Science is like looking through a keyhole: The closer you get to the keyhole, the more you see of the room on the other side.

-George Wald
1967 Nobel Laureate in Medicine

A Day in the Life of a Phagocytic Leukocyte
Selectin-mediated Adhesion is Weak and Promotes “Rolling” of Leukocyte Along Endothelia

Firm Adhesion is Triggered by Chemokine Activation of Leukocyte Integrins
Diapedesis: Crawling Through Endothelial Junctions and Into the Tissue

QuickTime™ and a Sorenson Video decompressor are needed to see this picture.

Movie, courtesy T. Springer

Leukocyte Migration, Start to Finish

<table>
<thead>
<tr>
<th>Rolling adhesion</th>
<th>Tight binding</th>
<th>Diapedesis</th>
<th>Migration</th>
</tr>
</thead>
</table>

Intravital Imaging of a Subset of Mouse Monocytes in Dermal Blood Vessels

CX3CR1-expressing cells express GFP in reporter mice, and dermal blood vessels are labeled with rhodamine-conjugated dextran.


A Subset of Monocytes "Patrol" the Vasculature, Primed for Diapedesis

The Innate Immune Response to Bacterial and Fungal Infections

Relative Risk of Death Associated With Death of a Biological Parent Before the Age of 50

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Relative Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>All causes</td>
<td>1.7</td>
</tr>
<tr>
<td>&quot;Natural causes&quot;</td>
<td>2.0</td>
</tr>
<tr>
<td>Infectious</td>
<td>5.8</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>4.5</td>
</tr>
<tr>
<td>Cancer</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Conclusion: Genes that determine responses to infectious agents have a disproportionate effect on mortality

### Distinctions Between Innate and Adaptive Immunity

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Innate immune system</th>
<th>Adaptive immune system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Germline-encoded</td>
<td>Somatic tissue-encoded</td>
</tr>
<tr>
<td>Kinetics</td>
<td>Rapid</td>
<td>Slow (requires clonal expansion)</td>
</tr>
<tr>
<td>Specificity</td>
<td>Recognizes non-self “pattern recognition”</td>
<td>Recognizes “altered self”</td>
</tr>
<tr>
<td>Effector Cells</td>
<td>All</td>
<td>Primarily lymphocytes, DCs, Mφ</td>
</tr>
</tbody>
</table>

### What Really Happens During the Lag Period Before the Acquired Immune Response?

![Chart showing innate and acquired immunity](chart.png)
Receptors Important in Innate Immunity

- **GPCRs** (G protein-coupled receptors)
- **TLRs** (Toll-like receptors)
- **Lectins**

Production of cytokines & chemokines

Phagocytosis of IgG-coated Targets by Macrophages

3 min 10 min
Extension of an F-actin-rich "Phagocytic Cup" Around Phagocytic Targets

Mast Cells Can Phagocytose Too!
**Most, but not all Leukocytes Can Perform Phagocytosis**

- Non-opsonic phagocytosis is typically mediated by cell surface receptors on leukocytes that recognize repeating carbohydrate subunits (comprising “molecular patterns”) on microbes.

- Opsonic phagocytosis is typically mediated by deposition of proteins (e.g., antibodies) on microbes that target them for recognition by specific phagocytic receptors on leukocytes.

(<Latin opsonare, to buy provisions> Greek opsonein, condiment

"Opsonin is what you butter the disease germs with to make your white blood corpuscles eat them."

- G.B. Shaw, The Doctor’s Dilemma

**Opsonic vs Non-opsonic Phagocytosis**
The Scavenger Receptor Superfamily

Non-opsonic Phagocytosis

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Opsonic Phagocytosis

- IgG
- Complement
What is complement?

Complement Proteins Deposit on Pathogen Surfaces, Triggering Phagocytosis, Inflammation, and Pathogen Lysis
Complement Activation Triggers
Opsonic Phagocytosis

**CLASSICAL PATHWAY**
Antigen:antibody complexes

**MB-LECTIN PATHWAY**
Lectin binding to pathogen surfaces

**ALTERNATIVE PATHWAY**
Pathogen surfaces

C3/C5 Convertase

Complement activation

C3α, C4α, C5α
C3b, C3bi
C5-9

Recruitment of inflammatory cells
Opsonization of pathogens
Killing of pathogens

The “Circuitry” of the Complement Pathway

Classical Pathway (IgG)

Lectin pathway (mannan-binding lectin)

Alternate pathway (pathogen surface)

C3b
C3a
C4b
C5b
Pathogen lysis
Opsonization
Chemoattraction
Elie Metchnikoff, 1845-1916

Metchnikoff is the First to Describe a Role for Phagocytosis in Immunity
QuickTime™ and a Cinepak decompressor are needed to see this picture.
Post-phagocytic Events: Phagosome-Lysosome Fusion

Phagocytosis of Bacteria is Followed by Phagosome-Lysosome Fusion

Granulomatous inflammation consists of epithelioid macrophages, giant cells, lymphocytes, plasma cells, and fibroblasts.

