Innate Immunity- First Line of Defense

What is the Innate Immune System?

- includes physical, chemical, and cellular barriers
- **physical** barriers include skin and mucus membranes
- **chemical** barriers include stomach acidity, secreted anti-microbial peptides
- **cellular** barriers include macrophages, neutrophils
- innate immune response activation occurs within minutes of pathogen recognition

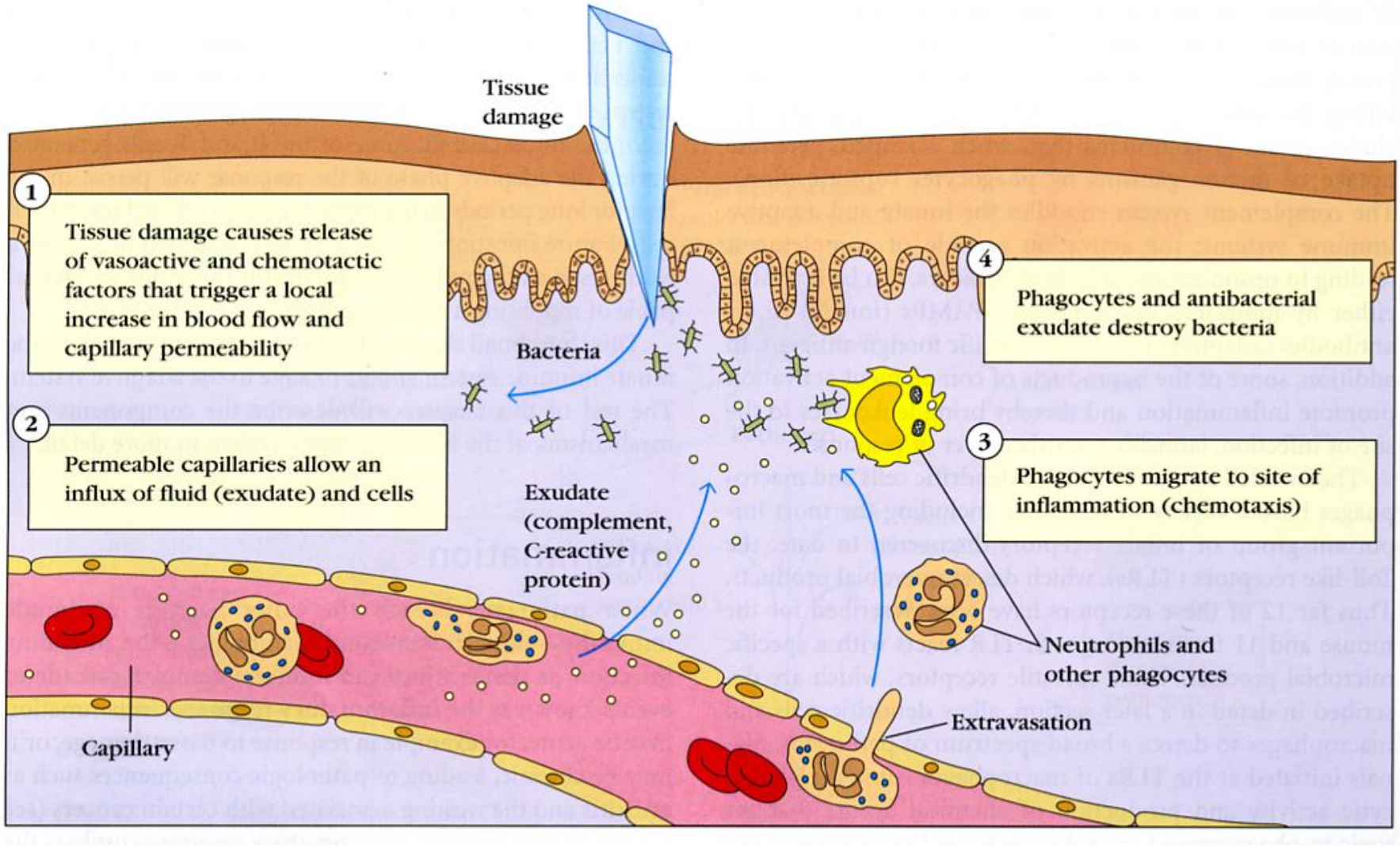
Epithelial defense mechanisms

Examples of chemical barriers:

- lysozyme, phospholypase A (saliva, tears)
- acid pH (stomach)
- anti-fungal peptides called alpha-defensins (intestinal tract)
- anti-microbial peptides called beta-defensins (respiratory, urogenital tract)
- surfactant-A and -D proteins opsonize pathogens for enhanced phagocytosis (lung)

Additional nonspecific defense mechanism is endogenous commensial (non-pathogenic) bacterial flora (microbiota)

What happens when the physical and chemical barriers are breached?



Innate Immunity- First Line of Defense

Characteristics:

- rapid
- does not generate immunologic memory
- dependent upon germline encoded receptors recognizing structures common to many pathogens

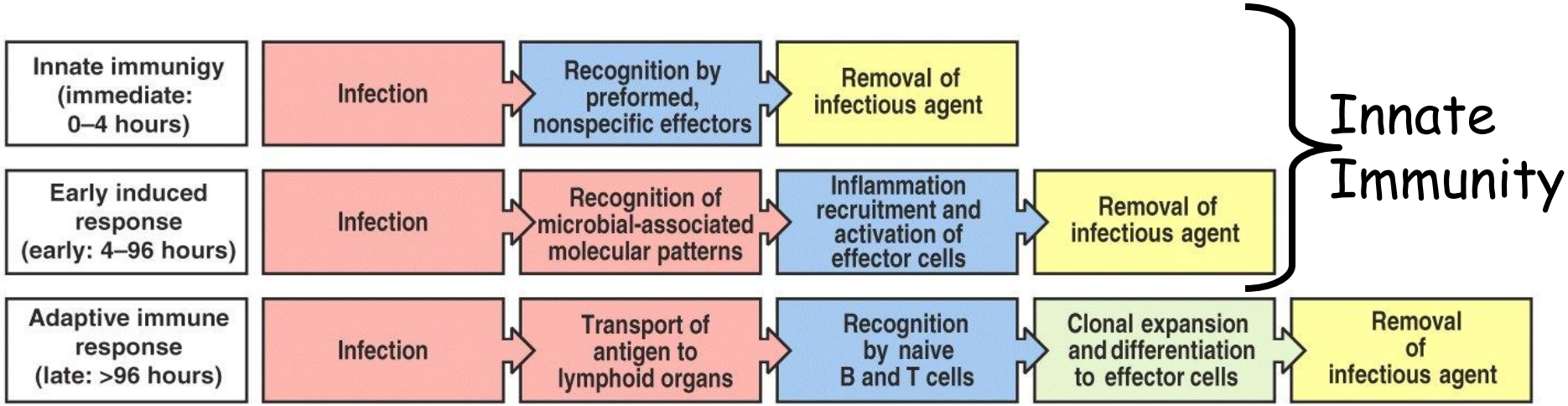
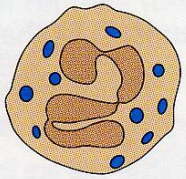
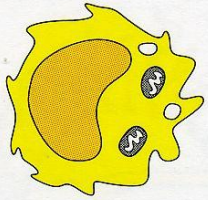
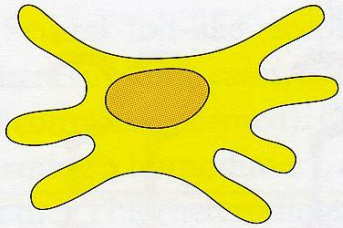
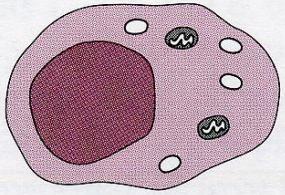


Figure 2-1 Immunobiology, 6/e. (© Garland Science 2005)

Leukocyte Players of Innate Immune Responses

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

Innate Immune Receptors

- Innate immune receptors are not clonally distributed
- Binding of receptors results in rapid response
- Innate immune receptors mediate three functions:
  - phagocytic receptors to stimulate pathogen uptake
  - chemotactic receptors that guide phagocytes to site of infection
  - stimulate production of effector molecules and cytokines that induce innate responses and also influence downstream adaptive immune responses

Pathogen Recognition

- Most microorganisms express repeating patterns of molecular structures termed **Pathogen Associated Molecular Patterns (PAMPs)**

- Innate immune system has evolved mechanisms capable of recognizing these repeating patterns termed **Pattern Recognition Receptors (PRRs)**

- Examples of Pattern Recognition Receptors:
  - Mannose-Binding Lectin (MBL)
  - Macrophage Mannose Receptor
  - Scavenger Receptors
  - Toll-like Receptors (TLRs)
  - Nod-like Receptors (NLRs)
  - RNA helicases (RIG-I, MDA-5)

There are **two functional classes** of pattern-recognition receptors:

1. **endocytic pattern-recognition receptors** – promotes phagocytosis.

Includes:

- **mannose receptor** (a sugar common on the end of bacterial polysaccharides, but not human ones)
- **scavenger receptor** (binds sialic acid-rich molecules)
- **CD14** – receptor of LPS and LPS-binding protein complexes

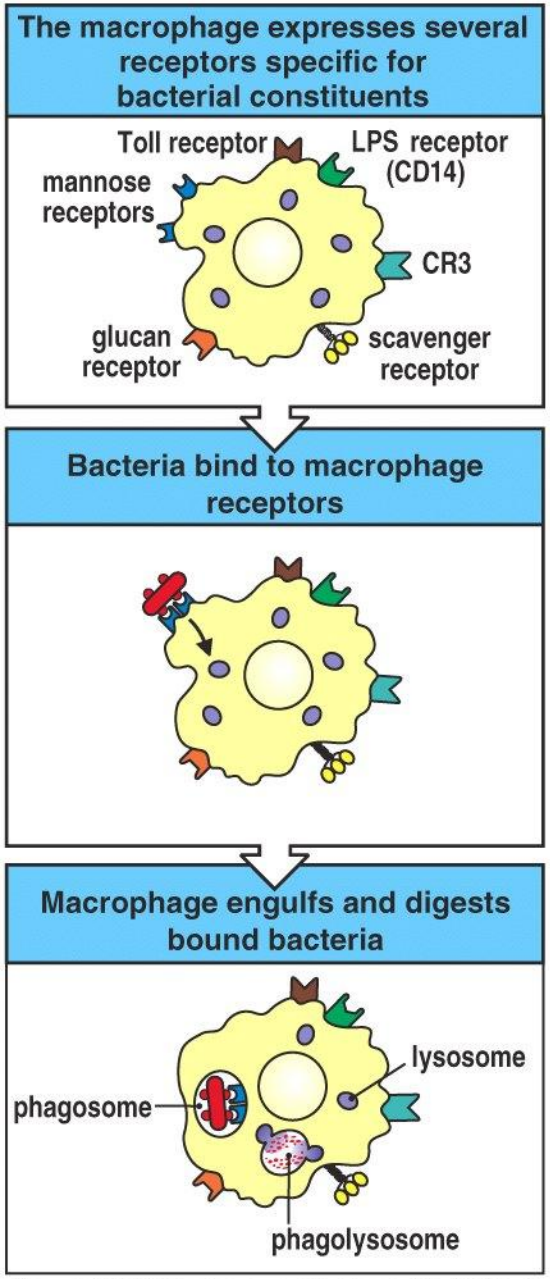


Figure 8-12 The Immune System, 2/e (© Garland Science 2005)

2. **Signaling pattern recognition receptors** – binding of ligand promotes production of cytokines. Includes a family of receptors referred to as **Toll-like receptors**.

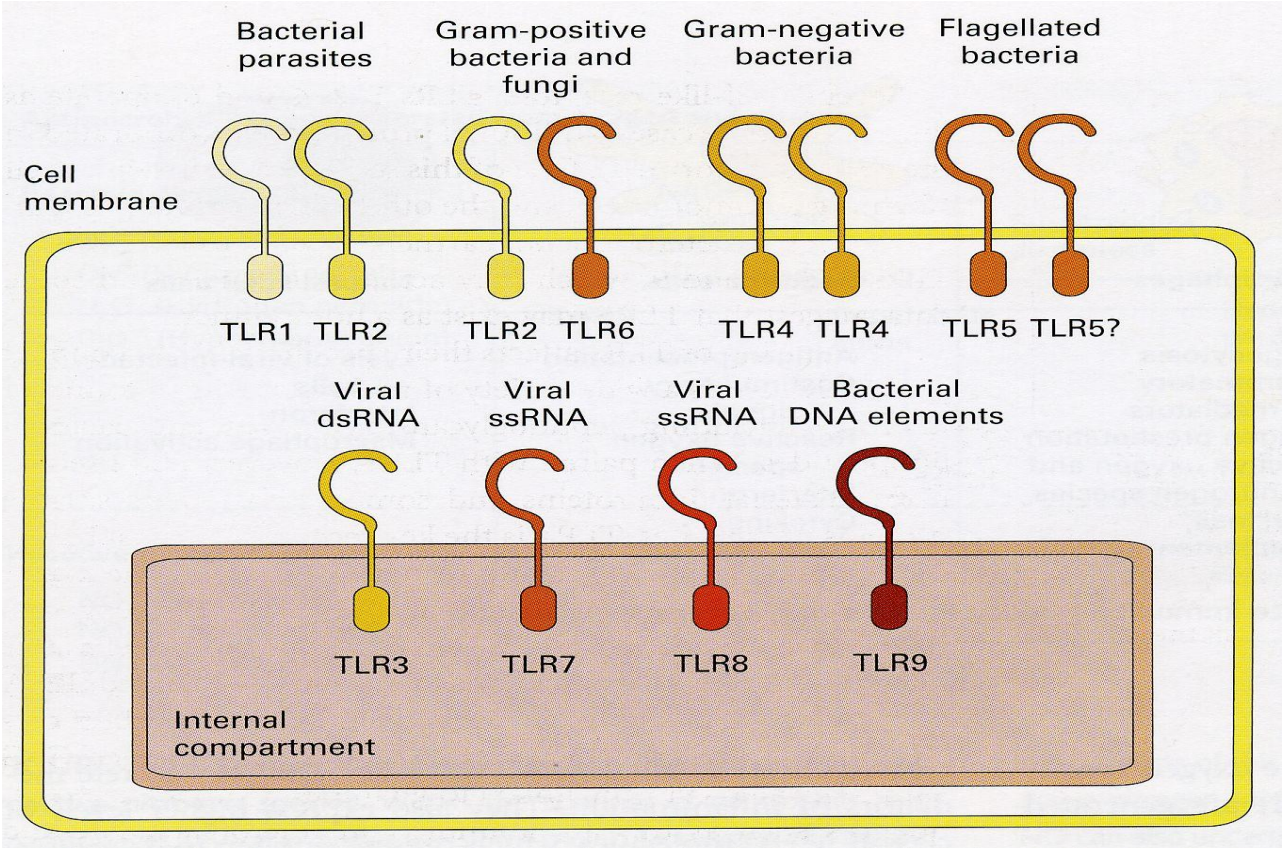
Humans have 10 different toll-like receptors. Each binds a different class of PAMP.

