IMMUNOLOGY

War Against The Pathogens

INTRODUCTION TO IMMUNE CELLS

- HEMATOPOIESIS
- CELLS OF CMP
- CELLS OF CLP

A successful immune response to a pathogen depends on fine interactions among diverse cell types, which include the innate immune cells and antigen presenting cells like phagocytes and dendritic cells; the B cells and the T cells. All the immune cells develop, within the bone marrow, from a single type of stem cell called the Hematopoietic Stem Cell (HSC). Stem cells are defined as cells having – i) the ability to regenerate or 'self renewal' and

ii) the ability to differentiate into all diverse cell types.

Embryonic Stem Cells (ESCs) have the capacity to generate every specialized cell type in an organism (i.e. pluripotent). The HSCs are considered as the paradigmatic adult stem cells because it can differentiate into all types of blood cells.

An HSC that is induced to differentiate loses its self-renewal capacity and differentiates into one of the two broad lineage commitment choices. It can either become a common myeloid-erythroid progenitor (**CMP**), which gives rise to all red blood cells (RBCs), granulocytes, monocytes and macrophages, or it can become a common lymphoid progenitor (**CLP**), which gives rise to B cells, T cells and NK cells (Owen *et al.* 2013). As HSCs progress along their chosen lineages they lose the capacity to contribute to other cellular lineages. Interestingly, both myeloid and lymphoid lineages give rise to dendritic cells, which are antigen presenting cells (**APCs**), with diverse features and functions that play an important role in initiating adaptive immune response.

