

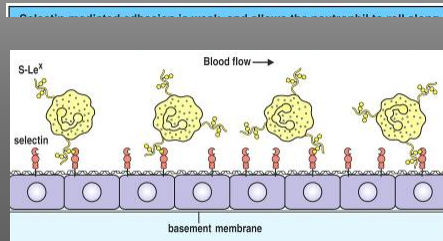
Science is like looking through a keyhole: The closer you get to the keyhole, the more you see of the room on the other side.

-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

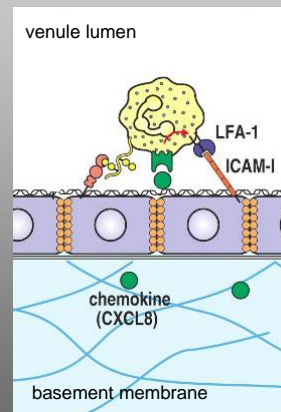
QuickTime™ and a Sorenson Video decompressor are needed to see this picture.



Movie, courtesy T. Springer

## Firm Adhesion is Triggered by Chemokine Activation of Leukocyte Integrins

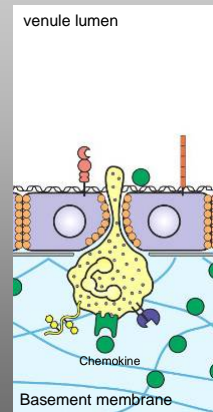
QuickTime™ and a Sorenson Video decompressor are needed to see this picture.



Movie, courtesy T. Springer

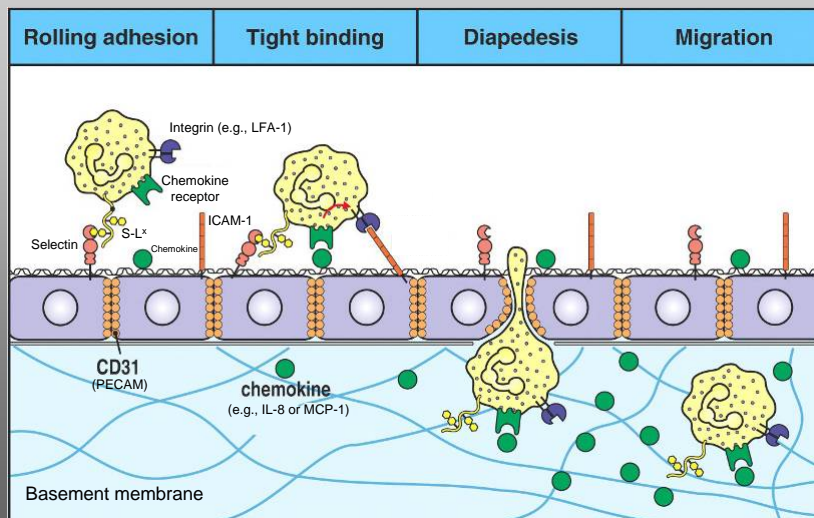
## Diapedesis: Crawling Through Endothelial Junctions and Into the Tissue

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



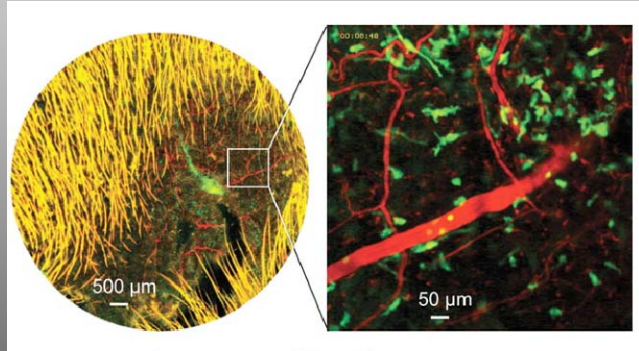
Movie, courtesy T. Springer

## Leukocyte Migration, Start to Finish



Modified from: Parham, *The Immune System, 2nd ed.* (Garland: New York), 2005

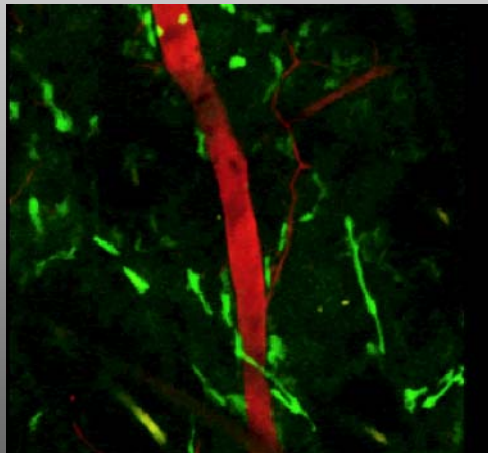
## Intravital Imaging of a Subset of Mouse Monocytes in Dermal Blood Vessels



