Path 203– Principles of Immunology
Lecture for undergraduate studies - Week 2

The innate immune response is the first mechanism for host defense found in all multicellular organisms. The innate immune system is more ancient than the acquired or adaptive immune response, and it has developed and evolved to protect the host from the surrounding environment in which a variety of toxins and infectious agents including bacteria, fungi, viruses and parasites are found

Innate immunity is comprised of different components including

1- physical barriers (tight junctions in the skin, epithelial and mucous membrane surfaces, mucus itself)

2-anatomical barriers; epithelial and phagocytic cell enzymes (i.e., lysozyme), phagocytes (i.e., neutrophils, monocytes, macrophages), inflammation-related serum proteins (e.g., complement, C-reactive protein, lectins such as mannose-binding lectin, and ficolins); surface and phagocyte granule antimicrobial peptides (e.g., defensins, cathelicidin, etc.);

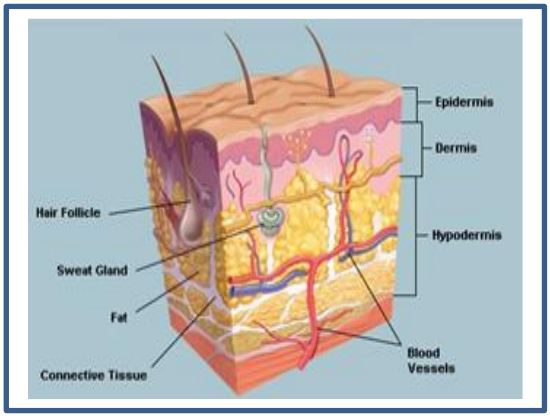
3- cell receptors that sense microorganisms and signal a defensive response (e.g., Toll-like receptors); and cells that release cytokines and inflammatory mediators (i.e., macrophages, mast cells, natural-killer cells). Once the interaction host-invader pathogen enters, a signaling cascade is initiated which enhances the immune response and activates specific mechanisms.

This natural immune response is designed to:

a) prevent infection,

b) eliminate invader pathogens, and

c) stimulate the acquired immune response.



Mechanism of innate immunity defense

1. Body covering (integumentary system) including skin and mucus membrane

The first part we describe is the anatomic barriers that protect the host against infection and examine the immediate innate defenses provided by various secreted soluble proteins. The simplest way to avoid infection is to prevent the microorganisms from gaining access to the body. The major line of defense is of course the skin which, when intact, is impermeable to most infectious agents; when there is skin loss, as for example in burns, infection becomes a major problem. Additionally, most bacteria fail to survive for long on the skin because of the direct inhibitory effects of lactic acid and fatty acids in sweat and sebaceous secretions and the low pH which they generate. An exception is Staphylo*coccus aureus* which often infects the relatively vulnerable hair follicles and glands.

What is the skins innate immune response?

The skin elicit a powerful defense reaction. The 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 skin is the largest organ of the body, with a total area of about 20 square feet. The skin protects us from microbes and the elements, helps regulate body temperature, and permits the sensations of touch, heat, and cold.

Skin has three layers:

- The epidermis, the outermost layer of skin, provides a waterproof barrier and creates our skin tone.
- The dermis, beneath the epidermis, contains tough connective tissue, hair follicles, and sweat glands.
- The deeper subcutaneous tissue (hypodermis) is made of fat and connective tissue.

The skin's color is created by special cells called melanocytes, which produce the pigment melanin. Melanocytes are located in the epidermis.

Keratinocytes are the predominant cells in the epidermis. They act as the first line of innate immune defense against infection. They express Toll-like receptors(TLRs) which are pattern-recognition receptors (PRRs) that detect conserved molecules on pathogens and trigger an inflammatory response.

i. Mucin

As part of their physiological function, many epithelial tissues in the human body are exposed to tribological shear stress. Examples include blinking, swallowing of food, and copulation. To prevent mechanical damage of the involved tissues during those processes and to guarantee an unimpeded functionality of the related body functions, proper lubrication and wear protection is needed. This is accomplished by several variants of mucus - a viscoelastic hydrogel that covers all mucosal membranes of mammalians. In addition to acting as lubricants, these mucus systems are also responsible for regulating the passage of molecules and particles towards the tissue in the gastrointestinal tract, protecting wet epithelial surfaces from dehydration, and serving as a defense layer against mucosal pathogens. The key constituent of such mucus hydrogels are mucins, a class of high molecular weight (~2- 3 MDa) glycoproteins, which exhibit a highly complex molecular architecture. During the past years,

