# Innate Immunity (Part 1)

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# Kuby Immunology

## SEVENTH EDITION

## CHAPTER 5

Innate Immunity

**Innate immunity**: most ancient line of defense, some form found in all multi cellular plants and animals.

**Adaptive immunity** : more recent evolutionary and evolved in jawed vertebrates. It complements innate immunity.

# Key Elements of Innate Immunity

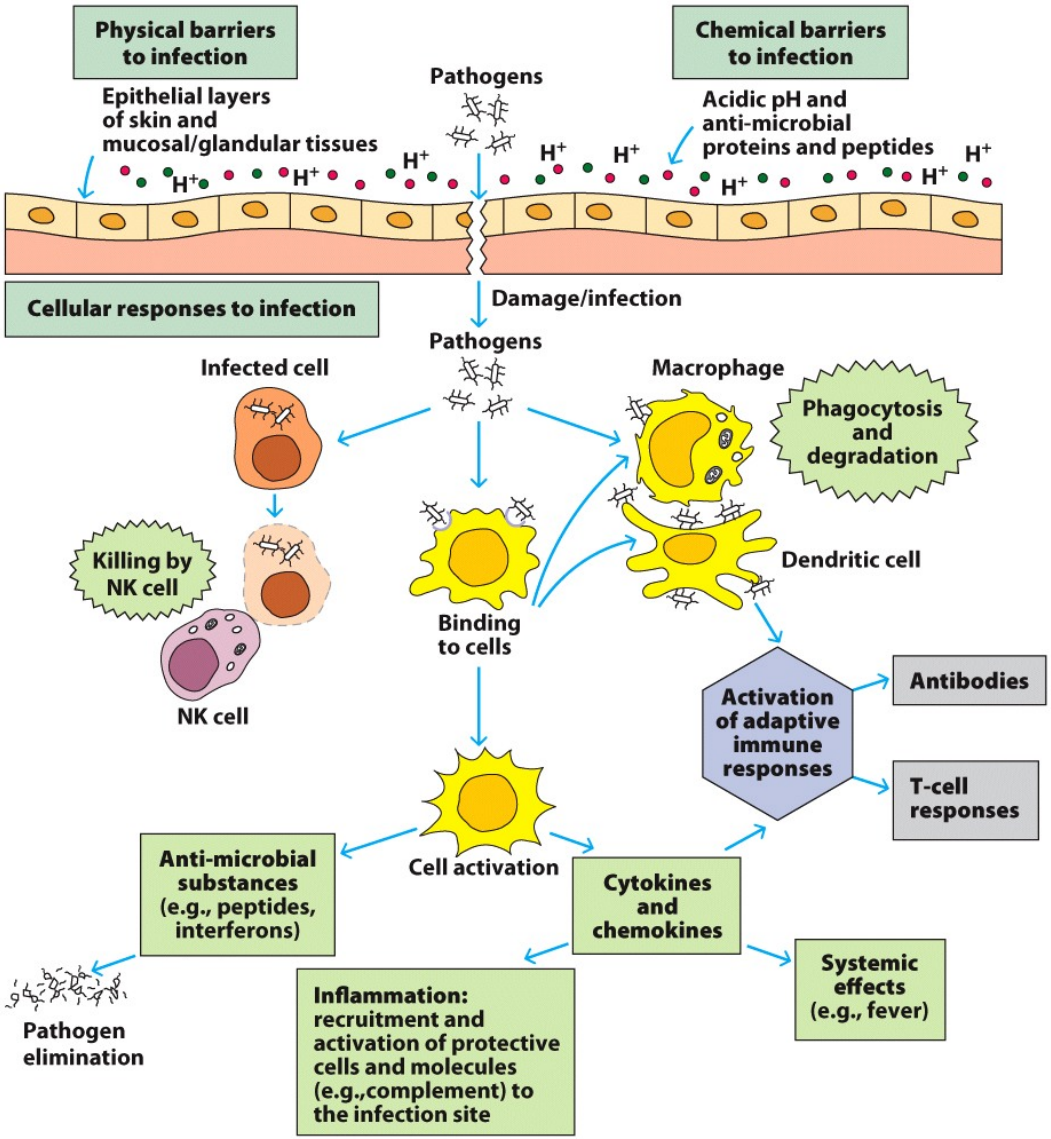


**Figure 5-1**
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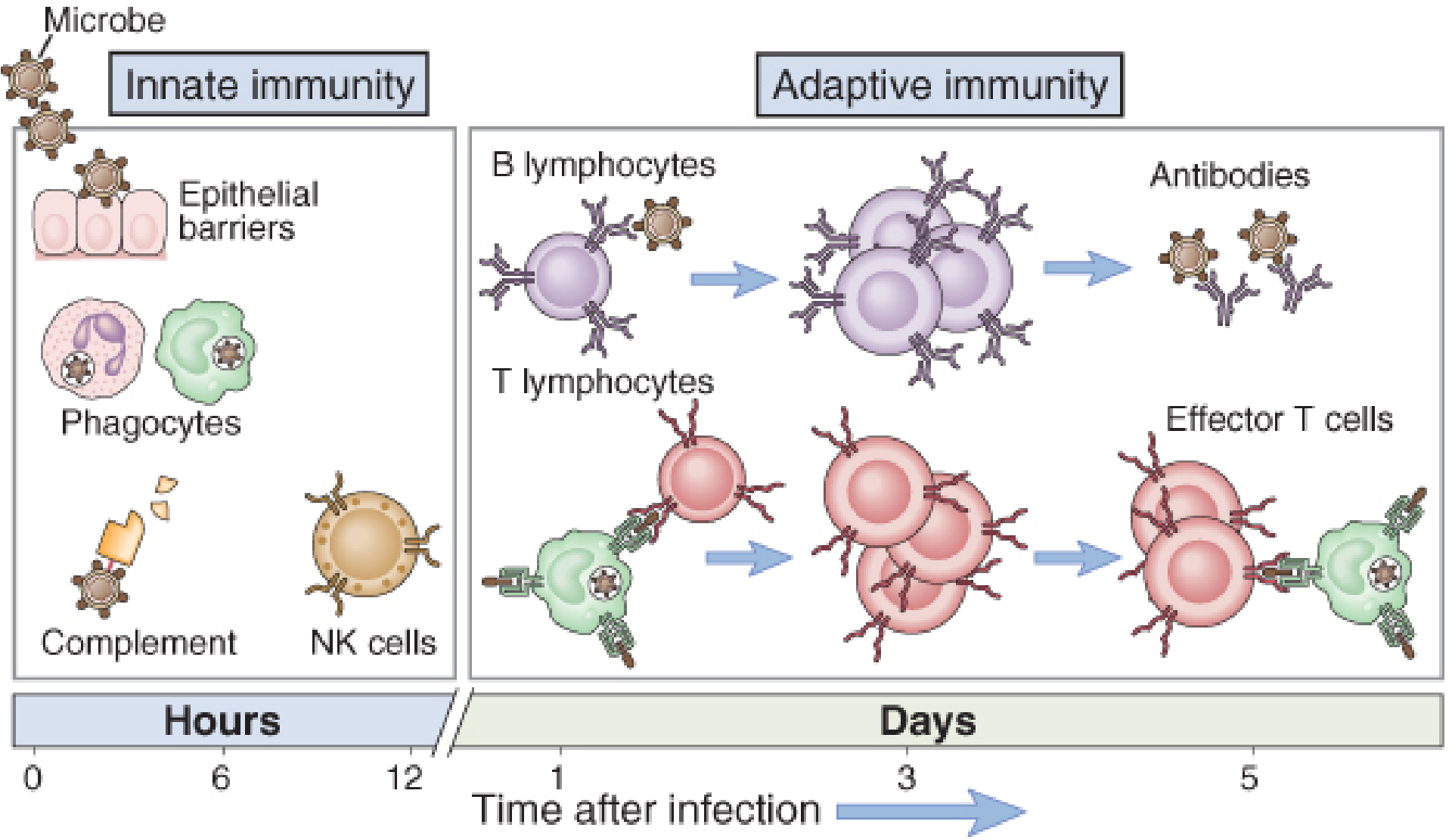

## TABLE 5-1 Innate and adaptive immunity

| Attribute | Innate immunity | Adaptive immunity |
|---|---|---|
| Response time | Minutes/hours | Days |
| Specificity | Specific for molecules and molecular patterns associated with pathogens and molecules produced by dead/damaged cells | Highly specific; discriminates between even minor differences in molecular structure of microbial or nonmicrobial molecules |
| Diversity | A limited number of conserved, germ line–encoded receptors | Highly diverse; a very large number of receptors arising from genetic recombination of receptor genes in each individual |
| Memory responses | Some (observed in invertebrate innate responses and mouse/human NK cells) | Persistent memory, with faster response of greater magnitude on subsequent exposure |
| Self/nonself discrimination | Perfect; no microbe-specific self/nonself patterns in host | Very good; occasional failures of discrimination result in autoimmune disease |
| Soluble components of blood | Many antimicrobial peptides, proteins, and other mediators | Antibodies and cytokines |
| Major cell types | Phagocytes (monocytes, macrophages, neutrophils), natural killer (NK) cells, other leukocytes, epithelial and endothelial cells | T cells, B cells, antigen-presenting cells |

**Table 5-1**
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Innate and adaptive immune systems have co-evolved and show a high degree of interaction and interdependence.

If innate immune response is poor, the adaptive immune response will be feeble. In other words, recognition by the innate sets the stage for an effective immune response.

Innate system includes: physical/anatomical, chemical and cellular barriers.

