

## IMPLICATION OF TOLL-LIKE RECEPTOR AND TUMOR NECROSIS FACTOR $\alpha$ SIGNALING IN SEPTIC SHOCK

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Received 6 Oct 2004; first review completed 15 Nov 2004; accepted in final form 19 Jul 2005

**ABSTRACT**—Septic shock is initiated by a systemic inflammatory response to microbial infection that frequently leads to impaired perfusion and multiple organ failure. Because of its high risk of death, septic shock is a major problem particularly for patients in the intensive care unit. In general, bacterial lipopolysaccharide (LPS) is a strong activator of various immune responses and stimulates monocytes/macrophages to release a variety of inflammatory cytokines. However, overproduction of inflammatory factors in response to bacterial infections is known to cause septic shock, similar to that induced by LPS. Studies of LPS-signaling pathways and downstream inflammatory cytokines may have critical implications in the treatment of sepsis. In recent years, there has been significant progress in understanding the signaling pathways activated by LPS and its receptor Toll-like receptor 4 (TLR4), as well as by tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), a potent inflammatory cytokine induced by LPS stimulation. This review briefly summarizes our current knowledge of these signaling pathways and critical signal transducers. Characterization of key signal transducers may allow us to identify tractable, novel targets for the therapeutic interventions of sepsis.

**KEYWORDS**—Septic shock, inflammation, tumor necrosis factor  $\alpha$ , toll-like receptor

### TOLL-LIKE RECEPTOR (TLR) SIGNALING

The ability of the mammalian innate immune system to defend against bacterial infection is essential for the first line of host defense. TLRs are evolutionarily conserved proteins that recognize pathogen-associated molecular pattern (PAMP) produced by bacteria, virus, or other pathogens (1). The Toll protein was originally identified in *Drosophila* as a multifunctional molecule that mediates an immune-like response (2). In mammals, at least 11 TLRs have been identified. Based on their cytoplasmic domains, mammalian TLRs are also homologous to members of the IL-1 receptor (IL-1R) family (3). Furthermore, the cytoplasmic domains of TLRs and IL-1R are homologous to domains in the plant R gene product, giving these regions the name of “TIR” domains (4). The signaling mechanisms mediated by TIR domains are remarkably similar in different organisms (2, 4). In *Drosophila*, the receptor-proximal signaling pathway downstream of Toll involves the adaptor proteins dMyD88 and Tube, which transmit signals to the serine-threonine kinase Pelle (5, 6). Pelle in turn signals via the Dorsal/Cactus complex in an equivalent manner as the transcription factor NF- $\kappa$ B in mammals (7).

Lipopolysaccharide (LPS), a structural component of the outer wall of gram-negative bacteria, is a PAMP that can activate monocytes/macrophages and induce endotoxic shock in mammals. Genetic mapping and mouse models have identified TLR4 as an essential receptor for LPS signaling (8, 9).

However, the TLR4 receptor alone is unable to confer LPS responsiveness (10). TLR4 appears to require the coreceptor protein CD14 plus a secreted protein MD2 to transmit the LPS signal. CD14 is required for the presentation of LPS to TLR4-MD-2 (11). CD14-deficient mice do not respond to LPS-induced shock, suggesting an essential role of CD14 in the LPS-binding process (11). MD-2 associates with the extracellular domain of TLR4 and supports the induction of NF- $\kappa$ B activation by TLR4 (11). Formation of the LPS and TLR4/MD-2 complex is a key step in activating cytoplasmic-signaling mediators.

Mammalian TLRs share similar cytoplasmic domains with the IL-1 receptor family, therefore they both use the same signaling pathway upon ligand binding (Fig. 1A). Several common proteins mediate both of their downstream signaling cascades, including the adaptor protein myeloid differentiation factor 88 (MyD88), IL-1 receptor-associated kinases (IRAKs), and TNF receptor-activated factor 6 (TRAF6) (12–14). For examples, MyD88 is recruited to IL-1R and TLR4 via TIR domain interactions, whereas Mal/TIRAP specifically associates with TLR4. Recruited by its death domain, IRAK-4 transduces signals mediated by MyD88. IRAK-1 appears to be an important substrate for the phosphorylation activity of IRAK-4. IRAK-1 and IRAK-4 associate with TRAF6 in the cytoplasm. TRAF6 forms a complex with TAB1/TAB2/TAK1 in which the kinase activity of TAK1 mediates downstream events such as the activation of the IKK complex and NF- $\kappa$ B (15). In addition to MyD88/Mal\_IRAK1/4\_TRAF6 pathway, TLR4 also uses TRIF and TRAM, two other MyD88 homologous proteins, to signal. TRIF/TRAM pathway contributes to NF- $\kappa$ B activation as well as establishes an antiviral response mediated by IRF-3 activation and induction of type I interferons.

