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Blockade of CD38 diminishes lipopolysaccharide-induced macrophage classical activation and acute kidney injury involving NF-κB signaling suppression

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ABSTRACT

The CD38, possessing ADP-ribosyl cyclase (ADPR-cyclase) and cyclic ADP-ribose hydrolase (cADPR-hydrolase), is able to regulate a variety of cellular activities. However, the role and mechanisms for CD38 in macrophage activation and sepsis-induced acute kidney injury (AKI) remain to be determined. Here we report that in cultured macrophages, Lipopolysaccharide (LPS) could upregulate CD38 expression in time and dose dependent manner. Knocking down or blockade of CD38 in macrophages could inhibit LPS-induced macrophage M1 polarization accompanied by diminished NF-κB signaling activation. In mouse model with LPS-induced acute kidney injury, blocking CD38 with quercetin could significantly relieve kidney dysfunction, kidney pathological changes as well as inflammatory cell accumulation. Similar to those in the cultured cells, quercetin could inhibit macrophage M1 polarization and NF-κB signaling activation in macrophages from kidneys and spleens in mice after LPS injection. Together, these results demonstrate that CD38 mediates LPS-induced macrophage activation and AKI, which may be treated as a therapeutic target for sepsis-induced AKI in patients.

1. Introduction

Acute kidney injury (AKI), a clinical syndrome characterized by a rapid decrease in renal function, suffers > 60% of patients during intensive-care-unit admission [1]. Currently, continuous renal replacement therapy is the most effective treatment for patients with AKI, but the mortality of the grievous AKI remains extremely high in the range of 50–60% [2]. In addition, even though patients survived from AKI, they may still have high possibility of being caught in short-term and long-term complications, including chronic kidney disease, end-stage renal disease and death [3].

AKI may be caused by a variety of pathogenic factors, such as renal hypoperfusion, obstruction of the urinary tract, rapidly progressive glomerulopathies, acute vasculitis, and acute interstitial nephritis. Among of them, sepsis is the most common one [4,5]. Lipopolysaccharide (LPS) has been identified as the major component that mediates sepsis-induced AKI [6]. The patients suffered from sepsis-induced AKI exhibit peritubular endothelial dysfunction, tubular cell injury and inflammatory cell infiltration [7–9]. Among the infiltrated inflammatory cells, activated macrophages may impair renal structure and function via producing inflammatory cytokines and reactive oxygen substrate [10,11]. In view of the crucial role for macrophages in the pathogenesis of AKI, exploring the underlying mechanisms of regulating macrophage accumulation and activation and finding efficient therapeutic strategy for retarding macrophage activation are quite necessary.

The CD38 is a 46-kDa type II transmembrane glycoprotein which contains a relatively long C-terminal extracellular domain, a transmembrane region and a short N-terminal cytoplasmic tail [12]. It was initially recognized as ADP-ribosyl cyclase (ADPR-cyclase) and cyclic ADP-ribose hydrolase (cADPR-hydrolase) [13,14]. Under acidic conditions, CD38 can catalyze the generation of nicotinic acid-adenine dinucleotide phosphate (NAADP +) from nicotinamide adenine dinucleotide phosphate (NADP +) [15]. Recently, it was demonstrated that CD38 could mediate diverse activities, including signal transduction, cell adhesion, activation, proliferation and differentiation, immune responses, cyclic ADP-ribose synthesis, muscle contraction, and hormone secretion [13,16,17]. Dysregulation of CD38 is involved in many types of disease, such as multiple myeloma, plasmablastic lymphoma and chronic lymphocytic leukemia [18–21]. However, the role and mechanisms for CD38 in regulating macrophage activation and acute kidney injury remain largely unknown.

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In this study, we found that LPS could time and dose dependently upregulate CD38 expression in RAW 264.7 cells, a well-used mouse monocyte/macrophage line that was established from a tumor induced by Abelson murine leukemia virus [22]. Knocking down or blockade of CD38 could largely inhibit LPS-induced NF-κB activation and macrophage M1 polarization. Furthermore, blockade of CD38 with quercetin, a CD38 inhibitor, could ameliorate LPS-induced AKI in mice. This study demonstrated that CD38 induction promotes macrophage activation and acute kidney injury in patients with sepsis.

2. Materials and methods

2.1. Mice and animal models

Male C57BL/6J mice weighing approximately 18–22 g were acquired from the Specific Pathogen-Free Laboratory Animal Center of Nanjing Medical University and maintained according to the guidelines of the Institutional Animal Care and Use Committee at Nanjing Medical University. For the model of acute kidney injury (AKI), mice were injected intraperitoneally (i.p.) with 15 mg/kg body weight of LPS in 300 μl PBS. Twenty-five mice were randomly divided into five groups:control group (n = 3), LPS group (n = 6), LPS + Quercetin (20 mg/kg, n = 6) group, LPS + Quercetin (40 mg/kg, n = 6) group, Quercetin group (n = 4). The mice within groups of LPS + Quercetin were begun to be treated with quercetin at 2 days before LPS administration. LPS group were pretreated with 5% DMSO as vehicle control. Quercetin (cat: Q4951-10 g, Sigma Aldrich, USA) was dissolved in 5% DMSO solution.

