

## 2

# Mechanisms of Innate Immunity in Sepsis

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The pathogenesis of the sepsis syndrome is critically dependent on activation of the innate immune response. Innate immunity plays a direct role in the development of sepsis and is also crucial for the activation and modulation of later antigen-specific adaptive immune responses. Nearly all of the clinical manifestations of sepsis and the systemic inflammatory response syndrome (SIRS) can be attributed to components of the innate immune response. However, this review focuses on the new and expanding field of innate immune activation by pathogen-responsive receptors, most importantly, the toll-like receptors (TLRs).

## The Innate Immune Response in Sepsis

The human innate immune system is the first line of defence against invading pathogens. Its remarkable ability to respond rapidly to a wide range of microorganisms is essential for survival. It combats and contains infection at the point of entry, signals danger to other systems, and allows time for the T and B cells of the more finely tuned, antigen-specific, adaptive immune system to become effective. In evolutionary terms, a vigorous innate immune response will have conferred a survival advantage to our hominid ancestors when the loss of the thick fur characteristic of other primates left the skin vulnerable to frequent injuries and contamination. Although the molecular and cellular components of the innate immune system differ little between mammalian

species, the human response to microbial components is one of the most sensitive. The disadvantage of such a vigorous response is an increased susceptibility to exaggerated systemic inflammation and shock when the innate immune system is activated systemically rather than locally.

The innate response is triggered by activation of cells equipped to respond to pathogens or specific pathogen components—cells of the macrophage/monocyte lineage, natural killer cells, dendritic cells, and endothelial cells. The activated cells secrete inflammatory mediators including cytokines (most importantly, tumor necrosis factor [TNF]- $\alpha$ , interleukin [IL]-1, and IL-6), chemokines (such as IL-8), prostaglandins, and histamine. These mediators act on vascular endothelial cells to cause nitric oxide-mediated vasodilatation, increased vascular permeability, and neutrophil recruitment into tissues. The coagulation cascade is activated locally with up-regulation of endothelial tissue factor, and decrease in thrombomodulin and its antithrombotic product, activated protein C.

In systemic sepsis, the local responses become widespread. Systemic vasodilatation causes hypotension, shunting, and reduced tissue oxygen delivery. Endothelial activation and apoptosis result in loss of vascular integrity, proteinaceous exudate, and edema. Disseminated intravascular coagulation produces small-vessel microthrombosis, depletion of clotting factors, and coagulopathy. Reactive oxygen species are generated from activated neutrophils, tissue effects of nitric oxide, and cytokine-induced alterations in cellular metabolism. The cumulative effect of these changes

is increasing severity of sepsis, with multiple organ dysfunction and worsening mortality.

## Triggering of Innate Immune Responses

Until recently, our understanding of sepsis lacked a description of the mechanism by which cells of the innate immune system recognize and respond to microbial threats. By definition, these cells lack the elegant (but relatively slow and energetically costly) antigen-specific receptor systems that characterize the adaptive immune response. In recent years, our understanding of immune reactivity has changed from the pure discrimination of “self” from “nonself” epitopes to an appreciation of the importance of specific “danger” signals in initiating, directing, and modulating the immune response. Danger signals can come from internal sources that indicate tissue damage or invasion, such as products of cell lysis, coagulation, or complement cascades, or from exogenous material, such as microbial surface molecules or genetic material. It is to these danger signals that the innate immune system responds. The fact that both microbial and internal danger signals can trigger the response explains the similarity of the sepsis syndrome to SIRS with a noninfective precipitant, such as trauma, burns, or pancreatitis.

Recognition of microbial danger signals requires a receptor system that responds to evolutionarily conserved structural components of microorganisms, so that an organism cannot use genetic variability to escape detection. The components that allow microorganisms to trigger the immune response are termed pathogen-associated molecular patterns (PAMPs). Typical PAMPs include lipopolysaccharide (LPS) and peptidoglycan from the cell walls of gram-negative and gram-positive bacteria, respectively, bacterial flagellin, and microbial DNA and RNA.

## Toll-Like Receptors

TLRs are the principal PAMP receptors in the innate immune system and obtained their unusual name because of their similarity to the receptor

Toll (German for “funky” or “cool”) in the fruit fly *Drosophila*. Toll was initially a cause for scientific excitement when it was found to be responsible for dorsoventral body patterning in *Drosophila*, but, in addition, was later shown to form part of the fly’s immune defense against fungal infections. This phylogenetically ancient system of pathogen detection is highly conserved in evolution, with similar receptors occurring not only in humans and invertebrates, but also in plants such as tobacco.

