Phagocytosis

The innate immune system provides a rapid, initial means of defense against infection using genetically programmed receptors that recognize these structural features of microbes that are not found in the host. Such receptors are known as pattern recognition receptors (PRRs), which are found on or in phagocytic cells, which bind to pathogen associated molecular patterns (PAMPs).

PAMPs are conserved, microbes-specific carbohydrates, proteins, lipids and/or nucleic acid (e.g. lipopolysaccharide, peptidoglycan, etc.). PRRs binding to PAMPs result in phagocytosis and enzymatic degradation of the infectious organisms.

Several types of cells in the immune system engulf microorganisms via phagocytosis.

•Neutrophils. Neutrophils are abundant in the blood, quickly enter tissues, and phagocytize pathogens in acute inflammation.

•Macrophages. Macrophages are closely related to monocytes in the blood. These longer-lived cells predominate in chronic inflammation. They also release some important inflammatory paracrines.

•Dendritic Cells and B Lymphocytes. Phagocytosis in these cells is important for the elaboration of a specific immune response rather than for directly destroying the pathogens.

- **Phagocytosis begins with the neutrophil or macrophage flowing around the pathogen and engulfing it so that it winds up enclosed in a phagosome (phagocytic vesicle). But this is only the first step, because the more challenging task of destroying the microorganisms remains. Indeed, some pathogens have special, effective mechanisms for frustrating this destruction step.**
- The next step is the fusion of lysosomes with the phagosome. The result is called a phagolysosome. Lysosome is derived from the Golgi apparatus, much like secretion vesicles, but their contents are focused on destroying microorganisms.

The following are important factors that help destroy microorganisms within a phagolysosome:

- **Oxygen Radicals. A complex of proteins called NADPH oxidase in the membrane of a phagolysosome generates oxygen radicals in the phagosome. These highly reactive molecules react with proteins, lipids and other biological molecules.**
- Nitric Oxide. Nitric oxide synthase synthesizes nitric oxide, a reactive substance that reacts with oxygen radicals to create further molecules that damage various