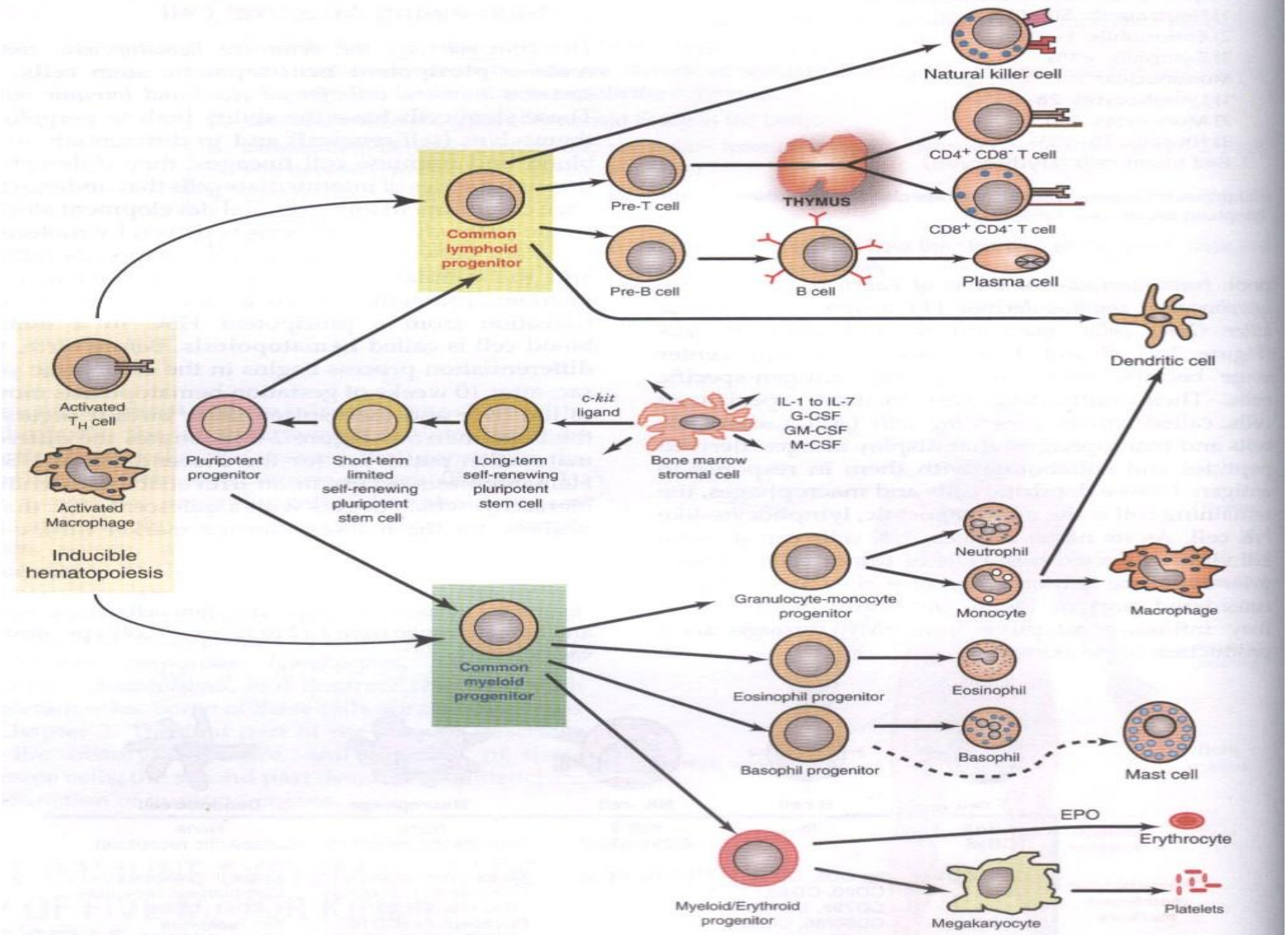
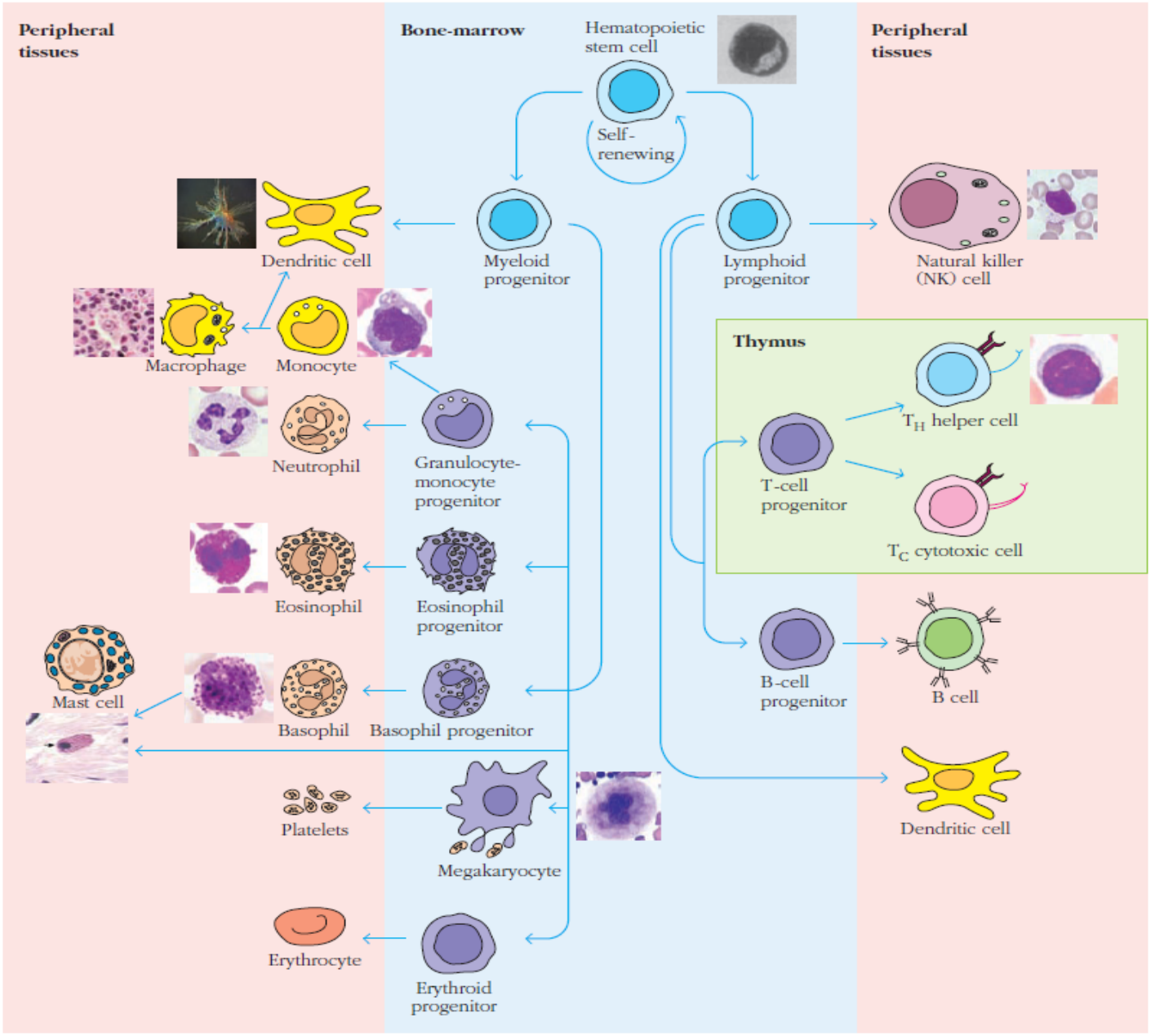


