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INNATE IMMUNITY - CELLULAR AND HUMORAL FACTORS

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We live in a potentially hostile world filled with various infectious agents of a different size, shape and composition. They would happily use us as a rich source for their propagation had we not also developed a series of defence mechanisms.

What is immunity?

It comes from the Latin “*immunitas*” – freedom from and describes the specific reactions of an organism against agents, carrying foreign information.

The body's defence against infectious diseases has two forms:

- ❖ Nonspecific resistance mechanisms
- ❖ Specific immune responses

The nonspecific resistance is called ***natural resistance*** or ***innate immunity***.

A sharp distinction should be drawn between the terms innate (1) and acquired (2) immune defences:

- Innate immunity confers resistance that does not involve production of antibodies.
- ***The major soluble factors*** of innate immunity are complement, acute phase proteins, interferons, lysozyme, while in acquired immunity these are antibodies:

- ✓ **The main cellular factors** of innate immunity are phagocytes and NK cells, where's in acquired immunity these are T lymphocytes.
- ✓ The first contact in innate immunity does not lead to **specific memory** and the second contact results in activation of the innate mechanisms with the same strength. The second contact in acquired immunity results in stronger and more powerful reaction and the immune response is also more rapid than the primary.

Innate immunity may be considered at the level of the species, race or individual.

- ***Species immunity*** means that the resistance or receptiveness to infections is characteristic for all members of a species (humans are resistant to plant pathogens). This immunity may be *absolute* (resistance at any circumstances) or *relative* (sometimes can be lowered – for ex. malnutrition).

- Within a species, different **races** may show differences in susceptibility to infections (some people are more susceptible to TBC than others, resistance to malaria in some areas in Africa).
- **Individual immunity** is resistance exhibited by different individuals in a race. It depends on:
 - ✓ **age** (newborns and senile are more susceptible to infections)
 - ✓ **endocrine disorders** (diabetes mellitus is associated with an enhanced susceptibility to infections)

- ✓ **sex** (females are more susceptible to infections during pregnancy, birth delivery)
- ✓ **malnutrition** (both humoral and cell mediated immune processes are reduced). It has also been reported that some viruses may not multiply in the tissues of severely malnourished individuals. Lack of proteins and vitamins increases susceptibility to infections too.

Nonspecific defences include physiological barriers

I. Defence against entry in the body

The first barriers that meet the infectious agents are skin and mucous surfaces.

1. **Skin** is normally *impermeable* to the majority of infectious agents (exception *Leptospira* spp. can pass through intact skin).

- a. direct inhibitory effect of ***lactic acid and fatty acids*** present in sweat and the lower pH to which they give rise. In cases of skin loss (for ex. burns) infection becomes a major problem.

- b. ***normal flora*** – associated with the phenomenon of bacterial antagonism suppresses the growth of pathogenic bacteria by competing for surfaces, nutrition, production of colicins (bactericidines) etc.

2. Membranes lining the inner surface of the body.

Role due to:

- a. secretion of mucus** which acts as a protective barrier inhibiting the adherence of bacteria to the epithelial cells
- b. microorganisms** trapped within the adhesive mucus may be removed mechanically by **ciliary action, coughing and sneezing**
- c. the flushing action of tears, saliva and urine** are other strategies which protect the epithelial surfaces

d. presence of ***microbicidal factors***, listed below are also protective mechanisms:

- ❖ lysozyme in tears
- ❖ nasal secretion and saliva
- ❖ acid in gastric juice
- ❖ lactoperoxidase in milk
- ❖ zink in semen

Mechanical barriers are highly effective and their failure often results in infection (for ex. defects in ciliary lining of the respiratory tract are associated with an increased susceptibility to lung infections – cystic fibrosis).

If microorganism overcomes the effectiveness of mechanical barriers it penetrates into the body. When this occurs two main defence strategies start to operate:

- ❑ Phagocytosis which involves engulfment and killing of microorganisms by specialized cells **(cellular factors of innate immunity)**
- ❑ Soluble chemical factors **(humoral factors of innate immunity)**

II. Cellular factors of innate immunity

1. **Phagocytosis** (literally “eating by the cell”). A process of adherence of the microorganisms to the phagocytic cells, engulfment and killing

Phagocytes – Elie Metchnikoff classified phagocytes in two cell families:

- ✓ *large* – macrophages
- ✓ *small* – polymorphonuclear leucocytes (microphages)

Macrophages originate as bone marrow promonocytes which develop into circulating blood monocytes and finally become mature macrophages, spread in the tissues, collectively termed “**mononuclear phagocyte system**”. They are in:

- ✓ the lung (alveolar macrophages)
- ✓ the brain microglia
- ✓ the liver (Kupffer cells)
- ✓ the bones (osteoclasts) etc.
- ✓ the lymph node medulla

Local tissue macrophages are called **histiocytes**.

