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Phagocytic Cells: Mechanisms of Bacterial Injury and Tissue Injury

M1 – Immunology Sequence
Joseph Fantone, MD
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 → Macrophages (tissues)

- Kupffer cells (lining liver sinusoids)
Peripheral Blood Smear

Neutrophil

Lymphocyte
Neutrophil
Monocyte
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

Tissue Injury (e.g. Bacterial infection)

endothelium

inflammatory mediators

- phagocytosis
- oxidant production
- lysosomal granules

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

Other receptors:
Toll-like receptor
Mannose receptor

G-protein tyrosine kinases

protein phosphorylation

phosphoinositide metabolism

↑ IP3
↑ Ca^{2+}

functional responses
Phagocytic Cell Functional Responses

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

- Protein recognized by phagocytic cell binds to bacteria surface
- Enhances phagocytosis
  - Antibody, IgM
  - Complement
  - Mannose binding protein

Fc receptors: IgG, IgM
C3b receptors
MBP receptors
Neutrophil Phagocytosis of Bacteria

Opsonization of Bacteria

Fc, C3b binding

Phagosome formation

Phagolysosome
Cell phagocytosis

Oxygen radicals

Elastase
Collagenase
Acid hydrolases
Respiratory Burst: NADPH Oxidase

Oxygen Levels (% of max.)

Time (minutes)

Stimulus added

Patient

Normal

J. Fantone
Reactive Oxygen Metabolites

Superoxide anion: $O_2^-$

$O_2 + e^- \rightarrow O_2^-$

Hydrogen peroxide: $H_2O_2$

$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

Hydroxyl radical: $OH$ .

$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^- + Fe^{3+}$

Hypochlorous acid: $HOCl$

$H_2O_2 \rightarrow HOCl + OH^-$

myeloperoxidase = MPO

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

L-arginine → NO radical → hydroxyl radical → peroxynitrites

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

a) unsaturated lipids: lipid peroxidation
   LOOH = 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**
Oxidants
Proteases

Anti-oxidants
Anti-proteases
Pneumonia and Abscess

J. Fantone
**Protective Mechanisms**

**Anti-oxidant: specific vs. non-specific**

**Specific enzymes:**

**Superoxide dismutase:** \( 2O2^- + 2H+ \rightarrow H2O2 + O2 \)

**Catalase:** \( 2H2O2 \rightarrow 2H2O + O2 \)

**Glutathione peroxidase:** \( H2O2 + 2GSH \rightarrow 2H2O + GSSG \)

\( LOOH + 2GSH \rightarrow H2O + LOH + GSSG \)

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

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

- $\alpha$-1- anti-protease (anti-trypsin):
  - plasma protein
  - binds proteases including elastase
  - inactivated by oxidants
- $\alpha$-2- macroglobulin
  - plasma protein
  - binds proteases
- TIMPs: tissue inhibitors of metalloproteininases
  - cell derived
**Synergism: Inactivation of alpha-1-anti-trypsin**

1. **HOCl Dependent**
   - PMNs → HOCL
   - HOCL → a-1-antitrypsin (active)
   - a-1-antitrypsin (inactive)

2. **Metalloproteinase Dependent**
   - PMNs → Metalloproteinase (collagenase)
   - 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 $\rightarrow$ $\beta_2$-Integrin/ICAM-1

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Tissue Injury (e.g. Bacterial infection)

endothelium

inflammatory mediators

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

- phagocytosis
- oxidant production
- lysosomal granules

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