top of surfaces of molecule.



Class II MHC molecules are also trans-membrane glycoprotein encoded by separate MHC genes. They contain two different α and β chains of 33 and 28 KD, respectively. These two chains are associated non-covalently (Fig. 22.20B).

Further, both chains fold to give two domains (β_1 and β_2 domains in other domain), one is membrane proximal domain and the second is membrane-distal domain. Like Class I MHC molecules, the class II molecules also contain trans-segment and a cytoplasmic anchor segment. Each chain of Class II molecule contains two external domains (α_1 and a_2 in one chain) and β_1 and β_2 domains in other chain.

Function of MHC Molecules:

MHC provides both cell mediated and humoral immune responses, while antibodies react only – with antigens, and most of the T cells recognise antigen only when it gets combined with an MHC molecule. Hence, MHC molecules act as antigen-presenting structure.

The MHC partly determines the response of an individual to antigens of infectious microorganisms. Therefore, it is implicated in susceptibility to disease and in the development of autoimmunity. Recently, it has been explained that the natural killer cells express receptors for MHC Class I antigens. The receptor-MHC interaction result in inhibition/activation.

Both Class I and Class II MHC molecules present the processed endogenous antigen to CD8 T cells. Class II molecules present the processed exogenous antigen to CD4 T cells. Class I molecules identifies mostly all the cells of the body as 'shelf. Also, they induce the production of antibodies which introduced into host with different Class I molecules. This is the basis for MHC typing when a patient is to undergo for antigen transplantation.

Class II molecules comprise of the D group of MHC. They stimulate the production of antibodies. But they are required for T cell communication with macrophage and B cells. Part of T cells receptor recognises Class II molecules on the adjacent cell before cytokine secretion by T cells. This is necessary for immune response.

Both Class I and Class II molecules recognise the microorganisms. They are also involved in the susceptibility of an individual to a specific non-infectious diseases e.g. multiple sclerosis, acute glomerulonephritis, tuberculoid leprosy, paralytic poliomyelitis, etc. The Class III molecules (e.g. C_2, C_{4a} and C_{4b}) participate in the classical pathway and factor B in the alternate pathway of the immune responses.

Gene Regulation of MHC Expression:

Regulation of MHC genes has not been studied much. Understanding of complete genomic map of the MHC complex hopefully will accelerate the identification and coding, and regulatory sequences. Transcriptional regulation of the MHC is mediated by both positive and negative elements e.g. MHC II trans-activator (cll TA) and transcription factor (RFX) binds to promoter region of Class II MHC gene. Any error in these transcription factor causes a type of disease in lymphocytes. Expression of MHC molecules is also regulated by many kinds of cytokines. Interferons and tumour necrosis factor increase the expression of Class I molecules on cells. Interferon-gamma induces the expression of cIITA.

Expression of MHC decreases after infection by certain viruses e.g. hepatitis B virus, and adenovirus 12, cytomegalovirus, etc. Adenovirus 12 causes a decrease in transcription of the transporter genes (TAP1 and TAP2). When these genes are blocked, class I molecules foil to assemble with β_2-micro-globulin.

Decreased level of Class I molecules promotes viral infection. Expression of Class II molecules by B cells is down-regulated by INF-gamma. Corticosteroids and prostaglandins decrease the expression of Class II molecules.

Antigen processing and presentation

Antigen processing and presentation is the process by which protein antigen is ingested by an antigen-presenting cell (APC), partially digested into peptide fragments and then displayed on the surface of the APC associated with an antigen-presenting molecule such as MHC class I or MHC class II, for recognition by certain lymphocytes such as T cells.

Antigen-presenting cells are a kind of cells capable of processing antigens and presenting antigen peptides to T cells in the form of antigen peptide-MHC molecular complexes, and play an important role in immune recognition, immune response and immune regulation of the body.