ii. longitudinal flow of air and fluid & movement of mucus by cilia such as ciliary movement, coughing and sneezing. Among other mechanical factors which help protect the epithelial surfaces, one should also include the washing action of tears, saliva and urine. Many of the secreted body fluids contain bactericidal components, such as acid in gastric juice, spermine and zinc in semen, lactoperoxidase in milk and lysozyme in tears ,nasal secretions and saliva. A totally different mechanism is that of microbial: Antagonism associated with the normal bacterial flora of the body. This suppresses the growth of many potentially pathogenic bacteria and fungi at superficial sites by competition for essential nutrients or by production of inhibitory substances. To give one example, pathogen invasion is limited by lactic acid produced by particular species of commensal bacteria which metabolize glycogen secreted by the vaginal epithelium. When protective commensals are disturbed by antibiotics, susceptibility to opportunistic infections by *Candida* and *Clostridium dificile* is increased. Gut commensals may also produce colicins, a class of bactericidins produced by bacteria which bind to the negatively charged surface of susceptible bacteria and insert a hydrophobic helical hairpin into the membrane.

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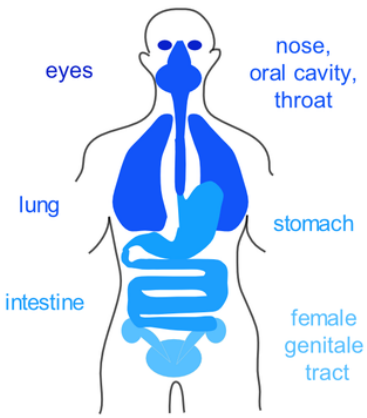

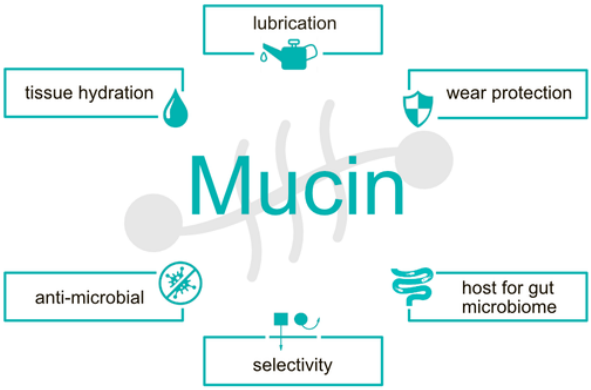

2. Tissue defense

A. Humoral factors in Innate Immune system

Once infectious agents have breached the anatomical barriers and penetrated tissues, . Humoral factors play an important role in inflammatory edema and the recruitment of these humoral factors are found in serum or they are formed at the site of infection.

Table of Humoral Factors in the innate immune system

Humoral factors	Effects
Complement	The complement system is the major humoral non-specific defense mechanism (see complement chapter). Once activated complement can lead to increased vascular permeability, recruitment of phagocytic cells, and lysis and opsonization of bacteria.
Coagulation	– 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.
beta-lysine	Antimicrobial substance, e.g. beta-lysine produce by platelate
Lysozyme	This is present in sweat, tear and many other secretions .It breaks down peptidoglycans in bacteria cell wall, thus damaging and killing the bacteria.
Interferons	Antiviral proteins
Defensins	Antibacteria , Antiviral proteins
Lactoferrin and	

transferrin	By binding iron, an essential nutrient for bacteria, these proteins limit bacterial growth.
Acids	
	Hydrochloric acid secreted by the stomach is lethal to many (though not all) bacteria .Commensal bacteria in the vagina produce lactic and proprionic acid producing allow pH ,which is inhibitory to the division of many bacteria.
Fatty acids	Sebaceous gland in the skin produce fatty acids which have antimicrobial properties.

Physical and chemical barrier are very effective at preventing pathogens from entering the body and they exclude more than 99.9% of the infectious organisms we are exposed to. However , organisms do infect the body and this can occurein a number of ways.

B. Cell defense

Cell defense includes the followings:

i. **Fever**
ii. **Inflammation**
iii. **Phagocytosis**

It is important before we study these aspects of cell defense to know the different types of immune cells and the important immune organs that might be involved in the immune response which will be explained below.