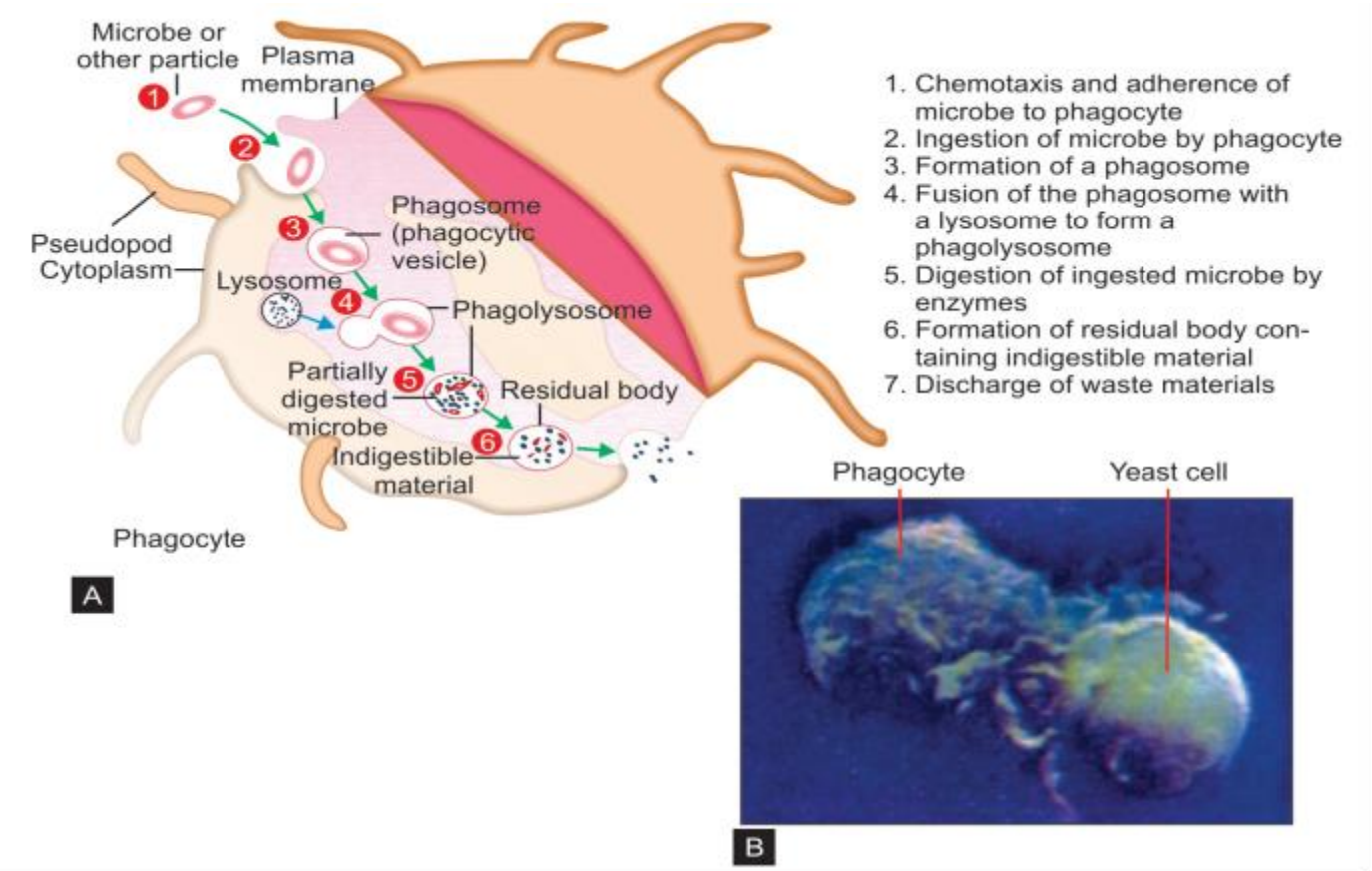
biological molecules. (But nitric oxide is also, remarkably enough, an important regulatory molecule elsewhere.

- Anti-Microbial Proteins. Lysosomes contain several proteases, including a broad spectrum enzyme,elastase, which is important or even essential for killing various bacteria. Another anti-microbial protein is lysozyme, which attacks the cell walls of certain (gram positive) bacteria.
- Anti-Microbial Peptides. Defensins and certain other peptides attack bacterial cell membranes. Similar molecules are found throughout much of the animal kingdom.
- Binding Proteins. Lactoferrin binds iron ions, which are necessary for growth of bacteria. Another protein binds vitamin B12.
- Hydrogen Ion Transport. Transporters for hydrogen ions acidify the phagolysosome, which kills various microorganisms and is important for the action of the proteases.
- In addition to destroying the microorganism, macrophages also release paracrines that alert other parts of the immune system that an infection is present. (Two important examples are IL-1 and TNF-alpha.) Among other things, these potent paracrines promote inflammation.

Difficult Pathogens

Sometimes phagocytes have a difficult time with certain pathogens. Tuberculosis is an especially important example. A macrophage can usually engulf the tuberculosis bacterium, but then the bacterium has a means for preventing the lysosomes from fusing with the phagosome. If the macrophage is not "activated" by paracrines from a specific immune response, the bacteria may remain alive for long periods within the macrophage. In this circumstance, other macrophages surround and wall off the infected macrophages, forming a type of chronic inflammation called a granuloma.

Some pathogenic bacteria are surrounded by a capsule that make phagocytosis difficult. Anthrax is one example. Anthrax spores entering the body from the lungs or a cut in the skin make their way first to lymph nodes, where they change to their "vegetative form" and begin dividing. But because they are difficult to destroy, they quickly become quite numerous and accumulate in the blood, causing septicemia. Not only do the bacteria release toxins, but also, as is the case in certain other dangerous infections, macrophages respond to the crisis by releasing enough IL-1 and TNF-alpha to cause inflammation systemically, that is, throughout the body. Indeed, this hyperinflammation by itself can quickly be fatal. It can include, for example, widespread formation of small blood clots, which is termed disseminated intravascular coagulation.