# Skin and other Epithelial Barriers

| Organ or tissue | Innate mechanisms protecting skin/epithelium |
|---|---|
| Skin | Antimicrobial peptides, fatty acids in sebum |
| Mouth and upper alimentary canal | Enzymes, antimicrobial peptides, and sweeping of surface by directional flow of fluid toward stomach |
| Stomach | Low pH, digestive enzymes, antimicrobial peptides, fluid flow toward intestine |
| Small intestine | Digestive enzymes, antimicrobial peptides, fluid flow to large intestine |
| Large intestine | Normal intestinal flora compete with invading microbes, fluid/feces expelled from rectum |
| Airway and lungs | Cilia sweep mucus outward, coughing, sneezing expel mucus, macrophages in alveoli of lungs |
| Urogenital tract | Flushing by urine, aggregation by urinary mucins; low pH, anti-microbial peptides, proteins in vaginal secretions |
| Salivary, lacrimal, and mammary glands | Flushing by secretions; anti-microbial peptides and proteins in vaginal secretions |

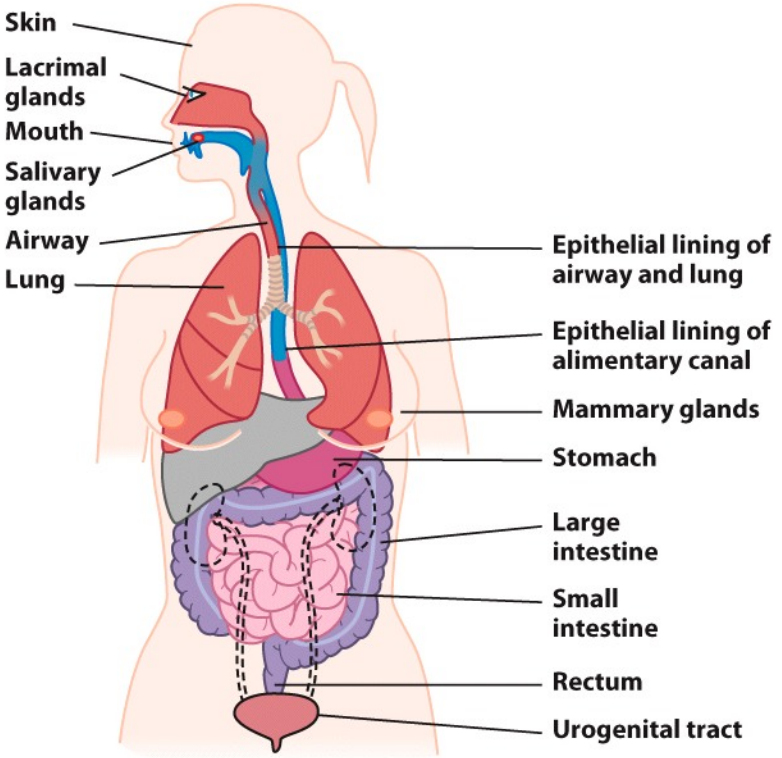


**Figure 5-2**
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Despite these surface barriers and molecules some pathogens have evolved ways to evade immune defenses.

Via fimbriae or pili (made up of pilin protein) the bacteria interact with glycoproteins or glyolipids only expressed by epithelial cells of the mucous membrane of particular organs.

eg. Influenza virus attaches firmly to respiratory tract cells expressing sialic acid residues of glycosylated receptor proteins via its hemagglutinin; *N. gonorrhorae* attaches to urogenital tract via its pili but also OPA protein which helps the bacteria to adhere within colonies but also adhere to host cells especially those that express CEA.

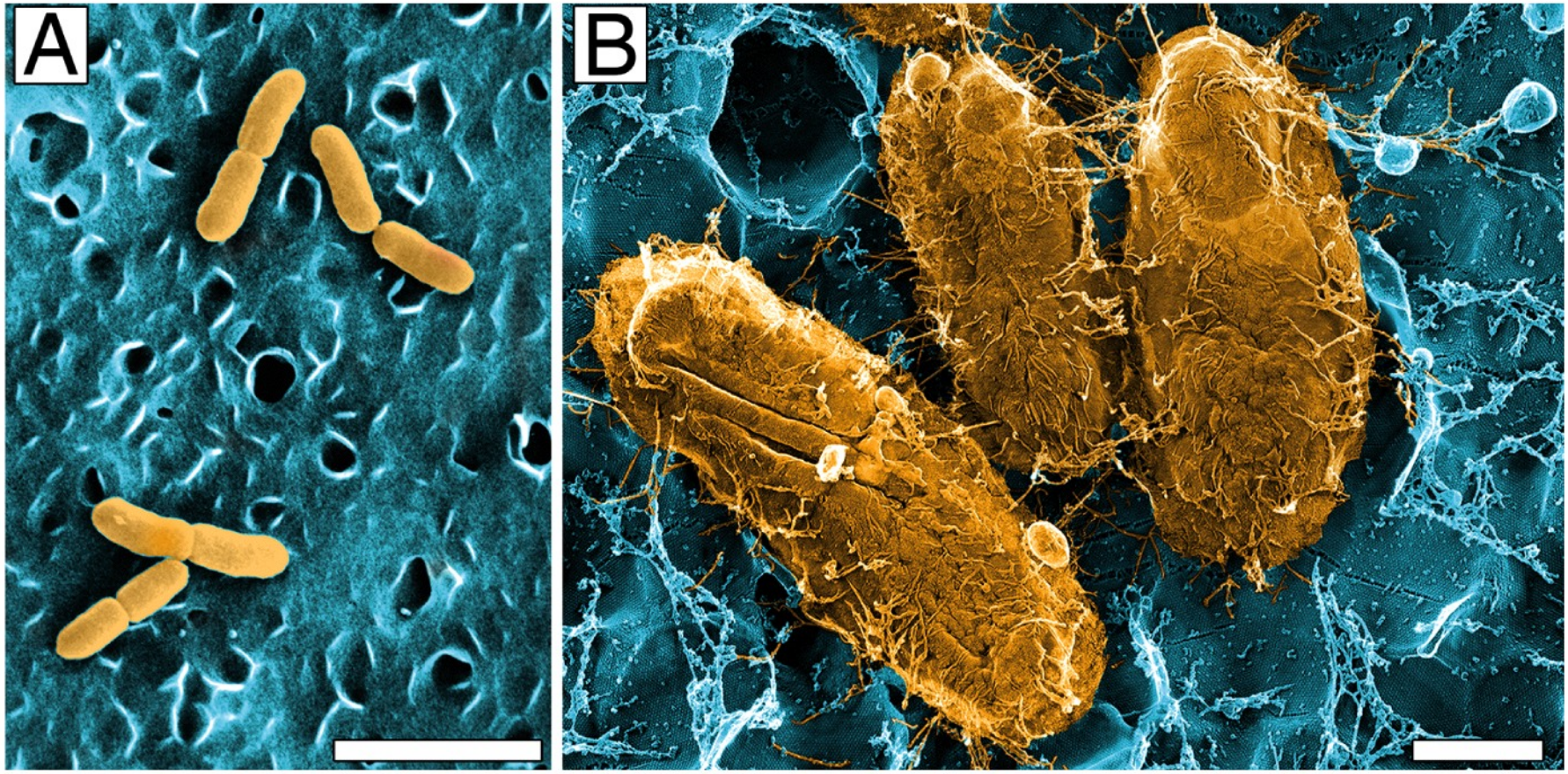


**Figure 5-3**
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# Effectors of Innate Responses to infection

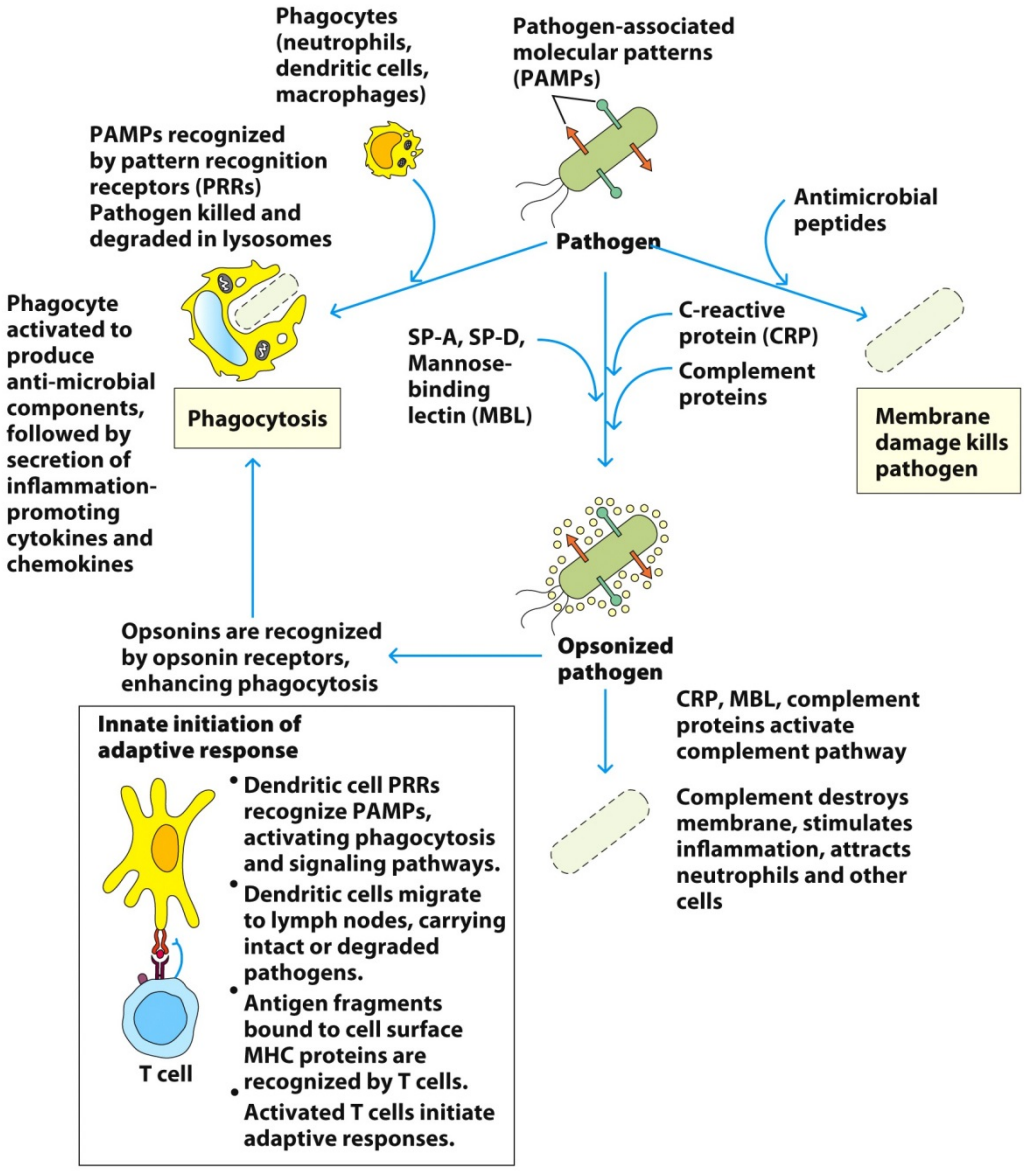


**Figure 5-6**
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The immune system:

1) Senses/detects the presence of a pathogen

2) Mounts a response

**Sensors:** Soluble or membrane bound molecules (receptors) that recognize *molecular patterns* or motifs absent in the host but present in the pathogen.

Pattern Recognition Receptors (**PRRs)** on host cell recognize Pathogen Associated Molecular Patterns ( **PAMPs**).

PAMPs: combination of sugars, lipoproteins and some nucleic acid motifs.

