Immunology

Cells and Organs of Immune System

Lokesh Chandra Mishra
Department of Zoology
University of Delhi
Delhi-110007

Correspondence address:
Room No: 119
Department of Zoology
University of Delhi
Delhi-110007

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Introduction

Immune system is the system of specialized cells and organs that protects an organism from any attack or invasion by any foreign body called antigen and responds accordingly. Immune system is made up of a complex and vital network of cells and organs. All these organs are called **lymphoid organs** because they are concerned with the growth, development, and deployment of lymphocytes. Thus, immune system is also sometimes called the **lymphoid system** and the tissues associated are called lymphoid organs. Depending upon the function, these can be of two types: primary lymphoid organs and secondary lymphoid organs. The Primary lymphoid organs include thymus and bone marrow, where maturation of lymphocytes takes place, whereas secondary lymphoid organs include lymph nodes, spleen, and various mucosal associated lymphoid tissues (MALT), which trap antigen and provide sites for interaction between mature lymphocytes and antigen.

The blood vessels and lymphatic vessels are important parts of the lymphoid system as they interconnect the organs of the immune system and carry the lymphocytes to and from the different areas in the body causing systemic immunity. On the other hand mucosal associated lymphoid tissues give mucosal immunity.

In this chapter, first part deals with the formation of blood cells, second and third part describes about the cells and organs of the immune system respectively.

Hematopoiesis

Like every living organism has limited life span and die with time, the body cells do age and die after a period of time. Blood cells also have a limited life span and to maintain homeostasis and for the normal functioning of the body, blood cells need to be renewed continuously. Hematopoiesis is a highly orchestrated process of blood cell development involving a complex interplay between the intrinsic genetic processes of blood cells and their environment. This interplay determines whether hematopoietic stem cells (HSCs), progenitors, and mature blood cells remain quiescent, proliferate, differentiate, self-renew, or undergo apoptosis. In simple words, it is the process of formation of red cells and leukocytes from pluripotent stem cells (HSCs). Hematopoiesis begins from the early embryonic development and continues throughout life. But there is remarkable shift in the hematopoietic site during the embryonic stage becoming stable by birth. In the first week of embryonic development, hematopoiesis takes place in the yolk-sac stem cells where it differentiates into primitive erythroid cells containing fetal hemoglobin. By the third month of gestation, the stem cells changes its location from yolk-sac stem cells to fetal liver and then to spleen where it remains till the seventh month of gestation. Towards the end of gestation, the site of hematopoiesis shifts to the bone marrow and finally by birth hematopoiesis ceases within the liver and spleen (Table 1).

The bone marrow is the definitive hematopoietic tissue containing the pluripotent hematopoietic stem cells that differentiates along all the blood cell lineages and also has the capacity of self-renewal. Hematopoiesis takes place in the extravascular compartment. The extravascular compartment consists of a stroma of reticular connective tissue and a parenchyma of developing blood cells. The stromal cells constitute fat cells, endothelial cells, fibroblasts, and macrophages. The high activity of the bone marrow is demonstrated by its daily output of mature blood cells: 2.5 billion erythrocytes, 2.5 billion platelets, 50-100 billion granulocytes. The numbers of lymphocytes and monocytes is also very large. Bone marrow is the site for

other important activities in addition to hematopoiesis. These include the removal of aged and defective erythrocytes and the differentiation of B lymphocytes. It is also the site of numerous plasma cells.

Table 1- Sites of hematopoiesis

Age of Animal	Hematopoiesis site
Embryo	yolk sac then liver
3rd to 7th month	Spleen
7th month	marrow cavity - erythrocytes
Birth	mostly bone marrow; spleen and liver when needed
Birth to maturity	number of active sites in bone marrow decreases but retain ability for hematopoiesis
Adult	Bone marrow of skull, ribs, sternum, vertebral column, pelvis, proximal ends of femurs

The stem cells are very few in number occurring with the frequency of one stem cell per 10^4 bone marrow cells but owing to their inherent capacity of self renewal it maintains a steady state level in the adult body. For the growth and maturation of hematopoietic cells of the adult bone marrow, some non-hematopoietic cells provide a suitable meshwork of stromal cells that support the growth and differentiation of these hematopoietic cells. The stromal cells are a group of various cells that include fat cells, endothelial cells, fibroblast and macrophages. These stromal cells act by providing a suitable **hematopoietic-inducing microenvironment** consisting of **cellular matrix** and either **membrane-bound or diffusible growth factors.** The differentiating stem cells acquire membrane deformability that allow them to pass through the sinusoidal walls and finally to enter into circulation.

During hematopoiesis, pluripotent stem cells may multiply to produce more uripotent stem cells, thus ensuring the steady and lasting supply of stem cells. Some of the pluripotent stem cells differentiate into precursor cells that are at least partially committed to become one type of mature blood cell (Fig. 1). This differentiation may take place along one of the two pathways- **lymphoid stem cell** or a **myeloid stem cell** (Fig. 1). Both the stem cell differentiates further into **progenitor cell** and thus becomes committed to a cell lineage and the capacity of the self renewal in lost at this stage. The lymphoid stem cell generates T and B progenitor lymphocytes. The myeloid stem cell generates progenitor for various cells that include red blood cells (erythrocytes), the various leukocytes (neutrophils, eosinophils, basophils, monocytes, mast cells) and the platelets. Congenial microenvironment for the progenitor cells is a must for its proliferation and differentiation. The type and the amount of growth factors regulate the differentiation of progenitor cells to give rise to the corresponding type of red or leukocytes.

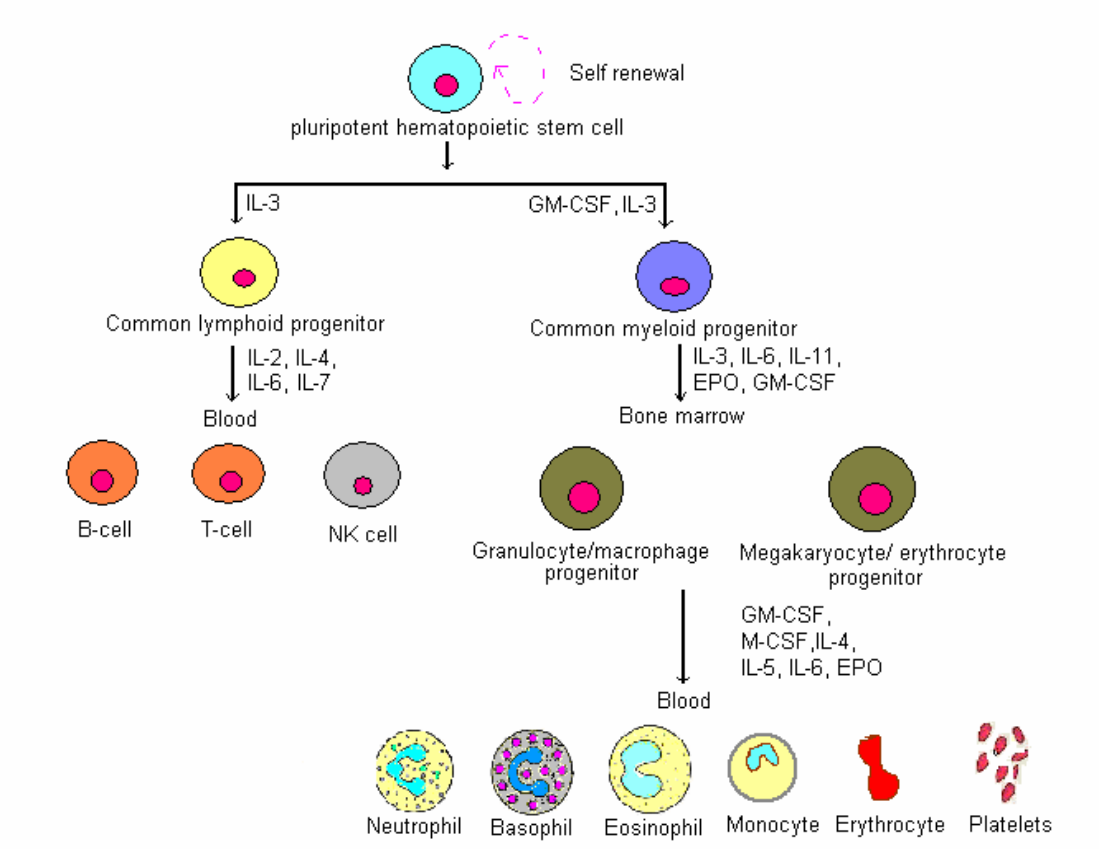

Fig 1. Differentiation pathways of hematopoietic stem cell and their fate

Hematopoietic Growth Factors

Hematopoietic growth factors (HGF) are the group of substances that regulate the proliferation, maturation and activation of the hematopoietic cells for hematopoiesis by interacting with developing immature cells. Development of cell-culture systems that can support the growth and differentiation of lymphoid and myeloid stem cells led to identification of numerous hematopoietic growth factors. Originally, these growth factors were detected in serum or in conditioned medium from in vitro cell cultures. These growth factors required for hematopoiesis exhibit the biological activity at concentrations as low as 10^{-12}. Due to the low physiologic concentrations of these factors, biochemical purification of these was hindered unless a major breakthrough in the form of cloning the gene encoding the hematopoietic growth factors came into practice. In vitro cell culture technique and gene cloning enabled the researcher's to take a leap in the field of identifying the various hematopoietic growth factors and their biological activity involved in hematopoiesis.

