

## Innate (Non-specific) Immunity

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Lecture: 1

### TEACHING OBJECTIVES:

1. Recognize the significance of the immune system in combating infection and disease.
2. Distinguish between non-specific (innate) and specific (adaptive) immune systems.
3. Understand the mechanisms of combating infection/disease (killing pathogens).
4. Know the humoral and cellular components of the non-specific immunity.
5. Comprehend the mechanism of action of the humoral and cellular components of non-specific immunity

READING: Roitt *et. al.*: Immunology (6th ed.).Ch. 1: pp. 1-2, 5-6, 9-10; ch. 15: pp.255-248.

Mechanisms of protection against infection and disease are diverse. Primarily they can be divided into two major categories: **non-specific** (innate) and **specific** (adaptive), which differ as follows:

Non-specific Immunity	Specific Immunity
Its response is <b>antigen-independent</b> .	Its response is <b>antigen-dependent</b> .
There is <b>immediate</b> response.	There is a <b>lag time</b> between exposure and maximal response.
It is <b>not antigen-specific</b> .	It is <b>antigen-specific</b> .
Exposure does <b>not</b> result in induction of <b>memory</b> cells.	Exposure results in induction of <b>memory</b> cells.

The elements of the innate immune system include anatomical barriers, secretory molecules and cellular components (Table 1).

**Anatomical barriers:** Skin, intestinal movement, oscillation of broncho-pulmonary cilia, etc. prevent pathogens from entering and/or getting a foothold in the body.

**Secretory molecules:** These include organic acids in skin secretions, lysozyme in oro-naso-pharyngeal and lacrimal secretions, thiocyanate in saliva, low molecular weight fatty acids in the lower bowel; bile acids and low molecular weight fatty acids in lower GI tract; transferrin, lactoferrin, lysozyme, interferons, fibronectin, complement, acute phase proteins, etc. in serum; Interferons and tumor necrosis factor (TNF) at the site of inflammation.

**Transferrin** and **lactoferrin** deprive organisms of iron. **Interferons** inhibit viral replication and activate other cells which kill pathogens. **Lysozyme**, in serum and tears, breaks down the bacterial cell wall (peptidoglycan); **fibronectin** coats (**opsonizes**) bacteria and promotes their rapid phagocytosis. **Complement** components and their products cause destruction of microorganism directly or with the help of phagocytic cells. Acute phase proteins (such as CRP) interact with the complement system proteins to combat infections. TNF-" suppresses viral replication and activates phagocytes.

Table 1. Physico-chemical barriers to infections

System/Organ	Active component	Effector Mechanism
Skin	Squamous cells; Sweat	Desquamation; flushing, organic acids
GI tract	Columnar cells	Peristalsis, low pH, bile acid, flushing, thiocyanate
Lung	Tracheal cilia	Mucociliary elevator, surfactant
Nasopharynx & eye	Mucus, saliva, tears	Flushing, lysozyme
Circulation and lymphoid organs	Phagocytic cells NK cells & K-cell LAK	Phagocytosis and intracellular killing Direct and antibody dependent cytotoxicity IL2-activated cytotoxicity
Serum	Lactoferrin and Transferrin Interferons TNF- $\alpha$ Lysozyme Fibronectin Complement	Iron binding Antiviral proteins Anti-viral, phagocyte activation Peptidoglycan hydrolysis Opsonization and phagocytosis Opsonization, enhanced phagocytosis and intracellular killing, inflammation

## Cellular Components:

### Phagocytic cells:

Neutrophils (PMN) and macrophages and monocytes are the most important cellular components of the non-specific immune system.

**Neutrophils** (polymorphonuclear: PMN) are most important cellular components in bacterial destruction. They are relatively large and most abundant white blood cells with lobed nucleus and cytoplasmic granules (lysosomes). They are identified by their characteristic morphology. In addition, monoclonal antibodies against characteristic cell surface protein, cluster differentiation marker, CD66 can be used to identify these cells.

PMN granules are of two kinds: primary (azurophilic) and secondary (specific).

**Primary azurophilic granules** are characteristic of immature and very young neutrophils. They contain NADPH oxidase co-factors, cationic proteins, defensins (small molecular weight proteins), proteases (elastase, cathepsin G, *etc.*), lysozyme and, characteristic for them, **myeloperoxidase**.

**Secondary granules** are more characteristic of (specific for) mature neutrophils. They contain lysozyme and NADPH oxidase cofactors, **lactoferrin** and **B-12-binding protein**, the last two are characteristic for these granules.

