Immunology

Lecture- 2
Phagocytosis

- **Phago**: eat  
  **Cyte**: cell

- A major mechanism used to ingestion and remove pathogens and dead cell performed by phagocytes. For example, when a macrophage ingests a pathogenic microbes, the pathogen becomes trapped in a phagosome which then fuses with a lysosome to form a phagolysosome. Within the phagolysosome, enzymes and toxic peroxides digest the pathogen. Bacteria, dead tissue cells, and foreign particles are all examples of objects that may be phagocytized.

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1. Unbound phagocyte surface receptors do not trigger phagocytosis.
2. Binding of receptors causes them to cluster.
Process of Phagocytosis

Steps of phagocytosis:

• 1- Chemotaxis
• 2- Recognition and attachment (adherence) of microbe to phagocytes
• 3- Engulfment and creation of phagosome
  (Phagosome: A membrane-bound vesicle formed in a cell by an inward folding of the cell membrane to hold microbe taken into the cell by phagocytosis)
• 4- Fusion of phagosome with lysosome to form a phagolysosome
  (Phagolysosome: cytoplasmic body formed by the fusion of a phagosome, or ingested particle, with a lysosome containing hydrolytic enzymes. After fusion, the pathogens contained within the phagosome are usually digested by the enzymes contained within the lysosome).
• 5- Destruction and digestion of ingested microbe by enzymes or super oxide
• 6- formation of residual body containing indigestible material
• 7- Residual body → Exocytosis (discharge of waste material)

Dead microbes are rapidly degraded in phagolysosomes to low molecular-weight components. Various hydrolytic enzymes are involved including lysozyme, proteases, lipases, nucleases. Neutrophils die and lyse after extended phagocytosis, killing, and digestion of bacterial cells. This makes up the characteristic properties of pus. Macrophages egest digested debris and allow insertion of microbial antigenic components into the plasma membrane for presentation to lymphocytes in the immunological response.
Phagocytosis Process

1. Microbe or other particle
2. Pseudopods
3. Plasma membrane
4. Phagosome (phagocytic vesicle)
5. Lysosome
6. Digestive enzymes
7. Phagolysosomes
8. Partially digested microbe
9. Residual body
10. Indigestible material
11. Phagocyte
The intracellular killing activities of phagocytes are usually divided into two main pathways:

### Pathways of Intracellular killing of microbe phagolysosome

<table>
<thead>
<tr>
<th>Oxygen-independent activity</th>
<th>Oxygen-dependent activity</th>
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#### Lysosomal granules

- **Lysosomal granules** contain a variety of **basic proteins (damage membrane)** that strongly inhibit bacteria, yeasts and even some viruses.
- The lysosomal granules of neutrophils contain **lactoferrin**, (iron-chelating), which holds iron needed for bacterial growth.
- The **pH of the phagolysosome** may be as low as 4.0 due to accumulation of lactic acid, which is acidic to prevent the growth of most pathogens. This acidic environment apparently optimizes the activity of many degradative lysosomal enzymes including **Lysozyme** (hydrolyse peptidoglycan), phospholipases, nucleases and hydrolytic enzymes such as protease (digest killed microbes).

#### Fc receptors

- Fc receptors (on neutrophils, monocytes or macrophages) and mannose receptors (on macrophages) increase **O₂** uptake, called the **respiratory burst (oxidative burst)**.
- These receptors activate a membrane-bound **NADPH oxidase** that reduces **O₂** to **O₂⁻** (superoxide). Superoxide can be reduced to **OH⁻** (hydroxyl radical) or dismutated to **H₂O₂** (hydrogen peroxide) by **superoxide dismutase**. **O₂⁻**, **OH⁻**, and **H₂O₂** are activated oxygen species (potent oxidizing agents) that affect a number of cellular structures including membranes and nucleic acids.
- In the case of neutrophils, these reactive oxygen can act with a lysosomal enzyme called **myeloperoxidase** (myeloperoxidase system), or MPO.
Phagocytic Oxidative Burst

1. NADPH oxidase (deficiency = chronic granulomatous disease)
2. Superoxide dismutase
3. Myeloperoxidase
4. Catalase/glutathione peroxidase
5. Glutathione reductase
6. Glucose-6-phosphate dehydrogenase (G6PD)

GSH/GSSG = glutathione (reduced/oxidized)
HOCl* = bleach (hypochlorite)
## Microbial Evasion of Phagocytosis

<table>
<thead>
<tr>
<th>Evasion Method</th>
<th>Bacterial Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibit adherence: M protein, capsules</td>
<td><em>Streptococcus pyogenes, S. pneumoniae</em></td>
</tr>
<tr>
<td>Kill phagocytes: Leukocidins</td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>Lyse phagocytes: Membrane attack complex</td>
<td><em>Listeriamonocytogenes</em></td>
</tr>
<tr>
<td>Escape phagosome</td>
<td><em>Shigella</em></td>
</tr>
<tr>
<td>Prevent phagosome-lysosome fusion</td>
<td>HIV</td>
</tr>
<tr>
<td>Survive in phagolysosome</td>
<td><em>Coxiella burnetti</em></td>
</tr>
</tbody>
</table>
Inflammation

Non specific response triggered by
- injury
- penetration of bacteria

5 major sings: Redness- Pain- Heat- Swelling (edema), loss of function.
Skin- respiratory, digestive, urinary tracts
Goals are: eliminate or destroy microbes or injuries (limit spread of infection)
- remove necrotic cells and tissues
- initiate the process of repair

Two main players
Histamine increase capillary permeability, dilates local blood vessels
Result is redness, heat and swelling

Heat - unfavorable to microbes
- mobilize white blood cell (monocytes)
- raise metabolic rate of surrounding cells

Complement chemotaxis agent- recruits in WBC to injury tissues.

