NROSCI/BIOSC 1070 and MSNBIO 2070

Block 4 - Immunology

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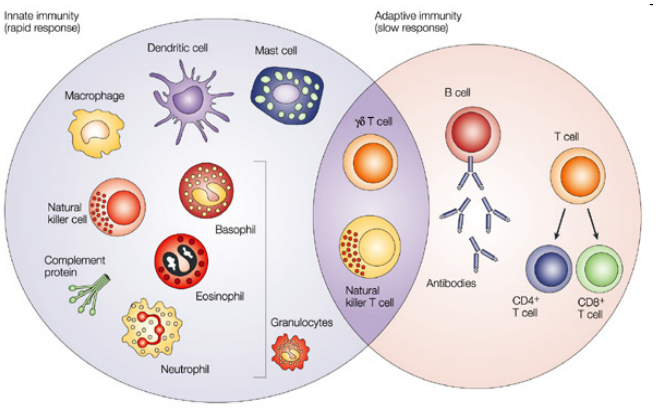


Dranoff G. Nat. Rev. Cancer. 2004. 4: 11-22

Lecture 1 - *Innate Immune System*

Chapter 1 - The Need for Self-Recognition

Introduction:

The human immune system:

- Is a collection of passive and active defensive mechanisms that keep out, identify, and neutralize threats to the body

- Consists of an "Innate" and "Adaptive" Immune System

- Must distinguish "non-self" from "self"
  - Bacteria
  - Virus
  - Foreign material

- Also has to recognize "bad self"
  - Cancer
  - Intracellular pathogens
  - Sterile tissue damage

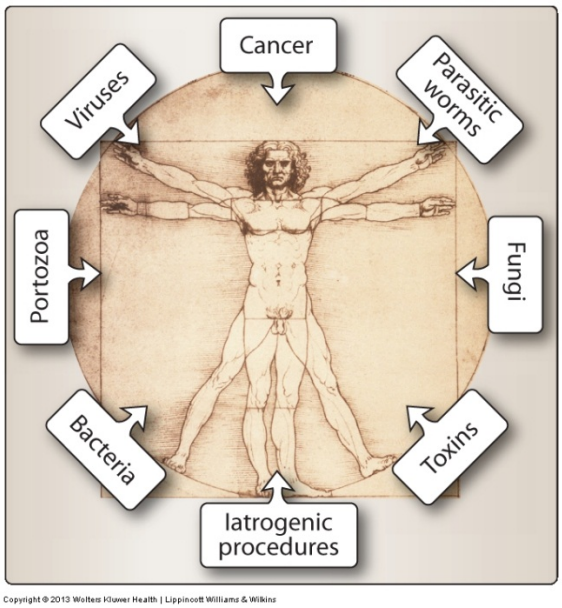


Fig. 1.1.

Chapter 3 - Barriers to Infection

Protective physical barriers and their associated inhospitable environments are our first line of defense against pathogens. These really represent our most important immune organ.

Example: Leading cause of death after burn injury is Sepsis (lethal inflammation due to systemic bacterial infection).

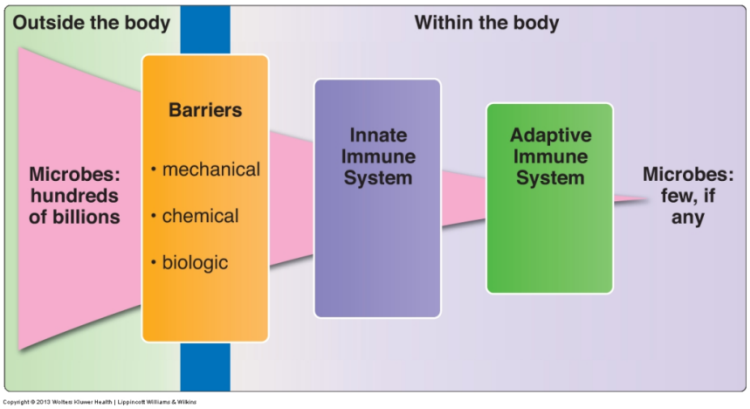


Fig. 1.2

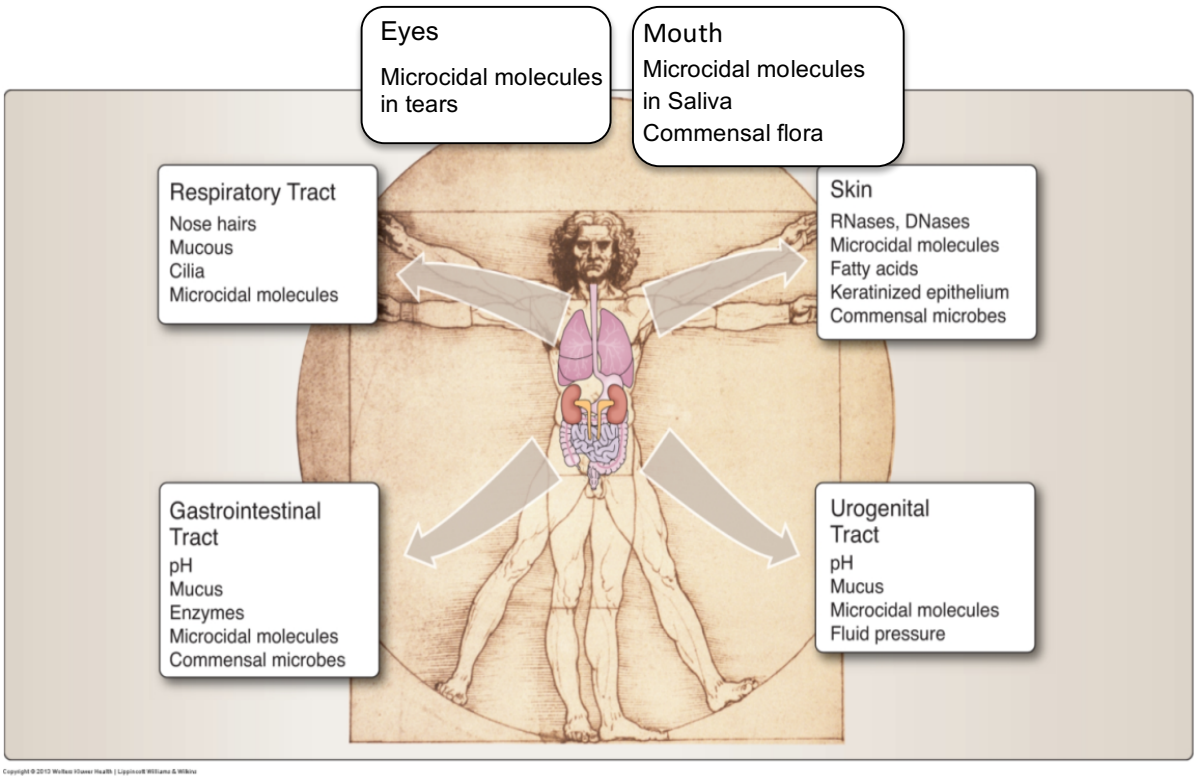


Figure 3.1
Protective barriers of the body. The barriers of the body represent the first line of defense and prevent or retard the entry cells and molecules into the body.

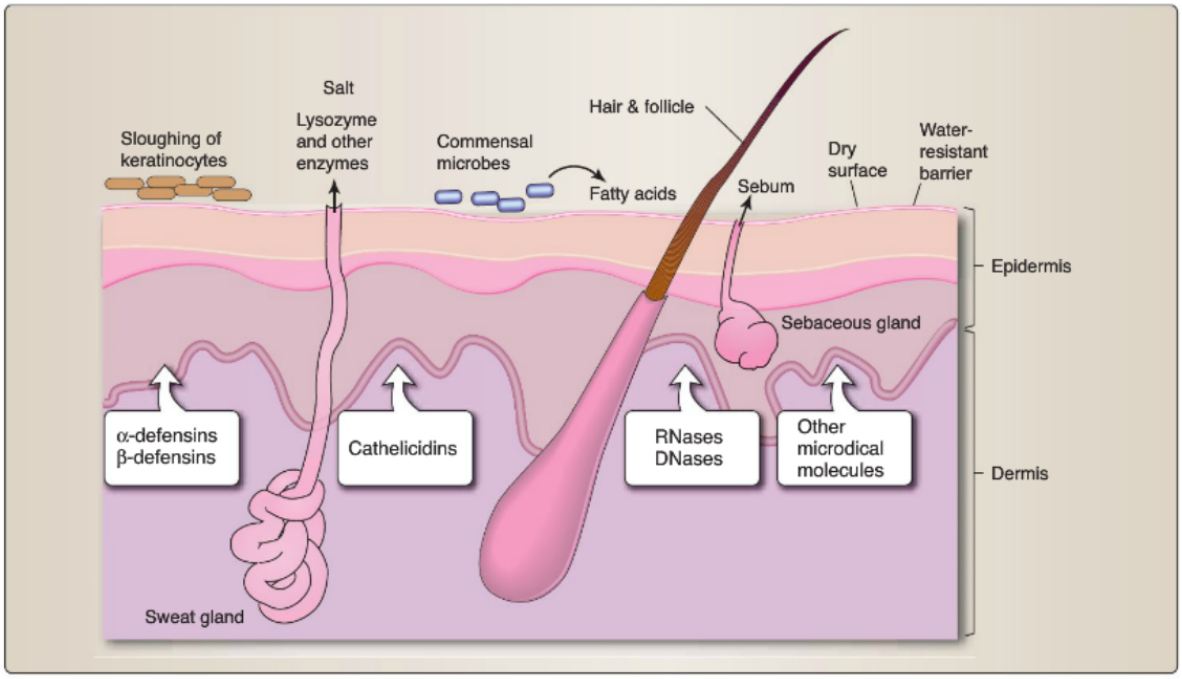


Fig. 3.2 Skin defense mechanisms.

Epidermis provides a dry, watertight barrier continually sloughing dead cells (keratinocytes).

Dermal glands bathe the epidermis with microcidal molecules as well as with sebum and sweat producing an acidic pH and deposit salt on the surface of the skin.

The dermis contains additional defense molecules and phagocytic molecules (e.g., neutrophils, macrophages) that attack invaders.

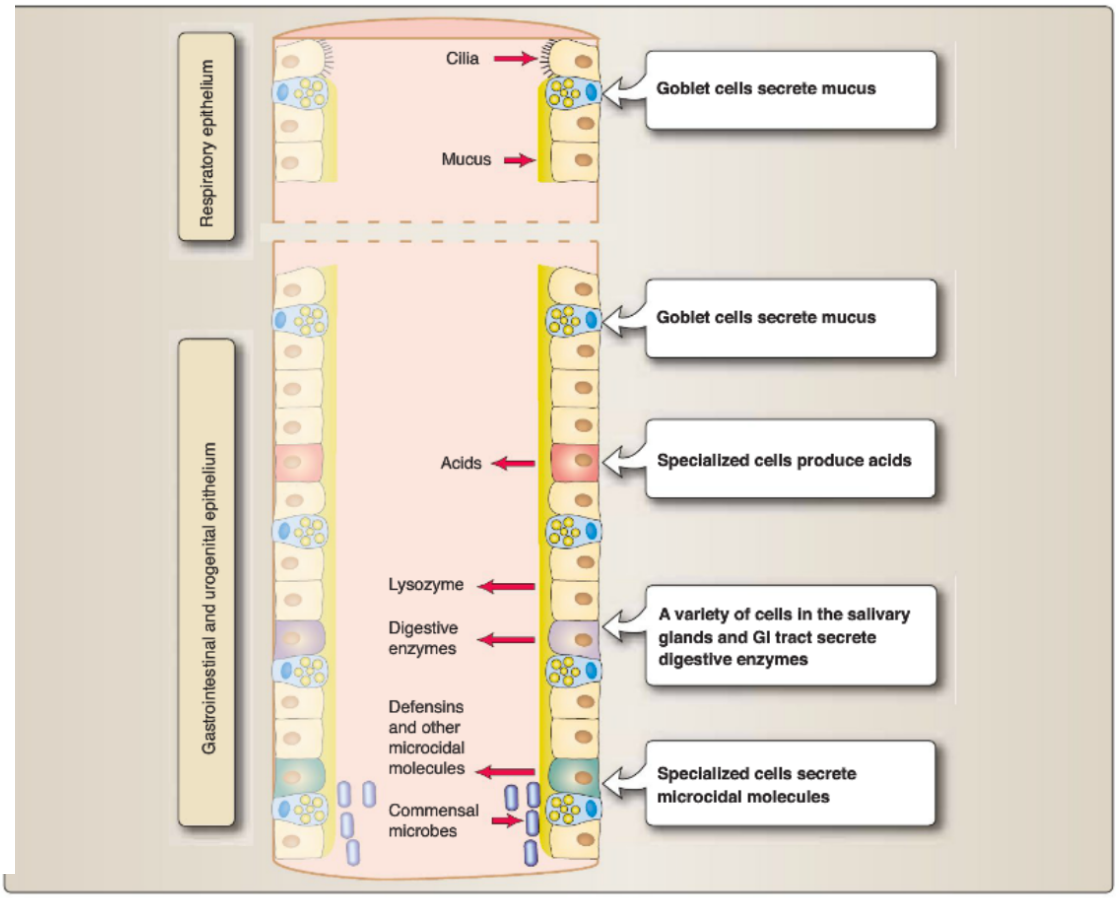
Commensal microbes secrete fatty acids that inhibit colonization by other microbes.

Fig. 3.3 Defense mechanisms of the mucous membranes.

Mucus entraps microbes and particulate matter (which, in the respiratory tract, is swept out by cilia).

Protective commensal microbes are present

Numerous microcidal molecules, enzymes and acids are produced.



Commensal Organisms

"Microbiota" refers to the collection of microorganisms that live in and on our bodies.

More bacterial cells then cells of our own body

Emerging evidence that these are not only serve as a competitive barrier, but influence important aspects of our physiology and health

- Cancer drugs

- Depression

Early colonization by microorganism the mother from during birth, skin, breast feeding, and food eaten.