The process of antigen processing and presentation mainly involves two processes. The first is antigen processing, which means that APC degrades and processes the exogenous antigen or the endogenous antigen produced by cytoplasm itself to a polypeptide fragment of a certain size, so that the antigen peptide is suitable for binding to the MHC molecule, and the complex is combined. Finally, the complex can be transported to the cell surface. The process is called antigen processing. Followed by antigen presentation, the antigen peptide-MHC molecular complex expressed on the surface of APC is recognized by T cells, thereby presenting antigen peptides to T cells and inducing T cell activation. Since T cells can only recognize antigenic peptides presented by APC, antigen processing and presentation play an important role in immune responses, especially cellular immunity.

Depending on the source of the presented antigen and MHC molecule, antigen-presenting cells can be divided into two categories:

1. APCs present exogenous antigens by MHC class II molecules.

2. APCs present endogenous antigens by MHC class I molecules. The former APC can take up, process exogenous antigens and present antigen peptides to $CD4^+$ T cells in the form of antigen peptide-MHC class II molecules complexes.

Both dendritic cells and monocytes/macrophages can express multiple receptors to recognize pathogenic microorganisms or antigen-antibody complexes, and take up antigenic substances through pinocytosis, phagocytosis and receptor-mediated endocytosis.

B cells take up antigen mainly by B cell receptors concentrate and internalize antigen or pinocytosis. DC will present the antigen to the initial T cells, promoting T cell activation, proliferation and differentiation. Monocytes/macrophages and B cells present antigens to T helper cells (Th), and T helper cells further activate macrophages and B cells and improve cellular and humoral immunity.

According to its cellular function, it can be divided into professional APC and non-professional APC.

- The former includes dendritic cells (DC), monocytes/macrophages, and B lymphocytes. They constitutively express MHC class II molecules, costimulatory molecules and adhesion molecules, and have the function of directly ingesting, processing and presenting antigens.
- Non-professional APCs mainly include endothelial cells, epithelial cells and fibroblasts. In non-professional APCs, MHC class II molecules are generally not expressed or under-expressed, but can be induced by inflammation or certain cytokines, and their ability to process and present antigens is weak.

For APCs, endogenous antigens are presented by MHC class I molecules, which degrade and process the endogenous antigen, and then present the antigen peptides to $CD8^+$ T cells in the form of antigen peptide-MHCI type molecular complexes. The endogenous antigen is mainly derived from an intracellular parasitic pathogen or mutational protein.

- **The process of antigen processing and presentation**

Depending on the nature and source of the antigen, APC processes and presents antigens through four pathways:

- The MHC class I molecular pathway
- The MHC class II molecular pathway
- The non-canonical antigen presentation pathway
- The CD1 molecular pathway of lipid antigens.

The MHC class I molecular pathway is an endogenous antigen presenting pathway. Endogenous antigens bind to transporter associated with antigen processing (TAP) after ubiquitinated endogenous antigens are degraded by the proteasome. TAP selectively transports 8-12 amino acid antigen peptides to the endoplasmic reticulum (ER).

The antigen polypeptide binds to the antigen-binding peptide groove of the MHC class I molecule assembled by the endoplasmic reticulum to form an antigen peptide-MHC I-like molecular complex. The complex is then transported to the cell membrane via the Golgi apparatus for recognition by $CD8^+$ T cells, thereby completing antigen presentation process.

The MHC class II molecular pathway is an exogenous antigen presenting pathway. The exogenous antigen is recognized and taken up by APC, and then APC will form endosomes or phagocytose lysosomes. After the endosomes or phagocytose lysosomes fuse with MHC class II compartment (MIIC), the antigen is degraded into a polypeptide.

- MIIC is the nonamer of MHCII/Ii, in which the Ii chain is degraded in MIIC, leaving class II-associated invariant chain peptide (CLIP) in the antigenic peptide binding groove of MHC class II molecules. Under the action of HLA-DM, the CLIP of the antigen peptide binding groove is replaced by the antigen peptide to form a stable antigen peptide-MHC class II molecules complex, which is then transported to the surface of the APC membrane for recognition by $CD4^+$ T cells, thereby completing the antigen presentation process.

