11.3B: Pattern-Recognition Receptors (PRRs)

Skills to Develop

1. State the function of the following as they relate to innate immunity.
   a. pattern recognition receptors (PRRs)
   b. endocytic pattern recognition receptors
   c. signaling pattern recognition receptors
   d. danger-associated molecular patterns
   e. danger recognition receptors
   f. inflammasome
   g. pyroptosis
2. Name 2 endocytic PRRs.
3. Name 2 signaling PRRs found on host cell surfaces.
4. Name 2 signaling PRRs found in the endosomes of phagocytic cells.
5. Name 2 signaling PRRs found on the host cell cytoplasm.
6. Briefly describe the major difference between the effect of the cytokines produced in response to PAMPs that bind to cell surface signaling PRRs and endosomal PRRs.

In order to recognize PAMPs, various body cells have a variety of corresponding receptors called pattern-recognition receptors or PRRs (see Figure 5) capable of binding specifically to conserved portions of these molecules. Cells that typically have pattern recognition receptors include macrophages, dendritic cells, endothelial cells, mucosal epithelial cells, and lymphocytes.
Many pattern-recognition receptors are located on the surface of these cells where they can interact with PAMPs on the surface of microbes. Others PRRs are found within the phagolysosomes of phagocytes where they can interact with PAMPs located within microbes that have been phagocytosed. Some PRRs are found in the cytosol of the cell.

There are two functionally different major classes of pattern-recognition receptors: endocytic pattern-recognition receptors and signaling pattern-recognition receptors.

Endocytic (Phagocytic) Pattern-Recognition Receptors

Endocytic pattern-recognition receptors, also called phagocytic pattern-recognition receptors, are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes leading to their subsequent engulfment and destruction. They include:

1. Mannose receptors

Mannose receptors on the surface of phagocytes bind to various microbial carbohydrates such as those rich in mannose or fucose, and to N-acetylglucosamine (NAG). Human glycoproteins and glycolipids typically have terminal N-acetylglucosamine and sialic acid groups. C-type lectins found on the surface of phagocytes are mannose receptors (see Figure 6).

It is now thought that mannose receptors may be quite important in removing potentially harmful mannose-containing glycoproteins such as lysosomal hydrolases that are produced in increased amounts during inflammation.

2. Dectin-1

Dectin-1 recognizes beta-glucans (polymers of glucose) commonly found in fungal cell walls.

3. Scavenger receptors

Scavenger receptors found on the surface of phagocytic cells bind to bacterial cell wall components such as LPS, peptidoglycan and teichoic acids (see Figure 7). There are also scavenger receptors for certain components of other types of microorganisms, as well as for stressed, infected, or injured cells. Scavenger receptors include CD-36, CD-68, and SRB-1.

4. Opsonin receptors

Opsonins are soluble molecules produced as a part of the body's immune defenses that bind microbes to phagocytes. One portion of the opsonin binds to a PAMP on the microbial surface and another portion binds to a specific receptor on the phagocytic cell.

- Acute phase proteins circulating in the plasma, such as:
  - mannose-binding lectin (also called mannose-binding protein) that binds to various microbial carbohydrates such as those rich in mannose or fucose, and to N-acetylglucosamine (NAG); and
C-reactive protein (CRP) that binds to phosphorylcholine portion of teichoic acids and lipopolysaccharides of bacterial and fungal cell walls. It also binds to the phosphocholine found on the surface of damaged or dead human cells.

- Complement pathway proteins, such as C3b (see Figure 8) and C4b recognize a variety of PAMPs.
- Surfactant proteins in the alveoli of the lungs, such as SP-A and SP-D are opsonins.
- During adaptive immunity, the antibody molecule IgG can function as an opsonin (see Figure 16).

5. N-formyl Met receptors

N-formyl methionine is the first amino acid produced in bacterial proteins since the f-met-tRNA in bacteria has an anticodon complementary to the AUG start codon (see Figure 17). This form of the amino acid is not typically seen in mammalian proteins. FPR and FPRL1 are N-formyl receptors on neutrophils and macrophages. Binding of N-formyl Met to its receptor promotes the motility and the chemotaxis of these phagocytes. It also promotes phagocytosis.

