Innate Immunity

All multicellular organisms have an internal mechanisms for combating microbial infections. This immunity acquired in born is called innate immunity. Innate immune system always is ready to recognize and eliminate microbes. Innate immunity can also be called as natural, or native immunity.

When microbes enter in the body, innate immune system respond within several hours. Innate immune response is first response to eliminate microbes and prevent infection.

Innate immunity provides the early defense against infections. Secondly, it also stimulates the adaptive immune system to respond microbes. The innate immune system respond in the same way to the repeated exposure of the same microbial antigen. Whereas the adaptive immune system respond more efficiently to each repeated exposure with the same microbial antigen. This is phenomenon of immunologic memory. This property is one major differences between innate and adaptive immune responses.

Components of innate immun system

The innate immune system has some components.

1. Anatomical barriers
2. Mechanical components
3. Normal flora
4. Antigen-Nonspecific Antimicrobial Molecules
5. Innate Immune system cells
6. Molecules such as complement proteins, acute phase proteins, and cytokines

1.-Anatomical Barriers

Anatomical barriers are the physical barriers that prevent the entry and colonization of many microbes.

a. the skin

It is dry, acidic, and has a lower temperature than body temperature. These conditions are not suitable for bacterial growth. Hair follicles and sweat glands produce lysozyme and toxic lipids that can kill bacteria. Epithelial cells also produce antimicrobial proteins to kill microbes, such as defensins and cathelicidins.

b. the mucous membranes

The respiratory tract, the gastrointestinal tract, and the genitourinary tract are lined by mucous membranes. Mucous membranes secrete mucus. The mucus traps microbes. Mucus also contains lysozyme to degrade bacterial structure. There are two other molecules lactoferrin and lactoperoxidase. Lactoferrin binds iron and keep it from microbes, and lactoperoxidase generates toxic superoxide radicals that kill microbes.

2. Mechanical removal

Many microbes are physically removed from the body.

a. Cilia

A cilium (plural cilia) is an organelle found in epithelial cells. They propel mucus and trapped microbes upwards towards the throat. Then the microbes are swallowed and are killed in the stomach. This is sometimes called the tracheal toilet. Also the epithelial cells of nose and sinuses contain cilia.

b. the cough and sneeze reflex

Coughing and sneezing remove mucus and trapped microbes.

c. vomiting and diarrhea

These processes remove pathogens and toxins from the gastrointestinal tract.

d. the physical flushing action of body fluids
Fluids such as urine, tear, saliva and sweat remove microbes from the body.

3. Normal Flora
Approximately 100 trillion bacteria and other microorganisms reside in or on the human body. These normal body flora keep away harmful microbes.

a. The normal flora can produce metabolic products such as fatty acids, bacteriocins. These metabolic products inhibit the growth of many pathogens.

b. The normal flora adhere to target host cells and cover them. By this way, they prevent pathogen colonization.

c. The normal flora also use nutrients essential for the growth of pathogens; and

d. It can non-specifically stimulate the immune system.

4. Antigen-Nonspecific Antimicrobial Molecules
There are many antigen-nonspecific antimicrobial chemicals produced by the body.

a. Hydrochloric acid and enzymes found in gastric secretions and they destroy microbes that are swallowed.

b. Lysozyme is found in tears, mucus, saliva, plasma, tissue fluid, etc. Lysozyme breaks down of peptidoglycan in bacteria and cause osmotic lysis.

c. Defensins form pores in the cytoplasmic membrane of a variety of microorganisms. Defensins are produced by leukocytes, epithelial cells, and other cells. They are also found in blood plasma and mucus.

d. Cathelicidins are proteins produced by skin and mucosal epithelial cells. The cathelicidins are also directly toxic to a variety of microorganisms.

e. Lactic and fatty acids, found in sweat and sebaceous secretions, inhibit microbes on the skin.

f. Lactoferrin and transferrin trap iron and prevent its use by microorganisms.

5. Innate Immune System Cells
a. Phagocytes
There are two types of phagocytes. The mononuclear phagocytes are circulating monocytes and tissue macrophages. The other family of phagocytes is polymorphonuclear granulocytes.

Monocytes and Macrophages
Monocytes normally make up 2-8% of the WBCs. They ingest microbes in the blood and in tissues. Monocytes enter extravascular tissues and survive in these sites for long periods. In the tissues, these monocytes differentiate into macrophages. Resident macrophages are found in connective tissues and in every organ in the body. Monocyte has a horseshoe-shaped nucleus and granules. These granules are called primary granule that are lysosomes. The lysosomes contain peroxidase and several acid hydrolases, which are important for killing phagocyted microorganisms.

