3-10 The Toll-Like Receptor Family of Innate Immune Receptors

Mammalian Toll-Like Receptors and their Ligands	
TLR2+TLR1	bacterial lipoproteins
TLR2+TLR6	bacterial lipoproteins, lipoteichoic acid, yeast cell wall mannans
TLR2+?	GPI anchors (parasites), bacterial porins, HMGB1
TLR3	dsRNA
TLR4	LPS, HSPs, HMGB1, some viral proteins
TLR5	bacterial flagellin
TLR7	ssRNA (viral)
TLR8	ssRNA (viral)
TLR9	CpG-containing DNA (viral and bacterial)
TLR10	unknown
TLR11	*Toxoplasma* profilin
TLR12	unknown
TLR13	unknown

Figure 3-30 Table showing the ligand specificity of mammalian TLRs TLR1–9 seem to be closely homologous between mice and humans. The human genome has TLR10 but not TLR11, 12 and 13, whereas the mouse genome has TLR11, 12 and 13 but not TLR10. TLR2 functions in complementary pairs with TLR1 or TLR6. The response to diacylated bacterial lipoproteins from *Mycoplasma* requires TLR2 and TLR6, whereas the response to triacylated bacterial lipoproteins requires TLR2 and TLR1. In addition to recognizing certain RNA species, TLR7 and TLR8 also recognize synthetic imidazoquinolines, compounds that have some utility in topical treatment of viral infections. dsRNA: double-stranded RNA, a replication intermediate of RNA viruses; HMGB1: high mobility group box 1 protein, a chromatin-binding protein released by necrosis; HSP: heat shock proteins, molecular chaperones; LPS: lipopolysaccharide; ssRNA: single-stranded RNA.

Recognition of conserved microbial components by Toll-like receptors leads to inflammation and activation of sentinel immune cells

Microbes that penetrate an epithelial barrier and enter a tissue site are encountered by the three types of sentinel immune cells in the tissues: tissue macrophages, mast cells and immature dendritic cells. These sentinels must be able to distinguish between apoptotic particles generated by normal tissue turnover and particles that are indicative of infection. The molecules mainly responsible for making this pivotal distinction are those of the family of **Toll-like receptors (TLRs)**. Stimulation of macrophages or mast cells through their Toll-like receptors leads to the synthesis and secretion of proinflammatory cytokines and lipid mediators, thereby initiating the *inflammatory response* that recruits both soluble immune components and immune cells from the blood, and which we describe in later sections. TLR stimulation of dendritic cells induces the initiation of an adaptive immune response, as we shall see in the next chapter. In this section and the next we shall focus on the structural and functional features of this family of receptors that enable it to detect the presence of infection and to signal an appropriate response.

The Toll-like receptors were named after the fruit-fly receptor Toll, which was first discovered because it has an important role in early fly development and was later recognized as contributing to innate immunity in adult flies. TLRs are characterized by an amino-terminal extracellular domain composed of repeated motifs high in leucine and known as **leucine-rich repeats (LRRs)**, followed by a single transmembrane domain and a globular cytoplasmic domain called the **Toll/interleukin 1 receptor (TIR) domain**, or **TIR domain** that is also found in IL-1 receptors as well as in adaptors of the TLR signaling pathway, as we shall see in the next section. Thirteen TLRs have been identified in mammals so far. TLRs recognize constituents of microbial cell walls or pathogen-specific nucleic acids (Figure 3-30). Most of the determinants recognized by these receptors are molecules essential to the integrity, function or replication of microbes or viruses, and therefore the infectious agent cannot readily escape detection by changing them. For example, lipopolysaccharide (LPS), the major ligand for TLR4, is a central component of the outer membrane of Gram-negative bacteria (see Figure 3-3), and mutations that ablate the enzymes required for synthesis of LPS are lethal to most species of Gram-negative bacteria. Similarly, double-stranded RNA, the ligand for TLR3, is a central replication intermediate for all RNA viruses, so evasion of TLR3 recognition by these viruses is not easily achieved.

Accessory molecules aid Toll-like receptor recognition of some ligands

A striking feature of TLR ligands is their molecular diversity: they include nucleic acids, proteins, lipids and polysaccharides. How one family of receptors can recognize all of these different types of molecules is not well understood. Some mammalian TLRs are thought to bind directly to their innate immune ligands, but in other cases recognition is greatly facilitated by accessory proteins: one example of this is LPS binding by TLR4, to which two accessory proteins and a soluble lipid-transfer protein contribute.

