

Activation of toll-like receptors by microbial lipoproteins: Role in host defense

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Conserved throughout evolution, mammalian toll-like receptors (TLRs) participate in innate immune response to microbial pathogens. The TLRs mediate activation by microbial ligands, resulting in cytokine activation as well as other host defense mechanisms. Activation of TLRs also can result in tissue injury including manifestations of septic shock and host cell apoptosis. In this manner, the activation of mammalian TLRs in the context of infectious disease can contribute to host defense and immunopathology. (J Allergy Clin Immunol 2001;108:S104-6.)

Key words: Toll, innate immunity, tuberculosis, IL-12

Effective host defense against invading microorganisms requires the detection of the foreign pathogens and the rapid deployment of an antimicrobial effector response. This type of immune response, known as innate immunity, depends on the recognition of molecular patterns displayed by microbial pathogens. Examples include LPSs of gram-negative bacteria, lipoteichoic acids of gram-positive bacteria, lipoproteins of bacteria and parasites, glycolipids of mycobacteria, mannans of yeast, and double stranded RNAs of viruses. These microbial patterns are detected by pattern recognition receptors present on host cells. Examples include the mannose receptor, which recognizes mannan, and CD14, which recognizes the lipid A portion of LPS. Pattern recognition receptors are nonclonal, that is, present on all cells of a class, eg, macrophages, and do not depend on immunological memory because they are germline encoded. In contrast, the adaptive immune response is slow, its receptors are highly specific, it requires germline rearrangement (ie, T-cell receptors), it is distributed clonally on subsets of particular cells, and it involves immunologic memory. Not only do the innate and adaptive immune responses complement each other,

Abbreviations used

NF- κ B: Nuclear factor- κ B

TLR: Toll-like receptor

they are interactive in that the innate immune response influences the type of adaptive response.

TOLL

Activation of *Drosophila* Toll leads to activation of nuclear factor- κ B (NF- κ B)-like kinases and induction of the antifungal peptide drosomycin. A human homologue of a *Drosophila* Toll was shown to lead to activation of NF- κ B and the up-regulation of cytokines and costimulatory molecules.¹ At least 9 human members of the mammalian Toll family (termed toll-like receptors or TLRs) have been reported.² TLR family members are similar in structure to other pattern recognition proteins, but they have a cytoplasmic signaling portion homologous to the IL-1 receptor.

TLRs AND THE RESPONSE TO LPS

A long-standing enigma in microbial immunology has been the mechanism by which cells are activated by LPS. Although CD14 was known to bind LPS, this pattern recognition receptor is anchored into the cell membrane by a glycosphosphatidylinositol linkage, suggesting it would have little signaling capacity. There now is clear evidence that mammalian TLRs mediate the response to LPS. Yang et al³ provided the first evidence that TLRs mediate responsiveness to microbial ligands by demonstrating that cells transfected with TLR2 can be activated by LPS leading to NF- κ B activity and cytokine induction. Further studies using transfectants, dominant negative constructs, and neutralizing antibodies suggested that both TLR2 and TLR4 could mediate LPS responsiveness.^{4,5} An accessory molecule, MD-2, facilitated the response of TLR4 to LPS.⁵

Despite these biochemical and molecular studies, strong genetic evidence implicates TLR4 in mediating responses to LPS. C3H/HeJ and C57BL/10ScCr mice have naturally occurring mutations in the TLR4 gene that renders them hyporesponsive to LPS.⁶ Takeuchi et al⁷ engineered and studied TLR2 and TLR4 gene knockout mice and demonstrated that TLR4 but not TLR2 mediated the response to LPS. Many LPS preparations contain a mixture of ligands and purified LPS activates TLR4.⁸

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The TLR signaling pathway involves an intracellular signaling cascade involving MyD88, IL-1 receptor accessory protein kinase, TNF-receptor associated factor 6, and NF- κ B-inducing kinase, leading to activation of NF- κ B and subsequent gene transcription.^{9,10} These findings are substantiated by the study of MyD88 gene targeted knockout mice that are hyporesponsive to LPS.¹¹

MICROBIAL LIPOPROTEINS TRIGGER HOST RESPONSES VIA TLRs

Mycobacteria are powerful adjuvants for immune responses.¹² This adjuvant activity may be due in part to their ability to trigger release IL-12¹³ and induce T_H1 responses¹⁴ required to eliminate intracellular pathogens,¹⁵⁻¹⁷ including *Mycobacterium tuberculosis*.^{18,19} Interestingly, patients with mutations in their IL-12R have increased susceptibility to mycobacterial infection.^{20,21} We found that the *M tuberculosis* 19-kD lipoprotein could activate IL-12 promoter activity and cytokine release.²² The 19-kD *M tuberculosis* lipoprotein is a member of a family of prokaryotic lipoproteins²³⁻²⁸ that triggers monocyte/macrophage activation.²⁹ The activity of the lipoprotein is dependent upon its N-terminal triacylated lipopeptide region.^{27,28,30-34} Previous studies indicated that bacterial lipoproteins induced IL-12 mRNA.^{28,35} Further analysis of the mechanism by which the 19-kD lipoprotein induced IL-12 release indicated a key role for TLR2.²² Aliprantis et al³⁶ also demonstrated that microbial lipoproteins induced NF- κ B activity via TLR2 as well as host cell apoptosis, indicating that microbial lipoproteins may induce both TLR-dependent activation of host defense and tissue pathology. This dual signaling pathway is similar to TNFR and CD40 signaling, which can induce both NF- κ B activation and apoptosis.³⁷ TLR2 gene knockout mice do not respond to microbial lipoproteins, and TLR ligand specificity was found to be precise enough to distinguish the natural stereoisomer of the lipid moiety of bacterial lipoproteins.³⁸

ROLE OF TLRs IN HOST DEFENSE

It is evident that TLRs play a prominent role in activating host defense mechanisms. Two important innate immune system responses to pathogen invasion include phagocytosis and gene modulation. Underhill et al³⁹ suggest that following phagocytosis, TLRs are recruited to the pathogen-containing phagosomes and discriminate between gram-positive and gram-negative bacteria. Activation of TLR2 by microbial lipoproteins induces activation of the inducible nitric oxide synthase promoter,²² which leads to the production of nitric oxide, a known antimicrobial agent in mice. The activation of *Drosophila* Toll leads to the production of antimicrobial peptides,⁴⁰ a phenomenon that is yet to be demonstrated in mammalian cells. It was shown that LPS induces β -defensin-2 in tracheobronchial epithelium,⁴¹ however, suggesting the conservation of the *Drosophila* Toll pathway of antimicrobial peptide induction. Therefore, TLRs can lead

to rapid and direct responses that characterize innate defenses against infectious agents.

The TLR activation also can be detrimental, leading to tissue injury. Mice that were administered LPS showed manifestations of septic shock, which is dependent on TLR4.⁶ TLR activation also can lead to apoptosis, which has been suggested as a mechanism of modulating the resulting immune response but also can lead to tissue damage.³⁷ Finally, Toll activation has been implicated in bone destruction.⁴²

The susceptibility to bacterial infection of mice with spontaneous mutations in TLRs implicates TLRs as critical receptors in mammalian host defense.⁴³⁻⁴⁷ TLR4 mutations also have been reported to be associated with LPS hyporesponsiveness in humans.⁴⁸ By inference, we anticipate that humans with genetic TLR alterations will present with increased susceptibility to certain microbial infections. It should be possible to develop strategies to activate TLRs to induce both innate and adaptive immunity as therapy or prophylaxis for a wide variety of microbial pathogens.

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