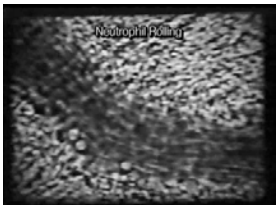
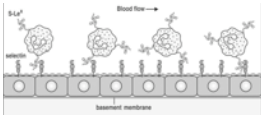
Science is like looking through a keyhole: The closer you get to the keyhole, the more you see of the room on the other side.

-George Wald
1967 Nobel Laureate in Medicine

A Day in the Life of a Phagocytic Leukocyte

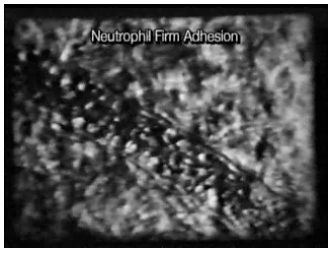
Selectin-mediated Adhesion is Weak and Promotes "Rolling" of Leukocyte Along Endothelia

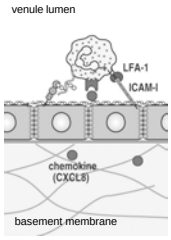




Movie, courtesy T. Springer

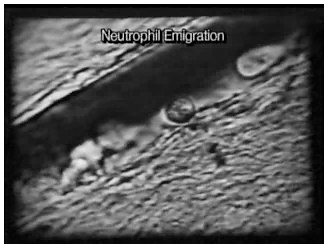
Firm Adhesion is Triggered by Chemokine Activation of Leukocyte Integrins

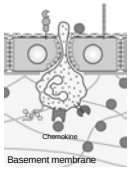




Movie, courtesy T. Springer

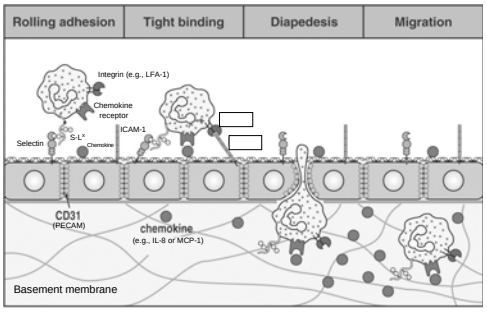
Diapedesis: Crawling Through Endothelial Junctions and Into the Tissue





Movie, courtesy T. Springer

Leukocyte Migration, Start to Finish



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

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

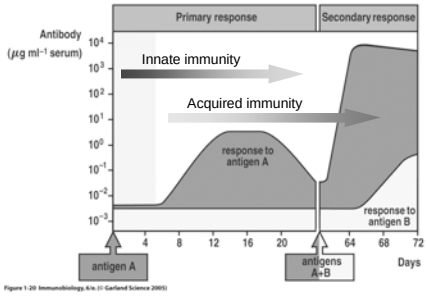
Conclusion: Genes that determine responses to infectious agents have a disproportionate effect on mortality

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

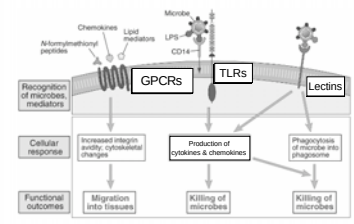
Distinctions Between Innate and Adaptive Immunity

	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, Mϕ

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

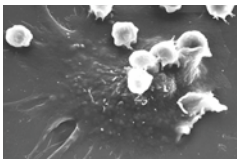


Receptors Important in Innate Immunity

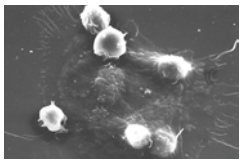


GPCR = G protein-coupled receptors
TLRs = Toll-like receptors
Lectin: A molecule that binds carbohydrates