Eleven different TLRs have been identified in mammals. The first to have its involvement in pathogen recognition demonstrated, and the most studied, is TLR4, which responds to the most powerful stimulant of innate immune responses, gram-negative bacterial endotoxin (LPS). This was established through study of two strains of mice that fail to mount a septic response to large doses of endotoxin and that were shown to have a loss-of-function mutation in the gene for TLR4. Subsequently, other TLRs and their ligands have been identified; these are summarized in Table 2.1. Some TLRs are able to respond to microbial ligands on their own, but, in many cases, the response depends on the interaction of several different molecules at the cell surface. TLR dimers are required for signaling through TLR4 (homodimers of two TLR4 molecules) and TLR2 (heterodimers with either TLR1 or TLR6, with the combination determining the ligand specificity of the receptor complex). In addition, LPS signaling through TLR4 requires the interaction of several other molecules at the receptor complex; LPS is delivered to the receptor by soluble LPS binding protein (LBP), and effective receptor activation requires the presence of at least two additional molecules, CD14 and MD2.

There is differential subcellular localization of individual TLRs. TLR2 and TLR4 are expressed on the cell surface, where they are most likely to encounter material from microbial cell walls. TLR3 and TLR9 are located within endosomes, where they are most likely to encounter their ligands in the lytic products of phagocytosed microorganisms.

At first, the triggering of immune responses by a relatively small range of receptors and ligands might seem crude. However, most microorganisms present more than one TLR ligand, therefore, it

**TABLE 2.1.** TLRs and their known ligands

TLR	Ligands
TLR1 (heterodimer with TLR2)	Triacylated lipopeptides, lipomannans from <i>Mycobacterium tuberculosis</i>
TLR2 (often dimer with TLR2 or 6)	Lipoproteins, peptidoglycans, lipoteichoic acids, yeast zymosan
TLR3	Double-stranded RNA
TLR4 (homodimer plus CD14 and MD2)	LPS, heat shock proteins, pneumolysin, respiratory syncytial virus coat proteins, heparan sulphate fragments, fibrinogen peptides
TLR5	Flagellin
TLR6 (heterodimer with TLR2)	Diacylated lipopeptides
TLR7	Responds to synthetic nucleosides and imidazoquinoline antivirals; native ligand is thought to be single-stranded RNA in endosomes
TLR8	Same as for TLR7
TLR9	Bacterial DNA—unmethylated CpG motifs
TLR10	Ligand unknown but TLR10 expressed in lung and B lymphocytes
TLR11	Uropathogenic bacteria in mice; absent in humans

is likely that microbes with differing patterns of molecular motifs can cause differential activation of a number of TLRs, allowing differential responses to various classes of pathogen.