Disruption of commensals by antibiotics can open up an individual to infection

- *Clostridium Difficile (C. Diff)*

Table 3.1
OUR MICROBIAL ENVIRONMENT

Location	Bacterial Load	
Skin	10^3 per cm^2	10^{12} total
Scalp	10^6 per cm^2	
Nasal mucus	10^7 per gram	
Saliva	10^8 per gram	
Mouth	—	10^{10} total
Feces	$>10^8$ per gram	
Alimentary tract	—	10^{14} total

Anti-microbial peptides

Small, cationic peptides (6-60 a.a.) typically found in mucosal surfaces, can directly kill microbes.

Antibiotics made by your body

Found in frogs, flies, humans, plants, etc.

Often work by disrupting target membranes, mechanisms not fully known

TABLE 3-2 Some antimicrobial peptides

Peptide	Typical producer species*	Typical microbial activity*
Defensin family α-Defensins	Human (found in paneth cells of intestine and in cytoplasmic granules of neutrophils)	Antibacterial
β-Defensins	Human (found in epithelia and other tissues)	Antibacterial
Cathelicidins	Human, bovine	Antibacterial
Magainins	Frog	Antibacterial; antifungal
Cercropins	Silk moth	Antibacterial
Drosomycin	Fruit fly	Antifungal
Spinigerin	Termite	Antibacterial; antifungal

*In many cases, production of the indicated antimicrobial peptide or family is not limited to the typical producer but is produced by many different species. Also, some members of the indicated peptide or family may have broader antimicrobial activity than the typical one indicated.

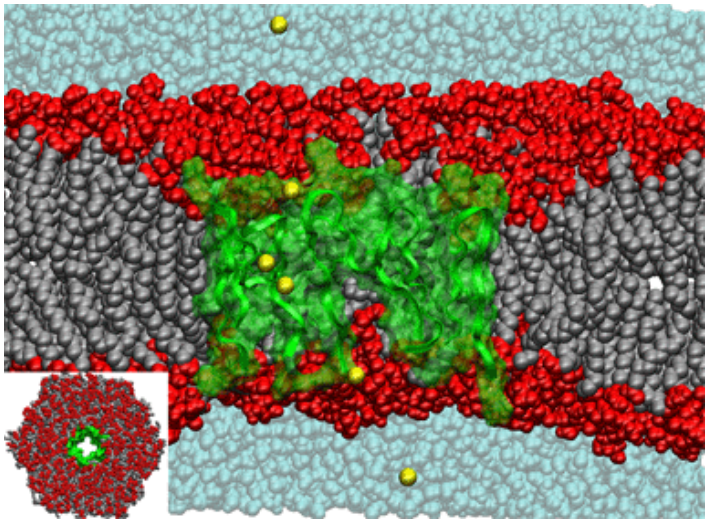
Table 3-2
Kuby IMMUNOLOGY, Sixth Edition

Example: Protegrin-1, an anti-microbial peptide

Why don't these kill our commensals?

Common commensals have evolved with us to express genes that provide resistance to our antimicrobial peptides

Ex. Commensal species from all three major phyla that colonize the gut could survive greater concentrations of the antimicrobial peptides than the pathogens could. Due to expression of enzyme that change the carbohydrates making up their cell wall (*Science* 9 2015).



Langham A., Sayyed-Ahmad A, Kaznessis YN. On the nature of antimicrobial activity: a model for Protegrin-1 pores. JACS, 2008, 130(13): 4338-4346.

Cells of the Innate Immune System - Chapter 4

There are two arms of the vertebrate immune system

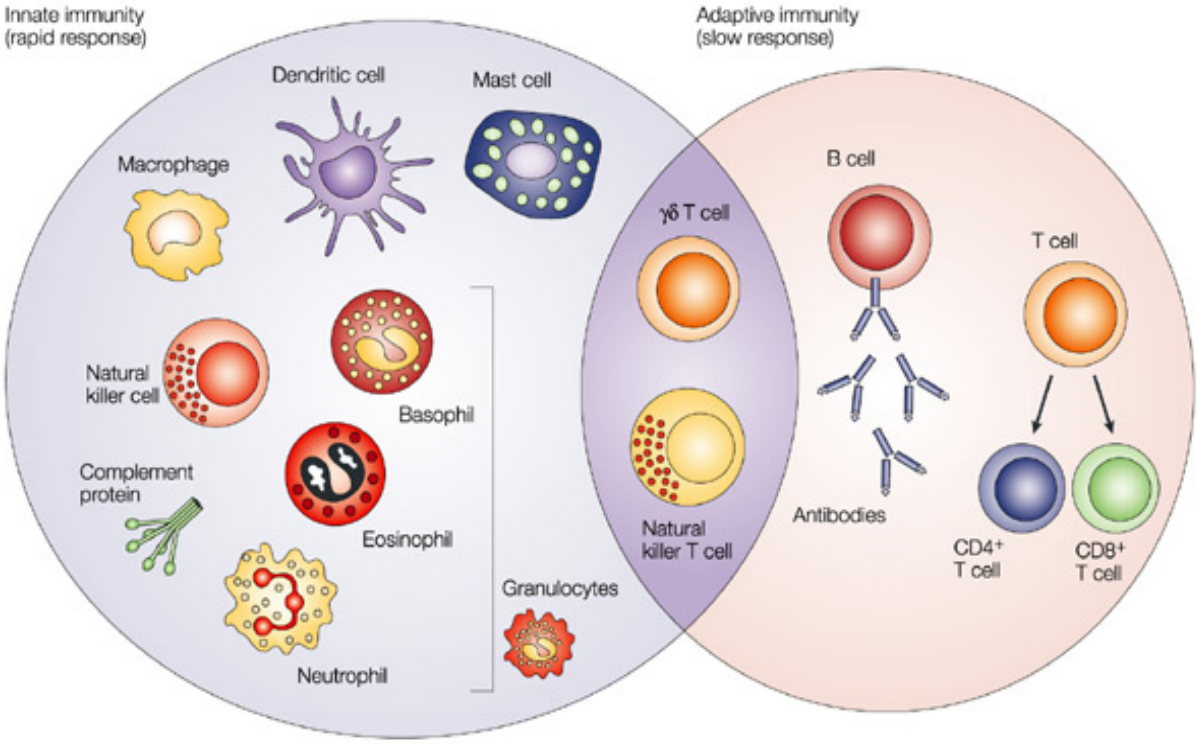
Innate: Evolutionary ancient set of cells and generalized mechanisms that counter pathogens

- Myeloid leukocytes

Adaptive: More recently developed set of immune cells (lymphocytes) and their products

- T lymphocytes
- B lymphocytes

The innate vs. adaptive immune systems



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Receptor Specificity:

Innate:

Limited and fixed in genome

Pattern recognition receptors (PRRs) that detect a conserved "molecular pattern" that are not found on host cells

Adaptive:

Diverse antigen-specific receptors (TCR, BCR)

See parts of specific antigens (peptides, proteins, carbohydrates)

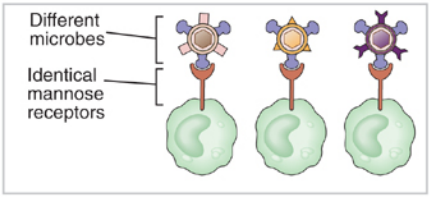
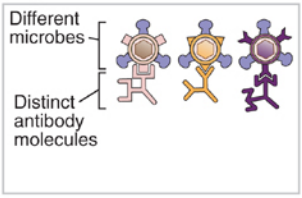
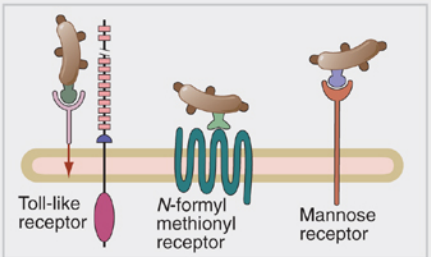
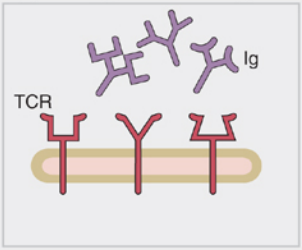
Receptor genetic encoding:

Innate:

Limited set of genes fixed in genome at the time of birth

Adaptive:

Receptors are diverse and generated via somatic gene rearrangement

	Innate immunity	Adaptive immunity
Specificity	For structures shared by classes of microbes ("molecular patterns") 	For structural detail of microbial molecules (antigens); may recognize nonmicrobial antigens 
Receptors	Encoded in germline; limited diversity 	Encoded by genes produced by somatic recombination of gene segments; greater diversity 
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Discrimination of self and nonself	Yes; host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on selection against self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

Receptor distribution:

Innate:

Non-clonal; all cells of a class identical

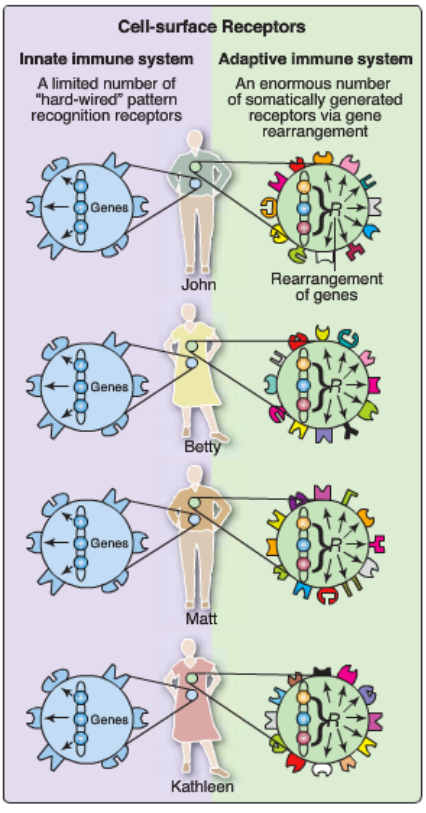
- Ex. Neutrophil 1 = Neutrophil 2

Adaptive:

Clonal: All cells of a class distinct

- Ex. T cell 1 ≠T cell 2

Figure 1.3
Innate pattern recognition receptors and adaptive somatically generated receptors. Each individual expresses pattern recognition receptors (innate immune system) and somatically generated receptors (adaptive immune system).



Receptor self/nonself discrimination:

Innate:

Selected and conserved over evolutionary time. "Self" is very broad (human vs. non-human)

Adaptive:

Selection required in individual to avoid self-reactivity. "Self" = you

The innate vs. adaptive immune systems, continued

	Innate	Adaptive
First response time	• Immediate activation of effectors; minutes to hours • Activation only requires a single signal • Response by actual activated cell	• Long response times; Days-weeks • Multiple permissive signals required • Stimulated cell can undergo clonal proliferation. Amplified immune responses.
Response to repeat stimuli	• Quick, but identical to primary response	• Much more rapid than primary responses. Generation of robust "memory"
Major components	• Physical barriers • Phagocytes • Granulocytes • Pattern recognition molecules	• T and B cells • Antigen-specific receptors • Antibodies

Immunologic memory

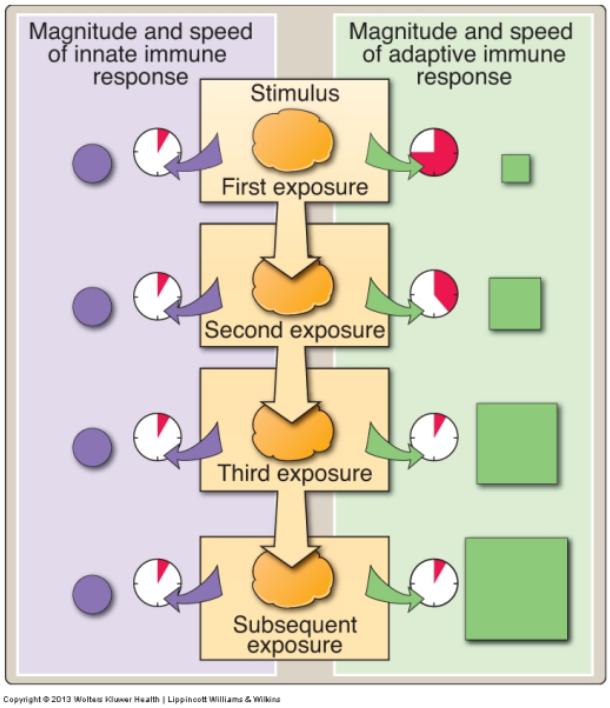


Fig. 1.5 Concept of immunologic memory.

The innate immune system reacts to a given stimulus with a consistent intensity, regardless of how many times it has been exposed to that stimulus.

The adaptive immune system can adapt and modify its response after each exposure to a given stimulus.

This is why vaccines work - generate strong adaptive immune responses

Innate Immune Cells -

All Immune cells are leukocytes = Greek origins

leuko- "white"
-cyte - "cell"

Hematopoietic Lineages:

All blood borne cells ultimately derive from **pluripotent hematopoietic stem cells**.

Pluripotent because each stem cell has the capacity to produce all leukocytes, red blood cells (erythroid lineage) and platelets (thrombocytic lineage).