FIGURE 2-3 Maturation of immune system cells. Myeloid and lymphoid cells develop in adults from pluripotent (many different potentials) stem cells in the bone marrow. This development is driven by colony-stimulating factors. Nonlymphoid stem cells give rise to *elements of the peripheral blood*, such as erythrocytes, platelets, granulocytes (basophils, eosinophils, or neutrophils), and *monocytes* (precursor cells for *macrophages* and some *dendritic cells*). Lymphoid stem cells can develop along two pathways. If these stem cells migrate through the thymus, they become *T lymphocytes* or *T cells*, represented by $CD4^+$ T and $CD8^+$ T cells. If the lymphoid stem cells mature in the bone marrow, the cells become a population of lymphocytes, called *B lymphocytes* or *B cells*.



Hematopoiesis. Self-renewing hematopoietic stem cells give rise to lymphoid and myeloid progenitors. Most immune cells mature in the bone marrow and then travel to peripheral organs via the blood. Some, including mast cells and macrophages, undergo further maturation outside the bone marrow. T cells develop to maturity in the thymus.

Cells of The Myeloid Lineage

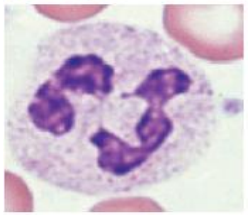
CMP gives rise to red blood cells (erythroid cells) as well as various types of white blood cells (myeloid cells). Myeloid cells are the first to respond to the invasion of a pathogen and communicate its presence to cells of the lymphoid lineage. The different types of myeloid cells include: -

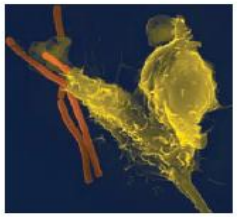
a) Granulocytes: These are at the front lines of the attack during an immune response and are considered as part of the innate immune system. These are the WBCs or leukocytes that are classified as neutrophils, basophil, mast cells or eosinophils on the basis of differences in cellular morphology and the staining behaviour of their characteristic cytoplasmic granules.