Cells and tissues of the Immune system

A: LYMPHOCYTES. These cells have receptors for antigen and confer specificity on an immune response. Lymphocytes express receptors with varying affinity for the antigen in question. The cell with the highest affinity for the most abundant antigen will have growth advantage and will preferentially generate progeny of itself. This process is called clonal expansion and is antigen driven. T and B cells that not interacted with antigen reffered to as Naïve ,after interaction with naïve lymphocytes induces these cells to progress through the cell cycle a lymphocytes enlarge in diammter cell called lymphoblast, these cells have a higher cytoplasmic to nucleus ratio and more organellar complexity than small lymphocytes.

Lymphoblast proliferate and differentiate into effector cells or memory cells.

Different lineage or maturational stage of lymphocytes can be distinguished by their expression of membrane molecules recognized by particular membrane molecule are grouped together as a cluster of differentiation (CD)

Lymphocytes sub divided into T, B, Natural killer cells

- B lymphocytes produce antibodies and some soluble mediators called cytokines. They arise in the bone marrow in adult mammals and bursa of fabricius in birds so known as the B –cells . B lymphocytes are found in blood, lymph nodes, spleen and tonsil and other mucosal tissues. About 5–25% of all human blood lymphocytes, which number 1000–2000 cells mm3, are B lymphocytes. B cells comprise a majority of the bone marrow lymphocytes, one-third to one-half of lymph node and spleen lymphocytes.

b-cells constitute 15-35% of lymphocytes pool which originate from fetal liver at 9- 10 wk & later from B.M.at first these are pre – B –CELLs characterized by intracytoplasmic μuta chain which from heavy chain constant region of IgM , when pre cells descend further they acquired light chain & then express IgM molecules on their surface ,B-cells during their passage the start to acquire IgD on its membrane – so they become mature B –cells produce Abs and some soluble mediators called cytokines.

- Plasma Cells

Plasma cells are the effector cells of the B lineage, are uniquely specialized to secrete large amount of Ig proteins to the surrounding milieu. Secreted immunoglobulins retain their ability to recognize and bind their specific ligands and are often referred to as antibodies. Binding of the antibodies to their specific ligands have a variety of effects that are beneficial to host cells. Plasma cells are oval or egg-shaped and have abundant cytoplasm and eccentrically placed round nuclei. Clumps of dark staining chromatin are often distributed around the inner aspect of the nuclear membrane in plasma cell, giving a cart-wheel or clock face appearance under light microscope. The cytoplasm contains abundant rough endoplasmic reticulum and a well-developed Golgi apparatus. Igs are not present in the surface of plasma cell, but produced in large amount in cytoplasm and are then secreted into the extracellular space. Plasma cells have relatively short span of life and are terminally differentiated.

- Memory B-cells

Have a longer life span than naïve cells and they express tha same membrane – bound Ab as their parent B –cell.

T lymphocytes arise in bone marrow but mature in the thymus. They do not produce antibody molecules but have surface receptors structurally related to Ig. T cells see antigen in a different way to B cells. They recognise peptide fragments of antigen complexed with cell surface MHC glycoproteins on neighbouring cells. The cell surface glycoproteins encoded by genes in the Major Histocompatibility Complex(MHC) bind fragments of antigen after it has been subjected to antigen processing.

- Natural killer cells (NK) are large ,granular lymphocytes that are part of innate immune response and do not express the set of surface markers that characterized Tand B. NK cells represent a first line of defence to infections, tumour growth and other pathogenic alterations of tissue homoeostasis. NK cells do not express antibodies or T cell receptors at their cell surface. They produce cytokines and express receptors for immunoglobulin. They also possess other receptor molecules which allow them to detect some infected host cells, including tumour cells, virus, or intracellular bacteria-infected cells.