The main properties of mononuclear phagocytes are listed below.

- The main role of the mononuclear phagocytes is to remove particulate matter of foreign origin or self origin.
- Macrophages are antigen-presenting cells in the adaptive immune responses.
- They produce a variety of cytokines that play numerous roles in body defense.
- They are long-lived and can multiply.
- They have special receptors for microbes, cytokines, opsonin molecules.

Polymorphonuclear granulocytes
There are three types of polymorphonuclear granulocytes. They are neutrophil, eosinophil and basophil. Polymorphonuclear granulocytes have irregular and lobed nucleus with several lobes. There are many granules containing enzymes and antimicrobial chemicals in cells.

Neutrophils

Neutrophils normally make up 54-75% of the WBCs. Neutrophils are the first cell type to respond to most infections, particularly bacterial and fungal infections. They can ingest microbes in the circulation, and they can rapidly enter extravascular tissues at sites of infection. At side of infection they also ingest microbes and die after a few hours. Human neutrophils have multilobed nucleus. The main properties of neutrophils are listed below.

- Neutrophils are important phagocytes.
- The neutrophils have two types of granule. Primary azurophil are lysosomes containing acid hydrolase, myeloperoxidase, muramidase (lysozyme), defensins, cathelisidines, seprocidins, and bactericidal permeability increasing protein (BPI). The lysosome fuses with phagosome. Then they become phagolysosomes. Secondary specific granules contain defense chemicals such as lysozyme, lactoferrin, collagenase, and elastase. These agents kill microbes intracellularly during phagocytosis. But they are also often released extracellularly. When they are released extracellularly, they kill microbes and injure surrounding tissue. This may be an important pathogenetic mechanism in immune complex diseases.
- They release the enzyme kallikrein and other enzymes. They catalyze the synthesis of prostaglandins and bradykinins. Prostaglandins and bradykinins promote inflammation by causing vasodilation, increasing vascular permeability, and increasing mucous production.
- Their life span is a few hours to a few days. They do not multiply. They circulate in the blood for around 6 hours and they undergo apoptosis. In tissue, they function for several hours and die.
- Neutrophils express adhesion molecules and receptors involved in responses to microbes. Neutrophils have the receptors for complement and antibody Fc.

b.-Cells that release inflammatory mediators

Eosinophils

Eosinophils normally make up 1-4% of the WBCs. Human eosinophils have bilobed nucleus and many cytoplasmic granules.

- Their granules contain acid phosphatase, peroxidases, major basic protein (MBP), RNase, DNases, lipase, and plasminogen. MBP is a potent toxin for helminth worms; induces histamine release from mast cells; activates neutrophils and platelets.
- They are capable of phagocytosis but primarily they release their contents into the surrounding environment to kill microbes extracellularly.
- They play specialized role in immunity to fungi, protozoa, and parasitic worms (helminths). In other words, pathogens that are too big to be entered by phagocytosis.
- They secrete leukotrienes, prostaglandins, chemicals that promotes inflammation. They also secrete various cytokines.
- Their life span is 8-12 days.

Basophils and Mast Cells
Basophils are found in very small numbers in the circulation and account for less than 0.2% of leukocytes. The mast cell is not found in the circulation. The function of basophils and mast cells are the same but there are some morphologic differences between them.

- Basophils release histamine, leukotrienes, and prostaglandins, that promotes inflammation. The granules of basophils also contain heparin and PAF. The stimulus for the degranulation of mast cell or basophil is often an allergen. As a result of basophil or mast cell degranulation, all contents of the granules are very rapidly released. Complement components (C3a and C5a) and immune complex can also cause degranulation of basophils and mast cells in innate immunity.
- They secrete IL4, and IL13
- Basophils life span is probably a few hours to a few days. But mast cells have longer life span that is weeks or months.

c. Lymphocytes
There are four populations of lymphocytes play important roles in innate immunity.

NK cells
NK cells are able to recognize and kill infected cells, cancer cells, and stressed cells. They also secrete inflammatory cytokines to promote an inflammatory response.

NK cells use a dual receptor system in determining whether to kill or not kill human cells. Some molecules called stress-induced glycoproteins are secreted by infected cells, cancer cells, or stressed cells. The first NK cell receptor, called the killer-activating receptor, recognizes these stress-induced molecules. These receptors stimulate the NK cell to kill the target cell.

Second receptor, called the killer-inhibitory receptor that recognizes MHC-I molecules. This signal prevents the NK cell activity.

Viruses, stress, and malignant transformation, inhibit the expression of MHC-I molecules. Without the signal from the killer-inhibitory receptor, the kill signal from the killer-activating signal is still activated. And then the NK cell kills the target cell.