Cross-presentation means that APC can uptake and process exogenous antigens to $CD8^+$ T cells via MHC class I molecular pathways or present endogenous antigens to $CD4^+$ T cells via MHC class II molecular pathways. The CD1 molecular pathway for lipid antigens is a pathway that specifically targets lipid antigens, mainly through the CD1 molecule on the surface of APC.

Monoclonal Antibodies

Monoclonal antibody (MAb) is a single type of antibody that is directed against a specific antigenic determinant (epitope). It was a dream of scientists to produce MAbs for different antigens. In the early years, animals were immunized against a specific antigen, B-lymphocytes were isolated and cultured in vitro for producing MAbs. This approach was not successful since culturing normal B-lymphocytes is difficult, and the synthesis of MAb was short-lived and very limited.

It is interesting that immortal monoclonal antibody producing cells do exist in nature. They are found in the patients suffering from a disease called multiple myeloma (a cancer of B- lymphocytes). It was in 1975. George Kohler and Cesar Milstein (Nobel Prize, 1984) achieved large scale production of MAbs. They could successfully hybridize antibody—producing B-lymphocytes with myeloma cells in vitro and create a hybridoma.

The result is that the artificially immortalized B-lymphocytes can multiply indefinitely in vitro and produce MAbs. The hybridoma cells possess the growth and multiplying properties of myeloma cells but secrete antibody of B-lymphocytes. The production of monoclonal antibodies by the hybrid cells is referred to as hybridoma technology.

Antibodies or immunoglobulins are protein molecules produced by a specialized group of cells called B-lymphocytes (plasma cells) in mammals. The structures, characteristics and various other aspects of immunoglobulin's (Igs) are described elsewhere. Antibodies are a part of the defense system to protect the body against the invading foreign substances namely antigens.

Each antigen has specific antigen determinants (epitopes) located on it. The antibodies have complementary determining regions (CDRs) which are mainly responsible for the antibody specificity. In response to an antigen (with several different epitopes), B-lymphocytes gear up and produce many different antibodies. These types of antibodies which can react with the same antigen are designated as polyclonal antibodies.

The polyclonal antibody production is variable and is dependent on factors such as epitopes, response to immunity etc. Due to lack of specificity and heterogenic nature, there are several limitations on the utility of polyclonal antibodies for therapeutic and diagnostic purposes.

Monoclonal antibody (MAb) is a single type of antibody that is directed against a specific antigenic determinant (epitope). It was a dream of scientists to produce MAbs for different antigens. In the early years, animals were immunized against a specific antigen, B-lymphocytes were isolated and cultured in vitro for producing MAbs. This approach was not successful since culturing normal B-lymphocytes is difficult, and the synthesis of MAb was short-lived and very limited.

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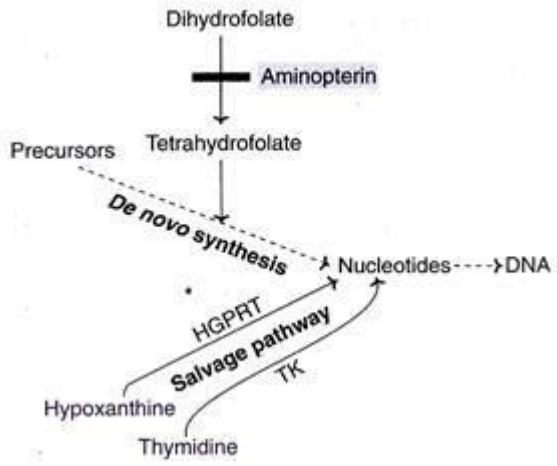
Principle for Creation of Hybridoma Cells:

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—de novo synthesis and salvage pathway.



