

TEACHING OBJECTIVES:

1. To recognize the significance of the immune system
2. To distinguish between the innate (nonspecific) and adaptive (specific) immune systems
3. To understand the mechanisms of combating infection/disease (killing pathogens)
4. To know the humoral and cellular components of the innate immune response
5. To recognize the mechanisms of action of the components of the innate immune response

INNATE (NONSPECIFIC) IMMUNE RESPONSE

1) Overview of the immune system

- a) We are constantly being exposed to infectious agents and yet, in most cases, we are able to resist these infections. It is our immune system that enables us to resist infections. The immune system is composed of two major subdivisions, the **innate or non-specific** immune system and the **adaptive or specific immune system** (Figure 1).

The innate immune system is our first line of defense against invading organisms while the adaptive immune system acts as a second line of defense and also affords protection against re-exposure to the same pathogen. Each of the major subdivisions of the immune system has both **cellular and humoral** components by which they carry out their protective function (Figure 1). In addition, the innate immune system also has anatomical features that function as barriers to infection. Although these two arms of the immune system have distinct functions, there is interplay between these systems (i.e., components of the innate immune system influence the adaptive immune system and vice versa).

- b) There are two phases to the immune response: **pathogen recognition and pathogen removal**. Although the innate and adaptive immune systems both function to protect against invading organisms, they differ in a number of ways. The adaptive immune system requires some time to react to an invading organism, whereas the innate immune system includes defenses that, mostly, are constitutively present and ready to be mobilized upon infection. Second, the adaptive immune system is antigen specific and reacts only with the organism that induced the response. In contrast, the innate system is not antigen specific and reacts equally well to a variety of organisms. Finally, the adaptive immune system demonstrates immunological memory. It “remembers” that it has encountered an invading organism and reacts more rapidly on subsequent exposure to the same organism. In contrast, the innate immune system does not demonstrate immunological memory.

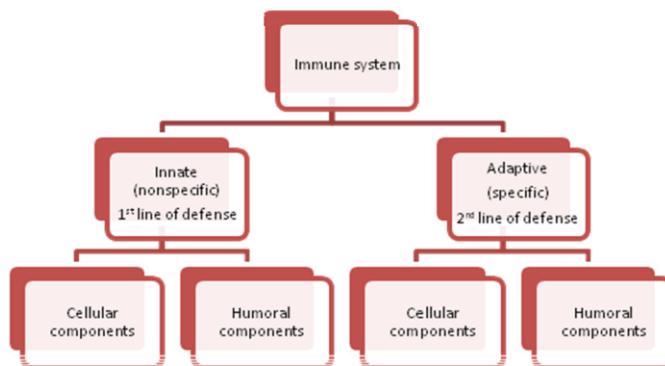


Fig. 1

cells of the immune system

- c) All cells of the immune system have their origin in the bone marrow. They include **myeloid** (neutrophils, basophils, eosinophils, macrophages, and dendritic cells) and **lymphoid cells** (B lymphocytes, T lymphocytes, and natural killer cells) (Figure 2).

