

Outline

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- Formation of blood cells
- Types of leucocytes
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- Phagocytosis and intracellular killing
- Cells of the adaptive immune system
- Cell-Mediated Cytotoxicity

Introduction

- Blood cells or leucocytes serve as sentinels and defenders against infection.
- They move around the body via the lymphatic and blood circulatory systems.
- Leucocytes are classified by morphology- number of nuclei lobes and presence or absence of cytoplasmic granules.
- Leukocytes may be found as individual cells throughout the body, as accumulations within lymphoid organs (e.g., spleen, lymph nodes) and at sites of infection or inflammation.

Formation of blood cells

- All bloodborne cells originate in the bone marrow.
- Pluripotent hematopoietic stem cell in the bone marrow give rise to two major lineages; **a myeloid lineage** and **a lymphoid lineage**.
- Cells of the myeloid lineage differentiate further into **platelets, erythrocytes, eosinophils, basophils (and mast cells), neutrophils, monocytes/macrophages, and some dendritic cells.**
- Cells of the lymphoid lineage differentiate further into **T and B lymphocytes, NK cells, and some**

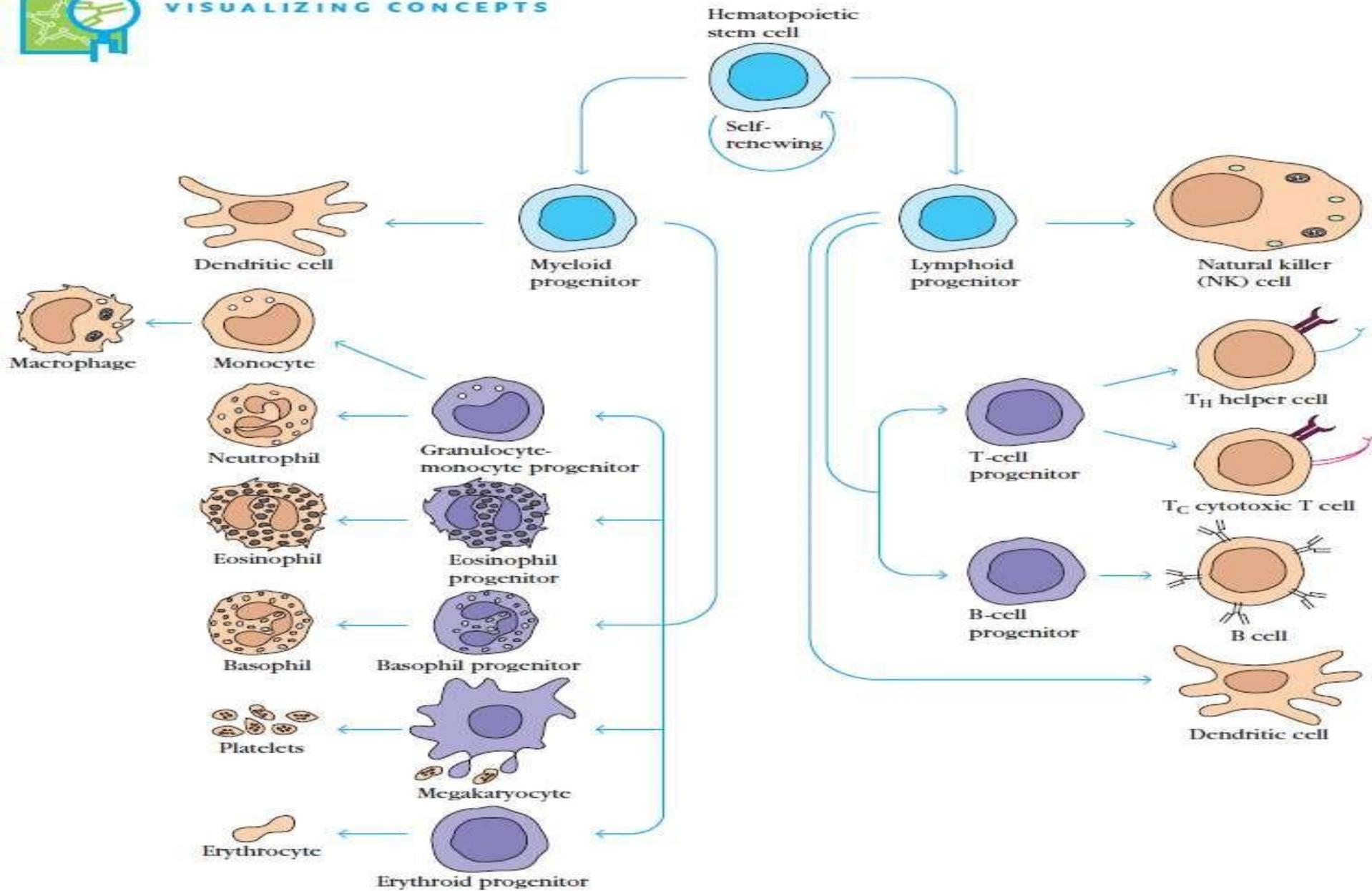


Fig 1: Hematopoiesis

Types of leucocytes

- White blood cells that have multilobed nuclei and contain conspicuous cytoplasmic granules are known as **granulocytes** .
- Others with a single, unlobed nucleus and cytoplasm that contains few or no granules are known as **agranular** leukocytes.
- Agranular leukocytes derive from lymphoid or myeloid lineage precursors and account for approximately 35% to 38% of the leukocytes in