Many genetically modified mouse models lacking key molecules involved in TLR4-mediated NF- $\kappa$ B signaling are

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W.-J.L. is a postdoctoral fellow supported by Prostate Cancer Training Grant at Princess Margaret Hospital, University of Toronto Health Network.

DOI: 10.1097/01.shk.0000180074.69143.77

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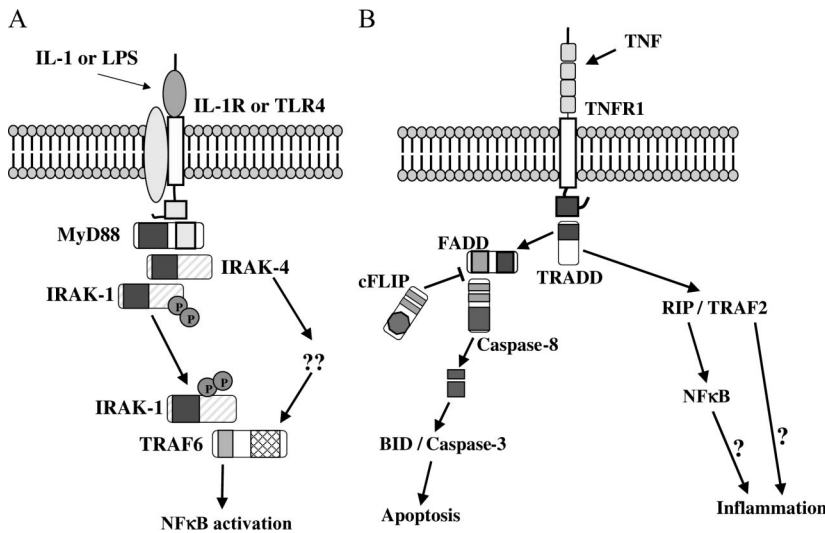


FIG. 1. **A simplified diagram of the TLR4 and TNF $\alpha$  signaling pathways.** (A) Activation of IL-1R or TLR4 initiates the recruitment of MyD88 to the receptor complex, which induces association of IRAK4 and IRAK1 followed by interaction with TRAF6. Subsequently, the signal from the receptor complex activates IKK complex and the activity of NF $\kappa$ B. (B) Engagement of TNF $\alpha$  with TNF $\alpha$ R1 induces the recruitment of TRADD, TRAF2, RIP, FADD, cFLIP, and caspase 8. Depending on distinct cell contexts, the formation of the receptor and downstream complex triggers downstream signaling, leading to apoptosis or NF $\kappa$ B activation, and induction of target genes, mediating inflammation.

protected from LPS-induced endotoxic shock. It is worth noting that LPS responses may vary depending on mouse genetic backgrounds (16). Because TLR activation is the initiating event after various pathogen infections, key proteins controlling this process may be the targets for therapeutic interventions (15, 17). For example, we found that IRAK-4-deficient mice are highly resistant to LPS-induced septic shock, suggesting that IRAK-4 may be an ideal therapeutic target to control septic shock. One concern is that innate immunity may be compromised by the ablation of IRAK-4 function because the knockout mice show difficulty in clearing *Staphylococcus aureus* infections (15). Recent reports on IRAK-4-deficient human patients reveal that these patients are capable of compensating for the loss of IRAK-4 in the long run, although they suffer recurrent bacterial infections during childhood.

Changes in TLR expression patterns have also been shown in leukocytes from patients with sepsis, suggesting that TLR expression on immune cells or other tissues may determine LPS responsiveness of cells at the surface level (18, 19). In addition to the TLR signal cascades, subsequent immune and inflammatory responses activated by TLRs against microbial invasion also play a crucial role in the development of sepsis. Upon TLR activation, many events are associated with apoptosis of immune effector cells and robust production of proinflammatory cytokines such as TNF $\alpha$ , IL-1, IL-6, and IL-8 (20–22). It has been well demonstrated that the TLR function plays a major role in the regulation of the apoptosis of macrophages in responding to PAMP molecules (21, 23, 24). The adaptor protein MyD88 mediates TLR2-induced apoptosis via protein-protein interactions with Fas-associated death domain (FADD) protein and caspase-8, suggesting that the crosstalk between the TLR and the death receptor signaling pathway may contribute to the various events triggered by microbial infection.

