INNATE IMMUNITY

- **THE IMMUNE RESPONSE OCCURS IN THREE PHASES**

A. **Immediate - 0-4 hours**

a. **Antimicrobial enzymes** (e.g., **lysozyme** that breaks down bacterial peptidoglycan)

b. **Antimicrobial peptides** (e.g., **defensins**)

i. Amphipathic molecules that disrupt pathogen membranes

ii. Secreted by epithelial cells

c. <u>Complement System</u>

i. series of plasma proteins that targets bacteria for destruction by phagocytosis and lysis

ii. complement proteins are produced as inactive pro-enzymes or zymogens by the liver and must be activated by proteolysis.

B. **Early Induced – 4-96 hours**

a. Pattern recognition by innate immune receptors or **Pattern Recognition Receptors (PRRs)**

i. Germline-encoded receptors that recognize broad classes of microbial molecules or **Pathogen-Associate Molecular Patterns (PAMPs)** (e.g., **lipopolysaccharides, lipoproteins, Flagellin, bacterial and viral DNA**, etc.)

b. Recruitment of effector cells (phagocytic cells) to destroy pathogens that survive phase one

i. Three major types of effector cells in this phase

1. **Macrophages** – mononuclear phagocytes – resident in the tissues and recruited from bloodstream as monoctyes upon infection.

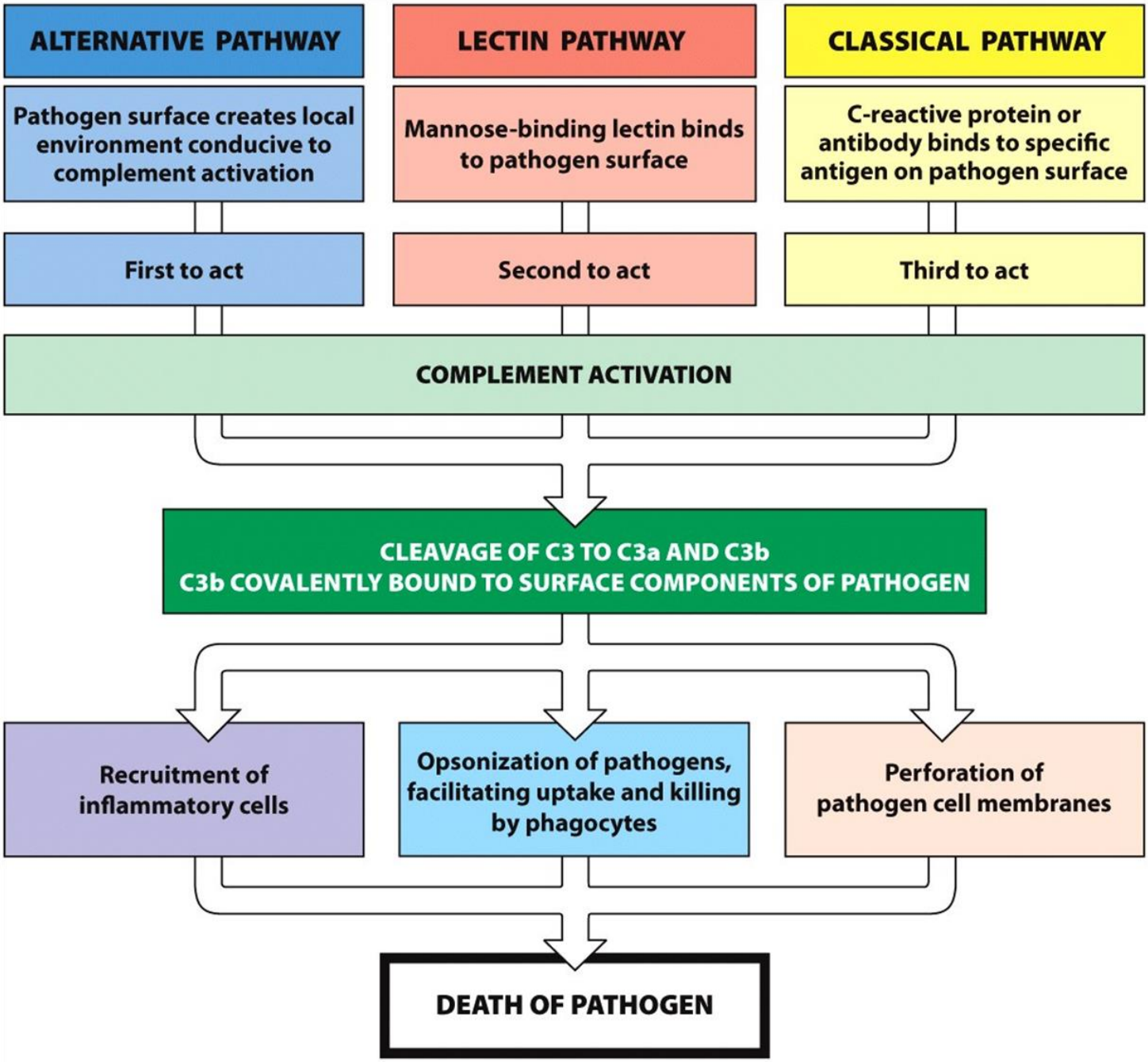
2. **Neutrophils** (polymorphonuclear leukocytes or PMNs) – generally the first inflammatory leukocytes recruited from the bloodstream during inflammatory responses following infection