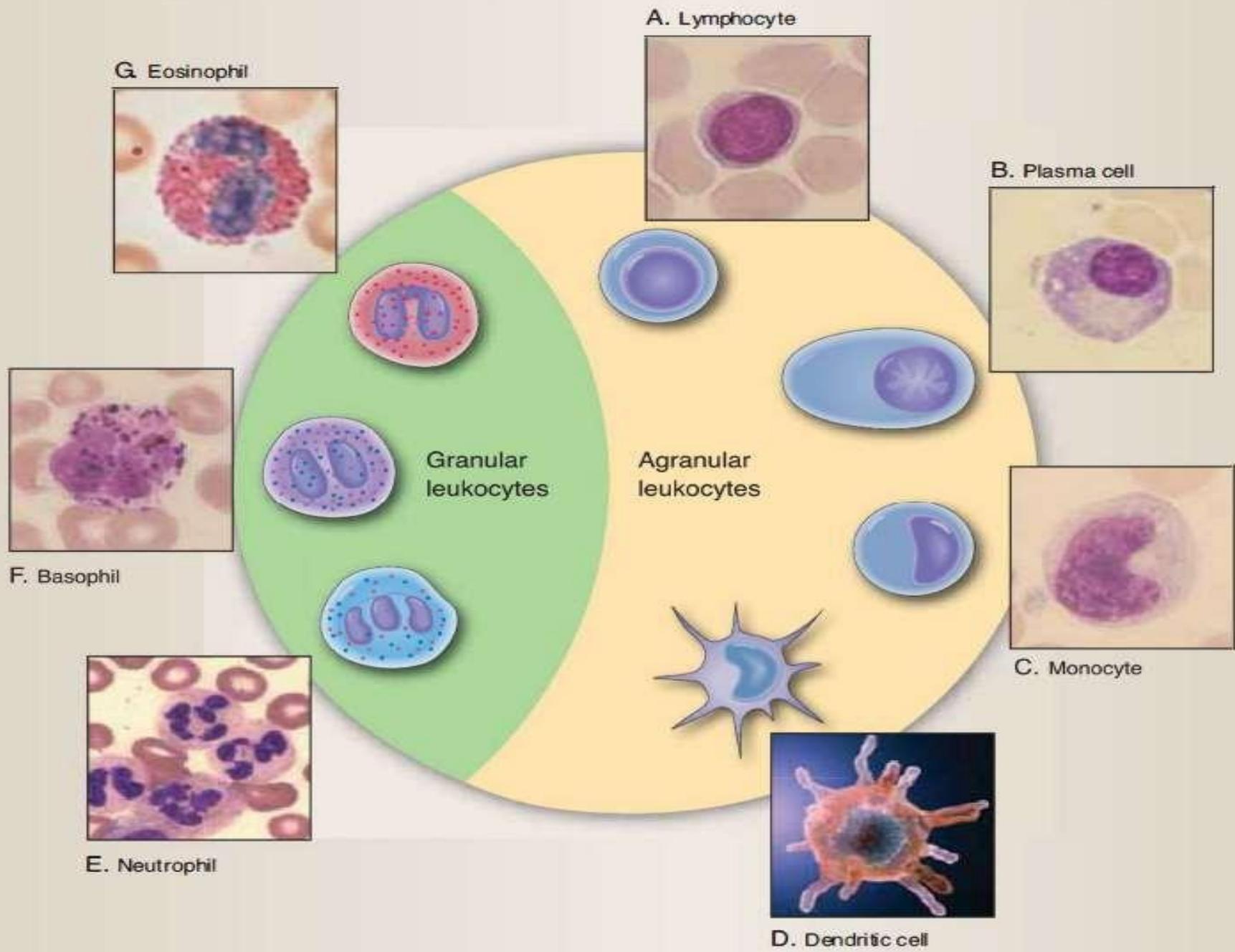


Fig 2: Types of leukocytes.

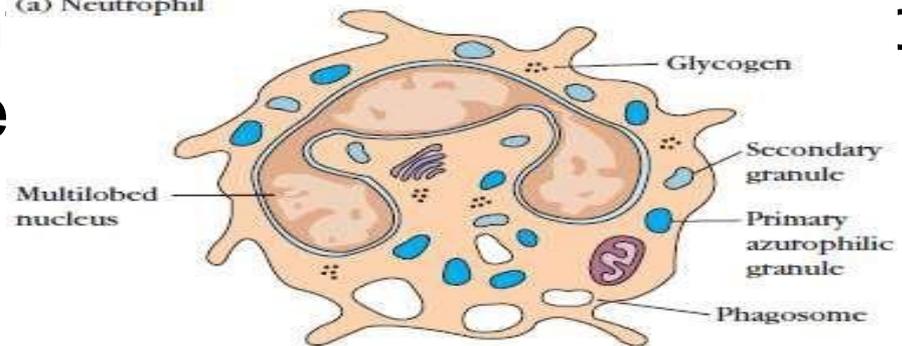
Cells of the innate immune system

- Myeloid Cells: First line of defense against invading organisms in non-specific innate immunity.
 - Neutrophils
 - Eosinophils
 - Basophils/Mast cells
 - Monocytes/Macrophages/Dendritic Cells
- Lymphoid Cells:

Neutrophils

- Comprises approximately 60% of the peripheral blood leukocytes, neutrophils are the most numerous leukocyte population.
 - Neutrophils have multi lobed nuclei (2-5) and cytoplasmic granules that stain with both acid and basic dyes.
 - often called polymorphonuclear cells (PMN's).
- **The neutrophil's main role is in inflammation.**
 - First to arrive at inflammation site
 - Leave blood/endothelium into tissue (extravasation).
- Neutrophils are attracted in factors stimulated by tissue
 - complement proteins, clotting proteins and T cell derived

(a) Neutrophil



- In the tissues, neutrophils are active phagocytes.
- They destroy ingested microorganisms via **oxygen-dependent** or **independent pathways**.
 - Produce myeloperoxidases to assist oxidated antimicrobial effects.
 - Produce lactoferrin and lysozyme as direct antimicrobial agents.
 - Produce leukotrienes and prostaglandins, products of the lipoxygenase and cyclo-oxygenase pathways, to mediate vascular functions.
- Deficiencies in pathways increase susceptibility to infections.

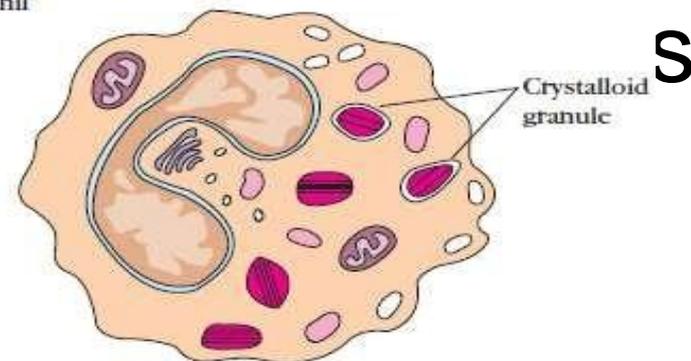
Characteristics of Neutrophil granules

Primary granules	Secondary granules
Azurophilic; young neutrophils	Specific for mature neutrophils
Contain: cationic proteins, lysozyme, defensins, elastase and	Contain: Lysozyme, NADPH oxidase components and
myeloperoxidase	Lactoferrin and B12-binding protein

Eosinophils

- Eosinophils have bilobed nuclei and cytoplasmic granules that stain with the acid dye eosin (hence its name).
 - Constitute 1%-3% of circulating leucocytes.
- Involved in asthma.
- Eosinophils are non-phagocytic, and are particularly active in parasitic infections.