Probably the best characterized environmental regulators of hematopoiesis are cytokines. Cytokines are a broad family of proteins that mediate positive and negative control on cellular quiescence, apoptosis, proliferation, and differentiation. Cytokines may also facilitate the interaction between stem cells and elements in the microenvironment. Hematopoietic cytokines are produced by bone-marrow stromal cells, activated T helper (T_H) cells and activated macrophages. Activated macrophages produce some factors like Interleukin 1 (IL-1) and Interleukin 6 (IL-6) which promotes inflammatory responces and fever and promotes innate immunity and elimination of pathogens respectively. Activated macrophages also produce interferon alpha which activates cellular genes, resulting in the production of

proteins that confer an antiviral state on the cell. Table 2 shows the functions of the some of the various hematopoietic cytokines.

Table 2 List of HGF with their functions

HGF	Function
Colony Stimulating Factor(CSF)	Induces the formation of distinct hematopoietic cell lineages
• Multilineage CSF (multi- CSF) or (IL-3)	Acts in the early differentiation stage to induce formation of all the non-lymphoid blood cells (erythrocytes, monocytes, granulocytes and megakaryocytes)
• Granulocyte macrophage CSF (GM-CSF)	Acts at a slightly later stage of differentiation of all the non-lymphoid blood cells
• Macrophage CSF (M-CSF)	Acts at a still later stage to induce the formation of monocytes
• Granulocyte CSF (G-CSF)	Acts at the same stage as that of M-CSF but induces the formation of neutrophils
Erythropoietin (EPO)	Induces terminal erythrocyte development and regulates red blood cell production.

Regulation of Hematopoiesis:

Hematopoiesis needs a regulating mechanism to maintain steady state of the variety of blood cells, yet with enough capability of production of blood cells in an enormous amount of ten-fold to twenty-fold in response to hemorrhage or any infection. Leukocytes rush to the site of infection to generate an inflammatory response to limit the infection and this is accomplished by the production of specific cell lineages to provide the necessary cells for such response. This kind of response is generally regulated by activated T_H cells and activated macrophages by the production of number of cytokines that stimulate proliferation and differentiation of different leukocytes involved in the immune response.

Regulation of hematopoiesis also occurs at the level of cytokine production by the bone-marrow stromal cells. These cells are known to produce GM-CSF, M-CSF, G-CSF, IL-4, IL-6 and IL-7. Multi-CSF (IL-3) is the earliest acting cytokine produced by only the activated T_H cells and in

the bone-marrow cells, some unidentified cytokine is thought be responsible for maintaining the constancy of the levels of the pluripotent stem cells.

Variable concentrations of cytokines or/and differential expression of the receptors for the various cytokines in different lineages results in the production of different hematopoietic lineages. But very little is known about the actual regulation by cytokines in different lineages as the in vitro studies on the stromal-cell matrix which provide a unique microenvironment to the developing hematopoietic cells are lacking and not understood completely. The binding of a CSF to its receptor causes some of the receptors to be internalized by the cell, which serves to down-modulate receptor expression by the cell. The cell becomes progressively less responsive to the CSF with scanty receptors on its membrane resulting in decline in its proliferation. For example, when GM-CSF binds to its receptors it induces the cell to down-modulate the expression of G-CSF and M-CSF receptors as well. This down-modulation causes the lineages bearing these receptors to become less responsive to these CSFs.

Regulation can be also brought about by degradation of a CSF following its binding to a receptor. Binding of M-CSF to its receptors results in degradation of the cytokine. With the increase in the monocytes number, there is a corresponding increase in M-CSF receptors resulting into increased degradation of M-CSF which in turn slows down the further proliferation and differentiation of this lineage as long as the number of monocytes remains high.

Expression of different set of lineage-specific genes at appropriate times and in the correct order is a must for the hematopoiesis to be carried out normally. The proteins specified by these genes form the critical components of regulatory networks that direct the differentiation of the pluripotent stem cells and its descendants. The studies pertaining to the genetic regulation of hematopoiesis is in its infancy as much needs to be done to understand. But, much of what has been known till date comes from the studies done by knocking out targeted genes that blocks the production of protein that is required in some step in hematopoiesis. Many transcription factors have been identified using this technique. Some of these transcription factors affect only a single lineage (example- GATA-1, Ikaros, Oct-2) and others affect multiple lineages (GATA-2, BM11). GATA-2 gene play a vital role in animals as it is involved in the formation of transcription factor GATA-2 which is required for the development of the lymphoid, erythroid and myeloid lineage.

Regulation of hematopoiesis is thus carried out in the following ways:

- At the level of cytokine production by bone-marrow stromal cells,
- At the level of cytokine production by other cell types having hematopoietic activity such as activated T cells and macrophages,
- At the level of receptors for hematopoietically active cytokines in stem cells progenitor cells,
- Removal of cells by apoptosis (discussed in details in the next section); and
- At the genetic level.

Apoptosis

The word ‘apoptosis’ is derived from Greek word (*apo* = from and *ptosis* = falling) meaning ‘falling leaves’ and was first used to describe new form of cell death distinct from necrosis in the early 1970s. This term was coined by John F. Kerr, Andrew H. Wyliie and A. R. Currie in 1972 to differentiate naturally occurring developmental cell death, from the necrosis that results from acute tissue injury (Fig. 2).

To maintain a steady-state level of various hematopoietic cells, along with its constant production, constant removal of blood cells is essential which is accomplished by a process called **programmed cell death.** In programmed cell death, cell undergoes distinctive morphologic changes, together known as **apoptosis** where the cell and its DNA both fragment. A cell undergoing apoptosis shows a pronounced decrease in cell volume and a characteristic morphology that can be seen under a microscope:

1. The cell becomes round as the protein structures conform the cytoskeleton are digested by specialized peptidases, called **caspases**, that have been activated inside the cell besides caspases also play major role in T-cell proliferation and cell differentiation. Initiator caspases (eg. CASP1, CASP2) cleave inactive pro-forms of effector caspases (eg. CASP3, CASP6) which in turn cleave other protein substrates with in the cell resulting in the apoptosis.
2. Chromatin undergoes gradual condensation into compact patches against the nuclear envelope. By this time, the specialized caspases have already advanced in the degradation of nuclear pore proteins and have begun to degrade the lamin that underlies the nuclear envelope. It must be noted that the initial stages of chromatin condensation is also observed in non-apoptotic forms of cell death but this advanced stage, called **pyknosis** is considered a hallmark of apoptosis.
3. The nuclear envelope becomes discontinuous and the DNA inside it gets fragmented (a process referred to as **karyorrhexis**) forming several discrete **chromatin bodies** or **nucleosomal units**.
4. Pronounced plasma blebbing occurs.
5. The cell breaks apart into several vesicles called **apoptotic bodies**, which are then phagocytosed by macrophages to ensure that their intracellular contents of proteolytic and other lytic enzymes, cationic proteins, and oxidizing molecules are not released into the surrounding tissue to avoid any toxin release and subsequently the inflammatory response.