The inflammatory response - start with release of histamin and other chemicals
- ends with WBC cleaning up the debris
Inflammation

- Acute-phase proteins activated (**complement**, **cytokine**, kinins)

- Vasodilation (**histamine**, kinins, prostaglandins, leukotrienes)

- Margination and emigration of WBCs

- Tissue repair
Components of the inflammatory process

- **White blood cells and plasma proteins**  Normally present in the blood
  
  **Cells**  Granulocytes (PMNs, Mast cells, etc)
  monocytes/macrophages
  Lymphocytes (Fibroblasts)
  
  **Proteins**  Complement, pentraxin, Ficolins, Coagulation, Kininogens, Proteoglycan

- **Chemical mediators produced by damaged host cells**
  
  **Cytokines** and other mediators

### Chemicals Released by Damaged Cells

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Histamine</td>
<td>Vasodilation, increased permeability of blood vessels</td>
</tr>
<tr>
<td>Kinins</td>
<td>Vasodilation, increased permeability of blood vessels</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Intensity histamine and kinin effect</td>
</tr>
<tr>
<td>Leukotrienes</td>
<td>Increased permeability of blood vessels, phagocytic attachment</td>
</tr>
</tbody>
</table>
Inflammation- steps

(a) Tissue damage

1. Chemicals such as histamine, kinins, prostaglandins, and leukotrienes (represented as blue dots) are released by damaged cells

2. Blood clot forms

3. Abscess starts to form (yellow area)

(b) Vasodilation and increased permeability of blood vessels
Inflammation - steps

4. Margination—phagocytes stick to endothelium
5. Emigration—phagocytes squeeze between endothelial cells
6. Phagocytosis of invading bacteria

(c) Phagocyte migration and phagocytosis
- Scab
- Blood clot
- Regenerated epidermis (parenchyma)
- Regenerated dermis (stroma)

(d) Tissue repair
- Bacterium
- Neutrophil
- Macrophage
Acute and Chronic Inflammation

**Acute**
- Fast: immediately following injury
  - Bacteria, virus, parasite pneumonia
  - Non specific response
    - Neutrophil
  - Mild and self limited
    - Occasionally necrosis
  - Vascular response (damage)
- Fluid production is part of response (little or no fibrosis/scar)
  - Exudation

**Chronic**
- Slow: persistent inflammation, prolonged duration
  - Prolonged exposure to toxic agent, tuberculosis, ulcer Rheumatoid arthritis
    - Specific response
      - Monocytes, macrophages, lymphocytes
      - Severe and progressive
        - Always necrosis
      - No vascular response
- Production of fibrosis tissues
- Repair with Angiogenesis (new vessel formation)
Acute and Chronic Inflammation

**Acute Inflammation**
- Vascular changes
- Neutrophil recruitment
- Mediators

**Resolution**
- Clearance of injurious stimuli
- Clearance of mediators and acute inflammatory cells
- Replacement of injured cells
- Normal function

**Chronic Inflammation**
- Angiogenesis
- Mononuclear cell infiltrate
- Fibrosis (scar)

**Fibrosis**
- Loss of function

**Injury**
- Infarction
- Bacterial infections
- Toxins
- Trauma
- Viral infections
- Chronic infections
- Persistent injury
- Autoimmune diseases
Fever: Abnormally high body temperature

- Hypothalamus normally set at 37°C
- Body temperature increases as a protective response to infection and injury.
- Gram-negative endotoxin cause phagocytes to release **interleukin 1 (IL-1)** as an endogenous **pyrogen**
- A part of the brain called the **hypothalamus** controls body temperature.
- Fever results from an actual resetting of the **hypothalamus's thermostat**.
- It is triggered by floating biochemical substances called **pyrogens**, which flow from sites where the immune system has identified potential trouble to the hypothalamus via the bloodstream.
- Some pyrogens are produced by body tissue; many pathogens also produce pyrogens. When the hypothalamus detects them, it tells the body to generate and retain more heat, thus producing a fever. Children typically get higher and quicker fevers, reflecting the effects of the pyrogens upon an inexperienced immune system.
- Hypothalamus releases prostaglandins that reset the hypothalamus to a high temperature
- **Rise in temperature can kill or inhibit some microbes, and increase iron withholding, T-cell production**
- **Body increases rate of metabolism and triggers the body’s repair process**
- When IL-1 is eliminated, body temperature falls.