3. **Dendritic cells** – critical for bridging the innate and adaptive phases of the immune response – resident and recruited by infection

C. **Late - Adaptive - >96 hours**

a. Transport of microbial antigens to secondary lymphoid organs (spleen and lymph nodes)

b. Activation of naïve antigen-specific B and T lymphocytes



c. Differentiation of B and T cells into effector lymphocytes that mediate humoral and cell-mediated immunity to eliminate infectious agent

- **THE COMPLEMENT SYSTEM AND COMPLEMENT ACTIVATION**

A. Complement is a system of plasma proteins that interact with components of the innate and adaptive immune systems to aid in the elimination of pathogens.

B. The most important function of complement is to facilitate the uptake and destruction of pathogens by phagocytic cells.

C. The main functional consequences of complement activation that facilitate the uptake and destruction of pathogens by phagocytic cells include:

   a. opsonization of pathogens

   b. recruitment of inflammatory cells

   c. direct killing of pathogens

D. Three different pathways associated with activation of the complement system

   a. Alternative pathway
      i. The first to act during the early phase of innate immune response
      ii. **Pathogen surfaces create local environment** conducive to complement activation

   b. Lectin pathway
      i. Second to act during the induced early phase
      ii. **Mannose-binding lectin** binds to pathogen surface and initiates activation of complement

   c. Classical pathway
      i. Third to act during the adaptive phase
      ii. C-reactive protein produced by liver during acute phase response or **antibody produced by B cells during adaptive response bind to antigen on pathogen surface and activate complement**

E. Regardless of the mechanism of activation, the complement cascade can be divided into two sequences of reactions:

   a. The **"early" events** are a series of proteolytic steps that generate an active protease called the **C3 convertase** that binds to the pathogen surface