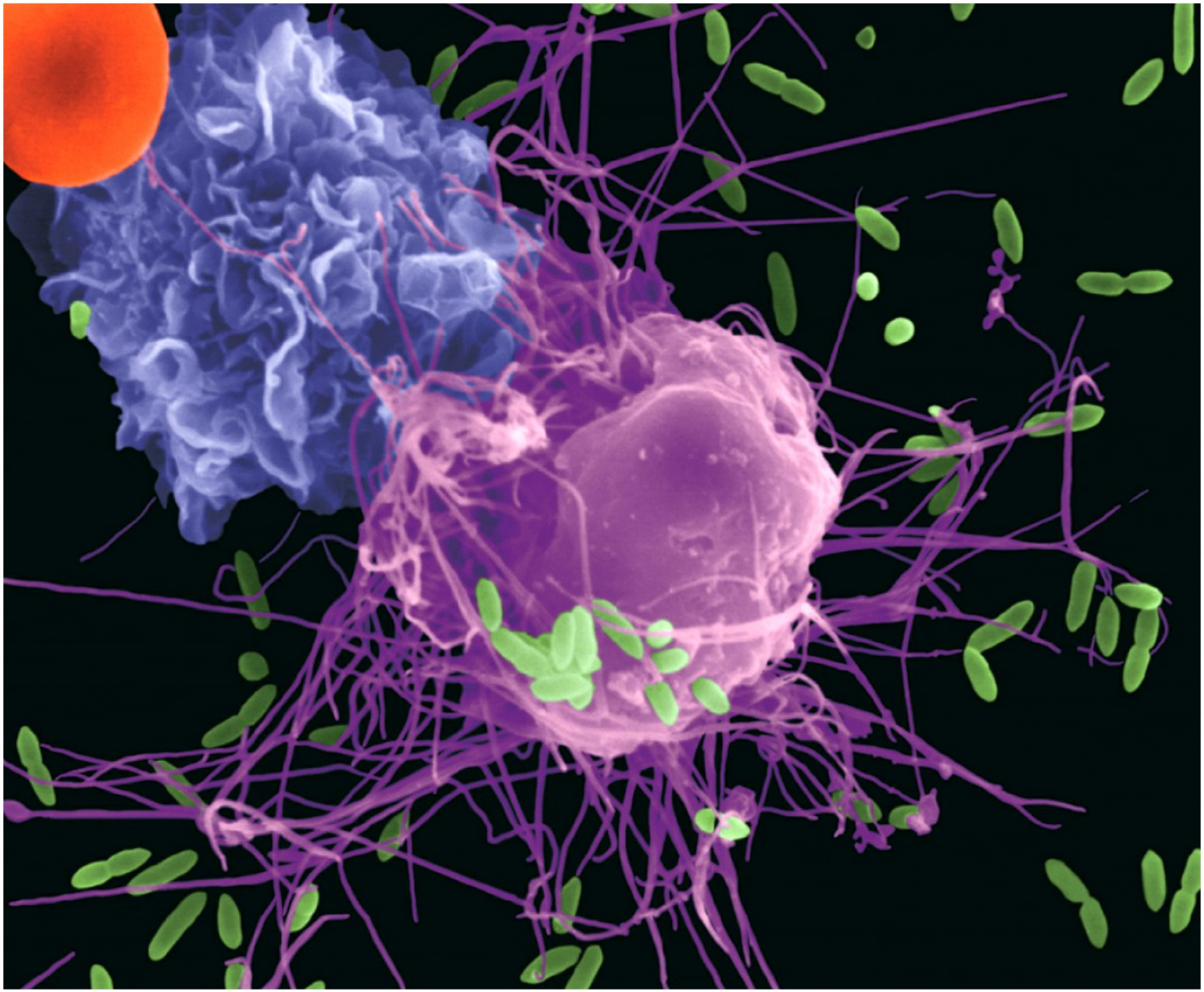

**Figure 3-6a**
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# Steps in the phagocytosis of a bacterium

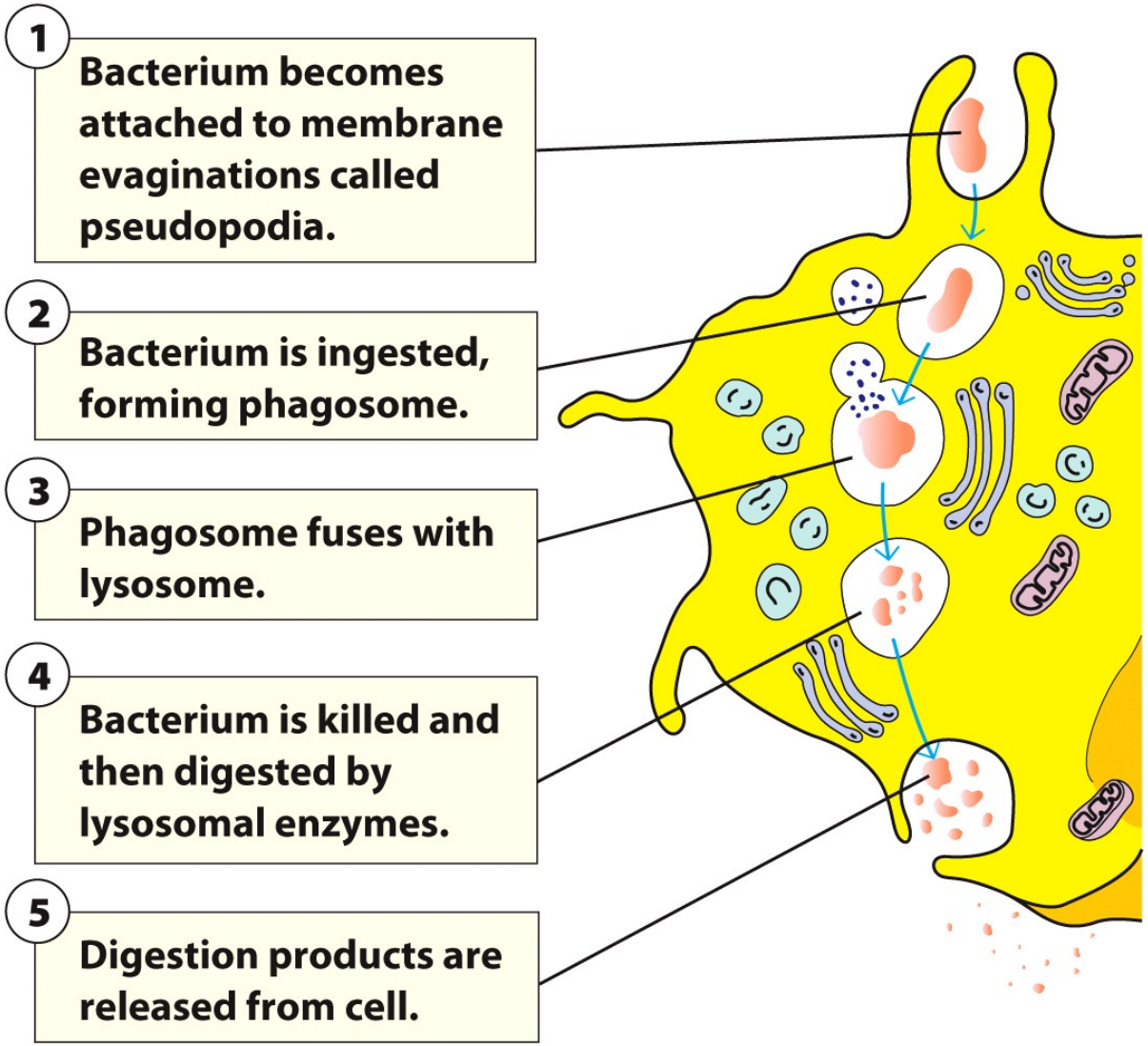


**Figure 5-5b**
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The activation of phagocytosis can also occur indirectly by the phagocyte recognizing soluble proteins (called opsonins) that have bound to the microbes surface thus enhancing phagocytosis.

This process is called **opsonization** (to make tasty).

Once opsonins are bound to the surface of the microbe the are recognized by opsonin receptors on the phagocyte activating phagocytosis.

## TABLE 5-3 Human receptors that trigger phagocytosis

| Receptor type on phagocytes | Examples | Ligands |
|---|---|---|
| **Pattern recognition receptors** | | **Microbial ligands (found on microbes)** |
| C-type lectin receptors (CLRs) | Mannose receptor | Mannans (bacteria, fungi, parasites) |
| | Dectin 1 | β-glucans (fungi, some bacteria) |
| | DC-SIGN | Mannans (bacteria, fungi, parasites) |
| Scavenger receptors | SR-A | Lipopolysaccharide (LPS), lipoteichoic acid (LTA) (bacteria) |
| | SR-B | LTA, lipopeptides, diacylglycerides (bacteria), β-glucans (fungi) |
| **Opsonin receptors** | | **Microbe-binding opsonins (soluble; bind to microbes)** |
| Collagen-domain receptor | CD91/calreticulin | Collectins SP-A, SP-D, MBL; L-ficolin; C1q |
| Complement receptors | CR1, CR3, CR4, CRIg, C1qRp | Complement components and fragments* |
| Immunoglobulin Fc receptors | FcαR | Specific IgA antibodies bound to antigen# |
| | FcγRs | Specific IgG antibodies bound to antigen;# |
| | | C-reactive protein |

* See Table 6-3 for specific complement components or fragments that are bound by individual receptors

# Opsonization of antibody-bound antigens is an adaptive immune response clearance mechanism

**Table 5-3**
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# Leukocyte Extravasation

- Rigorously controlled migration of leukocytes from the blood into the tissue.
- Regulated by small molecular mediators, including chemokines and complement proteins, and by cell adhesion molecules.

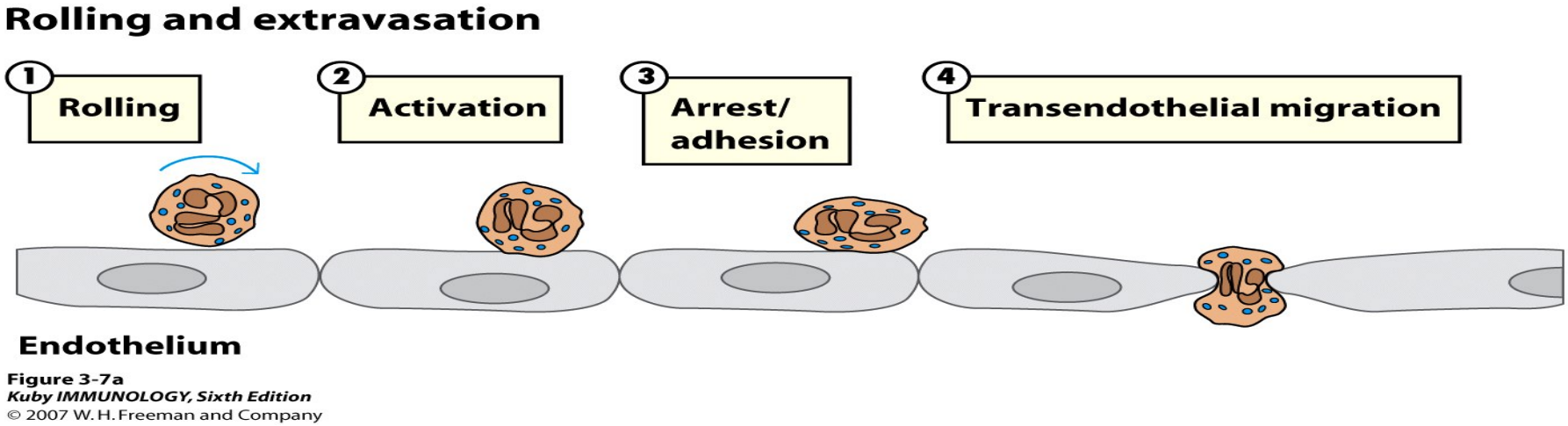


Figure 3-7a
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- This process will be covered in depth in a later chapter.

