Innate Immunity

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Learning Objectives

By the end of this lecture you will be able to:

① Describe anatomical and physiological barriers against microbial infections

② Define **P**athogen-**A**ssociated **M**olecular **P**atterns and **P**athogen-**R**ecognition **R**eceptors

③ Recognize the complement system

④ Explain the mechanism of inflammation

⑤ Describe acute-phase response

Innate Immune System

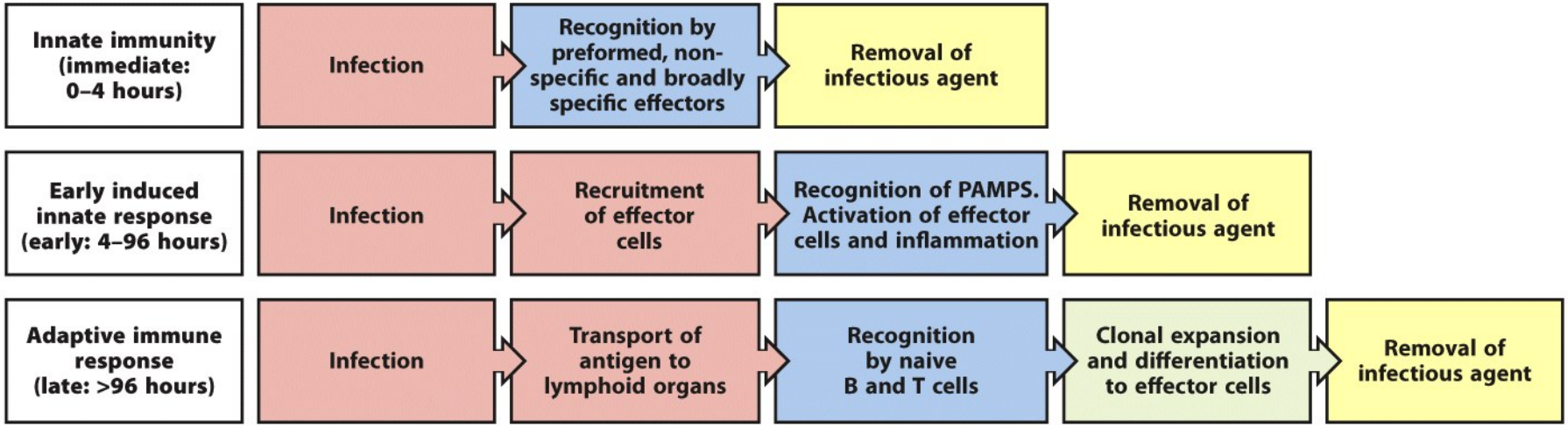


Figure 2.1 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Innate Immune System

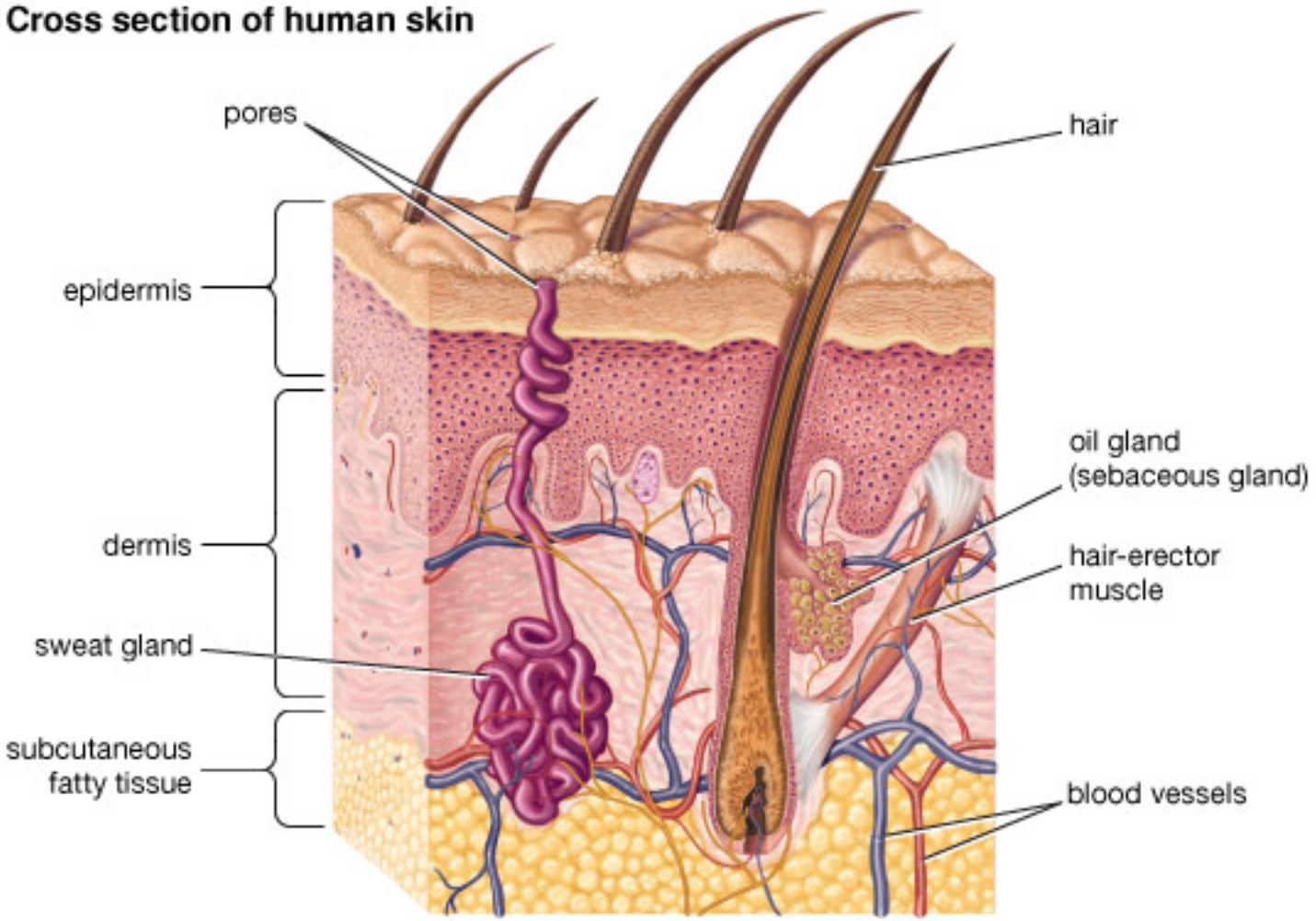
- The first hurdle for a pathogen includes host anatomical and physiological barriers
  - Skin
  - Respiratory Tract
  - Stomach and Intestine
  - Urogenital Tract
  - Tears/Saliva/Mucous Membranes
- The importance of these barriers becomes obvious when they are breached

Skin

- Inhospitable environment for most bacterial colonization except some bacteria such as *Staphylococcus aurous* and *Staphylococcus epidermidis*

- Bacteria cannot attach to the stratum corneum because:
  ① It consists of dead keratinocytes, keratin, ceramide, free fatty acids, and cholesterol
  ② Continually shed and renewed by layers pushing up from below

Skin


Cross section of human skin
pores
hair
epidermis
dermis
sweat gland
subcutaneous fatty tissue
oil gland (sebaceous gland)
hair-erector muscle
blood vessels
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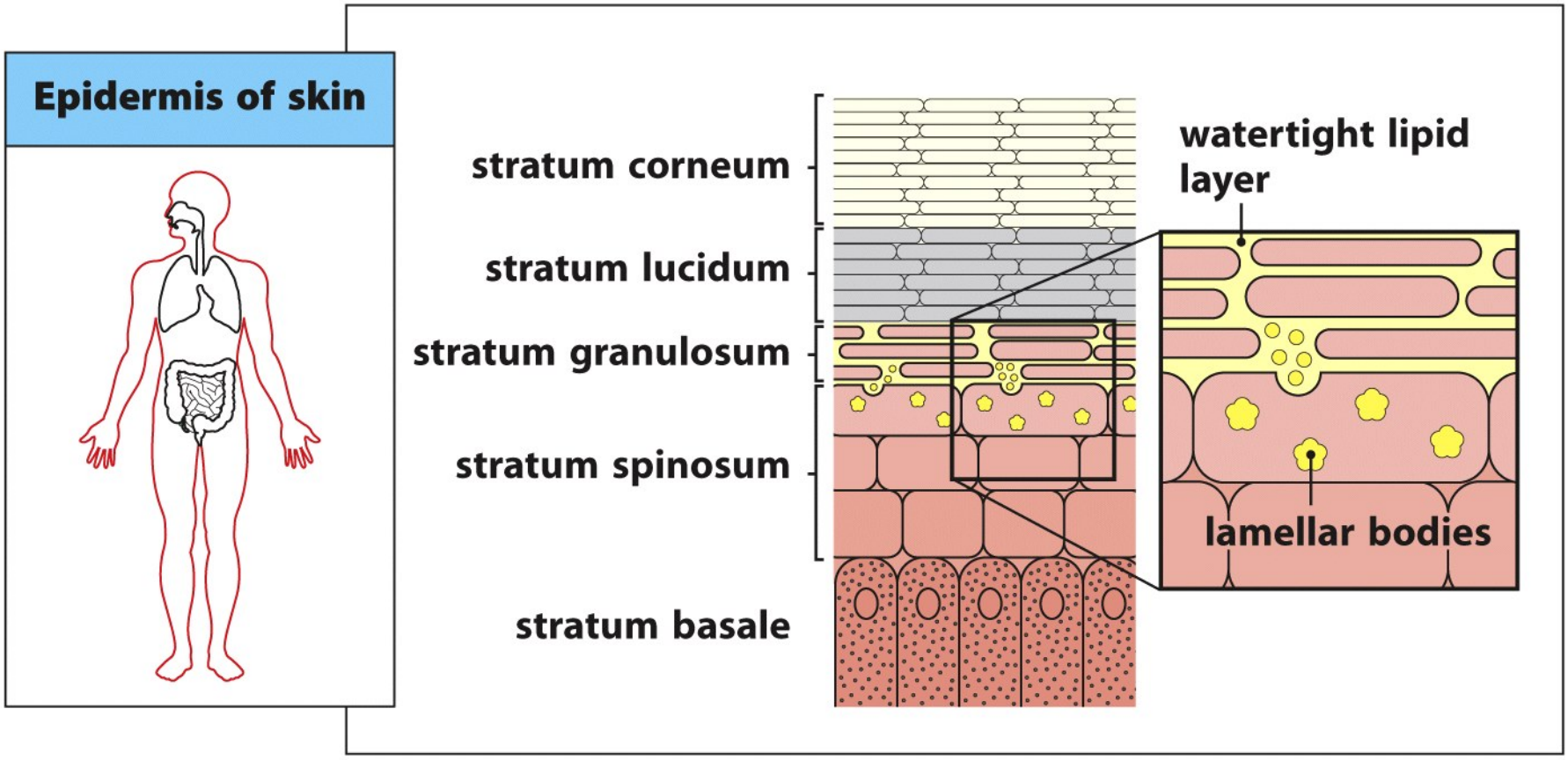
Skin

- **Psoriasin** produced by skin exhibits antibacterial activity to *Escherichia coli*
- Psoriasin helps when skin is scratched or cut to prevent infection
- Deposited salt by perspiration creates a hypertonic environment that inhibit bacterial growth
- Sebaceous gland produces **sebum** that contains lactic and propionic acid which reduce skin pH to 3-5

Skin



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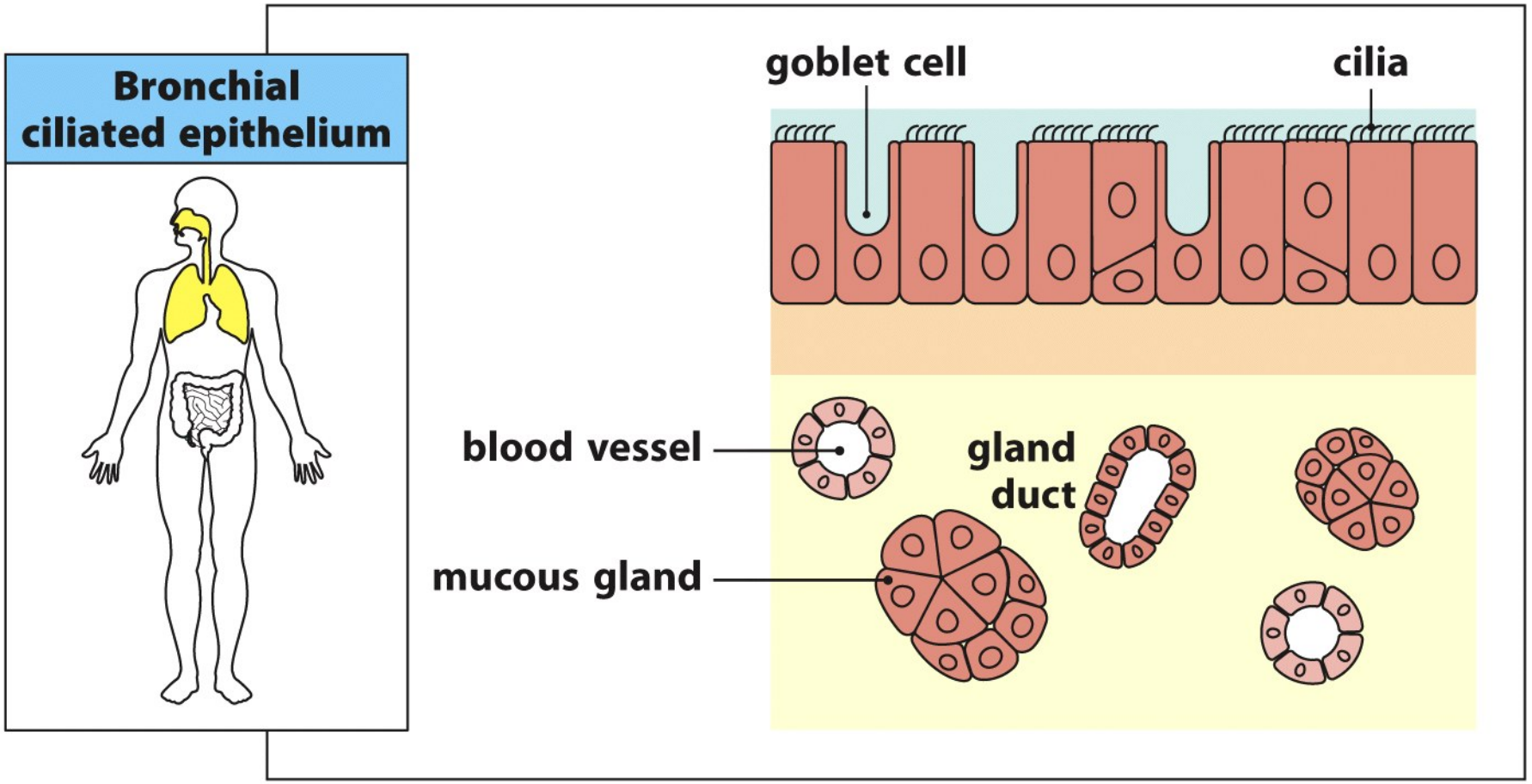
Respiratory Tract

- **Mucous** layer and ciliated epithelial cells
- **Mucus** traps particulate matter by its glycosylated protein **mucin**

- Coordinated beating of the cilia moves mucus upward (mucouciliary escalator)
  - Primary ciliary dyskinesia (PCD) is an inherited disease characterized by abnormal structure of cilia. Patients with PCD are prone to repeated respiratory infections
  - Smoking and drugs that destroy ciliated cells usually affect mucouciliary escalator
  - Cold and dry air create viscous mucus that cannot be moved

Respiratory Tract



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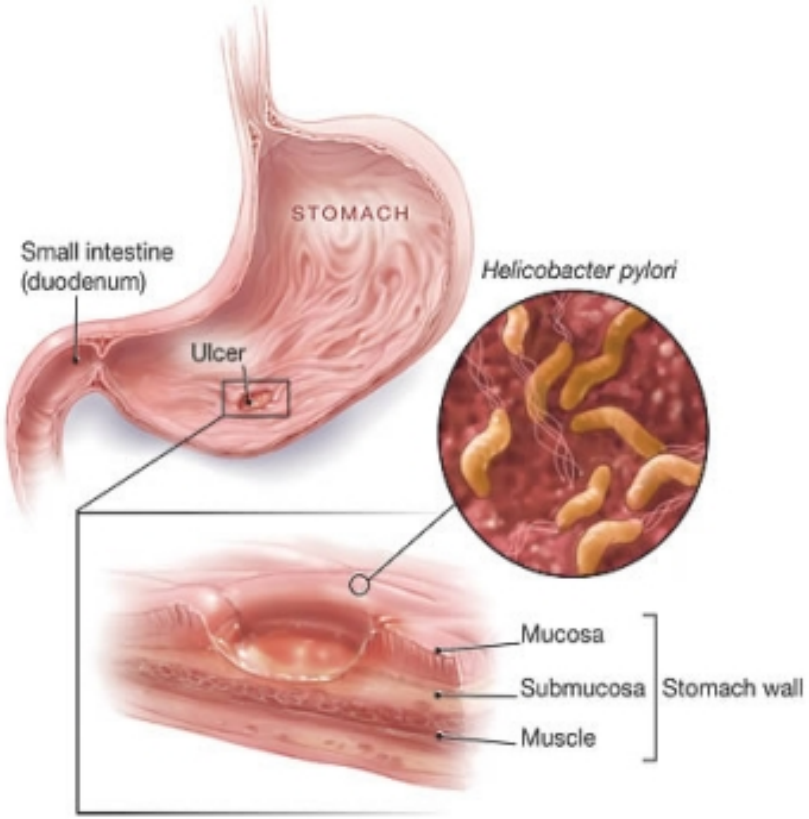
Stomach and Intestine

- Most pathogenic bacteria cannot survive in presence of stomach acid and enzymes except *Helicobacter pylori* which cause gastric and duodenal ulcers



Stomach and Intestine

- In the small intestine, digestive enzymes and antimicrobial peptides **(defensins)** inhibit pathogenic microbial growth

- Normal flora help to out-compete pathogens for space and nutrients

DRUG DEVELOPMENT RESEARCH •• : ••–•• (2013)

DDR

Research Overview

Gut Microbiota and Probiotics: Current Status and Their Role in Cancer Therapeutics