The NK cell then releases pore-forming proteins called perforins, proteolytic enzymes called granzymes, and chemokines. Granzymes pass through the pores and activate the enzymes that stimulate the apoptosis of the infected cell.

Cytokines such as interleukin-2 (IL-2) and interleukin-12 (IL-12) produced by respectively Th1 lymphocytes and macrophages activate NK cells. Once activated, NK cells themselves produce large amounts of IFN-gamma to activate macrophages so they can kill ingested microbes.

NK cells also play a role in adaptive immune responses. NK cells have roles in antibody-dependent cellular cytotoxicity (ADCC). They can kill antibody-bound cells.

NKT cells
NKT cells are a subset of lymphocytes. They have T-cell receptors (TCRs) on their surface. They recognize glycolipid antigen presented by CD1 by using TCR receptors. The main function of NKT cells is to comminicate between innate and adaptive immune systems by initiating of T cell response. NKT cells produce IL-4 and interferon gamma. NKT cells may likely produce IL-10.

Intraepithelial T-lymphocytes (γδ T cells)
γδ T cells T cells may protect mucosal surface the body. They are found in the epidermis of the skin and the mucosal epithelia. These lymphocytes are known as gamma delta T-cells. They can recognize
pathogen-associated molecular patterns (PAMPs) by using restricted TCRs. They function dominantly in innate immunity rather than adaptive immunity.

**B-1 lymphocytes**

B1 lymphocytes, or B-1 cells are found mostly in the peritoneal and pleural cavities. They respond to T-independent antigens, such as polysaccharide and lipid antigens of microbes, by producing antibodies. These antibodies are called natural antibodies.

**6.-Some molecules involved in innate immune response**

**The complement System**

The complement system is a series of proteins circulating in the blood and found in the fluids surrounding tissues. In normal conditions, the complement proteins are in inactive form. In response to the recognition, they are activated.

There are three activation pathways that form the complement system. These are the classical complement pathway, the lectin pathway, and the alternative complement pathway. The pathways produce a key enzyme called C3 convertase. The classical complement pathway is activated by antigen-antibody complexes. The lectin pathway is activated by the interaction of microbial carbohydrates with mannose-binding lectin (MBL). The alternative complement pathway is activated by microbe.

The result of complement activation is the promoting of innate immune responses by triggering inflammation, by promoting phagocytosis and by causing lysis of gram-negative bacteria. On activation, some components of complement system play role in activation of naive B-lymphocytes and in the removing of immune complexes from the body.

**Acute phase proteins**

Many of these plasma proteins increase rapidly after infection. This protective response is called the acute phase response to infection. The proteins, that are produced at acute phase of infection, are called acute phase proteins or acute phase reactants. Plasma MBL is a protein that recognizes microbial carbohydrates and can bind microbes for phagocytosis or activate the complement cascade by the lectin pathway. Surfactant proteins in the lung protect the airways from infection. C-reactive protein (CRP) binds to phosphorylcholine on microbes and coats the microbes for phagocytosis by macrophages, which express a receptor for CRP.

**Cytokines in Innate Immunity**

The cytokines that produced in innate responses are IL-1, IL-6, IL-8, IL-12, IL-15, IL-18, type-1 interferon, interferon gamma and TNF-alpha. TNF-alpha is the main cytokine that mediates acute inflammation. In excessive amounts it causes systemic complications such as the shock cascade.

**Recognition of Microbes by Innate Immune System Cells**

Innate immunity recognize molecules are not found in mammalian cells. These microbial molecules are called pathogen-associated molecular patterns or PAMPs.

In addition, stressed, injured, infected, or transformed human cells contain some molecules. These molecules also act as PAMPs. Heat-shock proteins and altered membrane phospholipids are examples of PAMP like molecules.

PAMPs are listed below.

- Lipopolysaccharide
- Bacterial lipoproteins and lipopeptides
- Porins

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- Peptidoglycan
- Lipoteichoic acids
- Lipoarabinomannan
- Mannose-rich glycans
- Flagellin
- Bacterial and viral nucleic acid
- N-formylmethionine
- Double-stranded viral RNA
- Single-stranded viral RNA
- Lipoteichoic acids, glycolipids, and zymosan
- Phosphorylcholine and other lipids

**Pattern-Recognition Receptors**

In order to recognize PAMPs, various body cells have a variety of receptors called **pattern-recognition receptors** or **PRRs**. Cells that typically have pattern recognition receptors include macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, and lymphocytes.

Many pattern-recognition receptors are located on the surface of these cells. Others PRRs are found within the phagolysosomes of phagocytes. These PRRs can interact with PAMPs which are located within the phagocytosed microbes. Some PRRs are found in the cytosol of the cell.