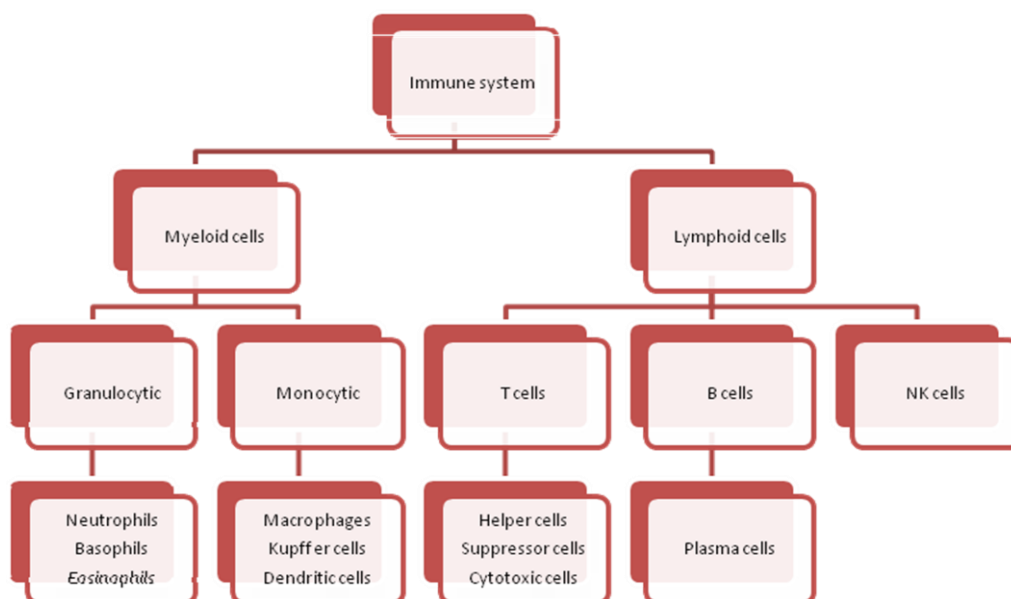


Fig. 2

functions of the immune system

- d) The main function of the immune system is self/non-self discrimination. This ability to distinguish between self and non-self is necessary to protect the organism from invading pathogens and to eliminate modified or altered cells (e.g. malignant cells). Since pathogens may replicate intracellularly (viruses and some bacteria and parasites) or extracellularly (most bacteria, fungi and parasites), different components of the immune system have evolved to protect against these different types of pathogens. It is important to remember that infection with an organism does not necessarily mean diseases, since in most cases the immune system will be able to eliminate the infection before disease occurs. Disease occurs only when the **bolus of infection is high, when the virulence of the invading organism is great or when immunity is compromised**. Although the immune system, for the most part, has **beneficial effects**, there can be **detrimental effects** as well. During inflammation, which is the response to an invading organism, there may be local discomfort and collateral damage to healthy tissue as a result of the toxic products produced by the immune response. In addition, in some cases the immune response can be directed toward self tissues resulting in autoimmune disease.

2) Innate host defenses against infection: Anatomical barriers

- a) **Mechanical factors**: epithelial surfaces form a physical barrier that is very impermeable to most infectious agents. Thus, the **skin** acts as our first line of defense against invading organisms. The desquamation of skin epithelium also helps remove bacteria and other infectious agents that have adhered to the epithelial surfaces. **Movement** due to **cilia** or **peristalsis** helps to keep air passages and the gastrointestinal tract free from microorganisms. The **flushing** action of **tears and saliva** helps prevent infection of the eyes and mouth. The **trapping effect of mucus** that lines the respiratory and gastrointestinal tract helps protect the lungs and digestive systems from infection.
- b) **Chemical factors**: fatty acids in sweat inhibit the growth of bacteria. **Lysozyme** and **phospholipase** found in tears, saliva and nasal secretions can breakdown the cell wall of bacteria and destabilize bacterial membranes. The low pH of sweat and gastric secretions prevents growth of bacteria. **Defensins** (low molecular weight proteins) found in the lung and gastrointestinal tract have antimicrobial activity. Surfactants in the lung act as **opsonins**.
- c) **Biological factors**: the **normal flora** of the skin and in the gastrointestinal tract can prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria for nutrients or attachment to cell surfaces.

3) Innate host defenses against infection: Humoral barriers

- a) Anatomical barriers are very effective in preventing colonization of tissues by microorganisms. However, when there is damage to tissues the anatomical barriers are breached and infection may occur. Once infectious agents have penetrated tissues, another innate defense mechanism comes into play, namely **acute inflammation**. Humoral factors play an important role in inflammation, which is characterized by edema and the recruitment of phagocytic cells. These humoral factors are found in serum or they are formed at the site of infection.

- b) The **complement system** is the major humoral non-specific defense mechanism. Once **activated complement can lead to increased vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.**
- c) Depending on the severity of the tissue injury, the **coagulation system** may or may not be activated. Some products of the coagulation system can contribute to the non-specific defenses because of their ability to increase **vascular permeability and act as chemotactic agents** for phagocytic cells. In addition, some of the products of the coagulation system are directly **antimicrobial**. For example, **beta-lysin, a protein produced by platelets** during coagulation can lyse many Gram positive bacteria by acting as a cationic detergent.
- d) By binding iron, an essential nutrient for bacteria, **lactoferrin and transferrin** limit bacterial growth.
- e) **Lysozyme** breaks down the cell wall of bacteria.
- f) **Cytokines** have various effects depending on the balance. **Interferons** are proteins that can limit virus replication in cells. Some **interleukins** induce fever and the production of acute phase proteins, some of which are antimicrobial because they can opsonize bacteria.