CX<sub>3</sub>CR1-expressing cells express **GFP** in reporter mice, and dermal blood vessels are labeled with **rhodamine-conjugated dextran**.

From: Auffray et al., *Science* 317:666, 2007

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



From: Auffray et al., *Science* 317:666, 2007

## 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

Cause of Death	Relative Risk
All causes	1.7
“Natural causes”	2.0
Infectious	5.8
Cardiovascular	4.5
Cancer	1.2

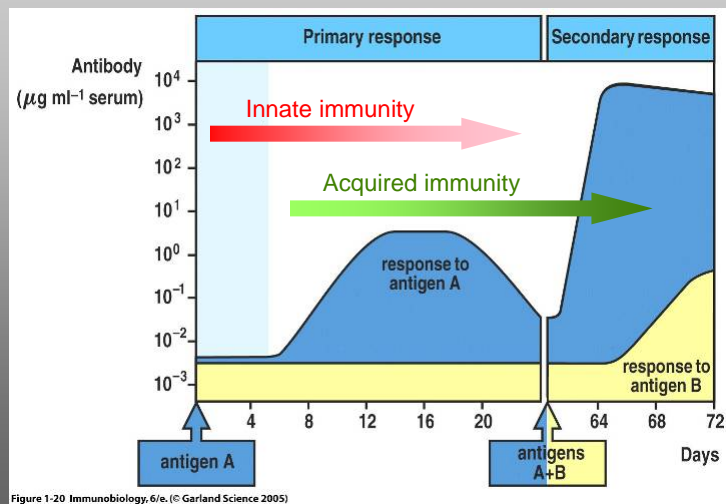
Conclusion: Genes that determine responses to infectious agents have a disproportionate effect on mortality

Source: Sorensen et al., *New Engl. J. Med.*, 318:727, 1988

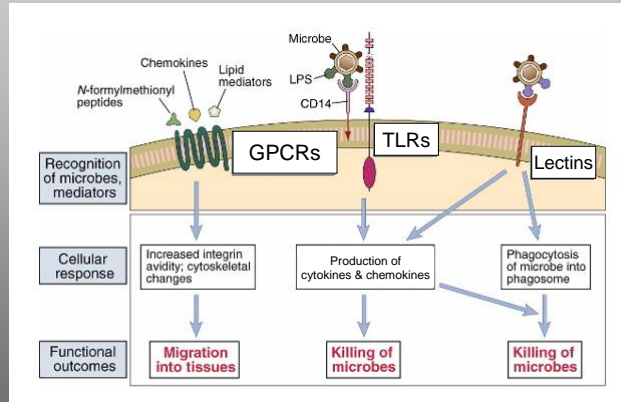
## Distinctions Between Innate and Adaptive Immunity

	Innate immune system	Adaptive immune system
Receptors	Germline-encoded	Somatically engineered
Distribution	Non-clonal	Clonal
Kinetics	Rapid	Slow (requires clonal expansion)
Specificity	Recognizes non-self "pattern recognition"	Recognizes "altered self" Primary structure (TCR) Higher order structure (Immunoglobulin; BCR)
Effector Cells	All	Primarily lymphocytes, DCs, M $\phi$

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

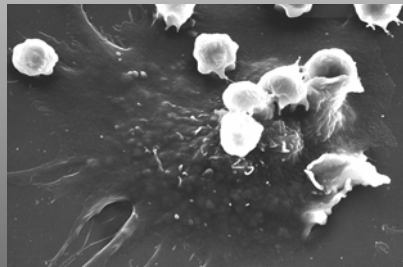


## Receptors Important in Innate Immunity

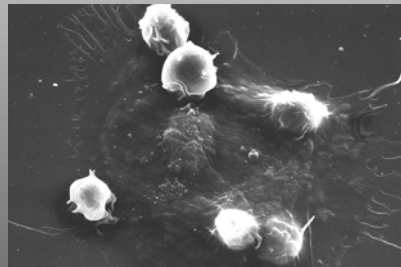


GPCR = G protein-coupled receptors  
TLRs = Toll-like receptors  
Lectin: A molecule that binds carbohydrates