## TABLE 5-2 Some human antimicrobial proteins and peptides at epithelial surfaces

| Proteins and peptides* | Location | Antimicrobial activities |
|---|---|---|
| Lysozyme | Mucosal/glandular secretions (e.g., tears, saliva, respiratory tract) | Cleaves glycosidic bonds of peptidoglycans in cell walls of bacteria, leading to lysis |
| Lactoferrin | Mucosal/glandular secretions (e.g., milk, intestine mucus, nasal/respiratory and urogenital tracts) | Binds and sequesters iron, limiting growth of bacteria and fungi; disrupts microbial membranes; limits infectivity of some viruses |
| Secretory leukocyte protease inhibitor | Skin, mucosal/glandular secretions (e.g., intestines, respiratory, and urogenital tracts, milk) | Blocks epithelial infection by bacteria, fungi, viruses; antimicrobial |
| S100 proteins, e.g.:<br>- psoriasin<br>- calprotectin | Skin, mucosal/glandular secretions (e.g., tears, saliva/tongue, intestine, nasal/respiratory and urogenital tracts) | - Disrupts membranes, killing cells<br>- Binds and sequesters divalent cations (e.g., manganese and zinc), limiting growth of bacteria and fungi |
| Defensins ($\alpha$ and $\beta$) | Skin, mucosal epithelia (e.g., mouth, intestine, nasal/respiratory tract, urogenital tract) | Disrupt membranes of bacteria, fungi, protozoan parasites, and viruses; additional toxic effects intracellularly; kill cells and disable viruses |
| Cathelicidin (LL37)** | Mucosal epithelia (e.g., respiratory tract, urogenital tract) | Disrupts membranes of bacteria; additional toxic effects intracellularly; kills cells. |
| Surfactant proteins SP-A, SP-D | Secretions of respiratory tract, other mucosal epithelia | Block bacterial surface components; promotes phagocytosis |

*Examples listed in this table are all produced by cells in the epithelia of mucosal and glandular tissues; examples of prominent epithelial sites are listed. Most proteins and peptides are produced constitutively at these sites, but their production can also be increased by microbial or inflammatory stimuli. Many are also produced constitutively in neutrophils and stored in granules. In addition, synthesis and secretion of many of these molecules may be induced by microbial components during innate immune responses by various myeloid leukocyte populations (monocytes, macrophages, dendritic cells, and mast cells).

**While some mammals have multiple cathelicidins, humans have only one.

**Table 5-2**
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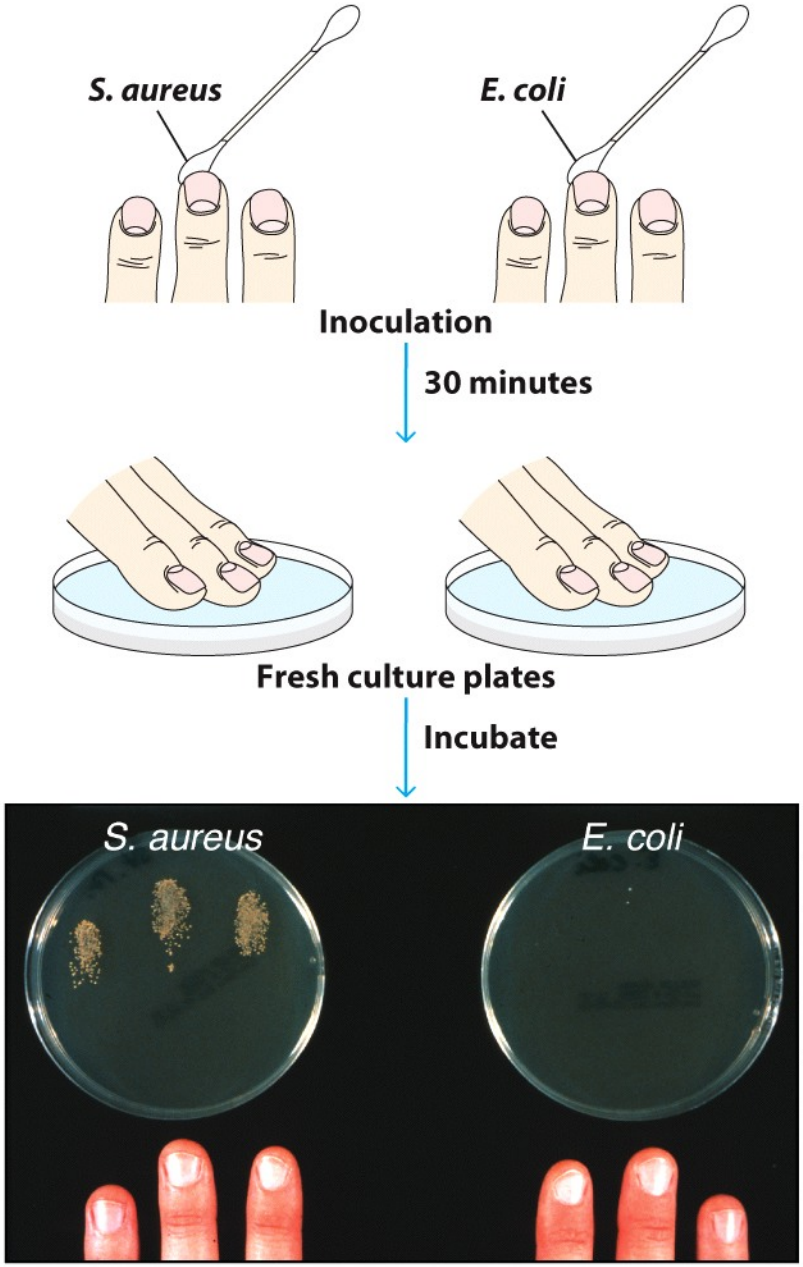


**Figure 5-4**
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Psoriasin (an anti-microbial protein) prevents colonization of skin by *E.coli*.

• Human Defensins: Cationic peptide, 29-35 residues, with 6 invariant cysteines that form disulphide bonds stabilizing the peptide into a relatively rigid three dimensional structure.

• Human defensins kill a variety of bacteria (E.coli, Streptococcus pneumoniae, Pseudomonas aeruginosa, and Hemophilus influenzae), and also attack the envelope of viruses like some herpes viruses and influenza virus.

• Made by paneth cells of the intestine, epithelial cells of the pancreas and kidney.

• Neutrophils are also a rich source of these peptides. Stored in granules where they kill phagocytosed microbes

• Defensins kill rapidly, within minutes.

• Even slowest acting anti-microbial peptide will kill within 90 mins.

# Severe fungal infection in a fruit fly: unable to synthesize antifungal peptide drosomycin

**Figure 3-8**
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# Components of Innate Immunity

Soluble molecules

- Antimicrobial peptides
- Cytokines
- Complement proteins
- Acute phase response proteins

Membrane receptors

- TLR
- NOD
- SR

Cytokines:

Cyto= *cell*

Kinein= *to move*

Cytokines bind to specific receptors on the membrane of target cells triggering signal transduction pathways

