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Neutrophil extracellular traps contribute to the intestine damage in endotoxemic rats

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ABSTRACT

Background: Sepsis is one of the most troublesome problems in critically ill patients and often accompanied with multiple organ dysfunction and high mortality. Gut injury or dysfunction may contribute to the pathogenesis of sepsis. Neutrophil extracellular traps (NETs) do not only kill microorganisms but also damage host cells during inflammatory response to infection. The aim of this study was to investigate whether NETs are capable of promoting the impairment of the gut in a rat model of lipopolysaccharide (LPS)-induced sepsis.

Methods: The sepsis model was induced in rats by intraperitoneal injection of LPS (10 mg/kg). All rats were divided into three groups as follows: 1) control group; 2) LPS group; and 3) LPS + DNase I group. The DNase I solution (10 mg/kg) was injected intravenously to disrupt NETs 30 min after the LPS treatment. The animals were sacrificed at 3 h and 24 h after LPS or saline challenge. The intestinal cell apoptosis was examined by detecting the level of cleaved caspase-3 and terminal deoxynucleotidyl transferase dUTP nick-end labeling assays. The length and morphology of Villi were assessed histologically through hematoxylin and eosin stain. The levels of tumor necrosis factor-alpha and interleukin-10 in serum and intestine were detected by enzyme-linked immunosorbent assay. Intestinal injury was evaluated with Chiu scoring system.

Results: A large number of neutrophils infiltrated were activated to release NETs in the intestine of LPS-induced septic rats. The disruption of NETs reduced the acute systemic inflammatory response and apoptosis of intestinal epithelial cells and alleviated histologic pathogenesis. Removal of NETs provided a beneficial effect on intestinal injury.

Conclusions: This study demonstrates that the release of NETs may contribute to the intestinal damage during sepsis.

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1. Introduction

Neutrophils are the most abundant type of leukocytes in mammals and play a key role in innate immune as a first line of defense against infection in the early sepsis phase. Besides phagocytosis and degranulation, a new mechanism was discovered for neutrophils to kill microorganisms through neutrophil extracellular traps (NETs) [1]. In response to interleukin (IL)-8, lipopolysaccharide (LPS), or phorbol 12-myristate 13-acetate, the stimulated neutrophils release NETs via a novel cell death program that was proved distinct from apoptosis and necrosis—NETosis [1,2].

NETs, an essential component of the antimicrobial repertoire, are composed of DNA in association with histones and granular proteins, such as myeloperoxidase (MPO) and several cytoplasmic proteases [1]. NETs can trap microorganisms to prevent their dissemination from the initial infection site and provide a high local concentration of antimicrobials to kill them [1]. However, the uncontrolled or excessive release of NETs may also injure surrounding cells and contribute to disease pathophysiology [3], including transfusion-related acute lung injury [4,5], septic liver damage [6], and systemic lupus erythematosus [7].

Sepsis is the systemic inflammatory response syndrome caused by the infection [8]. And severe sepsis is always accompanied with organ dysfunction or tissue hypoperfusion [9]. Notably, gut injury or dysfunction frequently occurs in sepsis. Gut is not only the victim but also the prime mover during sepsis [10]. The gut injury or dysfunction can trigger bacterial translocation and the abundant release of gut-derived inflammatory factors that reach the systemic circulation by lymphatic and portal pathways. These bacterial and nonbacterial factors may cause the development of the pathogenesis including epithelial lifting of villi and enterocyte necrosis and promote distant organ dysfunction [11]. This is the gut hypothesis of multiple organ dysfunction syndrome.

In sepsis, particularly severe sepsis, the infection with an overwhelming inflammatory response mostly contributes to the pathogenesis of this syndrome. The overactive immune cells, especially neutrophils, and the secreted abundant proinflammatory mediators may cause organ impairment [12]. It was demonstrated that the increased infiltration of activated neutrophils in the intestinal tissue may effect intestinal tissue damage leading to bacterial translocation after burn [13]. But whether NETs contribute to the intestinal injury during sepsis has not yet been investigated. Thereby, we present an LPS-induced severe sepsis model in rats to investigate the effects of NETs on the impairment of the gut.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 200 ± 30 g (from Experimental Animal Center, Jinling Hospital, Nanjing, China) were used for the experiments. All rats were maintained under special pathogen-free environment and had free access to food and water. All animal procedures were performed under local and national ethical guidelines and were approved by the Institutional Animal Care and Use Committee at Jinling Hospital, Nanjing University.

2.2. Experimental design

All rats were randomly divided into three groups as follows: 1) control group; 2) LPS group; and 3) LPS + DNase I group. In the LPS and LPS + DNase I groups, sepsis was induced in rats by an intraperitoneal injection of LPS (from *Escherichia coli* 055:B5; Sigma–Aldrich, St. Louis, MO; 10 mg/kg) dissolved in saline, whereas an equal volume of saline was injected in the control group. DNase I (Sigma–Aldrich, 10 mg/kg) dissolved in saline was injected via the tail vein 30 min after the LPS challenge for LPS + DNase I group or an equal volume of saline for control and LPS groups. At 3 and 24 h after LPS or saline solution injection, the blood of anesthetized rats was collected via the inferior vena cava, and the intestines (ileum) were removed and stored in liquid nitrogen for later assays. There were six rats in every group at each time point.

2.3. Immunofluorescence

The activated neutrophils can decondense the chromatin to release NETs. The histones citrullinated by peptidylarginine deiminase 4 mediate chromatin decondensation, which are extruded with DNA during NETs formation [14]. Thus, the CitH3-DNA complexes were chosen as the marker of NETs [15].

To identify NETs and neutrophils in the rat intestine, optimum cutting temperature-embedded frozen ileum was sectioned (7 μm). After blocked with the immunol staining blocking buffer (Beyotime, Haimen, China), the sections were incubated with antibody against Cit-Histone 3 or MPO (Abcam, Cambridge, MA) and then with species-specific secondary antibodies coupled with Alexa Fluor 488 Dyes. DNA was stained using 4′,6-diamidino-2-phenylindole. The sections were observed under the confocal laser-scanning microscope (FluoView FV10i; Olympus Corporation, Tokyo , Japan).

2.4. MPO-DNA enzyme-linked immunosorbent assay

To quantify NET levels in rats by a serological method, we used a capture enzyme-linked immunosorbent assay (ELISA) as descripted previously [5]. Antibody against MPO in 75 μL (5 μg/mL) was coated onto 96-well plates overnight at 4°C. The coating solution was then removed and 200 μL of incubation buffer (bottle 5 in Cell Death Detection ELISA kit; Roche, Mannheim, Germany) was added to each well for incubation for 30 min at room temperature. After three times wash with 300 μL of wash buffer, 40 μL of the sample were added to each well with 60 μL of incubation buffer. The plate was incubated for 2 h at room temperature. After three washes (300 μL each), 100 μL of incubation buffer containing a peroxidase-labeled anti–DNA monoclonal antibody (dilution 1:10) were added to each well. After three washes (300 μL each), 100 μL of peroxidase substrate (ABTS) were added. Absorbance at a wavelength of 405 nm was measured after 30-min incubation at room temperature in the dark. Values for soluble NETs formation are expressed as units relative to the control group.