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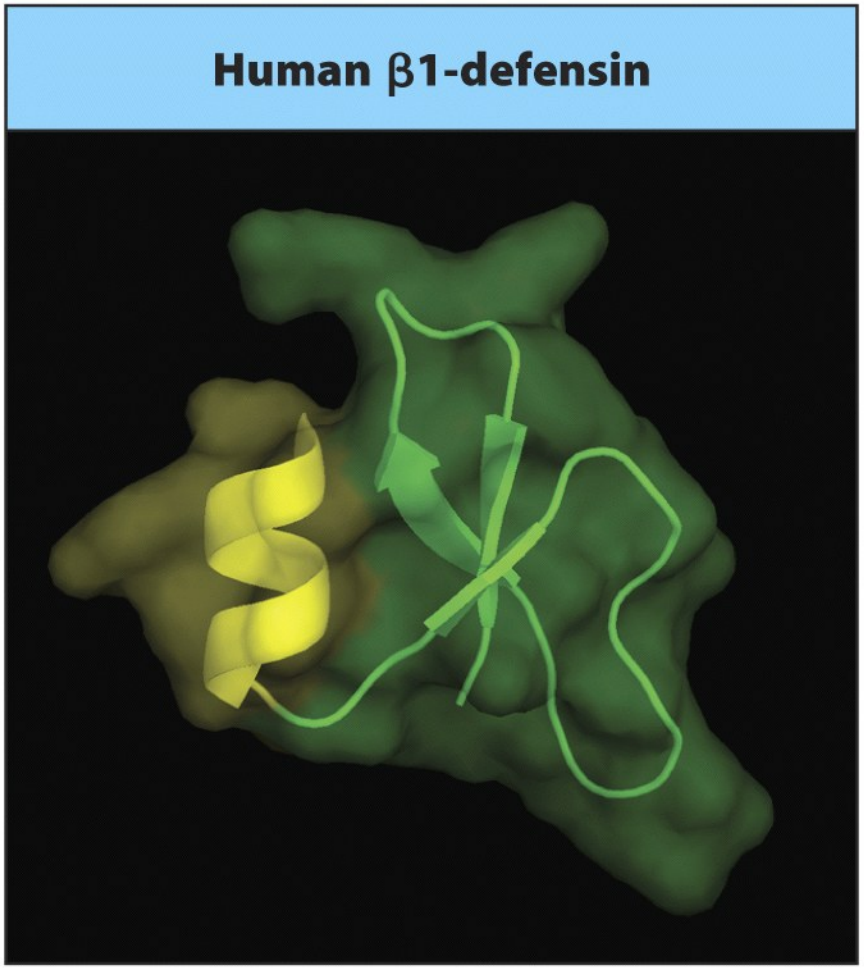
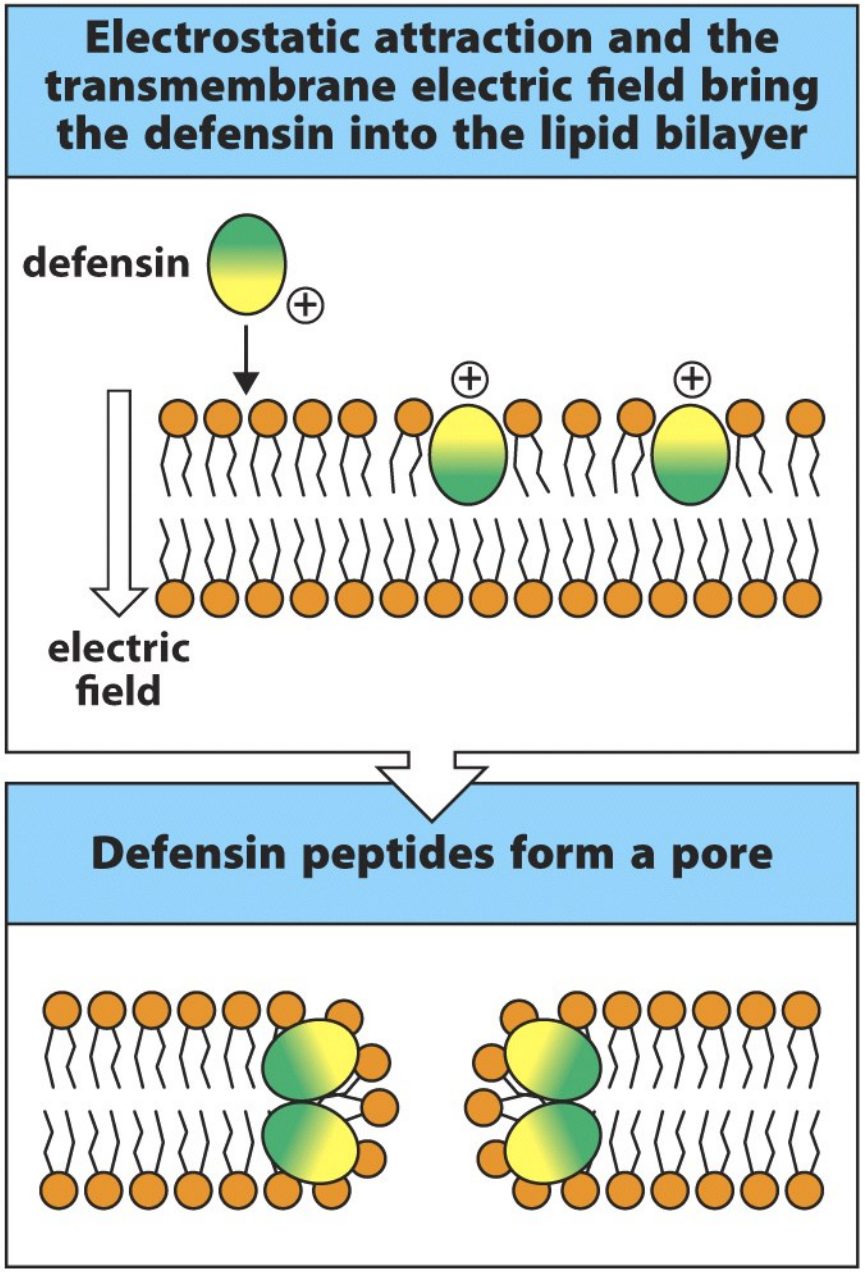
Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT The microbiome is a collection of all microbial species that coexist with an individual. These organisms influence several aspects of individual body functions. Probiotic organisms are generally beneficial components of microflora and confer normal health status. Usually, probiotics should be provided from the outside in the diet for maintaining proper health status. Probiotics can also have a significant impact on cancer management. While the results toward cancer management with probiotics are promising, careful risk assessment of probiotics use in cancer patients, who are usually immunocompromised due to radical therapy, comes as a great demand. This article provides an overview of the current research status of probiotics use in cancer patients and discusses the role of probiotics in cancer management. Drug Dev Res •• : ••–••, 2013.

Key words: probiotics; cancer; normal microflora; cancer management





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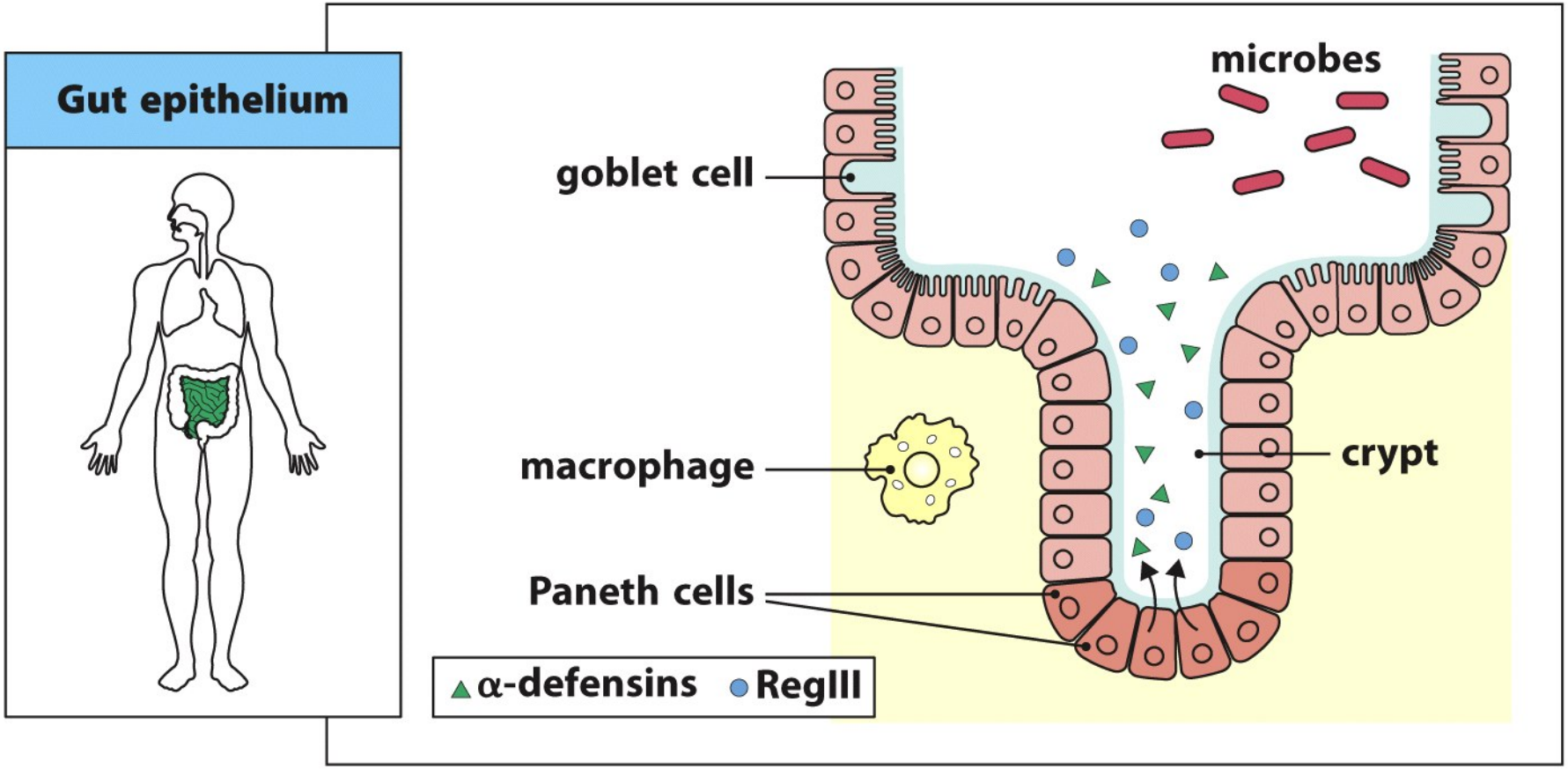
Stomach and Intestine



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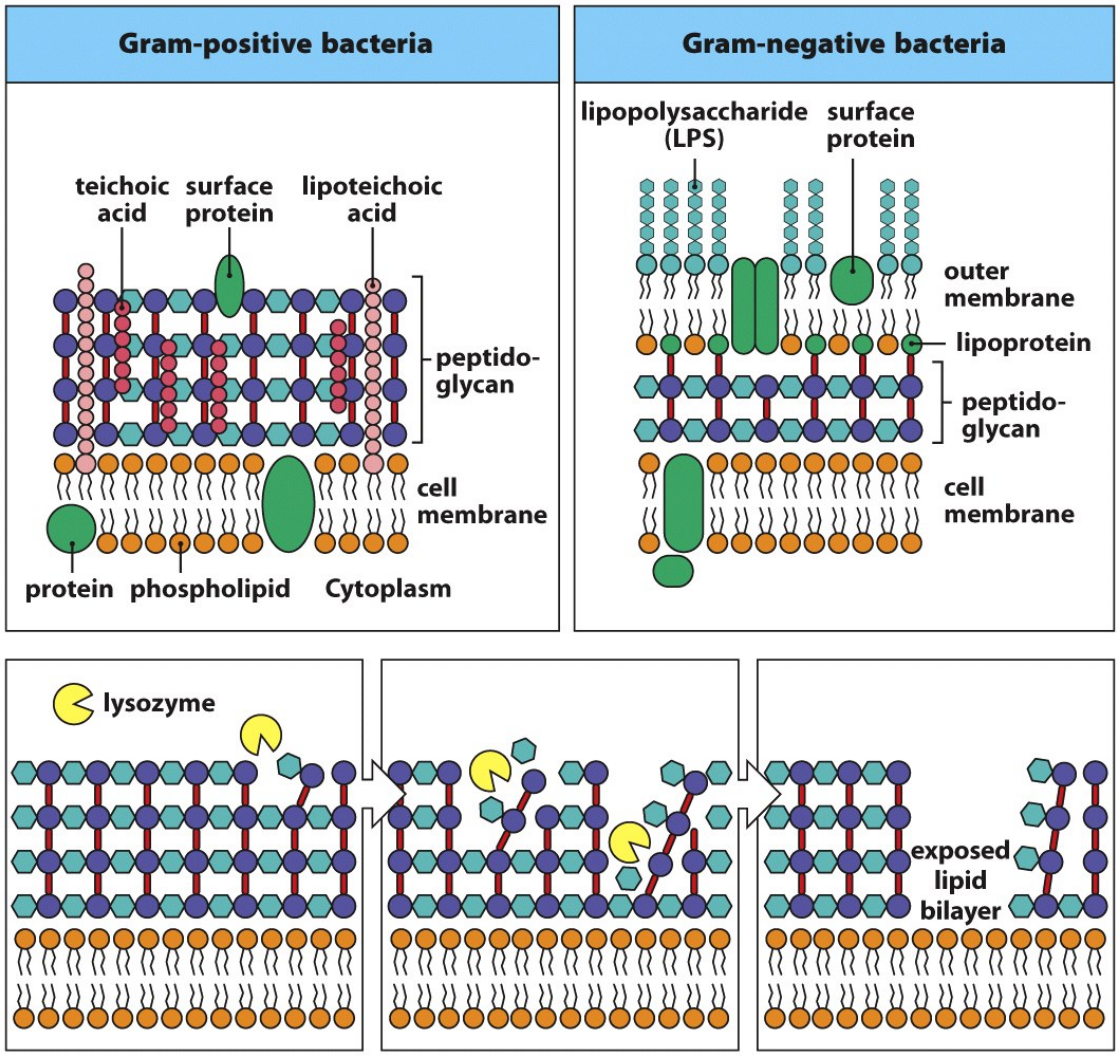
Urogenital Tract

- **Urethra:**
- The urethra is usually sterile because the persistent flushing of urine prevents bacterial attachment to urethral epithelium
- The acidity of the urine prevent bacterial colonization

- **Vagina:**
- Vaginal mucosa and normal flora prevents colonization by pathogenic microbes
- Acids produced by lactobacilli creates an environment that inhibits bacterial growth

Tears and Saliva

- Wash invaders away as well as contain antimicrobial peptides



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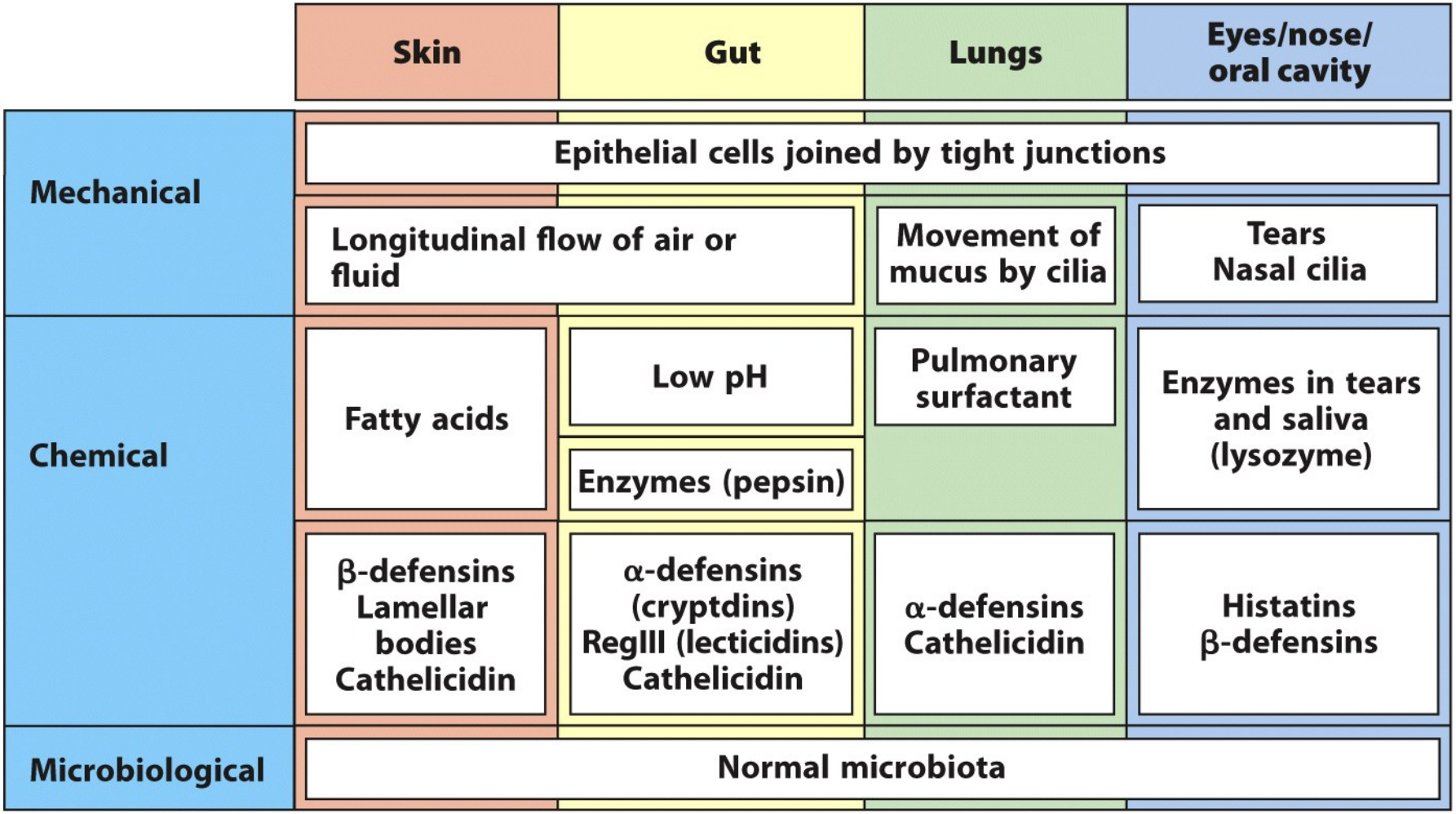

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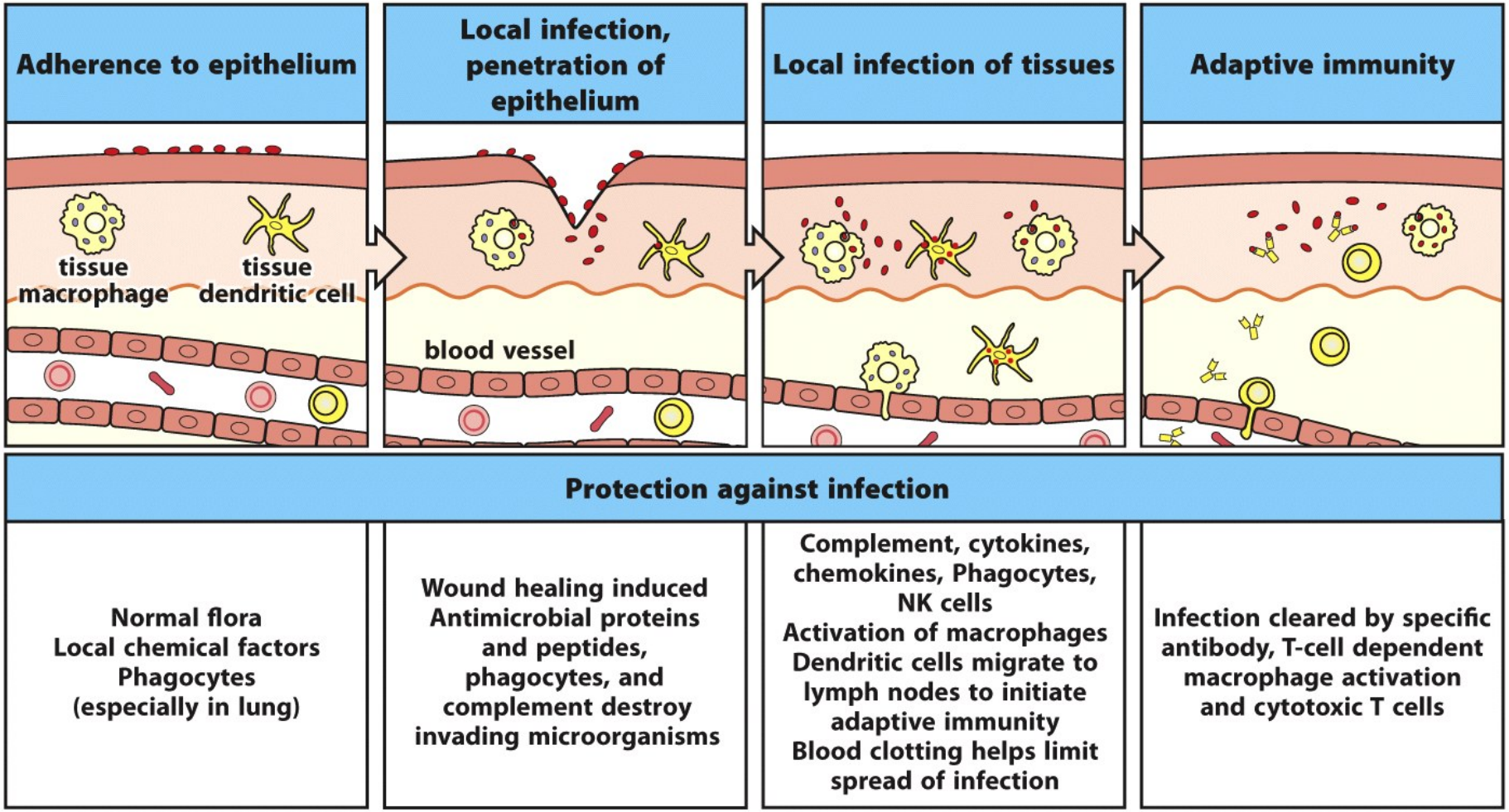
Connection between Innate and Adaptive Immune Response



