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CELLS AND ORGANS OF IMMUNE SYSTEM

Specific as well as non-specific immunity is maintained in the body the lymphoreticular system that is a complex organization of cells of diverse morphology and distributed widely in different parts of the body. Lymphoreticular cells include reticuloendothelial cells and lymphoid cells.

Reticuloendothelial system:
The reticuloendothelial system mainly comprise of phagocytic cells whose function is to engulf microbes, immune complex from blood and tissues and participate in inflammation. This way they contribute to non-specific immunity. These cells also participate in specific immunity by way of antigen presentation and cytokine secretions. The role of phagocytes was highlighted by Elie Metchnikoff. The deficiency of phagocytic system can lead to disorders such as Chronic Granulomatous Disease.

The major phagocytic cells are:

- Polymorphonuclear leucocytes (PMNLs), also called neutrophils, microphages
- Blood and tissue monocytes.

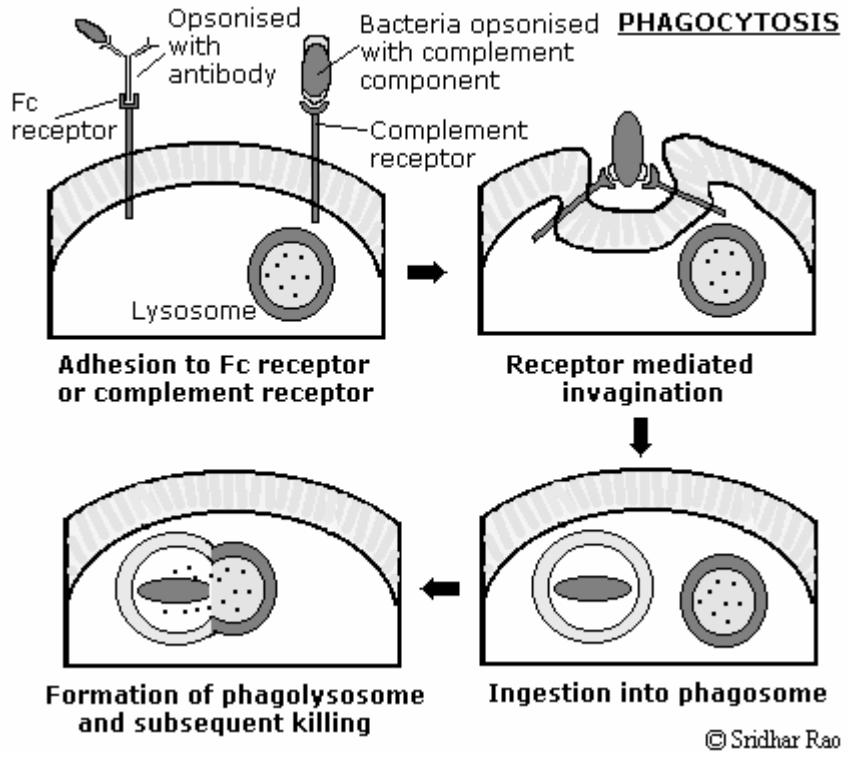
They both are derived form the bone marrow during hematopoiesis.

Neutrophils have short life span. They circulate in the blood for 6-7 hours, then migrate through the endothelial cell junctions and reside in tissue spaces where they live only for few days and do not multiply. Neutrophils are the most abundant of the leukocytes, normally accounting for 54-75% of the WBCs. An adult typically has 3,000-7,500 neutrophils/mm^3 of blood but the number may increase two- to three-fold during active infections. Adult body usually produces 10^{11} neutrophils per day. Some neutrophils may remain attached to endothelial lining of large veins and can be mobilised during inflammation. The nucleus of a neutrophil is segmented into 3-5 connected lobes, hence the name polymorphonuclear leukocyte. They are called neutrophils because their granules stain poorly with the mixture of dyes used in staining leukocytes. Because of the granules, they are considered as one of the granulocytes. There are two types of granules, the specific granules and azurophilic granules. Specific granules are present in abundance and contain proteolytic enzymes such as lysozyme, collagenase and elastase. They stain neither with acidic nor basic dyes. The azurophilic granules are actually lysosomes.

Monocytes have rounded or kidney-shaped nuclei with finely granular cytoplasm, measure 12-15 µm and have half-life of 3 days in circulation. Monocytes normally make up 2-8% of the WBCs (100-500/mm^3 of blood). Once monocytes leave circulation and enter tissue, they are called **macrophages**. There are two types of macrophages, one that wander in the tissue spaces and the other that are fixed to vascular endothelium of liver, spleen, lymph node and other tissue. Tissue macrophages survive for months and can multiply. Macrophages present in different organs have been given different names. They are Histiocytes (in tissue), Kupffer cells (in liver), Alveolar macrophages (in lungs), Peritoneal macrophages (in peritoneum), Microglial cells (in brain), Mesangial cells (in kidneys) and Osteoclasts (in bone). Some macrophages develop abundant cytoplasm and are called epitheloid cells. Macrophages can fuse to form multi-nucleated giant cells. Some mononuclear cells differentiate into dendritic cells. Functions of macrophage include killing of microbes, infected cells, tumor cells, secretion of immunomodulatory cytokines, antigen processing and presentation to T cells. Macrophages respond to infections as quickly as neutrophils but persist much longer; hence they are dominant effector cells in the later stage of infection.