The blood were collected by retro-orbital venous plexus and processed to prepare serum at 12 h and 24 h after LPS injection. The mice were euthanized at 24 h after LPS administration. Both kidneys and spleens were harvested. One portion of the sample was processed for histopathology; another portion was collected for cryosection. The remaining sample was snap-frozen in liquid nitrogen immediately and stored at − 80 °C for subsequent evaluation.

All experiments were performed in accordance with the approved guidelines and regulations by the Animal Experimentation Ethics Committee at Nanjing Medical University. All experimental protocols were approved by the Animal Experimentation Ethics Committee at Nanjing Medical University.

2.2. Cell culture and treatment

RAW 264.7 cells obtained from American Type Culture Collection were cultured in DMEM high glucose medium supplemented with 10% FBS (Invitrogen, Grand Island, NY). Cells were seeded on six-well culture plate and let the cells grow to 60–70% confluence in complete medium containing 10% FBS for 16 h, then changed to serum-free medium after washing twice with serum-free medium. LPS (*Escherichia coli* 0111:B4, Sigma Aldrich, USA) was added to the serum-free medium. CD38 and Scramble siRNAs (Integrated Biotech Solutions, Shanghai, China) were transfected into cells using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instruction. Quercetin (cat: Q4951-10 g, Sigma Aldrich, USA) dissolved in DMSO was added at 30 mins before LPS stimulation.

2.3. Serum Urea assay

Serum Urea was measured with the QuantiChrom Urea Assay kit (cat: DIUR-500, Hayward, CA) according to the manufacturer's instructions.

2.4. Histology

Kidney samples were fixed in 10% neutral formalin, embedded in paraffin. Three-μm-thickness sections were used for PAS and HE staining. Kidney injury was determined by a semi-quantitative scoring method based on tubular epithelial swelling, loss of brush border and vacuolar degeneration. Score 0 represents injury area < 10%, whereas 1, 2, 3 and 4 represent the injury involving 10–25%, 25–50%, 50–75% and > 75% of the tissue area, respectively. At least six randomly chosen fields under the microscope (400 ×) were evaluated for each mouse, and an average score was calculated. Slides were viewed with an Olympus Epi-fluorescence microscope equipped with a digital camera.

2.5. Immunofluorescent staining

Kidney and spleen cryosections at 3-μm thickness were fixed for 15 min in 4% paraformaldehyde, followed by permeabilization with 0.2% Triton X-100 in 1 × phosphate-buffered saline (PBS) for 5 min at room temperature. After blocking with 2% donkey serum for 60 min, the slides were immunostained with anti-CD38 (cat: sc-7048, Santa Cruz Biotechnology, USA), anti-F4/80 (cat: 14-4801, eBioscience, San Diego, CA), anti-Ly6B (cat:0715, Bio-Rad, California, USA), anti-p-NF-κB (cat: 3033, Cell Signaling Technology, USA), anti-NF-κB (cat: 3032, Cell Signaling Technology, USA). Slides were viewed with a Olympus Epi-fluorescence microscope equipped with a digital camera.

2.6. Western blot analysis

Cultural RAW 264.7 cells were lysed in 1 × SDS sample buffer. An equal amount of protein was loaded into SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The primary antibodies used as the following: anti-CD38 (cat: sc-7048, Santa Cruz Biotechnology, USA), anti-p-NF-κB (cat: 3033, Cell Signaling Technology, USA), anti-NF-κB (cat: 3032, Cell Signaling Technology, USA), anti-tubulin (cat: sc53646, Santa Cruz Biotechnology, USA). Quantification was performed by measuring the intensity of the signals with the aid of National Institutes of Health Image J software package.

2.7. RNA isolation and real-time quantitative reverse transcriptase-PCR

Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized with 1 μg of total RNA, ReverTra Ace (Vazyme, Nanjing, China), and oligo (dT) 12–18 primers. Gene expression was measured by real-time PCR assay (Vazyme) and 7300 real-time PCR system (Applied Biosystems, Foster City, CA). The relative amount of mRNA or gene to internal control was calculated using the equation $2^{\triangle CT}$, in which $\triangle CT = CT_{gene} - CT_{control}$.

2.8. Statistical analysis

All data examined are presented as mean ± s.e.m. Statistical analysis of the data was performed using the SPSS 21.0. Comparison between groups was made using one-way analysis of variance, followed by the Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. LPS upregulates CD38 expression in RAW 264.7 cells

RAW 264.7 cells, a mouse macrophage cell line, were treated with LPS (500 ng/ml) for different time duration and dosage as indicated. LPS could upregulate CD38 mRNA expression in a time dependent manner (Fig. 1A). The CD38 protein abundance was increased at as early as 1 h, and reached peak at 6 h after LPS treatment (Fig. 1B). RAW 264.7 cells were also treated with LPS at different dosage as indicated and harvested at 6 h after treatment. Western blotting analyses revealed that LPS could upregulate CD38 expression in a dose-dependent manner