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The effects of SOCS-1 on liver endotoxin tolerance development induced by a low dose of lipopolysaccharide are related to dampen NF-κB-mediated pathway

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Abstract

Background. Endotoxin tolerance is an important mechanism to maintain the homeostasis of liver. It was reported that suppressors of cytokine signalling-1 was a negative regulator of lipopolysaccharide-induced macrophages activation, however, the mechanism underlying endotoxin tolerance and suppressors of cytokine signalling-1 has not been fully elucidated.

Aim. Our aim here is to clarify whether suppressors of cytokine signalling-1 was involved in the mechanisms of endotoxin tolerance in liver through dampening nuclear factor-κB-mediated pathway.

Methods. Endotoxin tolerance models of C57BL/6J mice and isolated Kupffer cells were established by pretreating them with a low dose of lipopolysaccharide to observe the changes of suppressors of cytokine signalling-1 expression during endotoxin tolerance inducement. Moreover, a vector-based short hairpin RNA expression system was used to specifically inhibit suppressors of cytokine signalling-1 expression in RAW264.7 macrophage cells to further explore the role of suppressors of cytokine signalling-1 in endotoxin tolerance inducement. The expression of suppressors of cytokine signalling-1 was analysed by immunohistochemistry, reverse transcription-polymerase chain reaction and Western blotting, respectively. The responses to lipopolysaccharide were assessed by the activation of nuclear factor-κB and the production of tumour necrosis factor-α, which were analysed by ELISA.

Results. The histopathologic changes in the liver of the non-endotoxin tolerance group were more serious than those of the endotoxin tolerance group. The phagocytic activity of Kupffer cells were depressed and suppressors of cytokine signalling-1 expression in the endotoxin tolerance group obviously increased. Endotoxin tolerance also led to a hyporesponse of Kupffer cells to lipopolysaccharide with less activation of nuclear factor-κB, less production of tumour necrosis factor-α and more expression of suppressors of cytokine signalling-1 than those of non-endotoxin tolerance group. Moreover, the inhibitive effect was partly refracted in pSOCS-1-short hairpin RNA transfected RAW264.7 cells.

Conclusions. Endotoxin tolerance induced by lipopolysaccharide pretreatment was accompanied with upregulation of suppressors of cytokine signalling-1 and the silence of suppressors of cytokine signalling-1 by RNA interference obviously attenuated this inhibitive effect, indicating that the absence of suppressors of cytokine signalling-1 caused abnormal enhancement of inflammatory cytokine production and suppressors of cytokine signalling-1 was involved in endotoxin tolerance inducement through dampening nuclear factor-κB-mediated pathway. Therefore, suppressors of cytokine signalling-1 may be a new target for the clinical treatment of sepsis.

Keywords: Endotoxin tolerance; Kupffer cells; Lipopolysaccharide; Liver; SOCS-1

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1. Introduction

Endotoxin or lipopolysaccharide (LPS) can provoke macrophages to release magnanimous pro-inflammatory cytokines, such as interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α) and interferon (IFN), which can lead to systemic inflammatory response syndrome, multiple organ dysfunction syndrome and even death [1–3]. Many clinical conditions such as serious trauma, burn, infection and surgical operations can lead to the proliferation of Gram-negative bacteria in the intestine and the release of large quantity of LPS. Under these circumstances, the increased permeability of the intestinal wall allows more endotoxin absorption by portal vein, which results in intestinal endotoxemia (IETM) in various degrees. The shifting of endotoxin caused by IETM firstly insults the liver, a key detoxication organ in host. It is clear that the mechanisms of LPS in the liver are directly related to the response of host to LPS [4–6]. Kupffer cells (KCs) reside in hepatic sinusoid and constitute above 80% of all inherent macrophages in host, therefore, KCs obviously play an important role in defending invasion of bacteria from intestine [7–9]. Interestingly, different clinical manifestations were observed among patients after being infected with Gram-negative bacteria, suggesting that there existed different degrees of endotoxin tolerance (ET) in host. ET is a lower responsive state of host or cells to a lethal dose of LPS rechallenge after initial pretreatment with a sublethal dose of LPS [10,11]. However, the underlying mechanisms of ET are largely unknown.