Microbial killing by phagocytes:
Phagocytosis involves two steps namely attachment and ingestion. Following attachment of the organism, invagination of the phagocyte results in the formation of a phagosome. Some capsulated bacteria don't attach to the phagocyte, but they can still be phagocytosed if they are coated with opsonins such as IgG and complement component (C3b). The engulfed bacteria are held inside a vacuole called phagosome. The formation of phagosome triggers respiratory bursts and fusion of lysosome with phagosome to form phagolysosome.

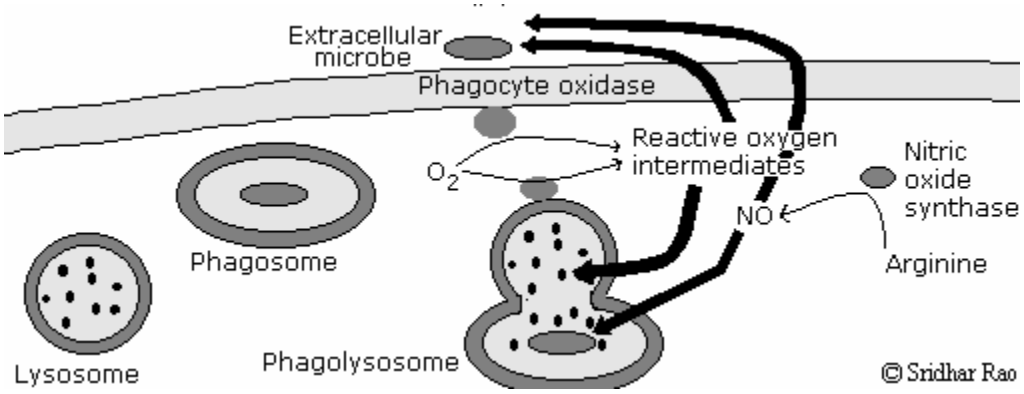
The phagocytes appear to kill engulfed bacteria by two pathways, oxygen independent pathway and oxygen dependent pathway. The microbicidal mechanisms of the respiratory burst are termed oxygen dependent and phagolysosome formations are termed oxygen independent.



Oxygen dependent mechanism involves catalytic conversion of molecular oxygen to oxyhalide free radicals, which are highly reactive oxidizing agents. The phagocyte oxidase present in the plasma membrane and phagolysosome reduce oxygen into reactive oxygen intermediates such as superoxide radicals. Superoxide is converted to H_2O_2, which is used by enzyme myeloperoxidase to convert unreactive halide ions to reactive hypohalous acids that are toxic to bacteria.

Oxygen independent mechanism involves release of lysosomal contents into phagolysosomes. The content of lysosome includes lactoferrin, cathepsin G, lysozyme and defensins etc.

In addition to the phagocyte oxidase system, macrophages have free-radical generating system, namely inducible nitric oxide synthase. This cytosolic enzyme is absent in resting macrophages but can be induced in response to bacterial lipopolysaccharides and IFN-γ. This enzyme catalyses the conversion of arginine to citrulline, and in the process releases nitric oxide gas. Nitric oxide may then combine with H_2O_2 or superoxide to form highly reactive peroxynitrite radicals that kill the microbes.



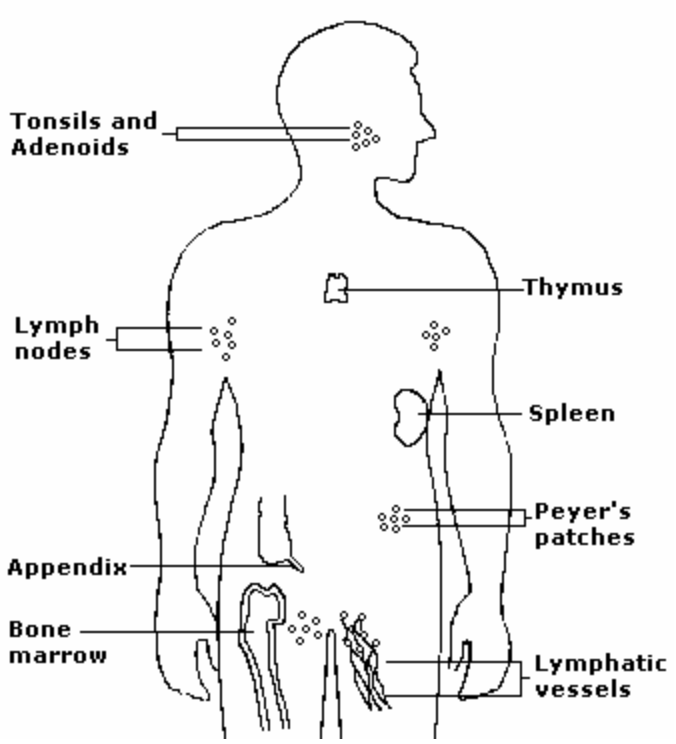
Dendritic cells:

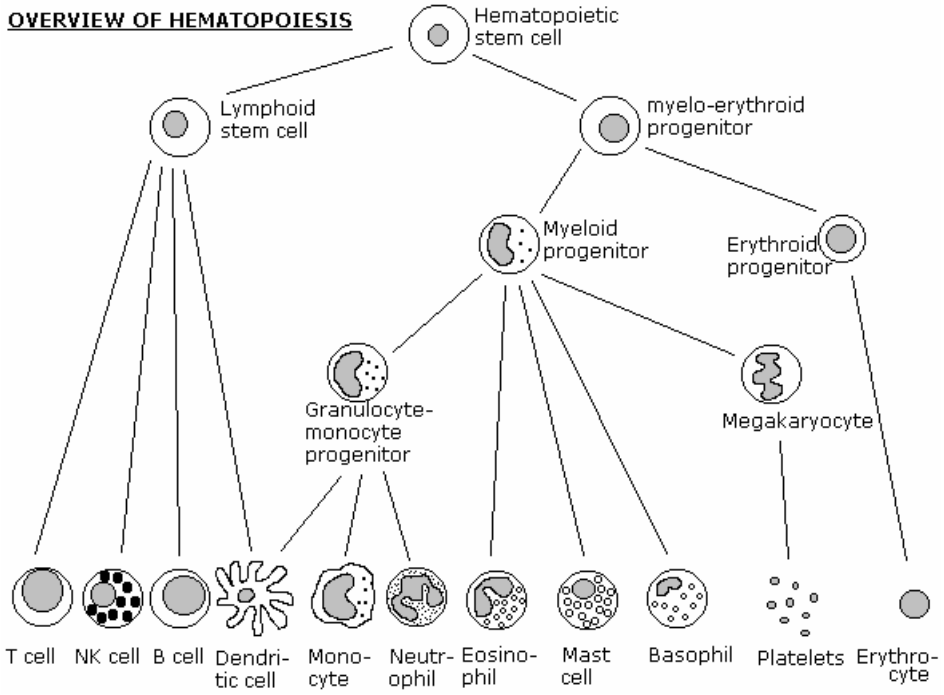
These cells are derived from myeloid progenitor in the bone marrow and are morphologically identified by spiny membranous projection on their surfaces. Immature dendritic cells are located in epithelia of skin, gastrointestinal tract and respiratory tract and are called langerhan cells. They express low levels of MHC proteins on their surface and their main function is to capture and transport protein antigen to the draining lymph node. During their migration to the lymph node, dendritic cells mature into excellent antigen presenting cells (APC). Mature dendritic cells reside in the T cell area (paracortex) of the lymph node. Here, they are referred as interdigitating dendritic cells. These cells are distinct from the dendritic cells that occur in the germinal centers of lymphoid follicles (follicular dendritic cells) in lymph node, spleen and MALT. The follicular dendritic cells are not derived from the bone marrow and their role is to present antigen-antibody complex and complement products to B cell.

Lymphoid system:

Lymphoid organs are stationed throughout the body and are concerned with the growth, development and deployment of lymphocytes. These structurally and functionally diverse lymphoid organs and tissues are interconnected by the blood vessels and lymphatic vessels through which lymphocytes circulate. The organs involved in specific as well as non-specific immunity are classified as primary (central) lymphoid organs and secondary (peripheral) lymphoid organs. The blood and lymphatic vessels that carry lymphocytes to and from the other structures can also be considered lymphoid organs. Recently, it has become accepted that the liver is also a hematopoietic organ, giving rise to all leukocyte lineages.

OVERVIEW OF LYMPHATIC SYSTEM





PRIMARY LYMPHOID ORGANS:

Also called central lymphoid organs, these are responsible for synthesis and maturation of immunocompetant cells. These include the bone marrow and the thymus.

BONE MARROW:

All the cells of the immune system are initially derived from the bone marrow through a process called hematopoiesis. During foetal development hematopoiesis occurs initially in yolk sac and para-aortic mesenchyme and later in the liver and spleen. This function is taken over gradually by the bone marrow. During hematopoiesis, bone marrow-derived stem cells differentiate into either mature cells or into precursors of cells that migrate out of the bone marrow to continue their maturation in thymus.

The bone marrow produces B cells, natural killer cells, granulocytes and immature thymocytes, in addition to red blood cells and platelets. It is both a primary and secondary lymphoid organ. The proliferation and maturation of precursor cells in the bone marrow are stimulated by cytokines, many of which are called colony stimulating factors (CSFs). The bone marrow also contains antibody secreting plasma cells, which have migrated from the peripheral lymphoid tissue.

THYMUS:

The thymus is a gland located in the anterior mediastinum just above the heart, which reaches its greatest size just prior to birth, then atrophies with age. This lymphoepithelial organ develops from ectoderm derived from the third branchial cleft and endoderm of the third branchial pouch.

Immature lymphocytes begin to accumulate in the thymus of human embryos at about 90-100 days after fertilization. Initially most of these immature lymphocytes have come from the yolk sac and fetal liver rather than the bone marrow. Cells from the bone marrow, later migrate to the thymus as precursors and develop into mature peripheral T cells. Once the immature lymphocytes have passed the blood-thymus barrier they are called thymocytes. Mature T cells migrate from the thymus to secondary lymphoid organs such as lymph node, Peyer's patches and spleen.