There are two major classes of pattern-recognition receptors; endocytic pattern-recognition receptors and signaling pattern-recognition receptors.

**A.-Endocytic Pattern-Recognition Receptors**

Endocytic pattern-recognition receptors are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes. They are mannose, scavenger and opsonin receptors.

1. **mannose receptors**

   Mannose are commonly found in microbial glycoproteins and glycolipids. C-type lectins found on the surface of phagocytes are mannose receptors.

2. **scavenger receptors**

   Scavenger receptors are found on the surface of phagocytic cells. They bind to bacterial cell wall components such as LPS, peptidoglycan and teichoic acids. There are also scavenger receptors on stressed, infected, or injured cells.

3. **opsonin receptors**

   Opsonins are soluble molecules. They bind the microbes to the phagocytes. The most important opsonin molecules are C-reactive protein, complement proteins such as C3b and C4b, and surfactant proteins such as SP-A and SP-D.

4. **N-formly methionine receptors**

   N-formly methionine is first amino acid produced in bacterial proteins. This form of the amino acid is not typically seen in mammalian proteins. N-formly methionine receptors are found on neutrophils and macrophages.

**B.-Signaling Pattern-Recognition Receptors**

Binding of microbial PAMPs to their these of PRRs promotes the synthesis and secretion of cytokines.

**Signaling PRRs found on cell surfaces**
A series of signaling pattern-recognition receptors known as toll-like receptors (TLRs) are found on the surface of a variety of defense cells and other cells. The binding of a microbial antigens to signalling TLRs, transmits a signal to the host cell's nucleus. This signal induces the expression of genes coding for the synthesis of cytokines.

Different TLRs directly or indirectly bind different microbial molecules.

a. **TLR-2** - recognizes peptidoglycan, bacterial lipoproteins, lipoteichoic acid (LTA), and porins;

b. **TLR-4** - recognizes lipopolysaccharide (LPS) of gram-negative cell wall, fungal mannans, viral envelope proteins, parasitic phospholipids, and heat-shock proteins;

c. **TLR-5** - recognizes bacterial flagellin;

d. **TLR-1/TLR-2 pairs** - bind uniquely bacterial lipopeptides and glycosylphosphatidylinositol (GPI)-anchored proteins in parasites;

e. **TLR-2/TL6 pairs** - bind lipoteichoic acid (LTA) from gram-positive cell walls, bacterial lipopeptides, and peptidoglycan.

Another cell surface PRR is CD14. CD14 is found on monocytes, macrophages, and neutrophils. CD14 promotes the binding ability of TLR-4 to LPS.

Many of the TLRs after binding to PAMPs stimulate to produce the inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-12 (IL-12), as well as chemokines such as interleukin-8 (IL-8), MCP-1, and RANTES. These cytokines trigger innate immune defenses.

T-independent antigens-1 are pathogens-associated molecule patterns (PAMPs) such as lipopolysaccharides (LPS) and lipoteichoic acids. These antigens activate B-lymphocytes to binding to their specific toll-like receptors.

TLRs also play role in adaptive immunity by providing secondary signals. When microbial antigens bins their TLRs on APC, APCs produce co-stimulatory molecules and cytokines. Without the interaction of the co-stimulatory molecules, the naive T4- or T8-lymphocytes are not activated and undergo apoptosis.

Signaling PRRs found in the membranes of the endosomes are TLR-3, TLR-7, TLR-8 and TLR-9. Most of the TLRs that bind to viral components trigger the synthesis of cytokines called interferons that block viral replication within infected host cells.

NOD proteins including NOD-1 and NOD-2, are cytosolic proteins that play role in the recognition of intracellular peptidoglycan components. **RIG-1** and **MDA-5** are cytoplasmic sensors for viral RNA. After activation, they stimulate the production of interferon.

In addition to the PRRs found on or within cells, there are also secreted pattern-recognition receptors. These PRRs bind to microbial cell walls. They are enable to activate the complement pathways and to promote phagocytosis. One of them is mannan-binding lectin (also known as mannan-binding protein).

**The results of PRRs-PAMPs binding are not only phagocytosis or secretion of cytokines. Another result is neutrophil extracellular traps (NETs)**. In response to pathogen associated molecular patterns such as LPS, and certain cytokines such as IL-8, neutrophils release DNA and antimicrobial granular proteins. **These NETs bind to bacteria, prevent them from spreading, and kill them with antimicrobial proteins**

**The response of innate immum system**

When effectors cells of innate immun system recognize microbial antigens, they respond by phagocytosis, by producing cytokines, by activating complement system, by extracellular killing, by synthetizing acute phase proteins and by homing of effector cells to infection side. All this responses of immune systems form the inflammation.
Diapedesis

In order to perform effector functions, the cells have to pass out the blood vessels. This process is called diapedesis.