A number of precious extracellular and intracellular negative signalling regulatory networks have been discovered, among which the newly discovered suppressors of cytokine signalling (SOCS) family is emerging as one of the most important feedback inhibitors. SOCS family is structurally characterised by a central SH2 domain and a conserved C-terminal motif named as the SOCS box. So far, eight members of the family including CISH (cytokine inducible SH2-domain containing) and SOCS-1–7, have been identified in mammals. Among them, the expression of SOCS-1 is earlier [12,13]. SOCS-1 could be induced in macrophages by various stimuli, including LPS, CpG-DNA, IFN-γ and IL-4 [14–20]. It was reported that SOCS-1 could inhibit overactivation of the Janus kinase (JAK)-mediated signal cascade and SOCS-1 deficient mice were shown lethal pathological alterations in various organs including fulminant hepatitis [16]. However, the mechanism underlying ET and SOCS-1 has not been fully elucidated [21].

In the study, we analysed SOCS-1 expression changes during ET induced in C57BL/6J mice liver and isolated KCs. In addition, we made use of a vector-based short hairpin RNA (shRNA) expression system, which overcome the limitations of transient and high cost in synthetic shRNAs, to specifically inhibit SOCS-1 expression in RAW264.7 macrophage cells to further explore the mechanism of SOCS-1 in ET inducement.

2. Material and methods

2.1. Reagents

Hanks' balanced salt solution, type IV collagenase, LPS (*E. coli* O111:B4), Percoll, TEMED, SDS, Leupeptin, Pepstatin and HEPES were purchased from Sigma Chemical (UAS). Rabbit anti-mouse SOCS-1 polyclonal antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG purchased from Santa Cruz (USA). DMEM cell culture medium, foetal bovine serums (FBS) were obtained from Hyclone (UAS). Nuclear Extraction Kit, Trans-AM NF-κB p65 Transcription Factor Assay Kit were obtained from Active Motif (USA). WizardR PureFection Plasmid DNA Purification System was purchased from Promega (USA). Lipofectamine 2000 was purchased from Invitrogen (USA).

2.2. Animals and treatment

The male C57BL/6J mice at 8–10 weeks of age, weighing between 19 and 21 g, were purchased from the Experimental Animal Centre of Chongqing University of Medical Sciences. They were kept at 24 °C, 55% humidity, 12 h day–night rhythm. All animals received humane care in accordance with the National Institutes of Health Guidelines and the legal requirements in China. The mice were divided randomly into the non-endotoxin tolerance (NETT) group ($n = 30$), which was pretreated with intraperitoneal injection sterile physiological saline (0.01 M), serving as control group and the endotoxin tolerance (ETT) group ($n = 30$), which was pretreated with intraperitoneal injection of 0.5 mg/kg LPS. All animals were intraperitoneally injected of 5 mg/kg LPS 24 h later [22]. Each group was divided into three subgroups (10 animals of each subgroup) at 1, 3 and 24 h. These mice were killed and the samples of the liver and blood (drawn from intrahepatic vena cava in sterile tubes, centrifuged at 1000 × *g* for 10 min at 4 °C and stored at −70 °C for TNF-α assay) were collected.

2.3. ET in KCs

KCs were isolated from mouse liver by collagenase digestion and differential centrifugation using Percoll as described elsewhere [23]. Briefly, the liver was excised after perfusion via the portal vein with Ca^{2+} and Mg^{2+} free Hanks' balanced salt solution containing 0.05% collagenase IV at 37 °C and cut into small pieces in collagenase buffer. The suspension was filtered through nylon gauze, and the filtrate was centrifuged at 450 × *g* for 10 min at 4 °C. Cell pellets were resuspended in buffer, parenchymal cells were removed by centrifugation at 50 × *g* for 3 min. Nonparenchymal cells were centrifuged on a 70%:30% Percoll gradient at 600 × *g* for 10 min. KCs concentrated at the interface of the 30% and 70% were collected and cultured in 24-well culture plates at a density of 1×10^6 cells/well in DMEM supplemented with 10% FBS and antibiotics (100 U/ml of penicillin G and