**Mononuclear phagocytes** are the other population of phagocytic cells and include monocytes in circulation, histiocytes in tissues, microglial cells in the brain, Kupffer

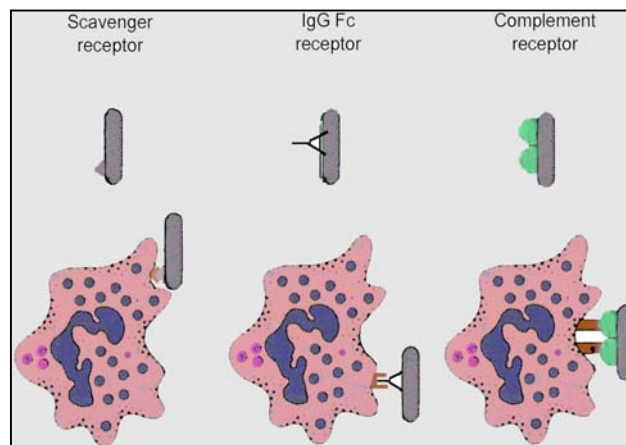


Figure 1: Adherence of bacteria via receptors

cells in the liver and macrophages in serous cavities and lymphoid organs, They also have granules similar to those in neutrophils, although not as abundant. They are recognized by their morphology, ability to adhere on glass/plastic surface, phagocytic property and CD14 marker.

All phagocytic cells have receptors for a variety of molecules (Figure 1). Most pertinent to non-specific immunity are Scavenger and TOLL-like receptors and receptors for the Fc part of IgG, complement, interferons, TNF and certain bacterial components. Interaction of organisms with these receptors promotes phagocytosis and phagocyte activation for a more efficient killing of pathogens.

### Phagocyte response to infection:

#### Chemotaxis:

Bacteria produce **N-formyl-methionine**-containing peptides which are powerful attractants (chemotactic) for phagocytic cells. Many bacteria also act on proteins of the complement and clotting systems to produce peptides that cause vasodilation, vascular permeability and expression of adherence molecules on vascular endothelial cells. They also induce, on phagocytic cells, expression of proteins (*e.g.*, integrins) that promote binding to endothelial cells. Phagocytic cells respond to chemotactic peptides of bacterial and host origin and migrate across the capillary wall (**diapedesis**) to the site of infection/inflammation.

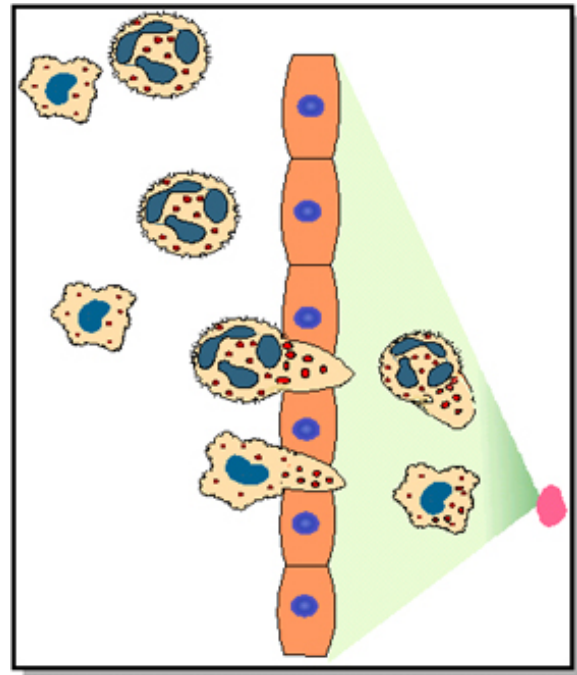


Figure 2: Chemotactic response to inflammatory stimulus

#### Attachment:

Once at the site of infection, phagocytes can attach to bacteria via a number of receptors for bacterial components (scavenger receptor, LPS receptor, mannose receptor, TOLL-like receptors, *etc.*) or host proteins that act as **opsonins** (proteins which aid Phagocytosis) such as fibronectin, complement and IgG antibody. This attachment triggers the activation of respiratory burst (hexose monophosphate shunt), internalization of the organisms (phagosome formation), phagosome lysosome fusion (phagolysosome) *etc.*

#### Respiratory burst and phagocytosis:

The process of attachment and phagosome formation is accompanied by the activation of the respiratory burst (hexose monophosphate shunt) which results in the production of superoxide anion, singlet oxygen, hydroxyl ion and hydrogen peroxide (Figure 3). These molecules are microbicidal and cause killing of organisms in the phagosome.