- Natural killer T (NKT) cells are a distinct subset of T cells. Activated NKT cells secrete IL-4 and IFN-gamma and may help regulate immune responses. NKT cells differ from NK cells in phenotype and certain functions.

There are two major subtypes of T cells: the killer T cell and the helper T cell. In addition there are regulatory T cells which have a role in modulating immune response.

- Killer T cells

Killer T cells are a sub-group of T cells that kill cells that are infected with viruses (and other pathogens), or are otherwise damaged or dysfunctional. As with B cells, each type of T cell recognizes a different antigen. Killer T cells are activated when their T-cell receptor (TCR) binds to this specific antigen in a complex with the MHC Class I receptor of another cell. Recognition of this MHC:antigen complex is aided by a co-receptor on the T cell, called CD8. The T cell then travels throughout the body in search of cells where the MHC I receptors bear this antigen. When an activated T cell contacts such cells, it releases cytotoxins, such as perforin, which form pores in the target cell's plasma membrane, allowing ions, water and toxins to enter. The entry of another toxin called granulysin (a protease) induces the target cell to undergo apoptosis. T cell killing of host cells is particularly important in preventing the replication of viruses. T cell activation is tightly controlled and generally requires a very strong MHC/antigen activation signal, or additional activation signals provided by "helper" T cells .

❖ Helper T cells

Function of T helper cells: Antigen-presenting cells (APCs) present antigen on their Class II MHC molecules (MHC2). Helper T cells recognize these, with the help of their expression of CD4 co-receptor (CD4+). The activation of a resting helper T cell causes it to release cytokines and other stimulatory signals (green arrows) that stimulate the activity of macrophages, killer T cells and B cells, the latter producing antibodies. The stimulation of B cells and macrophages succeeds a proliferation of T helper cells.

Helper T cells regulate both the innate and adaptive immune responses and help determine which immune responses the body makes to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly. They instead control the immune response by directing other cells to perform these tasks.

Helper T cells express T cell receptors (TCR) that recognize antigen bound to Class II MHC molecules. The MHC:antigen complex is also recognized by the helper cell's CD4 co-receptor, which recruits molecules inside the T cell (e.g., Lck) that are responsible for the T cell's activation. Helper T cells have a weaker association with the MHC:antigen complex than observed for killer T cells, meaning many receptors (around 200–300) on the helper T cell must be bound by an MHC:antigen in order to activate the helper cell, while killer T cells can be activated by engagement of a single MHC:antigen molecule. Helper T cell activation also requires longer duration of engagement with an antigen-presenting cell. The activation of a resting helper T cell causes it to release cytokines that influence the activity of many cell types.

❖ T cells

T cells develop from bone marrow stem cells that travel to the thymus, where they go through rigorous selection. There are 3 main types of T cell:

- Helper
- Regulatory (suppressor)
- Cytotoxic

In selection, T cells that react to self antigen presented by self MHC molecules or to self MHC molecules (regardless of the antigen presented) are eliminated by apoptosis. Only T cells that can recognize non self antigen complexed to self MHC molecules survive; they leave the thymus for peripheral blood and lymphoid tissues.

Most mature T cells express either CD4 or CD8 and have an antigen-binding, Ig-like surface receptor called the T-cell receptor (TCR).

Helper T (Th) cells are usually CD4 but may be CD8. They differentiate from Th0 cells into one of the following:

- **Th1 cells: In general, Th1 cells promote cell-mediated immunity via cytotoxic T cells and macrophages and are thus particularly involved in defense against intracellular pathogens (eg, viruses). They can also promote the production of some antibody classes.**

- **Th2 cells: Th2 cells are particularly adept at promoting antibody production by B cells (humoral immunity) and thus are particularly involved in directing responses aimed at extracellular pathogens (eg, bacteria, parasites).**

- Th17 cells: Th17 cells promote tissue inflammation.