Reside in the bone marrow and give rise to two lineages:

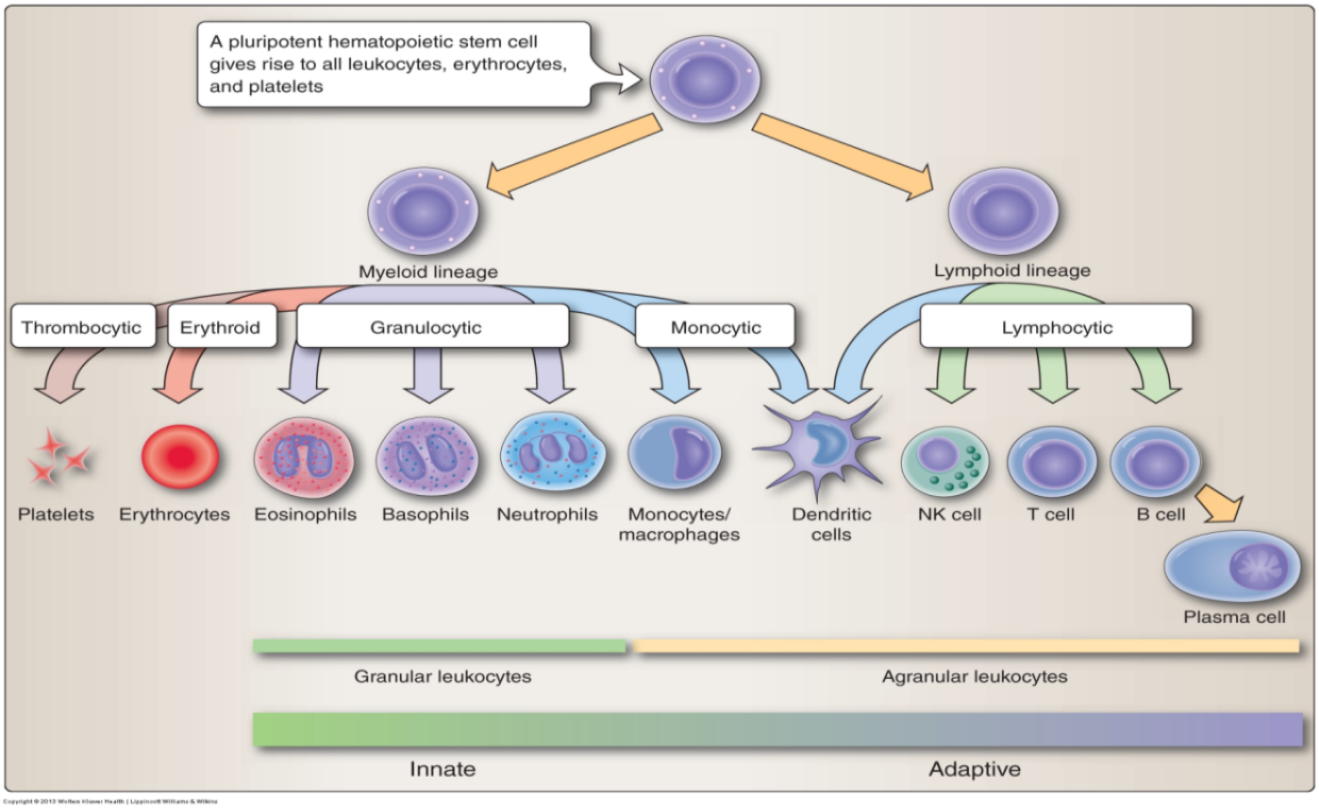
Lymphoid Lineage >Lymphocytes = Adaptive immune cells (T and B Lymphocytes; Plasma cells; 2nd Lecture)

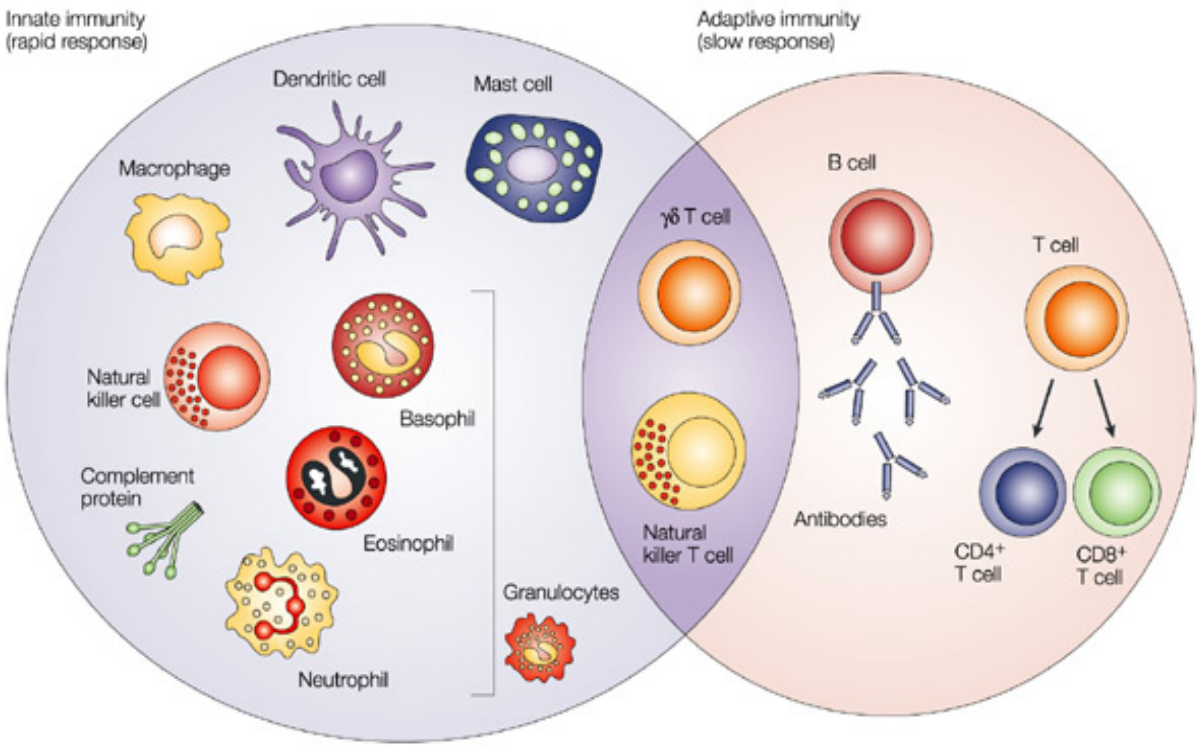
Lymphoid Lineage >Lymphocytes = Innate-like immune cells (NK Cells and NK T cells; Innate Lymphoid Cells)

Myeloid Lineage>Granulocytes or Granular leukocytes (eosinophils, basophils and neutrophils),

Myeloid Lineage>Agranular phagocytic cells (monocytes, macrophages, and dendritic cells) that use phagocytosis for clearance of pathogens and clear cell debris.

Myeloid Lineage>Cells and cell products involved in transport of oxygen and carbon dioxide (erythrocytes or red blood cells) and in blood clotting (platelets).





Dranoff G. Nat. Rev. Cancer. 2004. 4: 11-22

Cells making up the innate and adaptive immune systems: All leukocytes that can be leukocytes may be broadly classified by the absence (agranular) or presence (granular) of cytoplasmic inclusions or granules.

Adaptive lymphocytes include T, B, and natural killer (NK) cells and B cells that enlarge and differentiate into immunoglobulin secretors are known as plasma cells.

Innate cells include:

Monocytes are phagocytic cells in the circulation and are called macrophages when they enter tissues.

Dendritic cells are phagocytic cells that bare treelike cytoplasmic processes

Neutrophils have multilobed nuclei and cytoplasmic granules that stain with neutral (pH) dyes.

Basophils have bi-lobed nuclei and cytoplasmic granules that stain with basic (pH) dyes

Eosinophils have bi-lobed nuclei and cytoplasmic granules that stain with acidic (pH) dyes.

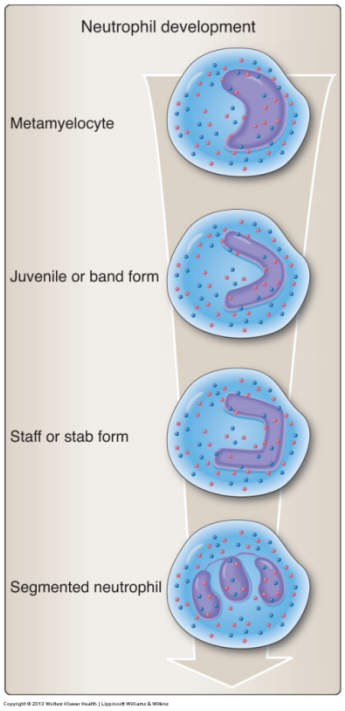
Granular Leukocytes

Leukocytes that contain conspicuous cytoplasmic granules are known as granulocytes. These cells have multilobed nuclei and cytoplasmic granules that contain amines (stained by basic dyes), basic proteins (stained with acidophilic or eosinophilic dyes), or both (neutral staining).

Typically blood stains are completed with Hematoxylin (Basic dye-blue) and Eosin (Acid dye - red)

Nuclei - Blue (- nucleic acids) vs. Muscle - Red (+ amino acids)

Neutrophils



Often called polymorphonuclear leukocytes (PMNs) because of their variable number of nuclear segments (two to five).

Most abundant leukocyte in the blood (4k-10k/ul)

40-60% of peripheral blood mononuclear cells (PBMC)

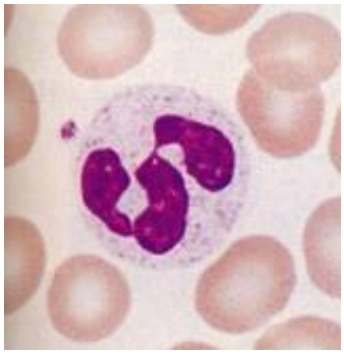
With a half-life of approximately 7 hours, more than 100 billion neutrophils enter the circulation daily in normal adults.

It takes about 2 weeks for metamyelocytes (an intermediate stage neutrophil with a kidney-shaped nucleus), to the staff or stab (German, meaning “staff”) form, and then to the segmented or mature stage

Neutrophils are the most numerous leukocytes and play a vital role in policing the body against microbial invasion.

Neutrophils are very effective at killing bacteria.

An increase in the number of peripheral blood neutrophils is often an indication of acute infection. Increases during infection (20k/ul)



As reserves of PMNs within the bone marrow become exhausted during an infectious disease, several metamyelocytes and juvenile forms increase in the circulation.

Paramount to effective responses to microbial invasion and tissue injury.

“first line of defense” - Earliest cells recruited to infected or wounded site

Immunological arsenal of neutrophils:

Phagocytosis

Anti-microbial cytotoxic substances and enzymes

Extracellular traps of DNA

Killing via “respiratory burst” generating reactive oxygen and nitrogen species

Myeloperoxidase (MPO)

MPO: Peroxidase enzyme produces hypohalous acids to carry out antimicrobial activity.

It is a lysosomal protein stored in azurophilic granules of the neutrophil and released into the extracellular space during degranulation.

MPO has a heme pigment, which causes its green color in secretions rich in neutrophils, such as pus and some forms of mucus.

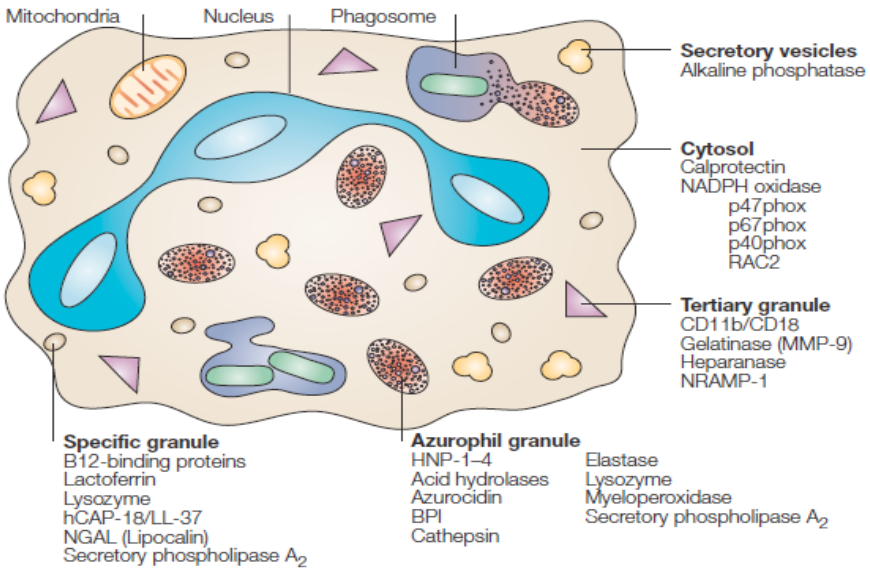


Figure 1 | **The antimicrobial mechanisms of polymorphonuclear leukocytes (PMNs).** Human PMNs contain four α-defensins (HNP-1–4) and hCAP-18/LL-37, a cathelicidin. The α-defensins are found within the azurophilic granules, together with several antimicrobial proteins, including lysozyme, azurocidin, bactericidal/permeability-increasing protein (BPI) and cathepsin G. hCAP-18/LL-37 is found within the smaller, specific granules, together with other proteins including lysozyme. In addition to this complement of antimicrobial peptides and proteins, the presence of NADPH oxidase and myeloperoxidase allows the generation of an antimicrobial respiratory burst when neutrophils are activated. Not drawn to scale.

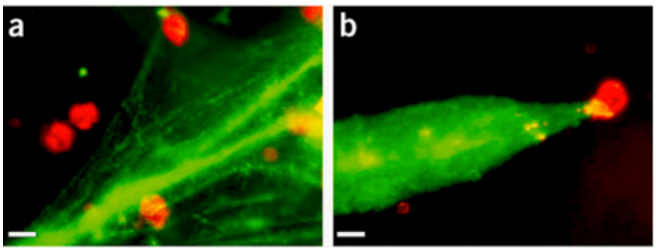
RI Lehrer. Nature Reviews Microbiology. 2004. 2, 727-738

NETosis

form of cell death that involves the release of decondensed chromatin and granular contents to the extracellular space.

Extracellular trap formation that acts to immobilize microbes and prevent their dispersal in the host.

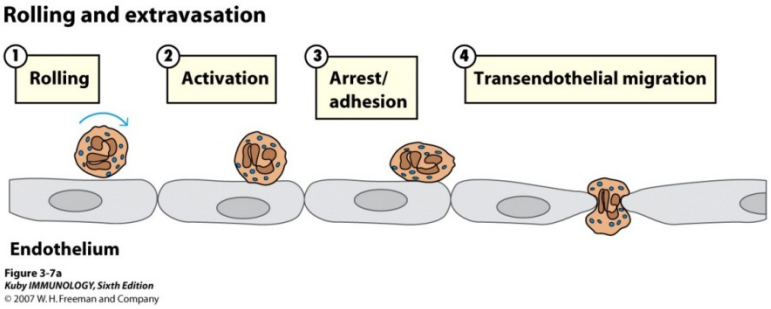
NETs serve for attachment of bactericidal enzymes including myeloperoxidase and leukocyte proteases.