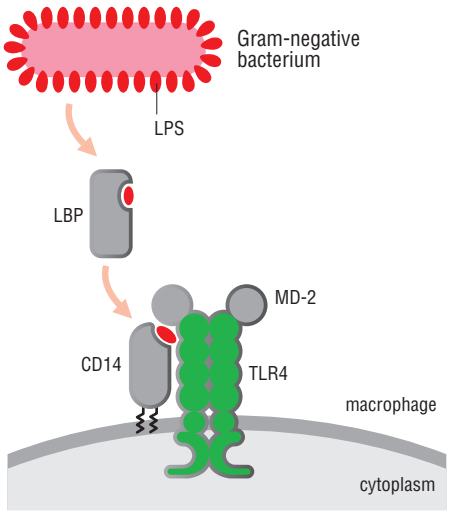

Figure 3-31 Recognition of bacterial lipopolysaccharide by innate immune cells Lipopolysaccharide (LPS) monomers are extracted from bacterial membranes by the serum protein LPS-binding protein (LBP) which transfers the LPS monomer to a lipid-binding site on CD14 in the membrane of phagocytes. CD14 promotes the binding of LPS to the TLR4–MD-2 complex, which signals to the cell interior. In the absence of CD14, TLR4–MD-2 can still function with some forms of LPS or with much higher LPS concentrations.

Definitions

CpG-containing DNA: DNA containing unmethylated C followed by G. Note that the sequence of bases adjacent to the CG motif also affects the stimulatory activity.

LBP: see **LPS-binding protein**.

leucine-rich repeat (LRR): unit of protein structure in which there are many repeats of a basic unit of approximately 25 amino acids.

lipoarabinomannan: a major immunostimulatory component of the lipid-rich mycobacterial cell wall, containing phosphatidylinositol linked to the carbohydrates mannose and arabinose.

LPS-binding protein (LBP): a lipid transfer protein of serum that can extract monomers of LPS from bacterial membranes and deliver them to the innate immune receptor CD14.

LRR: see **leucine-rich repeat**.

MD-2: a polypeptide that associates with the extracellular domain of the **Toll-like receptor** TLR4 and is required for LPS responsiveness.

The requirements for LPS recognition are illustrated in experiments in which the gene encoding TLR4 is introduced into cells that do not normally express it. TLR4 confers responsiveness to LPS on such cells only in the presence of a second polypeptide, called **MD-2**, which binds to the extracellular domain of TLR4 and enables it to bind to the relatively conserved inner lipid-containing region of the lipopolysaccharide (the outer polysaccharide region differs between different bacterial species).

Responses to forms of LPS from many but not all bacteria are also substantially increased by a second accessory molecule, CD14, a membrane protein expressed by monocytes, macrophages and neutrophils, that accepts LPS from a serum lipid transfer protein called **LPS-binding protein (LBP)**. Like most lipids, LPS is released from cells only in very small amounts. Efficient recognition of LPS by innate immune system cells therefore requires a mechanism for extracting LPS monomers and making them available to cells. This is the function of LBP, which exchanges monomers of LPS for other lipids present in its lipid-binding site and then carries the LPS monomer to cells where it is transferred to CD14, which presents it to the TLR4–MD-2 complex (Figure 3-31). Genetic removal of CD14 or blocking its function with monoclonal antibodies greatly reduces the sensitivity of macrophages or neutrophils to LPS, although some responses still occur with LPS from some bacteria. CD14 also promotes responses to other bacterial cell wall components, including lipoteichoic acid of Gram-positive bacteria, and **lipoarabinomannan** of *Mycobacterium tuberculosis*.

In other cases, phagocytic receptors contribute to the recognition for TLR signaling. For example, dimers of TLR2 and TLR6 respond to lipid-containing ligands such as lipoproteins and lipoteichoic acid, which they recognize with the help of a scavenger receptor called CD36, whereas the recognition of fungal cell wall polysaccharides by these TLRs is facilitated by the transmembrane C-type lectin molecule dectin-1.