Each cell type secretes several cytokines (see table Functions of T Cells). Different patterns of cytokine production identify other Th-cell functional phenotypes. Depending on the stimulating pathogen, Th1 and Th2 cells can, to a certain extent, downregulate each other's activity, leading to dominance of a Th1 or a Th2 response.

- **Regulatory (suppressor) T cells mediate suppression of immune responses and usually express the Foxp3 transcription factor. The process involves functional subsets of CD4 or CD8 T cells that either secrete cytokines with immunosuppressive properties or suppress the immune response by poorly defined mechanisms that require cell-to-cell contact. Patients with functional mutations in Foxp3 develop the autoimmune disorder IPEX syndrome (immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome).**
- Cytotoxic T (Tc) cells are usually CD8 but may be CD4; they are vital for eliminating intracellular pathogens, especially viruses. Tc cells play a role in organ transplant rejection.

Tc-cell development involves 3 phases:

- A precursor cell that, when appropriately stimulated, can differentiate into a Tc cell
- An effector cell that has differentiated and can kill its appropriate target
- A memory cell that is quiescent (no longer stimulated) but is ready to become an effector when restimulated by the original antigen-MHC combination

Fully activated Tc cells, like NK cells, can kill an infected target cell by inducing apoptosis.

Tc cells can secrete cytokines and, like Th cells, have been divided into types Tc1 and Tc2 based on their patterns of cytokine production.

Tc cells may be

•Syngeneic: Generated in response to self (autologous) cells modified by viral infection or other foreign proteins

•Allogeneic: Generated in response to cells that express foreign MHC products (eg, in organ transplantation when the donor's MHC molecules differ from the recipient's)

Some Tc cells can directly recognize foreign MHC (direct pathway); others may recognize fragments of foreign MHC presented by self MHC molecules of the transplant recipient (indirect pathway).

There are 2 basic types of T cell

Accessory Molecule	T cell	MHC restriction	Function
CD4	T helper	Class II	Cytokine production help for B cells and cytotoxic T cells
CD8	T cytotoxic	Class I	Cytokine production cytotoxic lysis of infected or tumour cells

B: MONONUCLEAR PHAGOCYTES

If you inject "vital" dyes into experimental animals they will be taken up by various cell types including macrophages (mf), microglial cells in the CNS, endothelial cells of vascular sinusoids and reticular cells of lymphoid organs. These are the cells of the Reticulo-Endothelial System (RES). These cells all take up dye by pinocytosis. Only cells of the monocyte-macrophage lineage take up large particulate antigens, pieces of tissue, senescent cells, bacteria etc. by phagocytosis.

These cells have important properties:

1. they express a myeloid receptor (CD14) which serves as a recognition molecule for a wide variety of bacterial envelope molecules, such as LPS from Gram -ve

2. organismsand components of Mycobacterial and Gram +ve cell walls. Ligation of this receptor leads to macrophage activation.

3. they can act as antigen presenting cells (APC) for T cells.

4. they are activated by T cell derived cytokines leading to increased phagocytosis and microbicidal activity (increased activity of degradative enzymes, nitrogen and oxygen free radical production and prostaglandins etc.).

5. they express receptors for antibody and complement which means that they bind immune complexes, especially if the antibody involved has complement components bound to it (if the antibody has fixed complement), and endocytose/phagocytose these rapidly.

6. they act as scavengers for cell debris and senescent cells (Kupffer cells in the liver bind "old" erythrocytes).

C: DENDRITIC CELLS

There are two cell types with similar names but different functions. Cells of the dendritic cell (DC) lineage are bone marrow derived. In the skin they are known as Langerhans Cells (LC). These cells efficiently process antigen but cannot present it to T cells. LC have been shown to pick up antigen in skin and carry it via afferent lymphatic vessels to lymph nodes. Dendritic cells in lymph are known as "veiled" cells. In lymph nodes the cells, now known as tissue dendritic cells or interdigitating cells, may efficiently present antigen if they encounter the right T cell. In fact these are the best APC.....far fewer DC are required to initiate an immune response than any other APC.