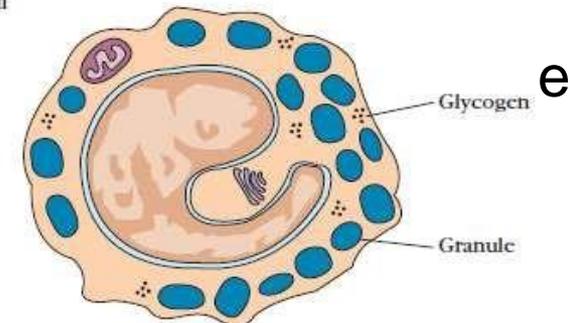
(b) Eosinophil



Basophils

- Basophils have bilobed nuclei and cytoplasmic granules that stain with the basic dye methylene blue.
 - Found in low numbers in the blood (<1%). Act like mast cells.
 - They are nonphagocytic
- Involved in allergic reactions (Type I hypersensitivity responses).
 - Have high affinity Fc receptors for IgE on their surface.
 - When an individual is exposed to an allergen, specific IgE is produced. This IgE binds to the surface of basophils.
 - Upon re-exposure to the allergen, the allergen binds to the surface of basophils resulting in degranulation.
 - Cross-linking of the IgE causes the basophil to release pharmacologically active mediators (histamine, prostaglandins, leukotrienes).

(c) Basophil



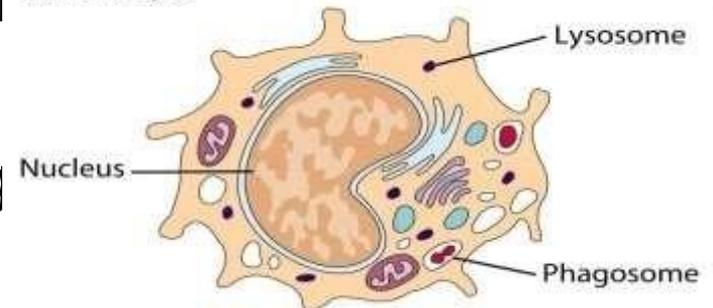
Mast cells

- Similar importance in allergic reactions as basophils, but only found in tissues.
- Contain granules with preformed mediators to be released after stimulation
 - histamine, prostaglandins
 - leukotrienes
- Stimulation of mast cells occurs by the anaphylatoxins (complement proteins C3a and C5a) or by cross-linking of surface immunoglobulin (IgE).

Monocytes/Macrophages

- Circulate in the blood after leaving the bone marrow.
- Survive only a day or so before they enter the tissue to mature into macrophages.
- Involved in phagocytosis and intracellular killing of microorganisms.
 - Generation of toxic metabolites through respiratory burst.
 - Production of nitric oxide, hydrogen peroxide, superoxide anion.
- Monocytes/Macrophages are antigen presenting cells.
 - Degradative enzymes in lysosomal granules
 - Chew ingested proteins.
 - Present to adaptive cells.

(a) Monocyte



Macrophages

- When monocytes enter the tissues and become macrophages:
 - Enlarge and increase production of intracellular lysozymes
 - Greater phagocytosis.
 - Can live for years in tissue; highly motile.
- Activation of these cells occurs by phagocytosis of antigens, or in response to T cell derived cytokines.
- Activated macrophages recognize and remove unwanted particulate matter including products

After activation, these cells secrete cytokines, chemokines, lysozymes and other factors to upregulate immune response. In chronic inflammation, macrophage scavengers can become “giant cells” or “foamy macrophages”

(b) Macrophage

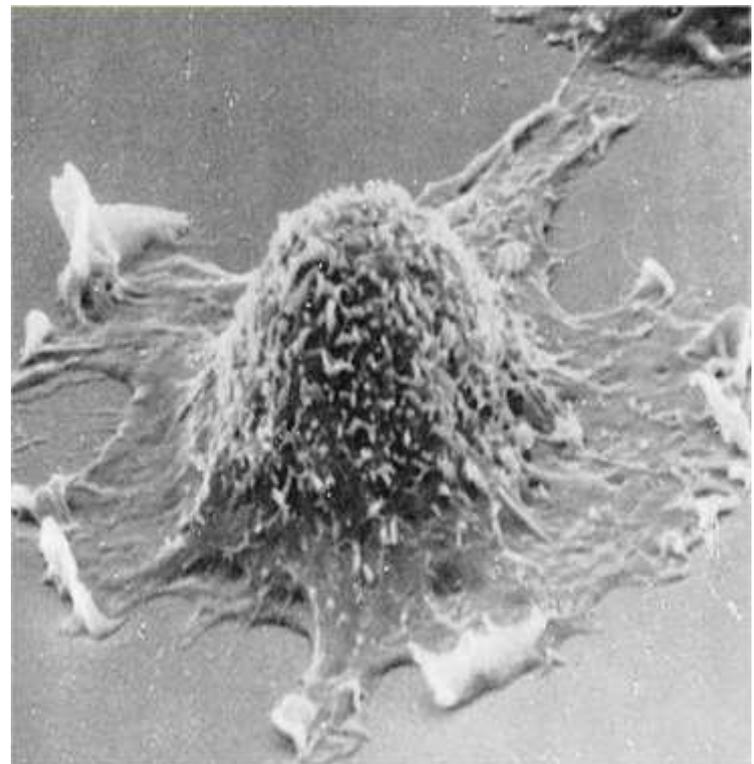
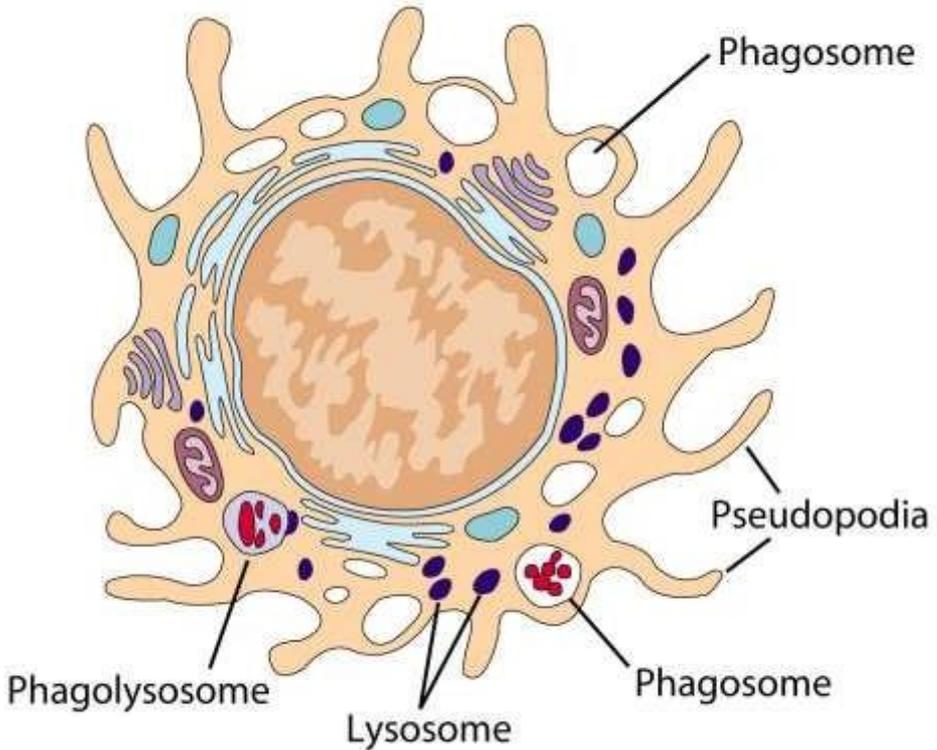
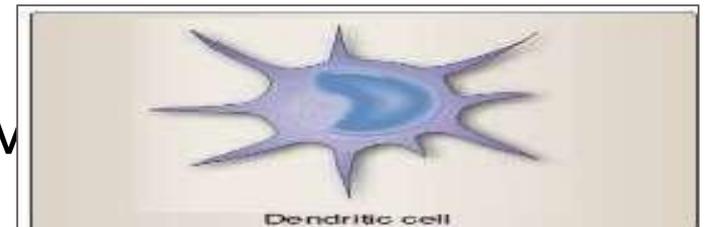