Different TLRs directly or indirectly bind different microbial molecules.

TLR-2 recognizes peptidoglycan and lipoproteins
TLR-3 recognizes double-stranded RNA
TLR-4 recognizes lipopolysaccharide
TLR-5 recognizes bacterial flagellin
TLR-9 recognizes bacterial DNA.

All toll like receptors signal through common pathways to regulate the expression of cytokine genes

Toll-Like Receptors (TLRs)



Cellular Localization:

- Lysosomal localization (i.e. subcellular) of TLR-3 and TLR7-9
- TLR-3 and 7-9 recognize viral/bacterial nucleic acids
- lysosomal expression isolates pathogen nucleic acid recognition away from potential cross-reaction with host mammalian nucleic acid motifs

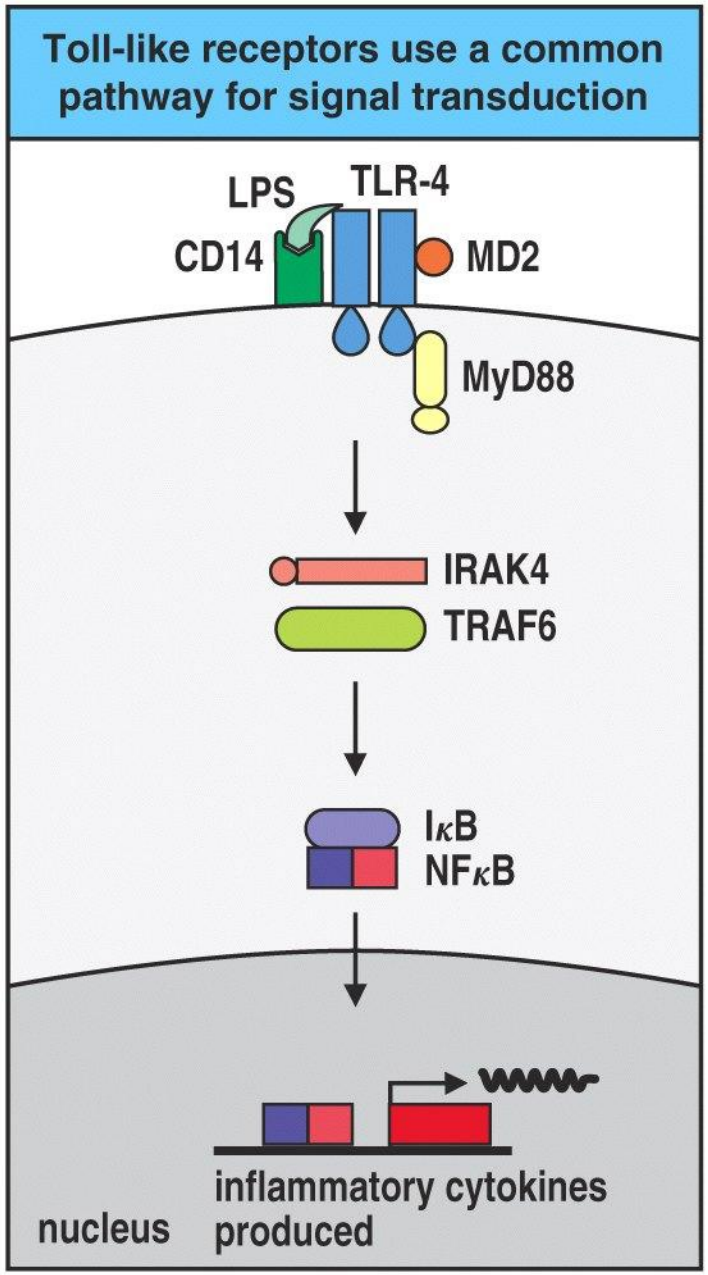
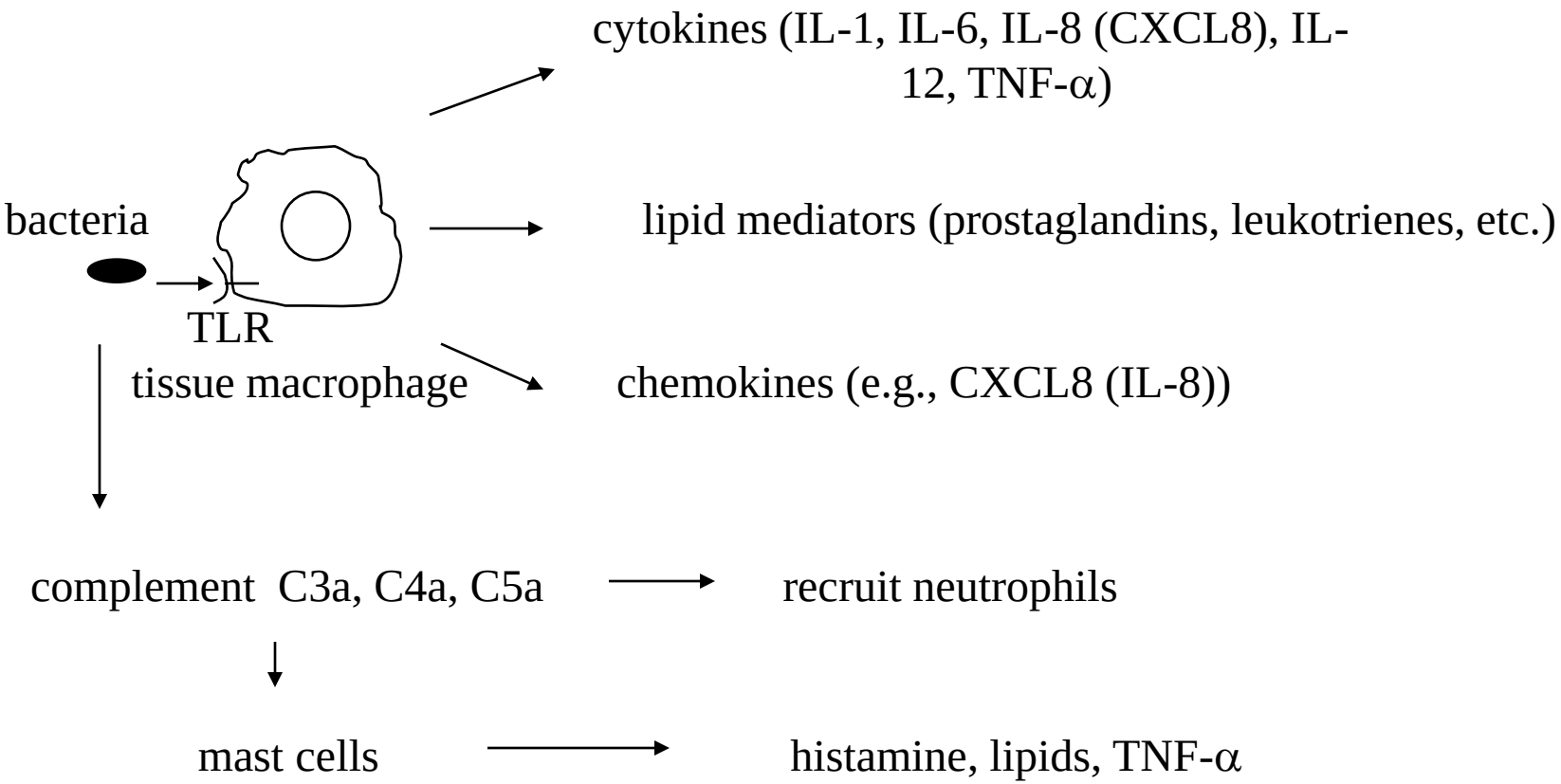


Figure 8-14 The Immune System, 2/e (© Garland Science 2005)

E. The activated macrophages secrete a battery of **cytokines** and **chemokines** that function to initiate the inflammatory response and to recruit other effector cells to the site of infection.



TNF-α acts on vascular endothelial cells to increase vascular permeability.

TNF-α induces vascular endothelial cells to make platelet-activating factor, which promotes blood clotting to block local blood vessels to restrict pathogen from entering blood.

The excessive release of TNF-α by macrophages into the blood stream, as can occur in response to blood-borne pathogens (e.g., a bacterial infection that enters the wider circulation), can produce a dangerous condition known as **sepsis**.

Instead of local blood vessel dilation, systemic vasodilation can occur with massive leakage of fluid into tissues and widespread blood clotting. Causes **septic shock** and can lead to organ failure and death.

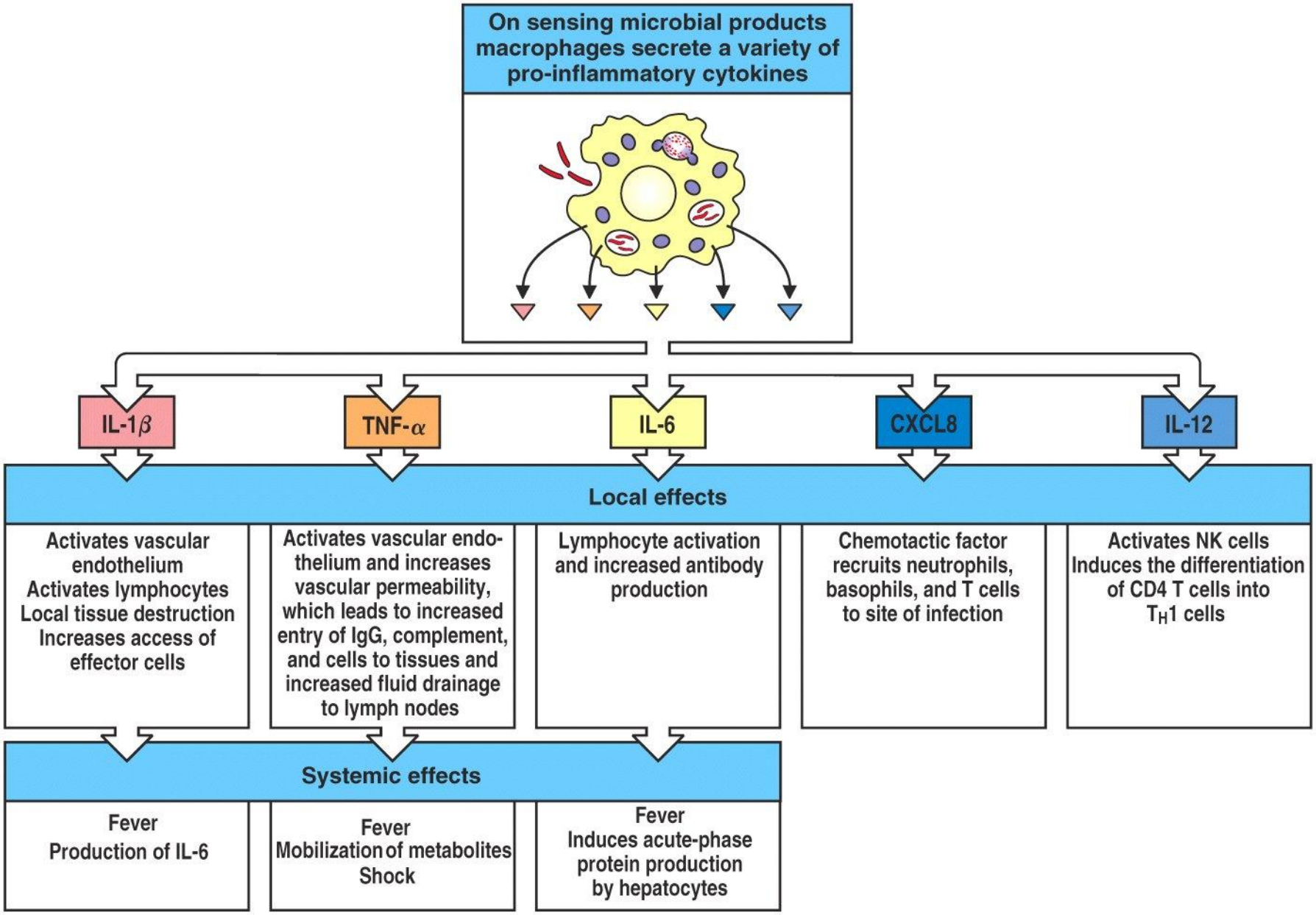


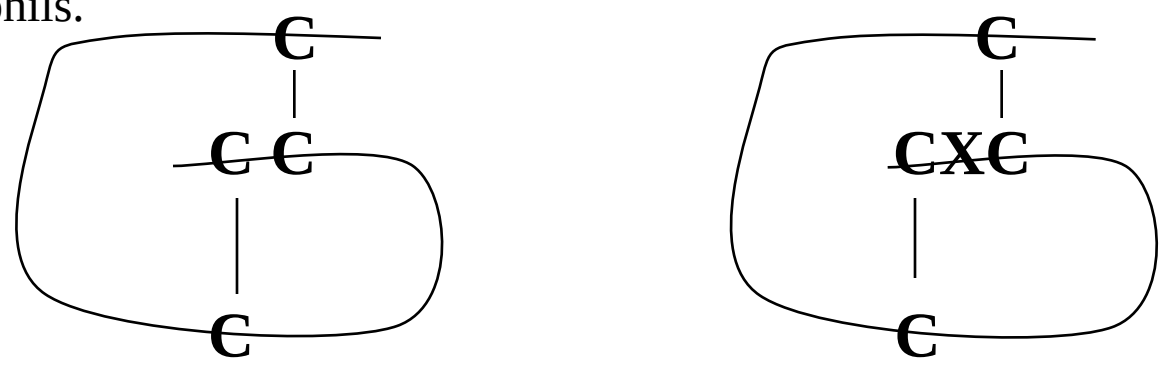
Figure 8-15 The Immune System, 2/e (© Garland Science 2005)