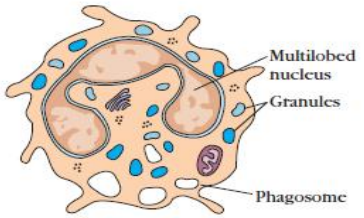
Neutrophils: They constitute the majority (50-70%) of circulating lymphocytes and are much more numerous than eosinophils (1-3%), basophils (<1%) or mast cells (<1%). After differentiation in the bone marrow neutrophils are released into the peripheral blood, where they circulate 7-10 hours before migrating to the tissues, where they have a life span of few days. The number of circulating neutrophils increases significantly in response to infections. They are recruited to the site of infection in response to inflammatory signals (*e.g.* chemokines) generated by innate cells that have engaged the pathogen. Once in the tissues, neutrophils phagocytose bacteria very efficiently and also secrete a range of proteins that have anti-microbial effects and tissue remodeling potential.

Basophils: These are non-phagocytic granulocytes that contain large granules filled with basophilic proteins. Basophils release the contents of their granules in response to binding of circulating antibodies. Histamine, one of the best known proteins in basophilic granules, increases blood vessel permeability and smooth muscle activity. Like neutrophils, basophils may also secrete cytokines that modulate the adaptive immune response.

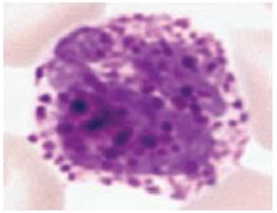
(a) Neutrophil

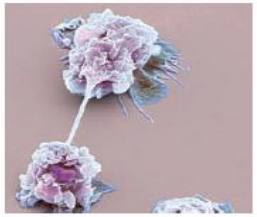


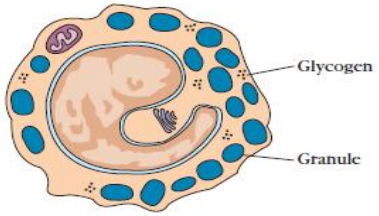




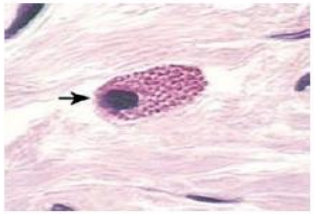
(b) Basophil

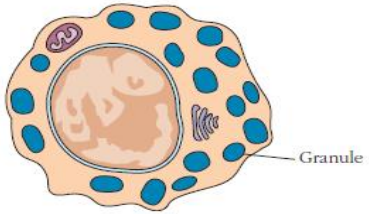




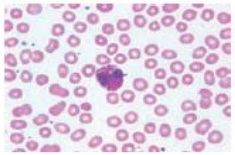


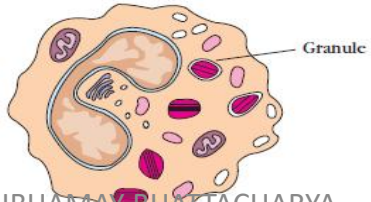
(c) Mast cell





(d) Eosinophil





Mast Cells: These cells are released from the bone marrow into the blood as undifferentiated cells. They mature only after leaving bloodstream. Mast cells can be found in a wide variety of tissues, including the skin, connective tissues of various organs and mucosal epithelial tissues of the respiratory, genito-urinary and digestive tracts. Like basophils, these cells also have large number of granules that contain histamine and other pharmacologically active substances.

Note: They also play an important role in development of allergies. They express high-affinity F_C receptors for IgE. The allergen-induced cross-linking of adjacent mast cell bound IgE causes the mast cell to release their granule contents, as well as start new synthesis and release off other mediators. (Elgert, 2009)

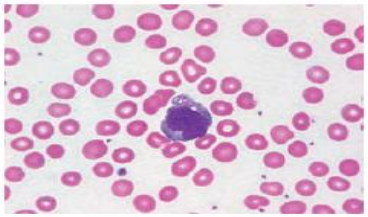
Eosinophils: These are motile phagocytic cells like neutrophils that can migrate from blood to tissue spaces. However, their phagocytic role is significantly less important than that of neutrophils. It is, therefore, thought that they play their most important role in defence against multicellular organisms including worms. Like neutrophils and basophils eosinophils may also secrete cytokines that regulate B & T lymphocytes, thereby influencing adaptive immune response.