**Signaling Pattern-Recognition Receptors**

Signaling pattern-recognition receptors bind a number of microbial molecules: LPS, peptidoglycan, teichoic acids, flagellin, pilin, unmethylated cytosine-guanine dinucleotide or CpG sequences from bacterial and viral genomes; lipoteichoic acid, glycolipids, and zymosan from fungi; double-stranded viral RNA, and certain single-stranded viral RNAs. Binding of microbial PAMPs to signaling PRRs promotes the production of:

- inflammatory cytokines, such as such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-12 (IL-12);
- antiviral cytokines called type-1 interferons (IFN), such as IFN-alpha and IFN-beta;
- chemotactic factors, such as the chemokines interleukin-8 (IL-8), MCP-1, and RANTES; and
- antimicrobial peptides, such as human defensins and cathelicidins.

These molecules are crucial to initiating innate immunity and adaptive immunity.

1. **Signaling PRRs found on cell surfaces** (see Figure 5):

A series of signaling pattern-recognition receptors known as toll-like receptors (TLRs) are found on the surface of a variety of defense cells and other cells. These TLRs play a major role in the induction of innate immunity and contribute to the induction of adaptive immunity.

Different combinations of TLRs appear in different cell types and may occur in pairs. Different TLRs directly or indirectly bind different microbial molecules. For example:
a. TLR-2 - recognizes peptidoglycan, bacterial lipoproteins, lipoteichoic acid (Gram-positive bacteria), and porins (gram-negative bacteria).
b. TLR-4 - recognizes lipopolysaccharide (Gram-negative bacteria), fungal mannans, viral envelope proteins, parasitic phospholipids, heat-shock proteins.
c. TLR-5 - recognizes bacterial flagellin;
d. TLR-1/TLR-2 pairs - binds to bacterial lipopeptides, lipomannans (mycobacteria) lipoteichoic acids (Gram-positive bacteria), cell wall beta glucans (bacteria and fungi), zymosan (fungi) and glycosylphosphatidylinositol (GPI)-anchored proteins (protozoa).
e. TLR-2/TLR6 pairs - also binds to bacterial lipopeptides, lipomannans (mycobacteria) lipoteichoic acids (Gram-positive bacteria), cell wall beta glucans (bacteria and fungi), zymosan (fungi) and glycosylphosphatidylinositol (GPI)-anchored proteins (protozoa).

Many of the TLRs, especially those that bind to bacterial and fungal cell wall components, stimulate the transcription and translation of inflammatory cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-alpha), and interleukin-12 (IL-12), as well as chemokines such as interleukin-8 (IL-8), MCP-1, and RANTES. These cytokines trigger innate immune defenses such as inflammation, fever, and phagocytosis in order to provide an immediate response against the invading microorganism (see Figure 9). Because cytokines such as IL-1, TNF-alpha, and IL-12 that trigger an inflammatory response, they are often referred to as inflammatory cytokines. Chemokines are a group of cytokines that enable the migration of leukocytes from the blood to the tissues at the site of inflammation. To counter inflammation, anti-inflammatory cytokines such as IL-1 receptor antagonist, IL-4, and IL-10 are produced.

Another cell surface PRR is CD14. CD14 is found on monocytes, macrophages, and neutrophils and promotes the ability of TLR-4 to respond to LPS. LPS typically binds to LPS-binding protein in the plasma and tissue fluid. The LPS-binding protein promotes the binding of LPS to the CD14 receptors. At that point the LPS-binding protein comes off and the LPS-CD14 bind to TLR-4. Interaction of LPS and CD14 with TLR-4 leads to an elevated synthesis and secretion of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-alpha, and platelet-activating factor (PAF). These cytokines then bind to cytokine receptors on target cells and initiate inflammation and activate both the complement pathways and the coagulation pathway (see Figure 9).

The signaling process for the CD14 and TLR-4 response to LPS is shown in Figure 15.

Flash animation illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.

html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LPS and TLR-4.