(Kubes - Nature Medicine 2007)

Neutrophil recruitment:

Neutrophils make up the first wave of cells that cross blood vessel wall to enter sites of pathology. Their recruitment to sites of inflammation, infection, injury are broken down into four steps and involve several groups of adhesion molecules.



http://www.youtube.com/watch?v=xKoncIMFPtg

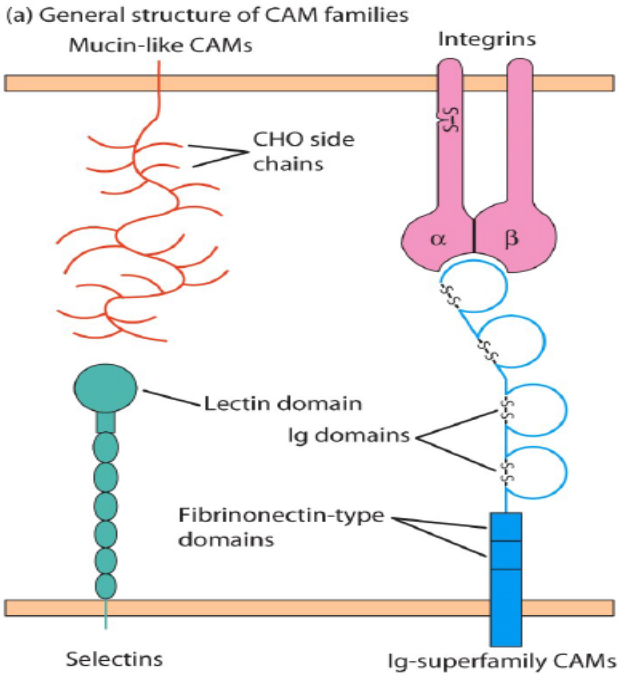
Selectins – resp. for initial contact between leukocytes and endothelial cells

- Bind to specific carbohydrate (CHO) groups (i.e., mucins)

Mucins – glycosylated proteins

Bind to selectins on endothelium

Bind to other mucins (CD34 and glyCAM) on endothelium of lymph nodes



Integrins – heterodimer proteins formed by all leukocytes. Need to be modified to an active form.

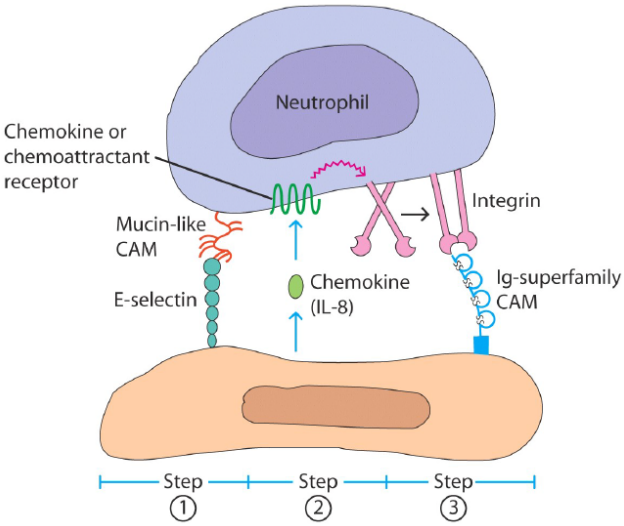
Bind to ICAM's along vasc. endothelium

ICAM's – CAM's with Ig domains on vasc. endothelia

Bind to integrins at Ig domain

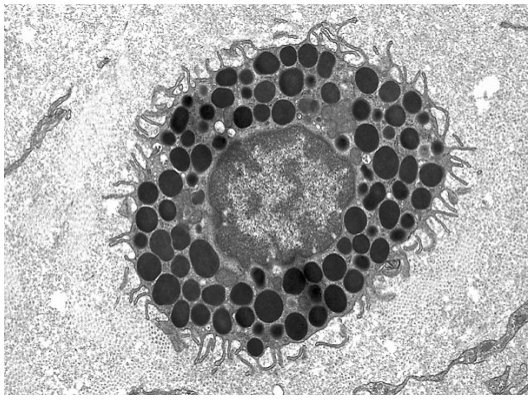
MadCAM's – have both IG and mucin-like domains; found on mucosal endothelia

Bind to integrins on lymphocytes



Recruitment Steps:

1. Rolling along vascular endothelium in blood steam. Mediated by low affinity interactions between neutrophil mucins and selectins on endothelium. Can not out compete shear force of blood stream.
2. Activation by a chemoattract release by damage or inflammation. Results in changes in integrins
3. Activated integrins interact with CAMs to arrest/adhere. Able to overcome shearing force of circulating blood.
4. Transendothelial migration of neutrophil



Mast cells

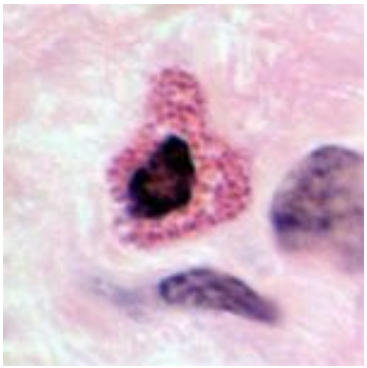
Low number in the blood (0-1%)

Long-lived in peripheral tissues, especially innervated and vascularized regions, as well as barrier tissues

Large cells with cytoplasm full of basophilic granules rich in histamine - stain blue

Degranulate when stimulated and granules increases vascular permeability and stimulates local nerves

http://www.youtube.com/watch?v=VT7knZ6_8rk



Generate lipid-derived messengers (leukotrienes) that module the local environment and promote chemotaxis

Crucial to role in allergy

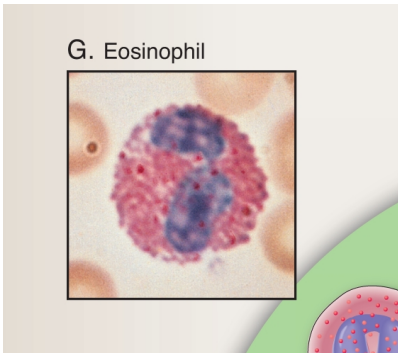
Eosinophils

1-3% of PBMC in non-allergic individuals

Bi-lobed nucleus and many cytoplasmic granules that stain red with acid dye eosin - why named “eosinophils”

Eosinophil specific granules

- Major Basic Protein - enzyme that targets parasites
- Eosinophil Peroxidase - formation of oxidizing hypohalous acids from hydrogen peroxide and halide ions
- Eosinophil Cationic Protein - ribonuclease and cytotoxic agent



Motile and Phagocytic

Main function is degranulation against large targets which cannot be phagocytosed, especially parasites (helminths)

Basophils

0.5% to 1% of circulating leukocytes

Granulocytic cells that circulate in the blood and are implicated in allergic and anti-parasitic responses

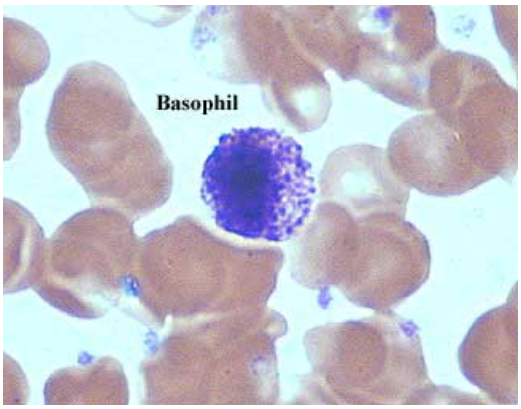
Lobed nucleus

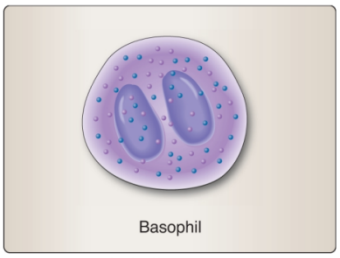
Stains with basic dyes

Nonphagocytic

Secretion of histamine, heparin, cytokines, and chemotactic agents

Role in allergic responses





Innate Lymphocytes

Cells that differentiate along one of several of the lymphocytic pathways are known as **lymphocytes**

Natural Killer (NK) Cells

Common lymphocyte (10% of PBMC) but distinct from T and B cells in that it lacks antigen-specificity

Large and granular

Activation determined by a balance between engagement of activating and inhibitory receptors

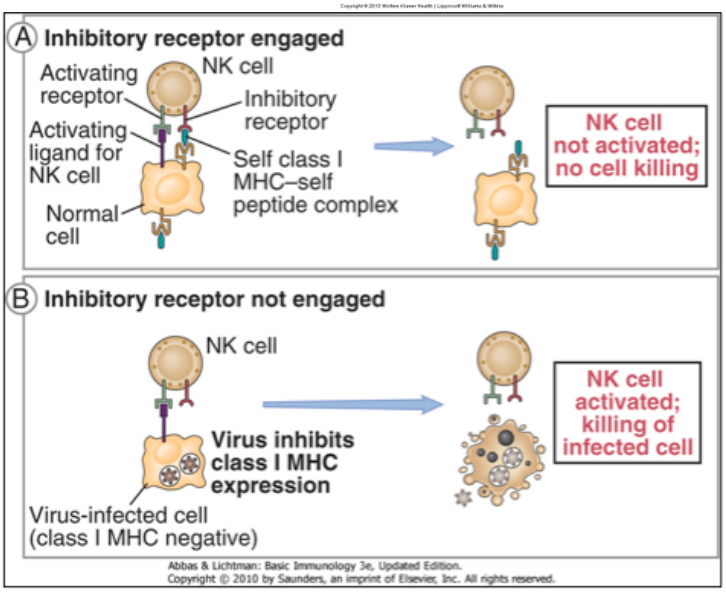
Recognize lack of MHC I via Killer inhibitor receptors (KIRs) to kill virally infected cells tumor cells

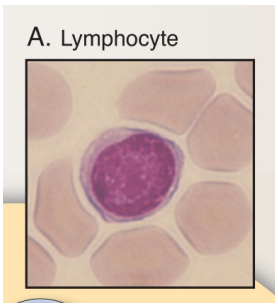
Can also remove stressed cells via Killer Activator Receptors (KARs) which recognize MICA and MICB.

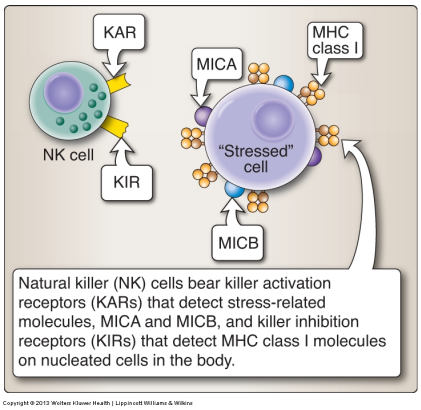
Produce high levels of cytokines that can shape the immune response

Granules released directly into the target cell. Contain enzymes (such as perforin and granzyme) that degrade target cell proteins and make holes in membranes.

Target dies by apoptosis, programmed cell death, while NK is unharmed







http://www.youtube.com/watch?v=84MlWh1XN0Q

Adaptive Lymphoid lineage

B lymphocytes or **B cells** reside in the bone marrow and are able to synthesize immunoglobulin molecules - "Antibodies" that make up the unique B cell receptors (BCR)

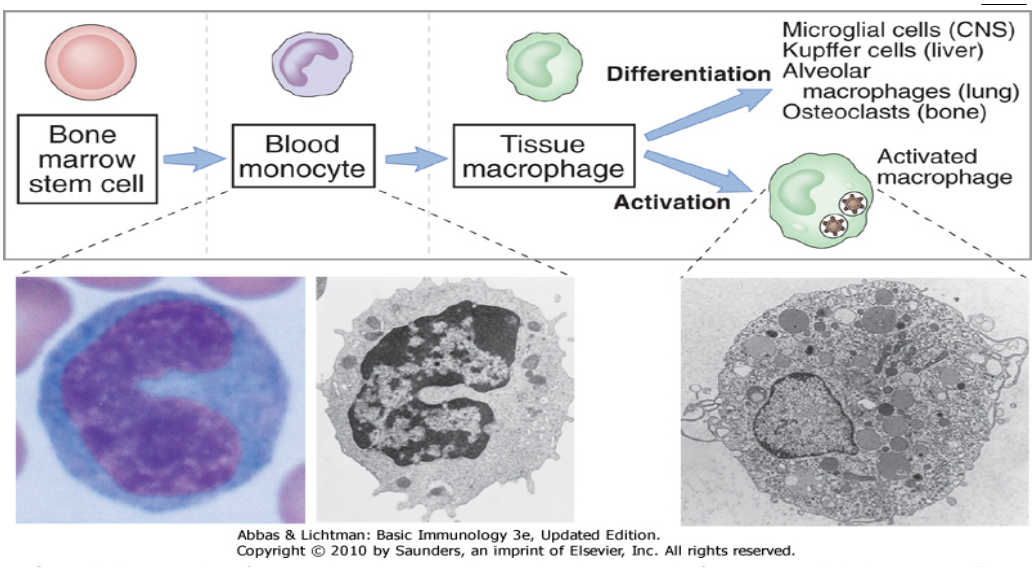
Other lymphoid lineage cells of bone marrow origin migrate to, differentiate, and are vetted within the environment of the thymus. Those cells (**thymocytes**) that exit the thymus are known as thymus-derived lymphocytes or **T lymphocytes (T cells)**. Each has a unique T cell receptor (TCR).