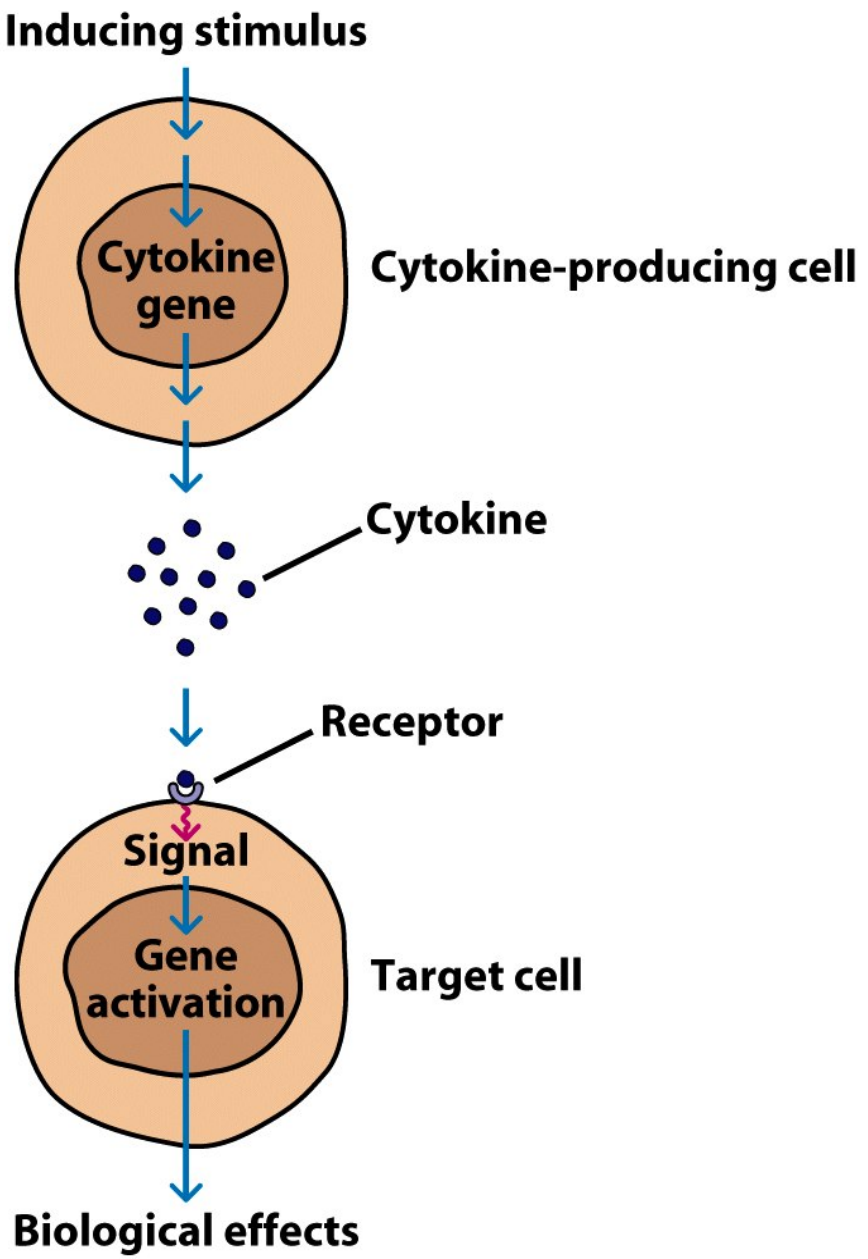


Figure 12-1a
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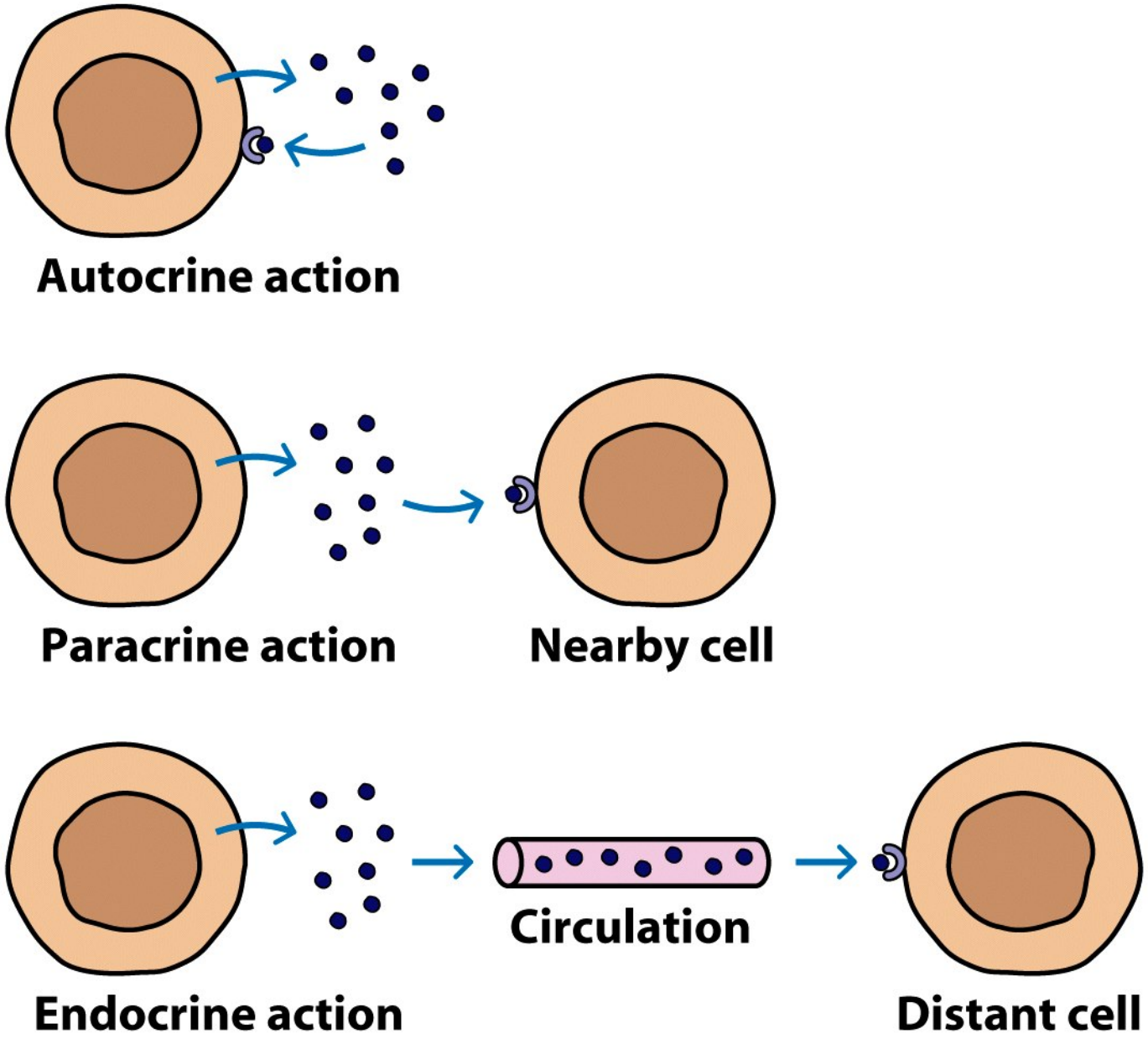


**Figure 12-1b**
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# Cytokines properties: pleiotropy, redundancy, synergy, antagonism and cascade induction

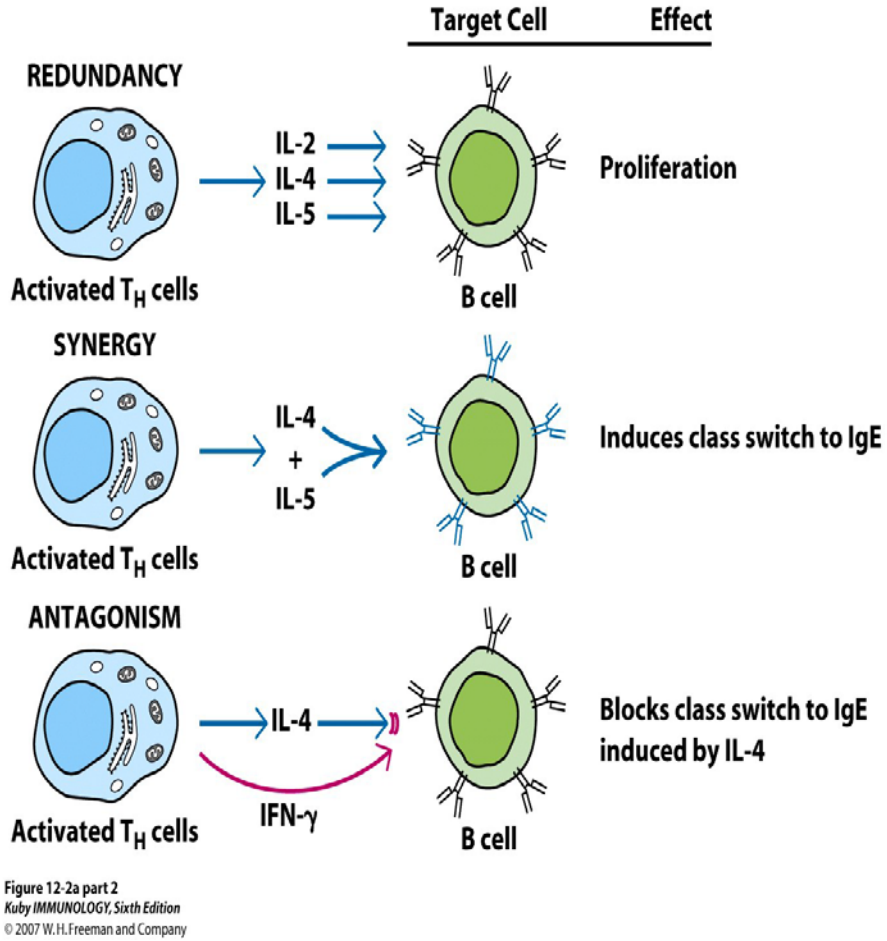


Figure 12-2a part 2
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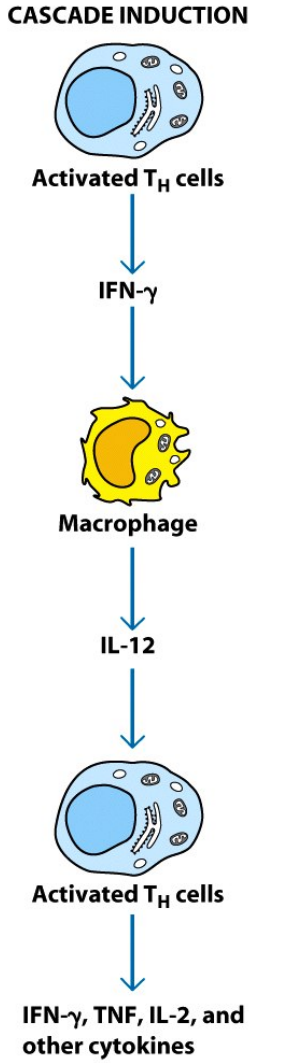


Figure 12-2b
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## TABLE 12-1 Functional groups of selected cytokines*

| Cytokine† | Secreted by‡ | Targets and effects |
|---|---|---|
| | **SOME CYTOKINES OF INNATE IMMUNITY** | |
| Interleukin 1 (IL-1) | Monocytes, macrophages, endothelial cells, epithelial cells | Vasculature (inflammation); hypothalamus (fever); liver (induction of acute phase proteins) |
| Tumor necrosis factor-α (TNF-α) | Macrophages | Vasculature (inflammation); liver (induction of acute phase proteins); loss of muscle, body fat (cachexia); induction of death in many cell types; neutrophil activation |
| Interleukin 12 (IL-12) | Macrophages, dendritic cells | NK cells; influences adaptive immunity (promotes $T_H1$ subset) |
| Interleukin 6 (IL-6) | Macrophages, endothelial cells | Liver (induces acute phase proteins); influences adaptive immunity (proliferation and antibody secretion of B cell lineage) |
| Interferon α (IFN-α) (this is a family of molecules) | Macrophages | Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells |
| Interferon β (IFN-β) | Fibroblasts | Induces an antiviral state in most nucleated cells; increases MHC class I expression; activates NK cells |
| | **SOME CYTOKINES OF ADAPTIVE IMMUNITY** | |
| Interleukin 2 (IL-2) | T cells | T-cell proliferation; can promote AICD. NK cell activation and proliferation; B-cell proliferation |
| Interleukin 4 (IL-4) | $T_H2$ cells, mast cells | Promotes $T_H2$ differentiation; isotype switch to IgE |
| Interleukin 5 (IL-5) | $T_H2$ cells | Eosinophil activation and generation |
| Transforming growth factor β (TGF-β) | T cells, macrophages, other cell types | Inhibits T-cell proliferation and effector functions; inhibits B-cell proliferation; promotes isotype switch to IgA; inhibits macrophages |
| Interferon γ (IFN-γ) | $T_H1$ cells, $CD8^+$ cells, NK cells | Activates macrophages; increases expression MHC class I and class II molecules; increases antigen presentation |

*Many cytokines play roles in more than one functional category.
†Only the major cell types providing cytokines for the indicated activity are listed; other cell types may also have the capacity to synthesize the given cytokine.
‡Also note that activated cells generally secrete greater amounts of cytokine than unactivated cells.

**Table 12-1**
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# Hallmarks of *Acute* Local Inflammation

- Tumor
  - Swelling
- Rubor
  - Redness
- Calor
  - Heat
- Dolar
  - Pain

Described by the Romans
>2000 years ago

- Functio laesa
  - Loss of function

Added by Galen

# Hallmarks of Acute Local Inflammation

- **Swelling**
  - Caused by increased vascular permeability, accumulation of fluid (edema) and extravasation of leukocytes into the area
- **Redness**
  - Caused by increased blood volume (vasodilation) and platelet leaking into the area
- **Heat**
  - Caused by increased blood volume

**Pain and loss of function**

Local inflammatory response is accompanied by a systemic response known as the **Acute Phase response.** This response is marked by the induction of fever, increased synthesis of hormones ACTH and hydrocortisone and increased production of leukocytes and the production of a large number of proteins by the liver called **Acute Phase Proteins**

Acute Phase Proteins made during the acute phase of the response to infection (preceding recovery or death).

eg. C-reactive protein levels increase 1000 fold during an acute phase response.

complement components (C3, factor B, factor D and properdin), mannose-binding lectin (MBL binds to mannose residues on the surface of bacteria, fungi and some viruses) are also acute phase proteins.