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Connection between Innate and Adaptive Immune Response

- The immune system reacts to invaders with two critical functions: **sensing** and **responding**

- If pathogens get past anatomical barriers, the innate immune system recognizes broad structural motifs of microbial species known as **Pathogen-Associated Molecular Patterns (PAMPs)**

- PAMPs are common pathogenic patterns that do not occur in the host such as: combinations of microbial sugars, proteins, lipids, and nucleic acids

Connection between Innate and Adaptive Immune Response

- PAMPs are recognized by different mechanisms including:
  ① **Pattern Recognition Receptors (PRRs)**
  ② **Acute Phase Proteins**
  ③ **Serum Complement System**

- PRRs precisely discriminate between self and nonself molecules

- Leukocytes express a variety of PRRs including **Toll-Like Receptors (TLRs)**, which recognize a wide range of microbial patterns

Pattern Recognition Receptors

① Scavengers Receptors:

- Binding of modified LDL, LPS, and some nucleic acids
- Phagocytosis of bacteria

② Opsonins:

- Attach to the surface of microbes making them more attractive to phagocytosis (**opsonization**)
- Receptors for opsonins are present on phagocytic cells

③ Toll-Like Receptors:

- Enhance activation of genes encoding cytokines and other antimicrobial molecules

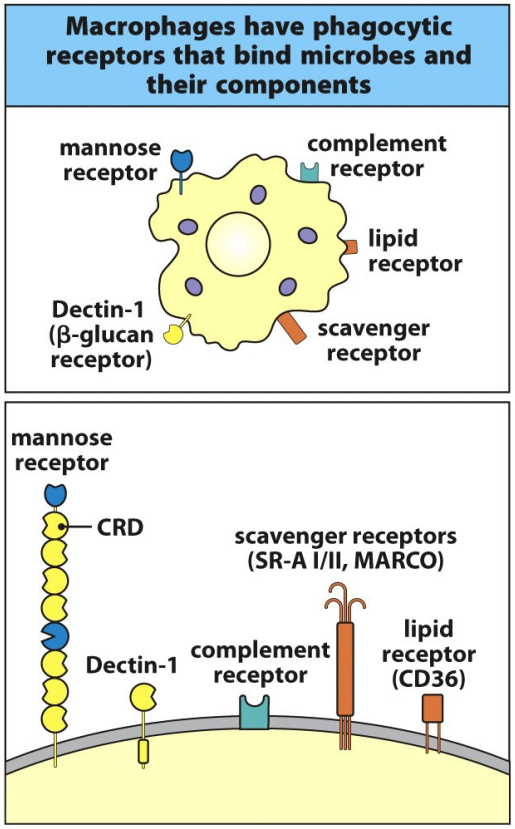

Figure 3.2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Toll-Like Receptors

- Membrane-spanning proteins
- Common extracellular elements:
  - Repeating segments (24-29 aa)
  - Containing sequence xLxxLxLxx
  - **Leucine-Rich Repeats (LRRs)**
- Intracellular domain:
  - **Toll/IL-1 Receptor (TIR)**
  - Three highly conserved regions
  - Box 1, Box 2, and Box 3
  - Serve as docking sites for intracellular signaling proteins

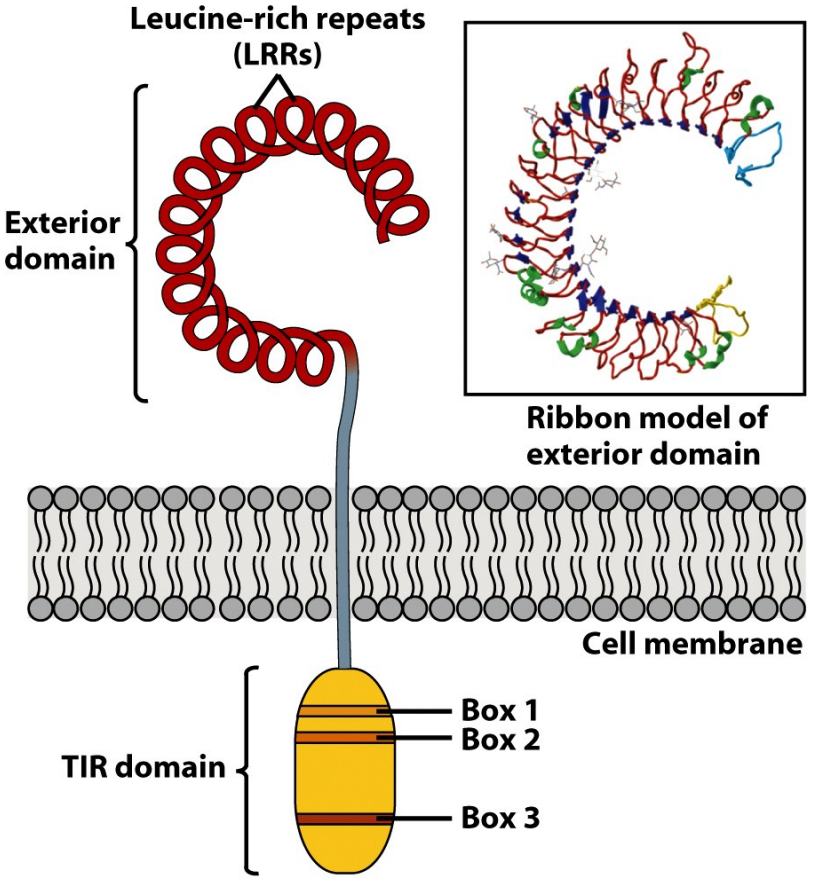

Figure 3-10
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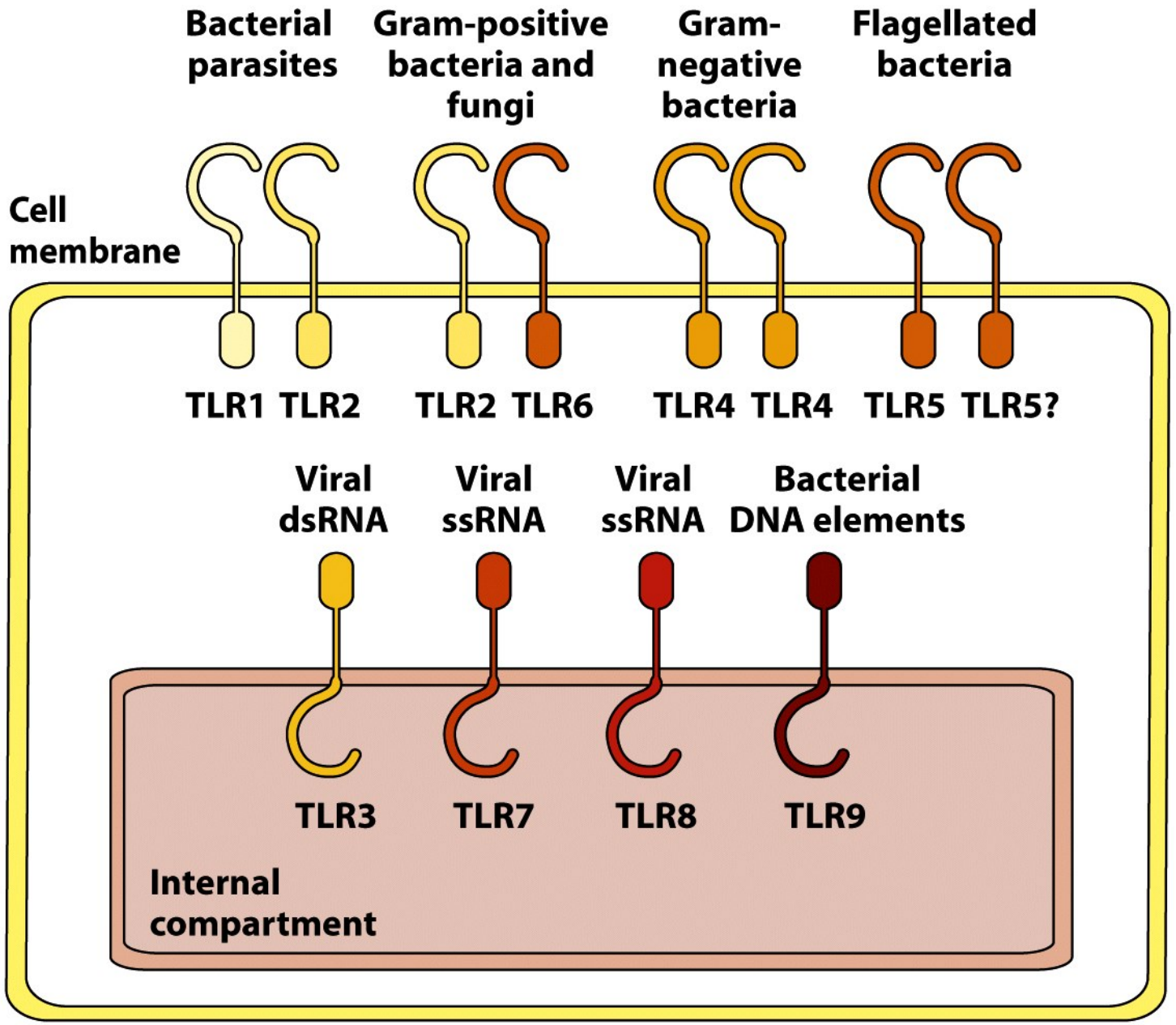

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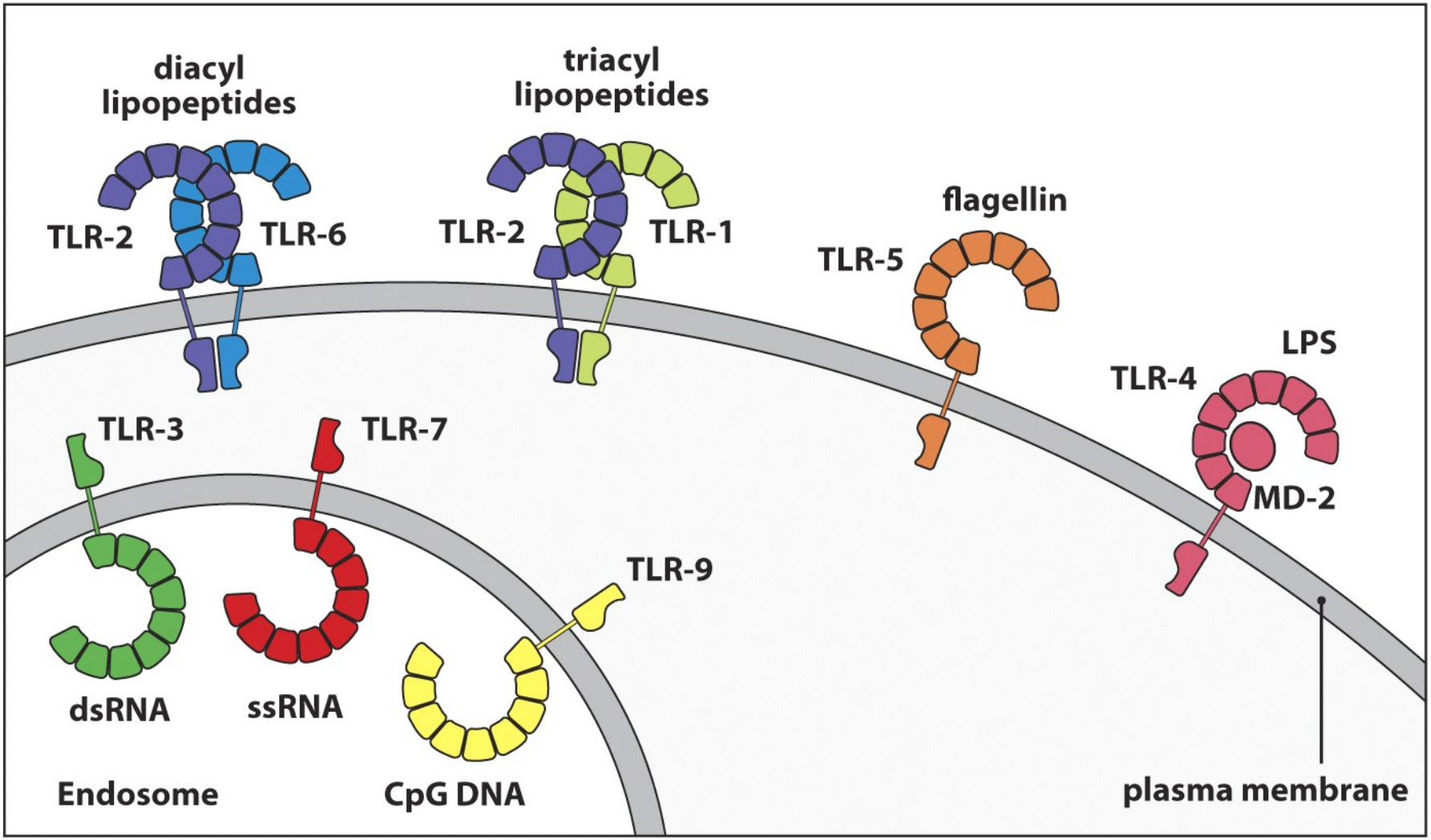

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Innate immune recognition by mammalian Toll-like receptors		
Toll-like receptor	**Ligand**	**Cellular distribution**
TLR-1:TLR-2 heterodimer	Lipomannans (mycobacteria) Lipoproteins (diacyl lipopeptides; triacyl lipopeptides) Lipoteichoic acids (Gram-positive bacteria) Cell-wall β-glucans (bacteria and fungi) Zymosan (fungi)	Monocytes, dendritic cells, mast cells, eosinophils, basophils
TLR-2:TLR-6 heterodimer		
TLR-3	Double-stranded RNA (viruses)	NK cells
TLR-4 (plus MD-2 and CD14)	LPS (Gram-negative bacteria) Lipoteichoic acids (Gram-positive bacteria)	Macrophages, dendritic cells, mast cells, eosinophils
TLR-5	Flagellin (bacteria)	Intestinal epithelium
TLR-7	Single-stranded RNA (viruses)	Plasmacytoid dendritic cells, NK cells, eosinophils, B cells
TLR-8	Single-stranded RNA (viruses)	NK cells
TLR-9	DNA with unmethylated CpG (bacteria and herpesviruses)	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-10	Unknown	Plasmacytoid dendritic cells, eosinophils, B cells, basophils
TLR-11 (mouse only)	Profilin and profilin-like proteins (*Toxoplasma gondii*, uropathogenic bacteria)	Macrophages, dendritic cells, liver, kidney, and bladder epithelial cells

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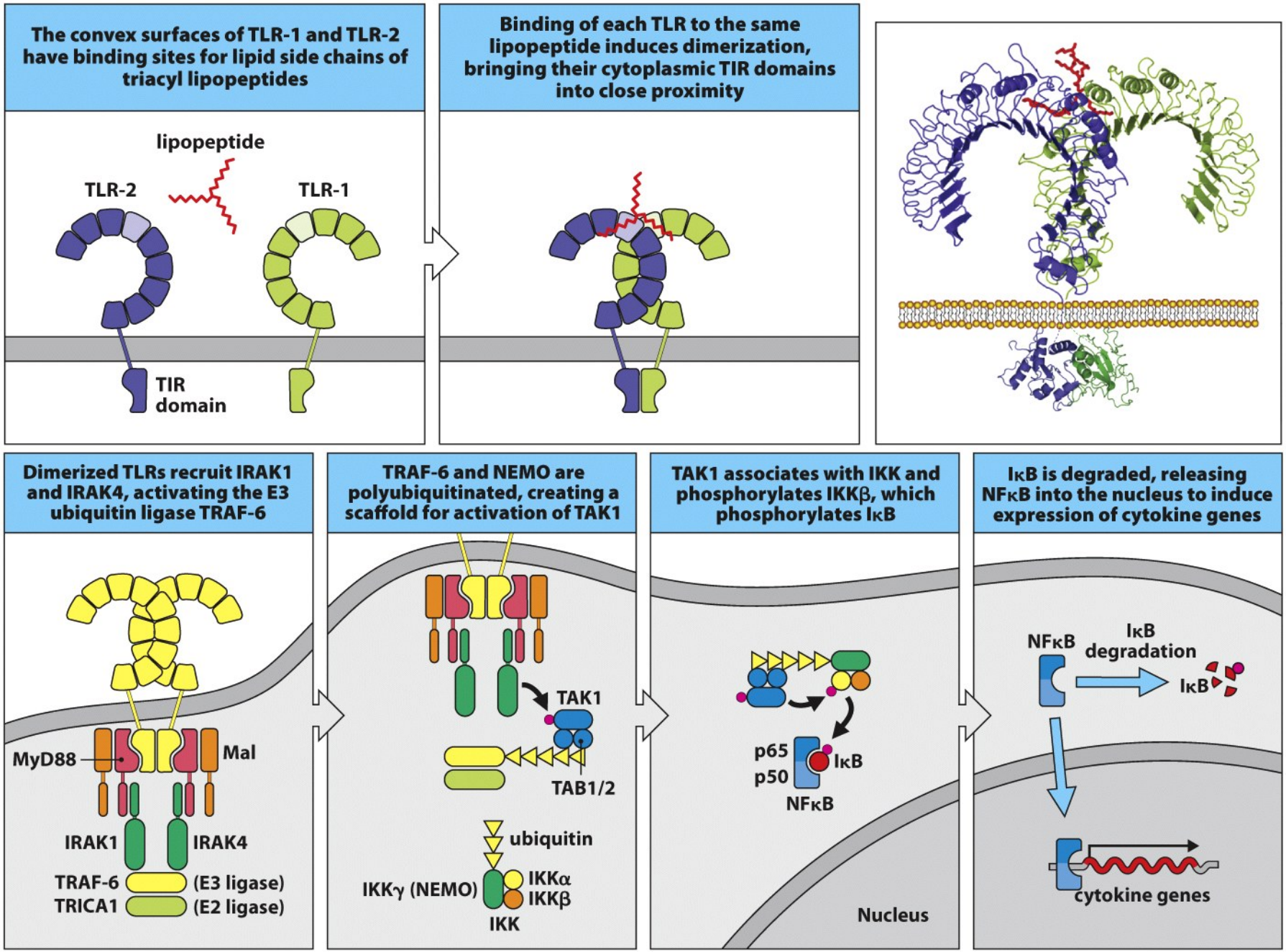

The convex surfaces of TLR-1 and TLR-2 have binding sites for lipid side chains of triacyl lipopeptides
lipopeptide
TLR-2
TLR-1
TIR domain
Binding of each TLR to the same lipopeptide induces dimerization, bringing their cytoplasmic TIR domains into close proximity
Dimerized TLRs recruit IRAK1 and IRAK4, activating the E3 ubiquitin ligase TRAF-6
MyD88
Mal
IRAK1
IRAK4
TRAF-6
(E3 ligase)
TRICA1
(E2 ligase)
TRAF-6 and NEMO are polyubiquitinated, creating a scaffold for activation of TAK1
TAK1
TAB1/2
ubiquitin
IKKγ (NEMO)
IKKα
IKKβ
IKK
TAK1 associates with IKK and phosphorylates IKKβ, which phosphorylates IκB
p65
p50
IκB
NFκB
Nucleus
IκB is degraded, releasing NFκB into the nucleus to induce expression of cytokine genes
NFκB
IκB degradation
IκB
cytokine genes