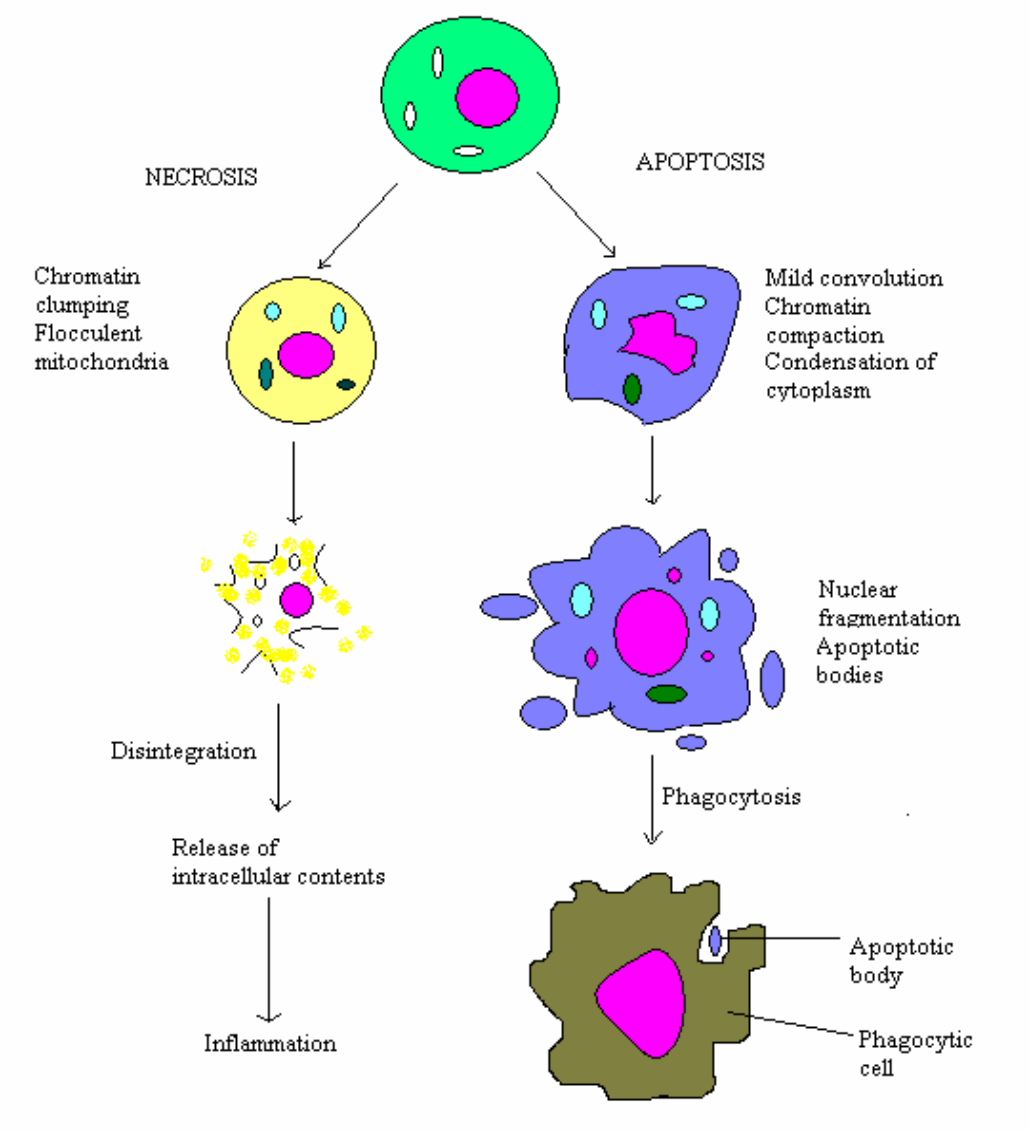

Fig. 2. Comparison of changes during necrosis and apoptosis

Apoptosis markedly differs from **necrosis**, the process of cell death induced by injury to body cells where the cell death results in the release of intracellular contents in the surrounding tissue which are cytotoxic and as a result an inflammatory response develops whereas The main function of apoptosis is to dispose of a cell without causing damage or stress to neighbouring cells. Apoptosis is a desirable activity for cells in a multicellular organism as it removes the old and dying out cells, virally infected cells and most importantly auto-aggressive T-cells to prevent auto-immune conditions.

This programmed cell death of the blood cells produced in the body coupled with its constant production by hematopoiesis maintains a steady-state level of these cells. If at any stage, programmed cell death fails to occur, a leukemic state may develop. Programmed cell death occurs not only in the committed cells but also can occur at the levels of hematopoietic progenitor cells to maintain its level too. For example, when colony stimulating factors are removed, progenitor cells undergo programmed cell death. Too much apopyosis can cause disease like, neurodegradation, autoimmune disease and ischaemia-asssociated injury. On the other hand, too little apoptosis can also cause diseases such as cancer. Since the beginning of the 1990s, research on apoptosis has grown spectacularly. In addition to its importance as a

biological phenomenon, defective apoptotic processes have been implicated in an extensive variety of diseases. Not all form of programmed cell death share the characteristic shapes and sequences of apoptosis, but all types of programmed cell death are highly-regulated processes.

Several genes were identified as being involved in apoptosis by mutational analysis and observations of its development. The expression of these genes regulates the process of apoptosis in leukocytes and other cells. Some gene products induce apoptosis and other gene products inhibit apoptosis. The *bcl-2* (B-cell lymphoma 2) gene, for example is a large family of proteins that inhibits apoptosis. Bcl-2 levels have been found to play an important role in regulating the normal life span of various hematopoietic cell lineages, including lymphocytes. Genes like *bcl-2* and *bcl-*x_L inhibit the apoptosis while *bax* and *bcl-*x_S genes promote the apoptosis. The *fas* gene is responsible for the initiation of the apoptosis. During acute infection, the lymphocyte count increases 4- 15 fold (of the normal 10^{10} lymphocytes). As the immune system can not sustain such an enormous increase in cell numbers for an extended period, the need for the removal of activated lymphocytes arises as soon as the antigenic threats subside. These activated lymphocytes have been found to express lower levels of *bcl-2* and thus more susceptible to induction of apoptosis. Details of some of the genes associated with apoptosis are listed in table 3. Apoptosis is a process of deliberate life relinquishment by an unwanted cell in a multicellular organism. It is the safe mode of disposal of cell corpses and fragments.

CELLS OF IMMUNE SYSTEM

Agranulocytes

The lymphocytes and mononuclear phagocytes (monocytes and macrophages) are referred to as non-granular leukocytes/agranulocytes as their cytoplasm does not contain lysosomal granules in the cytoplasm. Both monocytes and macrophages are active participants in immune and inflammatory response as they have the capacity of phagocytosis. They also ingest dead or defective cells- in particular the blood cells. Lymphocytes are the central cells of the immune system. They are round cells with little cytoplasm, large round nuclei, and chromatin arranged in coarse masses. Lymphocyte possessing the four cardinal features of diversity, specificity, memory and self-nonself recognition makes it central to the immune system. Lymphocytes represent 20-40% of the body's leukocytes and 99% of the cells in the lymph. They are found circulating between blood and lymphoid tissues and can localize in lymphoid organs. The lymphocytes can be broadly divided on the basis of function and cell-membrane components into three populations: B cells, T cells and null cells. All these cells are small, motile, non-phagocytic cells, which cannot be distinguished morphologically. Interaction of lymphocytes with antigens in the presence of cytokines (discussed later in chapter) induces these cells to enter the cell cycle. As they progress through the cell cycle, lymphocytes enlarge into 15μm diameter blast cells, called lymphoblast. Lymphoblasts proliferate and eventually differentiate into effector cells and memory cells.

Table 3 Genes regulating apoptosis and their functions

Gene	Function in apoptosis
Bcl-2	**Inhibits**
bax	**Promotes (opposes *bcl-2)***
Bcl-X_L	**Inhibits**
Bcl-X_S	**Promotes (opposes *bcl-X_L*)**
caspase	**Promotes**
fas	**Initiates**

B lymphocytes

B lymphocytes are so called as they were first studied and identified in the bursa of Fabricius in birds and it turned out to be apt for mammals too as its site of maturation is the bone marrow. As the B-cell matures they expresses membrane bound immunoglobulin or antibody receptors, which serves as receptors for antigen (Fig. 3).

Approximately, a single B cell bears on its surface 1.5 X 10^5 molecules of antibody bearing identical binding site for antigen. Antibodies are glycoproteins consisting of two identical heavy polypeptide chains and two identical light polypeptide chains. The chains are held together by disulfide bonds. The amino terminal ends of each pair of heavy and light chains form a cleft within which antigen binds. When a naïve B cell, which has not encountered antigen, first encounters the antigen specific for the antibody, the cell begins to divide rapidly into **memory B cells** and **effector B cells** called **plasma cells.** Memory B cells, as the name indicates, remains in the circulation for a long time and thus has a longer life-span. It continues to express the same membrane-bound antibody as the original parent naive B cell. Effector cells bring about the effect by secreting large number of antibody but they do not express the membrane-bound antibody. Though the plasma cells have a very short life span of few days but they can secrete more than 2000 molecules of antibody per second. These secreted antibodies are the major effector molecule of the humoral immunity. The secreted antibodies may be one of the five classes of antibody (IgG, IgA, IgM, IgD and IgE). All clonal progeny from a given B cell secrete antibody molecules with the same antigenic specificity.

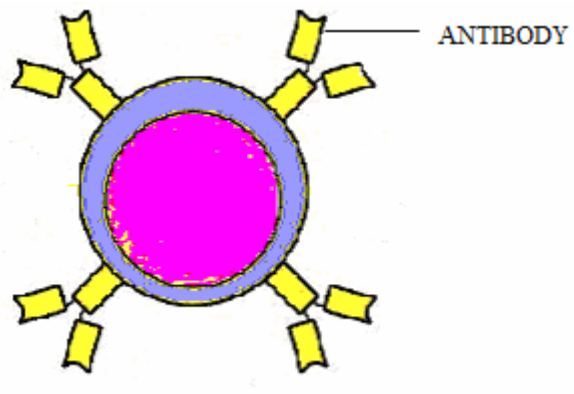

Fig. 3. Structure of a B-lymphocyte

Apart from the antibodies, other molecules are also expressed on the membrane of mature B cells (Fig. 4). These molecules include:

- B220 (CD45)-the earliest marker of the B cell lineage that appears during maturation on the precursor B cell
- Class II MHC molecules permit the B-cells to function as an antigen presenting cell
- CR1 (CD 35) and CR2 (CD21) are the complement receptors and enable B cells to bind to antigen-antibody-complement complexes
- FcγRII (CD 32) receptor specifically binds to the Fc portion of IgG
- CD19 and CD20 have a role in B-cell activation
- B7 is a co-stimulatory molecule that interacts with CD28 on T_H cells

T lymphocytes

T lymphocytes derive their name from their site of maturation in the thymus, the organ where pre-T cells, from the bone marrow, migrate to and mature in. During its maturation, it also expresses a unique antigen binding molecule on its membrane called the **T cell receptor**. T cells play a crucial role in the full expression of immunity by regulating antibody production, cellular immune reactions and killing of altered cells.