Macrophages are long-lived cells, containing lysosomes and unlike neutrophils, they have mitochondria. They also have several basic functions – phagocytosis, antigen presentation to T cells to initiate specific immune responses and secretion of cytokines to activate and promote innate and immune responses.

Macrophages express cell surface receptors for the Fc portion of immunoglobulin G (IgG), for the C3b product of the complement cascade, toll-like receptors and class II MHC antigen.

□ **Polymorphonuclear neutrophils (PMN)** are dominant cells in the blood stream. They are bone marrow derived too, but are short-lived cells. They have no mitochondria. The nucleus is segmented. Specific granules serve as reservoirs:

- **primary cytoplasm granules** contain myeloperoxidase, lysozyme cationic proteins
- **secondary** – lactoferrin, lysozyme
- **tertiary** – acid hydrolases

PMNs provide the major defence against pyogenic (pus forming) bacteria, while the macrophages provide defence against microorganisms, capable of living within the host cells (intracellular bacteria).

Severe neutropenia or neutrophil dysfunction is associated with life threatening infections caused by *S. aureus*, Gr(-) bacteria or fungi.

The process of phagocytosis – stages

Attachment – Phagocytes attach to microorganisms via nonspecific receptors. Many bacteria produce chemical substances which attract leucocytes, a process known as ***chemotaxis***. The attached agent is taken into the cell by the membrane and a phagosome is formed.

Lysozyme fusion – The cytoplasmic granules fuse with the phagosome, phagolysosome is formed and discharge their contents around the microorganism which becomes a target of killing.

Killing process involves:

- ❖ Oxygen-dependent mechanisms or
- ❖ Oxygen-independent mechanisms

❖ **Oxygen-dependent killing** – a burst of oxygen consumption as a result of a dramatic increase in the activity of hexose-monophosphate shunt. This reduces molecular oxygen and powerful microbicidal agents are produced: superoxide anion, hydrogen peroxide, single oxygen and hydroxyl radicals, all toxic for microorganisms. Peroxide in an association with myeloperoxidase generates a potent halogenating system from halide ions, which is capable of both killing bacteria and viruses. This mechanism is also called **“respiratory burst”**

❖ ***Oxygen-independent killing*** involves action of cathepsin, cationic protein, which adhere to microbial surface and damage it.

Other factors are lactoferrin and nitric acid (deprive bacteria from iron, which is a growth element for them).

Macrophages secrete also lysozyme and many proteolytic enzymes that degrade dead microorganisms.

Phagocytosis is promoted by opsonins – serum factors, IgG and complement are the best opsonins.

Mechanisms, avoiding phagocytosis

- Release of toxins that kill phagocytes (staphylococci, streptococci)
- Protein A (*S. aureus*) prevents interaction between opsonizing antibodies and phagocytic cells, thus inhibiting phagocytosis
- Capsule prevents contact with the phagocyte (*S. pneumoniae*, *Haemophilus* spp.)

- Inhibition of phagolysosome fusion (M. tuberculosis, M. leprae, Chlamydia spp.)
- Escape into the cytoplasm – microorganisms escape from phagolysosome and replicate within the phagocyte
- Resistance to killing by production of antioxidants – catalase (staphylococci)

2. *Extracellular killing*

- ✓ **NK cells** – a class of lymphocytes, called **Natural Killer cells**. They are important in nonspecific defence against viral infections, selectively killing virus infected cells and tumor cells as well. They are activated by interferons or mitogens.
- ✓ **Eosinophils** – peripheral blood cells that can kill parasites.

III. Humoral factors of innate immunity

1. Complement (Co)

Co is the name given to a complex of about 20 proteins, grouped in 9 components, which form one of the triggered enzyme systems in serum.

They produce a rapid amplified response to a stimulus, mediated by a cascade phenomenon where the product of one reaction is the enzymatic catalyst of the next.

Co components are designed with capital letter **C**, followed by a number. It is heat labile – it inactivates at 56°C for 30 min.

All Co components normally exist as inactive precursors, but once activated they react in a specific sequence. Each precursor is cleaved into two or more fragments.

The major fragment (usually designed **b**) has two biologically active sites:

- one for binding to cell membranes
- the other for enzymatic cleavage of the next component

The minor fragments have chemotactic activities.