Phagocytosis of IgG-coated Targets by Macrophages

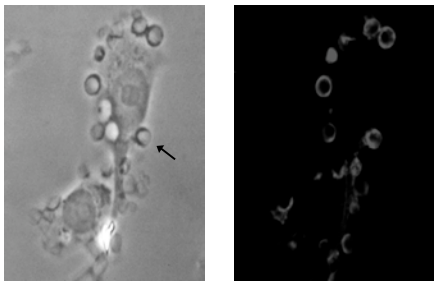


3 min

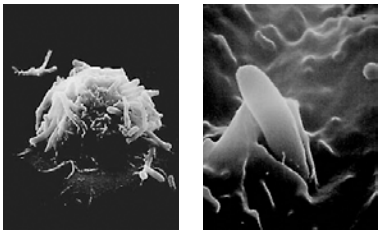


10 min

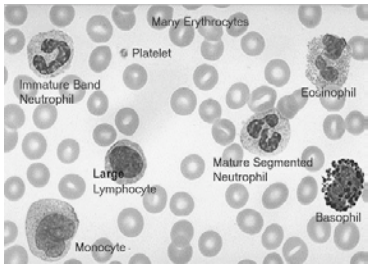
Extension of an F-actin-rich "Phagocytic Cup" Around Phagocytic Targets



Mast Cells Can Phagocytose Too!



Most, but not all Leukocytes Can Perform Phagocytosis



Opsonic vs Non-opsonic Phagocytosis

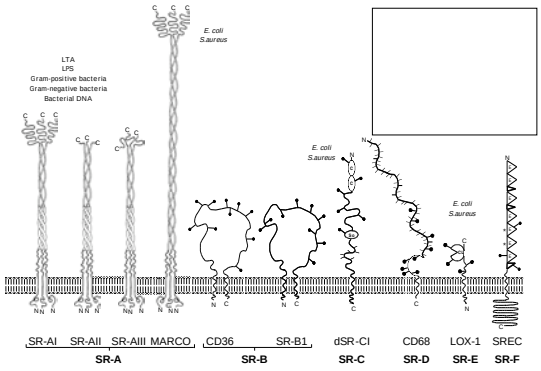
- Non-opsonic phagocytosis is typically mediated by cell surface receptors on leukocytes that recognize repeating carbohydrate subunits (comprising "molecular patterns") on microbes.

- 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 *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*

The Scavenger Receptor Superfamily



Examples of "Pattern Recognition Receptors" that Promote Non-opsonic Phagocytosis

Receptor	Ligand/Target	Expression
Integrins		
β2 Integrins	Invasin on *Yersinia*	Widespread
Scavenger Receptors		
SR-AI/SR-AII	Lipoteichoic acid on *Streptococcus* ? on Gram-negative bacteria	Mϕ
MARCO	? on *E. coli, S. aureus*	Mϕ
Lectins		
Dectin-1	β-glucan on *C. albicans*	Mϕ, DC

Non-opsonic Phagocytosis

Opsonic Phagocytosis

Y IgG

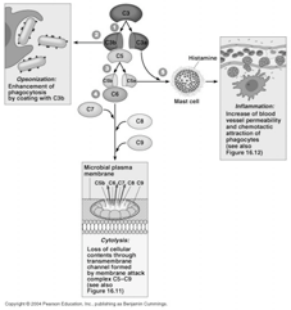
Opsonic Phagocytosis

Y IgG

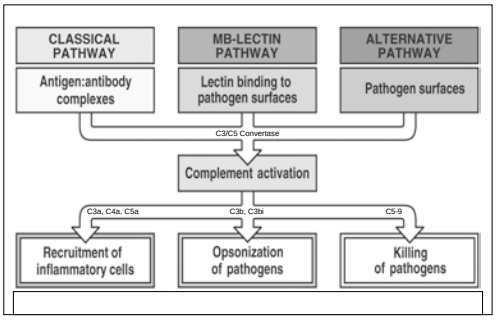
- Complement

What is complement?

