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M1 - Immunology, Winter 2008

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Phagocytic Cells: Mechanisms of Bacterial Killing and Tissue Injury

• Learning Outcomes:
  – To understand the pathophysiologic role of phagocytic cells in host defense.
  
  – To understand the role of reactive oxygen metabolites and lysosomal granules in phagocytic cell function
Phagocytic Cells

• Peripheral Blood Leukocytes (nrml. 4.5-11,000 cells/ul)
  – Lymphocytes (~ 30%)
  – Granulocytes (~ 70%)

• Granulocytes:
  – Neutrophils (~ 60% of total leukocytes in blood)
  – Eosinophils (~ 3%)
  – Basophils (<1%, rare)
  – Monocytes (~ 6%)
  – Monocytes \rightarrow \textbf{Macrophages} (tissues)

• Kupffer cells (lining liver sinusoids)
Peripheral Blood Smear

Neutrophil

Lymphocyte

Source: Undetermined
Lymphocyte

Platelets

Source: Undetermined
Neutrophil

Source: Undetermined
Neutrophils and Macrophages

• Function:
  – Ingest foreign material
  – Kill bacteria and other microbes
  – Degrade necrotic tissue and foreign antigens

• Tissue damage during prolonged inflammation
Neutrophil Recruitment

Selectins/Addressins $\rightarrow \beta_2$-Integrin/ICAM-1

flow $\rightarrow$ rolling $\rightarrow$ adhesion $\rightarrow$ transmigration

inflammatory mediators

endothelium

- phagocytosis
- oxidant production
- lysosomal granules

Tissue Injury (e.g. Bacterial infection)

chemoattractant (e.g. IL-8, C5a)
Phagocytic Cell Activation: Chemotactic Factors

C5a

plasma membrane

G-protein tyrosine kinases

protein phosphorylation

phosphoinositide metabolism

IP3

Ca^{2+}

functional responses

Other receptors:
Toll-like receptor
Mannose receptor
Phagocytic Cell Functional Responses

- Adhesion (localization)
- Chemotaxis (migration)
- Phagocytosis
- NADPH oxidase activation
- Lysosomal granule fusion: degranulation
Opsonization and Phagocytosis

- Fc receptors for antibody
- Complement receptors: (e.g. C3b)
- Other
  - receptors for collectins (e.g. mannose-binding protein)
NEUTROPHIL PHAGOCYTOSIS OF OPSONIZED BACTERIA

Source: Undetermined
cell phagocytosis

Oxygen radicals

Elastase
Collagenase
Acid hydrolases

Source: Undetermined
Respiratory Burst: NADPH Oxidase

![Graph showing oxygen levels over time for normal and patient conditions with stimulus added at time 0. Oxygen levels decrease over time, with the patient curve showing a more rapid decrease compared to the normal curve.]
**Reactive Oxygen Metabolites**

- **Superoxide anion:** \( \text{O}_2^- \)  
  \( \text{O}_2 + \text{e}^- \rightarrow \text{O}_2^- \)

- **Hydrogen peroxide:** \( \text{H}_2\text{O}_2 \)  
  \( 2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \)

- **Hydroxyl radical:** \( \text{OH}^- \)  
  \( \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{OH}^- + \text{Fe}^{3+} \)

- **Hypochlorous acid:** \( \text{HOCl} \)  
  \( \text{H}_2\text{O}_2 \rightarrow \text{HOCl} + \text{OH}^- \)  
  \( \text{MPO} \)

**myeloperoxidase = MPO**

**Chronic Granulomatous Disease of Childhood (CGD):** deficiency of NADPH Oxidase
Nitric Oxide (NO •) Synthase

L-arginine → NO • → hydroxyl radical
peroxynitrites

- Endothelial cell
- Macrophages (inducible): intracellular cytotoxic agent
- Nervous system
Oxidant Targets

a) **unsaturated lipids**: lipid peroxidation
   \[ \text{LOOH} = \text{lipid hydroperoxides} \]

c) **proteins**
   - sulfhydryl groups
   - methionine
   - tyrosine

d) **nucleic acids**
Degranulation

- Bactericidal proteins (e.g. defensins)
- Proteases
  - serine proteases (e.g. elastase)
  - metalloproteinases (e.g. collagenase, gelatinase)
- Acid hydrolases
Protective Mechanisms

Anti-oxidant: specific vs. non-specific

Specific enzymes:

Superoxide dismutase: \[ 2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

Catalase: \[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]

Glutathione peroxidase: \[ \text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2\text{H}_2\text{O} + \text{GSSG} \]

\[ \text{LOOH} + 2\text{GSH} \rightarrow \text{H}_2\text{O} + \text{LOH} + \text{GSSG} \]

LOOH = lipid hydroperoxides
GSH = reduced glutathione
GSSG = oxidized glutathione
Non-specific scavengers:

- Vitamin E
- Vitamin C
- Beta-carotene
Anti-proteases

- α-1- anti-protease (anti-trypsin):
  - plasma protein
  - binds proteases including elastase
  - inactivated by oxidants

- α-2- macroglobulin
  - plasma protein
  - binds proteases

- TIMPs: tissue inhibitors of metalloproteinases
  - cell derived
Synergism: Inactivation of alpha-1-Anti-trypsin

1. HOCl Dependent

PMNs → HOCl → a-1-antitrypsin (active) → a-1-antitrypsin (inactive)

2. Metalloproteinase Dependent

PMNs → Metalloproteinase (collagenase) → a-1-antitrypsin (active) → a-1-antitrypsin (inactive)
Case: A 3 year old boy is brought to the emergency department

- **CC:** a productive cough, fever (temp 102.1°C), and headache.
- **PEx:** healthy boy with rales present on auscultation of the left lower chest.
- **CxR:** intra-alveolar infiltrate in the left lower lobe.
- **Hx:** mother reports multiple episodes (approx. 5 per year) of recurrent bacterial infections including otitis media, sinusitis, pneumonia, and purulent skin lesions. These infections usually responded to antibiotic treatment.
List three different mechanisms that could account for this patient's increased susceptibility to bacterial infection:

1. ________________________________

2. ________________________________

3. ________________________________
Neutrophil Recruitment

Selectins/Addressins → $\beta_2$-Integrin/ICAM-1

- flow
- rolling
- adhesion
- transmigration

Tissue Injury (e.g. Bacterial infection)

Chemoattractant (e.g. IL-8, C5a)

- phagocytosis
- oxidant production
- lysosomal granules

Endothelium
Different mechanisms that could account for this patients increased susceptibility to bacterial infection:

1. Lack of neutrophils: leukopenia
2. Defective neutrophil function
   - Adhesion / migration
   - Phagocytosis
   - Bacterial killing
3. Lack of chemoattractants: deficiency
4. Lack of opsoninization of bacteria
   - antibody deficiency / complement def.
Additional References:

**Phagocytic Cells:**
Parham, The Immune System (2nd ed.): pgs. 15-17, 202-209.