Ultimately the thymus becomes an encapsulated and consists of many lobes, each divided into an outer cortical region and an inner medulla. The cortex contains mostly immature thymocytes, some of which mature and migrate to the medulla, where they learn to discriminate between self and non-self during foetal development and for a short time after birth. T cells leave the medulla to enter the peripheral blood circulation, through which they are transported to the secondary lymphoid organs. About 98% of all T cells die in the thymus.

The greatest rate of T cell production occurs before puberty. After puberty, the thymus shrinks and the production of new T cells in the adult thymus drops away. Children with no development of thymus suffer from DiGeorge syndrome that is characterized by deficiency in T cell development but normal numbers of B cells.

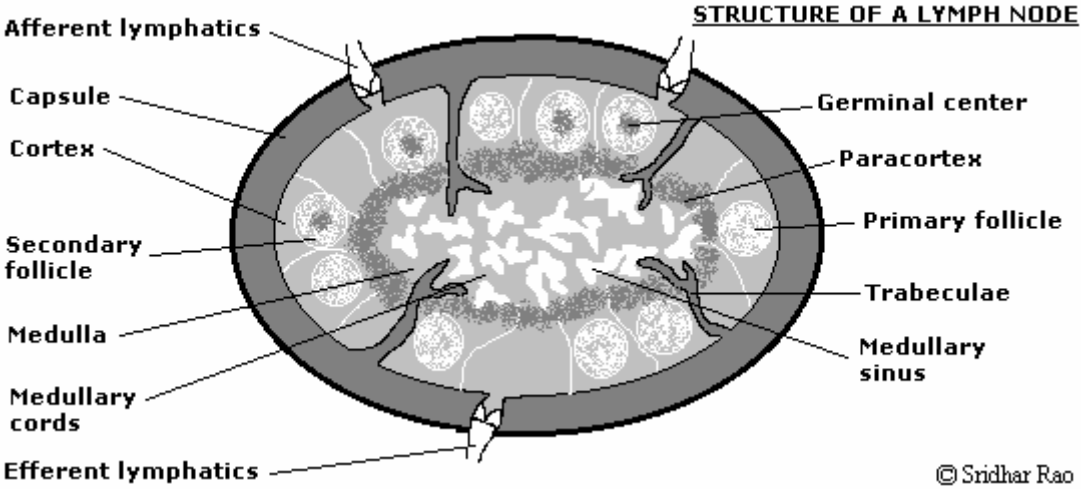
PERIPHERAL LYMPHOID ORGANS:

While primary lymphoid organs are concerned with production and maturation of lymphoid cells, the secondary or peripheral lymphoid organs are sites where the lymphocytes localise, recognise foreign antigen and mount response against it. These include the lymph nodes, spleen, tonsils, adenoids, appendix, and clumps of lymphoid tissue in the small intestine known as Peyer's patches. They trap and concentrate foreign substances, and they are the main sites of production of antibodies.
Some lymphoid organs are capsulated such as lymph node and spleen while others are non-capsulated, which include mostly mucosa-associated lymphoid tissue (MALT).

LYMPH NODE:

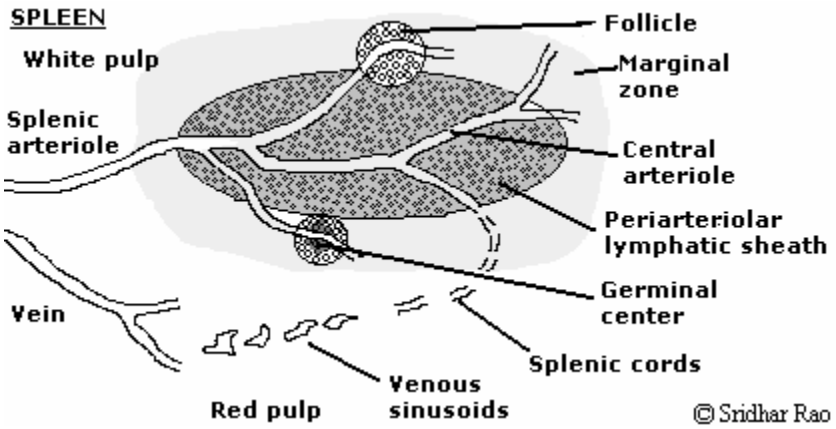
Clusters of lymph nodes are strategically placed in the neck, axillae, groin, mediastinum and abdominal cavity, where they filter antigens from the interstitial tissue fluid and the lymph during its passage from the periphery to the thoracic duct. The key lymph nodes are the axillary lymph nodes, the inguinal lymph nodes, the mesenteric lymph