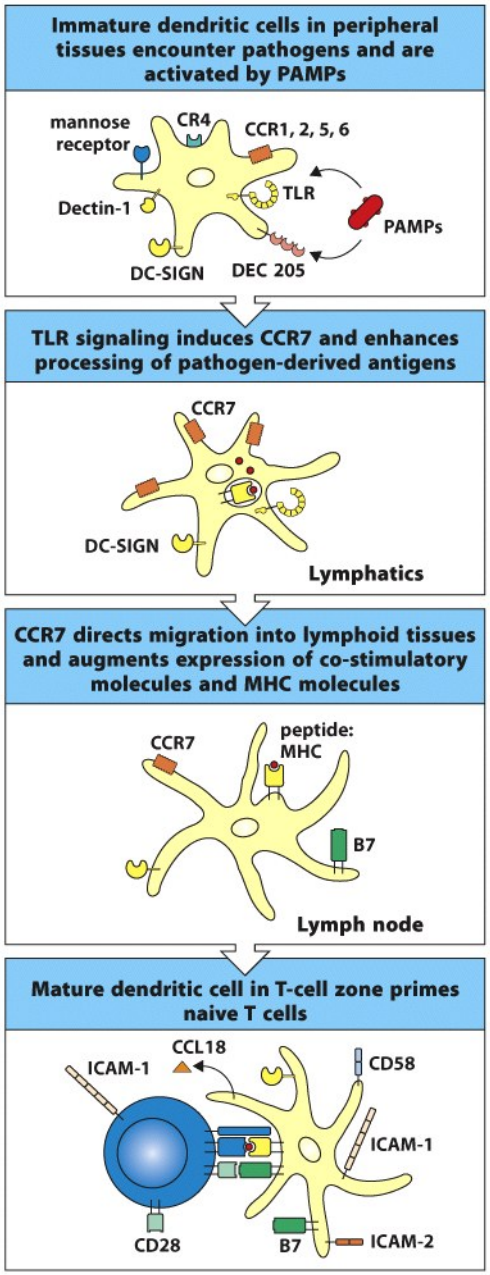
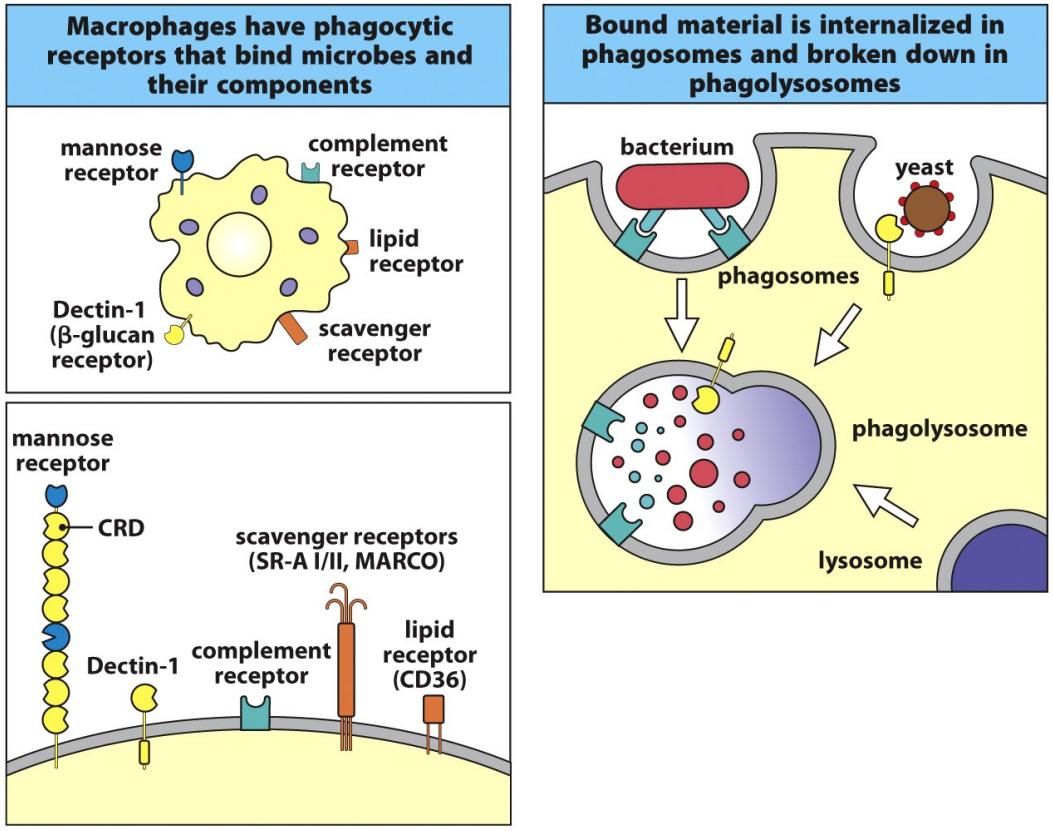


Figure 9.14 Janeway's Immunobiology, 8ed. (© G

Immature DCs at the site of infection internalize and process Ag and get activated by PAMPs through TLR stimulation. Then DCs mature and migrate to the draining lymph node, where they present antigens to T cells and secrete **cytokines** to initiate adaptive immune response. This activity is the bridge between innate and adaptive immunity

Inflammation

- When pathogens breach the anatomical barriers, the ligation of PAMPs with PRRs accelerates phagocytosis (especially by neutrophils and macrophages) and participate in the **acute inflammatory response** characterized by: **Hotness, Redness, Pain, Tenderness, Swelling**



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Inflammation

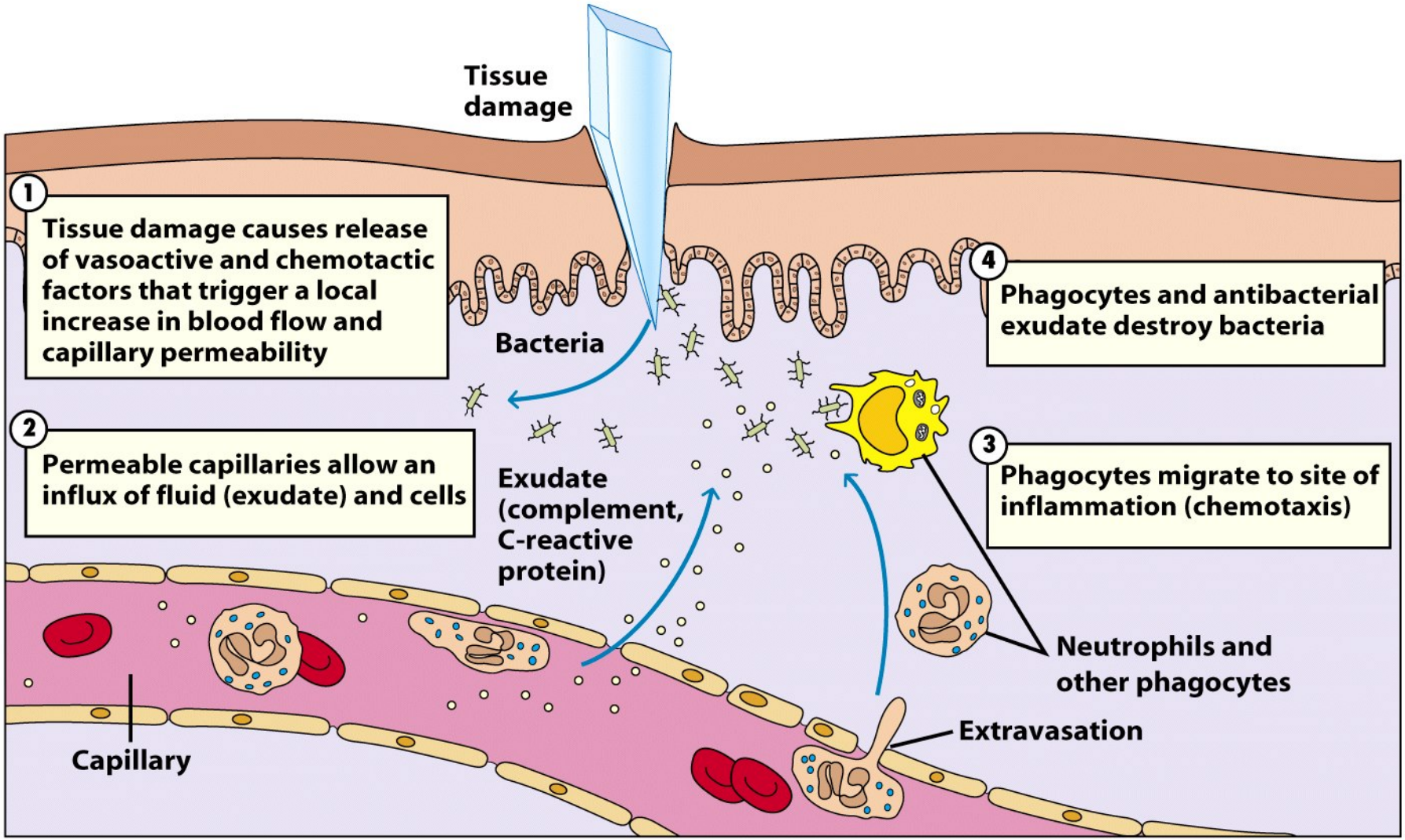

Figure 3-5
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Inflammation

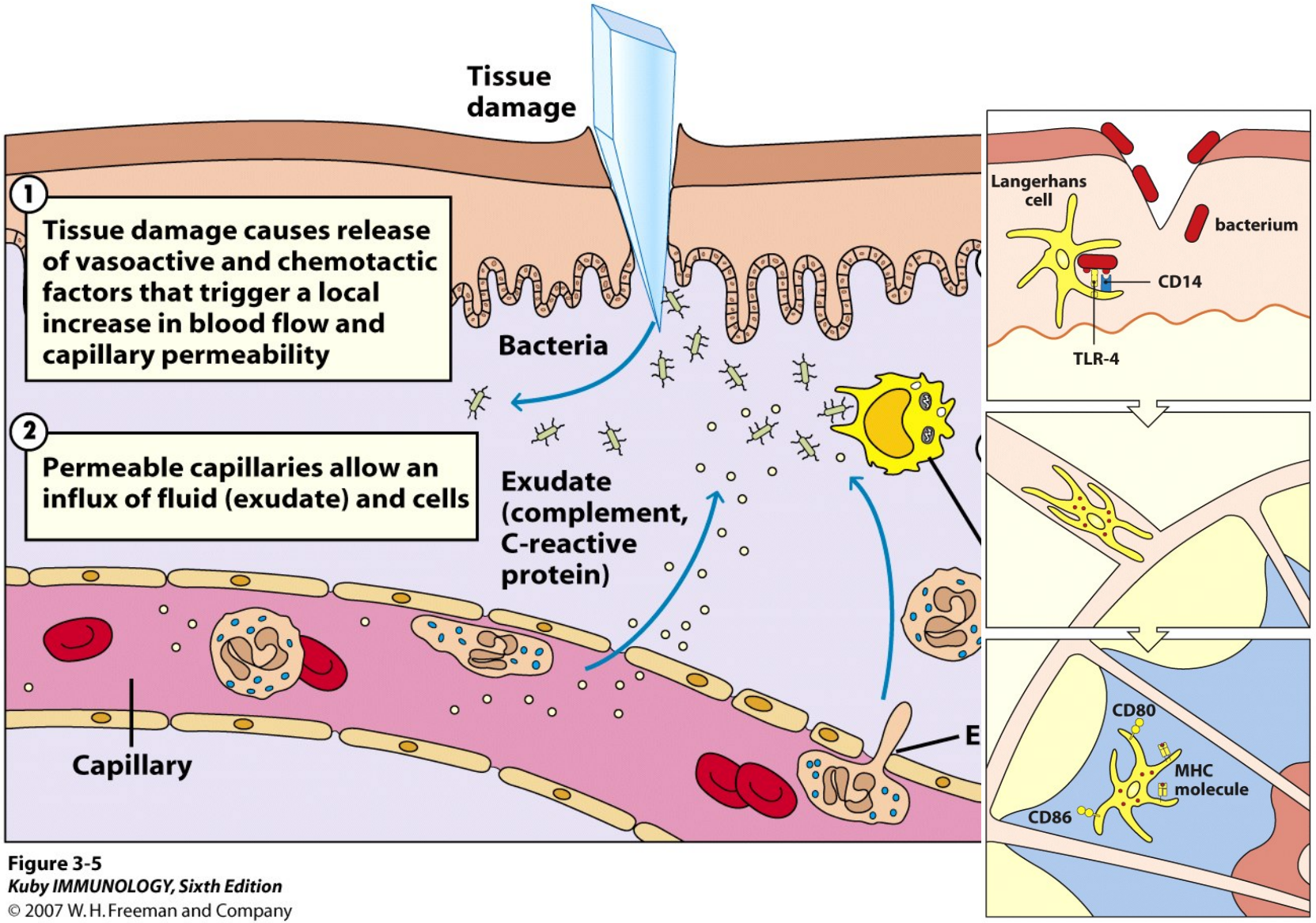

Figure 3-5
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Inflammation

- Events happening within minutes of tissue injury:
  - Vasodilation and **edema** caused by cytokines released from damaged tissue cells including macrophages

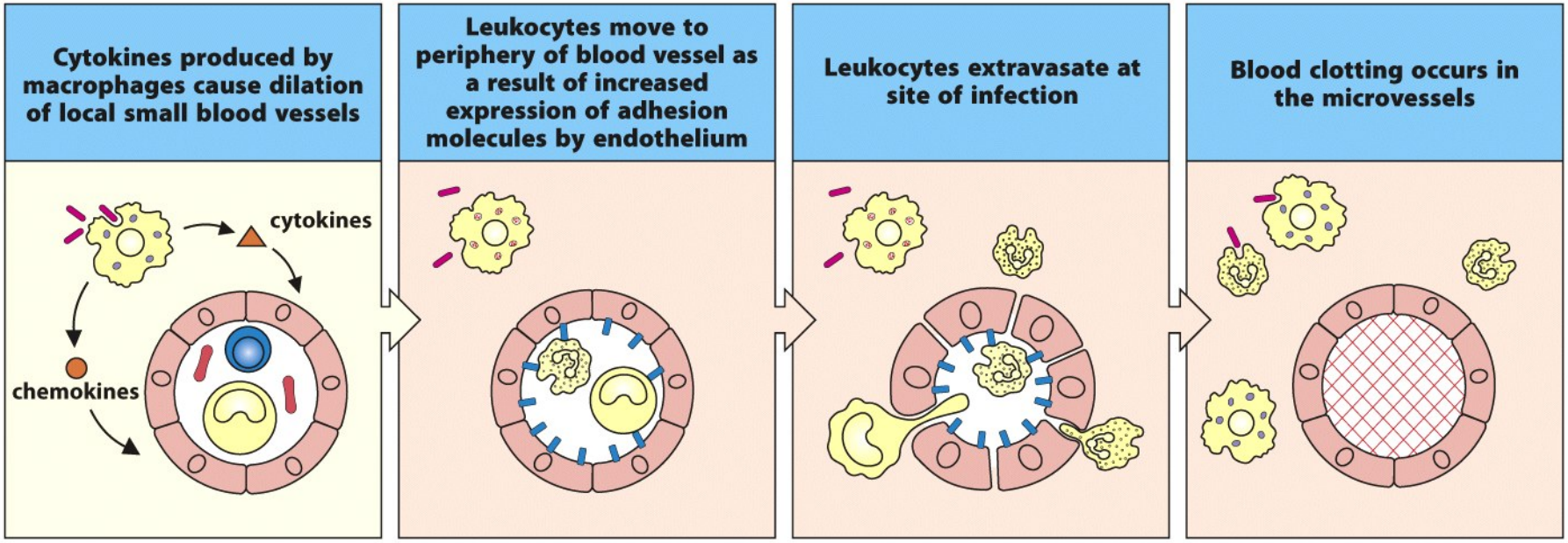
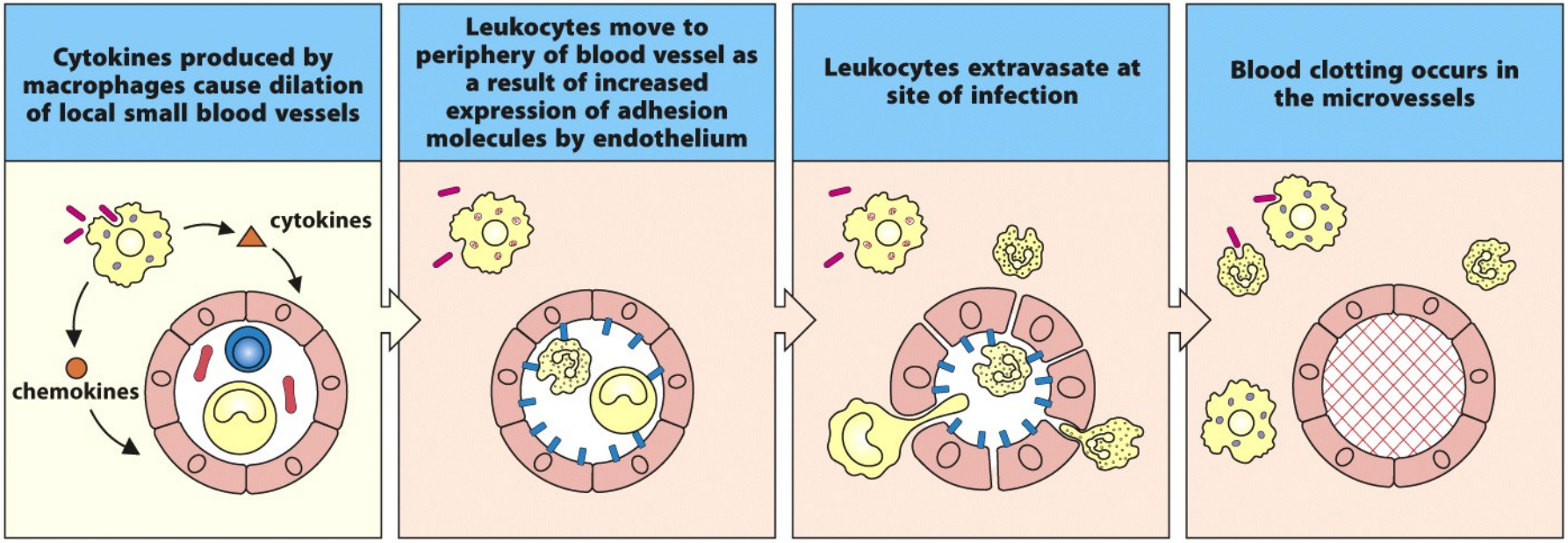


Figure 3.6 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Inflammation

- Events happening within a few hours of tissue injury:
  - Expression of adhesion molecules by endothelial cells



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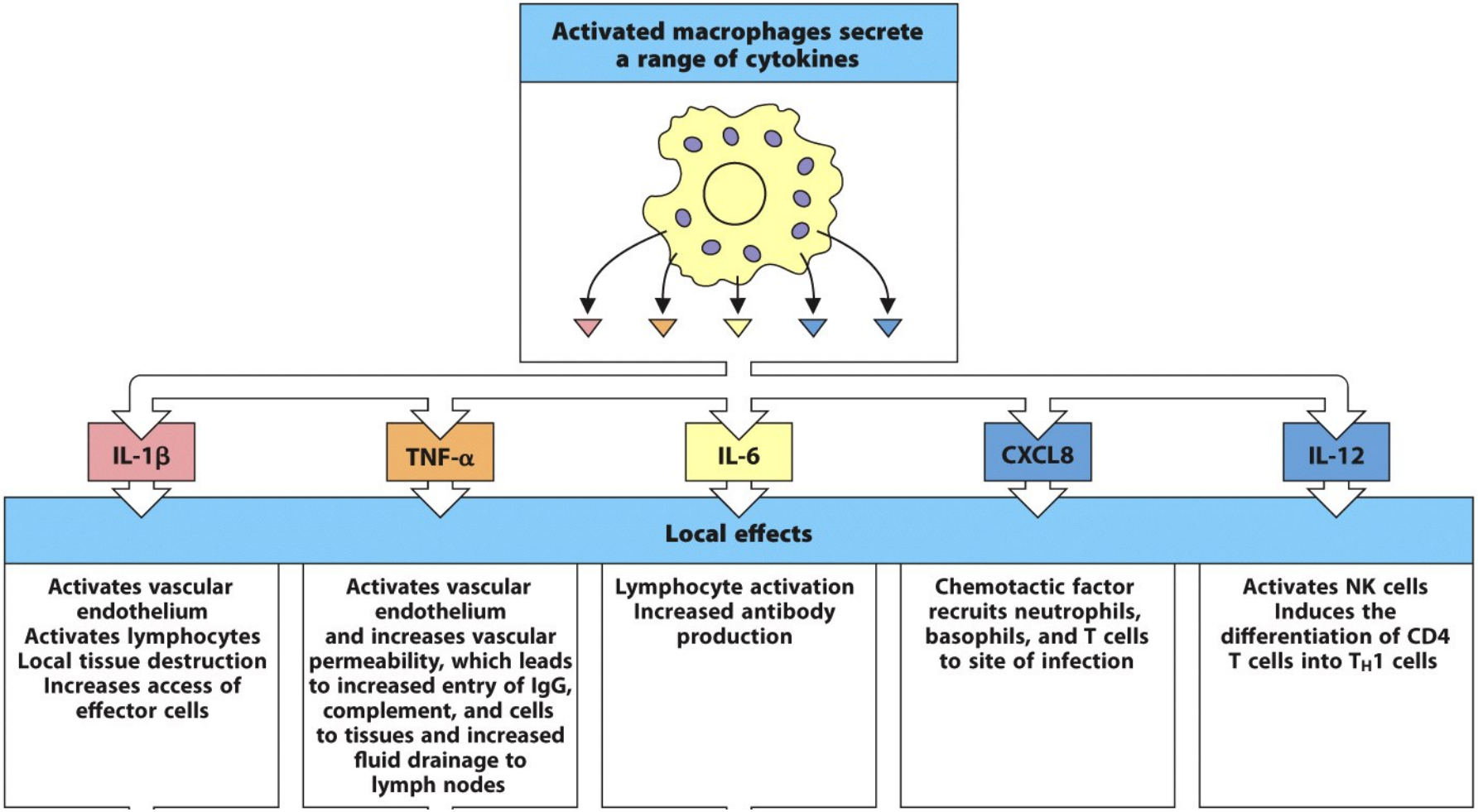
Activated macrophages secrete a range of cytokines
IL-1β
TNF-α
IL-6
CXCL8
IL-12
Local effects
Activates vascular endothelium
Activates lymphocytes
Local tissue destruction
Increases access of effector cells
Activates vascular endothelium
and increases vascular permeability, which leads to increased entry of IgG, complement, and cells to tissues and increased fluid drainage to lymph nodes
Lymphocyte activation
Increased antibody production
Chemotactic factor recruits neutrophils, basophils, and T cells to site of infection
Activates NK cells
Induces the differentiation of CD4 T cells into T_H1 cells