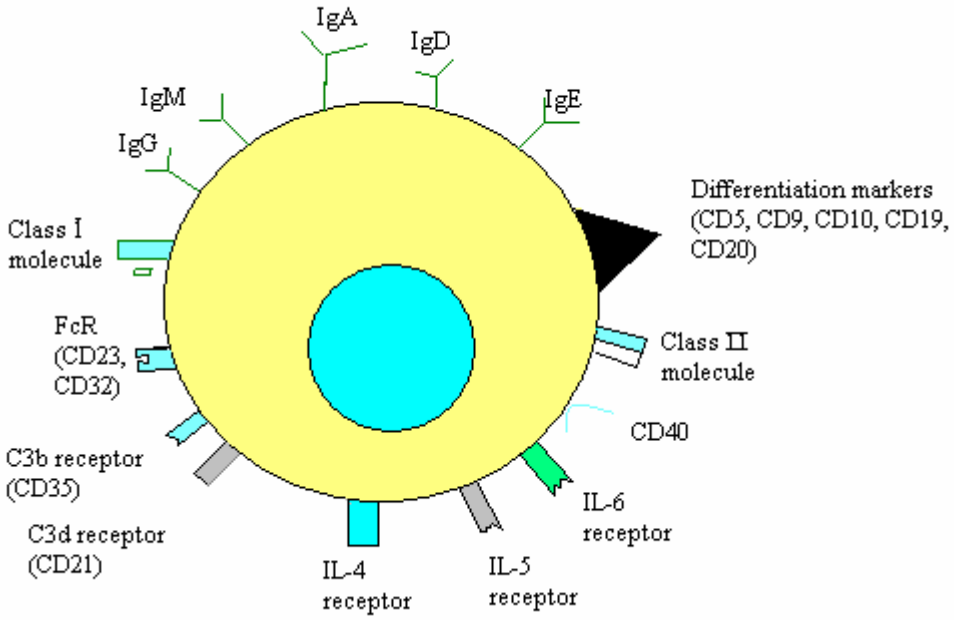

Fig. 4. Structure of a mature B cells with all its surface receptors

Unlike a B-cell, a T-cell can recognize antigens only when it is processed into antigenic peptides and is bound to cell membrane proteins called **major histocompatibility complex (MHC)**. This focusing of T-cell antigen recognition through MHC molecules is known as MHC restriction. The antigenic peptide must be displayed together with MHC molecules on the surface of antigen presenting cells, APCs (B cells, macrophages and dendritic cells) or on virus-infected cells, graft cells or cancer cells. Thus, the T-cells are developed to eliminate such altered self-cells that pose threat to the normal functioning of the body. T cells have two well-defined subpopulations: **T helper cells (T_H) cells** and **T cytotoxic (T_C) cells** (Fig. 5.)**.** Like B cells, T cells subpopulations also expresses the T cell receptor specific for each population. T_H cells and T_C cell expresses $CD4^+$ and $CD8^+$ glycoproteins on their surfaces respectively. Thus the ratio of T_H to T_C cells in a sample can be approximated by assaying the number of $CD4^+$ and $CD8^+$ T cells. This ratio is approximately 2:1 in normal human peripheral blood.

T_H cells get activated when it encounters an antigen complexed with MHC class II molecules presented on an APC. Following its activation, T cell secretes various growth factors known as cytokines which play a central role in activation of B cells, T_C cells and a variety of other cells that participate in immune response. Difference in the pattern of cytokine production elicits different activation of immune response. For example, T_H1 response is designated when T cytotoxic cells and macrophages are activated and T_H2 response designates the activation of B cells. T_H cell maintain memory and are the principal proliferative cells responding to foreign antigen associated with self class II MHC molecules.

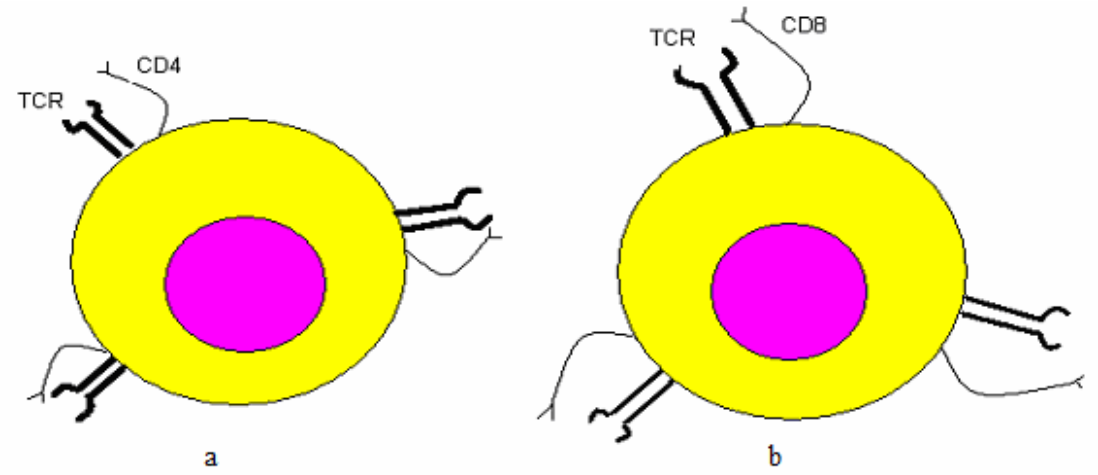

Fig. 5. Structure of a T lymphocytes- a) T_H Cell; and b) T_C Cell

T_C cells get activated when they interact with an antigen complexed with MHC class I molecules presented on an altered self-cells in the presence of appropriate cytokines. Activation results in the proliferation and differentiation of the T_C cells into an effector cells called a **cytotoxic T lymphocytes (CTL)**. CTLs recognize and eliminate altered self cells but secrete few cytokines. T_C cells are generally responsible for killing virus-infected cells, transplanted tissue and cancer cells.

The division of T cells into two sub-populations is not absolute as there is some functional ambiguity but these ambiguities are exceptions and not the rule. For example, $CD4^+$ cells sometimes function as cytotoxic and $CD8^+$ as helpers. Nonetheless, T_H cells are generally considered as being class II restricted and $CD4^+$; T_C cells as being class I restricted and $CD8^+$ restricted. In addition to these glycoproteins, all mature T cells express the following membrane molecules:

- Thy-1, the earliest marker of the T cell lineage expressed during maturation in the thymus
- CD3-a membrane complex associated with the T-cell receptor
- CD28-a receptor for the co-stimulatory B7 molecule present on antigen presenting cells
- CD45-a signal-transduction molecule

Natural killer cells

Natural killer (NK) cells are a small group of lymphocytes present in the peripheral blood that do not express any membrane receptors that distinguish the B- and T-cell lineages. As these cells lack antigen-binding receptors, they lack immunologic specificity and memory. Most members of the Natural killer cells are large, granular lymphocyte (Fig. 6) cells, constituting 5%-10% of the lymphocytes in human peripheral blood. One of the primary effector functions of the NK cells is the production of large amounts of interferon-gamma (IFN-γ) to fight of viral infections.

NK cells are probably best known for their natural ability to kill tumour cells. This is due to interaction between ligands on the tumor cell and a variety of receptors on the NK cell, leading to the release of the NK cell's cytotoxic granules.

NK cell have been shown to express both activating and inhibiting receptors. The extracellular structure of both types of receptors are similar but inside it is very different . When the activating receptors and their associated signal transducing molecules are cross linked by interaction with their ligand, a signal is send in to the cell that activates a series of kinases, eventually leading to activation of the cell's cytotoxic effector function and IFN-γ (production).

The inhibitory receptors have opposite effect. When activating and inhibitory receptors are crosslinked by their ligands on the surface of a potential target cell, the inhibitory signal delivered to the cell override the activating signal, and the target is spared. If only the activating receptor is bound, or if more activating receptors than inhibitory receptor are bound, the target is killed.

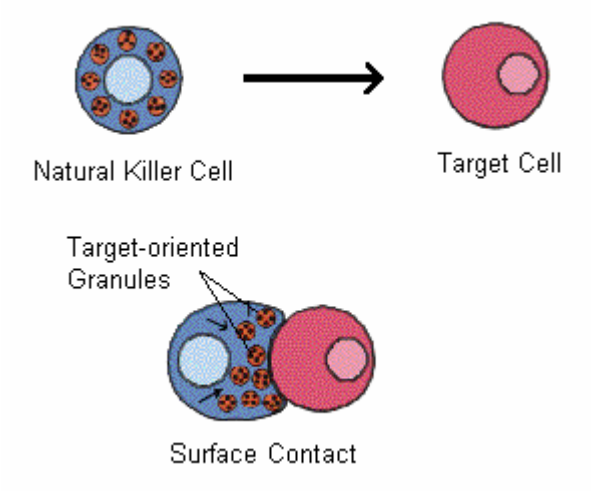

Fig. 6. A natural killer cell interacting with the target cell

The first evidence of natural killer cells came in 1976 when cytotoxic activity was displayed by certain null cells against a wide range of tumor cells even in the absence of prior exposure with the tumor. They are found to play an important role in host defense against tumors. They function as effector cells that directly kill certain tumors such as melanomas, lymphomas and viral-infected cells. NK cells are reported to be acting in two different ways. In some cases, they make a direct membrane contact with the tumor cell in a non-specific antibody independent way. But in some, there are reports on the involvement of surface receptors (CD16) which help in recognition of the F_C region of the IgG molecule and thus acting in an **antibody dependent cell-mediated toxicity (ADCC).** In this pathway, the NK cells bind with the antibodies bound to the surface of tumor cells and subsequently destroy the tumor cells.