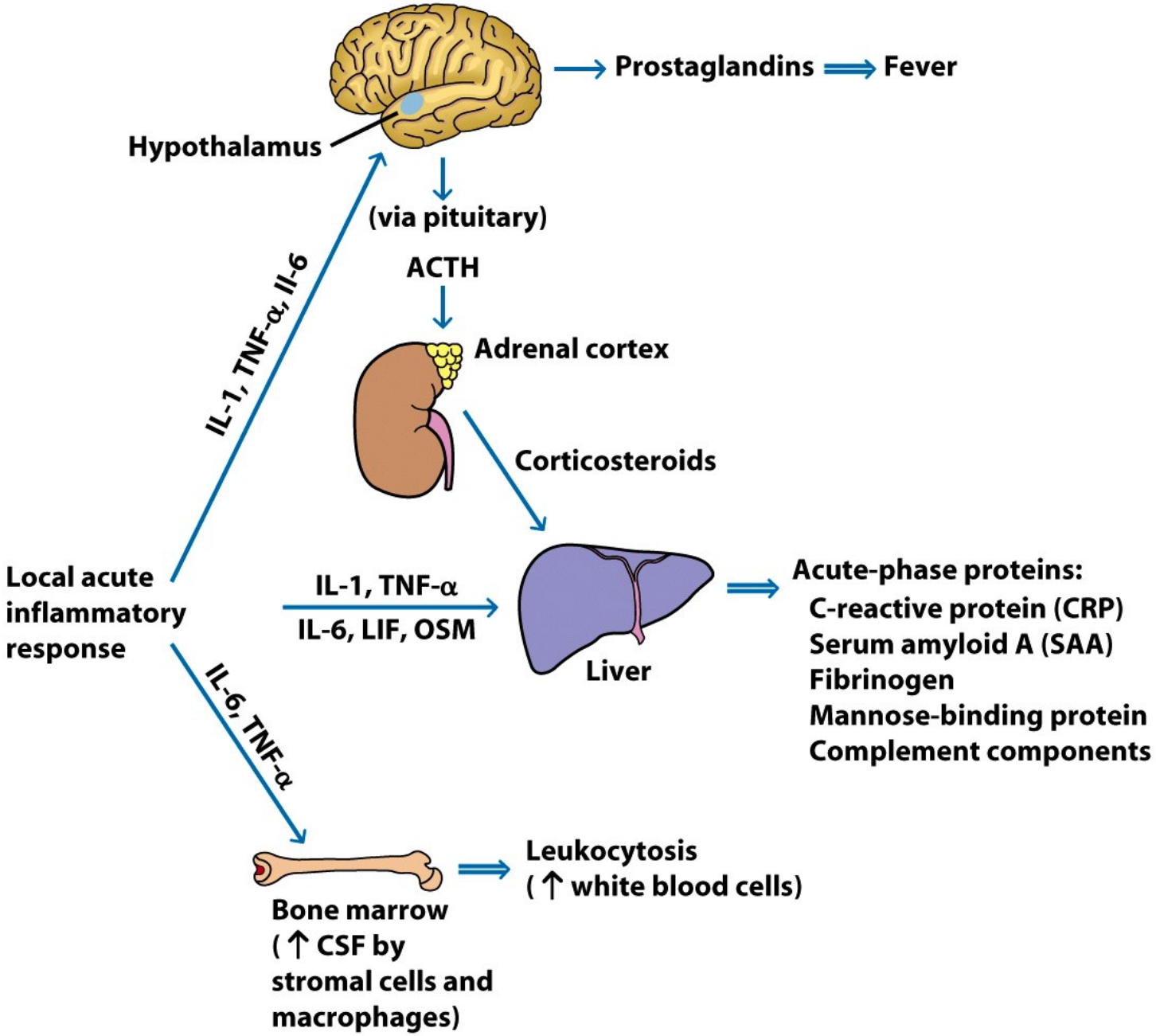


**Figure 13-14**
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A connection between inflammation, the immune system and artery disease was first suggested from studies in animals fed an artherosclerosis-inducing diet; lots of leukocytes found firmly attached to the arterial walls.

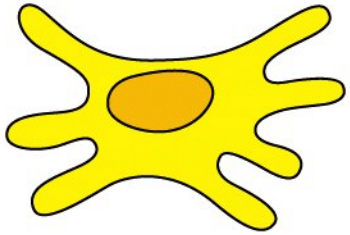
Now it is know that leukocytes are important in the development of artherosclerotic plaques.

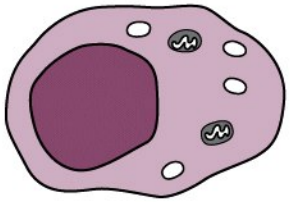
Examination of blood levels of inflammatory markers IL-6, TNF$\alpha$, CRP and the traditional risk markers (cholesterol, LDL, HDL) followed for 6-8 years in men and women.

Of the inflammatory markers only CRP levels were found to be associated with higher risk of coronary disease.

Statins which lower cholesterol levels also lower inflammation. Statins result in lowering levels of CRP.

# Cell Types of Innate Immunity





| Cell type | Neutrophils | Macrophages | Dendritic cells | Natural killer cells |
|---|---|---|---|---|
| Function | Phagocytosis<br>Reactive oxygen and nitrogen species<br>Antimicrobial peptides | Phagocytosis<br>Inflammatory mediators<br>Antigen presentation<br>Reactive oxygen and nitrogen species<br>Cytokines<br>Complement proteins | Antigen presentation<br>Costimulatory signals<br>Reactive oxygen species<br>Interferon<br>Cytokines | Lysis of viral-infected cells<br>Interferon<br>Macrophage activation |

**Figure 3-12**
***Kuby IMMUNOLOGY, Sixth Edition***

# Neutrophils

- First line of defense—first cell type that migrates from the blood to the site of infection.
- Essential for innate immunity against bacteria and fungi.
- Anti-microbial activity:
  - Phagocytosis; direct or by opsonization
  - Oxidative and nonoxidate killing
  - **Oxidative mechanism** : reactive oxygen species (ROS) and reactive nitrogen species (RNS)

    ROS include—superoxide ion $\bullet O_2^-$ , hydrogen peroxide($H_2O_2$), hypochlorus acid (HOCL). ROS generated by the NADPH phagosome oxidase (phox) enzyme complex
  - Reaction of nitric oxide with superoxide generates RNS
  - **Nonoxidative mechanism**: neutrophil granules fuse with phagosome releasing their anti-microbial peptides/ proteins (bactericidial permeability-increasing protein BPI), enzymes (proteases, lysozymes) that help to destroy the pathogen
- Increased expression of inducible nitric oxide synthetase (iNOS)

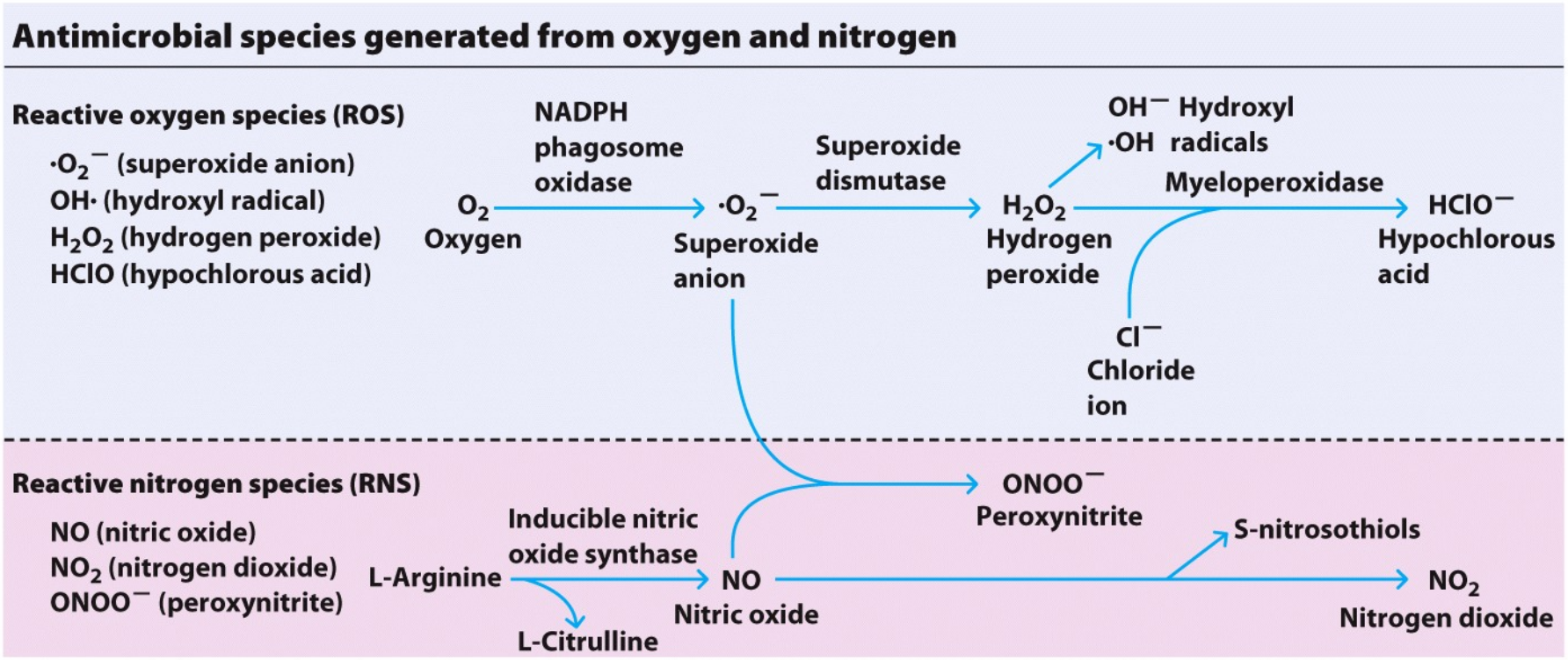



**Figure 5-8**
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# Macrophages

- Activated by TLR binding to their ligand
- **Activated macrophages exibit**:
  - Increased phagocytosis
  - Increased respiratory burst
  - Increased expression of inducible nitric oxide synthetase (iNOS). iNOS oxidizes L-arginine to L-citrulline and nitric oxide (NO)
  - Secrete cytokines IL-1, IL-6, and TNF-$\alpha$
  - Produce complement proteins
  - Express higher levels of MHC class 11 and thereby present antigens to T cells

# NK Cell

- Critical first line of defense against viral infections.
- Can distinguish between an infected and un-infected host cell. By killing the virally infected host cell they eliminate source of additional virus.
- Secrete cytokines IFN$\gamma$ and TNF$\alpha$

  - these cytokines stimulate maturation of dendritic cells.