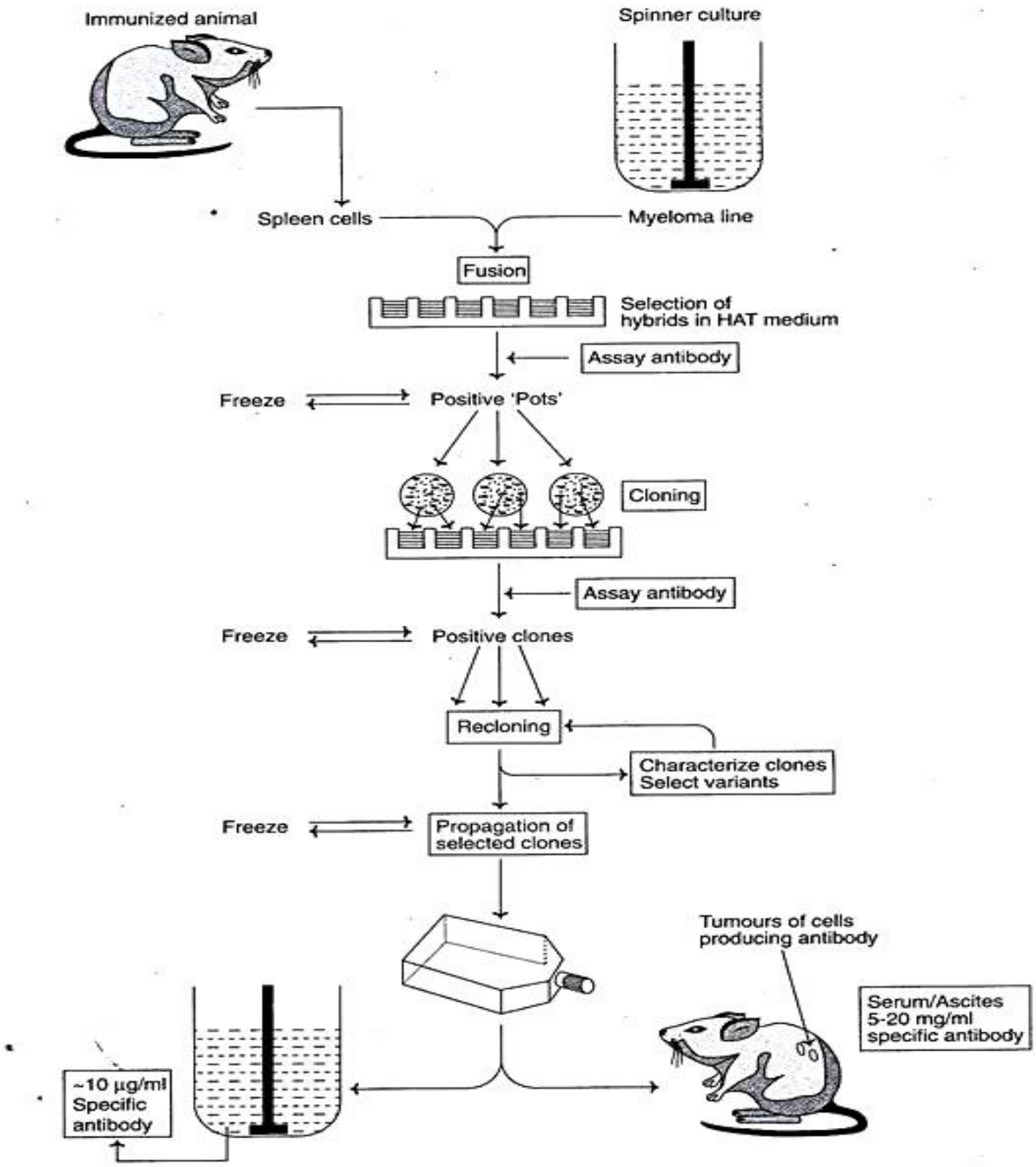
The de novo synthesis of nucleotides requires tetrahydrofolate which is formed from dihydrofolate. The formation of tetrahydrofolate (and therefore nucleotides) can be blocked by the inhibitor aminopterin. The salvage pathway involves the direct conversion of purines and pyrimidines into the corresponding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

It converts hypoxanthine and guanine respectively to inosine monophosphate and guanosine monophosphate. Thymidine kinase (TK), involved in the salvage pathway of pyrimidine'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 de novo synthesis of purine nucleotides (Note : Salvage pathway is not operative due to lack of HGPRT). Thus, cells lacking HGPRT, grown in HAT medium die.