Fig: Electron micrograph of macrophage

Dendritic cells

- Specialized phagocytic cells found in most tissues.
- Arise both from the myeloid and lymphoid lineages.
- Abundant at interfaces between the external and internal environments (skin, lining of the gastrointestinal tract), where they encounter invading pathogens.
- Actively motile; continuously sample surroundings by endocytic processes.
- Dendritic cells are very efficient at activating T cells.



Natural Killer cells

- Also known as large granular lymphocytes (LGLs)
- Functionally cytotoxic representing an innate population that kill virally infected or tumor target cells.
- Killing is nonspecific - they do not need to recognize foreign antigens presented on the target cell.

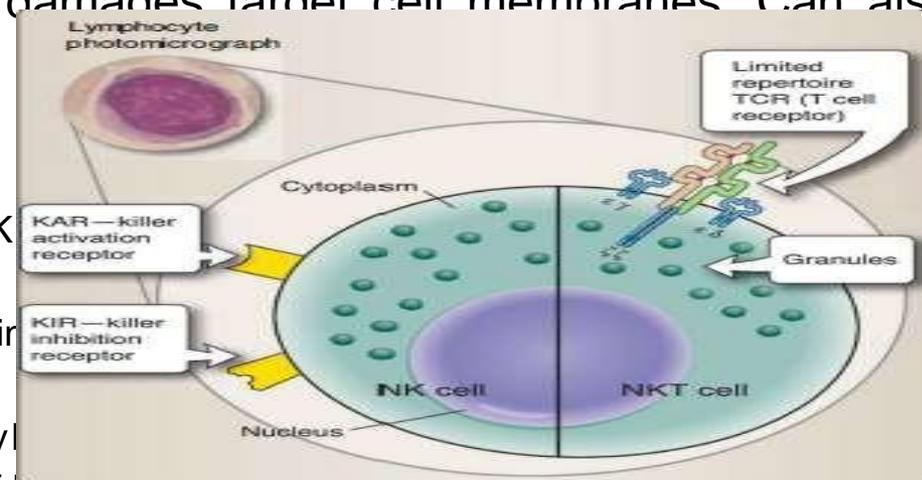
– NK cells do not have a specific cell receptor. Target recognition occurs by a Killer Inhibitory Receptor, KIR, which assess MHC I molecules on the target cell surface. MHC 1 molecule is lacking on infected and tumor targets.

- Kill targets by releasing perforin which damages target cell membranes. Can also induce apoptosis in the target cell.

- NK cells is different from NK T cells.

-NKT cells has some of the attributes of T cell and NK
Like T cells, NK1-T cells have T cell receptors (TCRs). Unlike most T cells, the TCRs of NK1-T cells interact with MHC-like molecules called CD1 rather than with class I or class II MHC molecules. Like NK cells, they have variable levels of CD16 and other receptors typical of NK

cells and they can kill cells



Phagocytosis and intracellular killing

- Phagocytosis is the engulfment and degradation of microbes and other particulate matter by cells such as macrophages/monocytes, dendritic cells, and neutrophils .

- Steps in phagocytosis-
 - 1) *Detection* of the foreign particle and movement of the phagocyte to the area by *chemotaxis*.
 - 2) *Attachment* of the foreign particle to the phagocyte.
 - 3) Engulfment or *ingestion* of the foreign particle into a vesicle called a phagosome.
 - 4) Fusion with lysosome and formation of the *phagolysosome*.
 - 5) Intracellular *killing and digestion*.
 - 6) In the case of macrophages, *exocytosis and antigen presentation*.

- Phagocytes detect microbes by the presence of N-formylated peptides, activated complement proteins and the mediators of inflammation.

