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MCPIP1 negatively regulates toll-like receptor 4 signaling and protects mice from LPS-induced septic shock

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ABSTRACT

Septic shock is one of leading causes of morbidity and mortality in hospital patients. However, genetic factors predisposing to septic shock are not fully understood. Our previous work showed that MCP-induced protein 1 (MCPIP1) was induced by lipopolysaccharides (LPSs), which then negatively regulates LPS-induced inflammatory signaling in vitro. Here we report that although MCPIP1 was induced by various toll-like receptor (TLR) ligands in macrophages, MCPIP1-deficient mice are extremely susceptible to TLR4 ligand (LPS)-induced septic shock and death, but not to the TLR2, 3, 5 and 9 ligands-induced septic shock. Consistently, LPS induced tumor necrosis factor α (TNFα) production in MCPIP1-deficient mice was 20-fold greater than that in their wild-type littermates. Further analysis revealed that MCPIP1-deficient mice developed severe acute lung injury after LPS injection and JNK signaling was highly activated in MCPIP1-deficient lungs after LPS stimulation. Finally, macrophage-specific MCPIP1 transgenic mice were partially protected from LPS-induced septic shock, suggesting that inflammatory cytokines from sources other than macrophages may significantly contribute to the pathogenesis of LPS-induced septic shock. Taken together, these results suggest that MCPIP1 selectively suppresses TLR4 signaling pathway and protects mice from LPS-induced septic shock.

1. Introduction

The innate immune system is the first line of defense against invading pathogens through an evolutionarily conserved system of pattern recognition [1]. Innate immune cells, including macrophages and dendritic cells, express a series of receptors known as toll-like receptors (TLRs), which bind to highly conserved sequences expressed by microorganisms [2,3]. In humans, more than 10 TLRs have been identified. TLR2 heterodimerizes with TLR1 or TLR6, leading to the recognition of Gram-positive bacterial components, such as lipoprotein [4]. TLR4, the first human TLR cloned [5,6], is engaged by lipopolysaccharide (LPS) found on Gram-negative bacteria, whereas TLR3, TLR5, TLR7, TLR8 and TLR9 recognize other bacterial and/or viral components, including dsRNA (TLR3), Flagellin (TLR5), ssRNA (TLR7 and TLR8), and CpG DNA (TLR9) [7]. All TLRs activate NF-κB and MAPKs, but a particular TLR may use distinct intermediate signaling molecules [8]. For example, cells deficient in the adaptor protein MyD88 are completely refractory to signaling through near all TLRs, with the notable exceptions of TLR3 and TLR4 [9,10]. TLR3 is completely dependent on TRIF but not MyD88, whereas TLR4 has two signaling pathways: one is MyD88 dependent, and the other is TRIF dependent. The engagement of TLR by these ligands results in a potent inflammatory response characterized by the release of proinflammatory cytokines, including TNFα, IL-1β, IL-6, IL-12, and IL-18. Activation of the innate immune system is important for subsequent activation of lymphocytes and other cell types and clearance of infectious organisms. However, exuberant production of proinflammatory cytokines leads to severe immunopathology such as endotoxic shock [11]. To prevent deleterious TLR activation, a number of signaling mechanisms are evoked. These mechanisms include the down-regulation of surface TLR expression, transcriptional induction of negative regulators such as IL-1 receptor-associated kinase (IRAK-M), suppressor of cytokine signaling 1 (SOCS1), and SH2-containing inositol phosphatase (SHIP), and production of anti-inflammatory cytokines, mainly IL-10 and TGF-β [12].

Abbreviations: MCPIP1, MCP-induced protein 1; TLR, toll-like receptor; TNF, tumor necrosis factor; NF-κB, nuclear factor-κB; IL-1β, interleukin 1β; MCP-1, monocyte chemotactic protein-1; LPS, lipopolysaccharide.

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MCP-induced protein 1 (MCPIP1, also known as ZC3H12A) is a recently identified gene in human peripheral blood monocytes treated with MCP-1 [13,14]. The gene undergoes rapid and potent transcription induction upon stimulation with LPS and other proinflammatory molecules, such as TNFα, MCP-1, and IL-1β [14–16]. Further studies showed that MCPIP1 plays an important role in both physiological and pathological processes related to inflammation [17–22]. In the experiments on cultured cells, MCPIP1 was proved to be a negative regulator of LPS-induced macrophage activation [14]. In recent reports on mice, MCPIP1 deficiency leads to a complex phenotype including severe anemia, autoimmune response and severe inflammatory response and most mice died within 12 weeks of birth [17,18]. These results suggest that MCPIP1 may be a resolution molecule that critically controls inflammation and immunity and would be a potential therapeutic target for treatment of human inflammatory diseases such as atherosclerosis and septic shock.

In this study, we examined the regulatory role of MCPIP1 in various TLR ligands-induced septic shock using MCPIP1-deficient mice and macrophage-specific MCPIP1 transgenic mice. Though MCPIP1 was significantly induced by various TLR ligands in macrophages, MCPIP1-deficient mice were extremely sensitive to LPS (TLR4 ligand)-induced septic shock, but not sensitive to the ligands for TLR2, 3, 5 and 9-induced septic shock. Consistently, LPS induced TNFα production in MCPIP1-deficient mice was 20-fold greater than that in their wild-type littermates. Moreover, macrophage-specific MCPIP1 transgenic mice were partially protected from LPS-induced septic shock, suggesting that inflammatory cytokines from the sources other than macrophages may significantly contribute to the pathogenesis of LPS-induced septic shock. These results suggest that MCPIP1 may selectively repress TLR4 signaling and protect mice from LPS-induced septic shock.