Mononuclear phagocytes

The mononuclear phagocytic system consists of monocytes circulating in the blood and macrophages in the tissue. Monocytes constitute 5-8% of WBCs in the blood. They remain in circulation for 8 h, during which time they enlarge and then migrate into the tissue and differentiate into specific tissue macrophage. Tissue macrophages are so called as they are seeded in a variety of guises throughout the body tissues, where they stay for 2-3 months. During the transition of monocytes to macrophages several changes takes place:

- Cell enlarges 5-10 fold
- Intracellular organelle increases in number and complexity
- Cells acquire increased phagocytic ability
- Increased secretion of several soluble factors

Some macrophages remain fixed to the tissues while others remain free and move throughout the tissues by amoeboid movements. Fixed macrophages are named according to their tissue location (Table 4).

Macrophages possess the following characteristics:

- They are mononuclear cells
- Show peroxidase and esterase activity
- Bears specific receptors for antibody and complement
- Show phagocytic abilities
- Remain in a resting state but can be activated by a variety of stimuli
- Possesses varied and prolific secretory activity

Activated macrophages are highly efficient in eliminating pathogens as they exhibit increased phagocytic ability and increased secretion of various cytotoxic proteins, higher expression of class II MHC molecules, thus making them efficient APCs and increased secretion of inflammatory substances (Fig. 7). Phagocytosis of foreign antigen acts as stimulus for activation of macrophages and can be further stimulated by interferon gamma (IFN-γ), the most potent activators of macrophages secreted by activated T_H cells. The activated macrophages are more effective in removing foreign pathogen than resting macrophages, as activated one express high level of Class II MHC molecules for presentation.

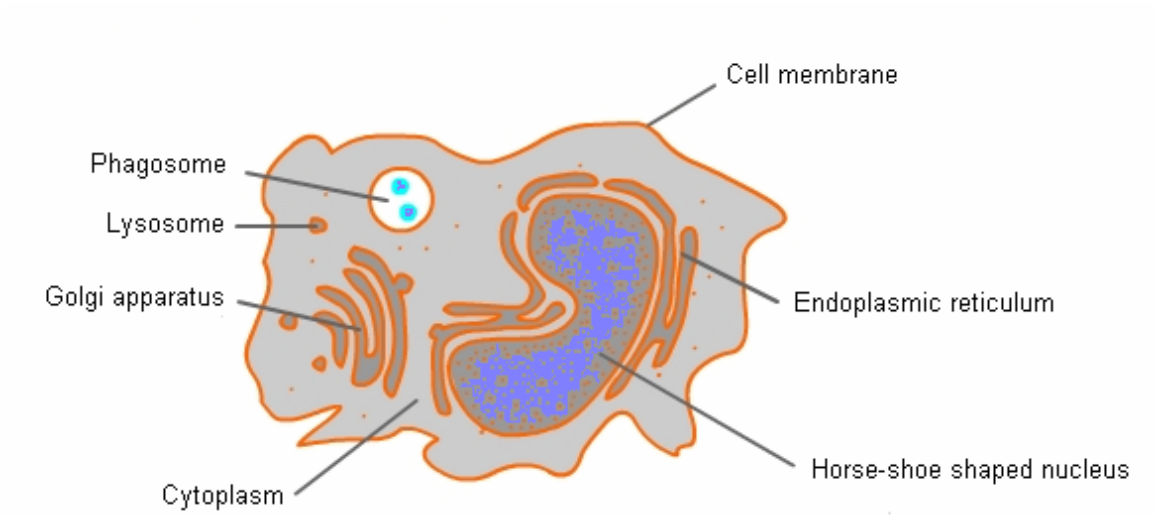

Fig. 7. Structure of a macrophage

Table 4. List of tissue macrophage

Tissue location	Macrophage name
Bone marrow	Histiocytes
Central Nervous System	Microglial cells
Connective tissue	Histiocytes
Liver	Kupffer cells
Lung	Alveolar macrophages
Kidney	Mesangial cells
Peritoneum	Peritoneal macrophage
Spleen, thymus, lymph node	Macrophages (free and fixed)

Macrophages have the capability to digest both exogenous (whole microorganism and insoluble particles) and endogenous antigens (injured or dead host cell) by attracting the antigens through chemotaxis and facilitating their adherence to macrophage cell membrane by developing pseudopodia. Pseudopodia extends around the antigen and takes it inside the cell in a membrane bound structure called a phagosome that fuses with the lysosome to further degrade the antigens to non-toxic form which is then finally eliminated by exocytosis. Phagocytosis is enhanced by the presence of antibody and complement receptors on its cell membrane as the there are some antibodies and complement component that act as opsonin (binding to both antigen and macrophage) thereby rendering antigen more susceptible to phagocytosis.

A number of cytotoxic substances produced by activated macrophages can destroy phagocytosed microorganisms. There are two types of mechanisms; oxygen dependent and oxygen independent killing mechanisms. In oxygen dependent mechanism activated macrophages produce a number of reactive oxygen intermediates that have potent antimicrobial activity. eg. Superoxide anion ($O_2^{\cdot -}$) , hydrogen peroxide (H_2O_2) etc. In oxygen independent mechanisms activated macrophages synthesizes lysosome and various hydrolytic enzymes whose activities do not require oxygen.

Granulocytes

Granulocyte cells are those that contain membrane bound granules in their cytoplasm. These granules contain enzymes capable of killing microorganisms and destroying debris ingested by the process of phagocytosis. There are three types of granulocytic cells: neutrophils, eosinophils and basophils. These cells are characterized by the presence of irregular segmented nuclei, specific granules and fully differentiated cells.

Neutrophils (polymorphonuclear leukocytes or polymorphs)

They are the most common of the leukocytes and represents about 55-70% of all the circulating leukocytes. They have a diameter of about 12 mm, segmented nucleus and cytoplasm packed with small specific granules that stains salmon pink colour after staining with Romanovsky type staining (Fig. 8). Neutrophils remain in circulation in the peripheral blood for about 7-10 h, after their production in the bone marrow.

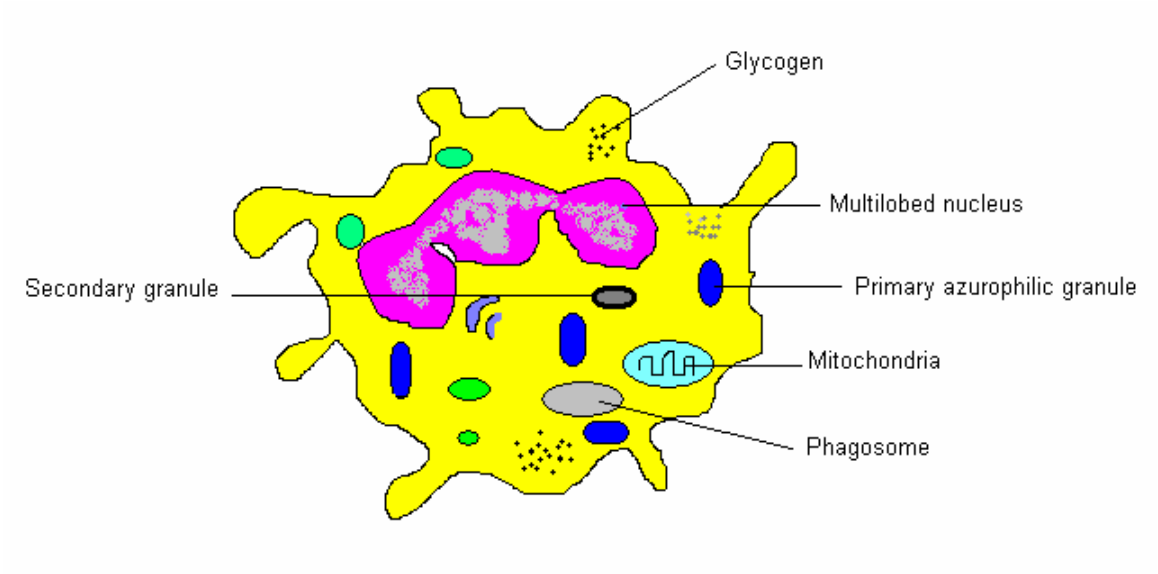

Fig. 8. Structure of a neutrophil

Then they migrate to their tissues where they have the life span of few days. They are involved as a first line of defence against invading microorganisms and are important in inflammation and at sites of injury or wounds. During infection, their production increases, a process called leukocytosis, to arrive at a site of inflammation which is medically used as an indicator of infection. Pus that develops in sites of infection is mainly composed of dead neutrophils. Like macrophages, neutrophils are active phagocytic cells. The difference lies in the lytic enzymes and bactericidal substances that are contained in primary and secondary granules in neutrophils. Primary granules are large and contain peroxidase, lysozyme, and various hydrolytic enzymes whereas secondary granules contain collagenase, lactoferrin, and lysozyme.

Eosinophils

Eosinophils form a small proportion of peripheral blood leucocytes (1-5%) but are more prevalent in tissues. They have a bilobed nucleus and a granulated cytoplasm that stains with acid dye eosin red, hence the name (Fig.10). They become more plentiful in circulation and in relevant tissues in allergic and parasitic diseases and their functions can be divided into effects on parasites and the inflammatory process.