- Phagocytes attach to microbes using opsonins, such as IgG and the complement protein C3b. In the absence of opsonins, phagocytes can

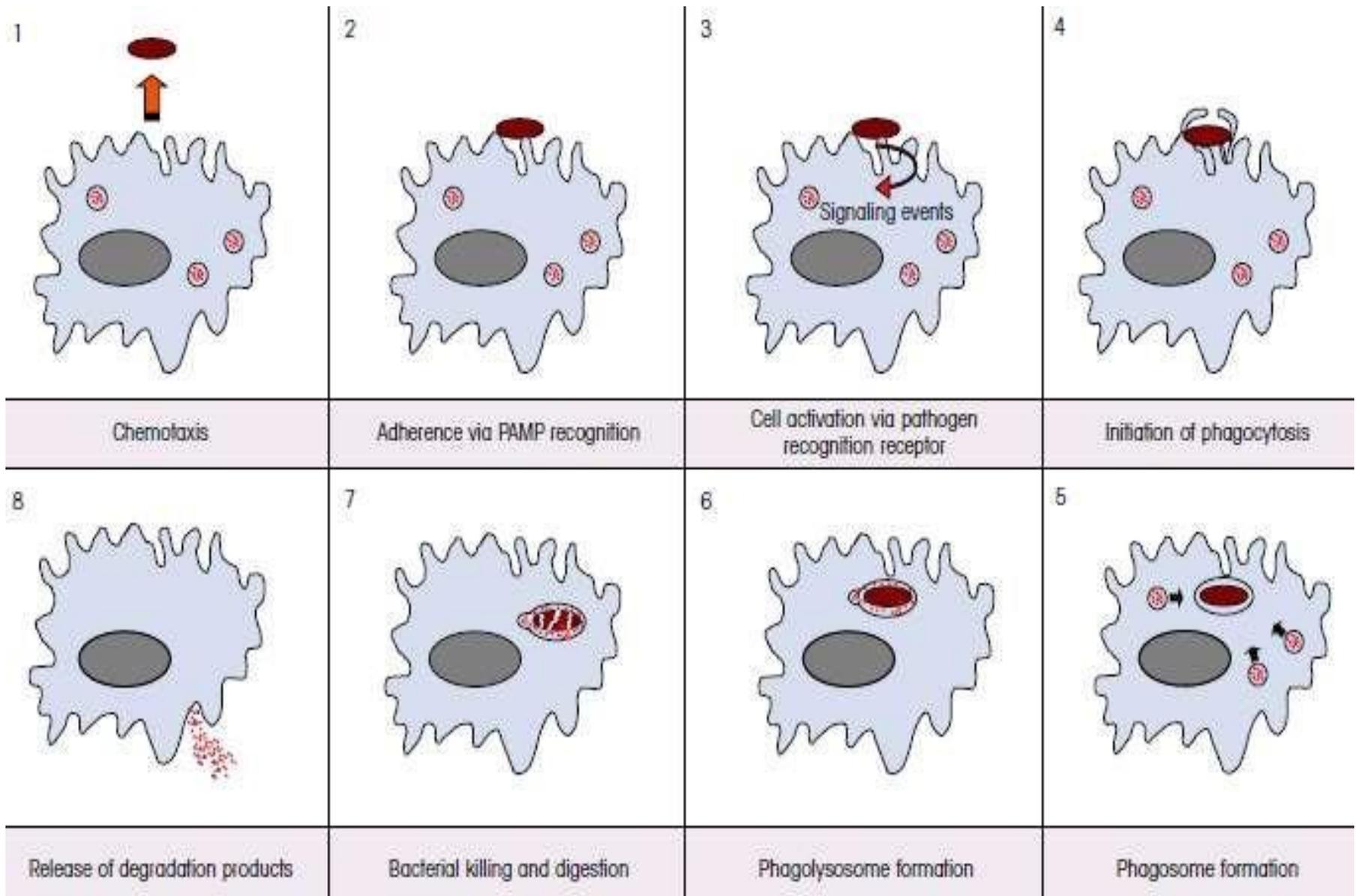


Fig: Steps in phagocytosis

Pathways of intracellular killing

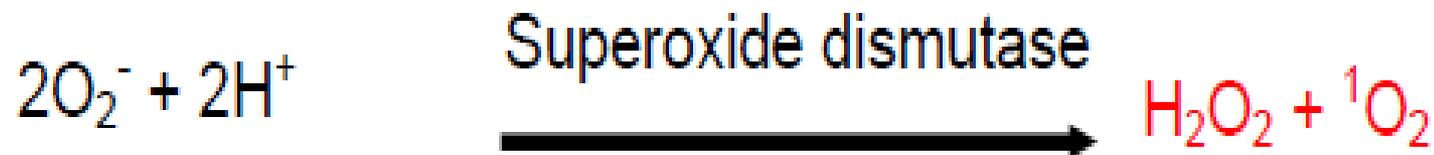
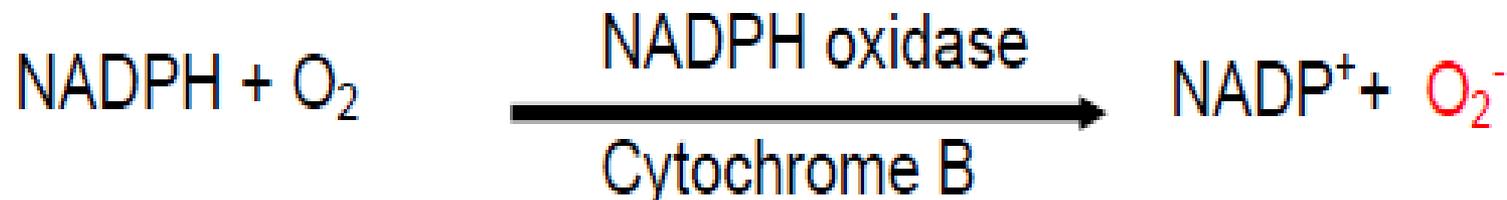
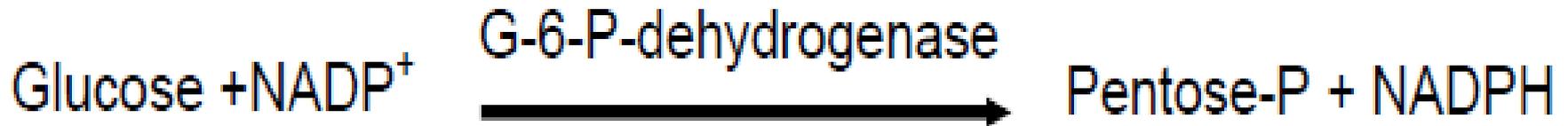
- Lysosomes employ multiple mechanisms for killing and degrading ingested microbe. These include;
 - Oxygen-independent killing
 - Oxygen-dependent-MPO independent killing
 - Oxygen-dependent-MPO dependent killing
 - Nitric Oxide mediated killing
- In oxygen-independent killing, activated phagocytes synthesize **lysozyme** and various **hydrolytic enzymes** whose degradative activities do not require oxygen.