We will address the differentiation and function of B cells, plasma cells, and T cells and their roles in adaptive immune function in the 2nd lecture

Phagocytes:

<u>Monocytes and macrophages</u>

- Monocytes 2-8% of PBMC
- Leave the blood and differentiate into tissue appropriate mononuclear phagocytes (macrophages)



Macrophages "see" bacteria or other microbes, and become "activated"

- Increased phagocytic activity and nitric oxide by up-regulating "iNOS" (inducible nitric oxide synthase)
- Process and present engulfed material in a form recognized by adaptive immune cells (T cells)
- Secrete inflammatory cytokines that support immune responses

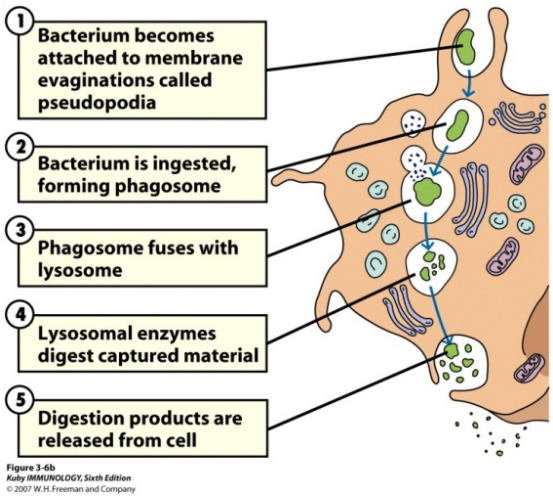


Figure 3-6b
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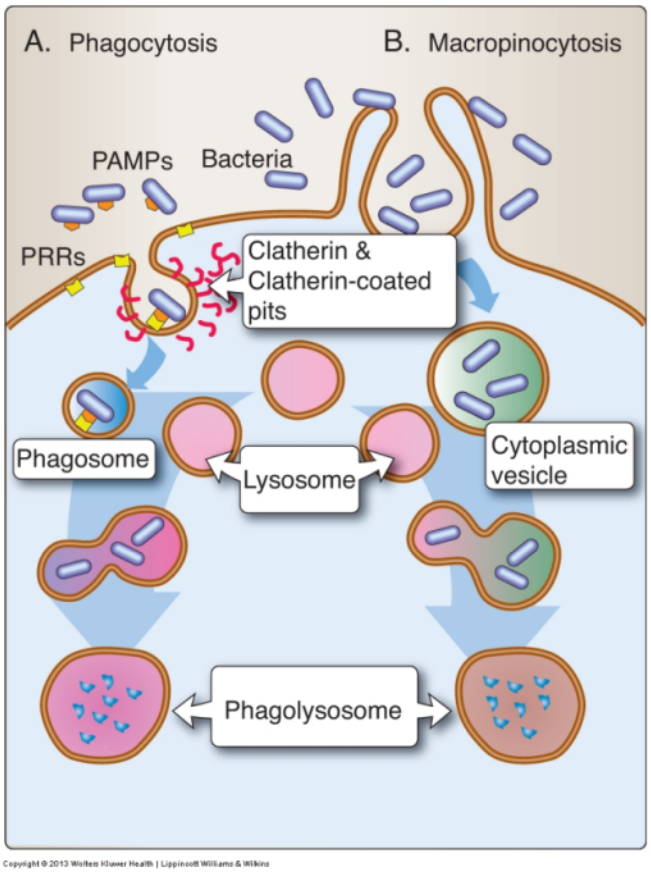
Phagocytosis in action

- Video of macrophage ingesting Shigella
- http://www.youtube.com/watch?v=UeuL3HPfeQw



Dendritic cells (DC)

Have many projections (look like nerve cell dendrites) so they can interact with a large number of cells

Rare, but ubiquitously-distributed mononuclear phagocytes

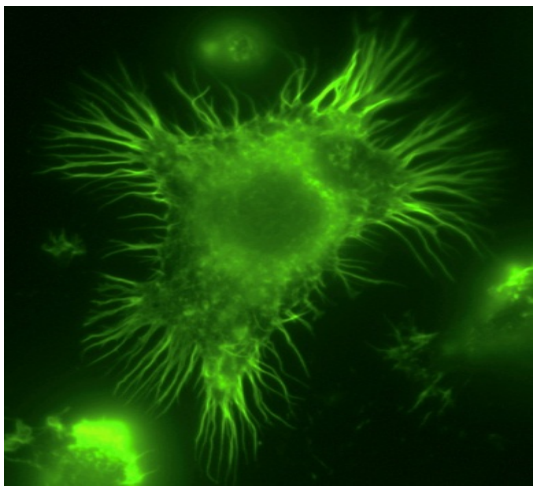
- Skin, solid organs, tissues

Both myeloid and lymphoid lineages

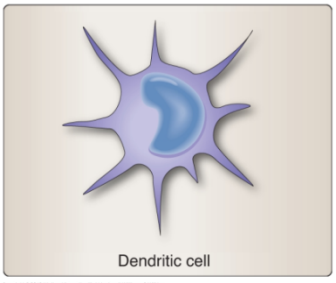
- Numerous subsets of DC

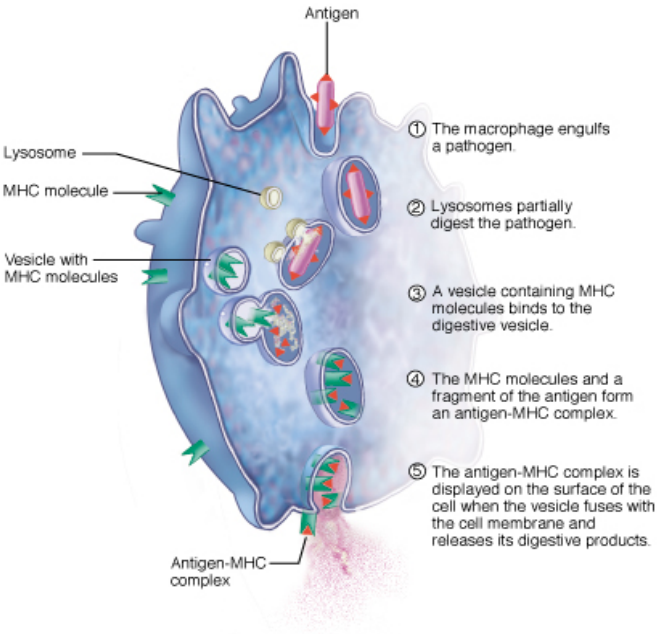
Highly phagocytic, but minor role in direct phagocytic removal of pathogens

Dominant role is to act as "Professional antigen presenting cells (APC)" which show pieces of pathogens to T cells in forms they can recognize.



Turnquist and Rosborough, Unpublished data





The Need for Self-Recognition - 1;
Antigens and Receptors - 2;
Innate Immune Function - 5

Immune responses are regulated by the interactions between a **ligand** and a **receptor** protein.

Immune receptors can be membrane bound and soluble

Ligands: A molecule that forms a complex with another molecule.

Ex. Peptide in MHCI

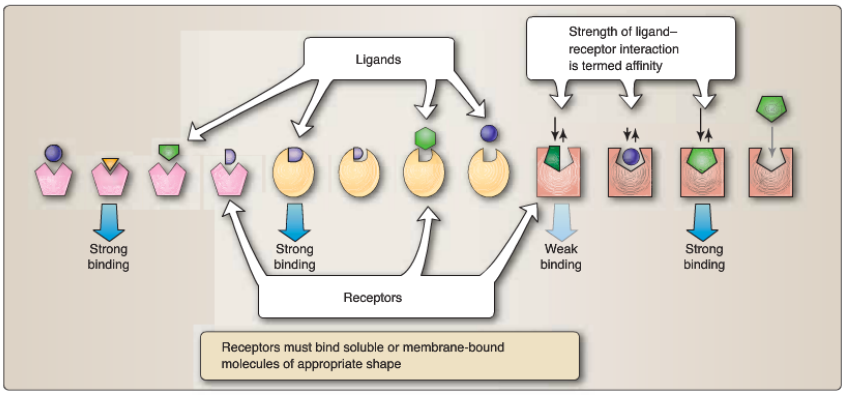
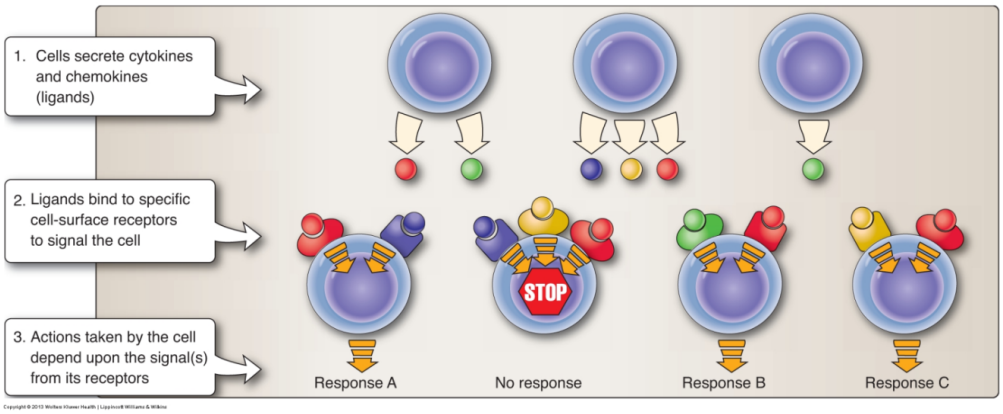


Figure 2.1
Receptor–ligand interactions. Receptors bind molecules or ligands that may be either soluble or bound to membranes. If the binding is sufficient, the receptor is able to provide a signal to the cell.

Affinity: How strong the non-convalent interaction between two molecules is.

Avidity: The collective affinity where multiple receptors are involved. Typically used to describe antibodies.

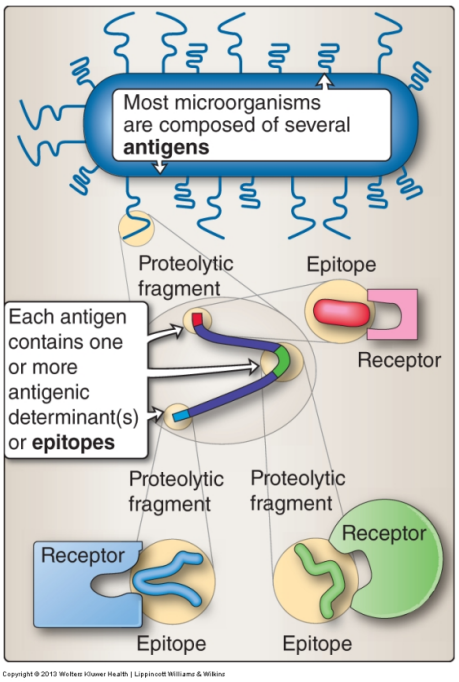


Cytokines/chemokines: Protein molecules that act as messengers between cells. Typically has an impact on cell functions or survival**.**

Ex. Interleukins (IL-37)

Antigen: (Ag) collection of ligands recognized by cells of both the innate and adaptive immune systems

Epitope: smallest individually identifiable part of an antigen that is bound by a receptor



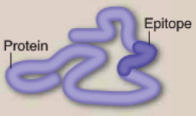
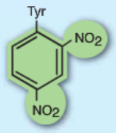
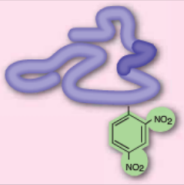
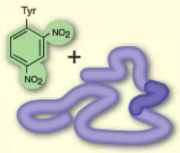
Immunogens: Material that contain epitopes that induce an immune response and are the target of that response

Ex. Chicken Ovalbumin (OVA), Bacterial cell walls, viral envelope proteins, proteins with point mutation

Haptens: Small, nonimmunogenic materials that can bind to immune receptors but not induce an immune response until bound to an immunogen

Immune repertoire: The sum of all the epitopes for which a given individual has immunological receptors to detect. Cells with receptors recognizing self molecules are selected out. Thus, individuals are "tolerant" to self material.

Table 2.1
IMMUNOGENS AND HAPTENS

Injection with	Structure	Response to protein epitope	Response to hapten	Comment
Immunogen or carrier		Yes	Not applicable	An injected protein (sometimes called a carrier) that elicits an immune response is called an immunogen
Synthetic epitope or hapten		Not applicable	No	Injection of a synthetic molecule, in this case 2,4-dinitrophenyltyrosine, by itself does not elicit an immune response and is called a hapten
Hapten–Carrier conjugate		Yes	Yes	Injection of a hapten chemically bound to a carrier elicits an immune response to both carrier epitope(s) and to the hapten
Hapten NOT conjugated to carrier		Yes	No	Injection of unconjugated hapten and carrier does not elicit a response

Tolerogens: induce adaptive immune unresponsiveness. Exposure to a tolerogen results in a diminished response rather than an enhanced one.

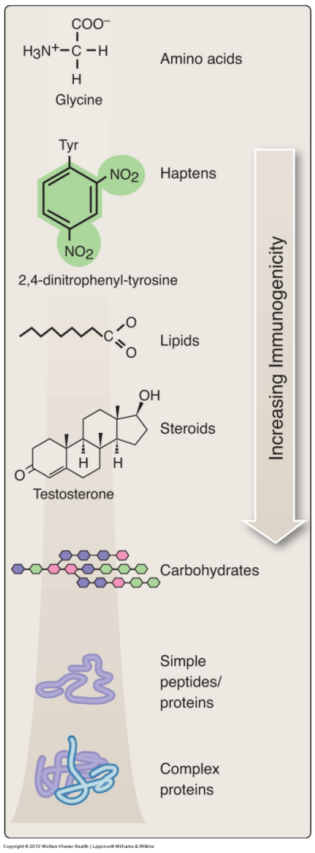
Factors governing immunogenicity: The greater the size and complexity of the antigen, the greater the variety of epitopes and the greater the immunogenicity

Size: Proteins > 10 kDa

Complexity: Complex proteins generating diverse epitopes.

Conformation: Epitopes must be "seen" by the immune system

Chemical properties: A protein must be cleavable after phagocytosis.



Immune Receptors:

The immune system is dependent on surface expressed and soluble receptors.

The binding of a ligand can lead to a variety of cellular activities. Depends on the ligand and the cell. The binding of a ligand to some receptors neutralizes the ligand and blocks immune response.

- Preformed receptors
  - Pattern recognition receptors (PRRs)/ Toll-like receptors (TLRs)
  - Killer activation receptors / Killer inhibition receptors
  - Complement receptors
  - Fc receptors
  - Cytokine receptors and cytokine antagonists
  - Integrins
  - Chemokine receptors

- Somatically generated receptors (Unique to adaptive immune cells)
  - B cell receptors (BCR; Antibodies)
  - T cell receptors (TCR)

Preformed receptors:

Innate and adaptive immune system use sets of highly conserved receptors that recognize conserved materials such as cytokines, pathogen parts, antibody regions, carbohydrates or protein domains.