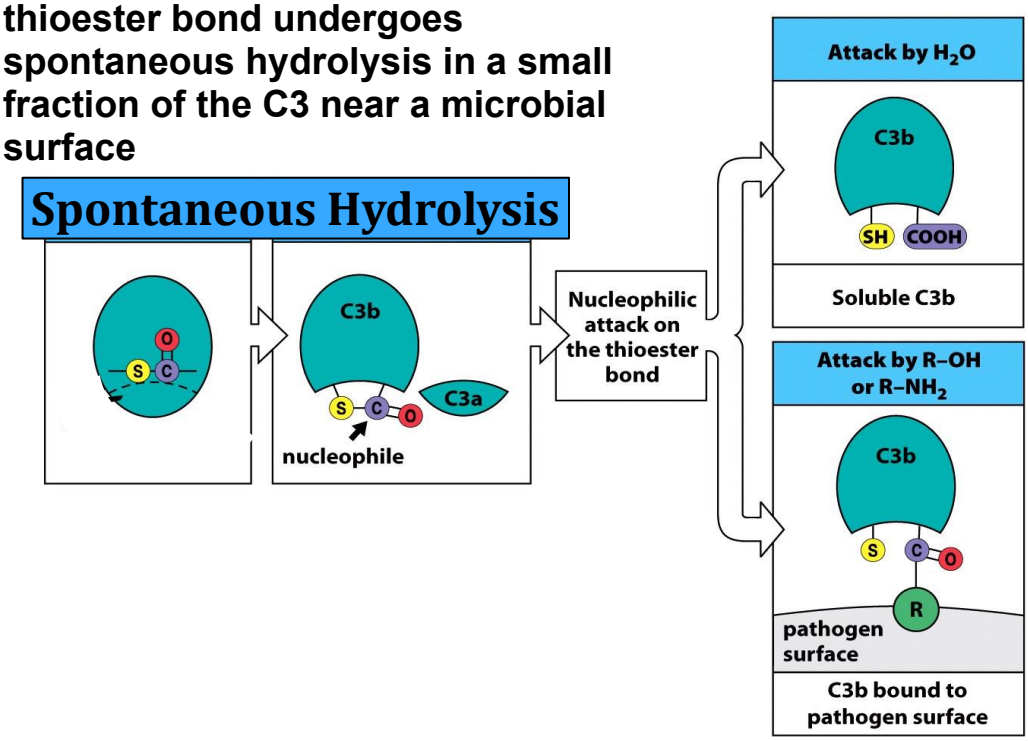
      i. The critical event is the cleavage of plasma C3 by C3 convertase into a large fragment called C3b that covalently attaches or "fixes" to the surface of microbes

      ii. C3 convertase is then bound to the pathogen surface and can catalytically generate large amounts of the **main effector molecule of the complement system C3b by cleaving C3**

      iii. The C3 convertase also generates several small fragments that serve as inflammatory mediators to activate endothelium and recruit phagocytes

   b. The **"late" events** comprise a sequence of polymerization reactions that result in the formation of a **membrane-attack complex (MAC)** that can directly damage the membranes of certain pathogens.

In the <u>Alternative Pathway</u>, the C3 thioester bond undergoes spontaneous hydrolysis in a small fraction of the C3 near a microbial surface



F. Early events in the **alternative pathway** - Generation of C3 convertase
   a. Spontaneous hydrolysis of the thioester in plasma C3 near a microbial surface results in some C3b fragments being bound to the microbial surface.
   b. These bound C3b molecules bind factor B, which is then cleaved by factor D to produce C3bBb, the surface-bound convertase of the alternative pathway.
   c. This enzyme cleaves C3 to produce further C3b fragments bound to the microbe and small soluble C3a fragments. The C3b fragments can be used either to make more C3 convertase, which amplifies the activation of C3, or to provide ligands for the receptors of phagocytic cells.

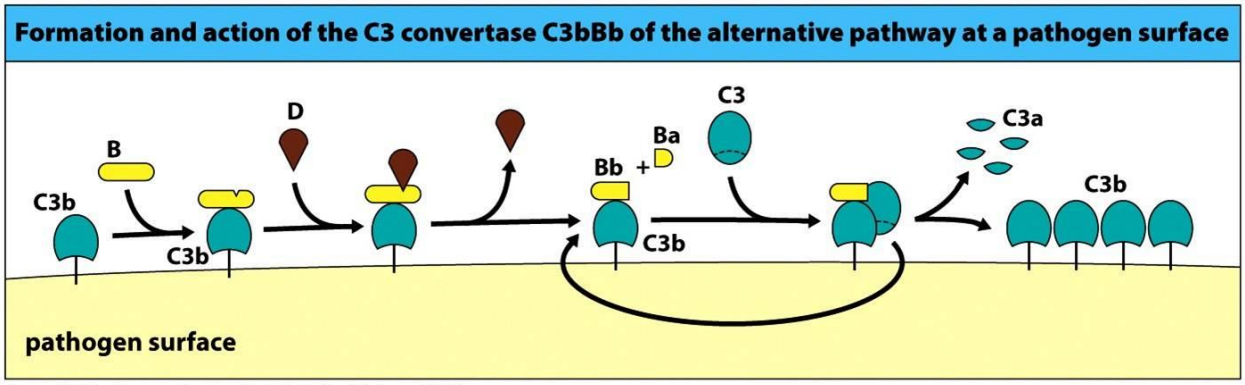


Figure 2.8 The Immune System, 3ed. (© Garland Science 2009)