## Phagocytosis of IgG-coated Targets by Macrophages

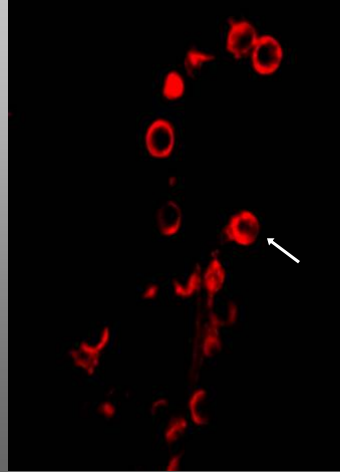
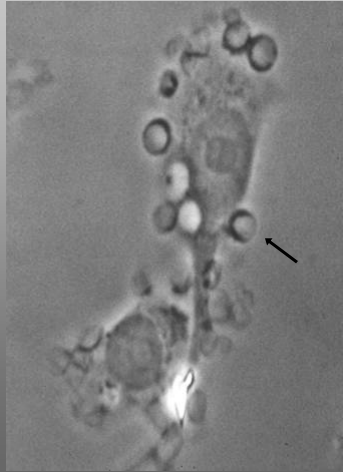


3 min

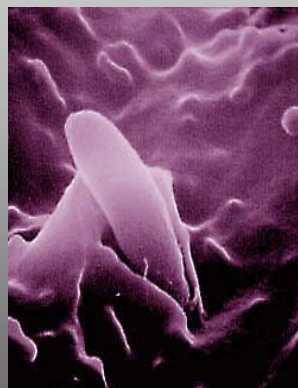
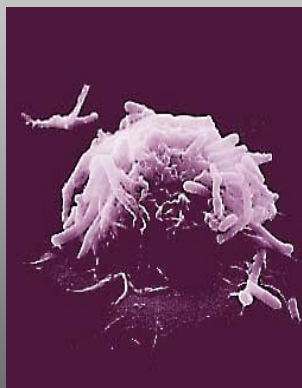


10 min

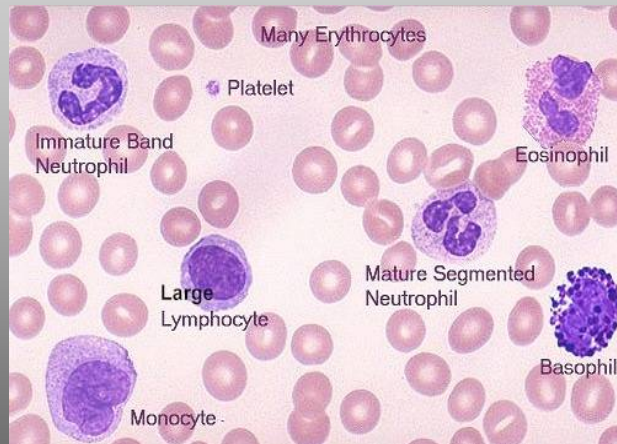
Extension of an F-actin-rich  
"Phagocytic Cup" Around Phagocytic Targets



Mast Cells Can Phagocytose Too!