Pattern recognition receptors (PRRs):

Receptors of the immune system that recognize structural motifs of pathogen ligands referred to as PAMPS

Pathogen-Associated Molecular Patterns (PAMPS): Patterns of molecules shared by microbes.

PRR binding to PAMPs triggers various inflammatory immune response intended to destroy the pathogen.

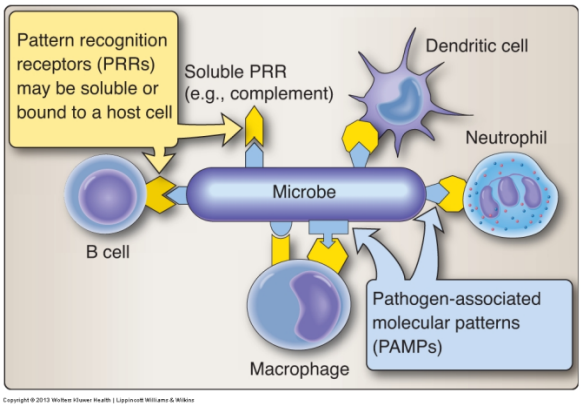


Figure 2.5. Pattern recognition receptors detect and bind PAMPS

Danger associated molecular Patterns (DAMPs):

Endogenous molecules when released from damaged human cells can be recognized and activate the innate immune system.

Also referred to as *"Alarmins"*

Described DAMPs are materials that should not be free of the cell.

Nuclear proteins: HBMGB1, DNA.

Cytosolic proteins: ATP, Hyaluronic acid.

Groups of PRRs:

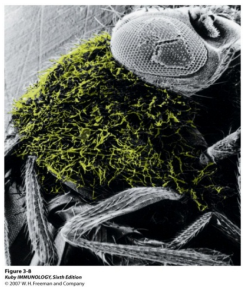
Membrane-bounds PRRs

KIR and **KARs** on NK cells

Toll-Like Receptors (TLRs): Class of membrane bound proteins that recognize structurally conserved molecules derived from microbes (PAMPs)

- may also recognize DAMPs

The gene for Toll was first found in Drosophila, where it directs dorsal-ventral axis formation ("weird")



Lemaitre et al, Cell 1996

Table 2.2
TOLL-LIKE RECEPTORS (TLRs)

TLR	Expressed on	Recognizes and Binds	Found on
TLR1	Monocytes/macrophages Dendritic cell subset B lymphocytes	Multiple tri-acyl lipopeptides	Bacteria
TLR2	Monocytes/macrophages Subset of dendritic cells Mast cells	Multiple glycolipids Multiple lipopeptides Multiple lipoproteins Lipoteichoic acid Peptidoglycan HSP70 Zymosan Numerous other molecules	Bacteria Bacteria Bacteria Bacteria Gram-positive bacteria Host cells Fungi
TLR3	Dendritic cells B lymphocytes	Viral DNA (double stranded)	Viruses
TLR4	Monocytes/macrophages Dendritic cell subset Mast cells Intestinal epithelium	Lipopolysaccharide Several heat shock proteins Fibrinogen (host cell product) Heparan sulfate fragments Hyaluronic acid fragments Numerous other molecules	Gram-negative bacteria Bacterial and host cells Host cells Host cells Host cells
TLR5	Monocytes/macrophages Dendritic cell subset Intestinal epithelium	Flagellin	Bacteria
TLR6	Monocytes/macrophages Mast cells B lymphocytes	Multiple lipopeptides (di-acyl)	Mycoplasma
TLR7	Monocytes/macrophages Dendritic cell subset B lymphocytes	Imidezoquinoline Loxoribine Bropirimine	Synthetic compound Synthetic compound Synthetic compound
TLR8	Monocytes/macrophages Dendritic cell subset Mast cells	Unknown	Unknown
TLR9	Monocytes/macrophages Dendritic cell subset B lymphocytes	CgG motif of bacterial DNA	Bacteria
TLR10	Monocytes/macrophages B lymphocytes	Unknown	Unknown
TLR11	Macrophages and liver Kidney Bladder epithelial cells	Unknown	Uropathogenic bacteria

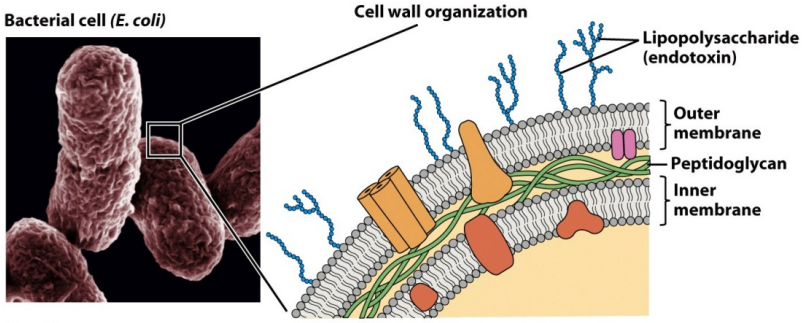


Figure 3-9
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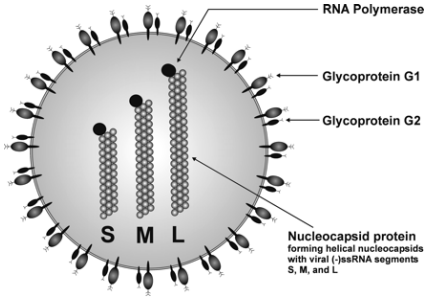


C-type Lectin Receptors (CLRs): comprise a large family of receptors that bind to carbohydrates in a calcium-dependent manner. The lectin activity of these receptors is mediated by conserved carbohydrate-recognition domains (CRDs).

Ex. Dectin-1; Dectin-1 plays an important role in antifungal innate immunity. Dectin-1 is a specific receptor for β-glucans

Mannose Receptor: A type I transmembrane protein, with an extracellular N-terminus and an intracellular C-terminus. The extracellular portion of the receptor is composed of multiple C-type CRDs. The cytoplasmic tail is not capable of signal transduction, but facilitates phagocytes.

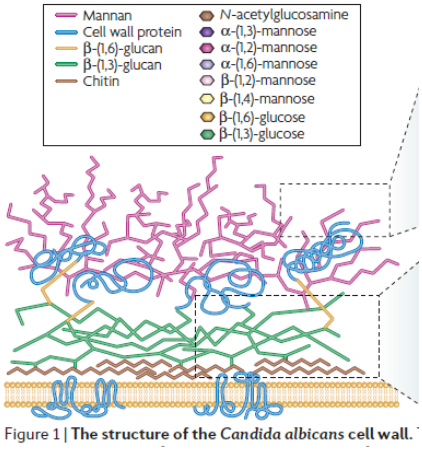


Figure 1 | The structure of the *Candida albicans* cell wall.

Fc Receptors: Many immune cells have Fc receptors on their surface. Can bind to Fc region of antibodies to aid antigen clearance by phagocytosis or stimulate immune cell functions.

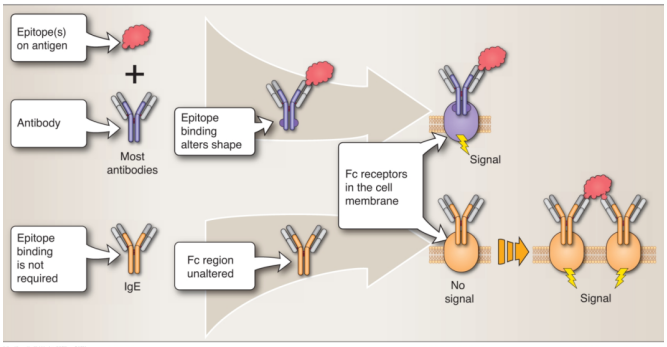
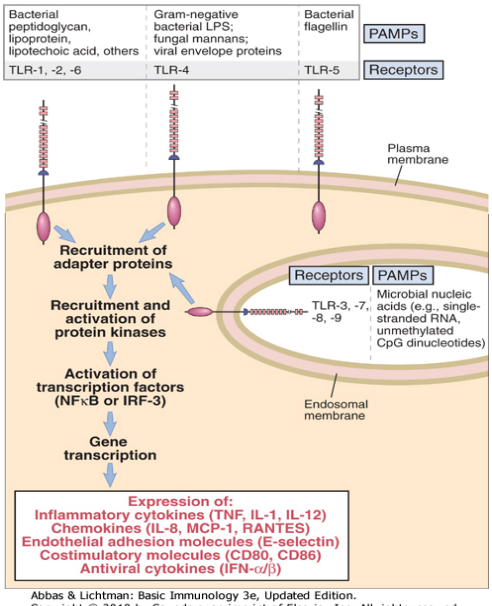


Fig. 2.8 Fc receptors. Like complement receptors, Fc receptors permit phagocytes to identify and ingest microbes and molecules that antibodies have previously tagged for destruction. The receptor for IgE is an exception, however, it binds free IgE and no cellular signaling occurs prior to the binding of antigen to the IgE.

PRR Signaling: Ligation of membrane bound PRRs and TLRs results in activation of transcription factors promote the expression of molecules that support immune responses.



Abbas & Lichtman: Basic Immunology 3e, Updated Edition.
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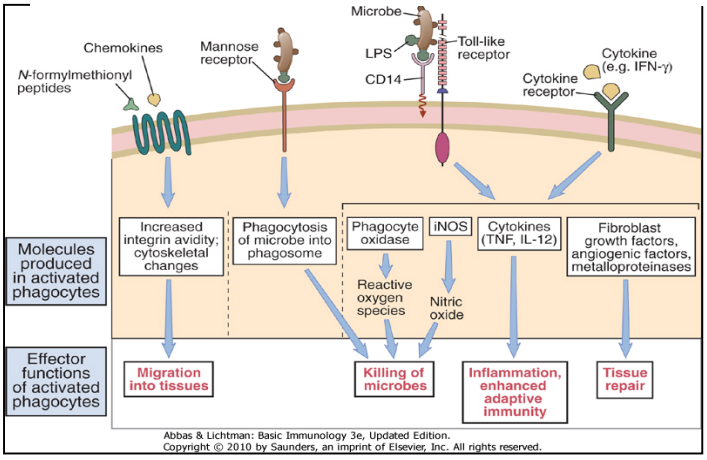
Specificities and functions of Toll-like receptors (TLRs). Different TLRs respond microbes. All of the TLRs activate similar signaling mechanisms, resulting in cellular re innate immunity. IFN, interferon; IL, interleukin; IRF-3, interferon response factor-3; LPS, and RANTES are two chemokines; NFκB, nuclear factor κB; PAMPs, pathogen-assoc TNF, tumor necrosis factor.



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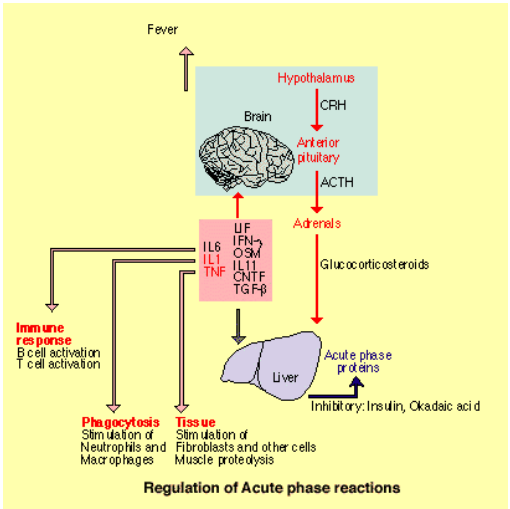
The acute phase response: Complex endocrine, metabolic, neurological, and immunologic changes induced rapidly after injuries or the onset of infections



Triggered by inflammatory cytokines (IL-1, IL-6, TNF)

Characterized by fever and increase in immune cells

Synthesis acute phase proteins including **soluble PRRs**:

Mannose-binding lectin (MBL)
C-reactive protein (CRP)
Complement pathway components

MBL: a major PRR of the innate immune system that binds to a wide range of bacteria, viruses, fungi and protozoa.

CRP: Coats bacterial cell wall components and mediates opsonization (marked for phagocytosis)

Routinely used as a clinical measure ongoing inflammation and tissue damage

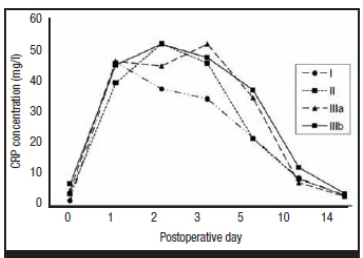


Figure 4: Graph demonstrating CRP levels in patients without postoperative surgical-site infection with the fractures graded according to the Gustilo-Anderson classification

Complement receptors and the complement pathway

Collection of circulating enzymes and proteins (in blood plasma) that "complements" the immune system.

Designated in the order they were discovered (C1-9) and not the order of reactions.

Many are cleaved into large (a) and small parts (b)

Also numerous associated enzymes, proteins, negative regulators.

Function in both the innate (Alternative and MBL pathways) and adaptive (Classical pathway) immune responses.

Example:

Alternative Pathway: C3 is unstable and is continually broken down to C3b. Microbial Ag is coated by C3b, which is activated by C3-convertase and then attract C5 convertase. C5 is converted to C5a that initiates formation of the membrane attack complex (MAC). The MAC consists of C6-C8, with multiple C9 molecules.

The MAC forms a pore in the surfaces of the microbes to which it is attached – membrane integrity is lost – unregulated flow of electrolytes – lytic death of the cell.

C3b also binds to the surface of microbes to opsonize pathogens (mark for phagocytosis)

Activated components of the complement system (C3a, C4a, C5a) are anaphylotoxins that also act as chemoattractants for phagocytes to further inflammatory response

Also associated circulating and cell-surface regulatory proteins keep the complement system in check.