Mediators of oxygen-independent killing in phagolysosome

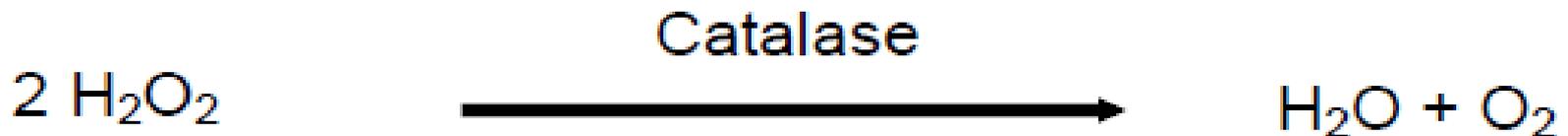
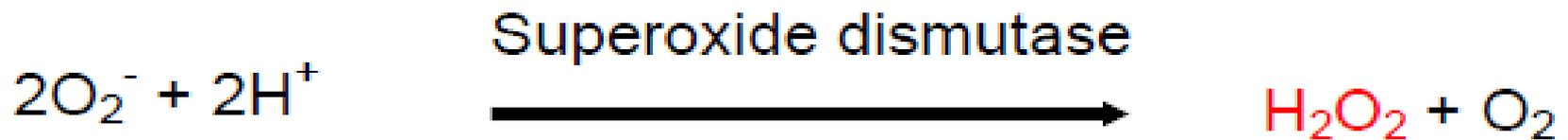
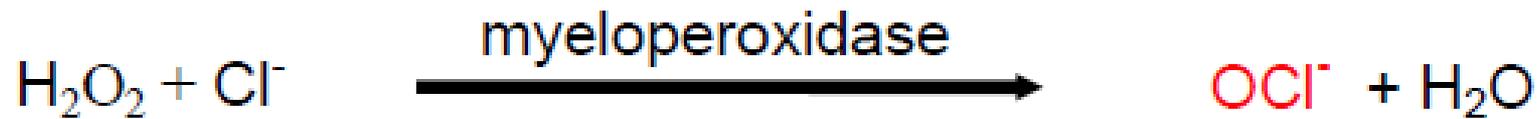
Effector Molecule	Function
Cationic proteins (cathepsin)	Damage to microbial membranes
Lysozyme	Hydrolyses mucopolysaccharides in the cell wall
Lactoferrin	Deprives pathogens of iron
Hydrolytic enzymes (proteases)	Digests killed organisms

- During phagocytosis there is an increase in glucose and oxygen consumption which is referred to as the **respiratory burst**.
- The consequence of the respiratory burst is that a number of oxygen-containing compounds are produced which kill the bacteria being phagocytosed. This is referred to as oxygen-dependent intracellular killing

Oxygen-dependent myeloperoxidase-independent reactions

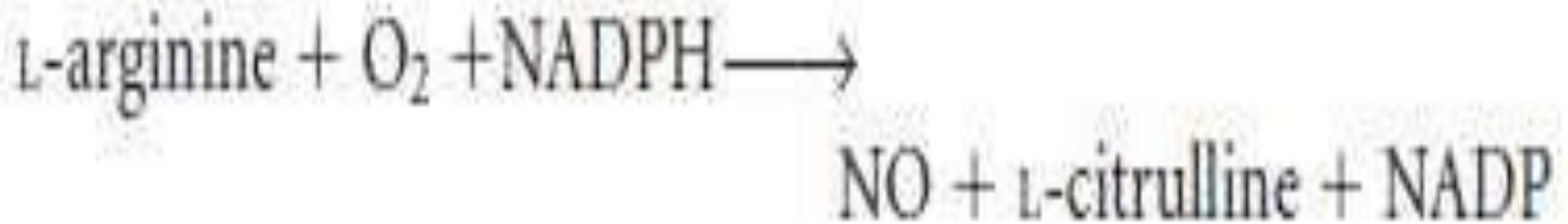


Oxygen-dependent myeloperoxidase-dependent reactions



Detoxification reactions

Nitric oxide-dependent killing



- Once microorganisms are destroyed, the unwanted organic material is expelled from the cell in a process called egestion.
- Egestion is the opposite of ingestion and the molecular mechanism is basically the reverse of phagocytosis with the microbial leftovers being dumped into the blood and lymph.
- Some of this microbial debris are not egested, but binds to special protein complexes (called Major Histocompatibility Complex molecules) on the membranes of macrophages for presentation to the immune system.

Cells of the adaptive immune system

- Adaptive immune responses are mediated by a specialized group of leukocytes, **the lymphocytes**, which include **T and B lymphocytes** (T cells and B cells) that specifically recognize foreign material or antigens.
- All lymphocytes are derived from bone marrow stem cells, but T cells then develop in the **thymus**, while B cells develop in the **bone**

B Lymphocytes

- Develop from stem cells in the bone marrow.
 - Produce **antibodies** with specificity for antigens and display it on their surfaces to function as **BCRs**.
 - Integral in humoral immunity
 - **Plasma cells** = terminally differentiated B cells that secrete immunoglobulins.
 - **Memory cells**- secondary immune response is swifter and stronger.
- Upon activation, a B cell can switch to produce a different class of antibody, with the same antigen specificity.
- Activation into antibody secreting cells is antigen-dependent.

T-Lymphocytes

- T lymphocytes develop in the thymus.
 - Regulate immune responses.
 - Integral in cell mediated immunity.
 - Critical in B cell-antibody production.
- Mature T cells display either **CD4** or **CD8**.
 - Cells with a CD4 marker are called helper T cells (Th cells).
 - CD8 marker positive cells are cytotoxic T cells (Tc cells).
- There are several different types of T cell, and they have a variety of functions :
 - **Type 1 helper T cells or TH1 cells** - interacts with mononuclear phagocytes and helps them destroy intracellular pathogens•
 - **Type 2 helper T cells or TH2 cells**; interacts with B cells and helps them to divide, differentiate, and make antibody•
 - **Cytotoxic T lymphocytes (CTLs or TC cells)**. responsible for the destruction of host cells that have become infected by viruses or other intracellular pathogens.
 - **Regulatory T cells or Tregs**, help to control the development of immune responses, and limit reactions against self tissues.