## Most, but not all Leukocytes Can Perform Phagocytosis



## Opsonic vs Non-opsonic Phagocytosis

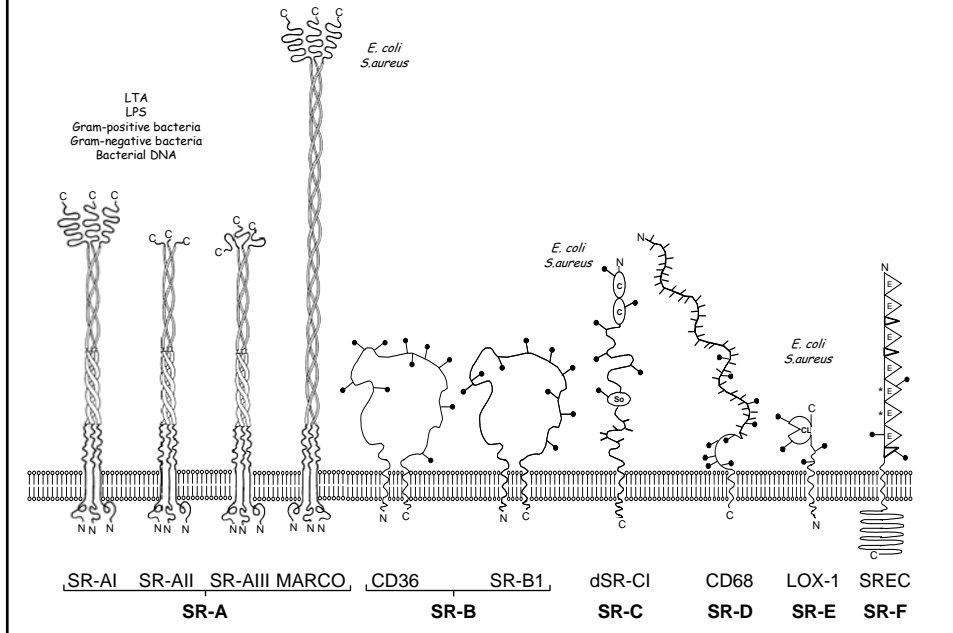
- Non-opsonic phagocytosis is typically mediated by cell surface receptors on leukocytes that recognize repeating carbohydrate subunits (comprising “molecular patterns”) on microbes.
- Opsonic phagocytosis is typically mediated by deposition of proteins (e.g., antibodies) on microbes that target them for recognition by specific phagocytic receptors on leukocytes.

(<Latin *opsonare*, to buy provisions<Greek *oponein*, 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*

## The Scavenger Receptor Superfamily



## Non-opsonic Phagocytosis

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

## Opsonic Phagocytosis

Y IgG

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

## Opsonic Phagocytosis

Y IgG

- Complement

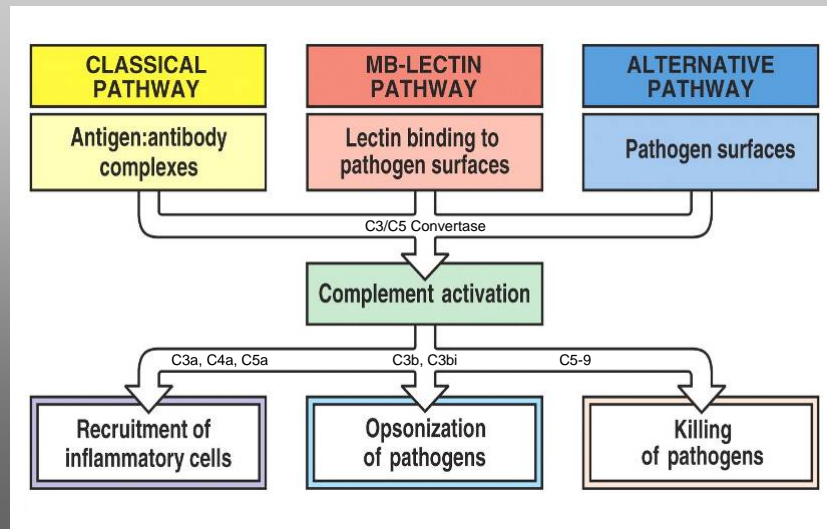
QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

What is complement?

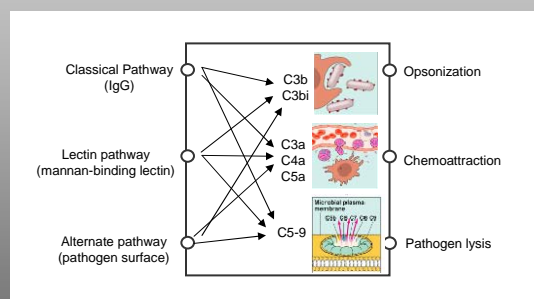
Complement Proteins Deposit on Pathogen Surfaces, Triggering Phagocytosis, Inflammation, and Pathogen Lysis