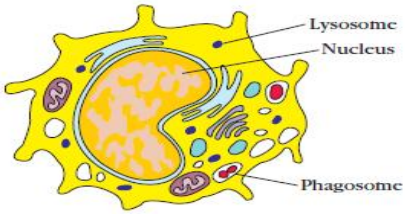
Note: It may also contribute to asthma and allergy symptoms in areas with low parasitic occurrence.

b) Myeloid Phagocytic Cells: Myeloid progenitors give rise to mononuclear phagocytes and polymorpho-nuclear phagocytes. The mononuclear phagocytic system consists of monocytes in blood and macrophages in tissues. Monocytes circulate in the bloodstream for about 8hrs, during which they enlarge; then migrate into the tissues and differentiate into specific tissue macrophages or into dendritic cells. Macrophage-like cells serve different functions in different tissues and are named according to their tissue location: **Alveolar macrophages** in the lung; **Histiocytes** in connective tissues; **Kupffer cells** in the liver; **Mesangial cells** in the kidney; **Microglial cells** in the brain; **Osteoclasts** in bone.

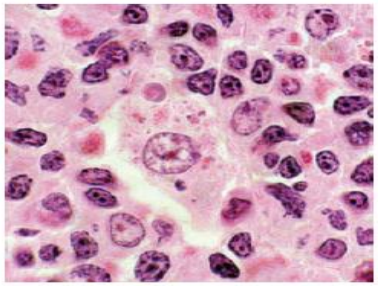
Note: Although, erythroid cells and megacaryotes belong to CMP cell lineage, they have very little role in immune response system.