The hybridoma cells possess the ability of myeloma cells to grow in vitro 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 antigen. The antigen, along with an adjuvant like Freund's complete or incomplete adjuvant is injected subcutaneously (adjuvants are non-specific potentiators of specific immune responses). The injections at multiple sites are repeated several times.

This enables increased stimulation of B-lymphocytes which are responding to the antigen. Three days prior to killing of the animal, a final dose of antigen is intravenously administered. The immune-stimulated cells for synthesis of antibodies have grown maximally by this approach. The concentration of the desired antibodies is assayed in the serum of the animal at frequent intervals during the course of immunization.

When the serum concentration of the antibodies 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 (a 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 (the principle described above), only the hybridoma cells grow, while the rest will slowly disappear. This happens in 7-10 days of culture. Selection of a single antibody 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 on an average one cell each. These cells, when grown in a regular culture medium, produce the desired antibody.

4. Screening the Products:

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

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

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-limiting dilution method and soft agar method.

Limiting dilution method:

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

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 MAbs.

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 MAbs are important. The cells (and MAbs) 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.

Human Monoclonal Antibodies:

The monoclonal antibodies produced by using mice are quite suitable for in vitro use. However, their administration to humans is associated with immunological complications, as they are foreign to human body. Production of human monoclonal antibodies is preferred. However, it is difficult to produce human MAbs by conventional hybridoma technology.

The following are the major limitations:

i. For ethical reasons, humans cannot be immunized against antigens.

ii. The fused human lymphocyte-mouse myeloma cells are very unstable.

iii. There are no suitable myeloma cells in humans that can replace mouse myeloma cells.

Advantages of Monoclonal Antibodies:

Monoclonal antibodies truly represent a homogeneous state of a single molecular species. Each MAb is specific to a given antigenic determinant. This is in contrast to the conventional antiserum that contains polyclonal antibodies. The wide range of applications of MAbs is described later.

Limitations of Monoclonal Antibodies:

Hybridoma technology is laborious and time consuming. MAbs are produced against a single antigenic determinant; therefore, they cannot differentiate the molecule as a

whole. Sometimes, they may be incapable of distinguishing groups of different molecules also.

The presence of retroviruses as a part of the mammalian chromosomes is a common occurrence. Mice used in MAb production carry several viruses (adenovirus, hepatic virus, retrovirus, reovirus, cytomegalovirus, thymic virus). The presence of some of these viruses has been detected in the hybridomas.

This poses a great danger, since there is no guarantee that MAb produced is totally virus-free, despite the purification. For this reason, US Food and Drug Administration insists that MAb for human use should be totally free from all pathogenic organisms, including viruses.

ELISA (Enzyme-linked immune sorbent assay)

ELISA is an antigen antibody reaction. In 1971, ELISA was introduced by Peter Perlmann and Eva Engvall at Stockholm University in Sweden. It is a common laboratory technique which is usually used to measure the concentration of antibodies or antigens 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 antibody 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), para nitrophenyl phosphate (for alkaline phosphatase) are used which are hydrolysed by above enzymes to give coloured end product.

Principle

ELISAs 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 antigen or antibody coated on to the solid surface. After some time, the plate is

washed to remove serum and unbound antibodies or antigens with a series of wash buffer. To detect the bound antibodies or antigens, 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 antibodies 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 antigens or antibodies present in the given sample. The intensity of colour/ optical density is measured at 450nm. The intensity of the colour gives an indication of the amount of antigen or antibody.

Types of ELISA

Frequently there are 3 types of ELISA on the basis of binding structure between the Antibody and Antigen.

1. Indirect ELISA
2. Sandwich ELISA
3. Competitive ELISA

1. Indirect ELISA

Antibody can be detected or quantitatively determined by indirect ELISA. In this technique, antigen is coated on the microtiter well. Serum or some other sample containing primary antibody is added to the microtiter well and allowed to react with the coated antigen. Any free primary antibody is washed away and the bound antibody to the antigen is detected by adding an enzyme conjugated secondary antibody that binds to the primary antibody. Unbound secondary antibody 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.