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

## Complement Activation Triggers Opsonic Phagocytosis



## The "Circuitry" of the Complement Pathway



## Metchnikoff is the First to Describe a Role for Phagocytosis in Immunity



Elie Metchnikoff, 1845-1916

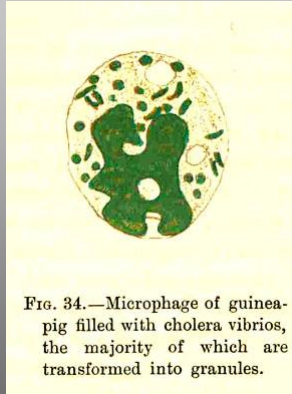
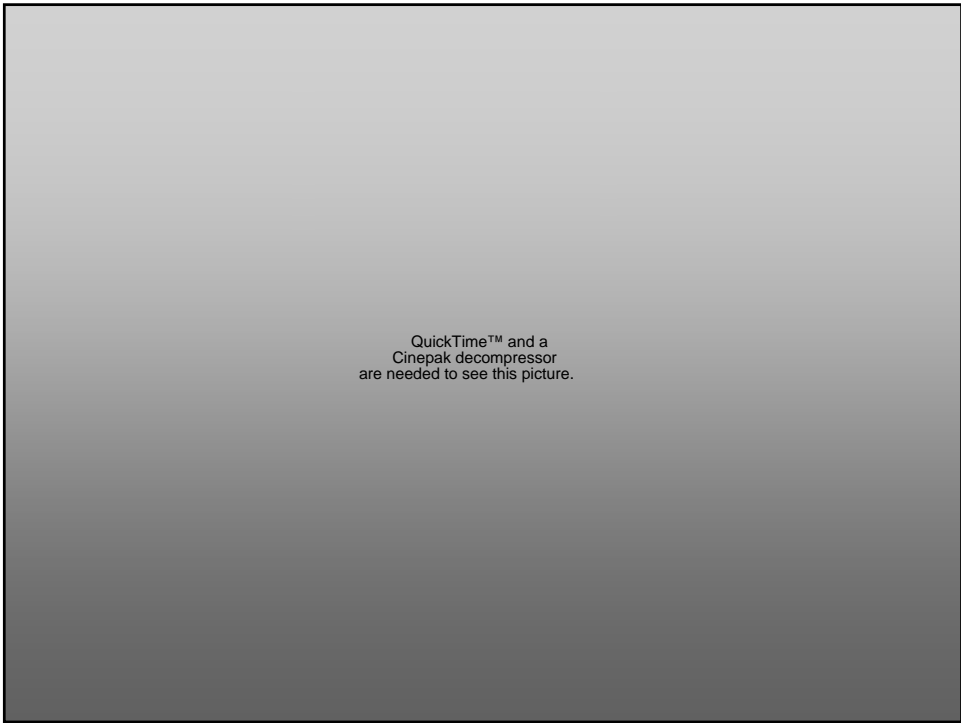
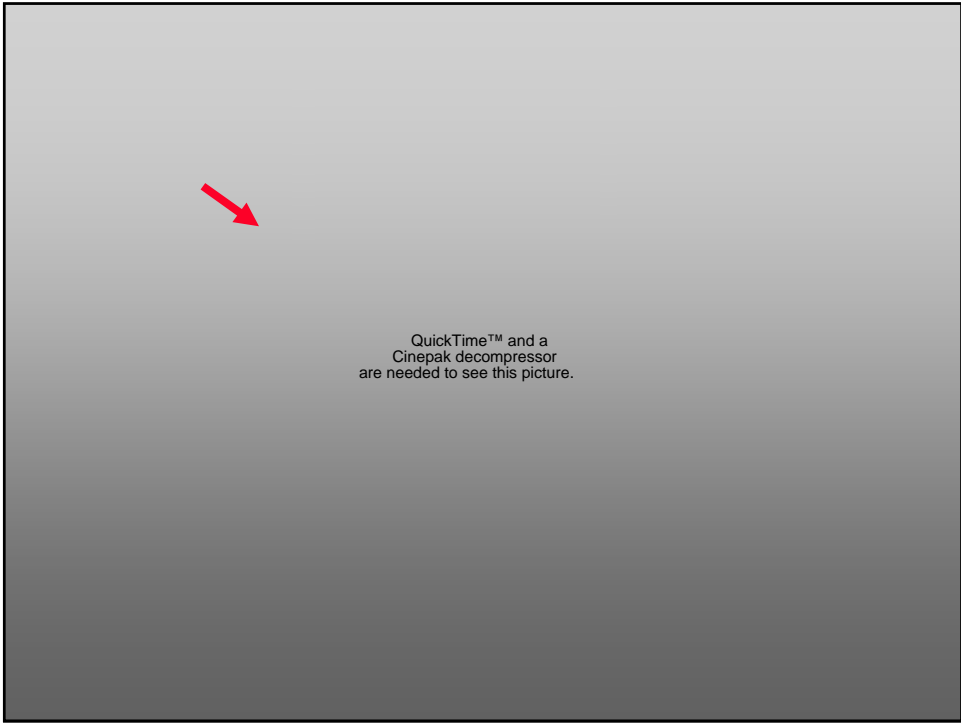