CXCL8 is an example of a chemoattractant or **chemokine.**

Small, 90-130 residue polypeptides. Some are produced all the time and some are produced selectively during an infection to help determine where a cell will go in the body. Over 50 chemokines and 15 chemokine receptors.

Classified based on the sequence around cysteines as the **CC** group (Cys's are adjacent) or the **CXC** group (one amino acid separates the two Cys) (there are also minor groups of C and CXXXC chemokines). Receptors for the CC group are termed **CCR's** (e.g., CCR1, CCR2, etc.) and for the CXC group are termed **CXCR's** (e.g., CXCR1, CXCR2, etc.). Chemokine receptors are G-protein coupled receptors.

IL-8 is a CXC chemokine that binds CXCR1 and CXCR2 to recruit neutrophils.



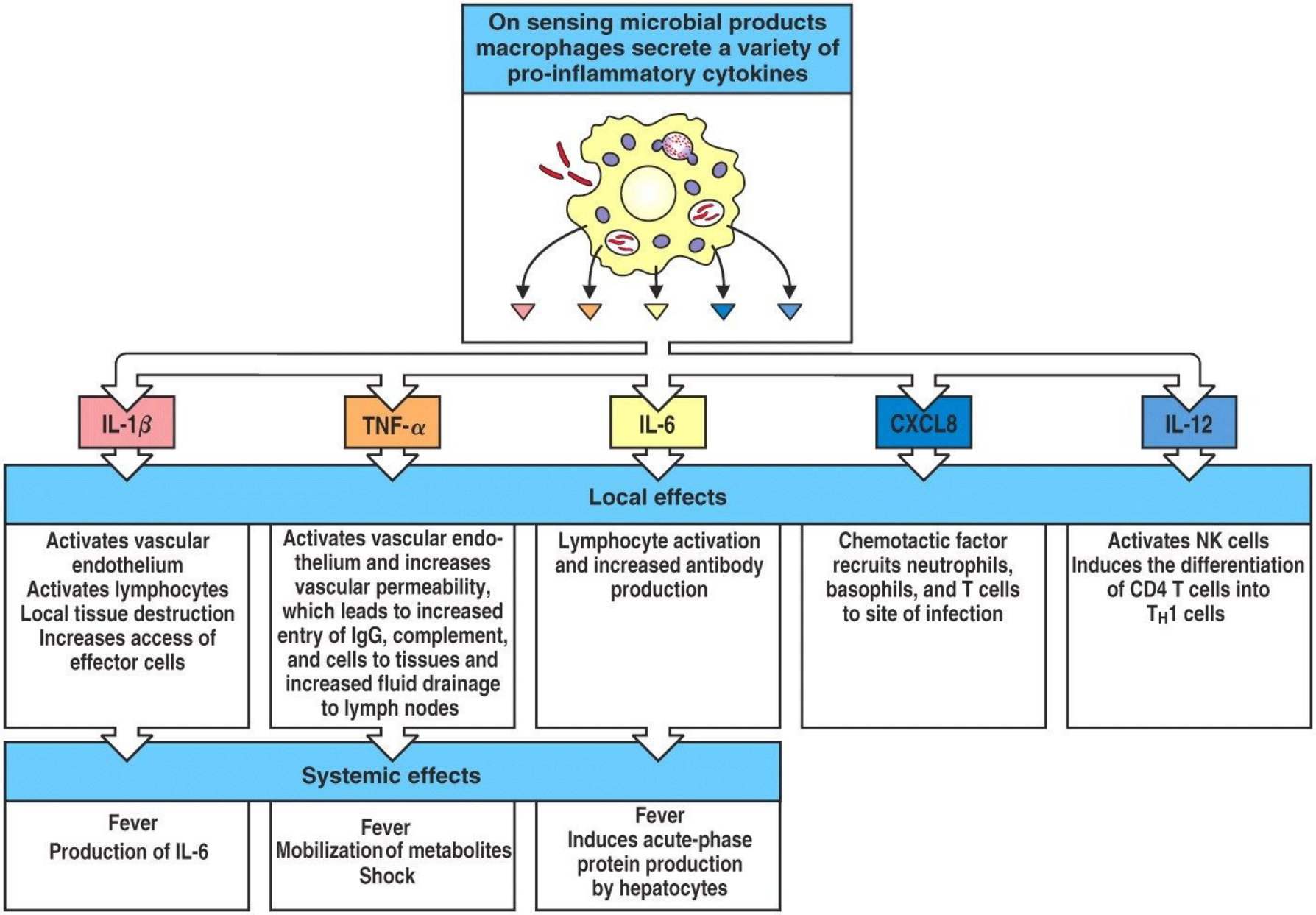
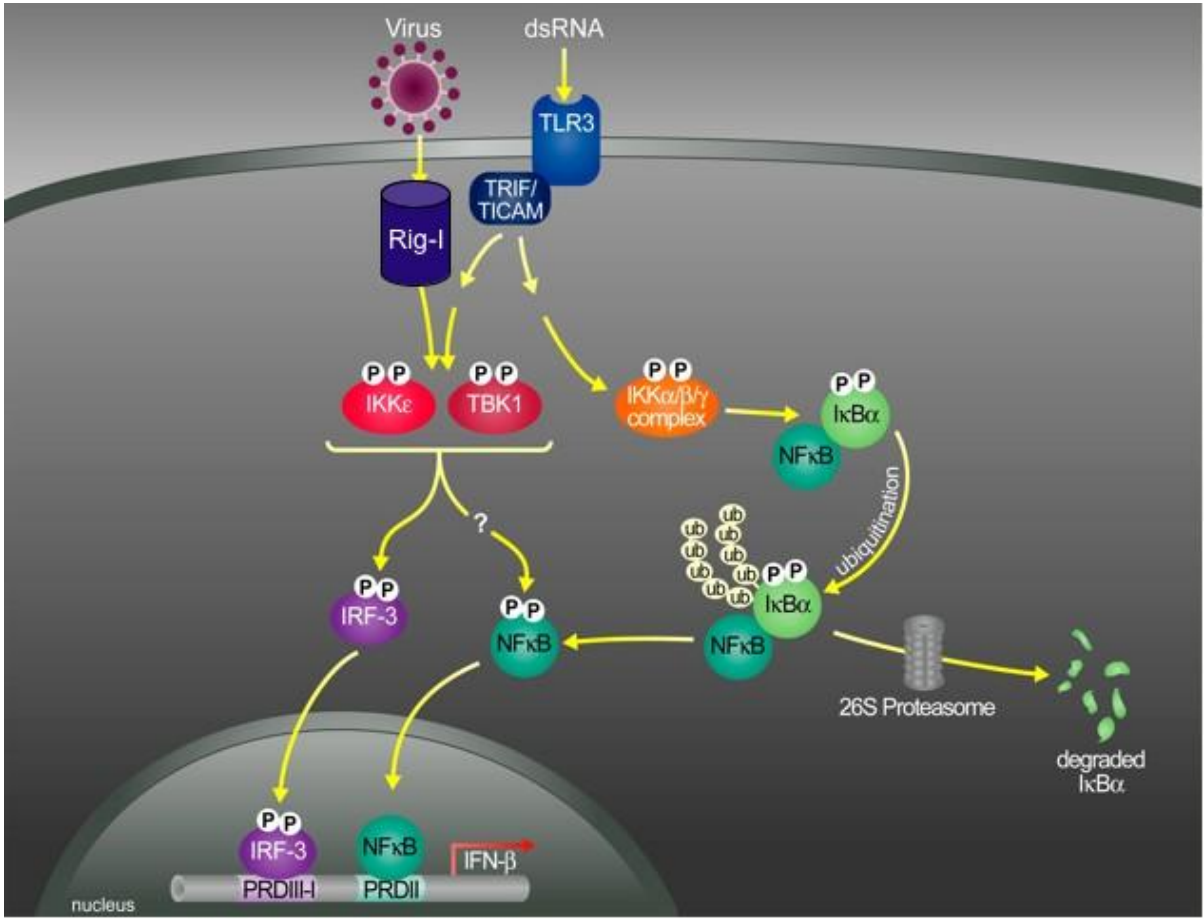


Figure 8-15 The Immune System, 2/e (© Garland Science 2005)

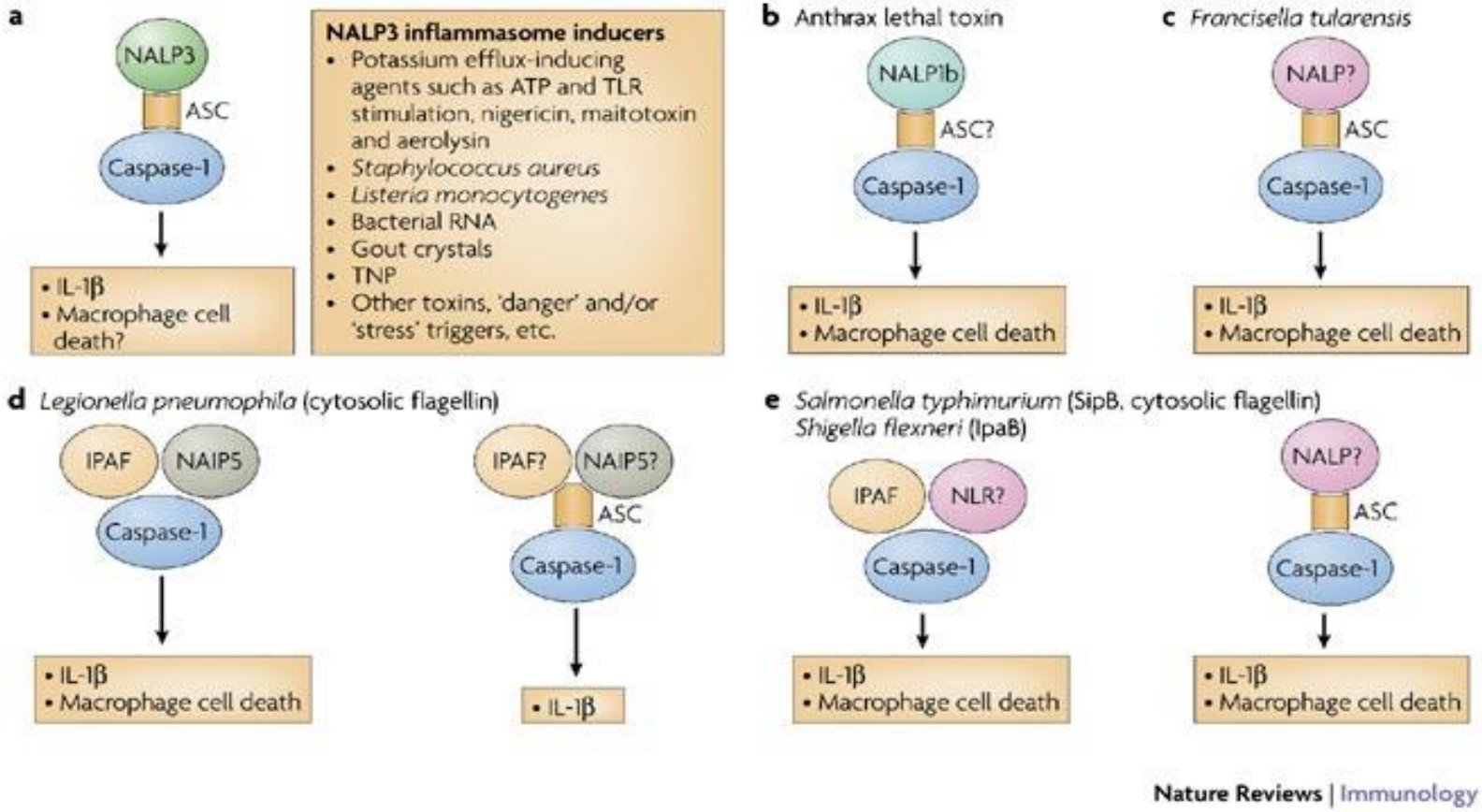
Cells also have cytoplasmic receptors to help sense viral nucleic acid