Co activation occurs via three routes:

- ❑ classical
- ❑ alternative
- ❑ lectin

➤ **Classical pathway** was the first to be described. It is activated by Ag-Ab complexes, where Ab is either IgG or IgM.

The classical Co cascade is initiated by binding to the Fc portion of Ab that is bound to cell surface antigens, or in an immune complex with soluble antigens.

The reaction of IgG or IgM with its Ag causes change in the Fc region of the Ab and this reveals a binding site for the first component **C1q**.

Once **C1q** is activated, **C1r** and **C1s** are sequentially bound to generate enzyme activity for **C4** and **C2**, splitting both into **a** and **b** fragments.

The complex **C4b2a** is known as **C3 convertase**. It cleaves **C3** into **C3a** (with anaphylatoxic and chemotactic activity) and **C3b** (with opsonic activity).

The interaction of **C3b** with **C4b2a** bound to the cell membrane produces **C4b3b2a** which is termed **C5 convertase** and initiates the final lytic pathway involving the sequential attachment of **C5**, **C6**, **C7**, **C8** and **C9** (known as **MAC** – membrane attack complex) and results in lysis of the target cell.

➤ **Alternative pathway** was described later but it is phylogenetically older than the classical one.

The central reaction is also the activation of **C3**. This pathway generates **C3 convertase** without Ag-Ab complex, **C1**, **C2** or **C4**.

The most important activators are endotoxins, as well as other factors.

The initial cleavage of **C3** happens continuously and spontaneously, generating low levels of **C3b**.

C3b is able to use factors **B** (properdin) and **D** (a serum enzyme) to produce the active enzyme **C3bBb**. It becomes stabilized in the presence of properdin to form **C5 convertase** of the alternative pathway.

Once **C5 convertase** is produced, the lytic cascade acts on the same way as in the classical route.

➤ **Lectin pathway** is also a bacterial and fungal defense mechanism. **Mannose-binding protein (MBP)** is a large serum protein that binds to nonreduced mannose, fucose and glucosamine on bacterial and other surfaces.

MBP resembles and replaces the **C1q** component and on binding to bacterial surfaces, activates the cleavage of mannose-binding protein – associated serine protease, which cleaves the **C4** and **C2** to produce the **C3 convertase**, the junction point of the complement cascade.

In conclusion:

- ❑ The major purpose of the Co is to destroy Ag, regardless whether or not it has become coated with Abs.
- ❑ Another important role of Co is the opsonization of microorganisms – coated with Co or Ig they are more easily recognized by macrophages and more readily bound and phagocytosed.
- ❑ Co also participates in the cytotoxic (type II) and immune complex (type III) hypersensitivity reactions.

Avoiding damage by Complement:

- ❑ capsules
- ❑ IgA coating (streptococci, *Campylobacter* spp.)

2. ***Lysozyme*** – in tears, saliva (acts against Gr/+/ bacteria)
3. ***Beta lysins*** – thermostable substances active against *Bacillus anthracis*
4. ***Leukins and plakins*** – basic polypeptides, extracted from leucocytes and platelets
5. ***Spermine*** – from kidneys, with antibacterial activity

6. Interferons (INFs) – They are a family of broad spectrum antiviral molecules, used for prevention and treatment of viral infections. They also have a non-specific activity on tumor cells.

There are three types of INFs:

- INF- α – produced by leucocytes
- INF- β – by fibroblasts and probably other cell types
- INF- γ – by immune lymphocytes and NK cells. It is a signalling molecule (cytokine) in acquired immune response

Inflammation

Inflammation is defined as a process of increased vascular permeability accompanied by an infiltration of *“inflammatory cells”*.

Vascular permeability may be increased by a number of agents such as **C3a**, **C5a**, **factor B** and **C2**. A lot of vascular permeability mediators are released. This allows exudation of fluid and plasma components, including more C_0 to the site of infection.

The inflammatory **cytokines** (IL-1, IL-6) also provoke an increased synthesis of particular serum proteins in liver, termed as acute **phase proteins (APP)**. Many of them such as **CRP** (C-reactive protein) increase dramatically, while others show more moderate rises (haptoglobin, alpha-1 antitrypsin).

In practice serum measurement of **CRP** gives an useful information about the extend and persistence of inflammation.

The process of capillary dilatation (redness or erythema), exudation of serum proteins and the accumulation of neutrophils are collectively termed **acute inflammatory response** and represent a highly effective way of focusing the phagocytic cells onto Co-coated microorganisms.

Inflammation may have a positive effect on self-control the infection, but in hypersensitivity and autoimmunity it may be a potential self damaging reaction.

Thank you for the attendance