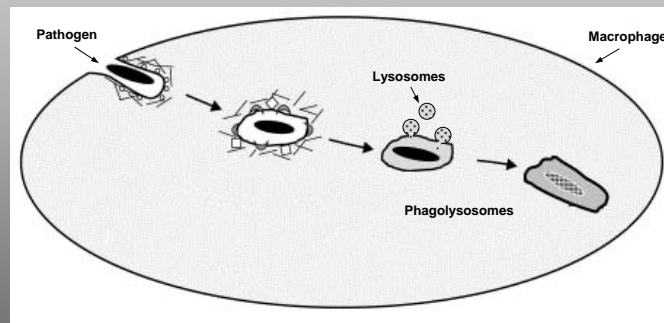
FIG. 34.—Microphage of guinea-pig filled with cholera vibrios, the majority of which are transformed into granules.

Phagosome-Lysosome Fusion?

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



0-3 min

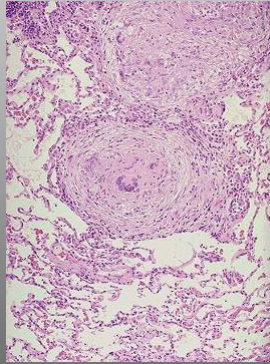
1-5 min

30 min-hrs

From: Allen et al., *J. Exp. Med.* 191:115, 2000

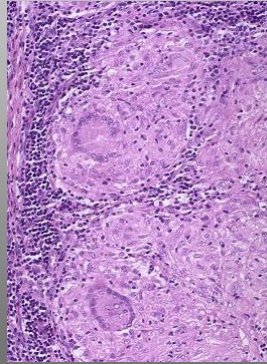
## The Granuloma: a Delayed Response to Indigestible Pathogens and Particles in Macrophages

Granulomas



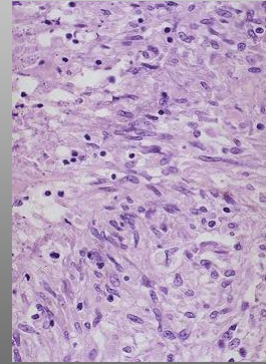
**Granulomatous inflammation** consists of epithelioid macrophages, giant cells, lymphocytes, plasma cells, and fibroblasts.

Langhans-type Giant Cells



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

Epithelioid Cells

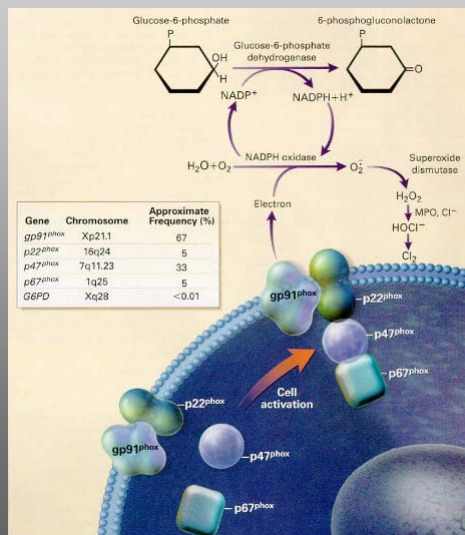


**Epithelioid cells** accumulate around the center of a granuloma. They get their name from the fact that they have pink cytoplasm similar to squamous epithelia.