- RIG-I Is a cytosoIic protein that detects viral RNA.
- 5'-trIphosphate and double-stranded (ds) RNA are two molecular patterns that enable RIG-I to discriminate pathogenic from self-RNA.
- Two N-terminal caspase activation and recruitment domains (CARDs) transmit the signal and the regulatory domain prevents signaling in the absence of viral RNA.
- MDA-5 works similarly



Induce IFN$\alpha\beta$

NLRs are cytoplasmic bacterial sensors that activate IL-1β "inflammasomes"



Mariathasan and Monack *Nature Reviews Immunology* **7**, 31–40 (January 2007) | doi:10.1038/nri1997

nature REVIEWS IMMUNOLOGY

Inflammatory Cytokines

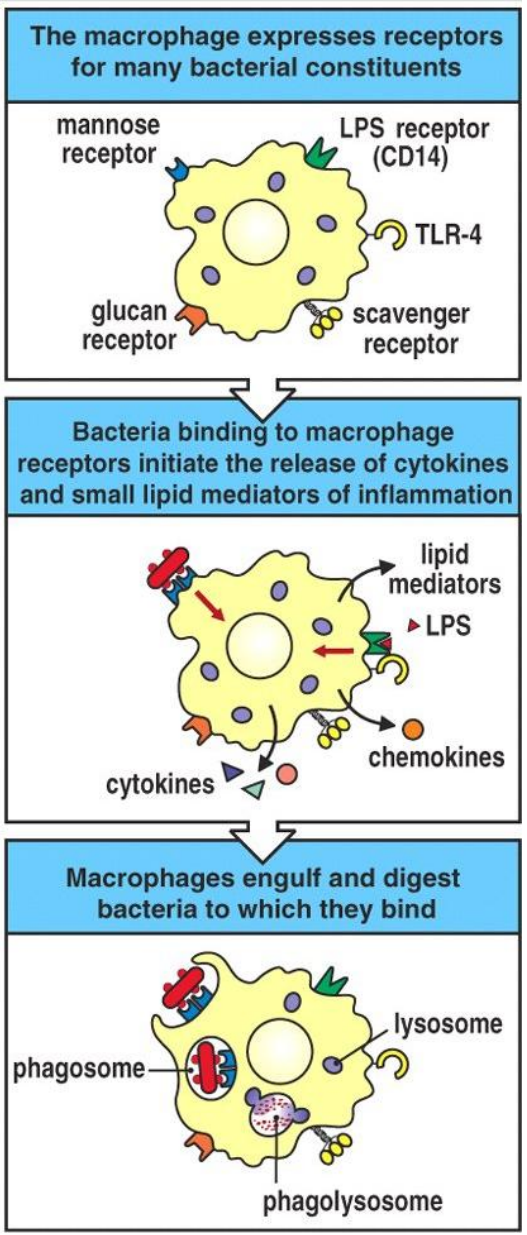
- Activation of TLRs, NLRs and RIG-I type molecules results in production of TNFα, IFN$\alpha\beta$ and IL-1β
- These cytokines are critical for host defense
  - TNFα activates macrophage and PMN phagocytosis and killing
  - IFN$\alpha\beta$ activates anti-viral mechanisms
  - IL-1 stimulates inflammation and fever

What happens next?

- If a pathogen is detected by a phagocytic cell type (macrophages and PMNs), the phagocytic cell will attempt to destroy the infection

Macrophage Microbial Killing

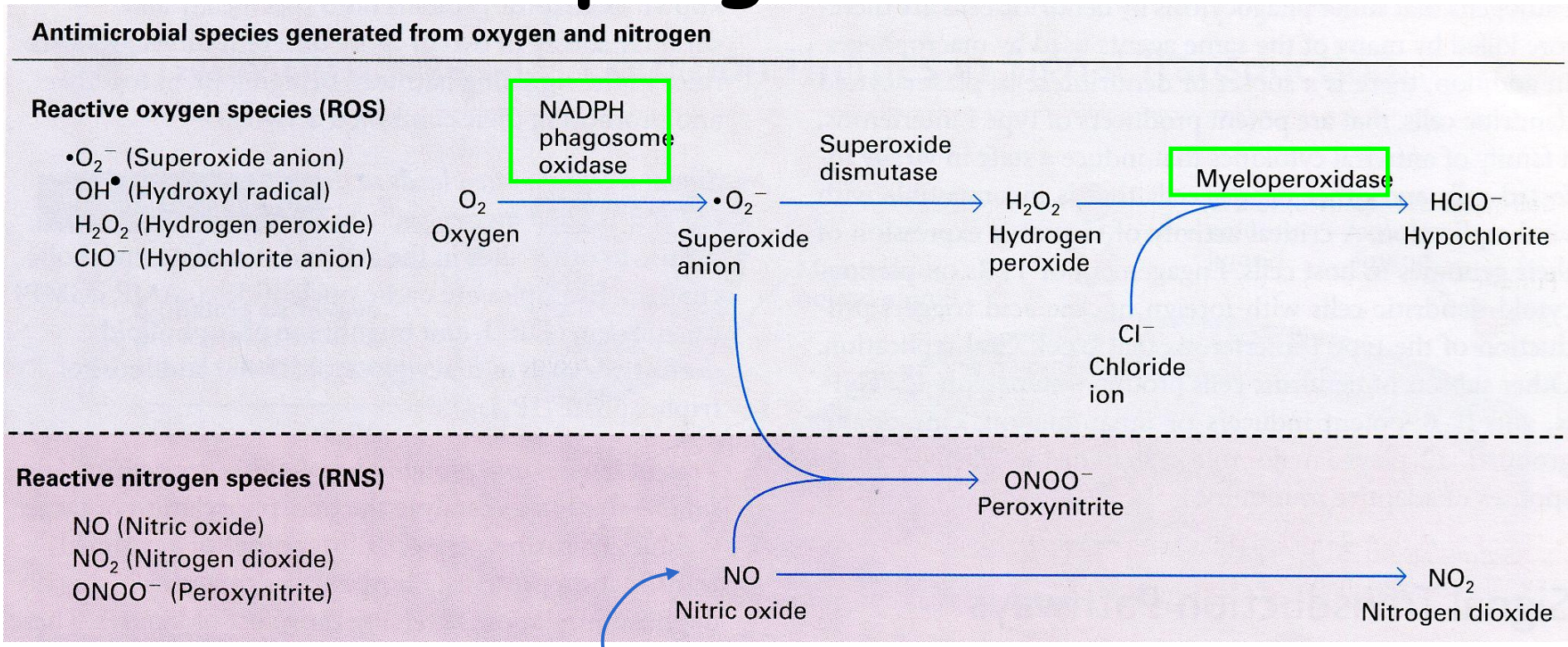
Once the PRRs are activated by the PAMPs, phagocytosis is initiated



Phagocytosis is active process:

- Internalization of pathogen into phagosome
- Acidification of phagosome
- Fusion of phagosome with lysosomes that contain anti-microbial compounds (**phagolysosome**)
- This may be sufficient to kill the pathogen
- If not, reactive oxygen and nitrogen species may need to be generated

Generation of Antimicrobial Species in macrophages and PMNs



L-arginine + O_2 + NADPH ⟶ NO + L-citrulline + NADP

Inducible nitric oxide synthetase (iNOS)

Chronic Granulomatous Disease (CGD):

- caused by mutation in NADPH oxidase enzyme complex
- most common form is X-linked
- heightened susceptibility to infections, particularly intracellular bacteria
- form granulomas due to inability to kill phagocytosed bacteria

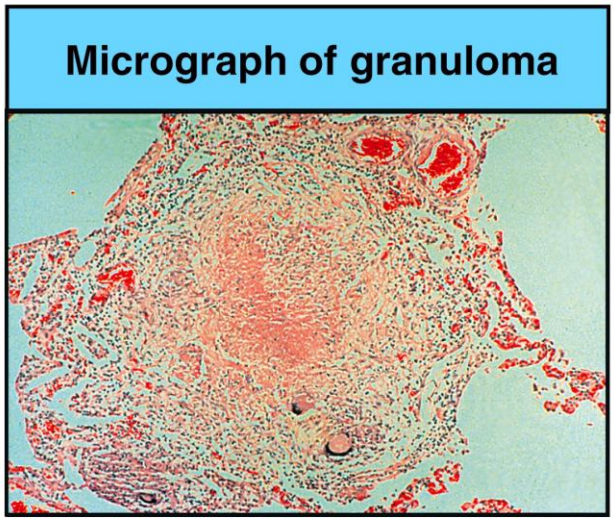


Figure 21-6 part 2 of 2 Case Studies in Immunology, 4/e (© Garland Science 2004)

Activated macrophages secrete proteins that drive innate response

Cytokines

- induce response by binding to specific receptors
- can function in autocrine or paracrine manner
- cytokines (and their receptors) are clustered according to structural similarities
- critical cytokines secreted by macrophages following activation include TNFα, IL-1, IL-6, IL-12 to stimulate inflammation and phagocytosis/killing

Chemokines

- diverse family of chemotactic cytokines, induce directed chemotaxis of cells
- all related in amino acid structure
- certain chemokines induce cell activation in addition to cell recruitment
- promiscuous in receptor usage, each can bind more than one receptor
- likewise, receptors are promiscuous

Clinical symptoms of inflammation: pain, redness, heat, swelling

1. Increased vascular diameter, increased blood flow (heat, redness)
2. Activation of vascular endothelium to express adhesion molecules, increases leukocyte binding
3. PMNs are first cell type recruited to site, followed later by monocytes
4. Increased vascular permeability results in local swelling and pain

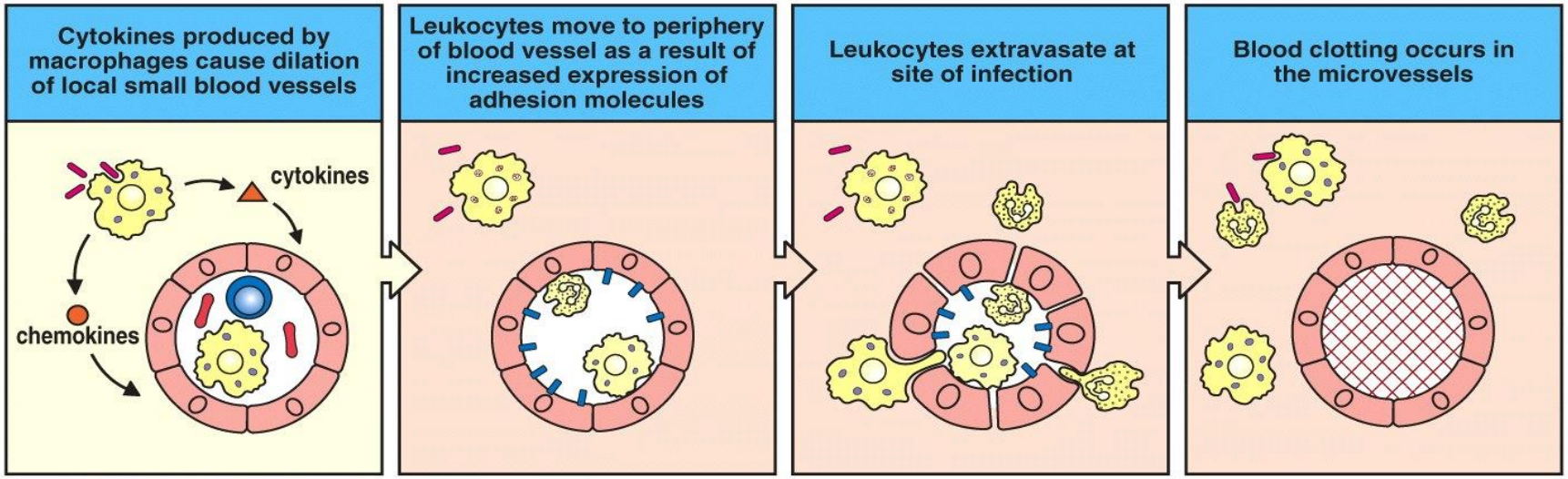
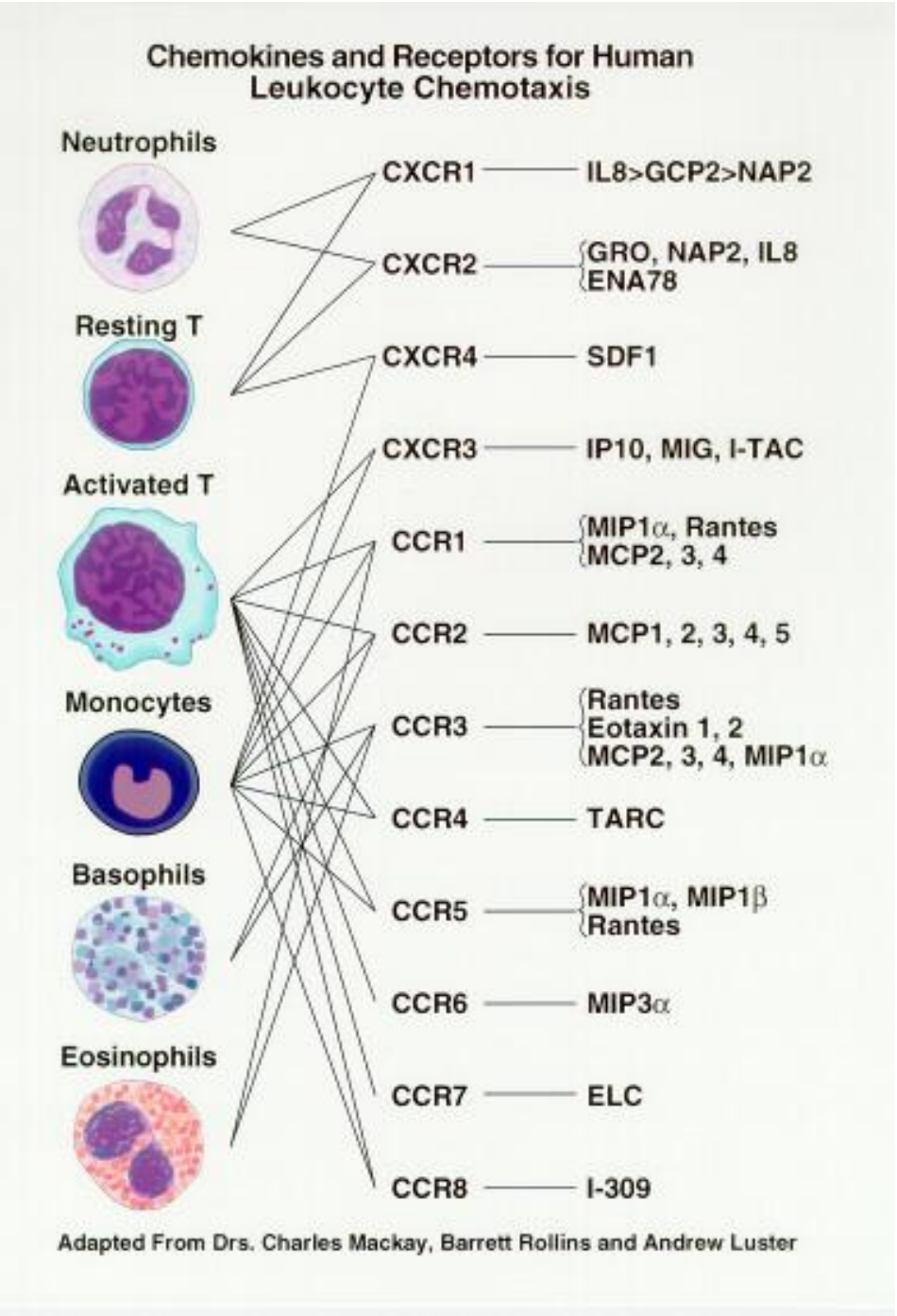