Follicular dendritic cells (FDC) are found in lymphoid follicles. They are called dendritic because of their morphology rather than any lineage relationship with DC. In fact, there is considerable uncertainty about their developmental origin [some evidence suggests they are long-lived bone marrow derived cells, other data that they are of epithelial origin]. FDC have receptors for immunoglobulin and complement and are able to trap antigen at their cell surface, in the form of antigen/antibody/C3d complexes, for long periods of time. They cannot present antigen to T cells but are important in developing responses by B cells.

D: GRANULOCYTES :There are three types of granulocyte distinguished according to their histological staining patterns.

•Neutrophils, also known as polymophonuclear leukocytes, express receptors for immunoglobulin and complement and are involved in the acute inflammatory response.

constitute 40 to 70% of total circulating WBCs; they are a first line of defense against infection. Mature neutrophils have a half-life of about 2 to 3 days. During acute inflammatory responses (eg, to infection), neutrophils, drawn by chemotactic factors and alerted by the expression of adhesion molecules on blood vessel endothelium, leave the circulation and enter tissues. Their purpose is to phagocytose and digest pathogens. Microorganisms are killed when phagocytosis generates lytic enzymes and reactive oxygen compounds (eg, superoxide, hypochlorous acid) and triggers release of granule contents (eg, defensins, proteases, bactericidal permeability-increasing protein, lactoferrin, lysozymes). DNA and histones are also released, and they, with granule contents such as elastase, generate fibrous structures called neutrophil extracellular traps (NETs) in the surrounding tissues; these structures facilitate killing by trapping bacteria and focusing enzyme activity.

- Eosinophils carry receptors for IgE, are involved in the destruction of IgE coated parasites, such as helminths, and contribute to the response to allergens. constitute up to 5% of circulating WBCs.Eosinophils are also a major source of inflammatory mediators (eg, prostaglandins, leukotrienes, platelet-activating factor, many cytokines).

- Basophils constitute < 5% of circulating WBCs and share several characteristics with mast cells, although the 2 cell types have distinct lineages. Both have high-affinity receptors for IgE called Fc-epsilon RI (FcεRI). When these cells encounter certain antigens, the bivalent IgE molecules bound to the receptors become cross-linked, triggering cell degranulation with release of preformed inflammatory mediators (eg, histamine, platelet-activating factor) and generation of newly synthesized mediators (eg, leukotrienes, prostaglandins, thromboxanes).

LYMPHOID TISSUE

The immune system includes several organs in addition to cells dispersed throughout the body. These organs are classified as primary or secondary lymphoid organs.

The primary lymphoid organs are the sites where white blood cells are produced and/or multiply:

- The bone marrow produces all the different types of white blood cells, including neutrophils, eosinophils, basophils, monocytes, B cells, and the cells that develop into T cells (T cell precursors).

- In the thymus, T cells multiply and are trained to recognize foreign antigens and to ignore the body's own antigens. T cells are critical for acquired immunity.

When needed to defend the body, the white blood cells are mobilized, mainly from the bone marrow. They then move into the bloodstream and travel to wherever they are needed

Primary		**Secondary**	
(Responsible for maturation of Ag-reactive cells)		(Sites for Ag contact and response)	
⬇	⬇	⬇	⬇
Thymus (T-cell maturation)	**Bone marrow**	**Lymph nodes**	**Spleen**
(T-cell maturation)	(B-cell maturation)	(Expansion of lymphatic system, separate from blood circulation. Deep cortex harbors mostly T-cells, superficial cortex harbors mostly B-cells)	(Similar to lymph nodes but part of blood circulation. Collects blood-borne Ags)