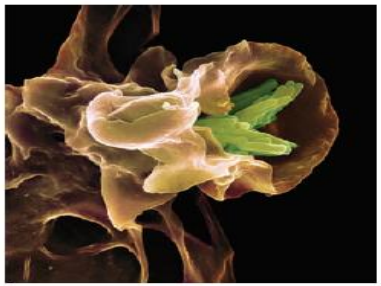
(a) Monocyte

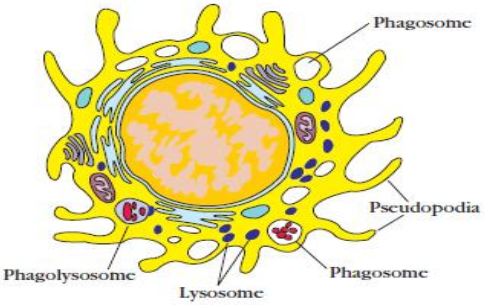




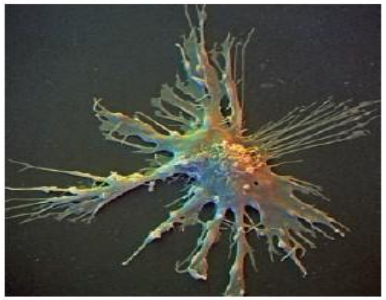
(b) Macrophage

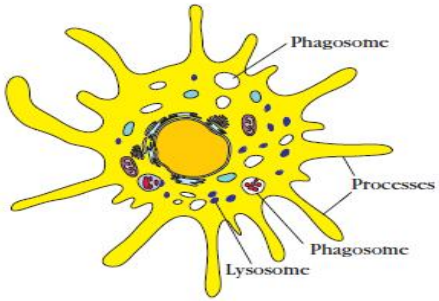




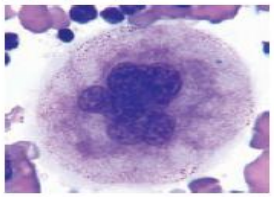


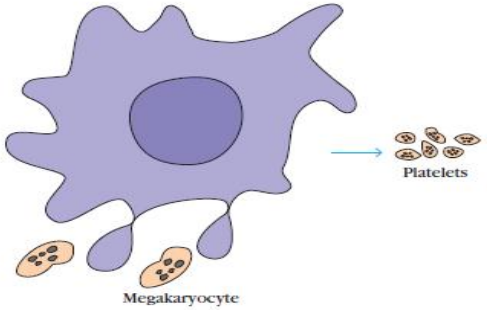
(c) Dendritic cell





(d) Megakaryocyte





Cells of The Lymphoid Lineage

Lymphocytes are the primary cells responsible for the adaptive immune system. They represent 22-40% of the body's leucocytes and are found circulating between blood and lymphoid tissues. They can be broadly sub divided into three major populations on the basis of functional and phenotypic differences: B lymphocytes (B cells), T lymphocytes (T cells) and Natural Killer (NK) cells. These non-phagocytic cells are morphologically indistinguishable but functionally different. Differentiation of lymphocyte sub-population is heavily dependent on expression of specific surface protein molecule. These surface proteins expressed by immune cells are referred to as the **cluster of differentiation (CD).** It is defined as the cluster of monoclonal antibodies that recognize different antigenic determinants on a particular lineage or differentiation stage of a cell. More than 325 human leukocyte CD designations has been described till date.

In addition to CD markers, each T cell or B cell also expresses antigen specific receptors, the B-Cell Receptor (**BCR**) and the T Cell Receptor (**TCR**) respectively. Mature B-cells and T-cells, though ready to encounter an antigen are considered naive until they do so. Contact with antigen induces naive lymphocytes to proliferate and differentiate into both **effector cells and memory cells.** Effector cells carry out specific functions to combat pathogens, while the memory cells persist in the host for future use. Upon re-challenge with same antigen the memory cells mediate immune response that is quicker and greater in magnitude. The first encounter with antigen is called the primary response and the re-encounter is called the secondary response.

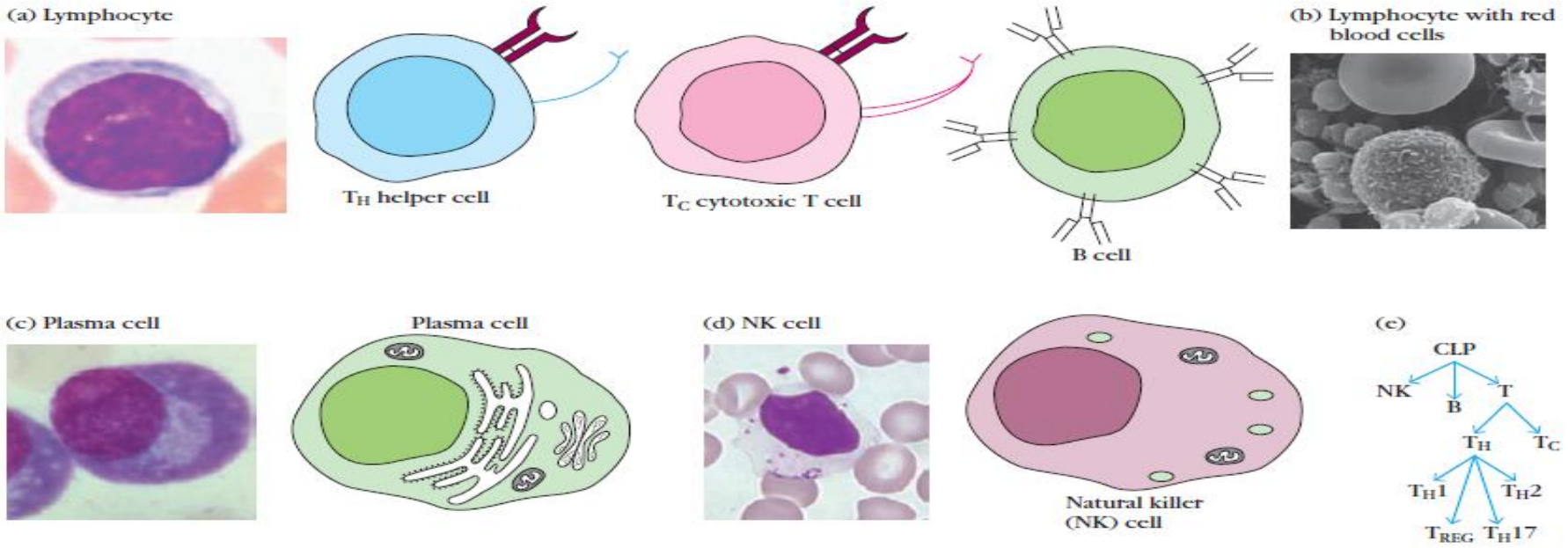
B Cells or B Lymphocytes: The B lymphocytes (or B cells) derived its later designations from its site of maturation; the *Bursa of Fabricius* in birds, in humans, mice and other mammals, its site of maturation is the **bone marrow**. Each B cell carries the genetic instruction to produce immunoglobulin (i.e. antibody) of unique antigen specificity. These immunoglobulins are initially expressed as membrane receptors or membrane bound immunoglobulins. Mature B cells do not secrete antibody, but readily differentiate into terminal non-dividing effector cells known as **Plasma Cells (PCs)**. Plasma Cells lose expression of surface immunoglobulin and become highly specialise for secreting antibody. Although, some long lived population of PCs are found in bone marrow, many die within one or two weeks.