4) **Innate host defenses against infection: Cellular barriers**

- a) Part of the inflammatory response is the recruitment of **polymorphonuclear eosinophils** and **macrophages** to sites of infection. These cells are the main line of defense in the non-specific immune system.
- b) **Neutrophils**, Polymorphonuclear cells (PMNs), are recruited to the site of infection where they phagocytose invading organisms and kill them **intracellularly**. In addition, PMNs contribute to collateral tissue damage that occurs during inflammation.
- c) Tissue **macrophages and newly recruited monocytes**, which differentiate into macrophages, also function in phagocytosis and **intracellular killing** of microorganisms. In addition, macrophages are capable of **extracellular killing** of infected or altered self target cells. Furthermore, macrophages contribute to **tissue repair and act as antigen-presenting cells**, which are required for the induction of specific immune responses.
- d) **Natural killer (NK) and lymphokine activated killer (LAK) cells** can nonspecifically kill virus infected and tumor cells. These cells are not part of the inflammatory response but they are important in nonspecific immunity to viral infections and tumor surveillance.
- e) **Eosinophils** have proteins in granules that are effective in killing certain parasites.

5) **Phagocyte response to infection**

- a) Circulating PMNs and monocytes respond to danger (SOS) signals generated at the site of an infection. SOS signals include **N-formyl-methionine containing peptides** released by bacteria, **clotting system peptides, complement products and cytokines** released from **tissue macrophages** that have encountered bacteria in tissue. Some of the SOS signals

stimulate endothelial cells near the site of the infection to express cell adhesion molecules such as **ICAM-1** and **selectins** which bind to components on the surface of phagocytic cells and cause the phagocytes to **adhere** to the endothelium. Vasodilators produced at the site of infection cause the junctions between endothelial cells to loosen and the phagocytes then cross the endothelial barrier by “squeezing” between the endothelial cells in a process called **diapedesis** (Figure 3). Once in the tissue spaces some of the SOS signals attract phagocytes to the infection site by **chemotaxis** (movement toward an increasing chemical gradient). The SOS signals also **activate** the phagocytes, which results in **increased phagocytosis and intracellular killing** of the invading organisms.

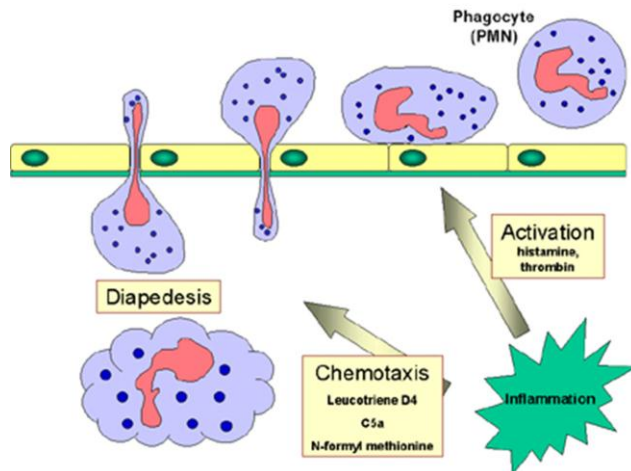


Fig. 3

- b) **Phagocytosis:** Phagocytic cells have a variety of receptors on their cell membranes through which infectious agents bind to the cells (Figure 4A). These include **Fc receptors, complement receptors, scavenger receptors, and toll-like receptors**. After attachment of a bacterium, the phagocyte begins to extend pseudopods around the bacterium (Figure 4B). The pseudopods eventually surround the bacterium and engulf it, and the bacterium is enclosed in a phagosome (Figure 4C). During phagocytosis the granules or lysosomes of the phagocyte fuse with the phagosome and empty their contents (Figure 4D). The result is a bacterium engulfed in a phagolysosome which contains the contents of the granules or lysosomes.