Procedure of Indirect ELISA

1. Coat the micro titer plate wells with antigen.
2. Block all unbound sites to prevent false positive results.

3. Add sample containing antibody (e.g. rabbit monoclonal antibody) to the wells and incubate the plate at 37°c.
4. Wash the plate, so that unbound antibody is removed.
5. Add secondary antibody conjugated to an enzyme (e.g. anti- mouse IgG).
6. Wash the plate, so that unbound enzyme-linked antibodies are removed.
7. Add substrate which is converted by the enzyme to produce a coloured product.
8. Reaction of a substrate with the enzyme to produce a coloured product.

Advantages

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

Disadvantages

- Cross-reactivity might occur with the secondary antibody, resulting in nonspecific signal.
- An extra incubation step is required in the procedure.

2. Sandwich ELISA

Antigen can be detected by sandwich ELISA. In this technique, antibody is coated on the microtiter well. A sample containing antigen is added to the well and allowed to react with the antibody attached to the well, forming antigen-antibody complex. After the well is washed, a second enzyme-linked antibody specific for a different epitope on the antigen is added and allowed to react with the bound antigen. Then

after unbound secondary antibody is removed by washing. Finally substrate is added to the plate which is hydrolyzed by enzyme to form coloured products.

Procedure of sandwich ELISA

1. Prepare a surface to which a known quantity of antibody is bound.
2. Add the antigen-containing sample to the plate and incubate the plate at 37°c.
3. Wash the plate, so that unbound antigen is removed.
4. Add the enzyme-linked antibodies which are also specific to the antigen and then incubate at 37°c.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme to produce a coloured product.
7. Reaction of a substrate with the enzyme to produce a coloured product.

Advantages

- High specificity, since two antibodies are used the antigen is specifically captured and detected.
- Suitable for complex samples, since the antigen 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 antigen in a sample.

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

Procedure of competitive ELISA

1. Antibody is incubated with sample containing antigen.

2. Antigen-antibody complex are added to the microtitre well which are pre-coated with the antigen.
3. Wash the plate to remove unbound antibody.
4. Enzyme linked secondary antibody which is specific to the primary antibody is added.
5. Wash the plate, so that unbound enzyme-linked antibodies are removed.
6. Add substrate which is converted by the enzyme into a fluorescent signal.

Advantages

- High specificity, since two antibodies are used.
- High sensitivity, since both direct and indirect detection methods can be used.
- Suitable for complex samples, since the antigen does not require purification prior to measurement.

Application of ELISA

1. Presence of antigen or the presence of antibody in a sample can be evaluated.
2. Determination of serum antibody 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 e.g. HIV, bird flu, common, colds, cholera, STD etc.

Complement system

The complement system is a part of the immune system, consists of a series of proteins that interact with one another in a highly regulated manner, in order to eliminate pathogens. It helps antibodies and phagocytic cells to clear pathogens and damaged cells; promote inflammation and attack pathogen's plasma membrane. Proteins that take part in the complement system are called complements that collectively work as a **biological cascade**; the sequence of reactions, each being the catalyst for the next.

Jules Bordet (1895) identified complements as heat-sensitive components in the blood, bearing non-specific antimicrobial activity.

Complements are soluble proteins and glycoproteins mostly **produced by hepatocytes**. More than 20 types of complements are present in serum, found circulating normally in human body in inactive forms *(called as zymogens or proenzymes)*. Complement activation is triggered by an antibody when it is bound to the antigen. It can also be triggered by some components of innate immunity. Thus, the **complement system works in both innate and acquired immunity.**

Complements are activated only during inflammatory reactions. During the inflammation, more amount of complements reaches to the interstitial area of the infected tissue through dilated blood vessels, which are then activated by proteolytic cleavage; this exposes the active site of the complements.

Complements are mainly **denoted by the capital letter *C*** with numbers; like, C1, C2, C3, and so on. Some have only alphabet, like, *B*, *D*. Some are simply represented by names, like, homologous restriction factor.