Epithelioid cells accumulate around the center of a granuloma. They get their name from the fact that they have pink cytoplasm similar to squamous epithelia.

Langhans-type giant cells represent fused macrophages. The nuclei are lined up around the periphery of the cell.

Oxidant-dependent Killing of Bacteria and Fungi
Chronic Granulomatous Disease (CGD), an Inherited Defect of the NADPH Oxidase Complex

Chronic Granulomatous Disease: Clinical Manifestations

- 1/250,000 live births in the US
- Characterized by recurrent infections with catalase-positive organisms, such as Staphylococcus, Burkholderia cepacia, Nocardia, Mycobacteria, Serratia, Klebsiella, Pseudomonas species, and fungi, especially Aspergillus species and Candida.
- Recurrent bacterial and fungal infections result in lymphadenitis, abscesses, and granuloma formation, with most patients presenting within the first 2 years of life.

From: Khanna et al., Radiographics 25:1183, 2005
What happens following pathogen ingestion?

Post-phagocytic Events
Post-phagocytic Events: “Phagosome-Oxidase Fusion”

Post-phagocytic Events: Generation of $H_2O_2$
Post-phagocytic Events: Myeloperoxidase Activity

Reactive oxygen species: $O_2^*$, HOCl, $H_2O_2$, $O_3$

Post-phagocytic Events: Peroxynitrite Production

Reactive oxygen species: $O_2^*$, HOCl, $H_2O_2$, $O_3$

Reactive nitrogen species: ONOO$^-$
Bacterial Virulence Factors Subvert Host Defenses

1. Ingestion phase impaired (Yersinia)
2. Phagosome maturation stalled (*M. tuberculosis*, *Legionella*)
3. Resistance to lysosomal degradation (*Salmonella*)
4. Modification of phagocytic receptors (*P. aeruginosa*)
5. Escape from phagosome into cytosol (*Listeria, Shigella*)

Non-oxidative Killing Mechanisms

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.
Non-oxidative Killing Mechanisms of Phagocytes

• Principally proteins within granules that are released upon cell stimulation

• These proteins include lysozyme, lactoferrin, proteases, defensins and other cationic proteins

Lysozyme
Disrupts peptidoglycan

HBD1
HBD2
HBD3
Permeablizes membranes

Epithelial Cells Express Defensins, Too

Phagocytosis: Not Just for Bugs

Phagocytosis is the Principal Mechanism of Disposal of Apoptotic Corpses

- Phagocytosis is the means of disposal of apoptotic corpses, and occurs continuously during the lifetime of an individual.

- In this setting, phagocytosis is not accompanied by inflammation, but rather leads to an "anti-inflammatory" signal (the production of TGF-β).

- As apoptotic corpses contain many potential self antigens, the lack of an appropriate anti-inflammatory signal has the potential to trigger autoimmunity.

Immunological Consequences of Phagocytosis

Appositions:
- Clearance of pathogens
  - Death of pathogenic microbe
  - Persistence of pathogenic microbe
  - Resolution of infection
  - Failure of resolution of infection

Clearance of apoptotic corpses
- Suppression of inflammation
- Inappropriate inflammation
  - Tolerance
  - Break in tolerance

Summary

1. Innate immunity represents the first-line of host defense. Its receptors are germline-encoded and recognize pathogen-associated “molecular patterns.”

2. Phagocytosis is a component of innate and acquired immunity. It is the principal means of destroying pathogenic bacteria and fungi. Phagocytosis initiates the process of antigen presentation.

3. Many phagocytic receptors recognize a diverse array of microbial pathogens. Some pathogens (e.g., S. pneumoniae) require opsonization by antibodies and complement for their clearance. However, bugs fight back.

4. Phagocytic leukocytes employ oxidative and non-oxidative means of killing. The NADPH oxidase generates reactive oxidants, such as superoxide anion and hypochlorous acid (bleach).

5. Innate immunity ushers in acquired immunity: innate immune activation of APCs results in up-regulation of co-stimulatory molecules and enhances the effectiveness of antigen presentation.

6. Phagocytosis is an essential component of development and tissue remodelling. Ingestion of apoptotic bodies is immunologically “silent” and is normally accompanied by a suppression of inflammation. Failure of this mechanism may result in autoimmunity.