Figure 2-8 Immunobiology, 6/e. (© Garland Science 2005)

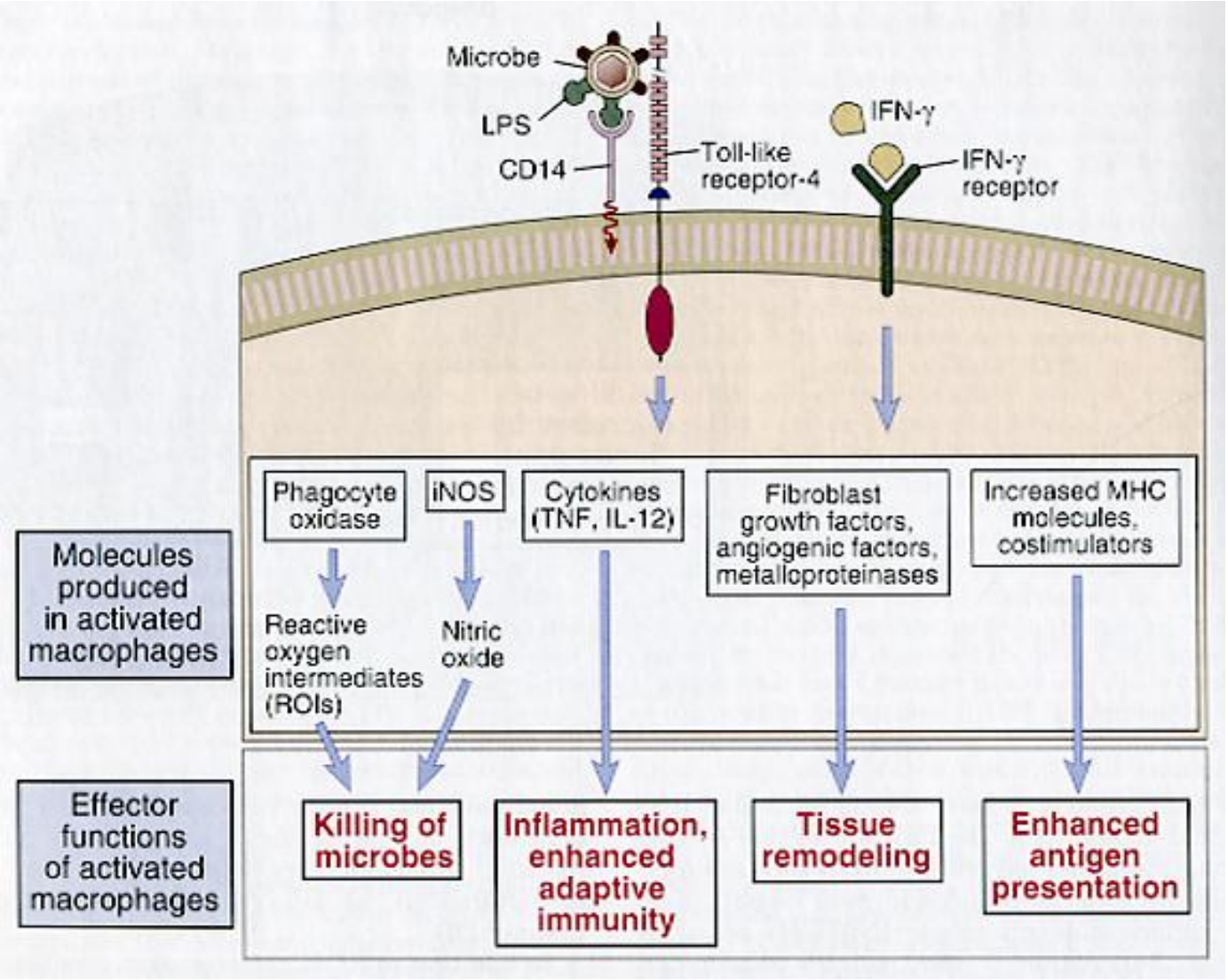
Microvascular coagulation helps prevent pathogen spread into bloodstream (physical barrier)



Chemokines

- Infection induces the release of various chemokines
- Theses substances bind specific and sometimes shared receptors to recruit various types of immune cells to the site of infection

Macrophage Effector Functions



Viruses induce Interferon-α/β production

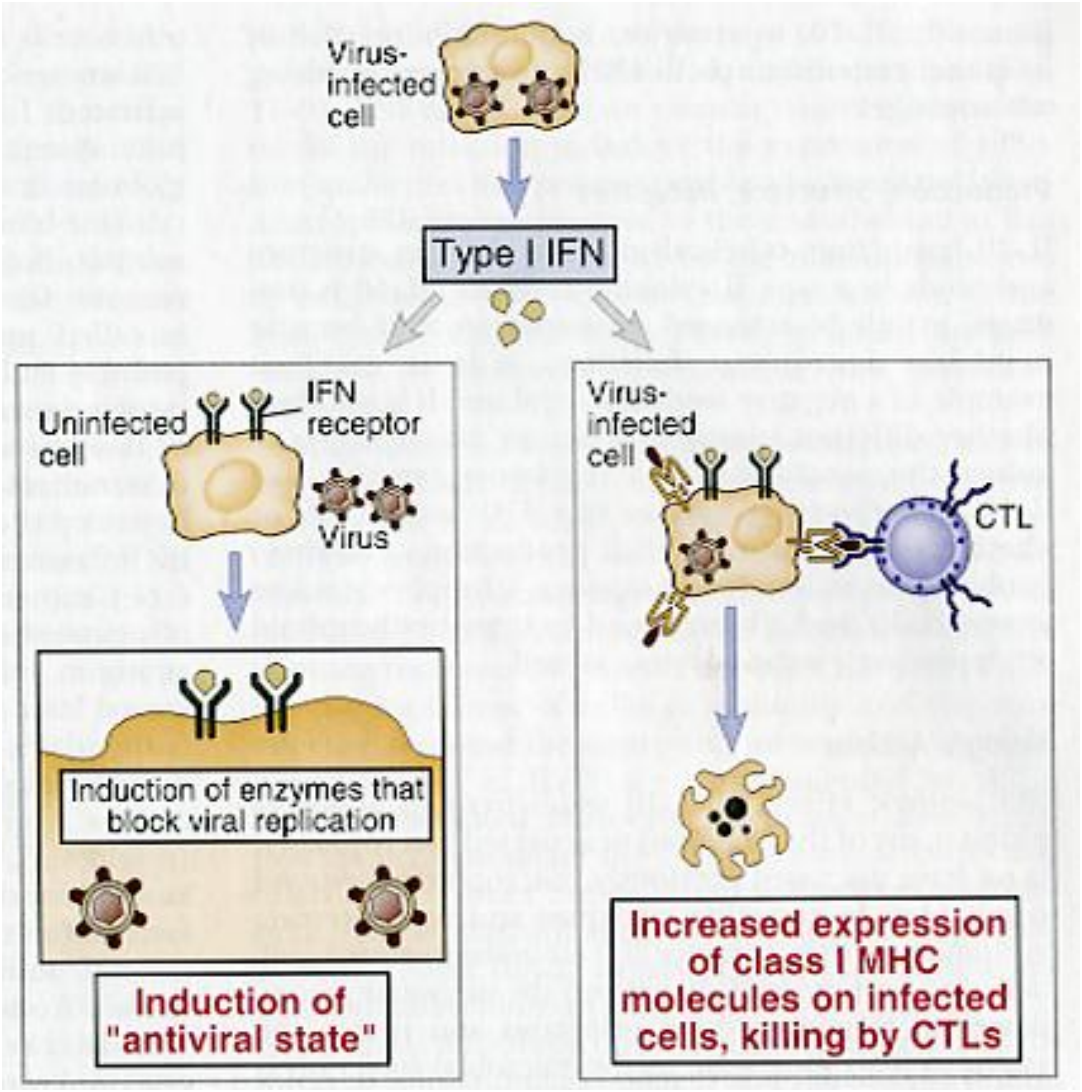
- Termed interferons because they "interfere" with viral replication.
- IFN-α/β produced by many diverse cell types following viral infection.
- Synthesized in response to dsRNA that is not found in mammalian cells.
- TLR-3, RIG-1, MDA-5 all recognize dsRNA.

IFN-α/β actions for viral defense:

- secreted IFNs bind to cell surface IFN-receptor in autocrine and paracrine fashion
- induce host cell proteins that inhibit viral replication
- enhance cellular immune responses against virus
- upregulate MHC class I molecules
- active Natural Killer (NK) cells to lyse infected cells and to secrete cytokines

- IFNs serve as a firebreak to prevent spread of virus in tissue

Protective Role of IFN-α/β during Viral Infection



Natural Killer (NK) Cells

- First identified by having the ability to lytically kill certain tumor cell lines without prior sensitization
- Kill target cell by release of cytotoxic granules containing granzymes and perforin which penetrate target cell membrane and induce programmed cell death
- Can mediate **A**ntibody-**D**ependent **C**ellular **C**ytotoxicity (ADCC)
- Kill virally-infected cells with missing MHC class I
- Activated by IFN-α/β or IL-12 (produced rapidly by activated macrophages)
- Activated NK cells secrete IFNγ, acts on macrophages to increase microbial phagocytosis and killing

Antibody-Dependent Cellular Cytotoxicity

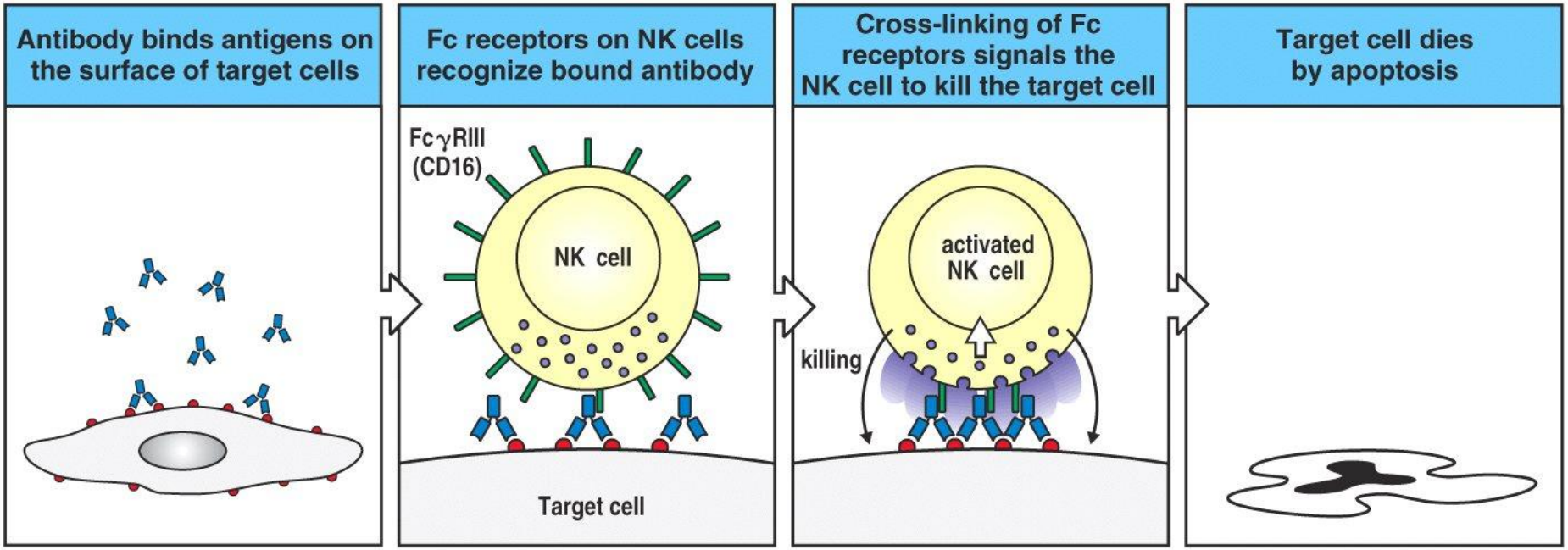


Figure 9-34 Immunobiology, 6/e. (© Garland Science 2005)