Cytokines

- Cytokines small proteins secreted by leukocytes and various other cells in response to stimuli. They play major roles in regulating the development and behavior of immune cells

- Cytokines belong to 4 families
  ① Hematopoietin family
  ② Interferon family
  ③ Chemokine family
  ④ Tumor necrosis family

- Cytokines mediate inflammatory response are called **proinflammatory cytokines**

Chemokines

- Chemokines are a major subgroup of cytokines that act as **chemoattractants** (agents that cause cells to move toward higher concentrations of the agent)

- Inflammatory chemokines are produced in response to proinflammatory cytokines

- Binding of chemokines to chemokine receptors on leukocytes triggers an activating signal that induces conformational changes that enhance cell adhesion

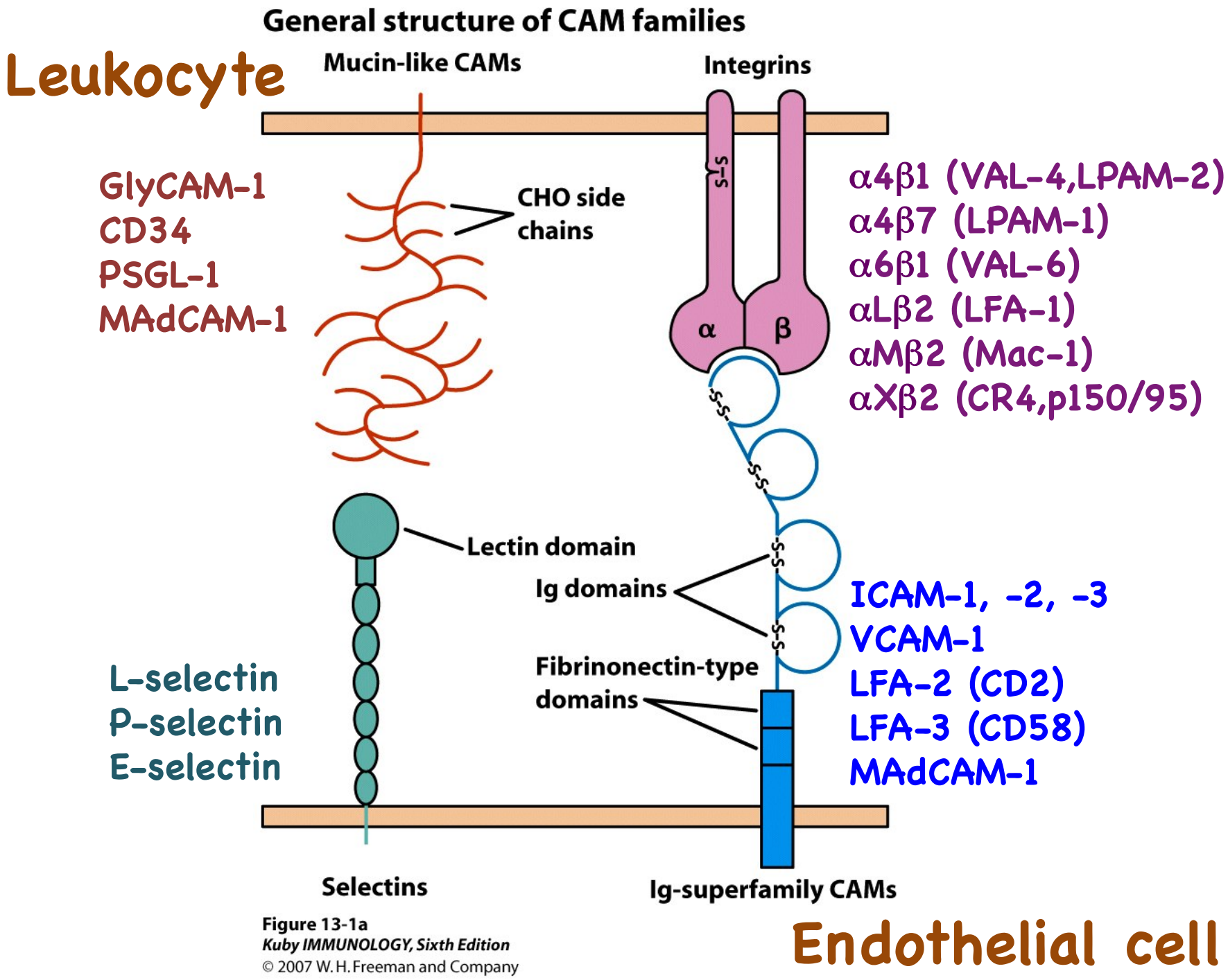

Figure 13-1a
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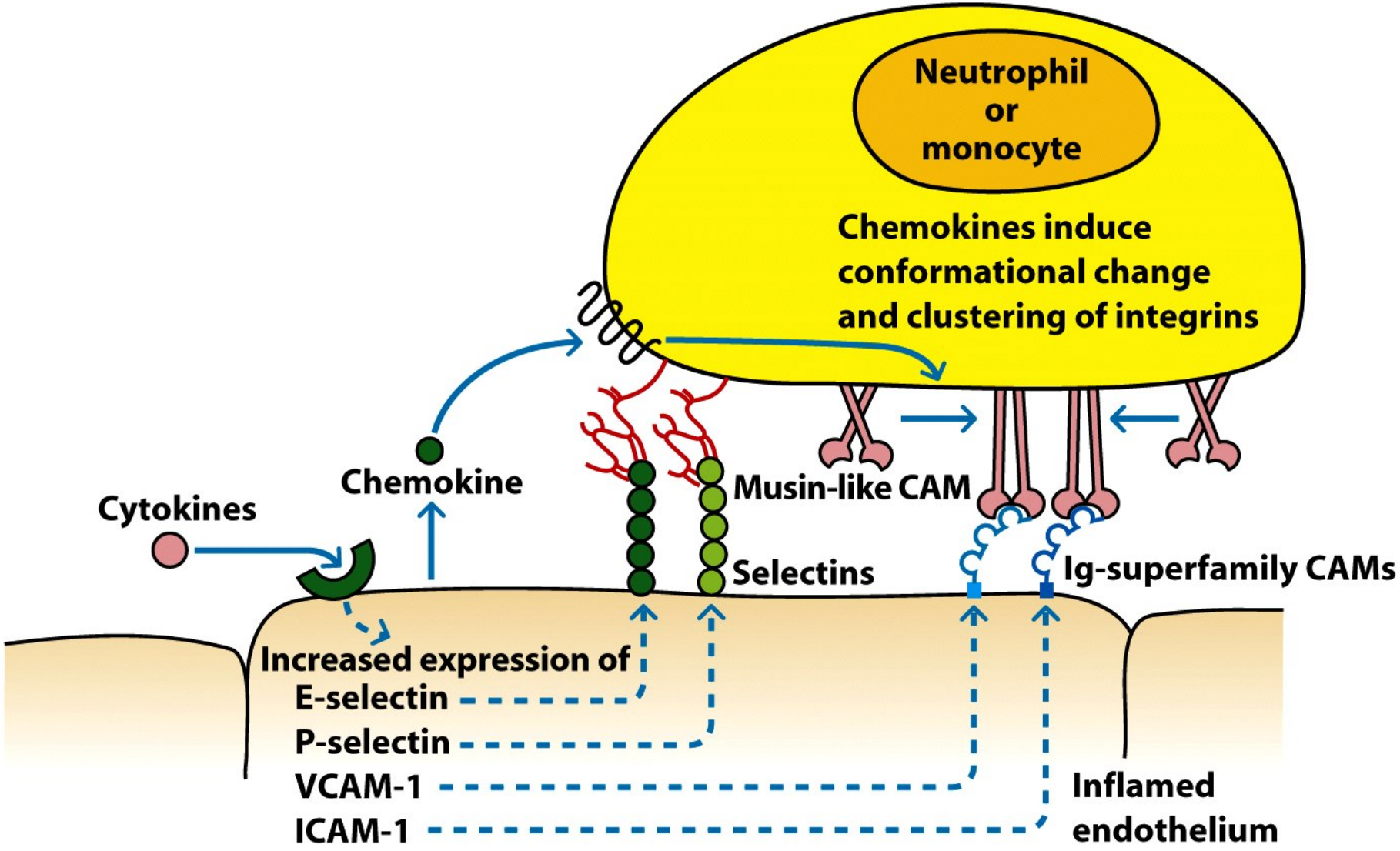

Figure 13-5b
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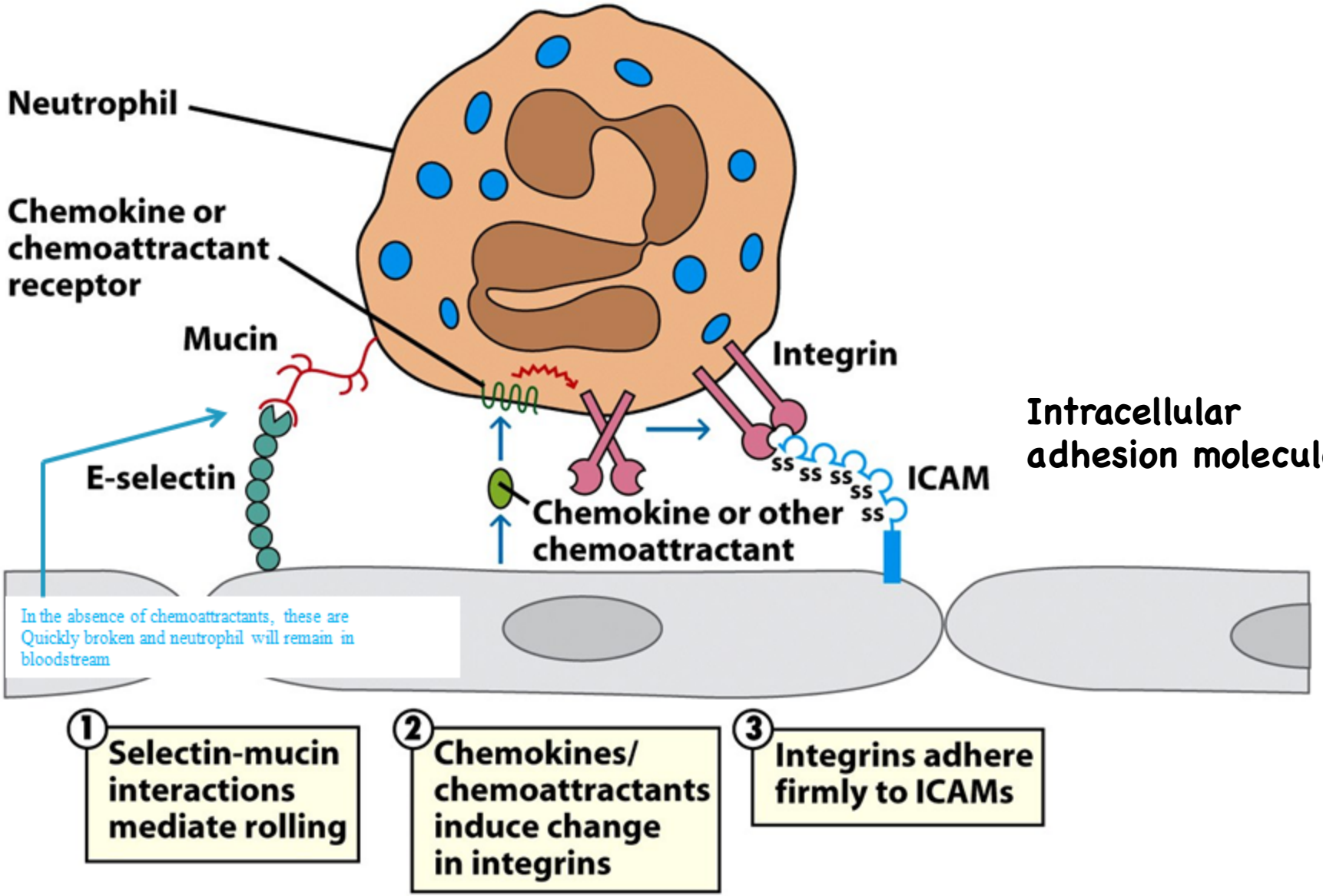
Initiation of extravasation



Intracellular adhesion molecules

Figure 3-7b
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Inflammation

- Events happening within hours of tissue injury:
  - Leukocytes (**neutrophils**) adhere to endothelial cells and pass through walls of capillaries into tissues (**Extravasation**)

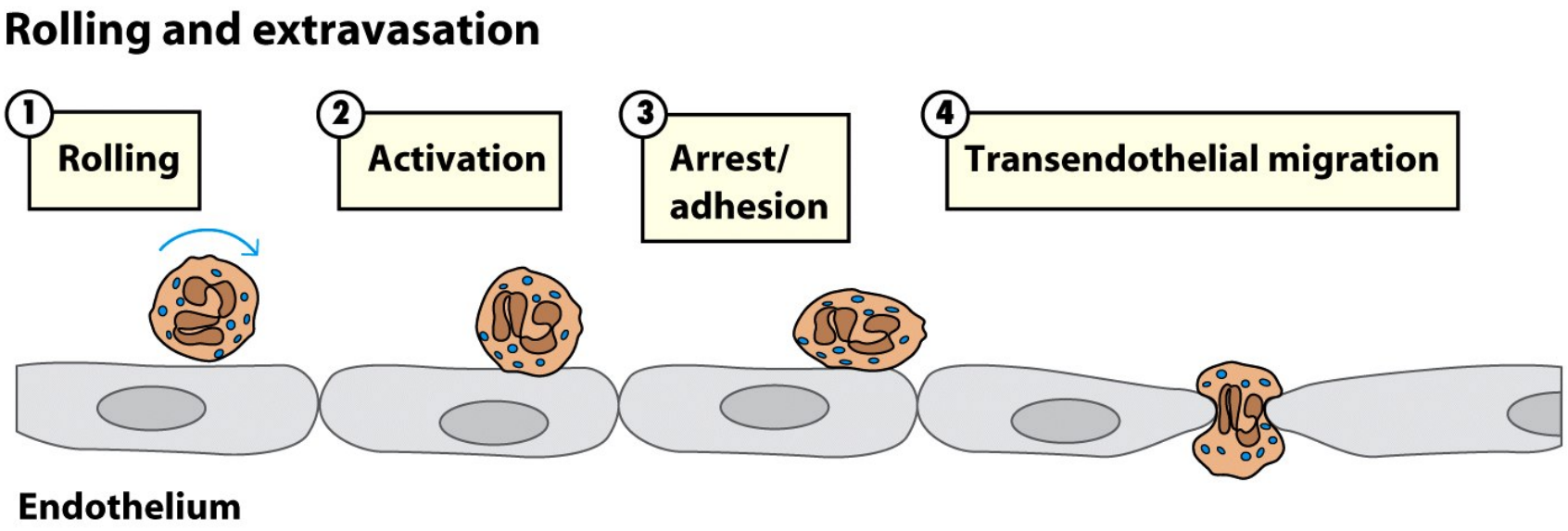

Figure 3-7a
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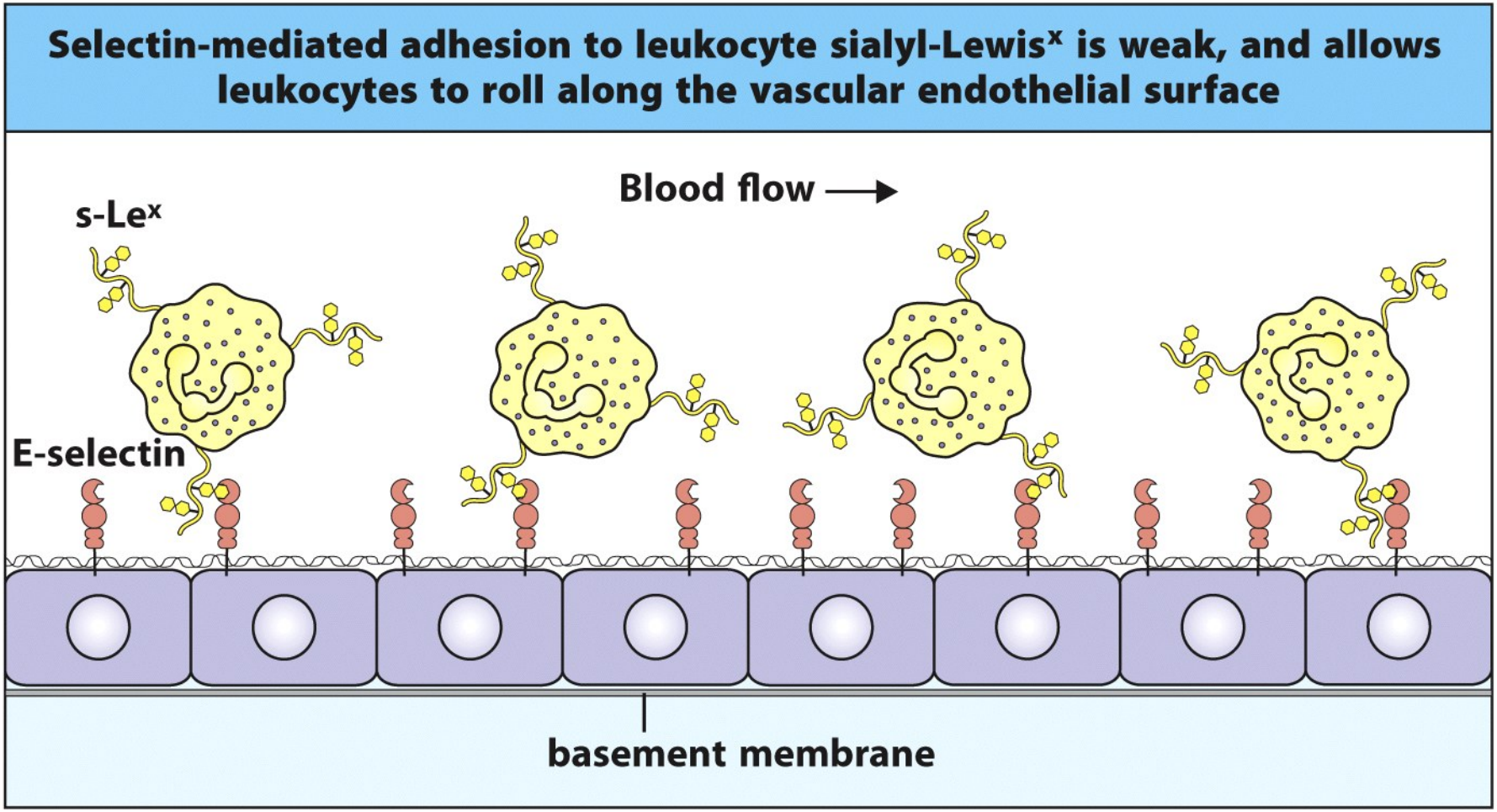