Cells of Immune response

Recognition of pathogens by cells of the innate immune system

When we say that cells of the innate immune system can 'recognise' pathogens, what do we actually mean? By recognition we mean that molecules or receptors, on cells of the innate immune system bind to other molecules that are present on

pathogens but not present on our own cells. In this way the immune system can distinguish foreign objects and respond to a foreign pathogen but not respond against our own tissue or cells.

It is a crucial feature of the immune system that it can target foreign objects. There are many receptors present on cells of the innate immune system **Pattern recognition receptors (PRR), immune sensors that discriminate self from non-self, link innate to adaptive immunity** and some of them are shown

-**The mannose receptor**. This binds to certain mannose components of carbohydrates (polysaccharides) found on the surface of many bacteria

-**Glycan receptor**. This can bind to polysaccharides of bacteria and yeast.

-**Scavenger receptor**. This recognizes sialic residues on bacteria and yeast.

-**CD14** the cell surface molecule called CD14 recognises lipopolysaccharide(LPS) found in the cell wall of Gram negative bacteria.

-Complement receptors 3 (CR3) and complement receptor4(CR4) these are receptors that recognize LPS , lipophosphoglycans and other structures found on bacteria and yeast.

Some of these receptors can also recognise new sugars that are exposed on dead or damaged host cells so that the phagocyte can remove the cell.

An important family of receptors that are able to recognise a wide variety of pathogen-associated molecules has been identified. These are the Toll-like receptors (TLRs), which were named because of their structural relationship to Toll, a protein involved in development of *Drosophila*, the fruit fly. There are about 10 TLRs that have been identified in human and similar numbers in other species.

The TLRs recognise a variety of microbial products such as double stranded RNA (found only in viral infection), lipopolysaccharide (LPS) from bacterial cell walls, bacterial lipoproteins, unmethylated DNA (indicating it is of bacterial origin) and flagellin, a component of bacterial flagella. Different TLRs recognise different microbial products, PAMPs are conserved molecular structures produced by microorganisms and recognized as foreign by the receptors of the innate immune systeme

The cellular response to recognition of microbial products

It can be seen that many cell types of the innate immune system have receptors on their surface that can recognise microbial products and therefore detect that we have been infected. Obviously recognition of infection is useful only if it results in a response to the infection that will ideally eliminate the pathogenic organism or at the very least limit the replication and spread of the pathogen. There are a number of ways in which different cells can respond to recognition of pathogens, and one of these is phagocytosis.

Lymphocytes are the central cells of the immune system, responsible for adaptive immunity and the immunologic attributes of diversity, specificity, memory, and self/nonself recognition. The other types of white blood cells play important roles,

engulfing and destroying microorganisms, presenting antigens, and secreting cytokines.

Types of cells involves in the immune response

1. Cells which are important in innate immunity

The important cells in the innate immunity includes all types of white blood cells (WBCs) which include all types of **Granulocytes.** Granulocytes are a subgroup of white blood cells characterized by presence of cytoplamsic granules. They are produced in bone marrow and consist of Basophils, Eosinophils, Neutrophils and mast cells.

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a) Neutrophils (50-70 % CBC)

- Neutrophils are granule-containing, polymorphonuclear leukocytes that develop in the bone marrow from myeloid precursors. They play a central role in the innate immune response by destroying foreign particles either intracellularly in phagosomes or extracellularly by releasing neutrophil extracellular traps (NETs), and promoting acute inflammation.
- In humans, neutrophils are the most abundant circulating leukocyte, accounting for 50-70% of white blood cells,
- In humans, neutrophils are distinguished from eosinophils and monocytes based on the expression of both CD15 and CD16/Fc gamma RIII on human neutrophils, along with the lack of expression of CD14. In addition, CD66b/CEACAM-8, CD11b/Integrin alpha M, CD33, and the cytoplasmic marker, myeloperoxidase, are other common markers that are used to identify human neutrophils.

Eosinophils, mast cells and basophils are major effector cells of innate immunity and play a fundamental role in the mechanisms of defense against bacterial, viral and parasitic infections. These cells are distinct in terms of development, maturation and location within tissues. Eosinophils and basophils fully mature in the bone marrow and under physiological circumstances circulate as blood cells. Mast cells leave the bone marrow as progenitors and reach their final maturation within peripheral tissues. However, these three types of cells are often involved together in a variety of pathological conditions when they are activated and accumulate in inflamed tissues where they release a wide spectrum of chemical mediators of inflammation.

