Lecture outline: Innate Immunity

Prof. N.P. Sunil-Chandra, Senior *Professor of Microbiology*, *Faculty of Medicine*, *University of Kelaniya*

07.08.2018

Innate Immunity

The innate immunity system is what we are born with and it is nonspecific; all antigens are attacked pretty much equally. It is genetically based and we pass it on to our offspring.

Innate immunity

- (a). **Barriers to infectious organisms** which include (i). Physical, (ii). Mechanical barriers

  (iii). Chemical barriers (iv). Normal bacterial flora
- (b). **Cell**s involved in innate immunity.
- (c **). Humoral components**: including Complement, acute phase proteins & Cytokines

Major components of the innate immunity

Cellular:

Phagocytic cells (macrophages,neutrophils)

NK cells (natural killer, spontaneously cytotoxic)

Humoral

Complement (classical & alternate pathway)

Acute phase proteins

Cytokines (interferons)

Barriers against infection

Simplest way to avoid infection:

Prevent microbes gaining access to the body

First line of defense against infection at the external surfaces of the body:

- 1. **Skin** barrier & fatty acids
- 2**. Cilia & mucus** of Upper RT
- 3. **Mucus, acid & normal bacterial flora** of the GIT.

Skin

Intact skin (i.e. keratin layer)-

Impermeable to most infectious agents.

Skin loss (i.e. in burns) - Infection become a major problem

Sweat & sebaceous gland secretions contain salts, lactic acid and fatty acids

Most bacteria fail to survive on skin for long due to

Direct inhibitory effects of lactic acid and fatty acids

Low pH generated by lactic acid and fatty acids

(Staphylococcus aureus *infection of hair follicles and glands is an exception)*

Mucus secretions by inner surfaces of the body

Mucus acts as a **protective barrier** to block adherence of bacteria to epithelial cells.

Microbes and other foreign particles are **trapped** in adhesive mucus.

Removed by mechanical methods

- Ciliary movement
- Coughing
- Sneezing
- Washing/flushing action of tears, saliva & urine

Chemical barriers - Bactericidal components of secreted body fluids:

Acids in gastric juice

Spermine and zinc in semen

Lactoperoxidase in milk

Lysozyme in in tears, nasal secretions & saliva

Microbial antagonism by normal bacterial flora

Suppress the growth of many potentially pathogenic bacteria & fungi at superficial sites. **How?**

- *By competition for essential nutrients*
- *By production of inhibitory substances*.

Even at this level survival is a tough game

Microbial antagonism

1. **lactic acid:** Produced by commensal bacteria (*Lactobacillus* species) which metabolize glycogen secreted by vaginal epithelium.

2. **Colicins** (a class of bactericidins): produced by Gut commensals

- *bind with negatively charged surfaces of susceptible bacteria.*
- *Kills bacteria by destroying the cell's energy potential.*

Commensals are disturbed by **antibiotics** & opportunistic infections by Candida & *Clostridium difficile* increased

If microorganisms do penetrate the body. Then what?

Two main defensive operations come to play.

1. Mechanism of **phagocytosis** (eating by the cell)

2. Destructive effects of **soluble chemical factors** (i.e. bactericidal enzymes).

Phagocytosis

Two types of phagocytic cells:

- Polymorphonuclear neutrophil (PMN)
- Macrophages (MQ)

Phagocytes:

Produced throughout life by the bone marrow. Scavengers – remove dead cells and microorganisms.

<u>Neutrophils /</u>Polymorphonuclear neutrophil (PMN)

60% of WBCs

Large numbers are released during infections

Short lived – die after digesting bacteria

Derived from Common haemopoetic stem cell precursor

Dominant white cell in blood

Non dividing , multi-lobed nucleus

Confined to blood stream except the sites of inflammation

Provide major defense against pyogenic bacteria

Array of granules unstained by H & E

Two types of granules

- Primary azurophil granules (develop early)
- Secondary specific granules

PMN granules

Primary azurophil granules (develop early) contain

(a) Myeloperoxidase,

(b). non-oxidative antimicrobial effectors

1. defensins
2. bactericidal/permeability increasing factor (BPI)
3. Cathepsin G

Secondary specific granules (Peroxidase negative) contain

(a). Lactoferrin

(b). Lysozyme

(c). Alkaline phosphatase

(d). Cytochrome b558 (Membrane bound)

- **Eosinophils**
- Attracted to cells coated with complement C3B,
- where they release major basic protein (MBP), cationic protein, perforins, and oxygen metabolites,
- all of which work together to burn holes in cells and helminths (worms). About 13% of the WBCs are eosinophils.
- Their lifespan is about 8–12 days. (Neutrophils, eosinophils, and macrophages are all phagocytes).

Macrophages (meaning "big eaters“)

- Larger than neutrophils.
- Found in the organs, not the blood.
- Made in bone marrow as **monocytes,** called macrophages once they reach organs.

  Initiate immune responses as they display antigens from the pathogens to the lymphocytes.
- Derived from bone marrow **promonocytes**
- Differentiate to circulating blood **monocytes**
- Mature & distribute throughout the body
- Settle as **tissue macrophages**
- Make up the mononuclear phagocytic system
- Long lived cells (unlike PMN)
- Best in combating those bacteria, viruses and protozoa <u>live within cells of the host</u>

Mononuclear phagocytic system

Brain	-	microglia
Lung	-	alveolar MQ
Bone	-	osteoclasts
Liver	-	Kuffer cells
Spleen sinusoids (lining)	-	splenic MQ
Joints	-	synovial MQ
Blood	-	monocytes precursors
Pleura	-	Pleural cavity MQ
Kidney glomerulus	-	mesangial cells
Lymph node medullary sinuses		lymphnode MQ
Resident connective tissue	-	histiocytes
Chronic inflammation	-	activated MQ,
	-	epithelioid & giant cells

Extravasation of MQ

- The non-fixed or *wandering macrophages* roam the blood vessels
- can even leave them to go to an infection site where they destroy dead tissue and pathogens.
- Emigration by squeezing through the capillary walls to the tissue is called **diapedesis** or **extravasation**. The presence of histamines at the infection site attract the cells to their source.