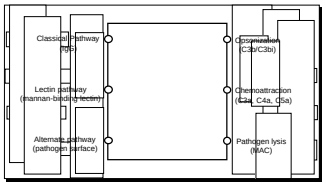
Complement Proteins Deposit on Pathogen Surfaces, Triggering Phagocytosis, Inflammation, and Pathogen Lysis



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

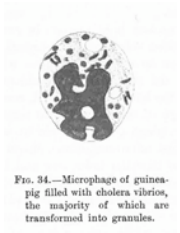


FIG. 34.—Microphage of guinea-pig filled with cholera vibrios, the majority of which are transformed into granules.

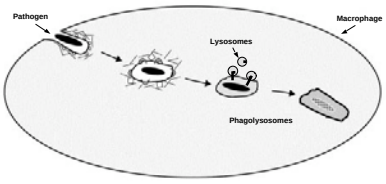
Phagosome-Lysosome Fusion?

MACROPHAGE:
Another white blood cell responsible for killing microbes is ingesting the yeast *Candida albicans*

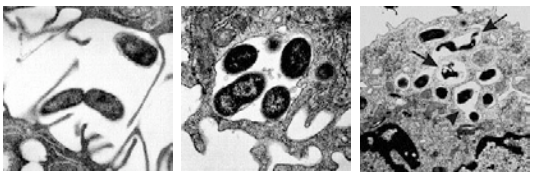
NECROSIS:
After a meal of these "leukotoxic" *Streptococcus pyogenes*, a white blood cell dies
Speed = x 3

BACTERIAL CAPSULE:
The slippery capsule of *Streptococcus pneumoniae* enables these bacteria to avoid being eaten by neutrophils

Post-phagocytic Events: Phagosome-Lysosome Fusion



Phagocytosis of Bacteria is Followed by Phagosome-Lysosome Fusion

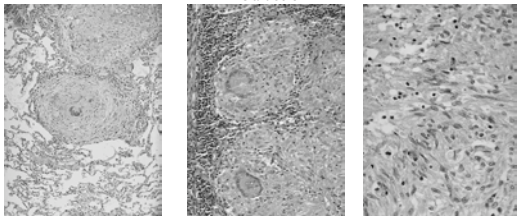


0-3 min | 1-5 min | 30 min-hrs

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

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

Granulomas | Langhans-type Giant Cells | Epithelioid Cells

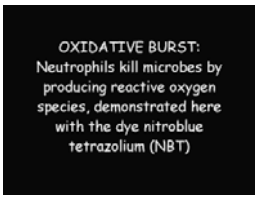


Granulomatous inflammation consists of epithelioid macrophages, giant cells, lymphocytes, plasma cells, and fibroblasts.

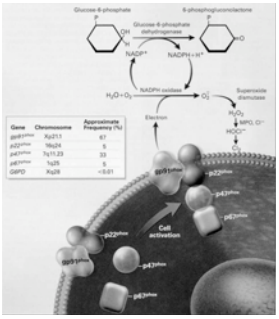
Langhans-type giant cells represent fused macrophages. The nuclei are lined up around the periphery of the cell.

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



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

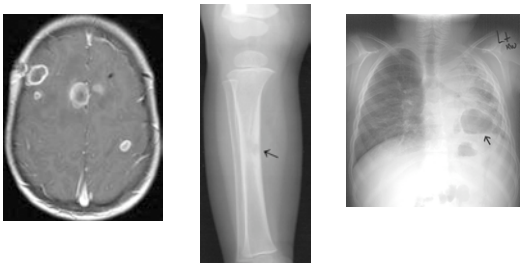


From: Lekstrom-Himes and Gallin, *N Engl J Med*, 343:1703, 2000

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.