Flash animation illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.

html5 version of animation for iPad illustrating signaling toll-like receptors on defense cells: LTA and TLR-2/TLR-6.
TLRs also participate in adaptive immunity by triggering various secondary signals needed for humoral immunity (the production of antibodies) and cell-mediated immunity (the production of cytotoxic T-lymphocytes, activated macrophages, and additional cytokines). Without innate immune responses there could be no adaptive immunity.

a. T-independent (TI) antigens allow B-lymphocytes to mount an antibody response without the requirement of interaction with effector T4-lymphocytes. The resulting antibody molecules are generally of the IgM isotype and do not give rise to a memory response. There are two basic types of T-independent antigens: TI-1 and TI-2. TI-1 antigens are pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) from the outer membrane of the gram-negative cell wall and lipoteichoic acids from the gram-positive cell wall. These antigens activate B-lymphocytes by binding to their specific toll-like receptors rather than to B-cell receptors (see Figure 11). Antibody molecules generated against TI-1 antigens are often called "natural antibodies" because they are always being made against bacteria present in the body.

b. The activation of naive T-lymphocytes requires co-stimulatory signals involving the interaction of accessory molecules on antigen-presenting cells or APCs with their corresponding ligands on T-lymphocytes. These co-stimulatory molecules are only synthesized when toll-like receptors on APCs bind to pathogen-associated molecular patterns of microbes (see Figure 12).

2. Signaling PRRs found in the membranes of the endosomes (phagolysosomes) used to degrade pathogens (see Figure 5):

a. TLR-3 - binds double-stranded viral RNA;
b. TLR-7 - binds single-stranded viral RNA, such as in HIV, rich in guanine/uracil nucleotide pairs;
c. TLR-8 - binds single-stranded viral RNA;
d. TLR-9 - binds unmethylated cytosine-guanine dinucleotide sequences (CpG DNA) found in bacterial and viral genomes but uncommon or masked in human DNA and RNA.

Most of the TLRs that bind to viral components trigger the synthesis of cytokines called interferons that block viral replication within infected host cells as well as inflammatory cytokines.

Flash animation showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.

html5 version of animation for iPad showing showing toll-like receptors (TLRs) recognizing viral double-stranded RNA.

GIF animation showing the antiviral nature of interferon.
3. Signaling PRRs and DRRs found in the cytoplasm (see Figure 5)

Pattern-recognition receptors or PRRs found in the cytoplasm include:

a. NODs (nucleotide-binding oligomerization domain)

NOD proteins, including NOD-1 and NOD-2, are cytoplasmic proteins that allow intracellular recognition of peptidoglycan components.

1. NOD-1 recognizes peptidoglycan containing the muramyl dipeptide NAG-NAM-gamma-D-glutamyl-meso dianaminopimelic acid, part of the peptidoglycan monomer in common gram-negative bacteria and just a few gram-positive bacteria.

2. NOD-2 recognizes peptidoglycan containing the muramyl dipeptide NAG-NAM-L-alanyl-isoglutamine found in practically all bacteria (see Figure 5).

As macrophages phagocytose either whole bacteria or peptidoglycan fragments released during bacterial growth, the peptidoglycan is broken down into muramyl dipeptides. Binding of the muramyl dipeptides to NOD-1 or NOD-2 leads to the activation of genes coding for inflammatory cytokines such as IL-1, TNF-alpha, IL-8, and IL-12 in a manner similar to the cell surface TLRs. Activation of NOD-2 also induces the production of antimicrobial peptides such as defensins as well as microbicidal reactive oxygen species (ROS).

b. CARD-containing proteins

CARD (caspase activating and recruitment domain)-containing proteins, such as RIG-1 (retinoic acid-inducible gene-1) and MDA-5 (melanoma differentiation-associated gene-5), are cytoplasmic sensors of viral RNA molecules that trigger the synthesis of type-1 interferons, antiviral cytokines that block viral replication within infected host cells in a manner similar to the endosomal TLRs. RIG-1 recognizes 5'-PPPs on viral RNAs. The 5'-PPPs on host cell RNAs are either capped or removed and are not recognized by RIG-1. Rig-1 and MDA-5 can also, through another regulatory pathway, stimulate the production of inflammatory cytokines.

Detection of PAMPs by PRRs in the cytosol trigger the formation of multi-protein complexes called inflammasomes which, in turn, leads to the activation of caspase-1. Caspase-1 triggers the formation of inflammatory cytokines and can also result in an inflammatory response-induced cell suicide called pyroptosis. Pyroptosis, unlike apoptosis, leads to the release of PAMPS as well as inflammatory cytokines from the lysed cell.