Ex. C3b Inactivator and Beta$_1$H-globulin regulate C3b

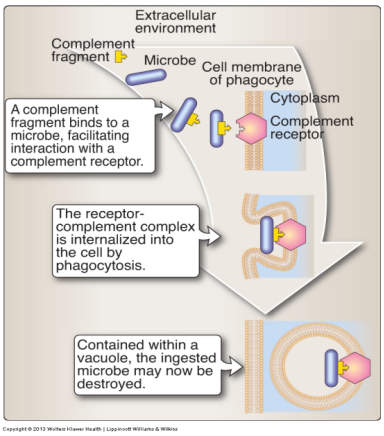


Fig. 2.7 Complement receptors. Binding by complement receptors on phagocytic cells facilitates binding, ingestion, and destruction of microbes.

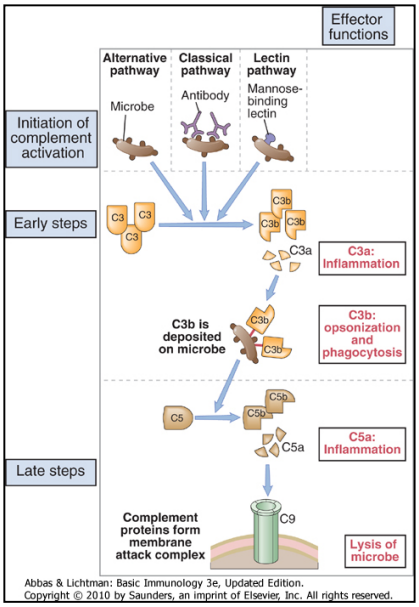


Table 5.1
ANAPHYLOTOXINS IN DECREASING ORDER OF POTENCY

Fragment	Acts on	Actions
C5a	Phagocytic cells	Increased phagocytosis
	Endothelial cells	Phagocyte activation
	Neutrophils	Activation of vascular endothelium
	Mast cells	Attraction/activation of neutrophils
		Mast cell degranulation
C3a	Phagocytic cells	Increased phagocytosis
	Endothelial cells	Phagocyte activation
	Mast cells	Activation of vascular endothelium
		Mast cell degranulation (release of cytoplasmic granules)
C4a	Phagocytic cells	Increased phagocytosis
	Mast cells	Mast cell degranulation

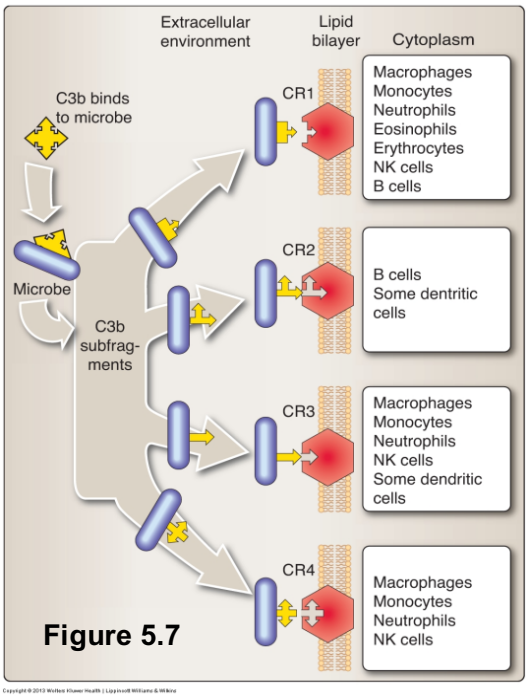


Figure 5.7

Innate Immune Functions - 5

Overview:

To respond quickly, components of the innate immune system are genetically programmed to recognize molecules associated with broad classes of pathogens.

Innate immune responses include the rapid destruction of an infectious organism, activation of phagocytic cells, and the localized protective response known as **inflammation**.

In inflammation, innate (and sometimes adaptive) cells and molecules are stimulated to isolate and destroy infectious agents and trigger tissue repair.

Innate Immune Cell Functions - Pathogen Recognition:

The innate immune system uses a limited number of **pattern recognition receptors (PRRs)** to recognize **pathogen-associated molecular patterns (PAMPS).** Because the host does not produce PAMPs, the innate immune system is able to discriminate between self and nonself.

Innate Immune Cell Functions - Abnormal Self Recognition:

Some viruses cause an infected host cell to reduce its expression of MHC class I molecules that are critical to the proper functioning of the adaptive immune system Similar changes sometimes occur in cells undergoing cancerous transformation. Host cells that become abnormal are detected by the killer activation receptors (KARs) of natural killer (NK) cells

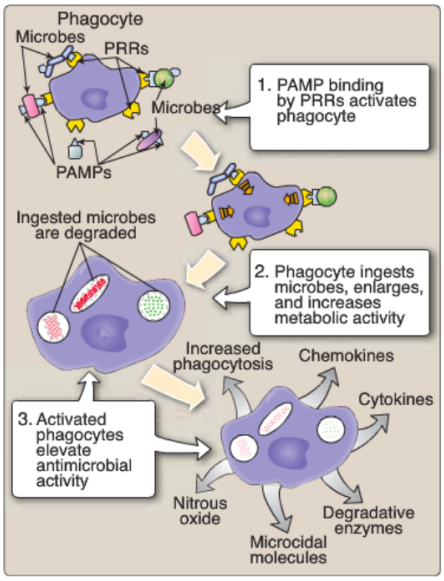


Fig. 5.1 PRR engagement activates phagocytes.

Binding of PAMPs on microbial surfaces by PRRs on the surfaces of phagocytes activates the phagocytes to ingest and degrade the microbes.

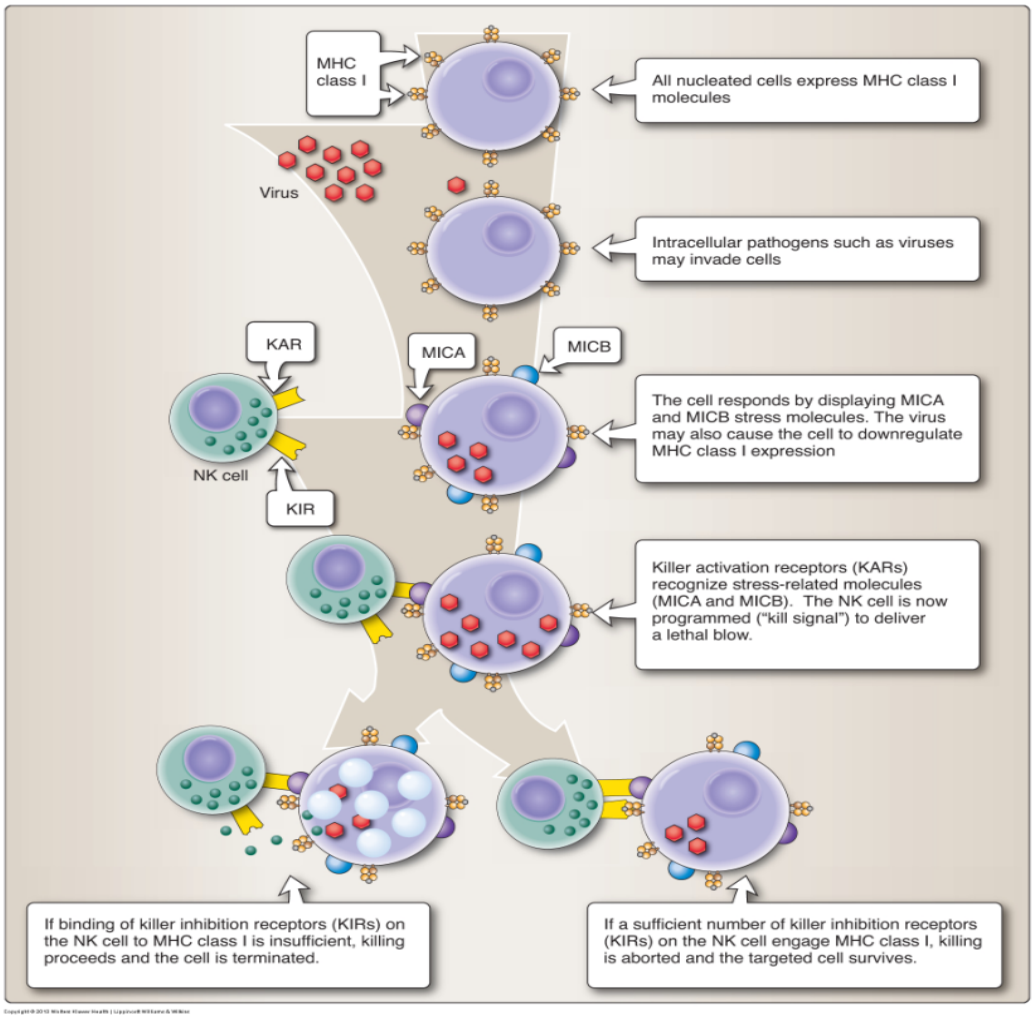


Fig. 5.14. Removal of virally infected cells by NK cells

Innate Soluble Defense Mechanisms

The activated innate immune system employs a variety of soluble molecules as weaponry for protection from viral infections, for lytic destruction of microbes, or for increasing the susceptibility of microbes to ingestion by phagocytic cells. These soluble molecules have potent impact on other immune and non-immune cells.

Microcidal molecules:

Lysozyme: Hydrolyzes the glycosidic linkages in the peptidoglycan of bacterial cell wall.

Myeloperoxidase: Discussed above.

RNases and DNases

Elastases: Breaks down outer membrane protein A of E. coli and gram-negative bacteria

Complement: Discussed above.

Cytokines of innate immune system:

Many are interluekins - (*inter-*) "communication between", and (*-leukin*) "leukocytes".

Group of small proteins that are used to communicate between immune cells and other cells (endothelial; nervous)

In response to pathogens and pathology innate immune cells express cytokines that mediate many of the cellular reactions of innate immunity

Typically secreted but can be surface bound

Short-lived, regulated by inhibitors and decoys

Type I Interferons

Type I **interferons** (IFNs) are produced by a subset of dendritic cells (**IFN**-α), by nonleukocytes such as fibroblasts (**IFN**-β), and by other cells in response to viral infection (Fig. 5.4). IFN-α and IFN-β are rapidly produced, within 5 minutes, by cells when viral PAMPs interact with certain PRRs.

Secreted type I IFNs induce both virally infected and non-infected cells to activate numerous antiviral defenses including RNA-dependent protein kinase (PKR) and apoptotic (programmed cell death) pathways.

In addition, IFN-α and IFN-β influence the activities of macrophages and dendritic cells.

Degrade polyA mRNA, which is made by viruses upon infection

Inhibit initiation of mRNA translation, preventing viral replication

Used as antiviral therapy

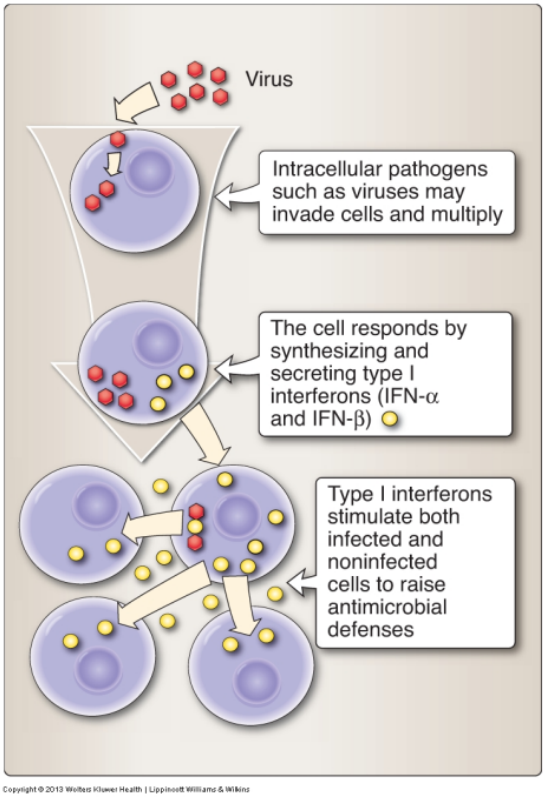
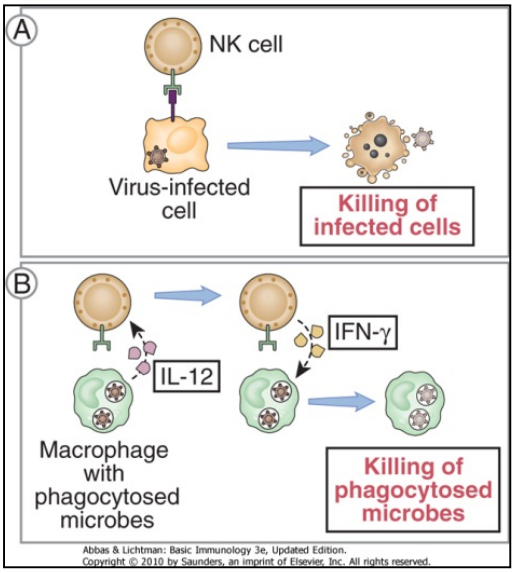


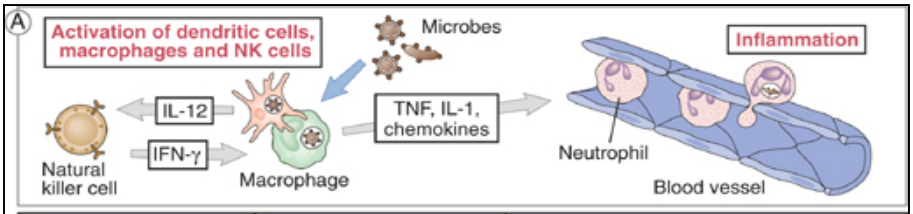
Fig. 5.4.

Cytokines and Chemokines associated with innate Immunity:

Cytokines act in an antigen-nonspecific manner and are involved in a wide array of biologic activities ranging from chemotaxis to activation of specific cells to induction of broad physiologic changes.

Chemokines are a subgroup of cytokines of low molecular weight and particular structural patterns that are involved in the **chemotaxis** (chemical-induced migration) of leukocytes.