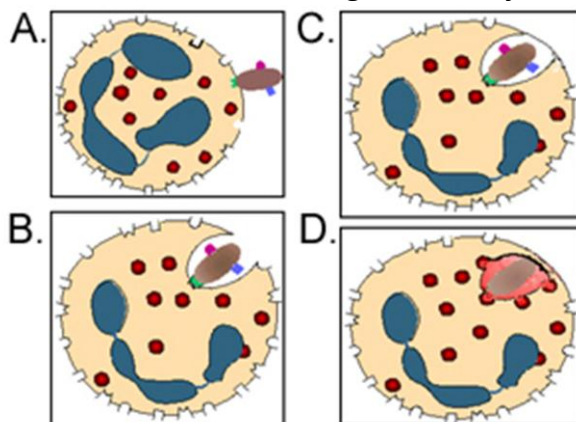
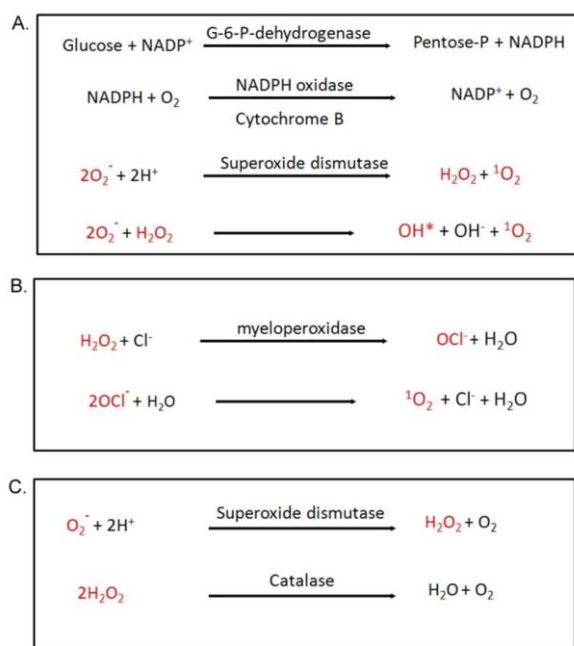


Fig. 4

Mechanisms of killing of engulfed bacteria by phagocytic cells

- c) **Respiratory burst.** 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**. In addition, bacteria can be killed by pre-formed substances released from granules or lysosomes when they fuse with the phagosome. This is referred to as **oxygen-independent intracellular killing**.
- Oxygen-dependent myeloperoxidase (MPO)-independent intracellular killing (Figure 5A). During phagocytosis glucose is metabolized via the pentose monophosphate shunt and NADPH is formed. Cytochrome B which was part of the granule combines with the plasma membrane NADPH oxidase and activates it. The activated NADPH oxidase uses oxygen to oxidize the NADPH. The result is the production of superoxide anion. Some of the superoxide anion is converted to H_2O_2 and singlet oxygen by superoxide dismutase. In addition, superoxide anion can react with H_2O_2 resulting in the formation of hydroxyl radical and more singlet oxygen. The result of all of these reactions is the production of the toxic oxygen compounds superoxide anion (O_2^-), H_2O_2 , singlet oxygen ($^1\text{O}_2$) and hydroxyl radical ($\text{OH}^\bullet$).
 - Oxygen-dependent MPO-dependent intracellular killing (Figure 5B). As the **azurophilic granules fuse with the phagosome**, myeloperoxidase is released into the phagolysosome. MPO utilizes H_2O_2 and halide ions (usually Cl^-) to produce hypochlorite, a highly toxic substance. Some of the hypochlorite can spontaneously break down to yield singlet oxygen. The result of these reactions is the production of toxic hypochlorite (OCl^-) and singlet oxygen ($^1\text{O}_2$).
 - Detoxification reactions (Figure 5C). PMNs and macrophages have means to protect themselves from the toxic oxygen intermediates. These reactions involve the dismutation of superoxide anion to hydrogen peroxide by superoxide dismutase and the conversion of hydrogen peroxide to water by catalase

Fig. 5



iv) Oxygen-independent intracellular killing. In addition to the oxygen-dependent mechanisms of killing there are also oxygen-independent killing mechanisms in phagocytes: **cationic proteins (cathepsin)** released into the phagolysosome can damage bacterial membranes; **lysozyme** breaks down bacterial cell walls; **lactoferrin** chelates iron, which deprives bacteria of this required nutrient; **hydrolytic enzymes** break down bacterial proteins. Thus, even patients who have defects in the oxygen-dependent killing pathways are able to kill bacteria. However, since the oxygen-dependent mechanisms are much more efficient in killing, patients with defects in these pathways are more susceptible and get more serious infections.

- d) **Nitric oxide-dependent killing.** Binding of bacteria to macrophages, particularly binding via Toll-like receptors, results in the production of **TNF-alpha**, which acts in an autocrine manner to induce the expression of the inducible nitric oxide synthetase gene (i-nos) resulting in the production of nitric oxide (NO). If the cell is also exposed to interferon gamma (**IFN-gamma**) additional nitric oxide will be produced. Nitric oxide released by the cell is toxic and can kill microorganism in the vicinity of the macrophage (Figure 6).