Myeloperoxidase Independent Reactions		
Reaction	Active Enzyme	Products
Glucose + NADP	Glucose-6-P-dehydrogenase	Pentose-P + NADPH
NADPH + O <sub>2</sub>	Cytochrome b-558	NADP <sup>+</sup> + O <sub>2</sub> <sup>-</sup>
2O <sub>2</sub> <sup>-</sup> + 2H <sup>+</sup>		H <sub>2</sub> O <sub>2</sub> + <sup>1</sup> O <sub>2</sub>
O <sub>2</sub> <sup>-</sup> + H <sub>2</sub> O <sub>2</sub>		.OH + OH <sup>-</sup> + O <sub>2</sub>

Figure 3. Respiratory burst:

**Phagosome-lysosome fusion:** Phagosome, soon after its formation, fuses with granules (**lysosomes**) to form a **phago-lysosome**. As mentioned earlier, lysosomes contain a variety of anti-microbial substances (*e.g.*, lysozyme, defensins, proteases, lactoferrin, transferrin, *etc.*) and phago-lysosome fusion results in the exposure of microorganisms to these substances and their destruction. Also, fusion of phagosome with primary granules (in newly recruited phagocytes) that contain **myeloperoxidase** results in the production of  $\text{OCl}^-$ , and **halogenation** of bacterial proteins and bacteriolysis (Figure 4).

### Three modes of intracellular killing:

It should be apparent from the preceding discussion that there are three pathways of killing by phagocytes (Figure 3-4 and Table 2): (1) by lysosomal antibacterial substances (lactoferrin, cationic proteins, lysozyme, defensins, proteases, *etc.*) without the requirement of respiratory burst (**oxygen-independent killing**: Table 2); (2) by products of respiratory burst (super-oxide, singlet oxygen, hydroxyl radical, hydrogen peroxide, *etc.*) without the need for myeloperoxidase (**oxygen-dependent, myeloperoxidase-independent killing**: table 3a); and (3) by halogenation of bacterial proteins catalyzed by myeloperoxidase (**oxygen-dependent, myeloperoxidase-dependent killing**: Figure 4). These processes occur simultaneously and act synergistically. A defect in any of these pathways, for example, a deficiency of NADPH oxidase (cytochrome b558) components (p91, p22, 947 p61-*phox*), myeloperoxidase, *etc.* would impair the killing activity of phagocytes and render the host more susceptible to pyogenic infections.

Myeloperoxidase dependent reactions			
Reaction	Active Enzyme	Products	
$\text{H}_2\text{O}_2 + \text{Cl}^-$	Myeloperoxidase	$\text{OCl}^- + \text{H}_2\text{O}$	
$\text{OCl}^- + \text{H}_2\text{O}$		$^1\text{O}_2 + \text{Cl}^- + \text{H}_2\text{O}$	
$2\text{O}_2^- + 2\text{H}^+$	Superoxide dismutase	$\text{O}_2 + \text{H}_2\text{O}_2$	
$\text{H}_2\text{O}_2$	Catalase	$\text{H}_2\text{O} + \text{O}_2$	

Figure 4 Respiratory burst:

Table 2. Oxygen independent mechanisms of intracellular killing

Effector Molecule	Function
Cationic proteins (including cathepsin) Lysozyme Lactoferrin Proteolytic and hydrolytic enzymes	Damage to microbial membranes Splits mucopeptide in bacterial cell wall Deprives proliferating bacteria of iron Digestion of killed organisms

Neutrophils also contain catalase and glutathion (GS) which detoxify excess  $\text{H}_2\text{O}_2$ . GS, in its reduced form (GSH), also recycles NADP to NADPH.

Interaction of phagocytic cells with certain humoral factors (*e.g.* interferons, TNF, C5a, IL-2, *etc.*) can increase their phagocytic function, respiratory burst and intra-cellular killing. Certain proteins secreted by various cells (cytokines) can also induce phagocytic cells, particularly macrophages, to produce nitric oxide (NO) that is toxic to microorganism and malignant cells (Figure 5). Thus certain bacterial products can cause macrophages to produce TNF that can, in turn, activate NO production. Likewise, interferon- $\gamma$  produced by NK (natural killer) cells (and T cells) can also activate the NO pathway of killing pathogens.