nodes and the cervical lymph nodes. Lymph nodes that protect the skin are termed somatic nodes, while deep lymph nodes protecting the respiratory, digestive and genitourinary tracts are termed visceral nodes.
Each lymph node is surrounded by a fibrous capsule that is pierced by numerous afferent lymphatics that drain lymph into marginal sinus. The lymph flows through the medullary sinus and leaves through efferent lymphatics. Each lymph node is divided into an outer cortex, inner medulla and intervening paracortical region. The cortex is also referred as B cell area, which mainly consists of B cells. The cortex is a high traffic zone where recirculating T- and B lymphocytes enter from the blood. Aggregates of cells called follicles are present in the cortex, which in turn may have central areas called germinal centers. Follicles without germinal centers are called primary follicles and those with germinal centers are called secondary follicles. Primary follicles are rich in mature but resting B cells. Germinal centers develop in response to antigenic stimulation and consist of follicular dendritic cells and reactive B cells. The medulla contains a mixture of B cells, T cells, plasma cells and macrophages. The medulla consists of medullary cords that lead to the medullary sinus. The cords are populated by plasma cells and macrophages. Between these two zones, lie the paracotex (T cell area) that contains T lymphocytes, dendritic cells and mononuclear phagocytes. Most of the T cells (70%) located there are CD4+ helper cells.



SPLEEN:

Situated in the left upper quadrant of the abdomen and weighing about 150 grams, spleen is the largest single lymphoid organ in the body. It has a dense fibrous capsule with muscular trabeculae extending inward to subdivide the spleen into lobules. It filters blood and is the major organ in which antibodies are synthesized and released into circulation. In addition to capturing foreign antigens from the blood that passes through the spleen, migratory macrophages and dendritic cells also bring antigens to the spleen via the bloodstream. Persons lacking spleen (eg. splenectomy) are highly susceptible to infections with capsulated bacteria such as pneumococci and meningococci. Spleen is the major site for phagocytosis of antibody coated bacteria and destruction of aged RBCs.



It is supplied by splenic artery, which pierces the capsule at hilum and divides into smaller branches that are surrounded by fibrous trabeculae. The spleen is composed of two types of tissue, the red pulp and the white pulp. The red pulp contains vascular sinusoids, large number of erythrocytes, resident macrophages, dendritic cells, granulocytes, few plasma cells and lymphocytes. It is the site where aged platelets and erythrocytes are destroyed. The white pulp contains the lymphoid tissue clustered around small arterioles and is known as a periarteriolar lymphoid sheath (PALS). PALS contain mainly T lymphocytes, about 75% of which are CD4+ helper T cells. Attached to this are lymphoid follicles, some of which contain germinal centers. Follicles and germinal center predominantly contain B cells. The PALS and follicles are surrounded by rim of lymphocytes and macrophages, called marginal zone. Marginal zone is composed of macrophages, B cells, and CD4+ helper T cells. The arterioles end in vascular sinusoids in the red pulp, which in turn end in venules that drain into splenic vein. Antigens and

lymphocytes enter the spleen through vascular sinusoids. Activation of B cells occurs at the juncton between follicle and PALS. Activated B cells then migrate to the germinal centers or into the red pulp.

MUCOSA ASSOCIATED LYMPHOID TISSUE (MALT):

Approximately >50% of lymphoid tissue in the body is found associated with the mucosal system. MALT is composed of gut-associated lymphoid tissues (GALT) lining the intestinal tract, bronchus-associated lymphoid tissue (BALT) lining the respiratory tract, and lymphoid tissue lining the genitourinary tract. The respiratory, alimentary and genitourinary tracts are guarded by subepithelial accumulations of lymphoid tissue that are not covered by connective tissue capsule. They may occur as diffuse collections of lymphocytes, plasma cells and phagocytes throughout the lung and lamina propria of intestine or as clearly organised tissue with well-formed lymphoid follicles. The well-formed follicles include the tonsils (lingual, palatine and pharyngeal), Peyer's patches in the intestine and appendix. The major function of these organs is to provide local immunity by way of sIgA (also IgE) production. Diffuse accumulations of lymphoid tissue are seen in the lamina propria of the intestinal wall. The intestinal epithelium overlying the Peyer's patches is specialized to allow the transport of antigens into the lymphoid tissue. This function is carried out by cuboidal absorptive epithelial cells termed "M" cells, so called because they have numerous microfolds on their luminal surface. M cells endocytose, transport and present antigens to subepithelial lymphoid cells.
Majority of intra-epithelial lymphocytes are T cells, and most often CD8+ lymphocytes. The intestinal lamina propria contains CD4+ lymphocytes, large number of B cells, plasma cells, macrophages, dendritic cells, eosinophils and mast cells. Peyer's patches contain both B cells and CD4+ T cells.

LYMPHOCYTES:

Lymphocytes are stem cells derived cells that mature either in the bone marrow or thymus. Together, the thymus and marrow bone marrow produce approximately 10^9 mature lymphocytes each day and the adult human body contains approximately 10^{12} lymphocytes. Lymphocytes comprise 20-40% (1000 - 4000 cells/µl) of all leukocytes. The lymphocytes are distributed to blood, lymph and lymphoid organs.

Typically, lymphocyte is small, round, cell with diameter of 5-10µm, spherical nucleus, densely compacted nuclear chromatin and scanty cytoplasm. Though the cytoplasm contains mitochondria and ribosomes, other organelles are not detectable. Such mature but resting lymphocytes are known as naïve cells. They are mitotically inactive but when stimulated can undergo cell division. Naïve lymphocytes have a short life span and die in few days after leaving bone marrow or thymus unless they are stimulated. Once the lymphocyte is activated (stimulated), they become large (10-12µm), have more cytoplasm and more organelles. Activated lymphocytes may undergo several successive rounds of cell division over a period of several days. Some of the progeny cells revert to the resting stage and become memory cells, but can survive for several years in the absence of any antigenic stimulus.