### TNF $\alpha$ SIGNALING

Excessive production of proinflammatory cytokines not only enhances immune responses fighting invading pathogens, but also has deleterious effects that perturb regular hemodynamic and metabolic balances. TNF $\alpha$  is one typical proinflammatory

cytokine that is produced at a high level in circulation during sepsis. In response to LPS challenge, TNF $\alpha$  is produced very quickly and the production peaks in 1.5 h (22). On the other hand, production of TNF $\alpha$  can affect the expression level of TLR4, suggesting that TNF $\alpha$  may regulate the inflammatory response by modulating TLR4 expression (25). Injection of neutralizing antibodies against TNF $\alpha$  was considered a promising therapeutic approach to block LPS-induced lethal inflammation (26, 27). However, blocking TNF $\alpha$  in patients with sepsis has not achieved the same therapeutic efficiency as observed in animal models. Furthermore, there appear to be unwanted side effects associated with neutralizing antibodies against TNF $\alpha$ . Therefore, characterization of TNF $\alpha$  downstream signaling cascades may help us find better therapeutic targets to modulate inflammatory and apoptotic cascades during the development of sepsis.

Two major signaling cascades, apoptotic and inflammatory, are induced by TNF $\alpha$ . The apoptotic signaling pathway is mediated by TNF receptor 1 (TNFR1), which is a member of so-called death receptors (DRs) that also include Fas, DR3-6. DRs are characterized by the presence of a motif called the death domain in their cytoplasmic tails (28). There are two basic models of DR-induced apoptosis signaling cascades: one exemplified by the engagement of Fas and the other by the engagement of TNFR1. The first event after the binding of the Fas ligand (FasL) to Fas is the direct recruitment of FADD to the cytoplasmic tail of Fas (29, 30). FADD is the common adaptor protein upon which almost all DR signaling pathways converge (31). FADD binds to Fas through the interaction of their homologous death domains, an event that unmasks the N-terminal death effector domain of FADD. The death effector domain allows FADD to recruit and activate caspase-8, which then triggers downstream caspase cascades through mitochondria-dependent or -independent mechanisms. TNFR1-mediated apoptosis basically models Fas-induced cascade, except that TNFR1 does not recruit FADD directly, but goes through an intermediate adapter called TRADD (Fig. 1B).

The inflammatory arm of TNFR1 signaling is also mediated through TRADD, which recruits TRAF2 and RIP to the TNFR1 complex (32–34). These molecules then trigger the recruitment

of additional mediators, such as NEMO and MEKK-3 (35), which ultimately promote NF- $\kappa$ B activation. NF- $\kappa$ B is a key transcription factor that, once activated, can lead to inductions of many target genes, including those critical for inflammatory responses and cell survival. Therefore, this arm of TNF $\alpha$  signaling is important for promoting inflammation as well as for antagonizing apoptosis. Another potential checkpoint of apoptotic signaling takes effect at the level of caspase-8. Recruitment of caspase-8 to FADD can be inhibited by cFLIP (structurally similar to caspase-8 but lacking the enzymatic activity), which is a critical regulator for Fas- and TNF $\alpha$ -mediated apoptosis (36).

Mice deficient in many key mediators of the TNF $\alpha$ -signaling pathways have been generated (37–41). The conclusions drawn from these studies mostly validate that these molecules play an essential role in promoting or inhibiting TNF $\alpha$ -induced apoptosis. Their functions in TNF $\alpha$ -induced inflammation, particularly during bacterial infections, remain to be elucidated because most of these mice die during embryonic stages. Interestingly, TRAF2-deficient mice were found to be particularly sensitive to TNF $\alpha$ -induced pathologies, including apoptosis and excessive inflammation, even though TRAF2 is required for the initial wave of signal transduction induced by TNF $\alpha$  (41). These results suggested that TRAF2 may be a built-in “toner” or regulator of TNF $\alpha$ -signaling, and that the molecular mechanisms associated with TRAF2 regulation may have strong implications in modulating inflammatory responses mediated by TNF $\alpha$ .

Recently another interesting study demonstrated that the Fas-FasL engagement enhances LPS-induced cytokine expression and promotes chronic inflammation (42). An interaction of MyD88 and FADD was implicated in this study, again suggesting a crosstalk between Fas and TLR signaling pathways. In support of this, other reports showed that Fas knockout mice are resistant to LPS-induced lethality (43), and that FasL enhances *in vivo* inflammation and induces dendritic cell maturation, as well as IL-1 $\beta$  production by neutrophils (44–47). These results suggest that there might be some common ground for TLR and TNF $\alpha$  signaling pathways that eventually collaborate to activate inflammatory cascades in response to bacterial infections.

## CONCLUSION

Sepsis frequently threatens the life of intensive care unit patients. Although much progress has been made, more effort is needed to understand the signaling and cascades involved in sepsis development. Many *in vivo* studies using animal models have provided us with insightful information important to the understanding of the roles of TLR and TNF $\alpha$  signaling during bacterial infection and sepsis development. Recently, studies suggest that these two inflammatory signaling cascades may be modulated at a molecular level. Moreover, the two pathways may crosstalk with each other, creating additional potential opportunities of therapeutic interventions and strategies.

## ACKNOWLEDGMENTS

We thank Debby Y. Chuang and Billie Au for editorial assistance.

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