  - IFN$\gamma$ mediates macrophage activation and regulates $T_H$ cell development.

# Dendritic Cells

- Critical cells for transition from innate to adaptive immunity.
- Binding of TLRs to recognize pathogens

  - this stimulates DC activation and maturation (increased production and surface expression of MHC Class 11 and co-stimulatory molecules).

  -activated DCs migrate to lymphoid tissue and present antigen to $T_H$ and $T_C$ cells
- DCs can generate ROS and RNS.
- Plasmacytoid DCs are potent producers of type 1 interferons that block viral replication.
- Myeloid DCs produce IL12, IL6 and TNF-$\alpha$, all potent inducers of inflammation.

The immune system:

1) Senses/detects the presence of a pathogen

2) Mounts a response

**Sensors:** Soluble or membrane bound molecules (receptors) that recognize *molecular patterns* or motifs absent in the host but present in the pathogen.

Pattern Recognition Receptors (PRRs) on host cell recognize Pathogen Associated Molecular Patterns ( PAMPs).

PAMPs: combination of sugars, lipoproteins and some nucleic acid motifs.

**1996: Jules Hoffman and Bruno Lamaitre** Cell 86: 973 reported that mutations in Toll made the fruit fly highly susceptible to lethal fungal infection.

**1997: Ruslan Medzhitov and Charles Janeway** showed that this pathway conserved between fruit flies and humans. Showed that a human protein ( TLR4) that they identified by homology of its cytoplasmic domain and that of Toll when transfected into a cell line activated the expression of immune response genes.

1998: Bruce Beutler mutant mice (lps ) gene encoded a mutant form of TLR4, resistant to fatal doses of LPS.

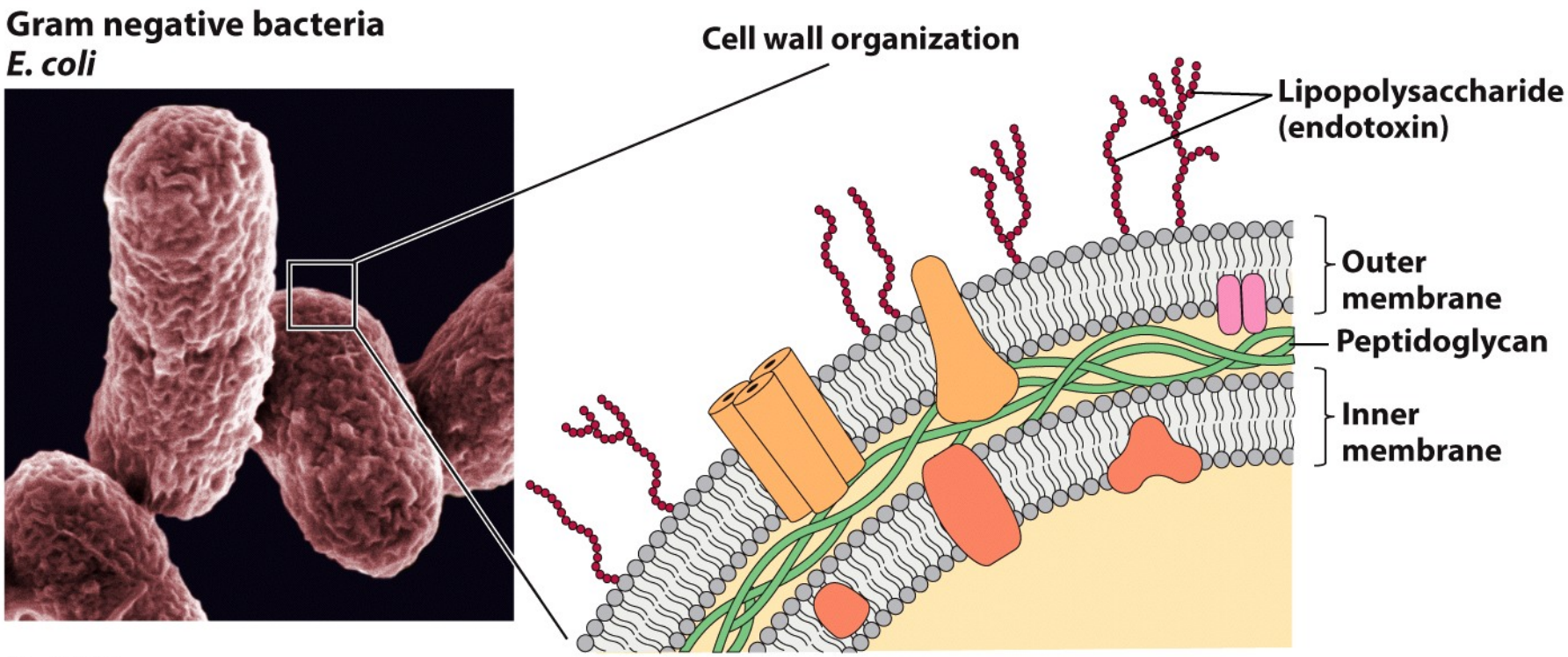


**Figure 5-10a**
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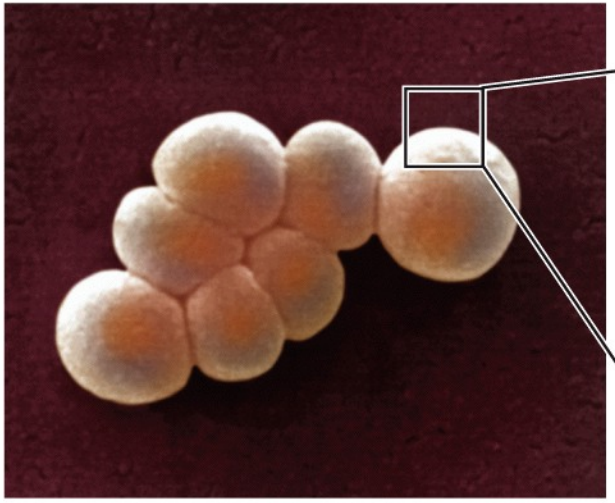
**Gram positive bacteria**
***S. aureus***



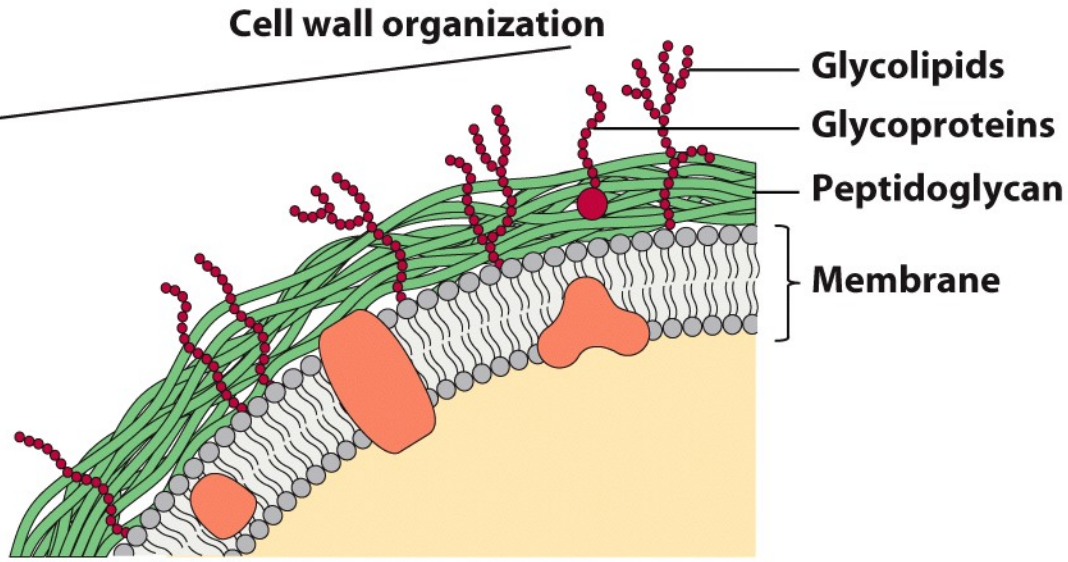



**Figure 5-10b**
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# TLRs

- **Membrane bound receptors**
- **Structurally Conserved**
  - Multiple leucine-rich regions in extra cellular region
  - Contain conserved TIR (Toll/IL1 receptor) in intercellular domain for signaling.