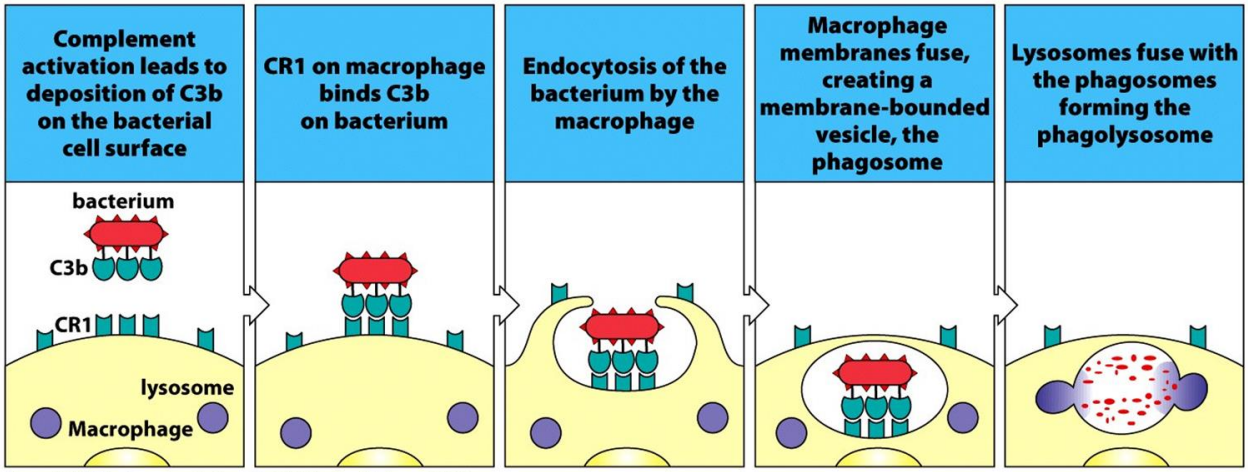


Figure 2.10 The Immune System, 3ed. (© Garland Science 2009)
Figure 2.12 part 2 of 2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

G. **Late events -** Complement-mediated **opsonization** and phagocytosis

   a. Complement components, especially bound C3b, bound on the surface of pathogens are recognized by specific **complement receptors** on phagocytes (macrophages, monocytes, neutrophils)

      i. Complement Receptor 1 (CR1) and CR3 are the most important complement receptors.
      ii. People with deficiencies in C3 have severe recurrent pyogenic infections, mainly caused by meningococci and pneumococci.

   b. The binding of C5a to its receptor on phagocytes is required for induction of phagocytosis by CR1 bound to microbial surface-bound C3b.

H. **Small complement fragments C3a, C4a and C5a** induce local inflammatory response - collectively known as **anaphylatoxins**

   a. induce smooth muscle contractions
   b. increase vascular permeability
   c. these changes increase extravasation of antibody and complement components into local tissues
   d. phagocytic cells are also recruited to the site, thereby increasing opportunity for phagocytosis of pathogen