There are three major types of lymphocyte, B lymphocyte, T lymphocyte and NK cells. Different lymphocytes are identified by certain protein markers on their surface called "cluster of differentiation" or "CD" system. One marker that all leukocytes have in common is CD45. The presence of the markers can be detected using specific monoclonal antibodies.

Distribution of lymphocytes

	Approximate %		
Tissue	**T-Cells**	**B-Cells**	**NK Cells**
Peripheral blood	70-80	10-15	10-15
Bone marrow	5-10	80-90	5-10
Thymus	99	<1	<1
Lymph node	70-80	20-30	<1
Spleen	30-40	50-60	1-5

B LYMPHOCYTE:

Also called B-cells, they are so called because in birds they were found to mature in bursa of fabricius. Humans don't have an anatomical equivalent to bursa, but the development and maturation of these cells occur in bone marrow.

Ontogeny:
In mammals, the early stages of B cell maturation occur in the fetal liver and bone marrow. B cell development begins in the fetal liver and continues in the bone marrow throughout life.
The stages in B cell development in the bone marrow are:
Stem cell > pro-B cell > pre-B cell > small pre-B cell > immature B cell > mature B cell.

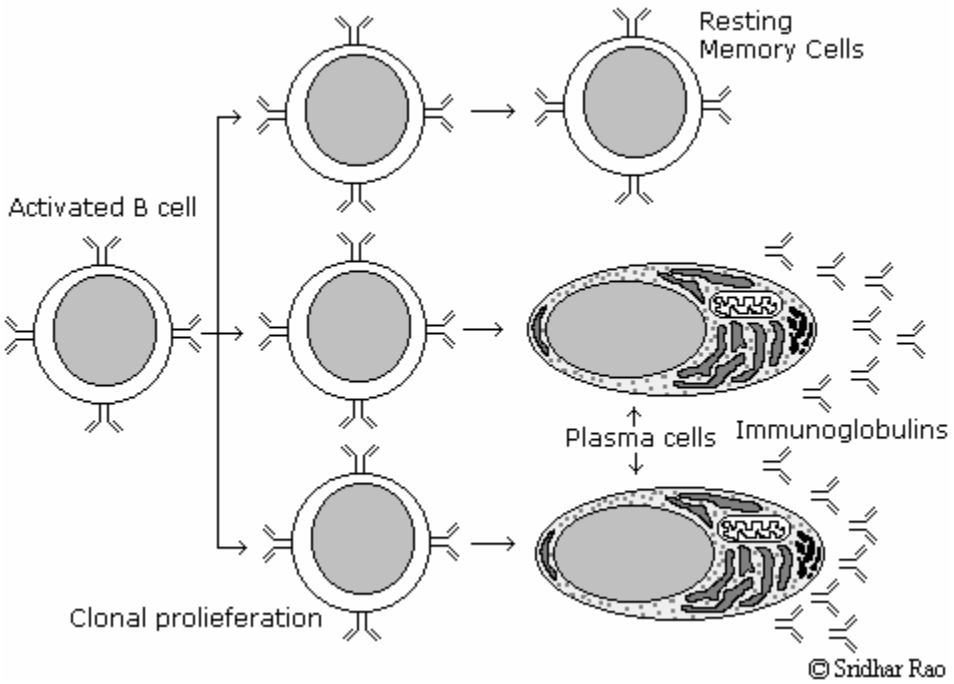
Distribution:
They account for 5-15% of lymphocytes (250 cells/µl) in circulation and 80-90% in bone marrow, 20-30% in lymph node and 50-60% in spleen.

Surface markers:
The most important surface marker on the surface of mature B cell is the surface immunoglobulin. The surface immunoglobulins are of IgM and IgD type. A B cell will have approximately 10^9 immunoglubulins of single specificity on its surface. Markers/Receptors on B cells are Surface Immunoglobulin (IgM and IgD), CD40, B7, ICAM-1, LFA-1, MHC II, CD32 (Ig Fc receptor), CD35 (Receptor for complement component) and additional markers that distinguish B cells such as CD19, CD20, CD21 and CD22.

Demonstration of B cells:
EAC (Erythrocyte Amboceptor Complement) Rosettes: When sheep RBCs coated with antibody and treated with complement and B cells, a rosette is formed due to the presence of complement receptor on B cells. B cells can be demonstrated by immunofluorescence with fluorescent-labelled monoclonal antibodies against surface markers such as surface immunoglobulin.
On stimulation by pokeweed mitogen, they undergo blast transformation.

Functions of B-cells:
Direct antigen recognition and Antigen presentation
B cells may differentiate into plasma cells (which secrete large amounts of antibodies) or into memory B cells. Memory cells can survive 20 years or more.