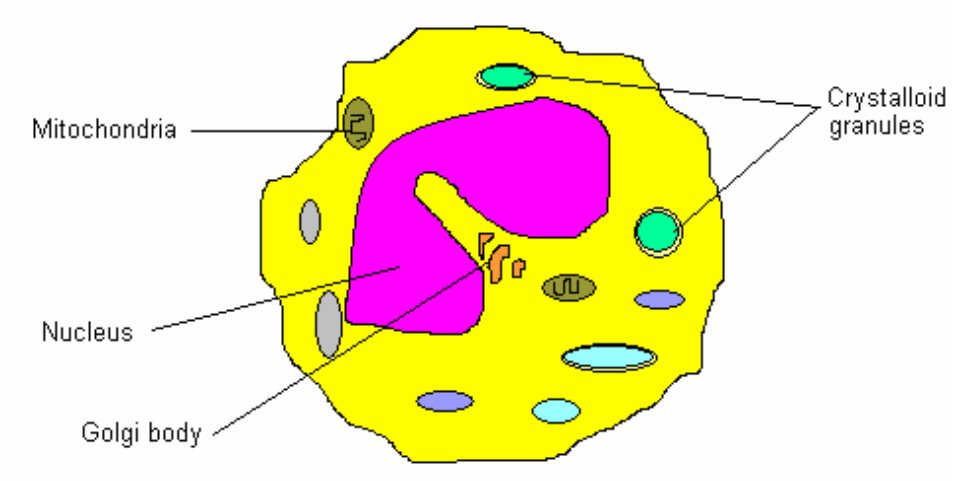

Fig. 9. Structure of an eosinophil

Like neutrophils, they are also motile and can move to the site of action. They phagocytose poorly but degranulate promptly in the presence of chemotactic factors and when membrane-bound IgG or IgE is cross-linked by antigen. Eosinophils have F_C receptors for both IgG and IgE isotypes. A prominent role of neutrophils is the intracellular digestion of microbes (e.g. bacteria) which are readily phagocytosed. They are more effective in the extracellular digestion of infectious agents that are too large to be engulfed (e.g. parasitic worms).

Basophils

These represent only about 1% of the leukocytes. Their nucleus is bilobed or S-shaped (Fig. 10). They have very large, irregular basophilic granules that stain with the basic dye methylene blue.

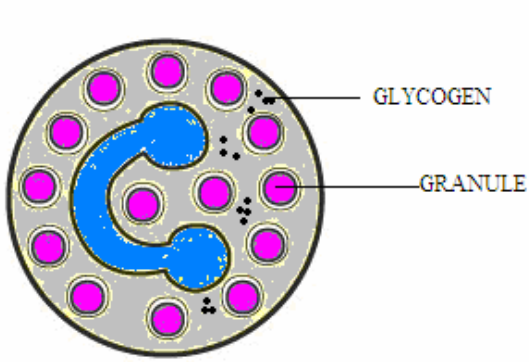

Fig. 10. Structure of a basophil

The granules contain histamine and heparin and release these contents in response to antigens and thus play a major role in allergic response. Basophils are circulating cells and possess surface F_C receptors with a high affinity for IgE.

Mast cells

Unlike basophil cells, mast cells are sessile cells present throughout the body but chiefly in perivascular connective tissue, lymph nodes and mucosal epithelial tissue of the respiratory, genitourinary and digestive tracts.

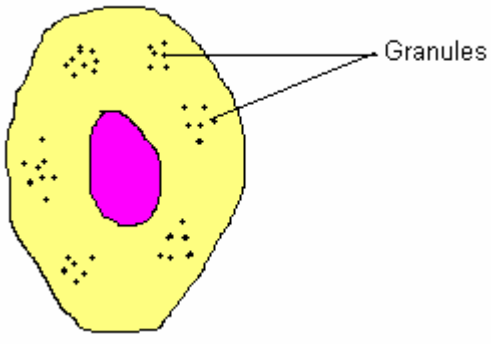

Fig. 11. Structure of a mast cell

They are released from the bone marrow into the blood during hematopoiesis as precursor cells which get differentiated only after reaching the tissue. Like basophil cells, these cells also possess large number of cytoplasmic granules containing histamine and other pharmacologically active substances and surface F_C receptors with a high affinity for IgE. Mast cells, together with basophil cells thus play a major role in the allergic responses (Fig. 11).

Dendritic cells

Dendritic cells are large, motile, weakly phagocytic antigen presenting cells possessing several elongated pseudopodia or processes that resembles dendrite of the nerve cells thus the name dendritic cells (Fig. 12). They comprise about 1% of the cells in the secondary lymphoid organs. These cells are found in different locations and are classified accordingly (Table 5). These different dendritic cells have different morphology and functions but constitutively express high levels of both class II MHC molecules and the co-stimulatory B7 molecules and hence are considered more potent APC than macrophages and B cells which require prior activation to function as APC.

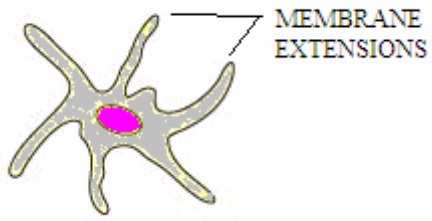

Fig. 12. Structure of dendritic cell

Follicular dendritic cells however, differ in function from APC dendritic cells as they do not express class II MHC molecules. But these cells express high levels of membrane receptors for antibody and complement system. Binding of antigen-antibody complexes is thought to activate the B-cell activation in the lymph nodes.

Table 5 Classification of Dendritic cells

Name of dendritic cell	Location
Langerhan cells	Epidermis and mucous membrane
Interstitial dendritic cells	Present in most organs like, heart, lungs, liver, kidney, gastrointestinal tract
Interdigitating dendritic cells	T-cell areas of secondary lymphoid tissue and the thymic medulla
Circulating dendritic cells	Blood (constitutes 0.1% of the blood leukocytes) and in the lymph (known as veiled cells)
Follicular dendritic cells	Follicles of the lymph node

The dendritic cells keep constant communication with other cells in the body. This communication can take the form of direct cell-to-cell contact based on the interaction of cell-surface proteins. An example of this includes the interaction of the receptor CD40 of the dendritic cell with CD40L present on the lymphocytes. However, the cell-cell interaction can also take place at a distance via cytokines. They also upregulate CCR7, a chemotactic receptor that induces the dendritic cell to travel through the blood stream to the spleen or through the lymphatic system to a lymph node.

ORGANS OF THE IMMUNE SYSTEM

The organs of the immune system can be divided on the basis of their function into **primary lymphoid organs** and **secondary lymphoid organs** (Fig. 13)**.** The functions of these lymphoid organs are:

- To provide an environment for the maturation of the immune system's immature cells
- To concentrate lymphocytes into organs
- To permit the interaction of different classes of lymphocytes
- To provide an efficient mode for the transportation of antibodies and other soluble factors

The primary lymphoid organs include the **thymus** and **bone marrow** where maturation of lymphocytes takes place and thus these organs provide special microenvironment where antigen-independent differentiation of lymphocytes takes place. In these organs the lymphocytes develop antigenic specificity during maturation. The mature lymphocytes are then exported through the blood and the lymphatic system to the secondary lymphoid organs that include lymph nodes, spleen and MALT. These secondary lymphoid organs trap antigen and as a result undergo antigen-dependent differentiation.

Primary lymphoid organs

The lymphocytes only after maturation in the primary lymphoid organs become committed to a particular antigen and the cell becomes immunocompetent. In mammals, T cells mature in the thymus and B cells in the bone marrow.

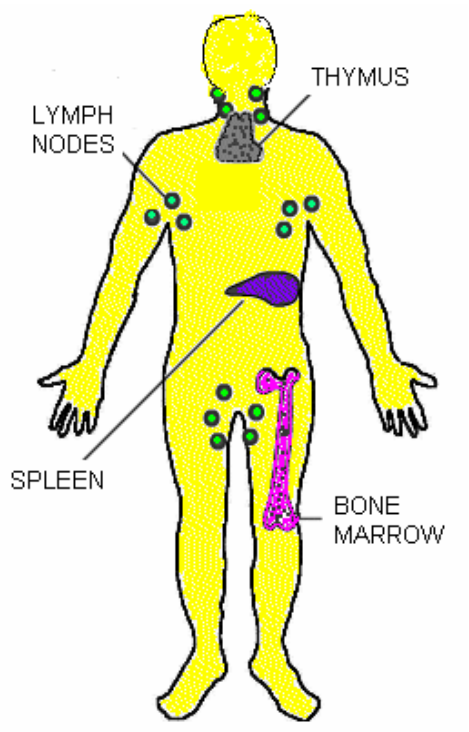

Fig. 13 The location of primary and secondary lymphoid organs

Thymus

It is a flat, bilobed organ situated above the heart and below the thyroid gland. At birth, it weighs around 15-20 g which grows more rapidly during the first two years and then more slowly reaching the maximum weight during puberty (40 g). Each lobe is surrounded by a capsule and is divided into lobules, which are separated from each other by strands of connective tissue called trabeculae. Each lobule is organized into two compartments: the cortex (outer compartment) the medulla (inner compartment). Cortex makes up 85 to 90% of the thymus (Fig. 14).