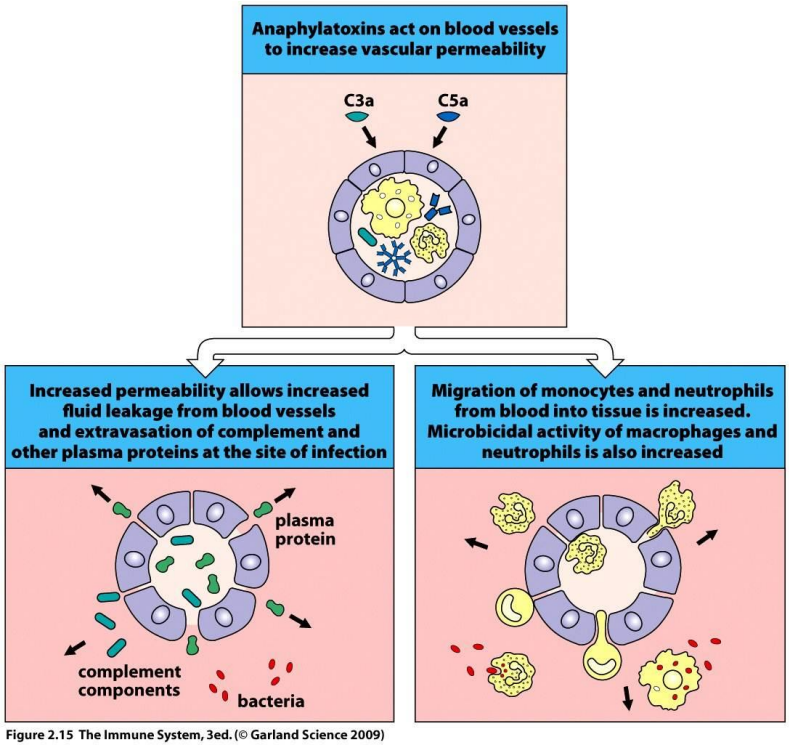


Figure 2.15 The Immune System, 3ed. (© Garland Science 2009)

I. **Membrane Attack complex**

a. Polymerization of complement components C5b, C6-C8 initiate the polymerization of C9 in the microbial membrane to form a pore

i. Up to 16 molecules of C9 form the pore
ii. *Neisseria* is one genus of bacteria affected by MAC; however, MAC formation is not the predominant mechanism for complement-mediated elimination of pathogens.

b. A protein called CD59 expressed on the surface of human cells binds to the C5b, C6, C7 complex to prevent pore formation by C9 in human cells.

J. **Complement activation must be tightly regulated**

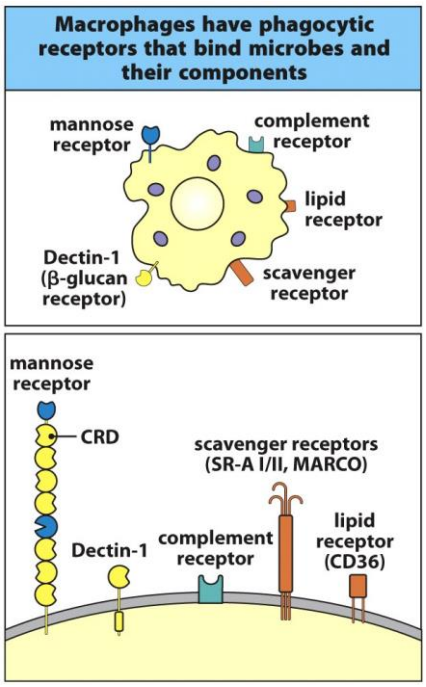
a. Positive regulation - the soluble plasma protein properdin binds to C3bBb and extends its lifetime on the microbial surface to insure that complement fixation continues as needed.
b. Negative regulation – soluble proteins factor H and factor I cleave and inactivates it so it cannot form a C3 convertase.
c. When C3bBb is formed on a human cell surface it is rapidly disrupted by the action of one of two membrane proteins: decay-accelerating factor (DAF) or membrane cofactor protein (MCP). In combination, these regulatory proteins ensure that much complement is fixed to pathogen surfaces and little is fixed to human cell surfaces.

- **INNATE IMMUNE RECEPTORS AND PATTERN RECOGNITION**

A. Many cell types express receptors known as Pattern Recognition Receptors (PRRs) that recognize broad classes of microbial molecules and induce the host cells to secrete cytokines and chemokines and activate antimicrobial mechanisms such as NOS and ROS.

a. Cytokines and chemokine responses induced by PRR recognition and binding of pathogen-associated molecular patterns (PAMPs) promote inflammation and ultimately effector mechanisms that kill pathogens.

i. Activation of endothelium lining blood vessels by cytokines and directly by PRRs promotes phagocyte extravasation into infected tissues and leakage of complement and antibodies into the tissues.
ii. Induction of fever
iii. Chemokines are chemoattractants for phagocytes
iv. Cytokines can influence that activation and nature of the adaptive immune response.



b. Multiple types of PRRs exist and can be classified according to their cellular location and function.