Plasma cells:
These are the effector cells of the B-cell lineage and are specialised in secreting immunoglobulins. When activated B cells divide, some of its progeny become memory cells and the reminder become immunoglobulin-secreting plasma cells. Plasma cells are oval or egg shaped, have eccentrically placed nuclei, have abundant cytoplasm containing dense rough endoplasmic reticulum (the site of antibody production), perinuclear Golgi body (where immunoglobulins are converted to final form and packaged). Unlike B cells, immunoglobulins are not present on the surface of plasma cells. They have a short life span of few days to few weeks.

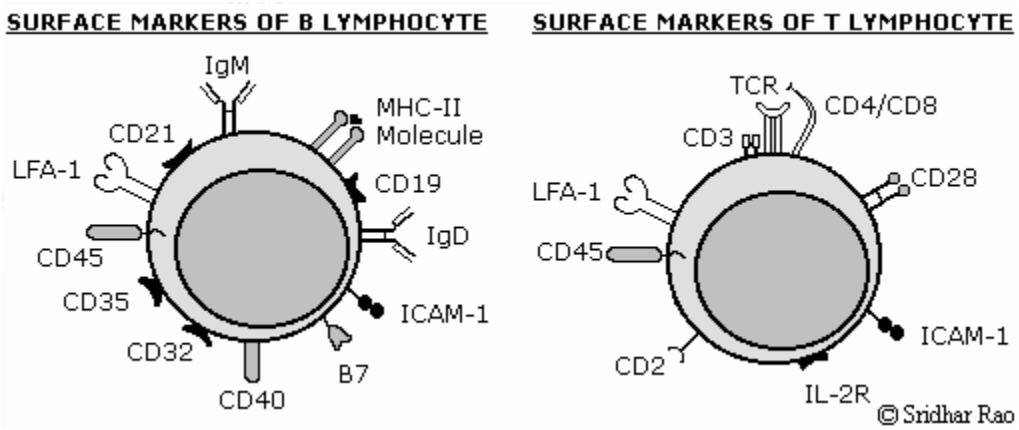


T LYMPHOCYTE:

Ontogeny:
The name "T-cell" is an abbreviation of "thymus dependent lymphocyte". T lymphocytes arise in the bone marrow as T-cell precursors, then migrate to and mature in the thymus. After entry into the thymus T-cell precursors are also referred to as "thymocytes".
In the thymus there are rearrangements at gene segments coding for the variable part of the TCR (T Cell Receptor) resulting in generation of diversity. T Cell Receptors are then expressed on the surface, which is followed by expression of either CD8 or CD4 surface molecules. Those cells expressing receptors that can interact with self MHC molecules are positively selected while those cells that express receptors that recognize peptides derived from self protein in association with self MHC are negatively selected. Such cells undergo clonal deletion or anergy.

Distribution:
T cell accounts for 70-80% (1500 cells/µl) lymphocytes in peripheral blood, 5-10% in bone marrow, 70-80% in lymph node and 30-40% in spleen.

Surface markers:
The most important surface receptor is TCR. TCR are polypeptides that belong to the immunoglobulin superfamily. There are two kinds of TCR, one composed of a α-β heterodimer (TCR2) and the other composed of a γ-δ heterodimer (TCR1). An individual T cell can express either α-β or γ-δ as its receptor but never both. 95% of T cells express the α-β heterodimer. The other markers/receptors present on the surface are IL-2R, IL-1R, CD2, CD3, CD4/CD8, CD28, ICAM-1 and LFA-1. Nearly all the mature T lymphocytes express both CD2 and CD3 on their surface. CD3, which is always found closely associated with TCR, is necessary for signal transduction following antigen recognition by the TCR.



Subsets of T Cells:
There are two major types of T cells, Helper (CD4) and Cytotoxic/Suppressor (CD8) T cells.
CD4 cells account for 45% (900/µl) of lymphocytes while CD8 cells account for 30% (600/µl).

- **Helper T cells (T_H)** secrete cytokines that promote the proliferation and differentiation of cytotoxic T cells, B cells and macrophages and activation of inflammatory leukocytes. T_H cells are identified by the presence of the CD4 marker. They recognize antigen when presented along with Class II MHC molecules. T_H cells are further subdivided into the T_{H1} and T_{H2} subsets on the basis of the kinds of cytokines they produce. T_{H1} cells produce interleukin-2 (IL-2), interferon-gamma (IFNγ), and tumour necrosis factor-beta (TNF-β) while T_{H2} cells produce IL-4, IL-5, IL-6, IL-10 and TGF-β.
- **Cytotoxic T cells (T_C)** lyse cells with foreign antigens, e.g. tumour cells, virus-infected cells, and foreign tissue grafts. T_C cells are identified by the presence of the CD8 marker. They recognize antigen presented when presented along with Class I MHC molecules. The suppressor T cells have a role in downregulation of immune response.