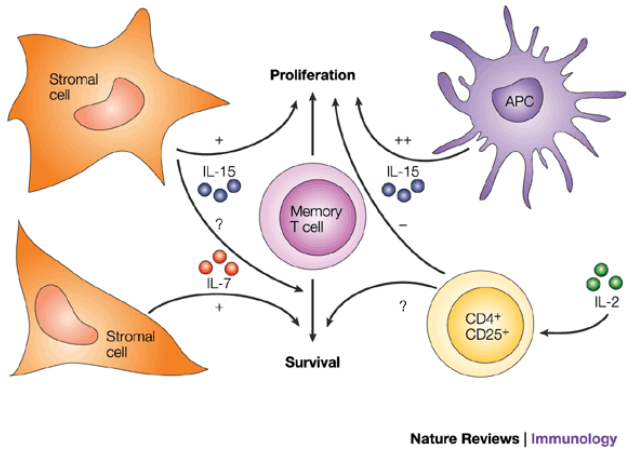


B

Cytokine	Principal cell source(s)	Principal cellular targets and biologic effects
Tumor necrosis factor (TNF)	Macrophages, T cells	Endothelial cells: activation (inflammation, coagulation) Neutrophils: activation Hypothalamus: fever Liver: synthesis of acute phase proteins Muscle, fat: catabolism (cachexia) Many cell types: apoptosis
Interleukin (IL-1)	Macrophages, endothelial cells, some epithelial cells	Endothelial cells: activation (inflammation, coagulation) Hypothalamus: fever Liver: synthesis of acute phase proteins T cells: T_H17 differentiation
Chemokines	Macrophages, dendritic cells, endothelial cells, T lymphocytes, fibroblasts, platelets	Leukocytes: Increased integrin affinity, chemotaxis, activation
Interleukin-12 (IL-12)	Dendritic cells, macrophages,	NK cells and T cells: IFN-γ production, increased cytotoxic activity T cells: T_H1 differentiation
Interferon-γ (IFN-γ)	NK cells, T lymphocytes	Activation of macrophages Stimulation of some antibody responses
Type I IFNs (IFN-α, IFN-β)	IFN-α: dendritic cells, macrophages IFN-β: fibroblasts	All cells: anti-viral state, increased class I MHC expression NK cells: activation
Interleukin-10 (IL-10)	Macrophages, dendritic cells, T cells	Macrophages, dendritic cells: inhibition of IL-12 production, reduced expression of costimulators and class II MHC molecules
Interleukin-6 (IL-6)	Macrophages, endothelial cells, T cells	Liver: synthesis of acute phase proteins B cells: proliferation of antibody-producing cells T cells: T_H17 differentiation
Interleukin-15 (IL-15)	Macrophages, others	NK cells: proliferation T cells: proliferation
Interleukin-18 (IL-18)	Macrophages	NK cells and T cells: IFN-γ production
TGF-β	Many cell types	Inhibition of inflammation T cells: differentiation of T_H17, regulatory T cells

IL-12: Tightly regulated production by activated macrophages and dendritic cells

Crucial to lymphocyte IFN-γ production and effective anti-microbe responses



IL-15: Produced by macrophages, dendritic cells, and stromal cells. Support lymphocyte activation and proliferation (T cell, NK cells, B cells)

Important for generation of T cell memory

Innate Cellular Defense Mechanisms:

Phagocytosis

Phagocytosis is the engulfment and degradation of microbes and other particulate matter by cells such as macrophages, dendritic cells, neutrophils, and even B lymphocytes (prior to their activation).

These cells are part of the body's "cleansing" mechanism. They not only defend the body by ingesting microbes, but also remove cellular debris and particulate matter that arise from normal physiologic functions.

Steps:

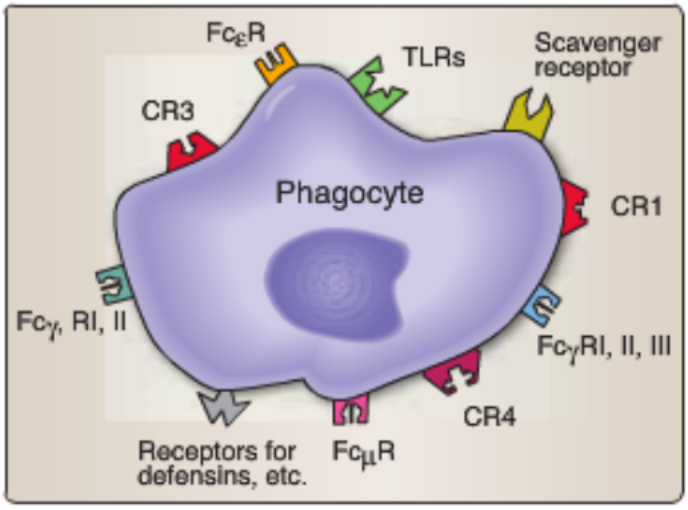


Fig. 5.11 Phagocyte receptors. Phagocytosis is initiated when any of several types of receptors on the phagocyte surface recognize an appropriate molecule that indicates the presence of a foreign cell or molecules.

1. Recognition and attachment of microbes by phagocytes: Phagocytosis is initiated when a phagocyte binds a cell or molecule that has penetrated the body's barrier. The binding occurs at various receptors on the phagocyte surface (Fig. 5.11). These include PRRs (including TLRs) that recognize microbe-related molecules, **complement receptors (CR)** that recognize certain fragments of complement (especially C3b) that adhere to microbial surfaces, Fc receptors that recognize immunoglobulins that have bound to microbial surfaces.

2. Ingestion of microbes and other material: Following attachment to the cell membrane, a microorganism or foreign particle is engulfed by **endocytosis**. Once internalized, the bacteria are trapped within **phagocytic vacuoles (phagosomes)** The attachment and ingestion of microbes trigger changes within the phagocyte. It increases in size, becomes more aggressive in seeking additional microbes to bind and ingest, and elevates production of certain molecules. Some of these molecules contribute to the destruction of the ingested microbes; others act as chemotactic agents and activators for other leukocytes.

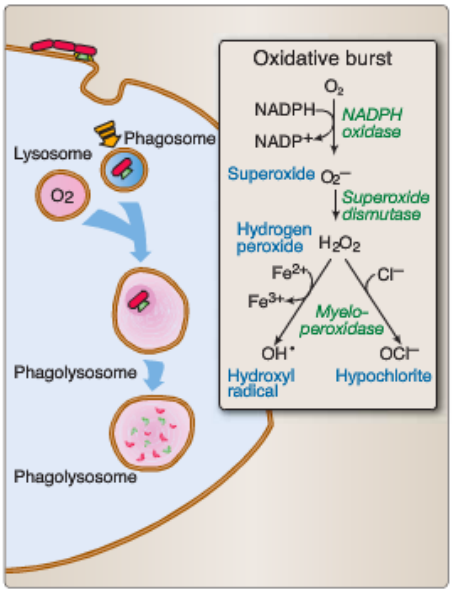


Figure 5.13
Oxidative burst. Phagolysosomes contain enzymes capable of generating free radicals that can efficiently kill microbes.

Inflammation:

Activated innate cells trigger the process of **inflammation**:

The cardinal signs of inflammation are **pain** (dolor), **heat** (calor), **redness** (rubor), **swelling** (tumor), and **loss of function** (function laesa).

Enlarged capillaries that result from vasodilation cause redness (**erythema**) and an increased in tissue temperature.

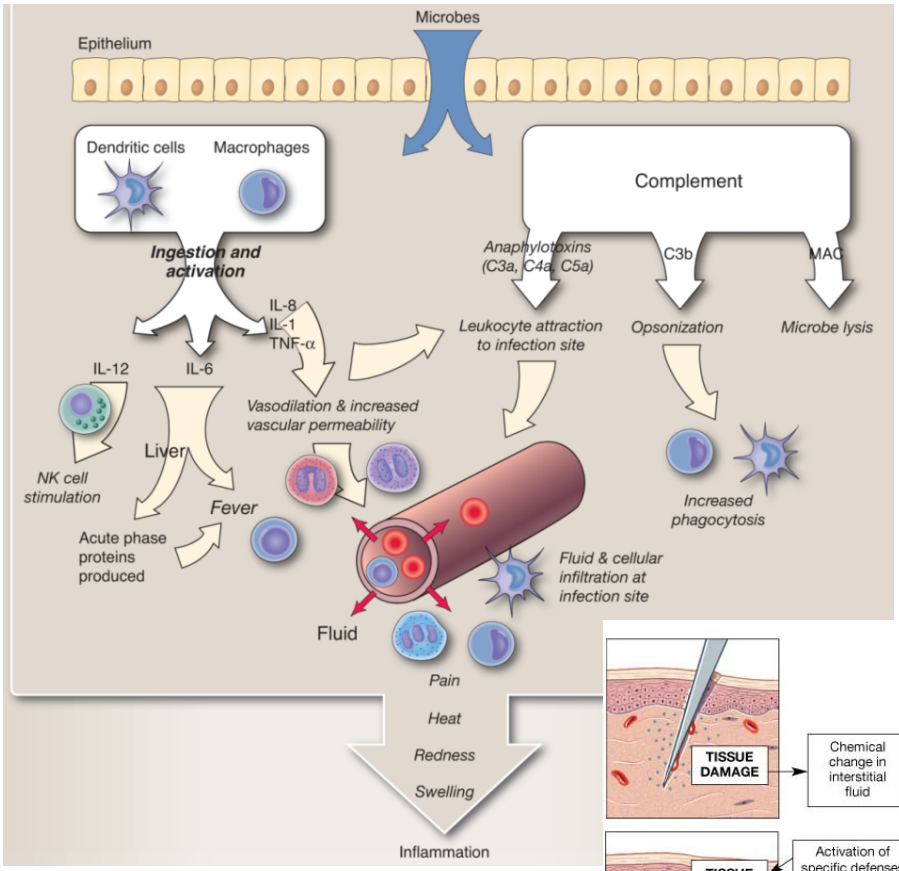
Increased capillary permeability allows for an influx of fluid and cells, contributing to swelling (**edema**).

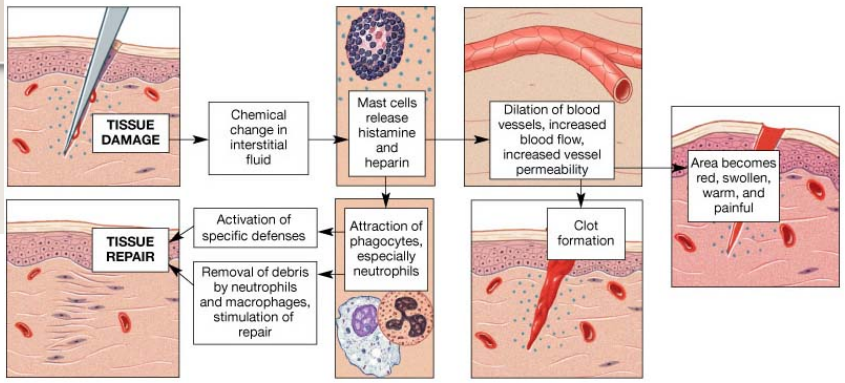
Phagocytic cells attracted to the site release lytic enzymes, damaging healthy cells. An accumulation of dead cells and fluid forms pus, whereas mediators released by phagocytic cells stimulate nerves and cause pain.

The innate immune system contributes to inflammation by activating the alternative and lectin-binding complement pathways, attracting and activating phagocytic cells that secrete cytokines and chemokines, activating NK cells, altering vascular permeability, and increasing body temperature

Local vs. systemic responses by innate immune system

- Local inflammatory response can be mild, short-lived
  - May not even involve adaptive immune response
  - If infection is not contained, leads to systemic response, with more extensive consequences





Lecture 1 - Summary Sheet

The immune system is the collection of molecules, cells, tissues and organ, which function in a coordinated fashion to protect and defend the host against invading pathogens and cancer.

Unlike invertebrates, vertebrates have both a primitive (innate) and a highly-evolved (adaptive) immune system.

The cells important in an immune response originate from a hematopoietic stem cell and terminally differentiate into cells with specific characteristics and functions.

Myeloid cells form the innate immune system, which use conserved receptors and effector mechanism to rapidly respond to pathogens.

The lymphocytes (T cells and B cells) are the pivotal cells found in the blood, tissues, and lymphoid organs, which are responsible for the adaptive immune response. They exhibit diversity, specificity, memory, and self/non-self recognition.

B cells mature in the bone marrow and contain membrane immunoglobulins on their surface, which bind soluble antigens. Following interaction with antigen, B cells can divide and differentiate into plasma cells or memory B cells.

T cells mature in the thymus and contain T cell receptors (TCRs) on their surface, which recognize antigen in the context of MHC. The T cells can differentiate into T effector or T memory cells. Three types of T cells exist based on function: T helper cells, T cytotoxic cells, and T suppressor cells.

NK cells are lymphocytes (unlike T cells and B cells), which provide cytotoxic (killing) activity against tumor cells and virally infected cells.

Monocytes and macrophages circulate in the blood and tissues, respectively. Macrophages are important in phagocytosis and in antigen presentation.

Granulocytes (neutrophils, eosinophils, and basophils) are differentiated, based on morphology and cytoplasmic staining. Neutrophils are the first cells to respond during innate immunity. Eosinophils have a role in eliminating parasitic infections. Basophils and mast cells have roles in allergies.

Antigen presenting cells include macrophages, B cells, and dendritic cells. Dendritic cells are the most potent antigen presenting cells.

Innate immunity is our most primitive and ancient defense against pathogens. The cellular components of the innate immune system recognize non-specifically and broadly patterns and classes of molecules on pathogens and eliminate these pathogens primarily by phagocytosis.

Innate immunity offers protection by anatomic, physiologic, phagocytic, and inflammatory barriers.

Inflammation is a protective response that counters pathogens and initiates tissue repair. It is caused by the release of chemical mediators into the tissue by invading pathogens. The resulting tissue damage causes vasodilation, increased capillary permeability and influx of phagocytes. Following elimination of the pathogen and the subsequent diminishing of the inflammatory response, tissue repair is initiated.

Innate immune cells are activated by conserved pathways that activate inflammatory pathways often involving NF-kB, IRFs, and MAP kinases that culminate in the secretion of cytokines that facilitate the functions of adaptive immune cells.