Mammalian Toll-like receptors recognize their ligands on the cell surface or intracellularly

Whereas TLRs that recognize bacterial and fungal cell wall components are localized to the cell surface, TLRs that recognize viral or microbial nucleic acids are localized to intracellular membranes and are thought to encounter their ligands in phagosomes or endosomes (Figure 3-32). This localization is thought to be an adaptation ensuring that these receptors detect nucleic acids released from apoptotic host cells, microbial cells or virions only after phagocytosis and partial digestion of the ingested particles has released the nucleic acids. Cell-surface TLRs are also targeted to nascent phagosomes and upon ligand binding can signal there.

TLRs participating in innate immunity to viruses recognize a variety of nucleic-acid ligands produced by viruses, although the mechanism by which they distinguish cellular nucleic acids from viral nucleic acids is not always evident. The basis for discrimination is clearest for TLR3. This TLR recognizes double-stranded RNA (dsRNA), which is an obligatory replication intermediate for viruses with RNA genomes, whereas vertebrate cells express very little dsRNA. TLR7 and TLR8 recognize single-stranded RNA that is rich in guanosine, and it has not yet been established how viral RNAs are distinguished from cellular RNAs. TLR9 recognizes DNA motifs containing the dinucleotide CG where the C is unmethylated (**CpG-containing DNA**). This dinucleotide is about 10-fold under-represented in vertebrate DNA, and most such dinucleotides are methylated in vertebrate genomes where CpG methylation is a gene-silencing mechanism.

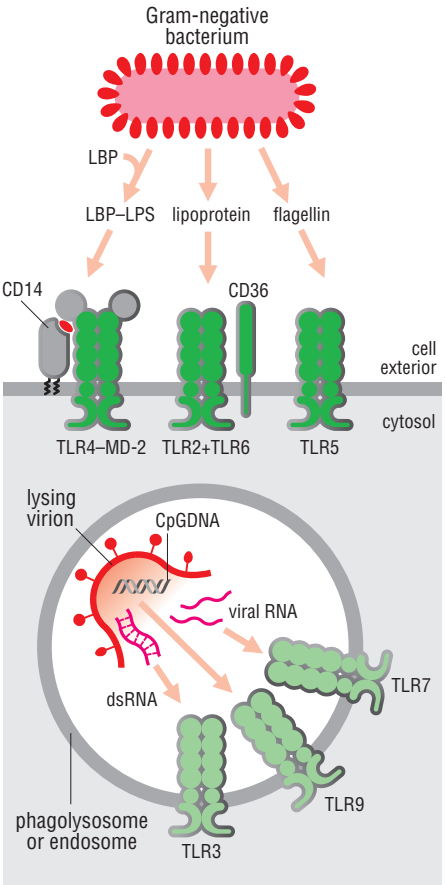

Figure 3-32 Cellular localization of TLRs TLRs that recognize bacterial and fungal cell wall components, such as TLR4–MD-2, TLR5 and the heterodimers TLR2+TLR1 and TLR2+TLR6, are localized to the plasma membrane and can recognize ligands there. They are also delivered to nascent phagosomes, apparently by a mechanism that is independent of ligand binding. In contrast, TLRs recognizing nucleic acids (TLR3, TLR7, TLR8 and TLR9) are primarily or completely contained in intracellular membranes and unavailable for interaction with extracellular ligands. It is believed that these TLRs are delivered to phagolyosomes or late endosomes. Ligand recognition by these TLRs can often be blocked by agents that block the acidification of endosomes or phagosomes, such as chloroquine.

TIR domain: see **Toll/interleukin 1 receptor (TIR) domain**.

TLR: see **Toll-like receptor**.

Toll/interleukin 1 receptor (TIR) domain: domain responsible for transmitting signals downstream of IL-1 receptors and **Toll-like receptors**.

Toll-like receptors (TLRs): family of receptors that have leucine-rich repeats in their extracellular domains and the **TIR domain** in their cytoplasmic domains.

References

Cook, D.N. *et al.*: **Toll-like receptors in pathogenesis of human disease.** *Nat. Immunol.* 2004, **5**:975–979.

Poltorak, A. *et al.*: **Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene.** *Science* 1998, **282**:2085–2088.

Takeda, K. *et al.*: **Toll-like receptors.** *Annu. Rev. Immunol.* 2003, **21**:335–376.

Takeuchi, O. *et al.*: **Differential roles of TLR2 and TLR4 in recognition of Gram-negative and Gram-positive bacterial cell wall components.** *Immunity* 1999, **11**:443–451.

Ulevitch, R.J. and Tobias, P.S.: **Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin.** *Annu. Rev. Immunol.* 1995, **13**:437–457.