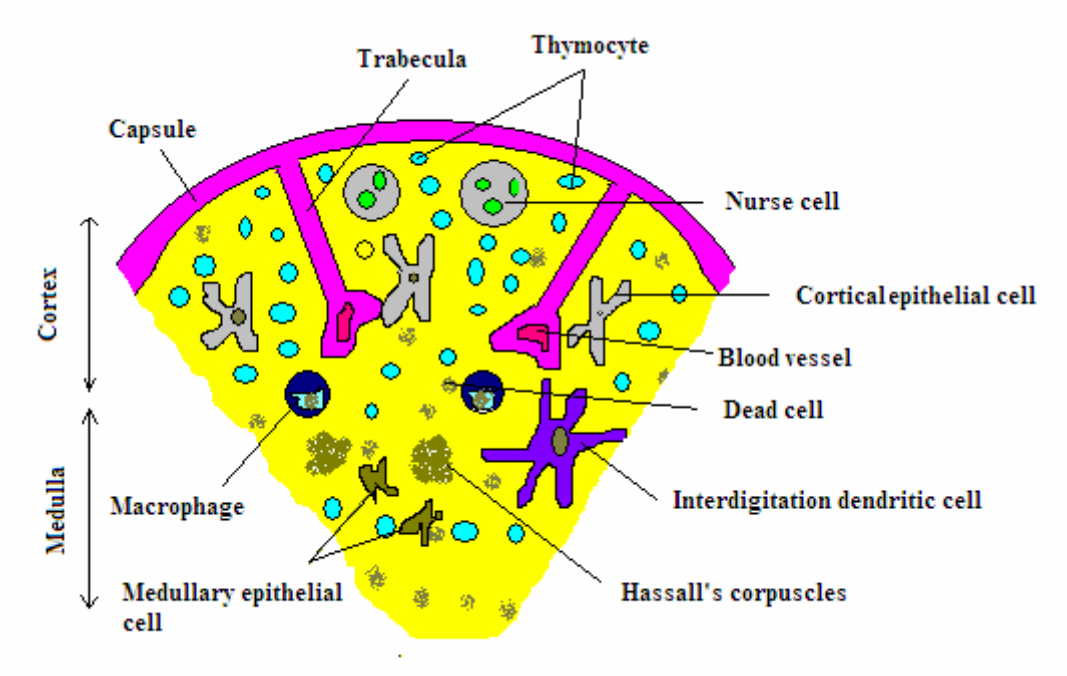

Fig. 14 Diagrammatic cross-section of a portion of the thymus.

The progenitor T cells formed during hematopoiesis in bone marrow enter into the thymus where they multiply rapidly in the cortex. Cortical thymocytes are phenotypically and functionally immature but progressively differentiate into T cells within the thymic cortex. Thymocytes then migrate to the medulla where selection and maturation are completed and finally enter into circulation. The small thymocytes in the cortex are in contact with the **nurse cells** that comprises dendritic cells, macrophages and large epithelial cells. All these cells express high levels of class I and class II MHC molecules. Only 2% of thymocytes leave the thymus each day, the remaining cells die. This high attrition rate of T cells is still unresolved.

For the differentiation and maturation of thymocytes into various types of mature T cells, several hormonal factors are required. The epithelial cells of the thymus serve as the source of these hormones namely, α_1-thymosin, β_4-thymosin, thymopoietin and thymulin. In the couse of thymocyte maturation within the thymus, the antigenic diversity of the T-cell receptor is generated by a series of random gene arrangements. After developing antigen binding receptors, the T cells are subjected to selection pressure whereby the thymocytes that cannot recognize foreign antigenic peptides displayed by self MHC molecules are removed and those thymocytes that recognize self-peptides displayed by self MHC molecules are also eliminated by programmed cell death.

The absence of thymus exhibits dramatic decrease in the circulating lymphocytes of the T cell lineage and hence the absence of cell-mediated immunity, a congenital birth defect in humans

called **DiGeorge's syndrome.** The decline in immunity with ageing could be correlated with the degeneration of both cortex and medullary cells. Until recently, the thymus was only thought to be the sole generator of T cells. Recent findings show that the **intestinal epithelium** is also a primary lymphoid organ that represents a site for thymu-independent T cell development. In fact, in adults it is the major site for T lymphopoiesis and contains the largest collection of T cells in the body.

Bone marrow

Bone marrow is the source for all immune cells except during early fetal development. Bone marrow is the soft tissue in the hollow shafts of the flat bones (iliac bones, ribs, sternum, and vertebrae) and weighs about 3 kg in an averaged-size adult. The cellular composition of normal bone marrow is 5-15% lymphocytes, 1% plasma cells, and 4% monocytes and megakaryocytes. Immature B cells proliferate and differentiate within the bone marrow by interacting with the various cytokines secreted by the stromal cells. During this maturation process, those B cells that develop self reactive antibody receptors or fail to develop functional antibody molecules are eliminated by the selection process. About 90% of differentiating B cells are believed to have this fate. B cells that survive this selection process leave the bone marrow through efferent blood vessels.

In birds, the bursa of Fabricius, a gut associated lymphoid tissue, is the primary site of B cell maturation. Though majority of mammals have bone marrow as the primary site of B cell maturation, yet some mammals have other organs as the B cell maturation site. For example, in cattle and in sheep, a large specialized Peyer's patch called the **ileal Peyer's patch** and in rabbit, gut associated tissue, such as appendix act as B cell maturation site. The selection process similar to that of the thymic selection during T cell maturation, eliminates B cells with self reactive antibody receptors.

Secondary lymphoid organs

Immune cells travel throughout the body by the circulatory and lymphatic systems. Lymphatic system serves to recover materials lost from the blood capillary network and antigens that enter the tissue spaces and return them to the blood. Sites where lymphocytes encounter antigen and interact with other cells of the immune system are called the secondary lymphoid organs. Such organs enlarge in response to antigenic stimulation. Various types of lymphoid tissues are located along the vessels of the lymphatic system. Some lymphoid tissue consists of the diffuse collection of lymphocytes and macrophages and others consist of more organized tissue called the **lymphoid follicles**. Latter, also known as **primary follicle** (|in the absence of antigen encounter)**,** comprises a network of follicular dendritic cells and small B cells. Primary follicles become large **secondary follicles** of concentrically arranged B cells when encounter an antigen.

Of all the secondary lymphoid tissue, lymph nodes and spleen are the most highly organized organs. Less organized lymphoid tissue, collectively called **mucosal associated lymphoid tissue (MALT)** occur in various body parts. The principal function of lymph nodes, spleen and MALT are to respond to antigens introduced into the tissues they drain, to respond to antigens in the blood and to protect against pathogens at major entry points respectively.

Lymph nodes

Lymph nodes are encapsulated bean-shaped structures, normally <1 cm in diameter, packed with lymphocytes, macrophages, and dendritic cells (Fig. 15). Lymph nodes are present at the junctions of lymphatic vessels and serve as the first organized structures to encounter most antigens. The major function of the lymph nodes is to filter antigen from the lymph. The lymph, along with many cells and particles, drain out of tissues and seep across the tiny single-cell-walled lymphatic vessels. The vessels transport lymph to the nodes where antigens are filtered

out. As the lymph is filtered, it is enriched with antibodies, cytokines and mainly T lymphocytes.

Morphologically, a lymph node can be divided into three regions: the **cortex, paracortex and medulla,** each of it providing a different microenvironment. Cortex is the outermost layer-contains mostly B lymphocytes, plus both follicular dendritic cells and macrophages all arranged in clusters called primary follicles. Following antigenic stimulation they primary follicles become secondary follicles consisting of concentric rings of densely packed lymphocytes and germinal central, macrophages, and dendritic cells. The germinal centers contain large proliferating B lymphocytes and plasma cells interspersed with macrophages and dendritic cells. It is a site of intense B-Cell activation and differentiation into plasma cells and memory cells. Paracortex is the region just beneath the cortex which is largely populated with T lymphocytes and also contains interdigitating dendritic cells. These interdigitating dendritic cells express high levels of class II MHC molecules. Paracortex is a thymus dependent area as compared to the thymus independent cortex region. Experiments on the neonatally thymectomized mice showed a severe depletion of the T lymphocytes in the paracortex region showing its dependence on the thymus. Medulla is the inner most region, more sparsely populated by cells. Many of the cells are plasma cells; activated T_H and T_C cells are also present.

In addition, there is a high concentration of immunoglobulin in this region due to the large population of plasma cells.