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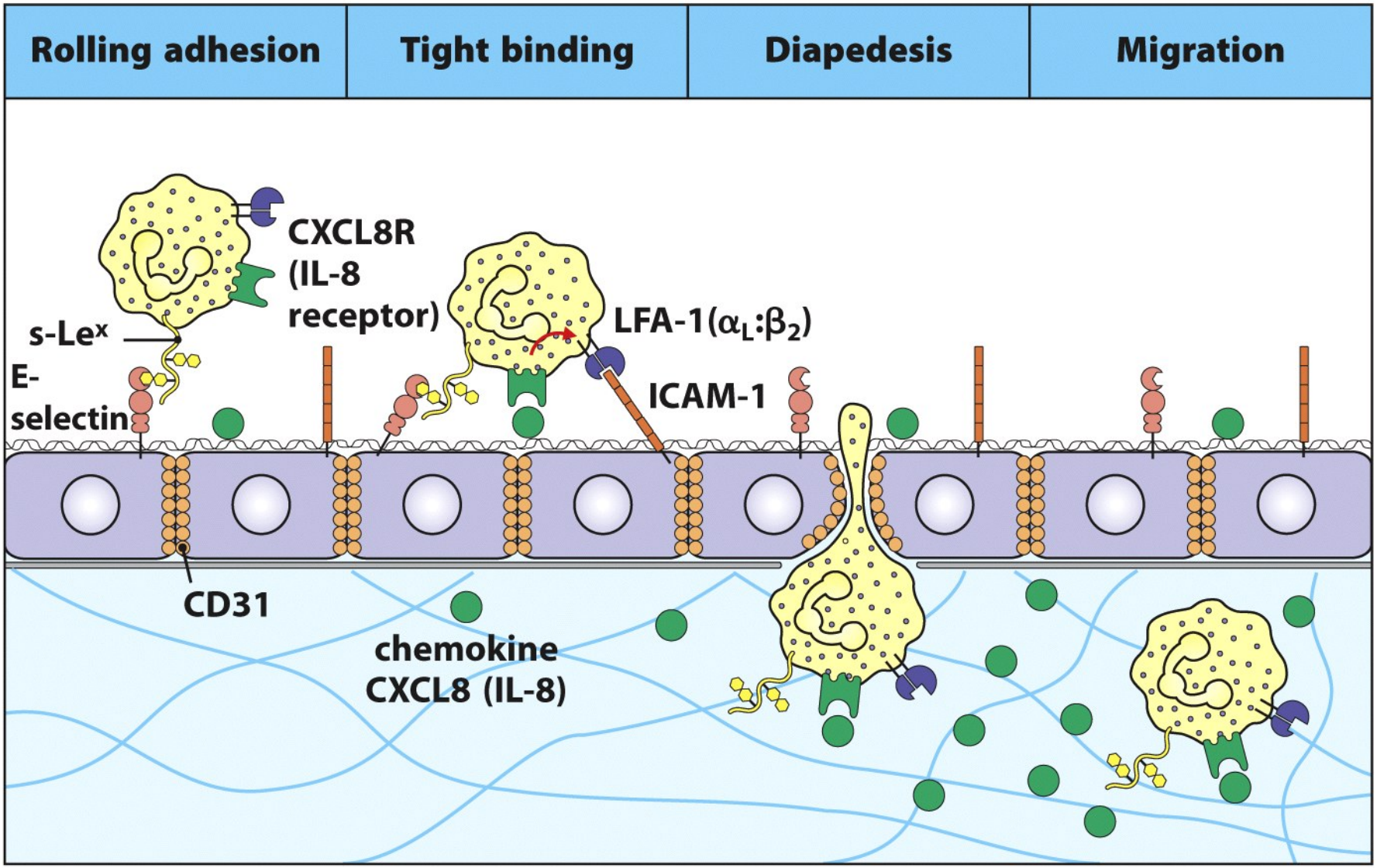

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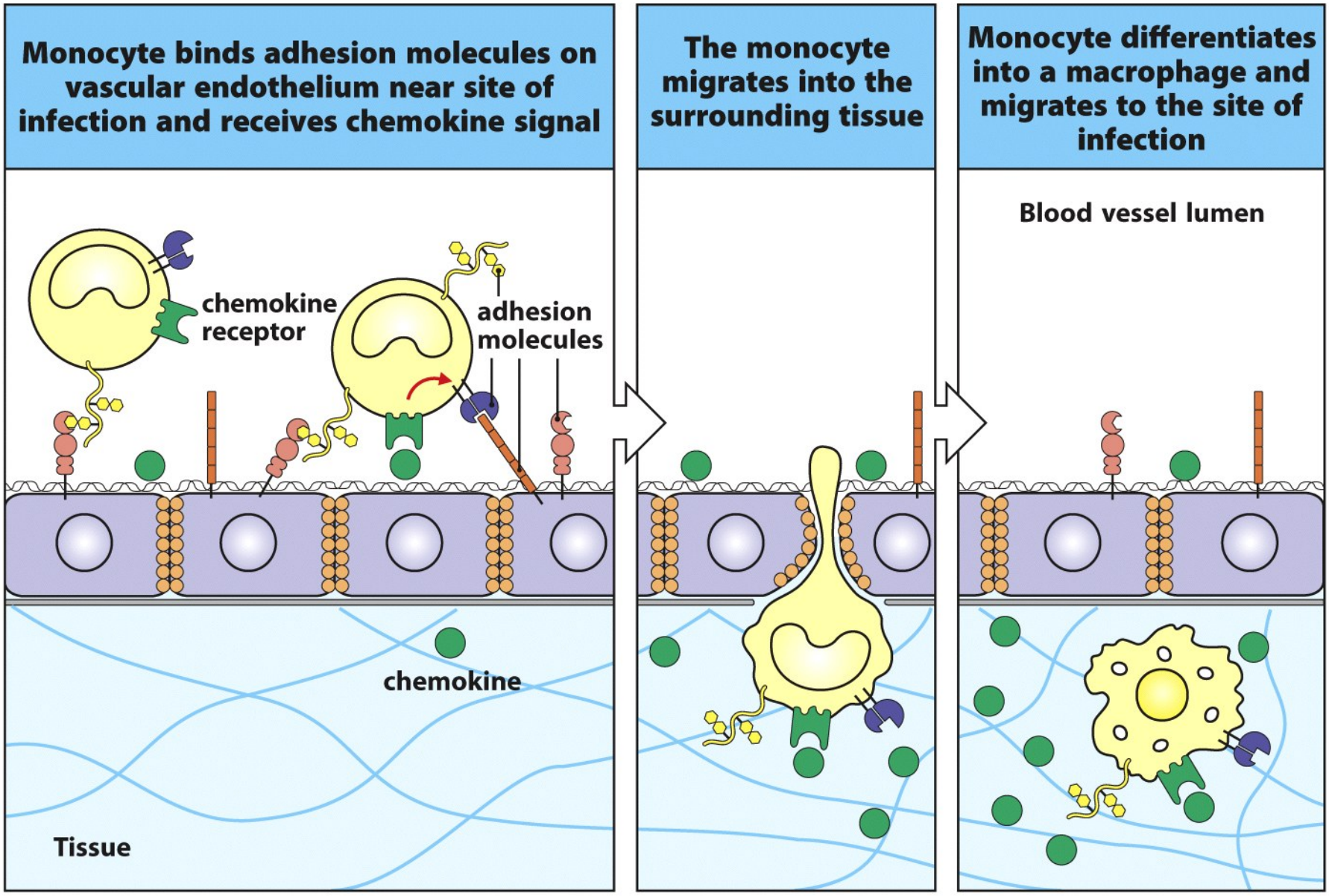
Extravasation

- Neutrophils arrives first
  - Bind to endothelium when E-selectin and P-selectin are expressed by endothelium

- Monocytes
  - Come in much later
  - This is because it takes time for inflamed endothelial cells to express CAMs (such as ICAM-1 and VCAM-1)



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Lymphocyte Extravasation

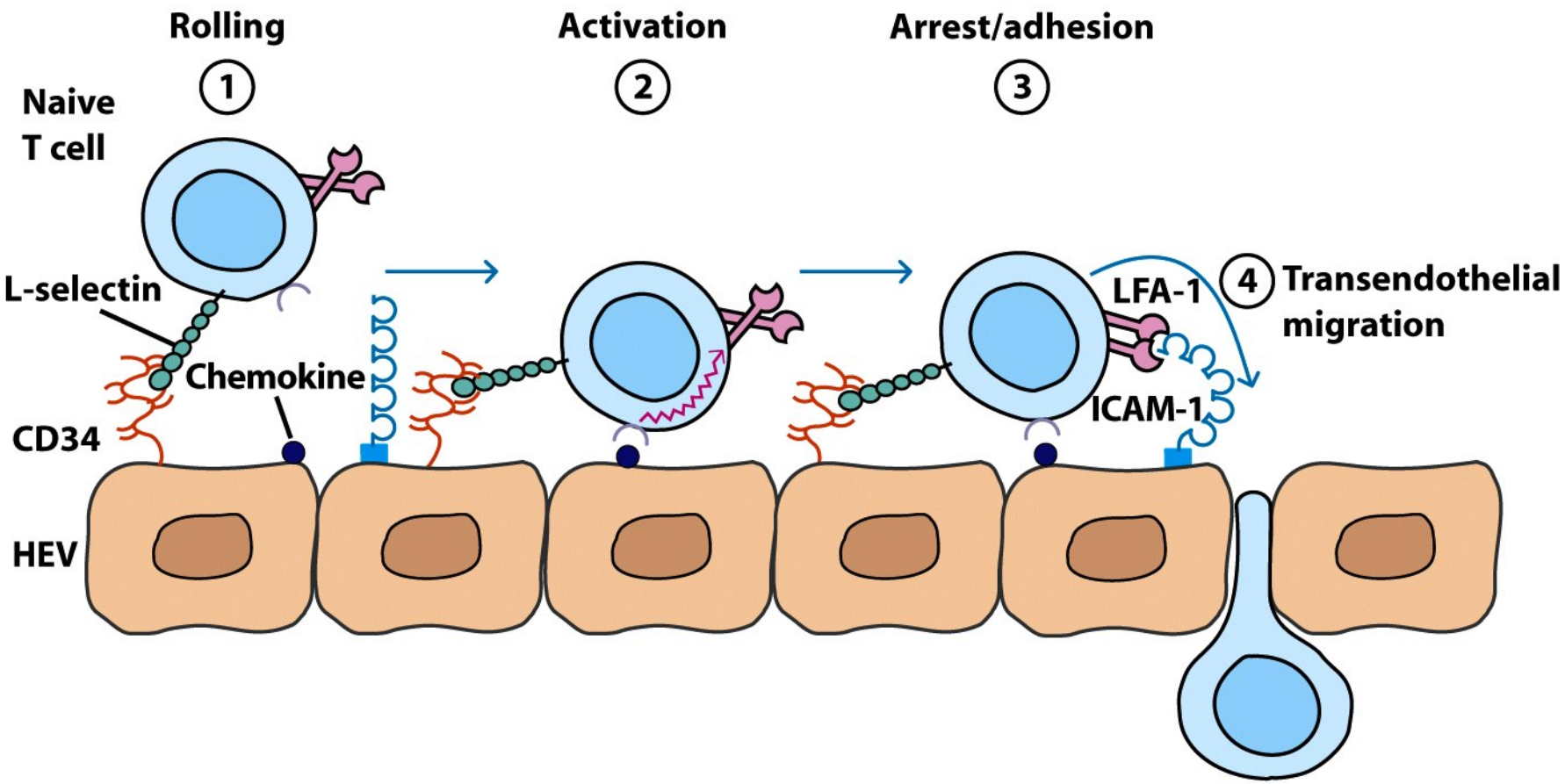

Figure 13-8
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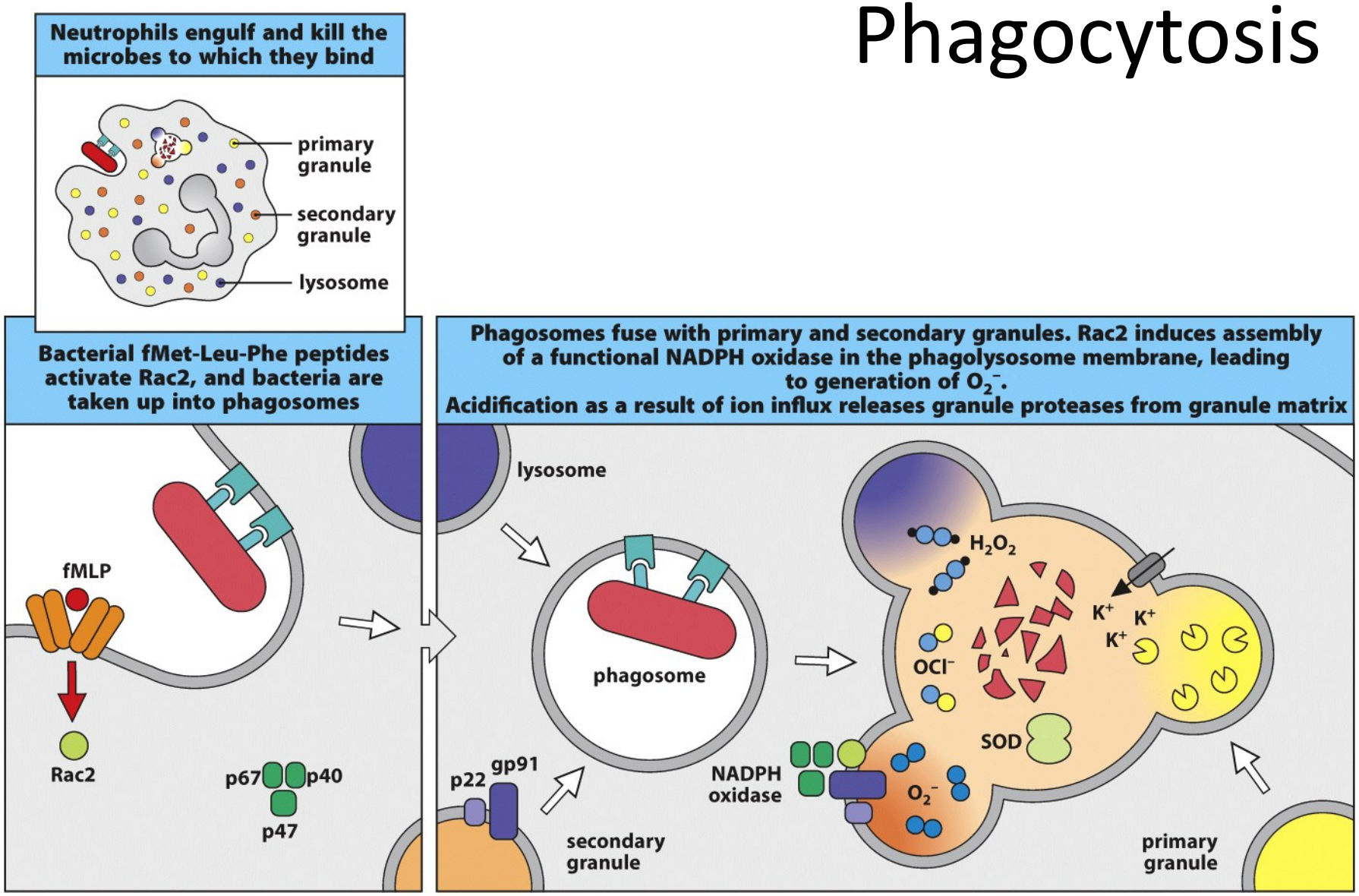
Phagocytosis



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Antimicrobial mechanisms of phagocytes

Class of mechanism	Macrophage products	Neutrophil products
Acidification	pH=~3.5–4.0, bacteriostatic or bactericidal	
Toxic oxygen-derived products	Superoxide O_2^-, hydrogen peroxide H_2O_2, singlet oxygen $^1O_2^{\bullet}$, hydroxyl radical $^{\bullet}OH$, hypohalite OCl^-	
Toxic nitrogen oxides	Nitric oxide NO	
Antimicrobial peptides	Cathelicidin, macrophage elastase-derived peptide	α-Defensins (HNP1–4), β-defensin HBD4, cathelicidin, azurocidin, bacterial permeability inducing protein (BPI), lactoferricin
Enzymes	Lysozyme: digests cell walls of some Gram-positive bacteria Acid hydrolases (e.g. elastase and other proteases): break down ingested microbes	
Competitors		Lactoferrin (sequesters Fe^{2+}), vitamin B_{12}-binding protein

Figure 3.4 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Antimicrobial Factors

① **Cathelicidins:**

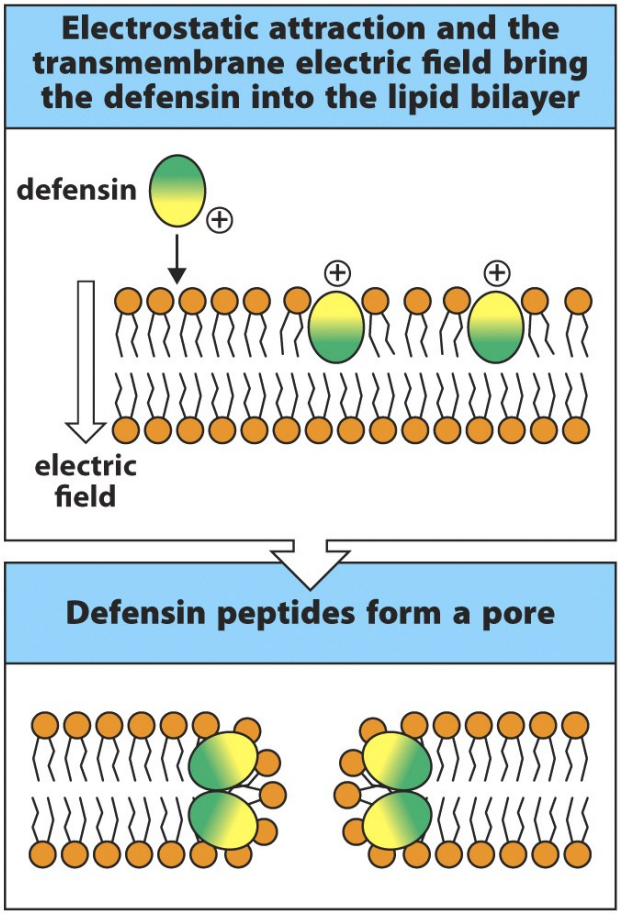
- Proteins produced by neutrophils, keratinocytes, and epithelial cells of the respiratory and GIT
  - In PMN: broad-spectrum antimicrobial activity
  - In epithelium: neutralization of LPS
  - In keratinocytes: chemotactic for phagocytes and T cells

- Important in host defense against Group A streptococci that cause necrotic skin lesions

Antimicrobial Factors

② **Defensins:**

- Small proteins produced by leukocytes and tissue cells (**α-defensins** in PMNs, Mϕ, and Paneth cells), (**β-defensins** in most leukocytes and epithelial cells)
  - Broad-spectrum antibacterial activity
  - Create voltage-dependent channels in bacterial membranes allowing water influx