NK cell receptors

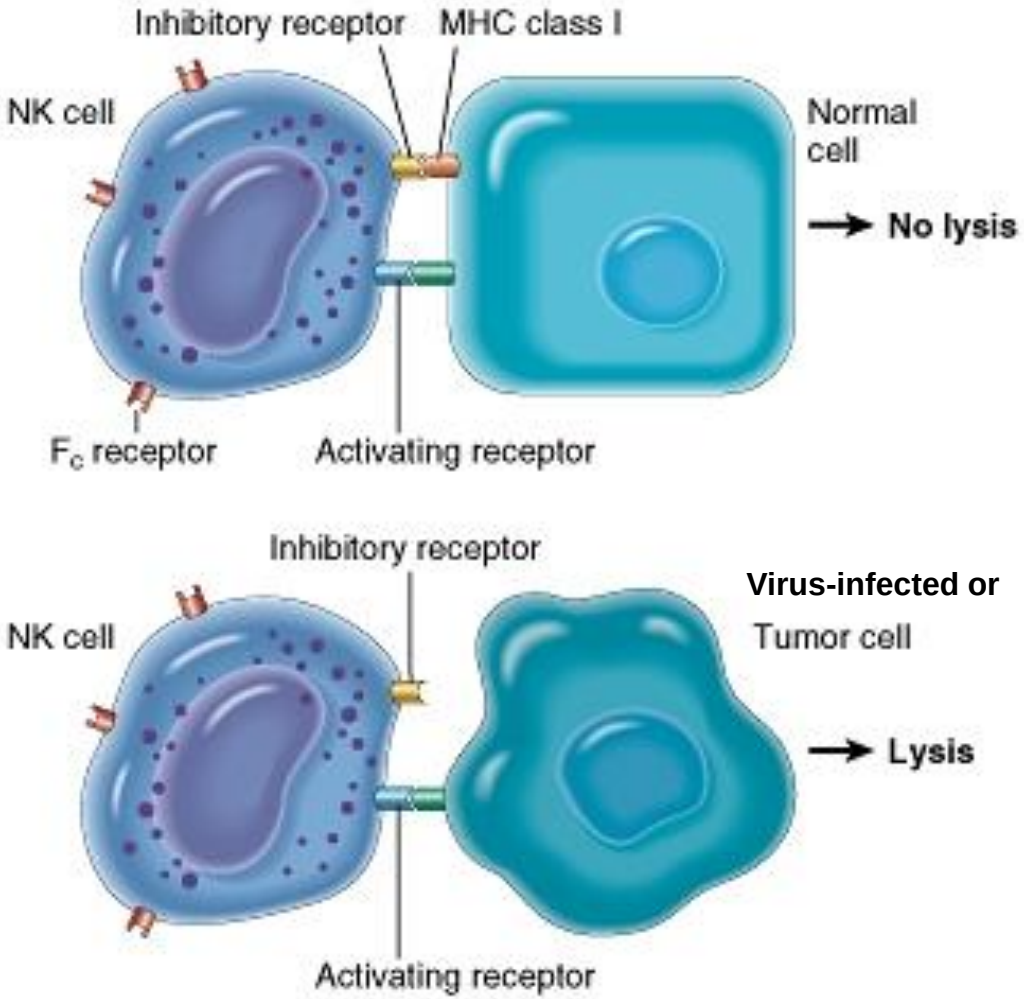
Inhibitory Receptors:

- germ-line encoded, no combinatorial diversity as seen with T/B cell receptors
- inhibit cytotoxicity to prevent killing of normal host cells
- specific for MHC class I alleles
- binding to class I sends inhibitory signal to NK cells

Activating Receptors:

- germ-line encoded
- recognize carbohydrate structures on self proteins

NK Cell Cytotoxicity



- Schematic representation of NK cell receptors and killing. Normal cells are not killed because inhibitory signals from MHC class I molecules override activating signals. In tumor cells or virus-infected cells, reduced expression or alteration of MHC molecules interrupts the inhibitory signals, allowing activation of NK cells and lysis of target cells.

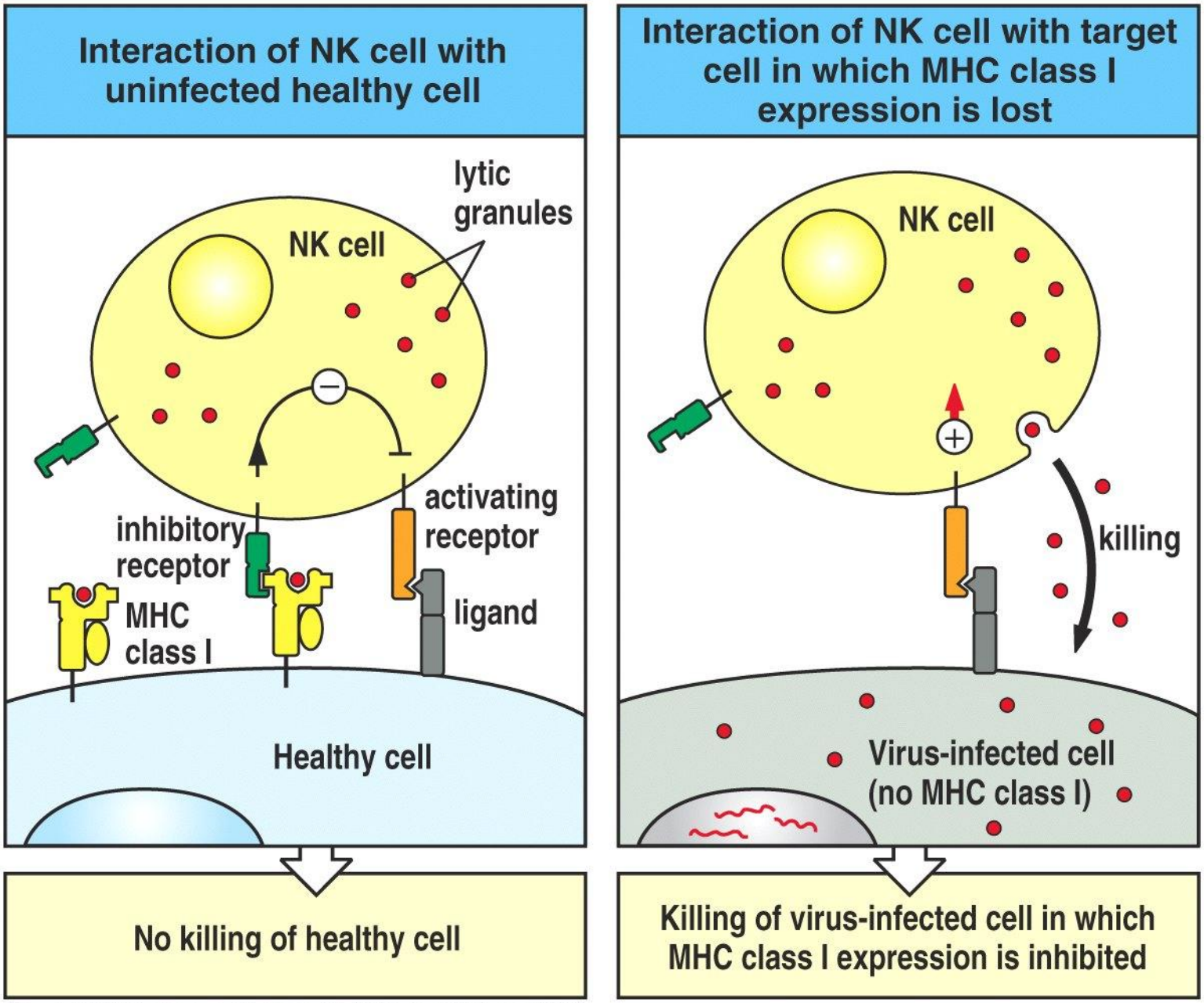


Figure 8-32 The Immune System, 2/e (© Garland Science 2005)

"Innate" Lymphocytes

- Unique, minor subsets of T and B lymphocytes that undergo receptor gene rearrangements to generate receptor diversity (unlike NK cells)
- These subsets express limited receptor diversity, utilizing only a small number of receptor gene segments
- Tend to found in specific locations in the body, usually sites that encounter exogenous antigens or pathogens

Innate-like lymphocytes		
B-1 cells	**Epithelial γ:δ cells**	**NK T cells**
Make natural antibody, protect against infection with *Streptococcus*	Produce cytokines rapidly	Produce cytokines rapidly
Ligands not MHC associated	Ligands are MHC class IB associated	Ligands are lipids bound to CD1d
Cannot be boosted	Cannot be boosted	Cannot be boosted

Figure 2-52 Immunobiology, 6/e. (© Garland Science 2005)

B-1 (CD5) B Lymphocytes

Distinguished from conventional B cells by expression of **CD5**

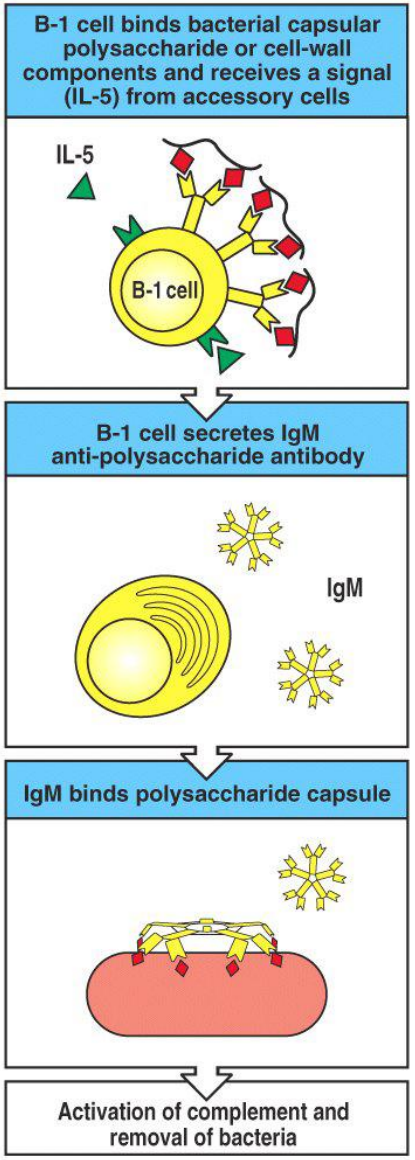
Likely to by the B-cell equivalent of $\gamma\delta$-T cells (arise very early in ontogeny, limited and distinctive gene rearrangements)

Found in distinct microenvironment (peritoneal cavity and pleural spaces)

Secrete IgM antibodies **without** need of T cell help (unlike conventional B cells), results in rapid response (within 48 hours); termed **natural antibodies**

Antibody responses to bacterial polysaccharide components of cell wall

No immunological memory generated



Gamma-Delta T cells

Generated very early in ontogeny (prior to birth). Represent the first T cell subsets generated in the thymus

Two subsets:

- one subset utilizes diverse $\gamma\delta$ T cell receptor rearrangements and are found in all lymphoid tissues
- one utilizes TCR of very limited diversity. Found in high numbers in mucosal linings (pulmonary, urogenital, gastrointestinal) and skin, termed **intraepithelial**$\gamma\delta$ T cells

T cell receptor ligands:

- self-proteins expressed by damaged, injured, stressed epithelium (heat shock proteins, unique MHC-associated molecules, phospholipids)
- products of bacterial metabolism and breakdown (small organic phosphates, alkylamines)

Ag recognition by γδ-T cells usually does not require MHC presentation, occurs directly (similar to antibody); detailed 3-D structure of γδ-TCR more closely resembles antibody than αβ-TCR

Direct recognition of TCR-ligand without requirement of antigen processing allows for rapid response