Several proteins and Fc receptors have been reported to be up- or down-regulated in one or more diseases when compared to normal .

b) Basophils (0- 0.5% in CBC)

Basophils are the least common of the classically described peripheral blood leukocyte populations. Because of their expression of FcεRI and easy accessibility in peripheral blood

- Granulocyte (granules stain dark blue)
- Defense against parasites
- Involved in allergic and inflammatory reactions
- Contain toxic granules used to destroy pathogens during phagocytosis
- basophils are typically identified by their expression of cell surface markers such as CD123, CCR3, or CRTH .
  Basogranulin was initially described as a high molecular weight granule protein that was a specific marker of basophils .Basogranulin is secreted from granules after both IgE- and non-IgE mediated stimuli. Basogranulin can be used as a specific immunohistochemical marker to identify basophils in tissue.
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c) Eosinophils (1-3 % in CBC)

- Granulocytes stain bright red
- Defense against parasites & allergic reactions
- Release toxic substances in granules to destroy pathogens
- Differentiate from myeloid precursor cells in response to IL-3, IL-5, & GM-CSF

e) Biologic activities of human eosinophil granule proteins

Granule protein	**Biologic activities**
Major Basic Protein (MBP-1)	causes histamine release from basophils and rat mast cells; neutralizes heparin; increases bronchial reactivity to methacholine; provokes bronchospasm; activates neutrophils
Major Basic Protein-2 (MBP-2)	Cytotoxin; causes histamine release and LTC_4 release from basophils; causes superoxide and IL-8 release from neutrophils

Granule protein	Biologic activities
Eosinophil Cationic Protein (ECP)	causes histamine release from mast cells; neutralizes heparin, alters fibrinolysis
Eosinophil –Derived Neurotoxin (EDN)	Neurotoxin, RNase activity, virucidal, weak helminthotoxin; inhibits cultures of peripheral blood lymphocytes; same as eosinophil protein X
Eosinophil Peroxidase (EPX)	kills microorganisms and tumor cells; causes histamine release and degranulation from mast cells; damages respiratory epithelium. Generates reactive oxidants and free radicals

f) Mast cells

- Generated in bone marrow
- similar to basophilic leukocytes
- two types of mast cells found: 1. Connective tissue cells (local allergic reaction) and 2. Mucosal cells (areas exposed to external environment like lung mucosa, GI tract).
- Release of granules containing

Histamine

Histamine is the main biogenic amine released upon IgE-receptor activation by human mast cells (3–8 pg/cell) and basophils

Heparin

Heparin is produced by mast cells and human lung mast cells contain approximately 2.4 to 7.8 micrograms of heparin per 10^6 cells

Tryptase

The human tryptase locus on chromosome 16 includes genes that encode α- and β-tryptases .which are major protein products of human mast cells, much lesser amounts being expressed by basophils

chymase is thought to participate in multiple inflammatory responses in the vasculature, including blood pressure regulation and plaque instability. Chymase degrades lipoproteins which promotes macrophage foam cell formation.

Carboxypeptidase A3

Carboxypeptidase was first identified in human mast cells in 1989. Mast cells containing Carboxypeptidase A3 (CA3) have been reported in association with allergic disease of both the lower and upper airways .

Prostaglandin D2 and cysteinyl leukotrienes

Prostaglandin D2 (PGD2) and cysteinyl leukotrienes (Cyst LTs) are the major lipid mediators synthesized after mast cell activation. They are released shortly after the granule contents as part of the immediate mast cell response. Prostaglandins and leukotrienes are synthesized from arachidonic acid (AA) which is released by the action of cytosolic phospholipase A2 on membrane phospholipids .

diseases, or detrimental, as in allergic disorders. Mast cells, eosinophils and basophils play a fundamental role in several inflammatory, allergic and autoimmune diseases and they contribute to defense against infectious organisms and to development of many types of cancer. The role of these cells in human diseases may be beneficial, for example in infectious

2. Bridge cells

These are the cell that act as a bridge between innate and acquired immunity and include the followings:

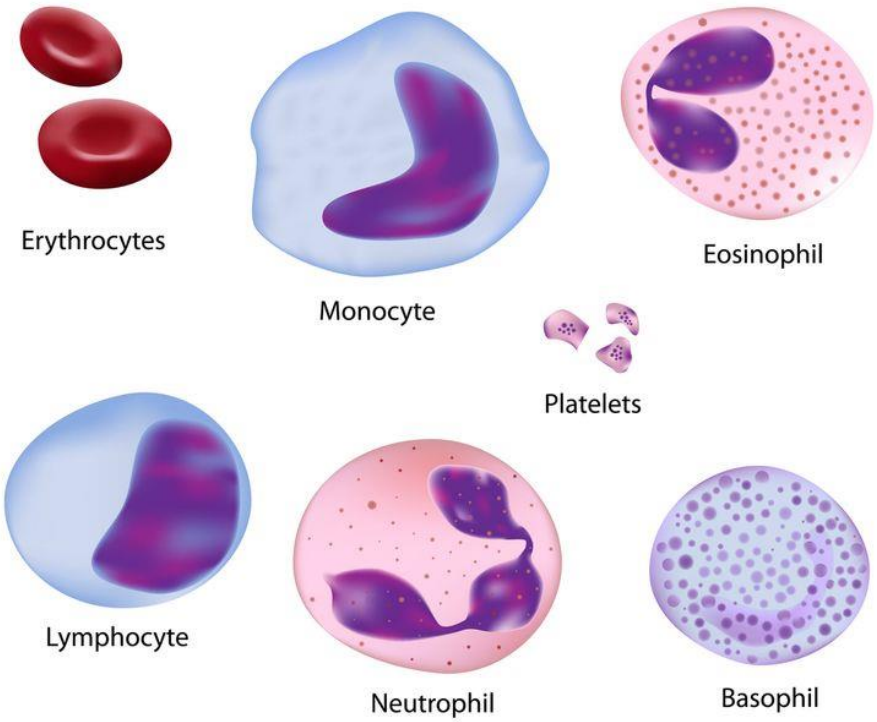
a) Monocyte (macrophage) (3-6 % in CBC)

- Circulate in the peripheral blood before entering tissues to replenish tissue-specific macrophage populations.
- "Big eater" - ingest and degrade bacteria.
- Can present antigens to lymphocytes.
- Important non-immune functions, such as recycling dead cells like red blood cells (housekeeping, happens w/out activation of immune response).

b) Dendritic cells

- specialized sentinel cells that constantly sense and respond to their immediate environment.
- Can matture at either bone marow, lymphoid, or non lymphoid tissues.
- Capture, process, and present antigens to T cells.
- Crucial for bridging innate and adaptive immunity as well as promoting self-tolerance.

dendritic cells after exposure/uptake of pathogens, these cells travel to secondary lymphoid organs to become potent T cell activators secrete large amounts of type I interferons upon activation through TLR7 and TLR9.



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c) Natural Killer Cells (NK cells)

- Innate immune cells (some have features of both innate & adaptive).
- Recognize and kill virus-infected cells or tumor cells.
- Contain granules filled w/ proteins that can form holes in target cells and cause apoptosis.
- function as cytolytic effectors and regulators of immune response.

γδ T cell

Gamma delta (γδ) T cells are the prototype of 'unconventional' T cells and represent a relatively small subset of T cells in peripheral blood. They are defined by expression of heterodimeric T-cell receptors (TCRs) composed of γ and δ chains.

Tissue-associated γδ T cell populations

γδ T cells often show tissue-specific localisation of oligoclonal subpopulations sharing the same TCR chains. For instance, human peripheral blood γδ T cells are largely Vγ9/Vδ2 cells (DETCs), are largely Vγ5/Vδ1+. In general, γδ T cells are enriched in epithelial and mucosal tissues where they are thought to serve as the first line of defense against pathogenic challenge.

Some γδ T cells also recognise markers of cellular stress, resulting from infection or tumor genesis. Stress surveillance performed by γδ T cells is thought to depend not only on their TCRs but also on co-stimulatory signals from, for instance, NK-type receptors. Finally, γδ TCRs have been shown to recognise lipid antigens presented by CD1 molecules, in particular CD1d.

- Interferons are small proteins released by macrophages, lymphocytes, and tissue cells infected with a virus. Strucurally, they are part of the helical cytokine family which are characterized by an amino acid chain that is 145-166 amino acids long
  When a tissue cell is infected by a virus, it releases interferon. Interferon will diffuse to the surrounding cells. When it binds to receptors on the surface of those adjacent cells, they begin the production of a protein that prevents the synthesis of viral proteins. This prevents the spread of the virus throughout the body.

- **Three types of interferons: alpha, beta and gamma.**
- Initially believed that T helper cell type 1 lymphocytes, cytotoxic lymphocytes and natural killer cells only produced IFNg, now evidence that B cells, natural killer T cells and professional antigen-presenting