## Oxidant-dependent Killing of Bacteria and Fungi

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

## Chronic Granulomatous Disease (CGD), an Inherited Defect of the NADPH Oxidase Complex



From: Lekstrom-Himes and Gallin, *N Engl J Med*, 343:1703, 2000

## 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.

## Chronic Granulomatous Disease: Clinical Manifestations

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

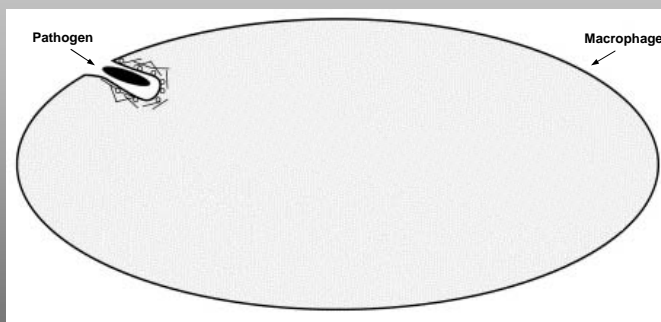
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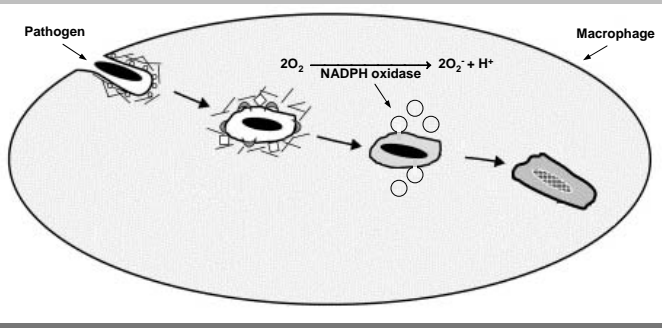
From: Khanna et al., *Radiographics* 25:1183, 2005

What happens following pathogen ingestion?

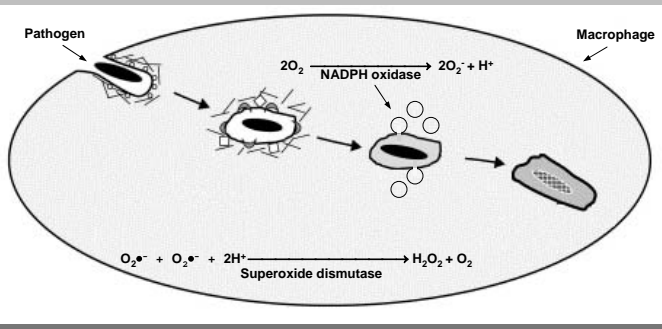
### Post-phagocytic Events



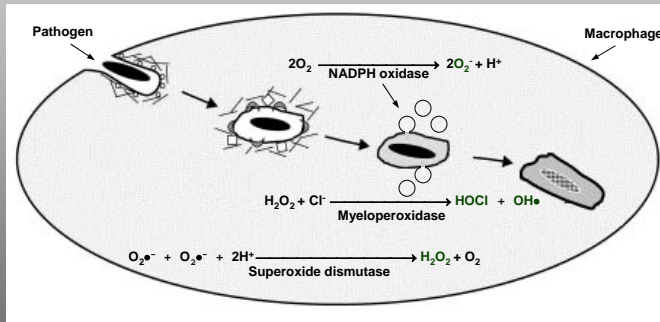
# Post-phagocytic Events: "Phagosome-Oxidase Fusion"



# Post-phagocytic Events: Generation of $H_2O_2$

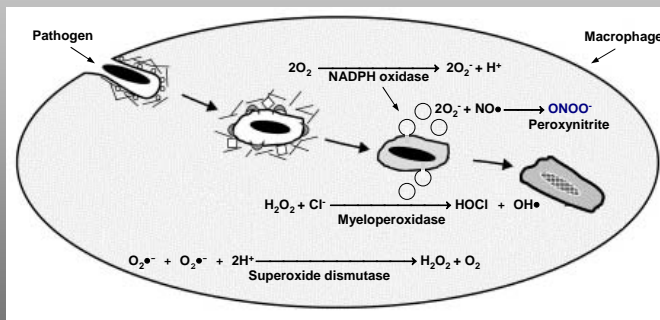


## Post-phagocytic Events: Myeloperoxidase Activity



Reactive oxygen species:  $\text{O}_2^{\bullet-}$ , HOCl,  $\text{H}_2\text{O}_2$ ,  $\text{O}_3$