i. Soluble receptors in serum
   1. **Complement (C1q)**
   2. **C-reactive protein (CRP)**
   3. **Mannose binding lectin (MBL)**

ii. Phagocytic receptors
   1. Mannose receptors
   2. Scavenger receptors

iii. Signaling receptors
   1. Membrane-bound
      a. **<u>Toll-like Receptors (TLRs)</u>**
         i. originally identified in Drosophila
         ii. Ancient system for recognizing infections
      b. Recognize microbial molecules found outside the host cell or within phagosomes

   2. Cytoplasmic signaling receptors
      a. NOD-like receptors (NLRs)
         i. Recognize microbial molecules found within the host cell cytoplasm
         ii. Also a very ancient system for recognizing infections that are homologous to resistance proteins found in plants
      b. RIG-like Helicases (RLHs)
         i. Recognize viral RNAs
         ii. Stimulate antiviral interferon expression

Recognition of microbial products through Toll-like receptors

Receptor	Ligands	Microorganisms recognized	Cells carrying receptor	Cellular location of receptor
TLR1:TLR2 heterodimer	Lipopeptides GPI	Bacteria Parasites e.g., trypanosomes	Monocytes, dendritic cells, eosinophils, basophils, mast cells	Plasma membrane
TLR2:TLR6 heterodimer	Lipoteichoic acid Zymosan	Gram-positive bacteria Yeasts (fungi)		Plasma membrane
TLR3	Double-stranded viral RNA	Viruses e.g., West Nile virus	NK cells	Endosomes
TLR4:TLR4 homodimer	Lipopolysaccharide	Gram-negative bacteria	Macrophages, dendritic cells, mast cells, eosinophils	Plasma membrane
TLR5	Flagellin	Motile bacteria having a flagellum	Intestinal epithelium	Plasma membrane
TLR7	Single-stranded viral RNAs	Viruses e.g., human immunodeficiency virus (HIV)	Plasmacytoid dendritic cells, NK cells, eosinophils, B cells	Endosomes
TLR8	Single-stranded viral RNAs	Viruses e.g., influenza	NK cells	Endosomes
TLR9	Unmethylated CpG-rich DNA	Bacteria Viruses e.g., herpes viruses	Plasmacytoid dendritic cells, B cells, eosinophils, basophils	Endosomes
TLR10 homodimer and heterodimers with TLR1 and 2	Unknown		Plasmacytoid dendritic cells, basophils, eosinophils, B cells	Unknown

B. Structure and Function of Toll-like Receptors (TLRs)

a. Ten TLRs identified in humans TLR1-TLR10
    i. TLRs form homodimers and heterodimers
    ii. Each TLR homo- or heterodimer recognizes distinct ligands expressed by broad classes of microorganisms.
    iii. TLRs can also recognize molecules released from host tissue as a result of cellular damage.
        1. These host-derived molecules that serve as TLR ligands are known as Damage-Associated Molecular Patterns (DAMPs).

b. TLRs are type I transmembrane proteins

    i. Include an extracellular recognition domain made up of leucine-rich repeats (LRRs) that forms a horseshoe shape
    ii. Include an intra-cytoplasmic domain known as the Toll-IL-1 receptor (TIR) domain
        1. TIR domain shares homology with a similar intra-cytoplasmic domain found in the IL-1 receptor.
        2. The TIR domain allows interaction with other TIR domain-containing proteins that serve as signaling adaptors for the TLRs.
    iii. Ligand binding by the extracellular TLR domains induces dimerization of the TLRs in the membrane.

c. The cellular location of the TLRs influences the type of ligands to which they respond and the type of signals that they send to the cell.

    i. TLR1/TLR2, TLR2/6 and TLR4 are expressed on the plasma membrane of the cell surface and these TLRs sense microbes outside the cell.
    ii. TLR3, TLR4, TLR7, TLR8 and TLR9 are expressed in endosomal vesicles where they can sense microbes that have entered the cell via phagocytosis, receptor-mediated endocytosis or macropinocytosis.
    iii. TLR4 may be unique in that it signals both from the cell surface and from within endosomal/phagosomal vesicles.

d. TLR signaling induces inflammatory cytokine and chemokine production by host cells