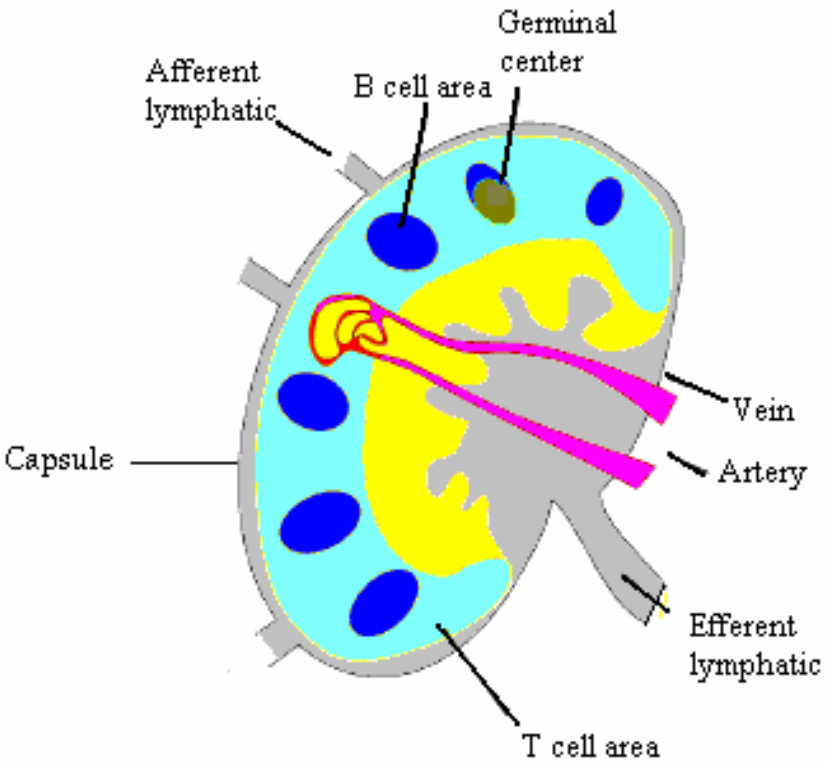

Fig. 15 Structure of a lymph node

Lymph enters the lymph node via afferent lymphatic vessel that pierce the capsule at numerous sites and empty lymph into the subcapsular sinus. This fluid flows through the cortex, paracortex and medulla allowing phagocytic cells and dendritic cells to trap antigen or any particulate matter (antigen-antibody complex), if any. It is then processed and presented together with class II MHC molecules resulting in T_H cell activation. B cell activation also takes place in T cell rich paracortex. Lymph leaves the capsule through a single exit, the efferent lymphatic vessel, which following infection is enriched with antibodies and also has 50 fold increase of the lymphocyte concentration than in the afferent lymph. This enormous increase in lymphocyte concentration could be attributed partly to the activation and proliferation of lymphocytes in response to antigen and largely to the T lymphocytes that travel from the blood into the node by passing between specialized endothelial cells lining the post capillary venules termed the **High Endothelial Venules (HEVs)** of the paracortex. Antigenic stimulation further increases this lymphocyte migration ten times resulting in visible swelling of the nodes.

Spleen

The spleen is a large, ovoid filtering organ situated high in the left abdominal cavity. Unlike lymph nodes which are specialized to trap localized antigens from regional tissue spaces, the spleen filters blood and trap blood borne antigen and thus responds to systemic infections. The spleen is surrounded by a capsule that sends projections known as trabeculae into the compartments. The compartment is of two types-red pulp and white pulp separated by diffused marginal zone. The red pulp consists of erythrocyte rich blood intermingled with many dendritic cells, macrophages, a few lymphocytes and plasma cells. It is the site where old and defective red blood cells are removed. The white pulp surrounds the splenic arteries forming a **periarteriolar lymphoid sheath (PALS)** populated chiefly by T lymphocytes. The marginal zone, located peripheral to PALS, is rich in B cells and macrophages which is organized into primary lymphoid follicles.

Blood borne lymphocytes and antigens enter the spleen through splenic artery which emties into the marginal zone. Sooner the antigen enters the marginal zone, the interdigitating dendritic cells traps them and presents it with class II MHC molecules to carry it to T_H cells. This further leads to the activation of B cells. The activated B cells, together with some T_H cells migrate to the primary follicle and develop into secondary follicles containing germinal centers like those in the lymph nodes.The spleen is the predominant organ in lymphocyte recirculation, moving more lymphocytes than all the lymph nodes combined.

Mucosal-Associated Lymphoid Tissue (MALT)

The non-encapsulated clusters organized lymphoid tissue with immune function is the mucosal associated lymphoid tissue. Approximately 400 m^2 surface area of the body are prone to attack by pathogens. These areas are the mucous membranes lining the digestive, respiratory and urinogenital tract. The defense of all these areas is provided by MALT. These tissue ranges from loose cluster or little organization of lymphoid cells in the lamina propria of intestinal villi to more organized structures such as the tonsils, appendix and Peyer's patches. The lamina propria contains large numbers of B cells, plasma cells, activated cells, and macrophages to impart better immunity than any other part of body. The epithelial cells of mucous membranes play an important role in promoting the immune response by delivering the antigen from the lumina of the respiratory, digestive and urinogenital tracts to the underlying MALT. This antigen transport is carried out by some specialized cells called **M cells** (Fig. 16). The M cells are flattened epithelial cells lacking the characteristic microvilli of the mucous epithelium and has a deep pocket or invagination in the basolateral plasma membrane filled with a cluster of B cells, T cells and macrophages. The transport of antigens by M cells activates the B cells. The activated B cells differentiated into plasma cells which secrete the IgA class of antibodies.

These antibodies then are transported across the epithelial cells and released as secretory IgA into the lumen where they can interact with antigen present in the lumen.

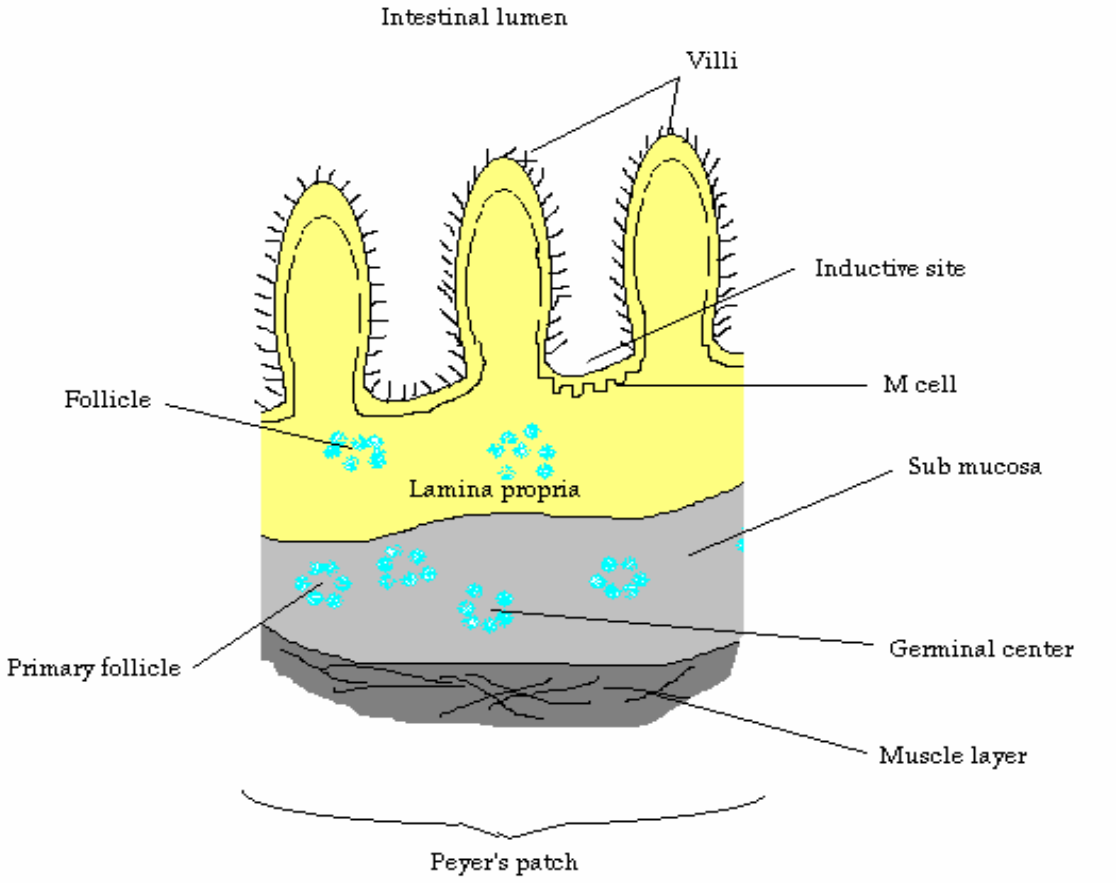

Fig. 16 Cross-sectional diagram of the mucous membrane lining the intestine showing the M cell region.

Summary

Immune system possesses diverse cells and organs to confer immunity to the body. The whole system is an intricate network of these cells and organs whose coordinated action is responsible for the various functions and the constancy in the internal environment of the body. The lymphocytes possess the immunologic attributes of specificity, diversity, immunologic memory and self/non-self recognition. Hematopoiesis generates the various blood cells required by the body and the location of the hematopoiesis varies initially but finally becomes constant with age and bone marrow becomes the key location for the process. Various factors, cytokines in particular are known to regulate the process of hematopoiesis. As the production of these cells is vital for the body, its destruction is equally important and body maintains the constancy in these cell numbers through the programmed cell death. Among all the cells of the immune system, B cell and T cells are the two major populations of lymphocytes. B cells mature in the bone marrow but T cells though originate from the bone marrow but mature in thymus. The primary lymphoid organs thus provide site where lymphocytes mature and become antigenically committed. The secondary lymphoid organs provide the site where lymphocytes interact with the antigen and undergo clonal proliferation and finally differentiation into the effector cells. Proper and balanced working of these cells and organs prevents us from succumb

to diseases to great extent and the understanding of functioning of these cells and organs are vital before understanding the other aspects of immunology.

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