T Cells or T lymphocytes: T lymphocytes (or T cells) derived their designations from their site of maturation, the **thymus**. Unlike B cells, the T cells can only recognize processed pieces of antigenic peptides bound to cell membrane proteins, called Major Histocompatibility Complex (**MHC**) molecules. MHC molecules are genetically diverse glycoproteins found on cell membranes of self cells. There are chiefly two types of MHC molecules; Class I MHC molecules, which are expressed by nearly all nucleated cells of vertebrate species and Class II MHC molecules, which are expressed by professional APCs and few other cell types.

T cells are divided in to two sub types – T helper cells (i.e. T_H cells) and T cytotoxic (i.e. T_C) cells. These can be distinguished from one another by the presence of specific CD markers. T cells displaying $CD4^+$ generally functions as T_H cells and recognize antigen bound to Class II MHC molecules, whereas those displaying $CD8^+$ generally functions as T_C cells and recognize antigen bound to Class I MHC molecules. Expression of specific CD markers is determined by a process called **thymic selection**, occurs during maturation in thymus.

Naïve $CD8^+$ T cells browse the surfaces of antigen presenting cells with their T-cell receptors. If and when they bind to an MHC-peptide complex, they become activated, proliferate, and differentiate into an effector cell called a **cytotoxic T lymphocyte (CTL).** The CTL has a vital function in monitoring the cells of the body and eliminating any cells that display foreign antigen complexes with class I MHC, such as virus-infected cells, tumor cells, and cells of a foreign tissue graft . To proliferate and differentiate optimally, naïve $CD8^+$ T cells also need help from mature $CD4^+$ T cells. Naïve $CD4^+$ T cells also browse the surfaces of antigen presenting cells with their T-cell receptors. If and when they recognize an MHC-peptide complex, they can become activated and proliferate and differentiate into one of a variety of effector T cell subsets (see Figure 2-4e). **T helper type 1 (T_H1) cells regulate the immune response to intracellular** pathogens, and **T helper type 2 (T_H2) cells regulate the** response to many extracellular pathogens. Two additional TH cell subsets have been recently identified. **T helper type 17 cells (T_H17), so named because they secrete IL-17, play an** important role in cell-mediated immunity and may help the defence against fungi. **T follicular helper cells (T_{FH})** play an important role in humoral immunity and regulate B-cell development in germinal centres. Which helper subtype dominates a response depends largely on what type of pathogen (intracellular versus extracellular, viral, bacterial, fungal, helminth) has infected an animal. Each of these $CD4^+$ T-cell subtypes produces a different set of cytokines that enable or "help" the activation of B cells, T_C cells, macrophages, and various other cells that participate in the immune response.