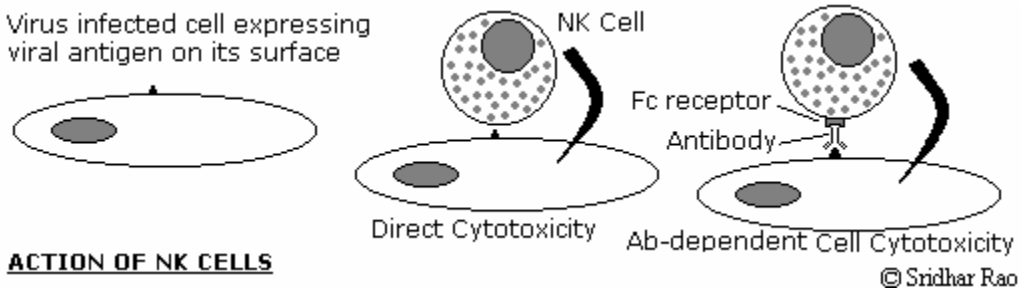
Demonstration of T cells:

- T cells can be demonstrated by immunofluorescence using fluorescent-labelled monoclonal antibodies against TCR or other surface markers.
- E-Rosette/ SRBC rosette: T cells bind to sheep RBCs at 37^{o}C forming rosettes.
- They undergo blast transformation on treatment with mitogens such as phytohemagglutinin (PHA) or Concanavalin A.

Functions of Helper T-cells (TH):
Promotes differentiation of B-cells and cytotoxic T-cells
Activates macrophages
Functions of Cytotoxic/Suppressor T-cells (CTL):
Kills cells expressing appropriate antigen
Downregulates the activities of other cells

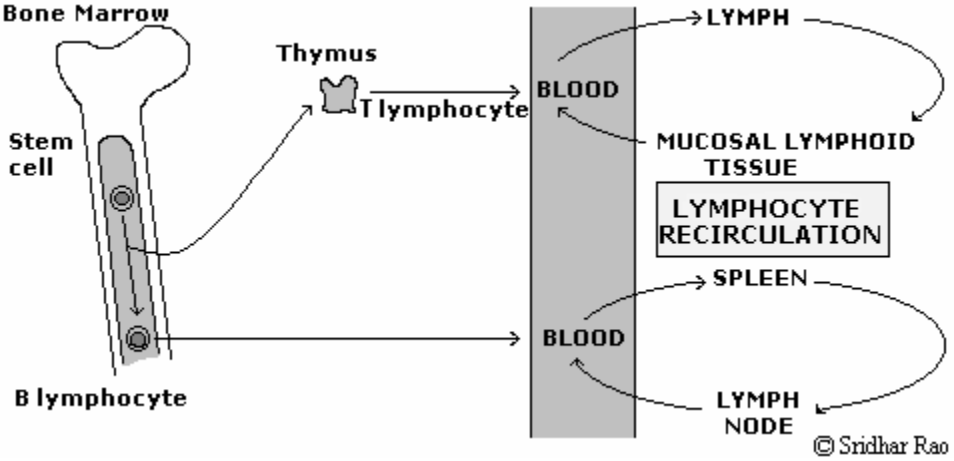
NK CELLS (LARGE GRANULAR LYMPHOCYTES):
Also called Large Granular Lymphocytes (LGLs), these are large lymphocytes containing azurophilic granules in the cytoplasm. NK cells derive form bone marrow but don't require thymus for development. NK cells are so called because they kill variety of target cells (such as tumour cells, virus-infected cells, transplanted cells) without the participation of MHC molecules. They can kill target cell without a need for activation unlike cytotoxic T lymphocytes. Hence they mediate a form of natural (innate) immunity.
Distribution:
They account for 10-15% of blood lymphocytes. They are rare in lymph nodes and don't circulate through lymph.
Surface markers:
NK cells lack any surface immunoglobulins, TCR or CD4 makers; instead they have CD16 (Immunoglobulin Fc receptor) and CD56. Approximately 50% of human NK cells express only one form of CD8. Other receptors include IL-2R, CD2, ICAM-1 and LFA-1.

Functions:
NK cells are activated by recognition of antibody-coated cells, virus infected cell, cell infected with intracellular bacteria and cells lacking MHC I proteins. Activation of NK cell results in cytolysis of target and cytokine secretion but no clonal expansion. Interestingly, NK cells are inhibited on contact with MHC I proteins.
NK cells can kill antibody-coated target cells, which is mediated through Fc receptor present on its surface. This is called antibody-dependent cell cytotoxicity (ADCC).



NK cells also participate in Graft vs Host reaction in recipient of bone marrow transplants. NK cells can be activated by IL-2 so that their cytotoxic capacity is enhanced. Such cells are called Lymphokine Activated Killer cells (LAK) and have been used clinically to treat tumours. LAK cells have enhanced cytolytic activity and are effective against wide range of tumour cells. Activated NK cells produce cytokines such as IFN-γ, TNFα, GM-CSF and CSF-1 all of which are immunomodulators.

LYMPHOCTE RECIRCULATION:



The movement of lymphocytes via the blood stream and lymphatics from peripheral tissue to another is called lymphocyte recirculation. Lymphocytes are migratory cells; mature lymphocytes continually migrate in and out of all peripheral lymphoid tissue. At an average each cell changes location once or twice each day. At any given point of time 1-2% of lymphocytes will be in transit. In most lymphoid organs, they enter through blood and exit through lymphatics, but in spleen they enter and leave directly through blood. As lymphocytes migrate, they can survey the body for foci of infection or presence of foreign antigens. Such a movement also helps to maintain a balance in distribution of lymphocytes in the body.