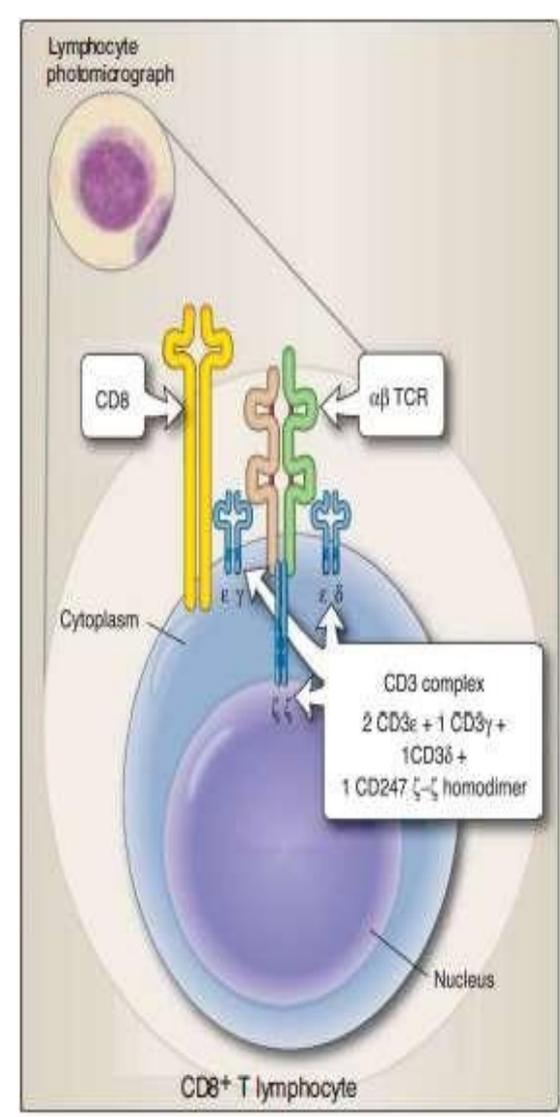
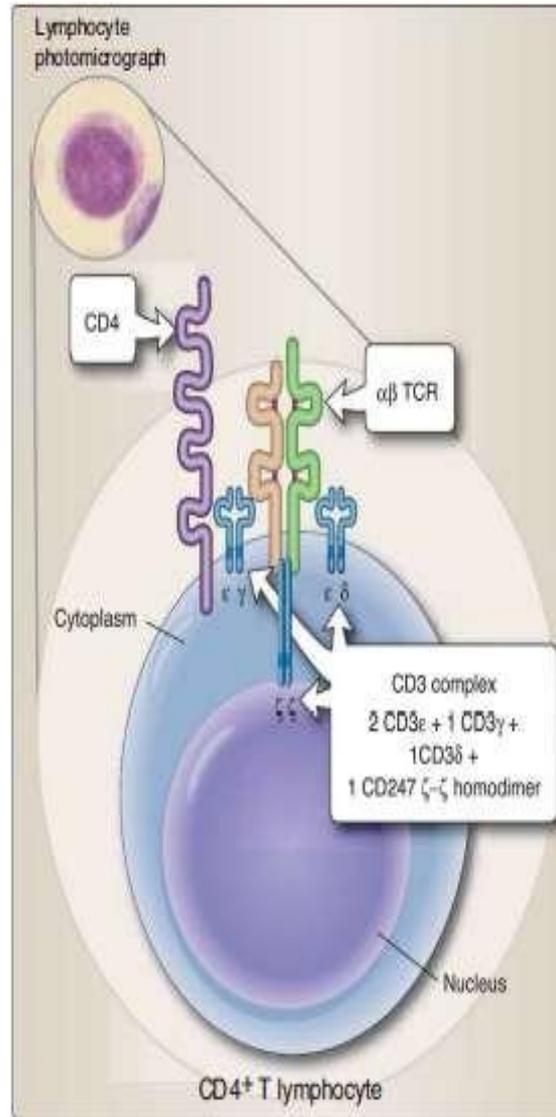
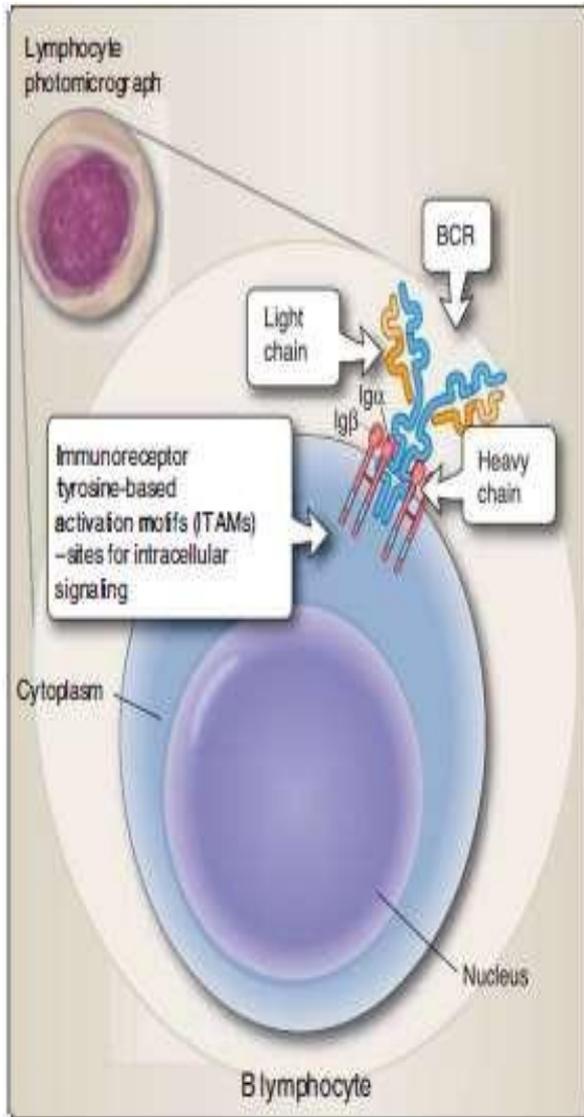
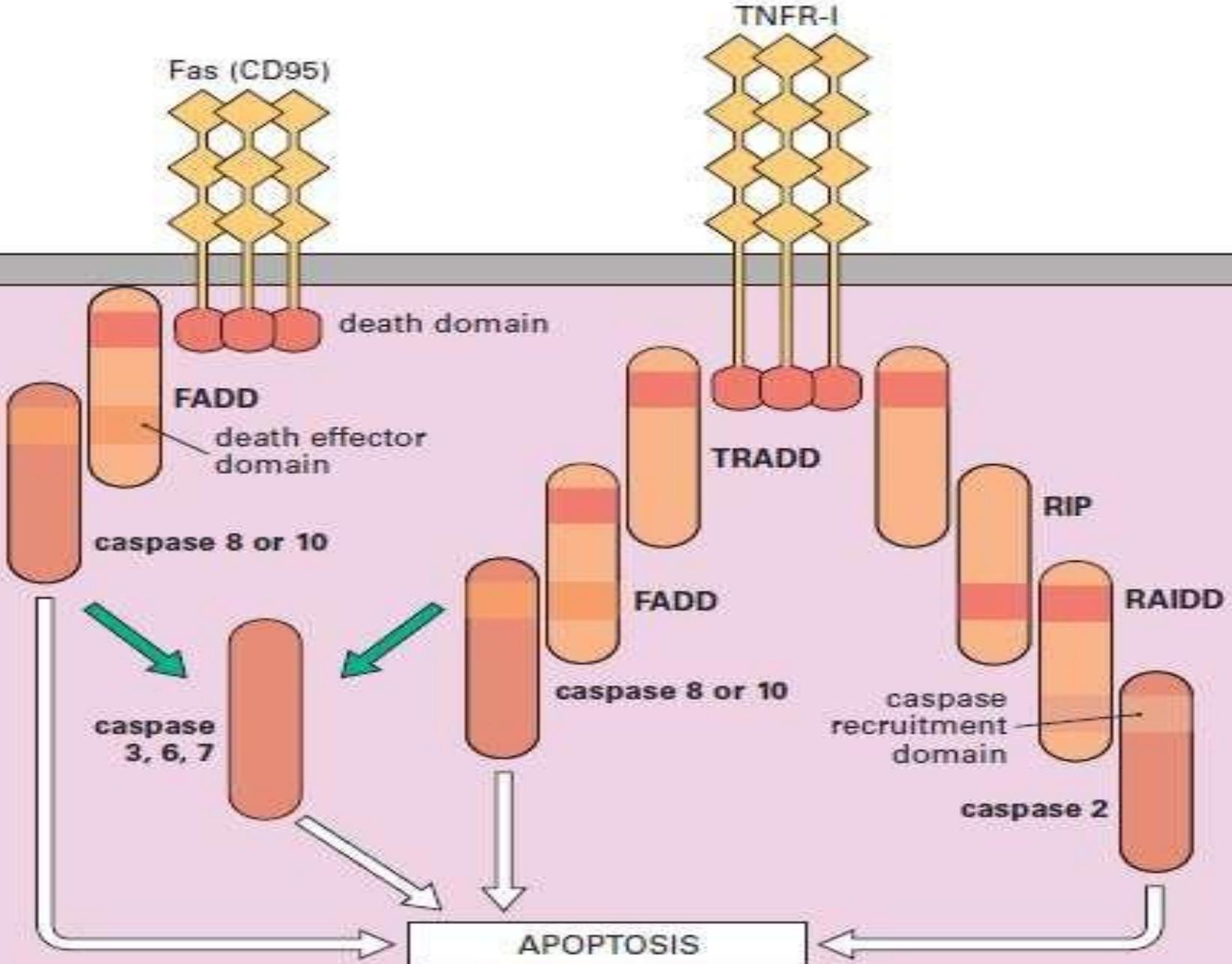