C1 has three sub-units; C1q, C1r and C1s. C2-C5 have two components, *a* and *b*. **Larger subunits are denoted by *b*** whereas the **smaller are denoted by *a*** *(except C2a, which is larger than C2b).*

The complement activation occurs via three pathways, which are:

1. **Classical pathway**
2. **Alternative pathway**
3. **Lectin pathway (or mannose binding lectin pathway)**

The early step of complement system varies in different pathways. However, all the pathways form enzyme complexes; C3 convertase, which cleaves C3 into C3a and C3b; and the C5 convertase, which cleaves C5 into C5a and C5b.C3b, thus formed, binds C3 convertase to form C5 convertase.

C5 convertase, generated by the alternative, classical, or lectin pathway, initiates the activation of late components of the complement system to form membrane attack complex (MAC) and ultimately kills the pathogen.

This occurs through three pathways; **Classical pathway**, activated by antigen-antibody reaction, **Alternative pathway**, activated on microbial cell surfaces, and **Mannose binding Lectin pathway**, activated by a plasma lectin that binds to mannose residues on microbes.

1. **Classical Pathway**

The classical pathway begins with the formation of antigen-antibody complex (immune complex). When an antigen enters the body, the antibody (IgM/IgG) binds to it. This induces conformational changes in the Fc portion of the antibody which exposes a binding site for C1 protein. Hence, the antibody activates the complement system only when bound to an antigen.

C1 is a large, multimeric, protein complex composed of one molecule of C1q and two molecules each of C1r and C1s subunits. C1q binds to the antigen bound antibody (Fc portion). C1r and C1s are proteases which help to cleave C4 and C2.

The immune complex bound to C1 calls another protein C4 which is cleaved into C4a and C4b. C4a goes away whereas activated C4b attaches to the target surface near C1q. Now, C4b attracts C2 which is also cleaved into C2a and C2b. C2a binds C4b forming the C4b2a complex whereas C2b goes away. The active C4bC2a activates C3. The C4b2a complex is also known as **C3 convertase** as this converts C3 into an active form by separating C3a and C3b. One molecule of C4b2a can cleave a large number of C3 molecules. C3b binds to the microbial surface or to the convertase itself.

C3b when binds to C3 convertase forms C4bC2aC3b (**C5 convertase**) which activates C5.

C5 convertase cleaves C5 into C5a and C5b. C5a diffuses away but C5b is stabilized by binding C6. Then C5bC6 binds to C7. C5bC6C7 complex is then inserted into the phospholipid bilayer of the cell membrane which further binds C8. These all (C5b678) activate C9 to form a macromolecular structure called the **membrane attack complex (MAC)**. This makes hole in the bacterium, as a result, the intracellular contents leak out and unwanted substances get in. Thus, the cell cannot

maintain its osmotic stability and the lysis occurs by an influx of water and loss of electrolytes.

This is more effective in Gram negative bacteria than in Gram positive bacteria because MAC formation is easy in the outer membrane in Gram negatives whereas it is difficult in the rigid thick layer of peptidoglycan in Gram positives.

Some of the C3b molecules do not associate with C4b2a; instead these molecules coat immune complexes or microbial cell surfaces and work as opsonin. This process is called opsonization in which opsonin molecule binds one side to the particulate matter i.e. in bacteria, tumour cell, RBC and on the other side they bind to the receptor of phagocytic cell(like, neutrophils and macrophages) which enhance the process of phagocytosis.

Smaller complement subunits diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors.

2. **Alternative Pathway**

Unlike classical pathway, alternative pathway, does not require Ag-Ab complex for the initiation of complement pathway. It is initiated by cell surface constituents that are foreign to the host. These surface molecules may be **lipopolysaccharide** etc.

When a bacterium enters the host body, as a result of inflammation, complements reach towards the site, where C3 molecules directly touch antigen and become active. In this pathway, serum C3 containing an unstable thioester bond undergoes slow spontaneous hydrolysis to yield C3a and C3b. C3b binds the surface of foreign cell and then binds to another serum protein called factor B. Now the factor B exposes the site which serves as the substrate for enzymatically active serum protein D. Then factor D cleaves B into Ba and Bb forming C3 convertase (C3bBb). C3 convertase then forms C5 convertase which ultimately forms a MAC as in classical pathway.