## TLR Signaling

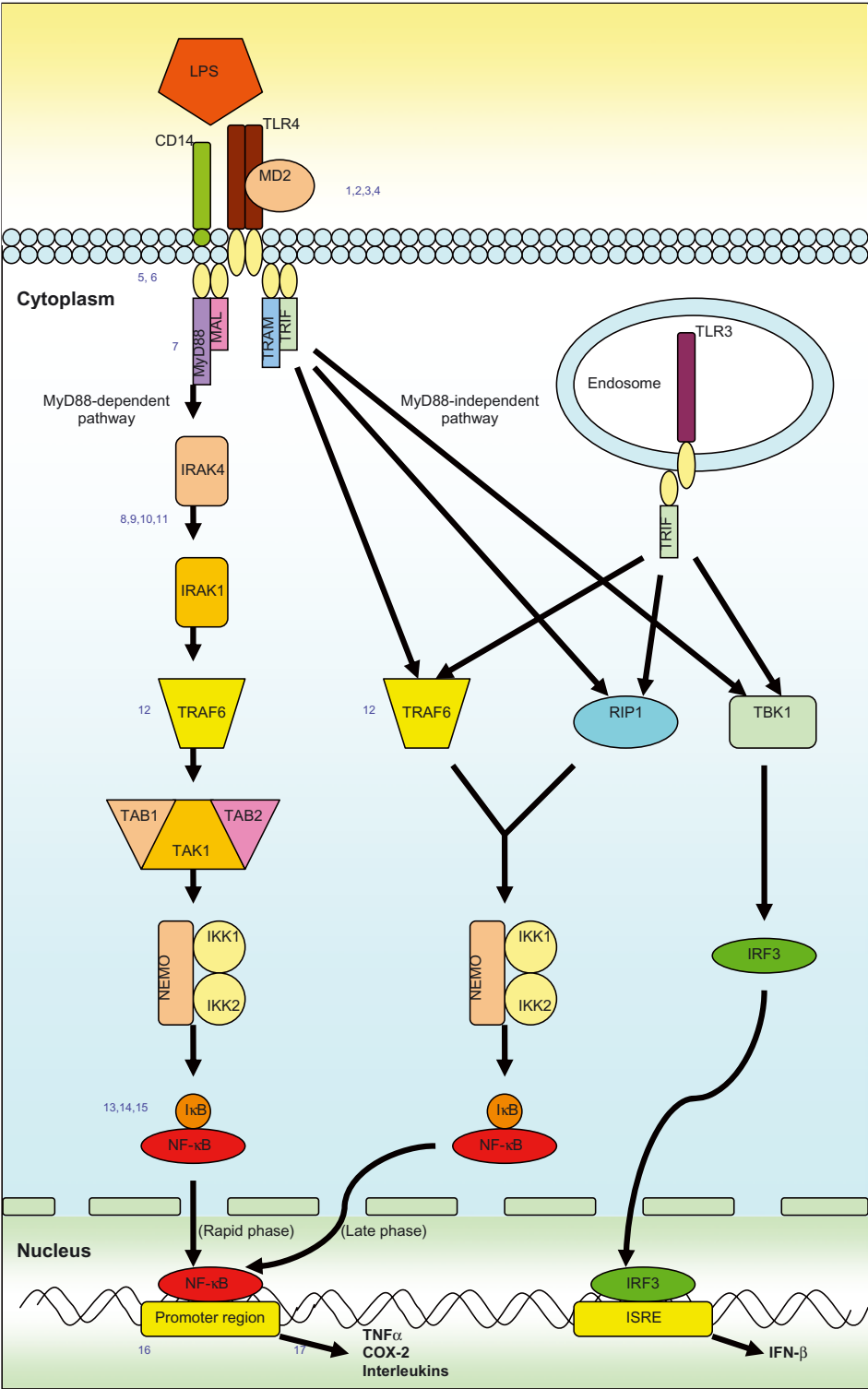
Understanding of the signaling pathway through which TLR ligation leads to activation of a cell and secretion of inflammatory mediators has advanced considerably in the last few years. The end product of intracellular signal transduction is activation of transcription factors, which translocate to the nucleus and modulate transcription of target genes. The principal transcription factor in inflammation is nuclear factor  $\kappa$ B (NF $\kappa$ B), which up-regulates transcription of genes for inflammatory mediators such as TNF $\alpha$ , ILs, and cyclooxygenase (COX)-2. Other transcription factors under TLR regulation induce proapoptotic, antiapoptotic, and even anti-inflammatory gene transcription, although how the differential effects of these pathways are modulated is not yet well understood.

Apart from TLR3, all TLRs signal down a common pathway accessed via the adaptor molecule, myeloid differentiation factor (MyD)-88. The various signaling intermediates have been

identified and are likely to be the targets of future immunomodulatory therapies in sepsis and inflammatory disease, and therefore, are summarized in Figure 2.1.

MyD88 recruits a kinase, IL-1 receptor-associated kinase (IRAK)-4, and facilitates its phosphorylation of IRAK-1. IRAK-1 then associates with TNF receptor-associated factor (TRAF)-6 to activate the transforming growth factor- $\beta$ -activating kinase (TAK)-1/TAK1 binding protein (TAB) complex, which, in turn, enhances the activity of the inhibitor of NF $\kappa$ B (I $\kappa$ B) kinase (IKK) complex. NF $\kappa$ B is held inactive in the cytoplasm by its inhibitor, I $\kappa$ B. The IKK complex phosphorylates I $\kappa$ B, leading to its degradation and the release of free NF $\kappa$ B, which can translocate to the nucleus. There, NF $\kappa$ B undergoes phosphorylation and associates with other transcription regulators to activate inflammatory gene transcription.

TLR3 and TLR4 can also access a separate MyD88-independent pathway to inflammatory gene transcription using the adaptor molecules toll/IL-1 receptor domain-containing adaptor-inducing interferon- $\beta$  (TRIF) and TRIF-related adaptor molecule (TRAM). This pathway leads to a slower activation of NF $\kappa$ B and also to transcription of genes for the type 1 interferons via a different transcription factor, interferon regulatory factor (IRF)-3.



**FIGURE 2.1.** Initiation of inflammation through TLRs. Intracellular signaling through other TLRs uses the MyD88-dependent pathway with small variations. Numbers in blue indicate the site of action of the regulators of TLR signaling summarized in Table 2.2.

MAL, MyD88 adaptor-like; NEMO, NFκB essential modulator; RIP1, receptor-interacting protein 1; TBK1, TRAF-family member-associated NFκB activator-binding kinase 1; ISRE, IFN-stimulated response element.