Fig: B and T Lymphocytes

Cell-Mediated Cytotoxicity

- Cytotoxicity describes the ways in which leukocytes recognize and destroy other cells.
- Cell-mediated cytotoxicity is an essential defense against:
 - intracellular pathogens, including viruses;
 - some bacteria;
 - some parasites.
 - Tumor cells, eukaryotic pathogens, and even cells of the body may also become the target of cytotoxic cells.
- Several types of cell have cytotoxic activity including:
 - cytotoxic T lymphocytes (CTLs);
 - natural killer (NK) cells
- CTLs and NK cells use a variety of different mechanisms to kill their targets. These include:
 - direct cell–cell signaling via surface molecules; and
 - granule-associated killing

Mechanisms of cell killing



Immunopathology

- **Autoimmune disease** - When the immune system reacts against 'self' components, for example rheumatoid arthritis or pernicious anemia.
- **Immunodeficiency**- If any elements of the immune system are defective, the individual may not be able to fight infections adequately.
 - primary immunodeficiencies are hereditary and start to manifest shortly after birth; eg chronic granulomatous disease (CGD) and leukocyte adhesion deficiency (LAD).
 - Secondary immunodeficiencies develop later in life, for example the acquired immune deficiency syndrome (AIDS).
- **Hypersensitivity**- Sometimes immune reactions are out of all proportion to the damage that may be caused by a pathogen. The immune system may also mount a reaction to a harmless antigen, such as a food molecule causing

Table 1. Characteristic infections in primary immunodeficiencies.

component	primary pathogen	primary site	clinical example
T-cells	intracellular, bacteria viruses, protozoa, fungi,	Many	SCID, DiGeorge
B-cells	<i>Haemophilus, Pneumococcus, Streptococcus</i>	lung, skin, CNS	Bruton's hypogammaglobulinemia IgG, IgM deficiency
	enteric bacteria and viruses and protozoa	GI, nasal and other mucosal tissues, eye	IgA deficiency
phagocytes	Staphylococcal, <i>Klebsiella</i> <i>Pseudomonas</i> ,	lung, skin, regional lymph node	chronic granulomatous disease (CGD)

Conclusion

- The immune system has evolved to protect us from pathogens.
- Phagocytes and lymphocytes are key mediators of immunity. Phagocytes internalize pathogens and degrade them. Lymphocytes (B and T cells) have receptors that recognize specific molecular components of pathogens and have specialized functions. B cells make antibodies (effective against extracellular pathogens), cytotoxic T lymphocytes (CTLs) kill virally infected cells, and helper T cells coordinate the immune response by direct cell–cell interactions and the release of cytokines.
- The immune system may fail (immunopathology). This can be a result of immunodeficiency, hypersensitivity, or dysregulation leading to autoimmune diseases

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