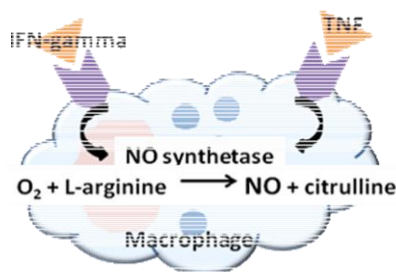


Fig. 6

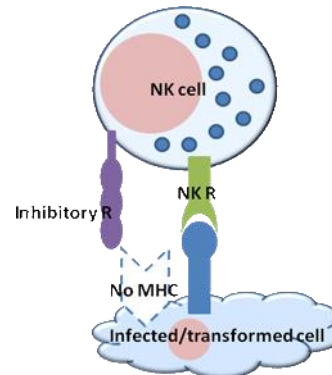


Fig. 7

6) Non-specific killer cells

- a) Several different cells including **NK cells, activated macrophages, eosinophils, and mast cells** are capable of killing foreign and altered self target cells in a non-specific manner. These play an important role in the innate immune system.
- b) **Innate response to virus infection and altered self (transformed cells):** NK cells have two kinds of receptors on their surface, **NK receptor and inhibitory receptor** (Figure 7). When the NK receptor encounters its ligand on a target cell, the NK cell is signaled to kill. However, if the inhibitory receptor also binds its ligand (MHC class I) then the killing signal is repressed. Normal cells constitutively express MHC class I on their surface, however virus infected and transformed cells down regulate expression of MHC class I. **Thus, NK cells selectively kill virus-infected and transformed cells while sparing normal cells.**
- c) **Innate response to extracellular microorganisms (parasites):** **eosinophils** are a specialized group of cells with the ability to engage and damage large extracellular parasites, such as schistosomes. Activated eosinophils release their granule components including major basic protein, eosinophil peroxidase (a cationic hemoprotein), and eosinophil cationic

protein (a ribonuclease that is an eosinophil-specific toxin that is very potent at killing many parasites).

7) Determinants recognized by the innate immune response

- a) Determinants recognized by components of the innate (nonspecific) immune system differ from those recognized by the adaptive (specific) immune system. Antibodies, and the B and T cell receptors recognize discrete determinants and demonstrate a high degree of specificity, enabling the adaptive immune system to recognize and react to a particular pathogen. In contrast, components of the innate immune system recognize broad molecular patterns found in pathogens but not in the host. Thus, they lack a high degree of specificity seen in the adaptive immune system. The broad molecular patterns recognized by the innate immune system have been called **PAMPS** (pathogen associated molecular patterns) and the receptors for PAMPS are called **PRRs** (pattern recognition receptors). A particular PRR can recognize a molecular pattern that may be present on a number of different pathogens enabling the receptor to recognize a variety of different pathogens. Examples of some PAMPS and PRRs are illustrated in Figure 8.

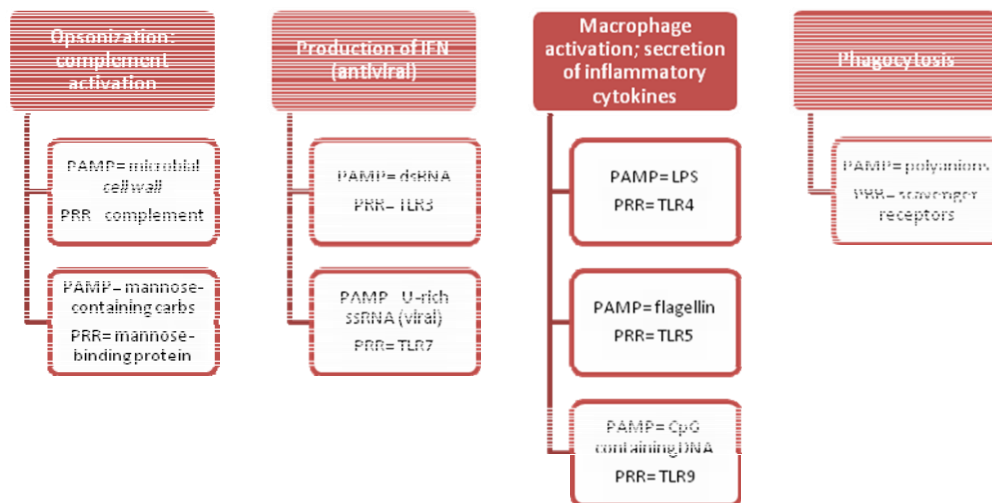


Fig. 8