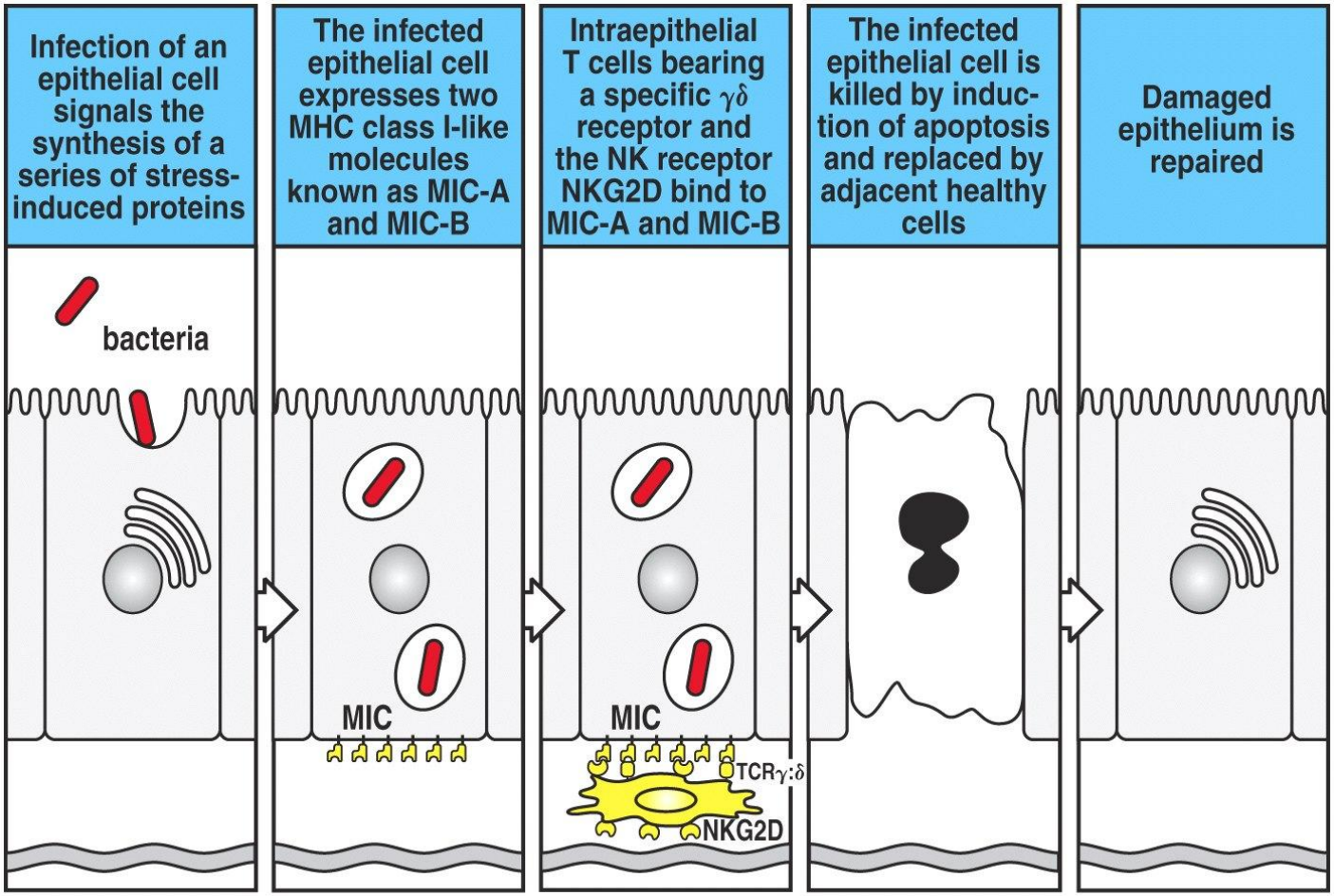


Figure 8-35 The Immune System, 2/e (© Garland Science 2005)

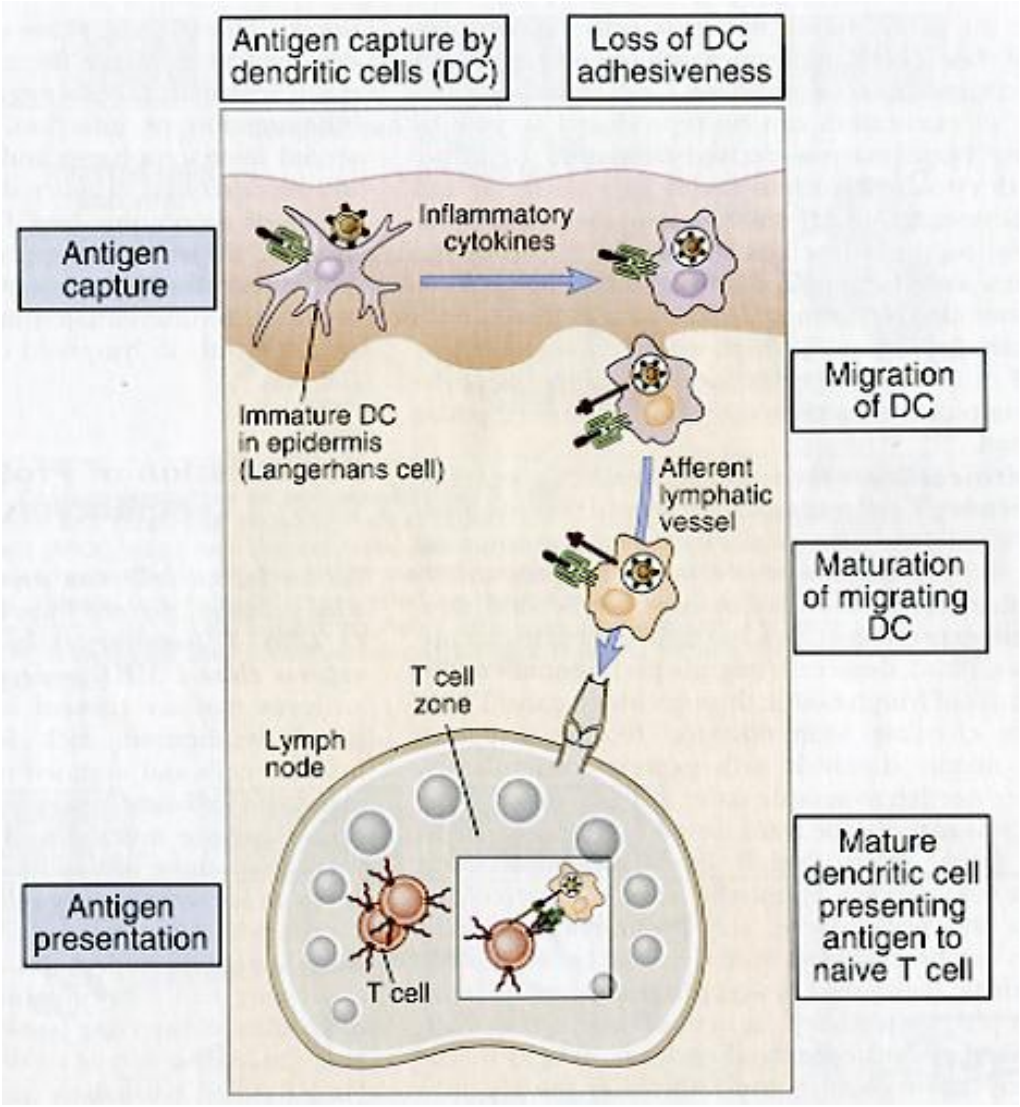
Natural Killer-T Cells (NK-T Cells)

- Minor subset of $\alpha\beta$-T cells originally described by expression of NK-cell associated markers
- Majority express <u>invariant TCR</u> ($V\alpha14$-$J\alpha18/V\beta8.2$), remaining express diverse TCR
- Rapidly release large amounts of IL-4 and IFNγ, can interact with/influence other "innate immune" lymphocytes (NK cells, $\gamma\delta$-T cells)
- Recognize self and foreign glycolipids presented by CD1
- Crystal structure analysis of CD1d indicates the presence of an MHC-like fold with a large, hydrophobic binding groove
- Due to the unique glycolipid antigen binding ability of CD1 molecules, it has been speculated that CD1 acts as an alternative mechanism for surveillance of foreign and altered-self glycolipids that would otherwise escape conventional class I and II pathways

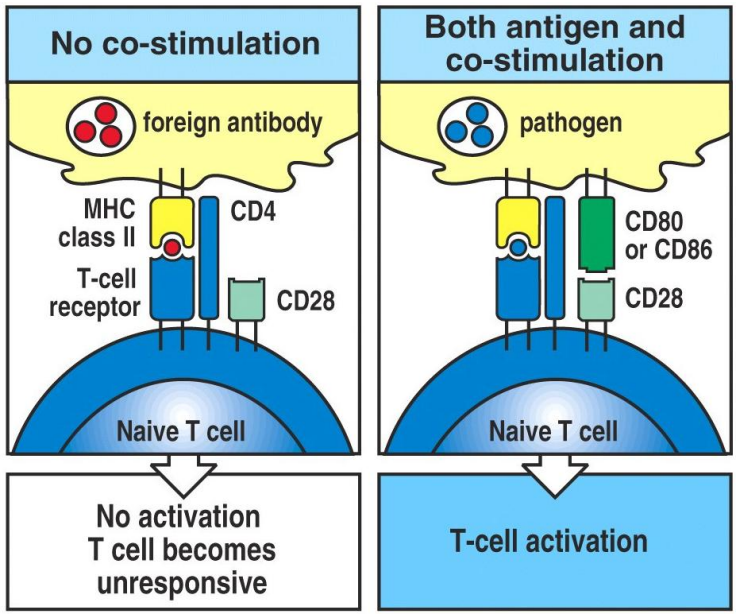
Dendritic Cells

- DCs link innate and adaptive immunity
- DCs are immature as they circulate waiting to encounter pathogens
- At this point, they are highly phagocytic, but not good stimulators of adaptive T cell responses
- Once they are activated by pathogens and activation of their PRRs, they secrete cytokines to initiate inflammation and then they migrate to lymph nodes and mature
- As mature DCs they are excellent APCs for T cell stimulation

Dendritic Cell Activation: an overview



DC migration and antigen presentation: interface between innate and adaptive immunity



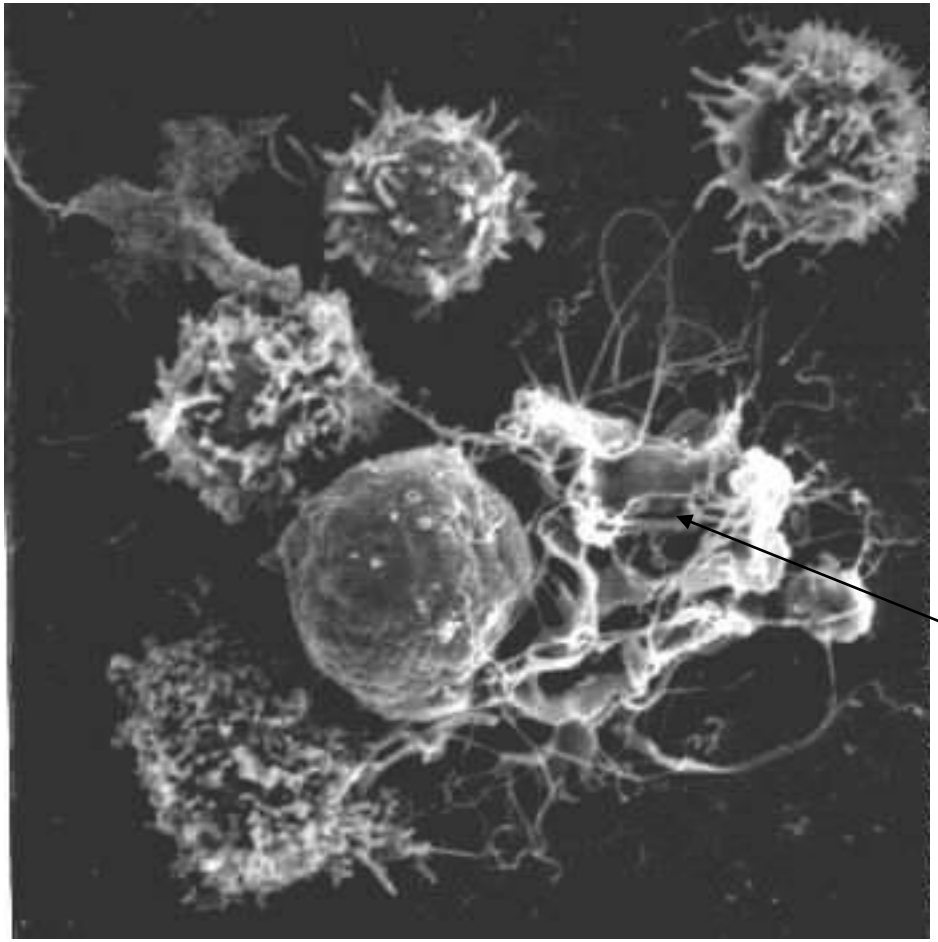
Immature DC

- principal function is antigen capture
- highly phagocytic
- low T cell stimulating potential
  - low MHC class II
  - low CD80/86 expression

Mature DC

- principal function is antigen presentation
- low phagocytic capacity
- high T cell stimulating potential
  - high MHC class II
  - high CD80/86 expression