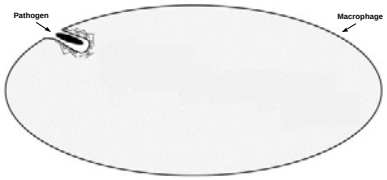
Chronic Granulomatous Disease: Clinical Manifestations



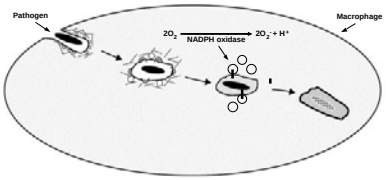
From: Khanna et al., *Radiographics* 25:1183, 2005

What happens following pathogen ingestion?

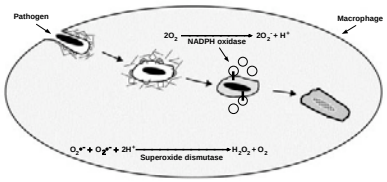
Post-phagocytic Events



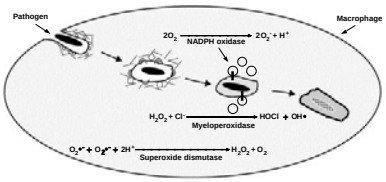
Post-phagocytic Events: "Phagosome-Oxidase Fusion"



Post-phagocytic Events: Generation of H_2O_2

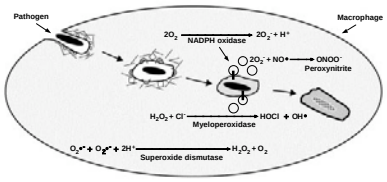


Post-phagocytic Events: Myeloperoxidase Activity



Reactive oxygen species: $O_2^{-\bullet}$, HOCl, H_2O_2, O_3

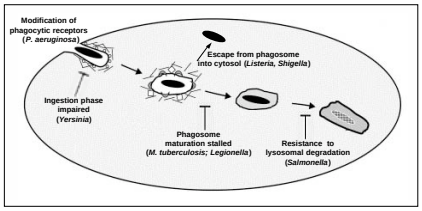
Post-phagocytic Events: Peroxynitrite Production



Reactive oxygen species: $O_2^-\bullet$, HOCl, H_2O_2, O_3

Reactive nitrogen species: $ONOO^-$

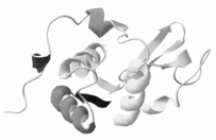
Bacterial Virulence Factors Subvert Host Defenses



Non-oxidative Killing Mechanisms

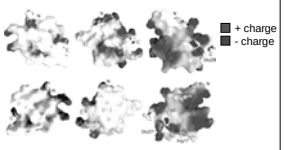
Non-oxidative Killing Mechanisms of Phagocytes

- Principally proteins within granules that are released upon cell stimulation
- These proteins include lysozyme, lactoferrin, proteases, defensins and other cationic proteins



Lysozyme

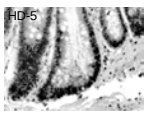
Disrupts peptidoglycan

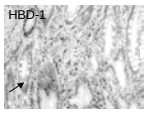


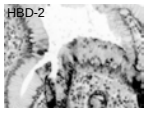
HBD1 HBD2 HBD3

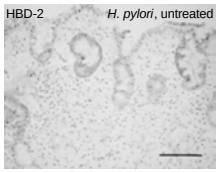
Permeablizes membranes

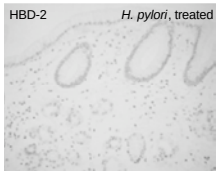
Epithelial Cells Express Defensins, Too







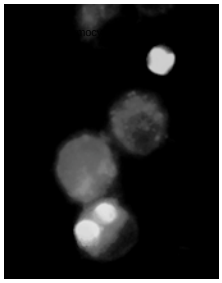




From: Wehkamp et al., *J. Clin. Path.*56:352, 2003; Hamanka et al., *Gut* 49:481, 2001

Phagocytosis: Not Just for Bugs

Phagocytosis is the Principal Mechanism of Disposal of Apoptotic Corpses



- 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.

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 aquired 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.