Defensins

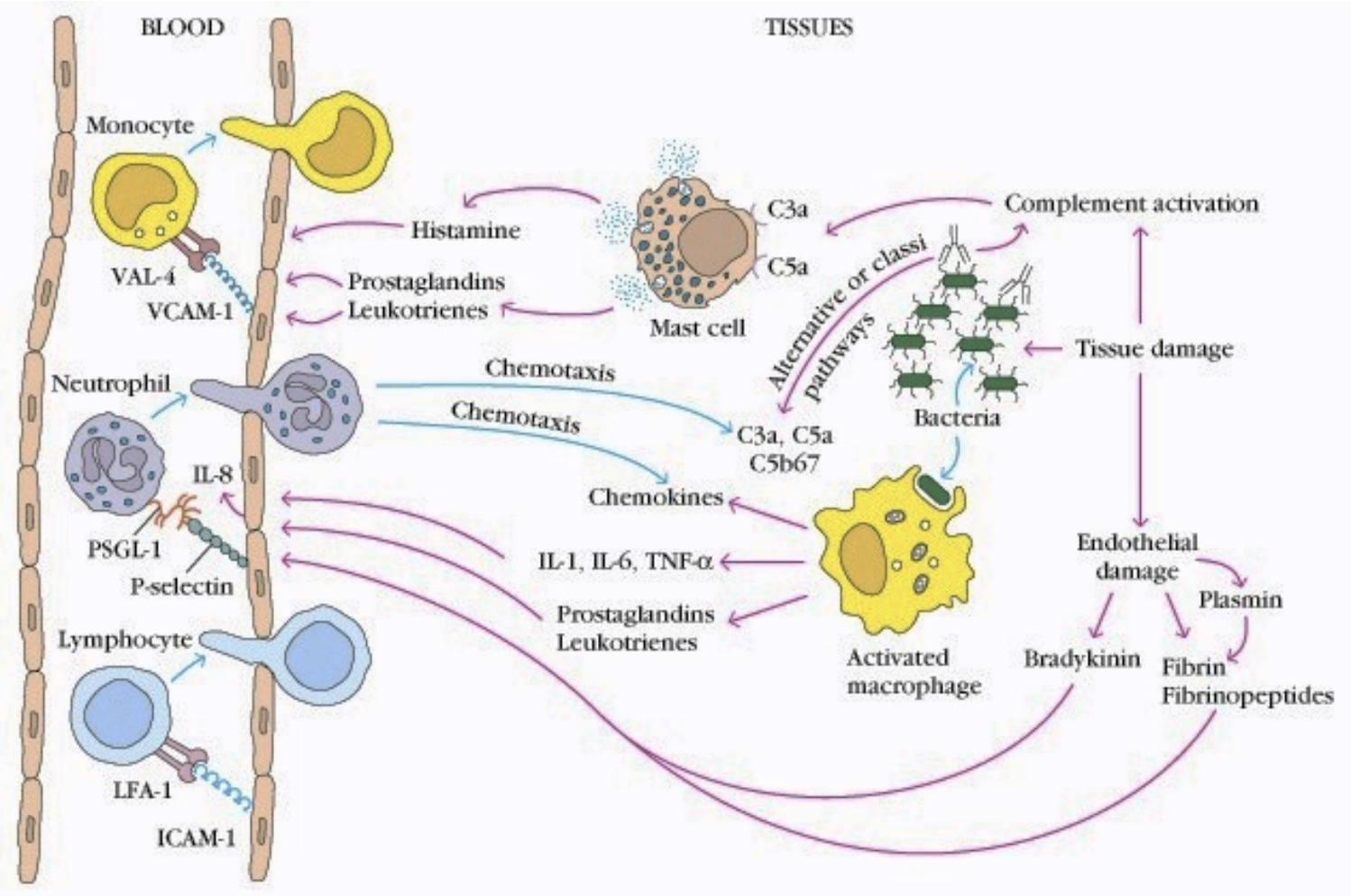
- ***Crypticidins*** (secreted by Paneth cells) serves to reduce the number of bacteria in the intestinal lumen

- ***Tracheal antimicrobial peptide*** in the airway prevents infection by virulent and opportunistic pathogens.
  - Patients with **cystic fibrosis** have tracheal antimicrobial peptide inactivated by high salt concentration in the respiratory mucosa allowing for respiratory infection by opportunistic pathogens such as ***Pseudomonas aeruginosa***

Antimicrobial Factors

③ **Nitric Oxide:**

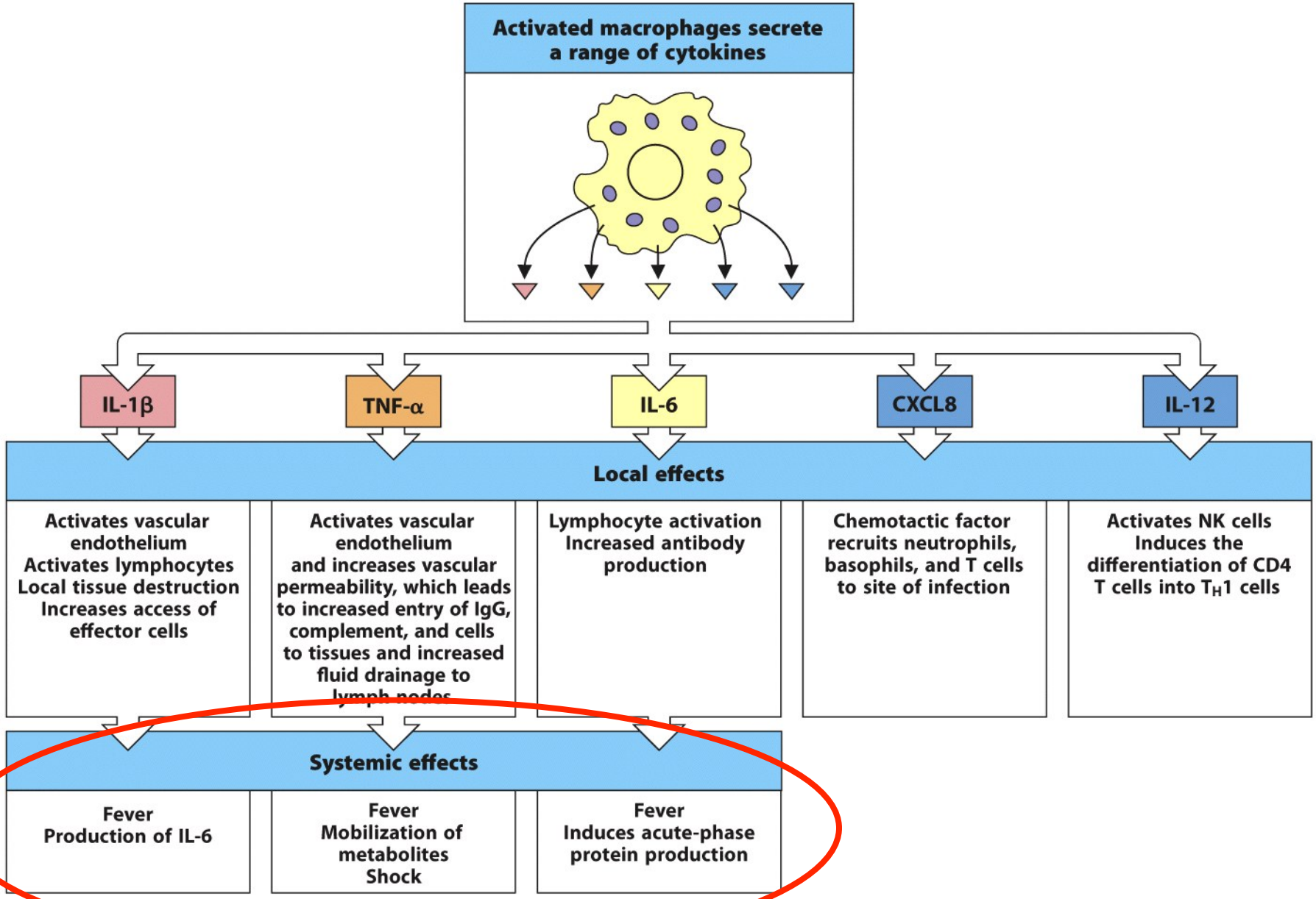
- Highly-reactive molecule produced by neutrophils and macrophages following TLR interaction with PAMPs
- Nitric oxide synthase oxidizes L-arginine to L-citrulline and NO
- Exposure to NO is cytotoxic to bacteria, fungi, parasites, and tumor cells
- NO inhibits DNA synthesis and mitochondrial respiration
- Reaction between NO and O_2 forms NO_2 (nitrogen dioxide)


BLOOD
TISSUES
Monocyte
VAL-4
VCAM-1
Histamine
Prostaglandins
Leukotrienes
Mast cell
C3a
C5a
Complement activation
Alternative or classi
pathways
Tissue damage
Bacteria
Neutrophil
Chemotaxis
Chemotaxis
C3a, C5a
C5b67
IL-8
Chemokines
PSGL-1
P-selectin
IL-1, IL-6, TNF-α
Prostaglandins
Leukotrienes
Activated
macrophage
Endothelial
damage
Plasmin
Bradykinin
Fibrin
Fibrinopeptides
Lymphocyte
LFA-1
ICAM-1

Inflammation Responses

- Localized Inflammatory Response
  - Duration and intensity must be carefully regulated to control tissue damage

- Systemic Acute Phase Response
  - Localized accompanied by acute phase response
    - Induction of fever
    - Increased production of WBCs



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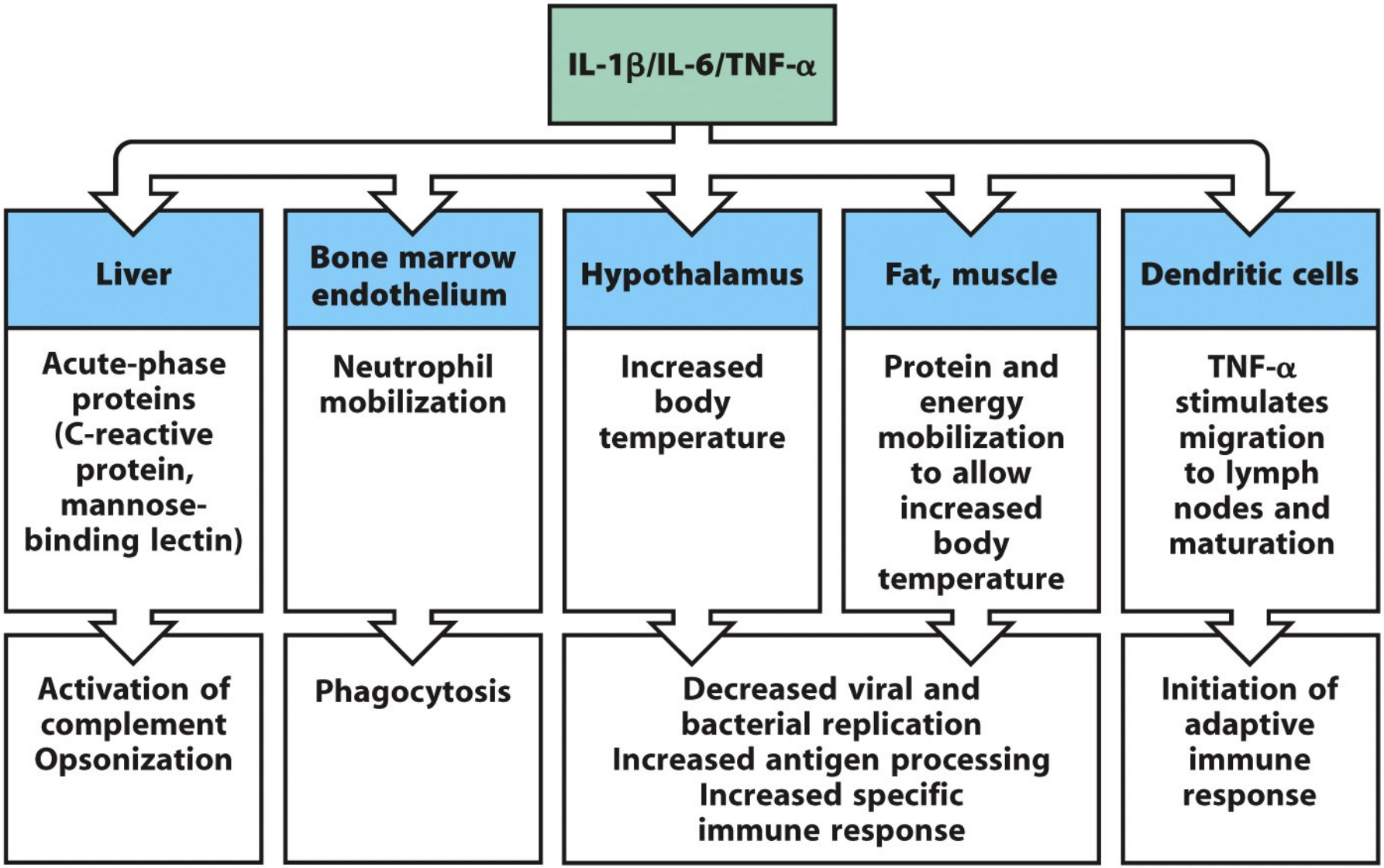
Acute Phase Response

- Besides local inflammation, tissue injury or infection initiate an **acute phase response (APR)**, which is a collective serum changes characterized by **fever**, **demargination of PMNs**, increased synthesis of **hormones** such as **ACTH and hydrocortisone**, increased **WBC production** by the bone marrow, and the synthesis of **APR proteins**

APR Proteins

- Proinflammatory cytokines produced by activated macrophages such as TNF-α, IL-1, and IL-6 increase local blood flow to the affected area

- IL-6 induces the liver to produce APR proteins such as:
  - C-reactive protein (CRP)
  - Type I interferons
  - Fibronectin
  - Fibrinogen
  - Ferritin
  - Ceruloplasmin
  - Haptoglobin
  - Complement system components
  - Serum amyloid protein



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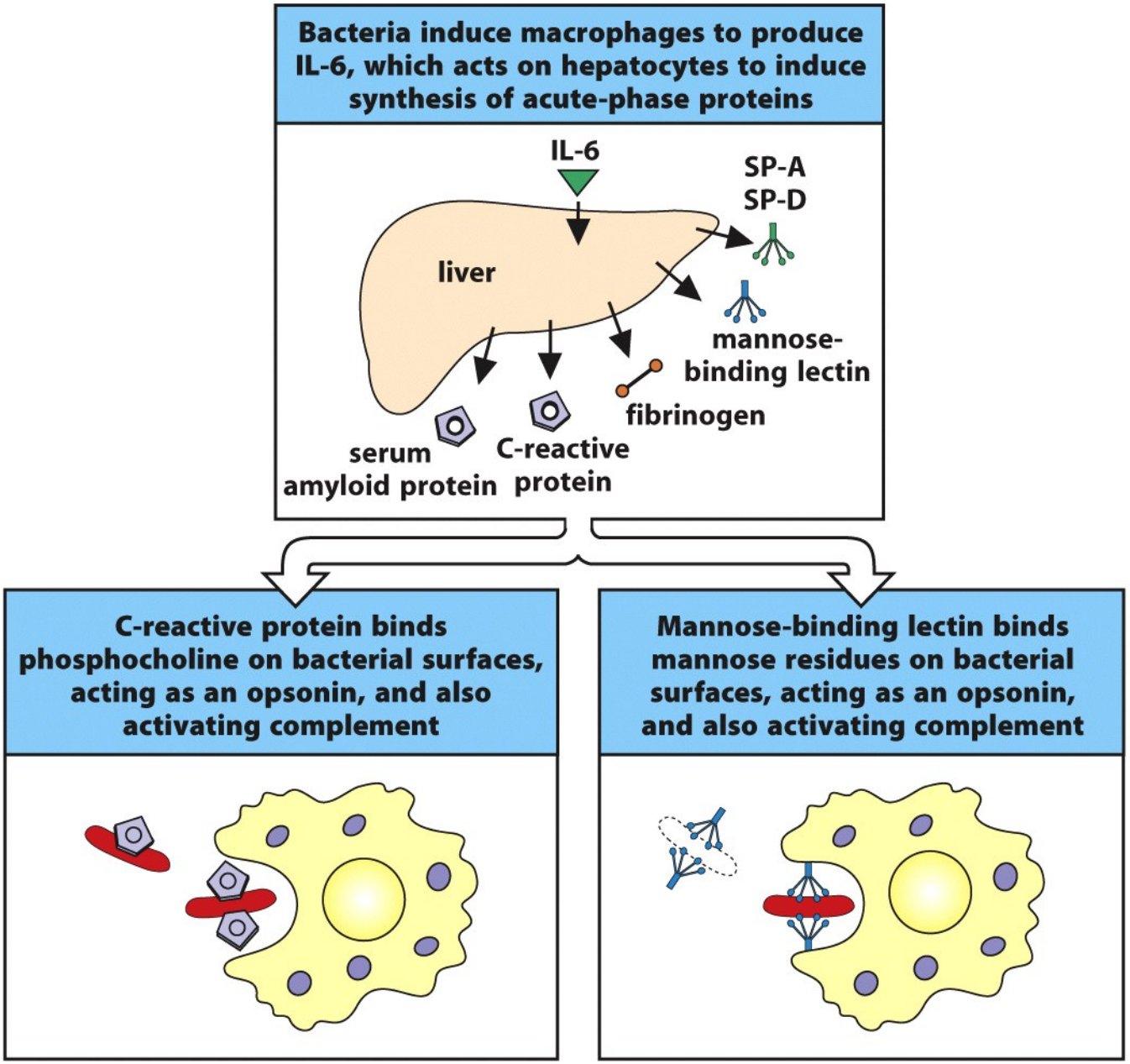

Figure 3.28 part 1 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

APR Proteins

- Acute-phase proteins inhibit bacterial growth and activate complement system cascade
- APR proteins inhibit the spread of infectious organisms
- Some APR proteins can act on the hypothalamus causing hyperthermia and fever

C-Reactive Protein

- CRP is a representative acute-phase protein
- CRP blood levels increases 1000 folds within 24 to 48 hours
- Composed of 5 identical polypeptides held together by non-covalent interactions

C-Reactive Protein

- CRP binds to a wide variety of microorganisms and activates complements
  - Disposition of opsonins on bacterial surface

- CRP is a key marker of cardiovascular risk
- CRP is associated with higher risk of coronary heart disease
- Administration of **statins** reduces CRP levels

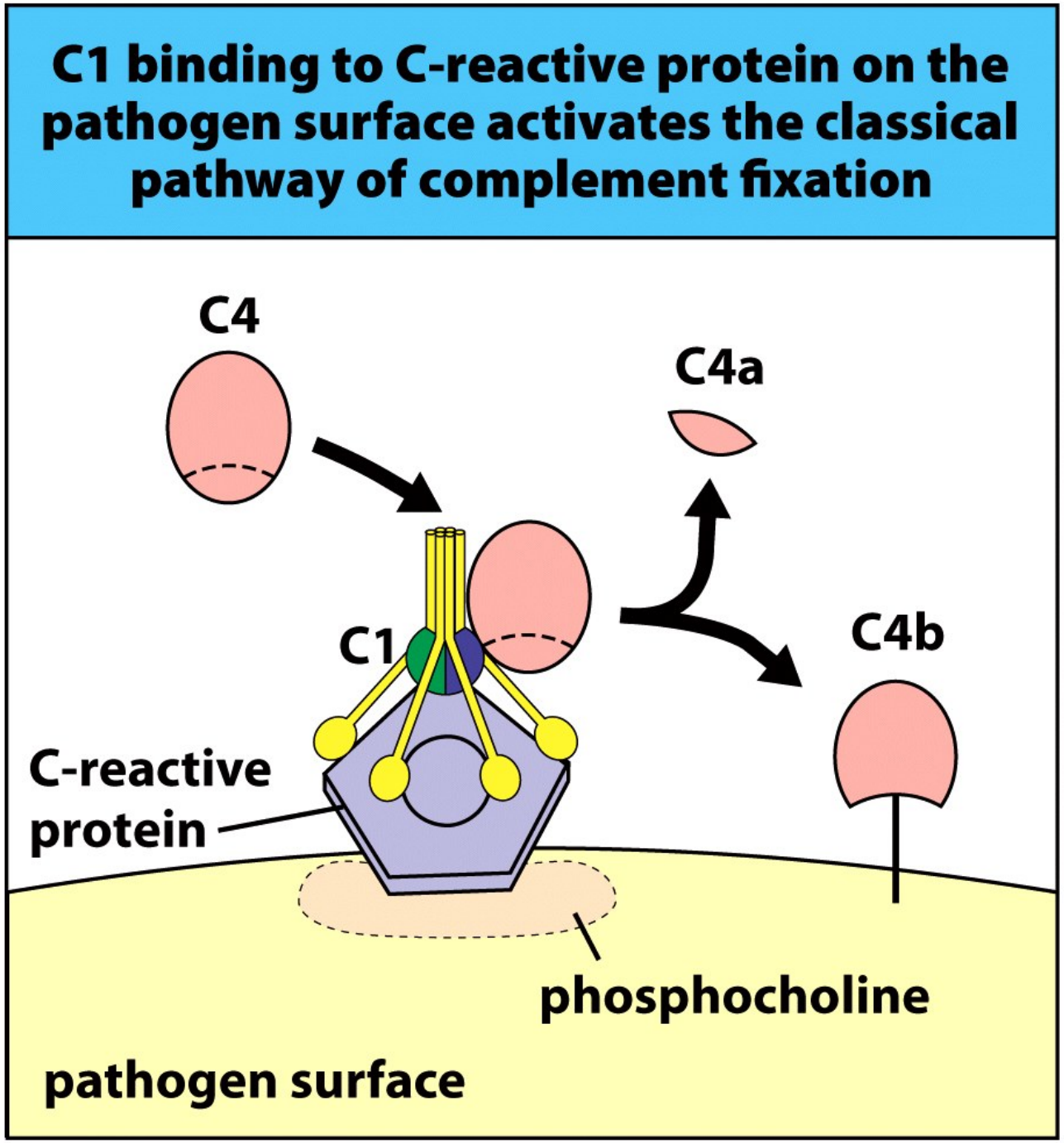

Figure 2.43 The Immune System, 3ed. (© Garland Science 2009)