Pyroptosis is initiated by PAMPs binding to pattern-recognition receptors (PRRs) on various defense cells which then triggers the production of inflammatory cytokines and type-1 interferons. Other PRRs, called nod-like receptors (NLRs) located in the cytosol of these defense cells recognize PAMPs and DAMPs that have entered the host cell’s cytosol. Some NLRs trigger the production of inflammatory cytokines while others activate caspase 1-dependent pyroptosis of the cell causing the release of its intracellular inflammatory cytokines (see Figure 1). The binding of PAMPs or DAMPs to their respective NLRs triggers the assembly of multiprotein complexes called inflammasomes in the cytosol of the host cell. It is these inflammasomes that activate caspase 1 and induce inflammation and pyroptosis. Pyroptosis results in production of proinflammatory cytokines, rupture of the cell’s plasma membrane, and subsequent release of proinflammatory intracellular contents. It plays an essential role in innate immunity by...
promoting inflammation to control microbial infections. At highly elevated levels, however, it can cause considerable harm to the body and even death.

c. Danger recognition receptors or DRRs

Danger recognition receptors or DRRs found in the cytoplasm recognize danger-associated molecular patterns (DAMPs) in the cytosol such as altered membrane phospholipids, and materials released from damaged phagosomes and damaged lysosomes, including antibodies bound to microbes from opsonization. DAMPs are also produced as a result of tissue injury during cancer, heart attack, and stroke. Detection of DAMPs by DRRs in the cytosol also triggers the activation of inflammasomes, release of inflammatory cytokines, and pyroptosis.

4. Secreted signaling PRRs found in plasma and tissue fluid

In addition to the PRRs found on or within cells, there are also secreted pattern-recognition receptors. These PRRs bind to microbial cell walls and enable them to activate the complement pathways, as well as by phagocytes. For example, mannan-binding lectin -also known as mannan-binding protein - is synthesized by the liver and released into the bloodstream as part of the acute phase response discussed later in Unit 4. Here it can bind to the carbohydrates on bacteria, yeast, some viruses, and some parasites (see Figure 6). This, in turn, activates the lectin complement pathway (discussed later in Unit 4) and results in the production of a variety of activated complement proteins that are able to trigger inflammation, chemotactically attract phagocytes to the infection site, promote the attachment of antigens to phagocytes via enhanced attachment or opsonization, and cause lysis of gram-negative bacteria and infected or transformed human cells.

Other secreted PRRs include C-reactive protein (CRP), surfactant protein A (SP-A), surfactant protein D (SP-D), collectin liver 1 (CL-L1), and ficolins.

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Exercise: Think-Pair-Share Questions

1. Compare and contrast the functions of endocytic pattern-recognition receptors and signaling pattern-recognition receptors.

2. Compare and contrast signaling pattern-recognition receptors found on cell surfaces with those found in the membranes of endosomes (phagolysosomes).
Summary

1. Early induced innate immunity begins 4 - 96 hours after exposure to an infectious agent and involves the recruitment of defense cells as a result of pathogen-associated molecular patterns or PAMPS binding to pattern-recognition receptors or PRRs and danger-associated molecular patterns or DAMPs binding to danger-recognition receptors or DRRs.

2. Endocytic pattern-recognition receptors are found on the surface of phagocytes and promote the attachment of microorganisms to phagocytes leading to their subsequent engulfment and destruction. They include mannose receptors, scavenger receptors, and opsonin receptors.

3. Binding of microbial PAMPs to signaling PRRs promotes the production of inflammatory cytokines, antiviral cytokines called type-1 interferons (IFN), chemotactic factors, and antimicrobial peptides. They include toll-like receptors (TLRs) and NODs.

4. PRRs found on the surface of the body's cells typically bind to surface PAMPs on microbes and stimulate the production of inflammatory cytokines.

5. PRRs found within cellular phagolysosomes (endosomes) typically detect nucleic acid PAMPs released during the phagocytic destruction of viruses and stimulate the production of antiviral cytokines called type-1 interferons.

6. PRRs and DRRs found within the cytoplasm of host cells typically trigger the formation of multi-protein complexes called inflammasomes which, in turn, triggers the formation of inflammatory cytokines and can also leads to an inflammatory response-induced cell suicide called pyroptosis.

7. PRRs circulating in the blood and tissue fluid activate the complement pathways and may function as opsonins.

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