Phagocytosis

- Microorganism must 1^{st} adhere to PMN or MQ
- Primitive recognition mechanism involving CHO
- Particle attachment initiate ingestion phase
- Activate actin- myosin contractile system
- Extend pseudopodia around particle
- Particle is enclosed in a phagosome

- Cytoplasmic granules fuse with phagosome (within 1 minute)
- Discharge contents around ingested microbe
- Microbes are subjected to a formidable battery of microbicidal mechanisms

Killing by phagocytes

- 1. By reactive oxygen intermediates (ROI)
- 2. By reactive nitrogen intermediates
- 3. By preformed antimicrobials

Reactive oxygen intermediates (ROI)

- Invading microbe initiate phagocytosis
- (a). Increase activity of hexose monophosphate (HMP) shunt generating NADPH
- Electron pass from NADPH --- FAD containing membrane flavo – protein ----- plasma membrane cytochrome b558
- Cyt b 558 has very low midpoint redox potential of -245mV
- **This reduce molecular oxygen (O_2) to superoxide anion**
- This key reaction is catalyzed by **NADPH oxidase**
- ***NADPH + O_2 --- (oxidase) -- = $NADP^+ + O_2^-$ superoxide anion***
- **O_2^-** anion undergo conversion to **H_2O_2 by the enzyme superoxide dismutase**
- H_2O_2 is converted to (.OH) **hydroxyl radicals**. (.OH) is one of the most reactive free radical known
- **O_2^-, and (.OH) are potent microbicidal agen**ts.
- (b). The combination of peroxide, myeloperoxidase and halide ions form a potent halogenating system capable of killing bacteria & viruses.
- H_2O_2 , and halohenated not active as free radicals but more stable, can diffuse further so - toxic to **microbes in extracellular fluid**

Reactive Nitrogen intermediates

- Production of Nitric oxide within MQ & possibly in PMN generate a powerful antimicrobial system.
- Mechanism of action by
  - Degradation of Fe-S prosthetic groups of some electron transport systems.
  - Production of (.OH) radicals.
- ***L- Arginine + O_2 ---(NO-synthatase) -- = NO + citrulline***

Preformed antimicrobials

  - contained within PMN granules
  - contact the ingested microbe when fusion with phagosome occurs
- Dismutation of superoxide consume H+ leading to gentle rise of pH allowing a family of cationic proteins and peptides known as **defensins** to function optimally

Preformed antimicrobials include

- **Defensins**
  - insert into microbial membranes to form destabilizing voltage regulated ion channels (similar to bacterial colicins)
  - act as disinfectants against a wide range of gram +ve and gram –ve bacteria, many fungi and number of envelop viruses
  - has remarkable selectivity to prokaryotes, eukaryotic microbes relative to the host (microbe from 'self')
- If above is not enough Further damage is inflicted on bacterial membranes by
  - **Cathepsin G** (neutral protinase
  - **permeability increasing factor** (BPI)
  - **Low pH, Lysozyme and Lactoferrin** are oxygen independent bacteriostatic and bactericidal factors which can perform under anaerobic conditions
- Finally killed microbes are digested by hydrolytic enzymes and degradation products are released to the exterior

Evasion of phagocytic activity

Antimicrobial activity of phagocytes depend on

- attraction of phagocytes to microbes
- Ability to adhere to microbes
- Ability to respond by membrane activation that initiate engulfment

Continuous mutation above defenses may be useless.

Human body has solved it with few million years of evolution by developing **complement system**

- If microorganisms do penetrate the body. Then what?

Two main defensive operations come to play. 1. Mechanism of phagocytosis (eating by the cell)

2. **Destructive effects of soluble chemical/humoral factors**

- complement system
- acute phase proteins
- interferons

Inflammatory barriers

five characteristic signs: redness, swelling, heat, pain, loss of function

Causative events: vasodilation, increased capillary permeability, emigration of phagocytes

Extracellular killing

Virally infected cells can be killed by large granular lymphocytes with NK activity (NK cells).

Extracellular killing by C3b bound eosinophils in parasitic infections.

Refer: acute phase proteins and role of NK cells

Natural killer cells (**NK cells):**

- **Natural killer cells** move in the blood and lymph to lyse (cause to burst) cancer cells and virus-infected body cells.
- They are large granular lymphocytes that attach to the glycoproteins on the surfaces of infected cells and kill them.
- Refer for role of NK cells

Defense against infection

The innate immunity provide the first and 2nd Line of Defense against Infection

Complement system

A major triggered enzyme plasma system.

It coats microbes with molecules that make them more susceptible to engulfment by phagocytes.

Vascular permeability mediators increase the permeability of the capillaries to allow more plasma and complement fluid to flow to the site of infection.

They also encourage polys to adhere to the walls of capillaries (**margination**) from which they can squeeze through in a matter of minutes to arrive at a damaged area.

Once phagocytes do their job, they die and their "corpses," pockets of damaged tissue, and fluid form pus.

Figure:

Defense against infection