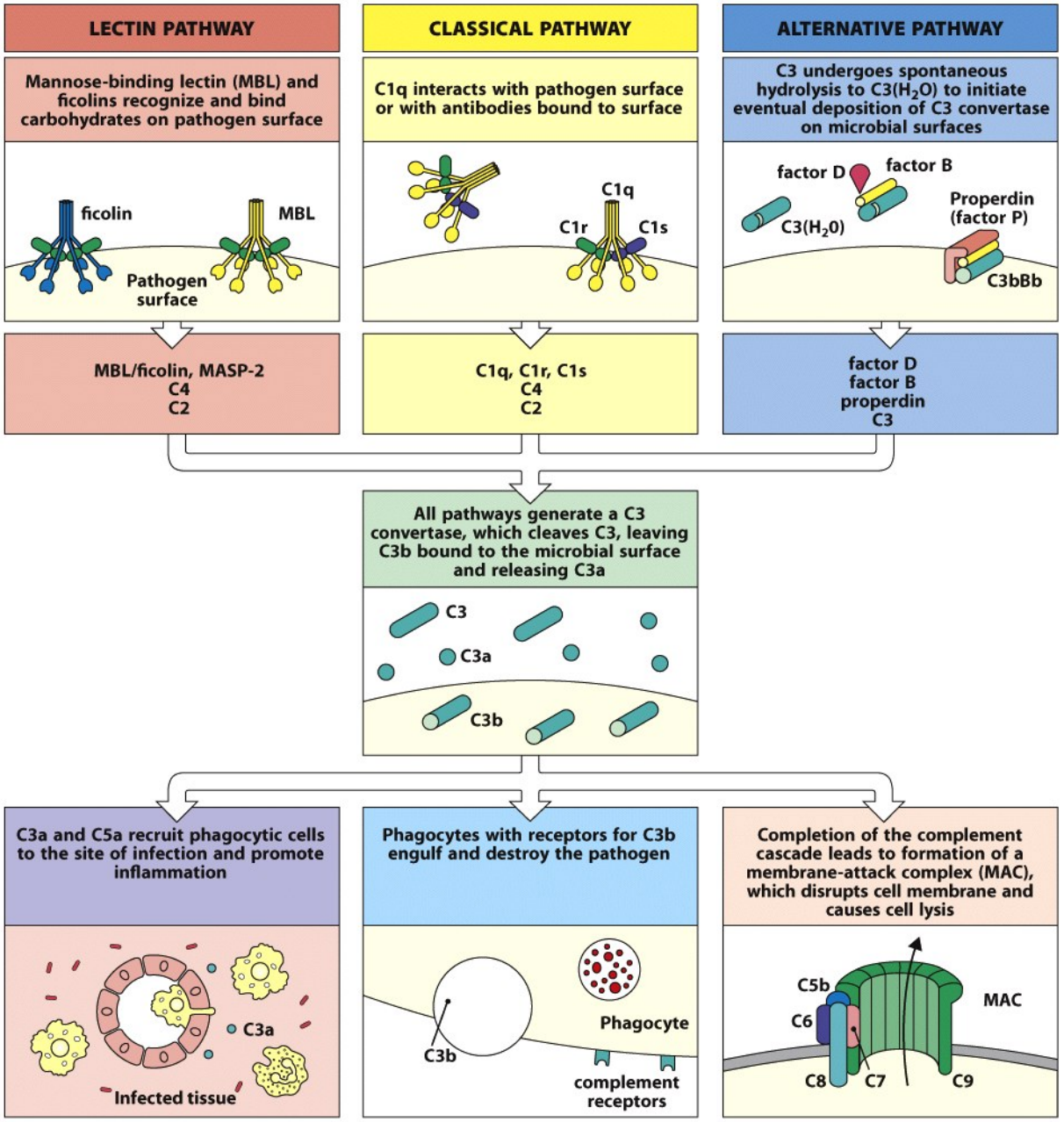

Figure 2.12 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

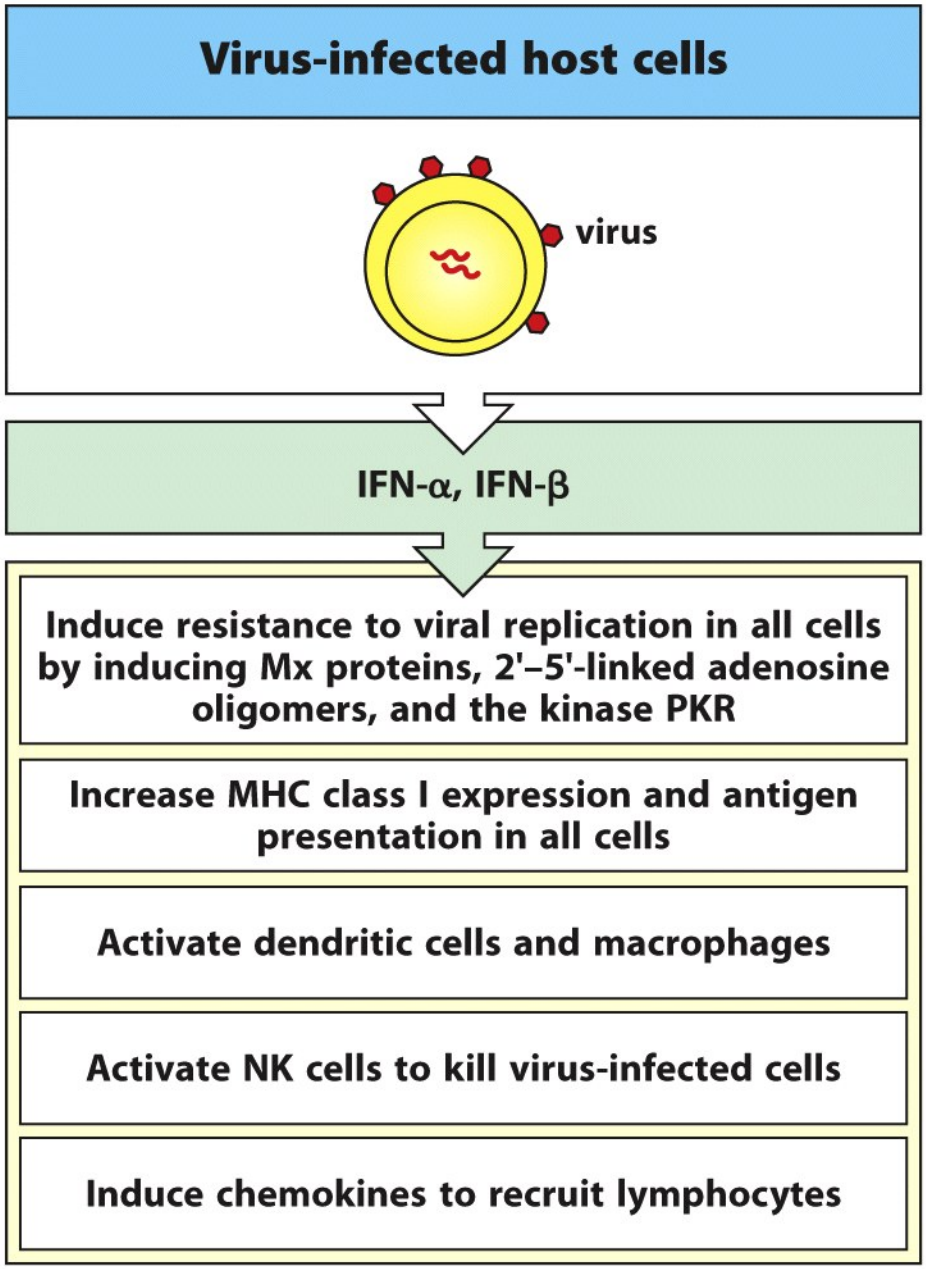

Figure 3.29 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

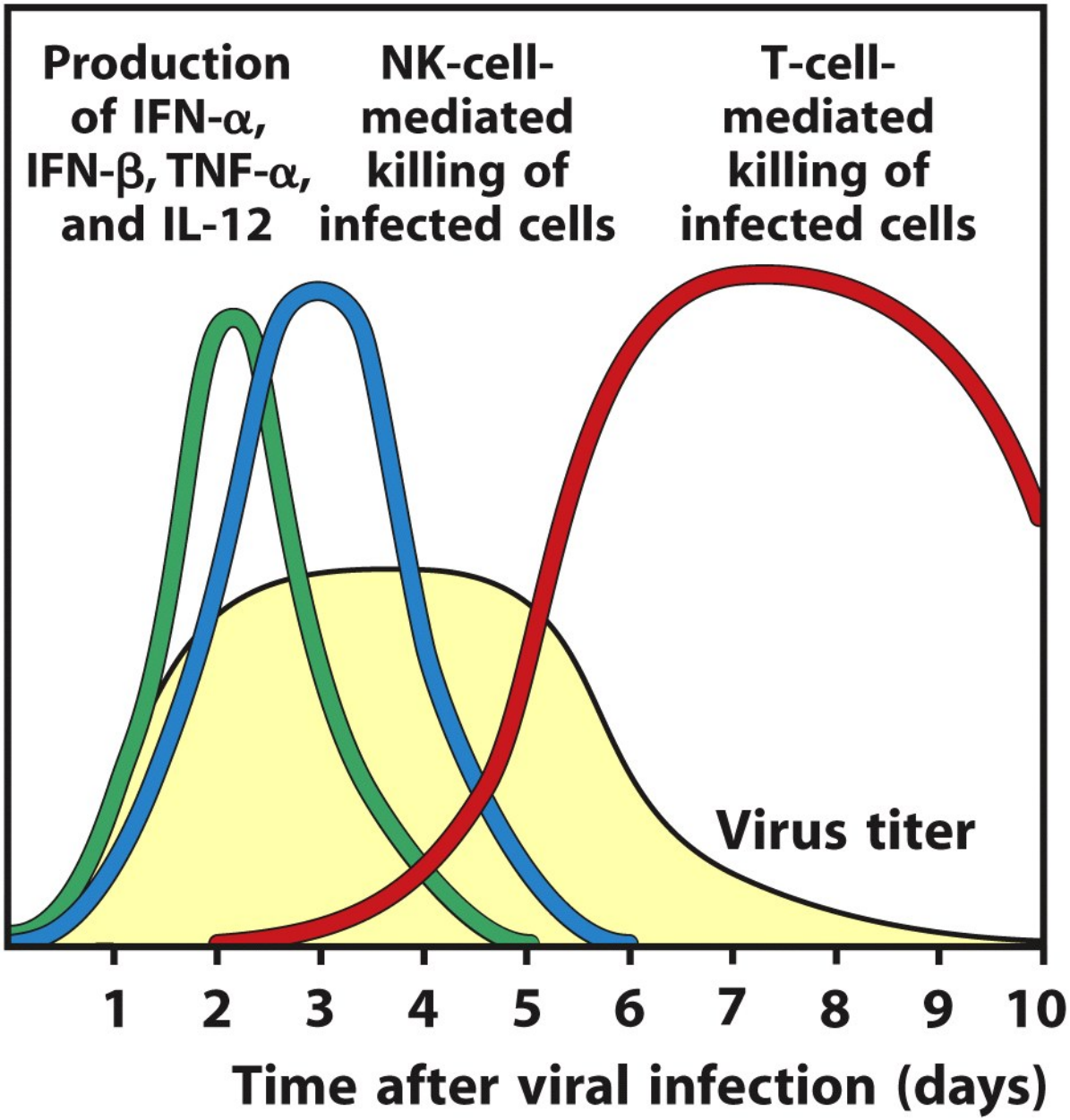

Figure 3.30 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

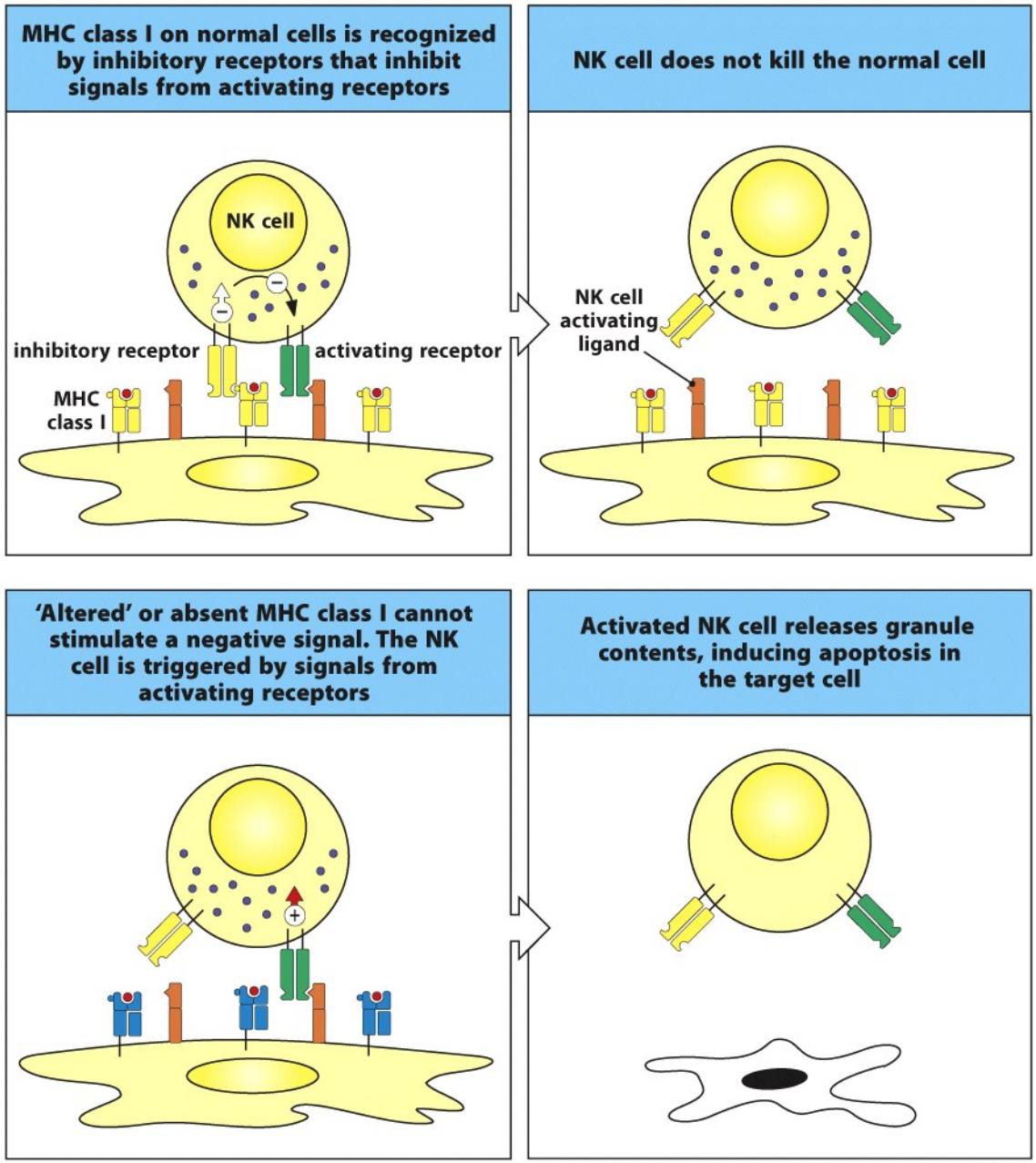

Figure 3.31 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

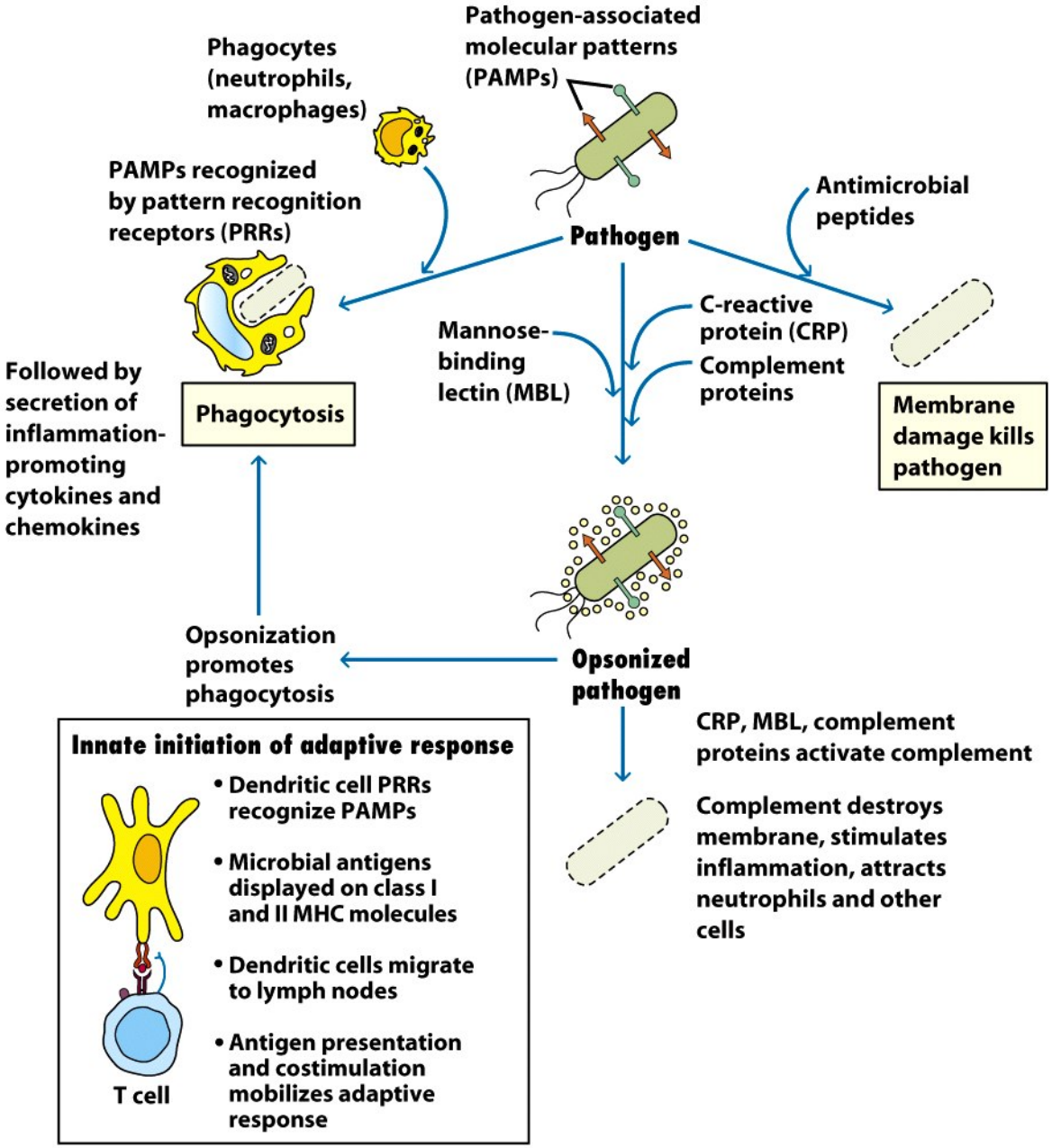

Figure 3-4
Kuby IMMUNOLOGY, Sixth Edition

You are now able to:

- ✓ Describe anatomical and physiological barriers against microbial infections
- ✓ Define **P**athogen-**A**ssociated **M**olecular **P**atterns and **P**athogen-**R**ecognition **R**eceptors
- ✓ Recognize the complement system
- ✓ Explain the mechanism of inflammation
- ✓ Describe acute-phase response