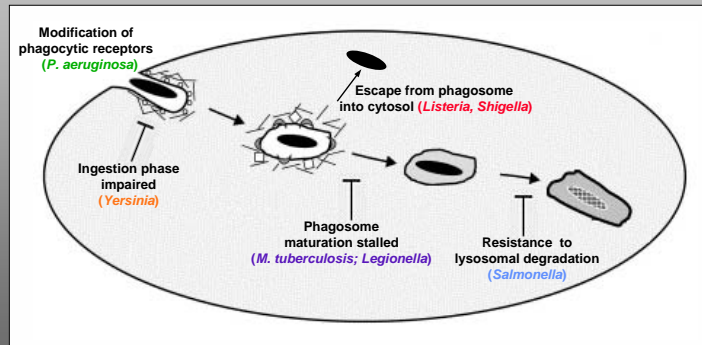
## Post-phagocytic Events: Peroxynitrite Production



Reactive oxygen species:  $\text{O}_2^{\bullet-}$ , HOCl,  $\text{H}_2\text{O}_2$ ,  $\text{O}_3$

Reactive nitrogen species:  $\text{ONOO}^-$

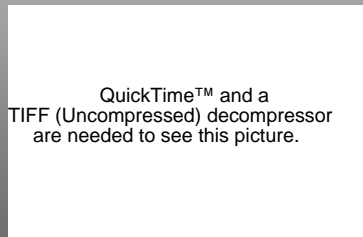
## Bacterial Virulence Factors Subvert Host Defenses



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



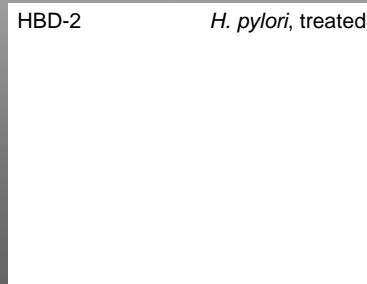
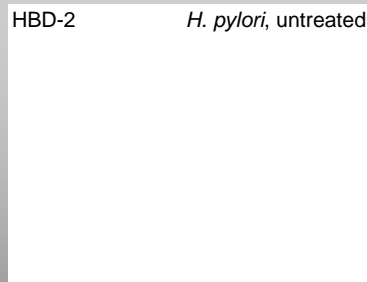
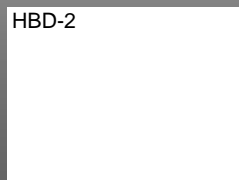
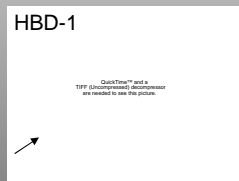
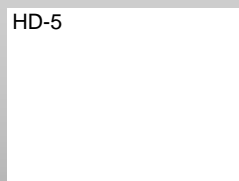
**Lysozyme**  
Disrupts peptidoglycan



**HBD1 HBD2 HBD3**  
Permeabilizes membranes

■ + charge  
■ - charge

## Epithelial Cells Express Defensins, Too



From: Wehkamp et al., *J. Clin. Pathol.* 56:352, 2003; Hamanka et al., *Gut* 49:481, 2001

## Phagocytosis: Not Just for Bugs

### Phagocytosis is the Principal Mechanism of Disposal of Apoptotic Corpses

Macrophage  
Apoptotic Thymocyte

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

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

From: Jennings et al., *Am. J. Resp. Cell Mol. Biol.* 32:108, 2005

## Immunological Consequences of Phagocytosis

### Clearance of pathogens

Death of pathogenic microbe  
Resolution of infection

Persistence of pathogenic microbe  
Failure of resolution of infection

### Clearance of apoptotic corpses

Suppression of inflammation  
Tolerance

Inappropriate inflammation  
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 remodeling. Ingestion of apoptotic bodies is immunologically "silent" and is normally accompanied by a suppression of inflammation. Failure of this mechanism may result in autoimmunity.