2. Materials and methods

2.1. Mice

MCPIP1-deficient ($MCPIP1^{-/-}$) mice and their wild-type ($MCPIP1^{+/+}$) littermates on a C57BL/6 background were generated as described previously [17]. Two lines of macrophage-specific MCPIP1 transgenic mice were generated and used in this study (see Fig. 5). All mice were housed in the Laboratory Research Animal Center at the University of Missouri-Kansas City. All mice were maintained in sterilized filter-top cages and fed with autoclaved food and water under specific pathogen-free conditions. All mice used were between the ages of 6 and 8 weeks, unless indicated otherwise. Experimental procedures were approved by the Animal Care and Use Committee of University of Missouri-Kansas City.

2.2. Cell culture and reagents

Raw264.7 cells were obtained from the American Type Culture Collection (ATCC) and grown as a monolayer in DMEM (Invitrogen) containing 10% FBS, 2 mM L-glutamine, with 100 U/ml penicillin and streptomycin in 5.0% CO_2. The ligands for TLR1-9 were purchased from Invivogen. Protein isolation and Western blot were essentially performed as described previously [17].

2.3. TLR ligands-induced death and cytokine production in mice

Age-matched $MCPIP1^{+/+}$ and $MCPIP1^{-/-}$ mice were injected intraperitoneally with various ligands for TLRs: Pam3CSK4 (2.5 mg/kg body weight), Pam3CSK4 plus D-galactosamine (1 g/kg body weight), poly(I:C) (20 mg/kg body weight), LPS (5 mg/kg body weight), FLA-ST (1 mg/kg body weight), and CpG DNA(5 mg/kg body weight). Survival was monitored every 2–8 h for 72 h. Spleen RNA was isolated from LPS and poly(I:C)-challenged mice after 75 min and the expression of inflammatory cytokines was measured by QPCR. Blood was obtained from LPS-challenged mice at 75 min and the serum concentration of TNFα and IL-6 was measured by ELISA. Two lines of macrophage-specific MCPIP1 transgenic mice and wild-type littermates were injected intraperitoneally with LPS (40 mg/kg body weight). Survival was monitored every 8 h for 72 h. The expression of inflammatory cytokines in spleen and lungs was measured by QPCR.

2.4. Histological analysis

Lungs were obtained from LPS-challenged mice at 2 h. Tissue samples were fixed in 10% neutral buffered formalin for 1 day, subsequently routinely processed, and embedded in paraffin. Sections of 5 μm thickness were stained with hematoxylin and eosin (H&E).

2.5. Statistics

Data were expressed as mean ± SD. For comparison between two groups, the unpaired Student's test was used. For multiple comparisons, analysis of variance followed by unpaired Student's test was used. A value of $p<0.05$ was considered significant.

3. Results

3.1. MCPIP1 was remarkably induced by various TLR ligands in Raw264.7 cells

Previously we showed that MCPIP1 was significantly induced by LPS and other inflammatory cytokines in macrophages [14]. To examine whether MCPIP1 is also induced by other TLR ligands, Raw264.7 cells, a murine macrophage cell line, were stimulated with or without various TLR ligands for 1 and 2 h. QPCR was performed to measure the expression level of MCPIP1 mRNA. As showed in Fig. 1A, MCPIP1 expression was significantly induced by the ligands for TLR1/2, 4, 6 and 9 in a time-dependent manner, but not affected by the ligands for TLR3, 5 and 7/8. To verify these results by Western blot, Raw264.7 cells were stimulated with the ligands for TLRs for 8 h. The cell lysates were isolated and MCPIP1 protein level was detected by Western blot with MCPIP1 antibody (Santa Cruz Biotech.). As shown in Fig. 1B, MCPIP1 protein was up-regulated not only by TLR1/2, 4, 6, and 9, but also by TLR5 and TLR7/8. To further confirm these results, Raw264.7 cells were stimulated with the ligands for TLR2, TLR4, TLR5 and TLR7/8 for 0, 3, 8 and 24 h. As shown in Fig. 1C, MCPIP1 protein level was increased by the ligands for TLR2 (Pam3CSK4), TLR4 (LPS) and TLR5 (Flagellin), but not by the ligand for TLR7/8 (ssRNA40) in a time dependent manner. Taken together, these results suggest that MCPIP1 was induced by various TLR ligands and may play a role in the regulation of inflammatory response to various TLR-ligands in innate immune cells.

3.2. MCPIP1 deficient mice are extremely sensitive to LPS-induced septic shock

To further investigate whether MCPIP1 is also essential for controlling TLR signaling in vivo, we challenged MCPIP1-deficient mice and their wild-type littermates with sub-lethal dose of ligands for TLR2, 3, 4, 5 and 9, respectively. The mice were closely monitored for 72 h. As shown in Fig. 2, after intraperitoneal injection with 2.5 mg/kg body weight of Pam3CSK4 (TLR2 ligands), the survival rate of $MCPIP1^{+/+}$ mice was 100% within 72 h, however, the survival rate of $MCPIP1^{-/-}$ mice was 66% after 72 h of treatment. As reported that D-galactosamine can increase the toxicity of TLR2 ligands, we further treated the mice with 2.5 mg/kg body weight of Pam3CSK4 plus 1 g/kg body weight of D-galactosamine. Both $MCPIP1^{-/-}$ mice and their wilt-type littermates developed septic shock and died with similar curves within 72 h after treatment. These results suggest