3. **Mannose binding Lectin (MBL) Pathway**

Some bacteria can activate complement system without having antibody and endotoxin. This occurs through MBL pathway which is activated when circulating

lectin (MBL) binds to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms. Microorganisms inducing MBL pathway are bacteria, such as Salmonella, Listeria, and Neisseria strains, some fungi and some viruses including HIV-1. MBL is an acute phase protein and its concentration increases during inflammation. The lectin recognizes and binds the carbohydrate of the target cell which then activates complements.

MBL pathway resembles classical pathway as it proceeds through the action of C4 and C2 to produce activated proteins of the complement system. MBL works same as C1q which it resembles in structure.

After the MBL binds to carbohydrate residues on the surface of a cell or pathogen, two components, MASP-1 and MASP-2 bind to MBL. MASP stands for MBL-associated serine proteases. Two proteases form a tetrameric complex similar to the one formed by C1r and C1s and cleaves C4 and C2 forming C3 convertase. The process now continues to form of C5 convertase and the MAC as in classical pathway.

Functions of Complements

Function of Complement Pathway

Some major functions of complements are:

1. **Opsonization and phagocytosis**

C3b, bound to immune complex or coated on the surface of pathogen, activate phagocytic cells. These proteins bind to specific receptors on the phagocytic cells to get engulfed.

2. **Cell lysis**

Membrane attack complex formed by C5b6789 components ruptures the microbial cell surface which kills the cell.

3. **Chemotaxis**

Complement fragments attract neutrophils and macrophages to the area where the antigen is present. These cell surfaces have receptors for complements, like C5a, C3a, thus, run towards the site of inflammation, i.e. chemotaxis.

4. **Activation of mast cells and basophils and enhancement of inflammation**

The proteolytic complement fragments, C5a, C4a, and C3a induce acute inflammation by activating mast cells and neutrophils. All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine. These peptides are also called anaphylatoxins because the mast cell reactions they trigger are characteristic of anaphylaxis. Binding to specific complement receptors on cells of the immune system, they trigger specific cell functions, inflammation, and secretion of immunoregulatory molecules.

5. **Production of antibodies**

B cells have receptor for C3b. When C3b binds to B-cell, it secretes more antibodies. Thus, C3b is also an antibody producing amplifiers which converts it into an effective defence mechanism to destroy invading microorganism.

6. **Immune clearance**

The complement system removes immune complexes from the circulation and deposits them in the spleen and liver. Thus, it acts as anti-inflammatory function. Complement proteins promote the solubilization of these complexes and their clearance by phagocytes.

Complement regulation

The complement system has the potential to be extremely damaging to host tissues; hence regulatory mechanisms are required to restrict the complement pathway. Various plasma and cell membrane proteins regulate complement activation by inhibiting different steps in the cascade.

The membrane of most mammalian cells has a high level of sialic acid, which contributes to the inactivation of complements.

Complement related Diseases

Diseases associated with complements can be due to the deficiencies in any of the protein components or in regulatory components.

Some examples of complement protein deficiencies are:

Deficiency of C2 and C4 can cause systemic lupus erythematosus; deficiency of C3 and factor D can cause pyogenic bacterial infection; and deficiency of C5-C9 (or MAC deficiency) may lead to the Neisserial infections like, gonorrhea and meningitis.

Deficiencies of regulatory proteins lead to too much activation of complements in wrong time and place which leads to unwanted inflammation and cell lysis. Pyogenic bacterial infection and glomerulonephritis are the results of such deficiencies.

Mutations in the complement regulators factors may lead to atypical hemolytic uremic syndrome, age-related macular degeneration, hereditary angioedema, etc.

Complement system can also be stimulated by abnormal stimuli, like persistent microbes, antibody against self-antigens or immune complexes deposited in tissues. Even when the system is properly regulated and activated, it can cause significant tissue damage.

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