**TABLE 2.2.** Known regulators of TLR signaling<sup>a</sup>

	Regulator	Action
1	Soluble TLRs	Bind ligand and prevent interaction with cell-surface TLR2 and TLR4; soluble TLR4 can also bind MD2 making it unavailable to the LPS receptor complex
2	Triad3A	Ubiquitylates TLRs, marking them for degradation
3	RP105	Inhibits ligand binding to TLR4
4	MD2B	Inactive variant of MD2
5	Single immunoglobulin IL-1-related receptor (SIGIRR)	Sequesters adaptor molecules MyD88 and MAL
6	ST2	Sequesters adaptor molecules
7	MyD88s (short)	Splice variant of MyD88, antagonizes MyD88
8	Suppressor of cytokine signaling (SOCS)-1	Inhibits IRAK
9	Phosphatidylinositol 3 kinase (PI3K)	Mechanism still unknown
10	IRAK-M	Inhibits IRAK phosphorylation
11	Toll-interacting protein (TOLLIP)	Inhibits phosphorylation and facilitates degradation of IRAK
12	A20	Inhibits TRAF6
13	IκB	Activated NFκB increases transcription of its own inhibitor, providing negative feedback control
14	TNF-related apoptosis-inducing ligand receptor (TRAILR)	Stabilizes IκB, so more NFκB is retained in the cytoplasm
15	Nucleotide-binding oligomerization domain (NOD)-2	Activated by bacterial muramyl dipeptide and suppresses NFκB; defective in Crohn's disease
16	Inhibitors of transcription	Inhibitory forms of NFκB and IκB $\epsilon$ block NFκB DNA binding; chromatin remodeling (changes in methylation and acetylation) alters rate of transcription
17	Reduced stability of messenger RNA	Reduces synthesis of inflammatory compounds

<sup>a</sup>Sites of action are marked with blue numbers in Figure 2.1.

## Regulation of TLR Signaling

TLR activation can trigger a rapid and vigorous inflammatory response, therefore, it is not surprising that TLR signaling is subject to regulation at multiple levels. Some regulatory molecules are constitutively expressed in tissues and plasma, whereas others are induced by activation of the TLR signaling pathway and, thus, provide negative feedback regulation. There is negative feedback within the signaling pathway itself with the gene for the inhibitor IκB being under direct control of a NFκB-binding promoter sequence, thus, NFκB activation results in increased IκB concentration and subsequent down-modulation of the NFκB effect. Known regulators of TLR signaling are summarized in Table 2.2 and their site of action marked on Figure 2.1.

## Endotoxin Tolerance

Repeated observations have demonstrated that the natural history of an episode of sepsis or SIRS consists of the initial inflammatory phase of

vigorous innate immune responses, and then a period of relative immune suppression in which the individual is at increased susceptibility to further infections. These secondary infections tend not to elicit as vigorous an inflammatory response as the initial infection and can insidiously become widespread. This is paralleled by the responses of isolated monocytes, which, after an initial stimulation with LPS, show diminished proinflammatory cytokine responses to repeat stimulation. This phenomenon of “endotoxin tolerance” has also been demonstrated with other TLR responses, with previous exposure to a TLR ligand producing diminished responses to the same TLR ligand (termed “homotolerance”) and, in some cases, to other TLR ligands (“heterotolerance”).

The mechanism of TLR tolerance is still being investigated and some of the regulators of TLR signaling mentioned above have been implicated in its etiology. Other mechanisms may involve down-regulation of surface TLRs or nuclear events that suppress the transcription of proinflammatory genes. Recent work has suggested that

endotoxin tolerance is not simply an all-or-nothing “off switch” for inflammation, but rather a state of immune “reprogramming”—a switch to more anti-inflammatory cytokine profiles with modulation of LPS sensitivity, so that markedly increased doses can still induce an inflammatory response. It is becoming clear that the surrounding cytokine milieu can modulate the effect of tolerance with, for instance, interferon- $\gamma$  restoring LPS sensitivity in some systems.

Endotoxin tolerance may have developed as a protective mechanism to avoid death from the cytokine storm associated with severe sepsis but, in the age of intensive care, it puts the postseptic patient in danger of later infective complications. Treatments designed to reverse tolerance and “reboot” the innate immune response might give hope of improving survival after sepsis. However, these will have to be developed with caution because endotoxin tolerance may be significant in situations other than sepsis. Organ systems, such as the gut and liver, that are exposed to tonic levels of TLR ligands from commensal microbes may rely on the tolerance mechanisms to physiologically elevate their threshold for activation and prevent unwanted inflammation.

## Clinical Directions

The coming years will see increasing relevance of TLR signaling to clinical practice. Polymorphisms of genes for TLRs and components of the signaling pathway have already been shown to influence severity of sepsis and susceptibility to invasive bacterial disease. Increased understanding of the “inflammatory” and “tolerant” phases of the septic response may help novel anti-inflammatory and immunostimulatory therapies to be used appropriately.

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<http://www.springer.com/978-1-84628-938-5>

Sepsis

Baudouin, S.V. (Ed.)

2008, VIII, 103 p. 60 illus., Softcover

ISBN: 978-1-84628-938-5