Another type of CD4 $^{+}$T cell, the **regulatory T cell (T_{REG}),** has the unique capacity to inhibit an immune response. These cells can arise during maturation in the thymus from auto-reactive cells (natural T_{REG}), but also can be induced at the site of an immune response in an antigen-dependent manner (induced T_{REG}). They are identified by the presence of CD4 and CD25 on their surfaces, as well as the expression of the internal transcription factor FoxP3. T_{REG} cells are critical in helping us to quell auto-reactive responses that have not been avoided via other mechanisms. In fact, mice depleted of T_{REG} cells are afflicted with a constellation of destructive self-reactive inflammatory reactions.



Examples of lymphocytes. (a, c, d) H&E stain of blood smear showing typical lymphocyte. Note that naïve B cells and T cells look identical by microscopy. (b) Scanning electron micrograph of lymphocytes and red blood cells. Cartoons depicting the typical morphology of the cells indicated accompany each image (including three different lymphocytes that would all have the same appearance). Note the enlarged area of cytoplasm of the plasma cell, which is occupied by an extensive network of endoplasmic reticulum and Golgi—an indication of the cell's dedication to antibody secretion. The NK cell also has more cytoplasm than a naïve lymphocyte; this is full of granules that are used to kill target cells. (e) A branch diagram that depicts the basic relationship among the lymphocyte subsets described in the text.

Natural Killer Cells

Natural killer (NK) cells are lymphoid cells that are closely related to B and T cells. However, they do not express antigen specific receptors and are considered part of the innate immune system. They are distinguished by the expression of a surface marker known as NK1.1, as well as the presence of cytotoxic granules. Once referred to as "large granular lymphocytes" because of their appearance under a microscope, NK cells constitute 5% to 10% of lymphocytes in human peripheral blood. They are efficient cell killers and attack a variety of abnormal cells, including some tumor cells and some cells infected with virus. They distinguish cells that should be killed from normal cells in a very clever way: by "recognizing" the absence of MHC class I, which is expressed by almost all normal cells, but is specifically down-regulated by some tumors and in response to some viral infections. NK cells express a variety of receptors for self MHC class I that, when engaged, inhibit their ability to kill other cells. When NK cells encounter cells that have lost their MHC class I, these receptors are no longer engaged and can no longer inhibit the potent cytotoxic tendencies of the NK cell, which then releases its cytolytic granules and kills the abnormal target cell. NK cells also express receptors for immunoglobulins and can therefore decorate themselves with antibodies that bind pathogens or proteins from pathogens on the surface of infected cells. This allows an NK cell to make a connection with a variety of target cells (independently of their MHC class I expression).

NKT Cells

Another type of cell in the lymphoid lineage, **NKT cells,** received a great deal of recent attention and share features with both conventional T lymphocytes and NK cells. Like T cells, NKT cells have T-cell receptors (TCRs), and some express CD4. Unlike most T cells, however, the TCRs of NKT cells are not very diverse and recognize specific lipids and glycolipids presented by a molecule related to MHC proteins known as CD1. Like their innate immune relatives, NK cells, NKT cells have antibody receptors, as well as other receptors classically associated with NK cells. Activated NKT cells can release cytotoxic granules that kill target cells, but they can also release large quantities of cytokines that can both enhance and suppress the immune response. They appear to be involved in human asthma, but also may inhibit the development of autoimmunity and cancer. Understanding the exact role of NKT cells in immunity is one research priority.

Antigen Presenting Cells:

A group of phagocytic cells (chiefly monocytes, macrophages and dendritic cells) that have professional antigen presenting cell (**APC**) function. These are considered as cellular bridges between the innate and adaptive immune systems. They make contact with a pathogen at sight of infection and communicate this encounter to T lymphocytes in the lymph node (i.e. "antigen presentation").