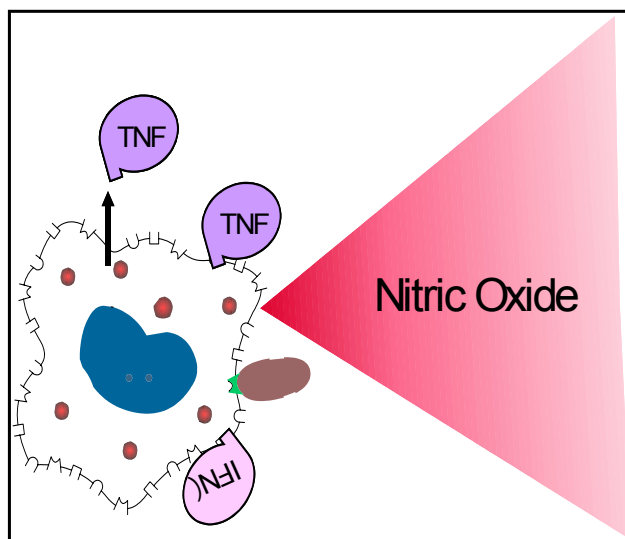


Figure 5: Nitric oxide production by phagocytes

### Other cells:

A number of other cells are also involved in non-specific resistance: they include NK), antibody dependent cytotoxic cells (ADCC) also referred to as K-cells and lymphokine (proteins secreted by lymphocytes) activated killer (LAK) cells and eosinophils.

**NK cells** are important in defense against viral infections and malignancies. They resemble lymphocytes in morphology but are larger and granular, hence also known as large granular lymphocytes (LGL). The granules contain cytolytic proteins such as perforin. NK cells recognize the difference between normal and malignant or virus-infected cells in a nonspecific manner via sugar-lectin interaction and kill them following intimate contact. They also have low affinity Fc (III) receptor (CD16) by which they can interact with antibody-coated cells and cause their death. NK cells also have receptors for interleukin-2 (**IL-2**) and interferon- $\gamma$  and interaction with these cytokines leads to their activation (Figure 6). The presence of CD56 & CD16 and absence of CD3 is currently used as characteristic markers for NK cells.

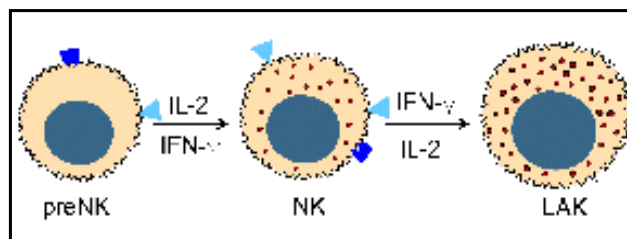


Figure 6. NK cell activation

Interaction with IL-2 (or interferon- $\gamma$ ) causes activation of NK cells. These activated cells are referred to as **LAK** (lymphokine-activated killer) cells. LAK cells function the same way as NK cells except that they are more potent killers and are capable of killing not only malignant but also transformed cells.

**K-cells** are morphologically undefined cells attach to target cells coated with IgG antibody via the **Fc receptor** and cause their lysis; thus, they require interaction of specific antibody with target. Macrophages can also function as K cells since they also have Fc receptors. Macrophages, when activated by a variety of cytokines, can kill malignant cells without the aid of antibody.

**Eosinophils** have Fc receptor for IgE and cause cytotoxicity to large multicellular parasites coated with specific IgE antibody, analogous to K cells.

There is no memory or specificity in the components of non-specific immunity. However, cells of the non-specific immune system become functionally more efficient following exposure to a pathogen because of interaction with products of the specific immune system (e.g., antibodies and cytokines).

Table 3. Characteristics of cells involved in non-specific resistance

Effector cell	Identifying marker(s) and/or function				
	CD3	Ig	Fc	CD	Phagocytosis
Neutrophil	-	-	IgG	CD66	+
Macrophage	-	-	IgG	CD14, CD68	+
NK cell	-	-	IgG	CD56	-
K-cells	-	-	IgG	?	-
LAK cell	-	-	?	?	-
Eosinophil	-	-	IgE	CD66	-

At this time you should know the following:

1. Differences between the non-specific and specific immune functions.
2. Humoral components of the non-specific immune system and their action.
3. Cellular components of the non-specific immune function and their action.
4. **Pathways of intracellular killing of bacteria by phagocytes and their characteristic features.**
5. Effect of humoral components such as interferon, TNF, IL-2, complement etc. on cellular components of the non-specific immune system.