# TLR structure

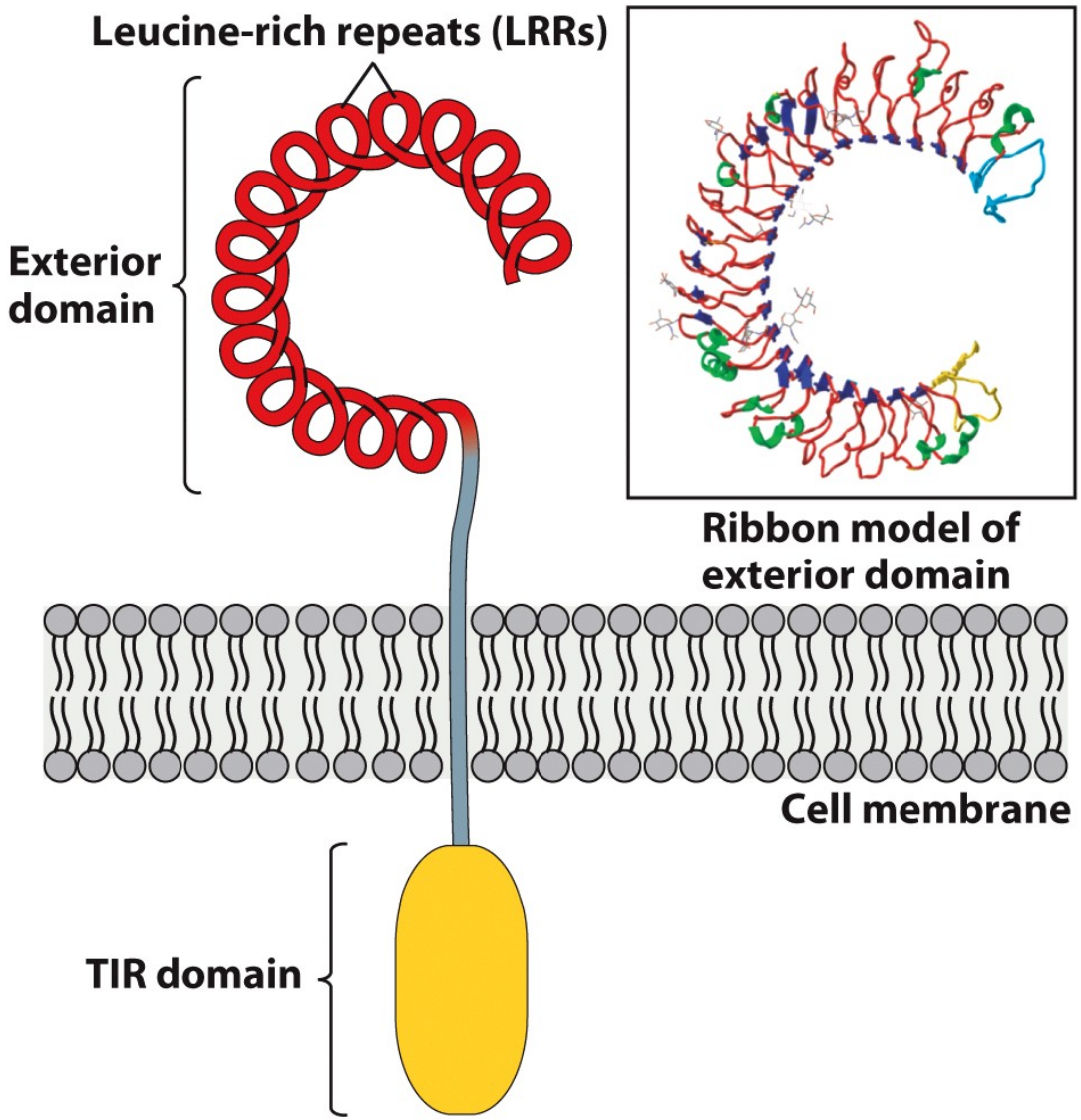


**Figure 5-11a**
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## TABLE 5-4 TLRs and their microbial ligands

| TLRs* | Ligands | Microbes |
|---|---|---|
| TLR1 | Triacyl lipopeptides | Mycobacteria and Gram-negative bacteria |
| TLR2 | Peptidoglycans | Gram-positive bacteria |
| | GPI-linked proteins | Trypanosomes |
| | Lipoproteins | Mycobacteria and other bacteria |
| | Zymosan | Yeasts and other fungi |
| | Phosphatidlyserine | Schistosomes |
| TLR3 | Double-stranded RNA (dsRNA) | Viruses |
| TLR4 | LPS | Gram-negative bacteria |
| | F-protein | Respiratory syncytial virus (RSV) |
| | Mannans | Fungi |
| TLR5 | Flagellin | Bacteria |
| TLR6 | Diacyl lipopolypeptides | Mycobacteria and Gram-positive bacteria |
| | Zymosan | Yeasts and other fungi |
| TLR7 | Single-stranded RNA (ssRNA) | Viruses |
| TLR8 | Single-stranded RNA (ssRNA) | Viruses |
| TLR9 | CpG unmethylated dinucleotides | Bacterial DNA |
| | Dinucleotides | |
| | Herpes virus components | Some herpesviruses |
| | Hemozoin | Malaria parasite heme byproduct |
| TLR10 | Unknown | Unknown |
| TLR11 | Unknown | Uropathogenic bacteria |
| | Profilin | Toxoplasma |
| TLR12 | Unknown | Unknown |
| TLR13 | Unknown | Vesicular stomatitis virus |

*All function as homodimers except TLR1, 2, and 6, which form TLR2/1 and TLR2/6 heterodimers. Ligands indicated for TLR2 bind to both; ligands indicated for TLR1 bind to TLR2/1 dimers, and ligands indicated for TLR6 bind to TLR2/6 dimers.

**Table 5-4**
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**Function as either hetero or homodimers**

- Pairing affects specificity
  - TLR1/2
  - TLR2/6

**Location reflects ligands:**

- TLRs that recognize extracellular ligands are found on the cell surface
- TLRs that recognize intracellular ligands are found on the endosome

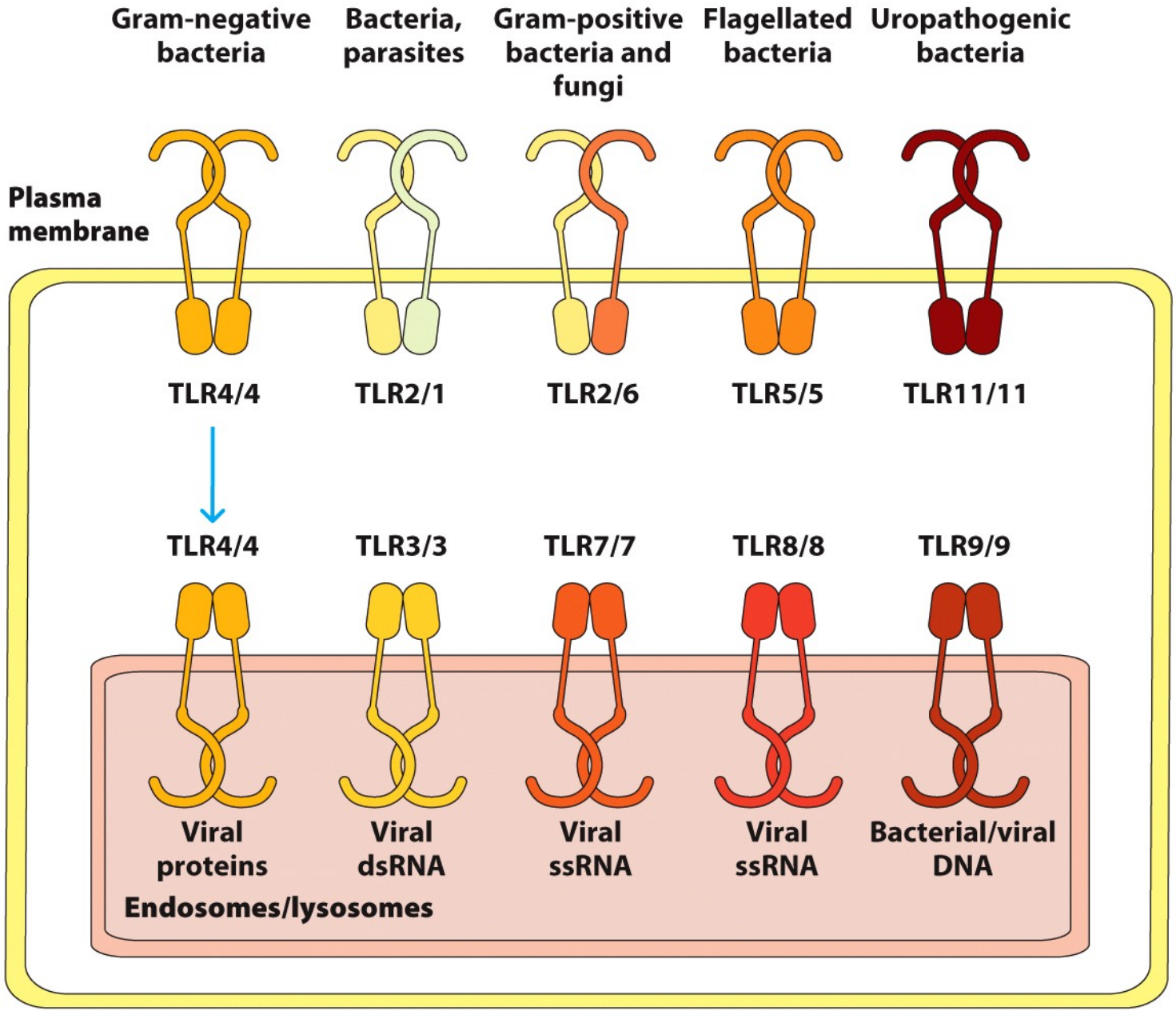


**Figure 5-12**
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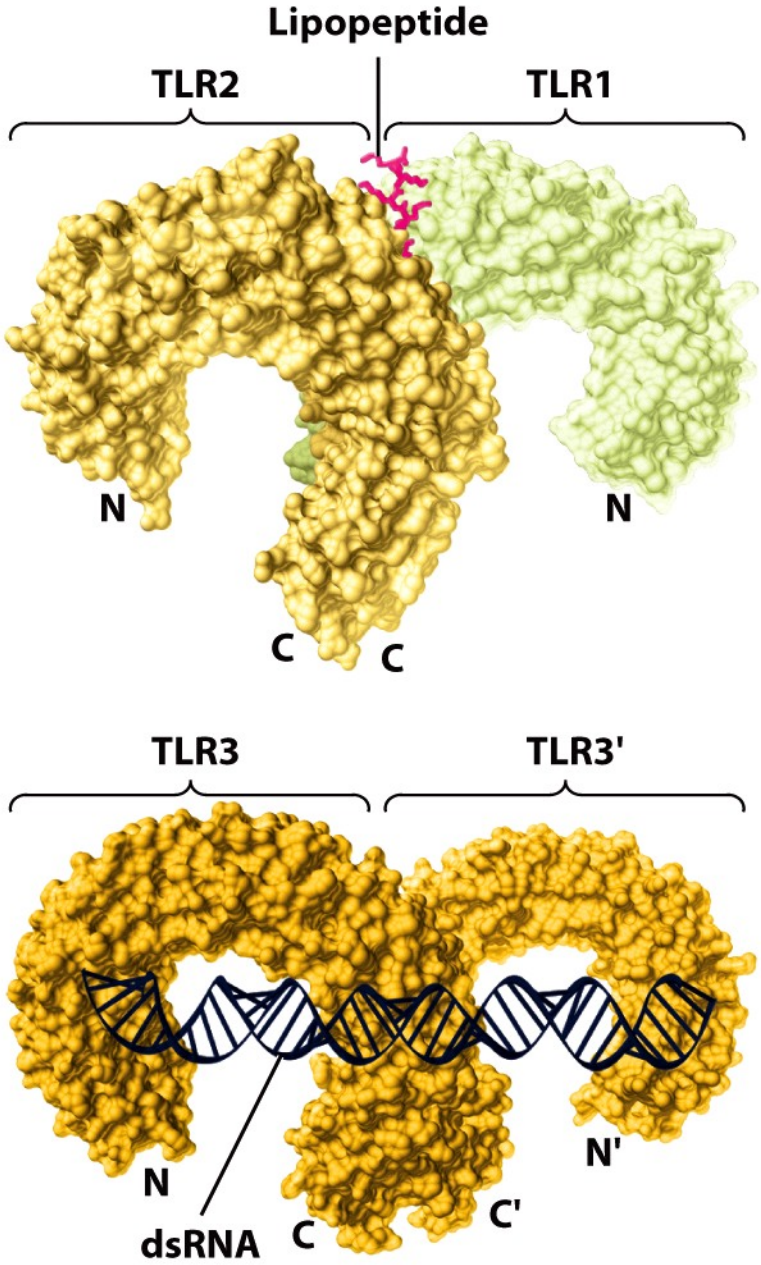


**Figure 5-11b**
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