The resident macrophages recognize the microbe and produce soluble proteins called cytokines. Two of these cytokines, called tumor necrosis factor (TNF) and interleukin-1 (IL-1) stimulate the endothelial cells for the expression the adhesion molecules and the production of chemokines. The endothelial adhesion molecules are called E-selectin, P-selectin and ligands for integrins.

Circulating neutrophils and monocytes express ligands for selectins. These ligands bind weakly to the selectins. The neutrophils become tethered to the endothelium. Following the leucocytes begins to roll on the endothelial surface. The other molecules named integrins are present in a low-affinity state on unactivated leukocytes. The endothelial cells produce chemokines. These chemokines stimulate to increase the affinity of the leukocyte integrins for their ligands on the endothelium. The integrins leukocytes bind firmly to their ligands. The cells pass out the blod vessels and club at the infection site.

Chemokines also stimulate the motility of leukocytes. As a result, the leukocytes migrate along the chemokine concentration gradient to the site of infection.

The phases of the migration of blood leukocytes to an extravascular site of infection are the selectin-mediated rolling, the integrin-mediated firm adhesion, and the chemokine-mediated motility. All these phases are completed within minutes after the infection.

Phagocytosis

The recognition of microbes by neutrophils or macrophages induces the phagocytosis of the microbes and to kill the ingested microbes. In phagocytosis process, the phagocyte membrane zip up around the recognized microbe. And the membrane closes up and corners the particle. At the end the target particle is ingested in a membrane-bound vesicle, called a phagosome. The phagosome fuses with lysosome to form phagolysosomes.

At the same time several enzymes in the phagolysosomes are activated. One of these enzymes, called phagocyte oxidase, converts molecular oxygen into superoxide anion and free radicals. These substances are called reactive oxygen intermediates (ROIs). They are toxic to the ingested microbes. A second enzyme, called inducible nitric oxide synthase, catalyzes the conversion of arginine to nitric oxide (NO), also a microbicidal substance. The third set of enzymes are lysosomal proteases, which break down microbial proteins.

All these microbicidal substances are produced mainly within lysosomes. They act on the ingested microbes but do not damage the phagocytes. In strong reactions, the same enzymes may be liberated into the extracellular space and may injure host tissues.

Evasion of innate Immunity by bacteria

Some bacteria are able to resist microbicidal mechanisms. They use different ways for this aim. These mechanisms are listed below.

- The escaping from phagocytosis by covering of the microbial antigens and by forming coagulase mediated fibrin.
- The inhibition of the fusing of endosome to phagosome.
- The escaping into cytoplasm before the fusing of endosome to phagosome.
- The resistance to and destroying of microbicidal enzyme in phagolysosome.
• The inhibition of complement activation.
• The production of enzyme for killing of the phagocytes.
• The stimulation of apoptosis of macrophages.
• The removing of MAC complex by elongating of LPS

**The stimulation of adaptive immune system by innate immunity**

Full activation of antigen specific lymphocytes requires two signals. An antigen is "signal 1," and microbes or innate immune responses to microbes may provide "signal 2"

Microbes, or IFN-γ produced by NK cells in response to microbes, stimulate dendritic cells and macrophages to produce two types of second signals that can activate T lymphocytes. First, the dendritic cells and macrophages express surface molecules called **costimulators** (B7 proteins), which bind to receptors on naïve T cells and function together with antigen recognition to activate the T cells. Second, the dendritic cells and macrophages secrete the cytokine IL-12, which stimulates the differentiation of naïve T cells into the effector T cells.

Blood-borne microbes activate the complement system by the alternative pathway. C3d covalently attached to the microbe. When B lymphocytes recognize microbial antigens by their antigen receptors, at the same time the B cells recognize the C3d bound to the microbe by a receptor for C3d (CR2). The recognition of antigen and Cd3 together activate B lymphocyte to differentiate into antibody-secreting cells. Thus, a complement product serves as the second signal for humoral immune responses.

The second signals also guide the nature of the adaptive immune response. Intracellular and phagocytosed microbes need to be eliminated by cell-mediated immunity. T cell play major role in cell mediated immun responses. Microbes that are ingested by or live in macrophages induce the production of the second signals that stimulate T cell responses. In contrast, blood-borne microbes are only eradicated by antibodies. Blood-borne microbes activate the plasma complement system for differentiations of B cells into plasma cells.