A view of DC-T cell interactions

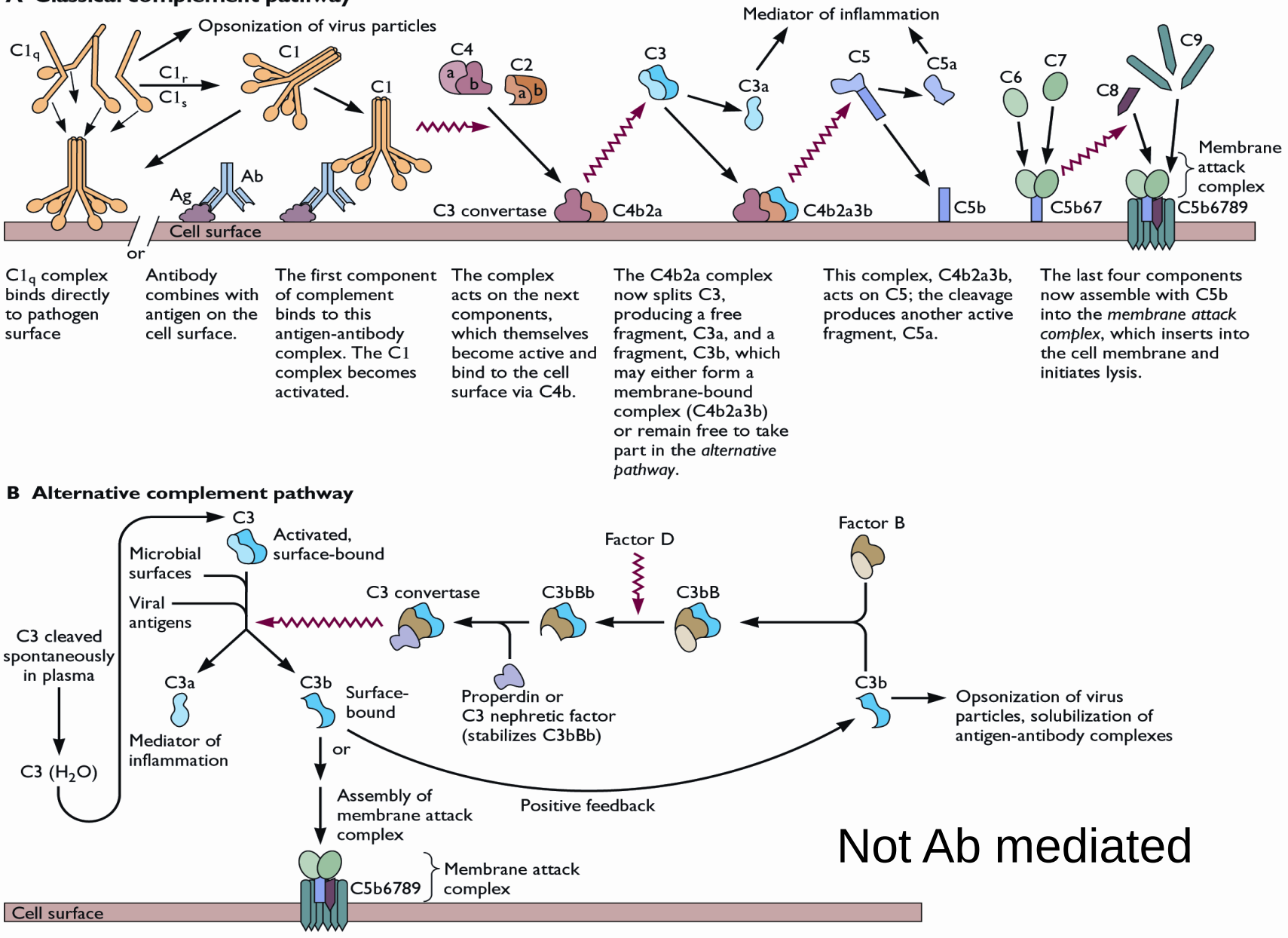


DC: Note dendrites

Complement

- There is a system of >30 proteins that collectively make up the complement system (originally named because they complement Ab functions)
- These proteins can recognize some pathogen surfaces intrinsically, or can recognize Ab molecules bound to pathogen-infected cells
- The recognition by and activation of the complement system results in pore-forming complexes being created on infected cells which results in lysis of infected cells
- Other byproducts of complement activation are the recruitment of more immune effector cells by the C3a and C5a components
- There are classical and alternative pathways of complement activation, but they lead to the same outcome

Ab-mediated



Not Ab mediated

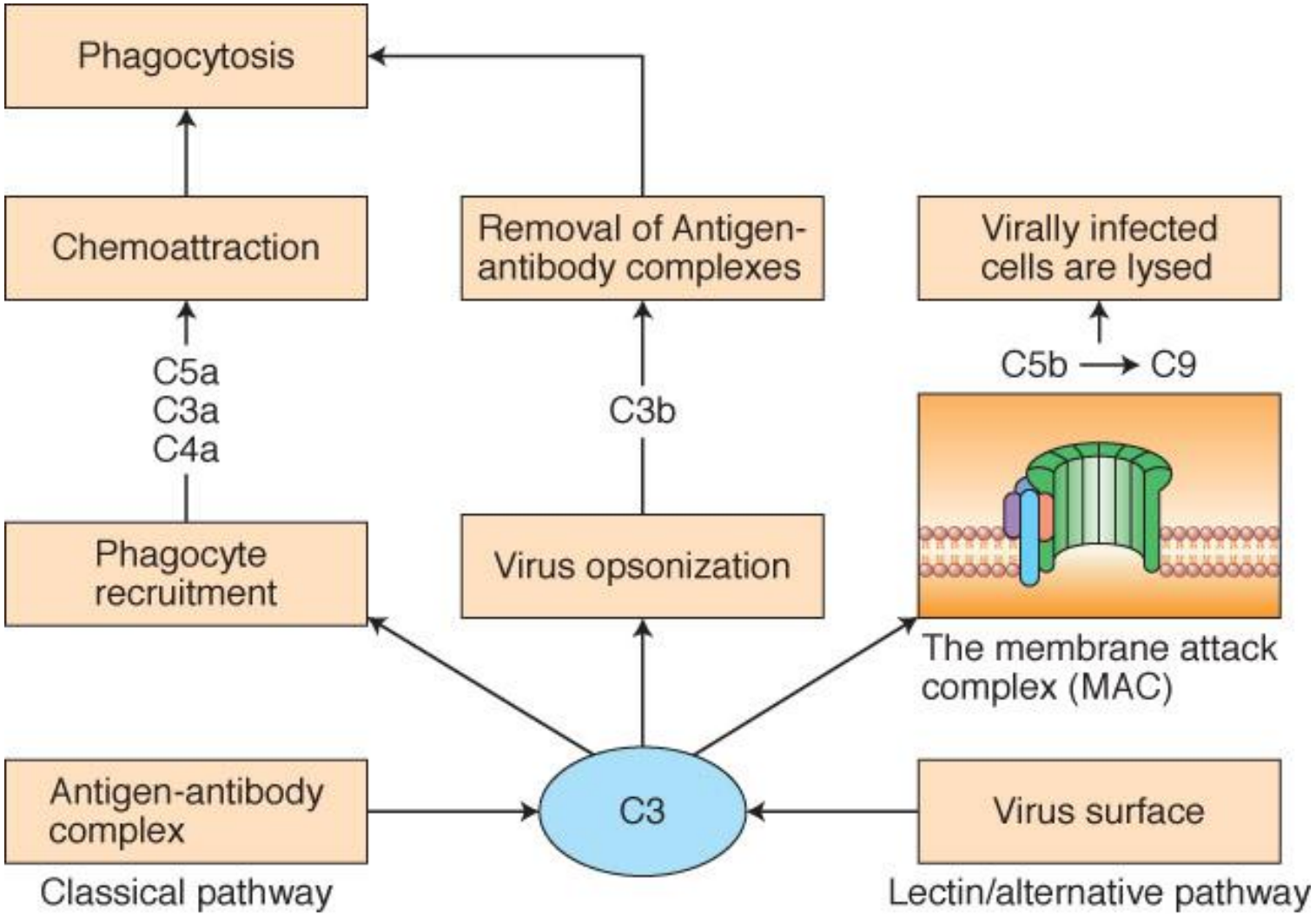
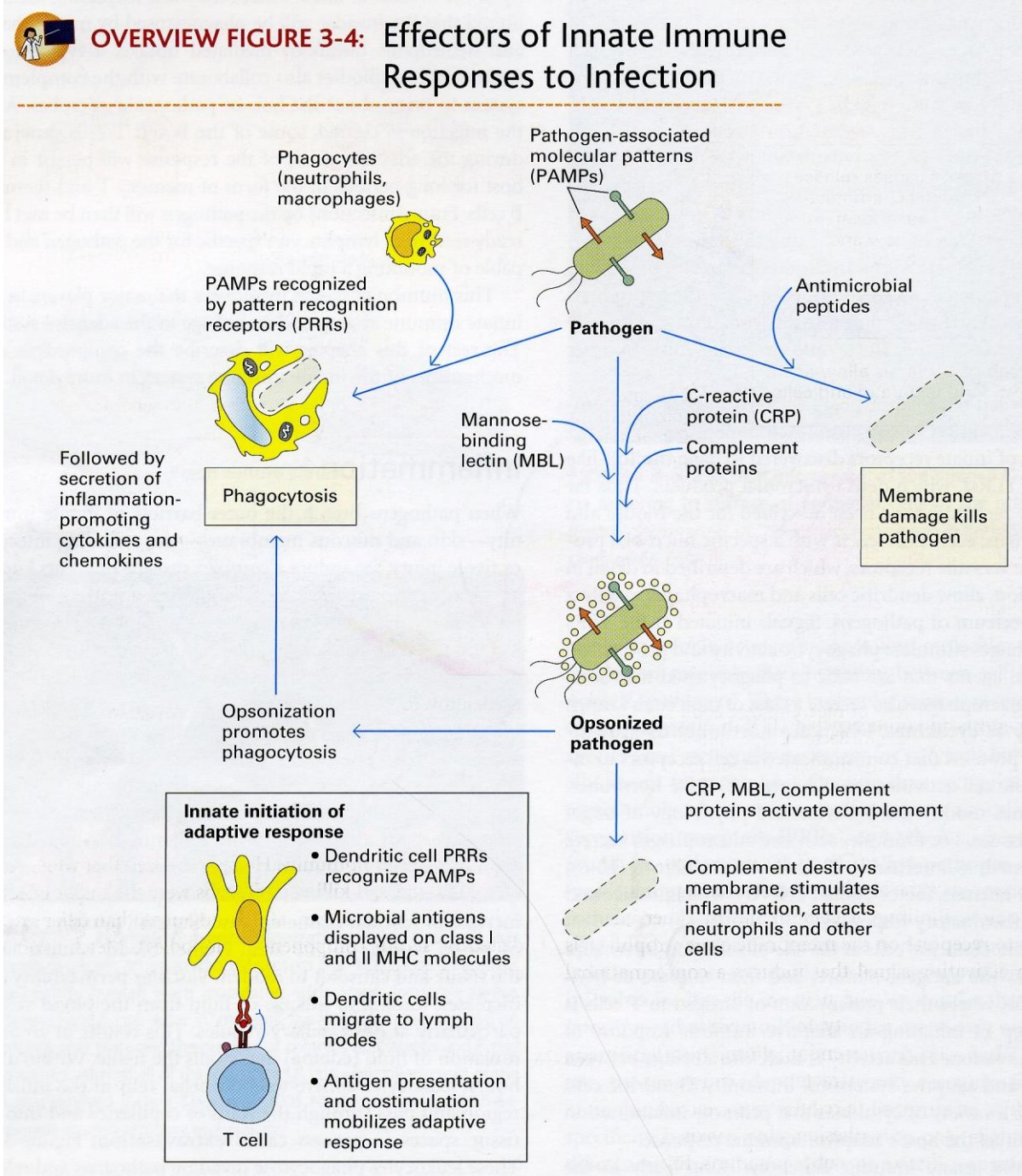


Figure 7.11: Complement cascade pathways.

Adapted from S. Smith. “Immunologic Aspects of Organ Transplantation.” *Organ Transplant*. Medscape, 2002.

Summary and Review of Innate Immune Responses



The body's defenses against infection

To combat infections, the body uses both the **innate** and **adaptive** immune systems.

- the innate immune system contains the initial infection while the adaptive immune system clears the infection.

- It is probable that the innate immune system eliminates the vast majority of infections before any symptoms begin.

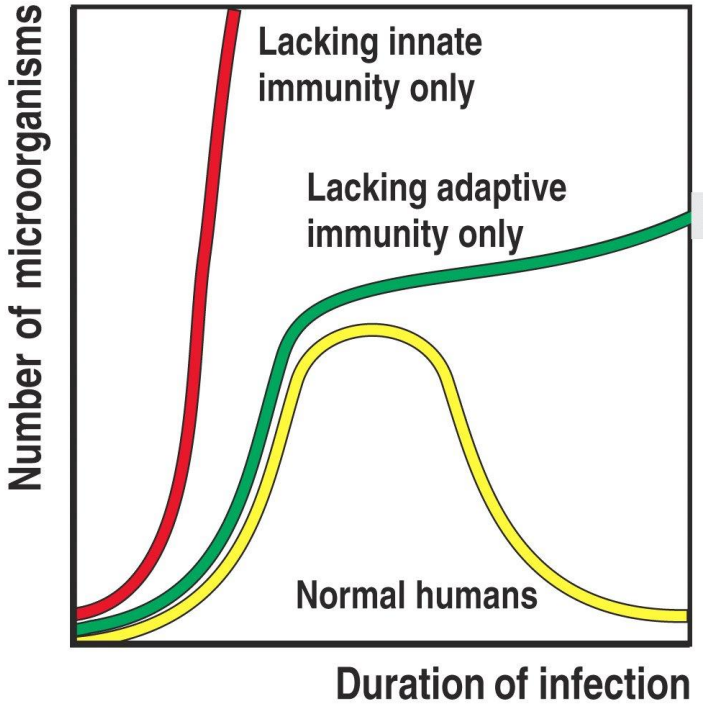


Figure 8-1 The Immune System, 2/e (© Garland Science 2005)