Veterinary Immunology and Immunopathology 146 (2012) 143–149

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

Kinetics of IL-6 and TNF-α changes in a canine model of sepsis induced by endotoxin

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ARTICLE INFO

Article history:
Received 8 December 2011
Received in revised form 16 January 2012
Accepted 23 February 2012

Keywords:
Sepsis
IL-6
TNF-a
Gene expression
Experimental septic model

ABSTRACT

Sepsis is a major cause of death in veterinary medicine, although a better prognosis can result from an early diagnosis. To speed the diagnosis, the biomarkers TNF-α and IL-6 can provide valuable information regarding systemic inflammatory response. The purpose of this study was to investigate the changes in cytokine levels in an experimental model of sepsis using ELISA and real-time PCR. Ten adult Beagles were studied; seven received an IV bolus of high dose lipopolysaccharide solution (1 mg/kg) to induce sepsis. The remaining three beagles were the control group. Blood samples were collected before and 1, 3, 6, 12, 24 and 48 h after administering LPS. Serum IL-6 level peaked at 3 h (1.89 ± 0.10 ng/ml) and serum TNF-α peaked at 1 h (1.11 ± 0.01 ng/ml). The expression of IL-6 mRNA in peripheral blood mononuclear cells (PBMC) increased 62-fold compared to the control group at 1 h; TNF-α mRNA increased by 4.5-fold at 1 h. The expressions of IL-6 and TNF-α mRNA in PBMCs changed more rapidly than serum IL-6 and TNF-α concentrations. In addition, TNF-α mRNA levels in PBMCs remained elevated longer than serum TNF-α. Our study establishes the basis for future work aimed at a better understanding of the systemic inflammatory response to infection and sepsis in canine patients.

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1. Introduction

Sepsis, which comes from Greek word meaning 'to make putrid,' is the systemic inflammatory response to infection (SIRS) (Bone et al., 1992). Sepsis is a common cause of morbidity and mortality in both veterinary medicine and human medicine. In United States, the incidence rate of sepsis has increased over the past few decades, and it is now the tenth leading cause of death in human beings (1.5–8% per year). The incidence of sepsis in dogs similarly increased from 1 per 1000 hospital cases in 1988 to 3.5 in 1998 (Rau et al., 2007). In dogs, the estimated incidence of sepsis was 6–10%, and the median estimate of survival was 25–50% (Otto, 2007). In dogs and cats, bacterial infections are the most common cause of sepsis, with *Escherichia coli* being the most common isolate (Paterson and Webster, 2000). However, any microbial organism (*e.g.*, fungus, parasite, and virus) can cause sepsis. In spite of new antimicrobial agents, the mortality rate of sepsis remains desperately stable. In other words, mortality in septic patients is not a result of the bacteria, but a result of the non-adapted host's response to the bacteria. Humans are considered septic if they are positive for four of seven criteria, including heart rate (HR), respiratory rate (RR), platelet count, body temperature (BT), white blood cell (WBC) count, presence of a septic focus, and decreased blood pressure (Hauptman et al., 1997). These human criteria are the basis for the clinical criteria used to diagnose sepsis in veterinary medicine: HR, RR, BT and WBC count. In human medicine, various parameters have been studied for their use in judging the severity and prognosis of serious disease, and treatment is based on these parameters. In veterinary medicine, the diagnostic criteria have been used to determine the severity of illness and prognosis in

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0165-2427/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetimm.2012.02.008

critically ill animals (Okano et al., 2002). However, estimates of septic level based on these data are inaccurate, thus further investigation is needed. The sequence of events leading to sepsis is complex and not completely understood. Inflammation due to sepsis results from activation of the cytokine network (Rau et al., 2007). Among the cytokines involved, TNF-α is produced mainly by activated macrophages and mast cells. TNF-α plays a role in antibacterial immunity and is an essential mediator of inflammation. In inflammation that results from a stimulant, TNF-α is secreted as the most proximal mediator of the cytokine cascade (Creasey et al., 1991). Serum TNF-α level is increased within 1–2 h after injecting animals with endotoxin, so it may be involved in sepsis (Bienvenu et al., 2000). Secreted TNF-α induces marked vasodilatation leading to tissue hypoperfusion, shock, and organ failure.

Macrophages and mast cells also produce IL-6, a prominent activator of the acute phase response. IL-6 production is stimulated by bacterial endotoxins, IL-1, and TNF-α (Nemzek et al., 2001). IL-6 has a longer plasma half-life than TNF-α or IL-1β, and its concentration is significantly elevated in the plasma of septic patients (Rau et al., 2007). Previous studies have found an association between high IL-6 level in septic patients and mortality (Watanabe et al., 2005); thus, IL-6 may be a good marker of the severity of a systemic bacterial infection (Rau et al., 2007). Pro-inflammatory mediators, such as TNF-α and IL-6, could be valuable biomarkers, providing information on the pathogenesis, diagnosis, and prognosis of systemic inflammatory response. Thus, studying changes in these cytokines may help to evaluate the therapeutic response and the prognosis of sepsis in veterinary medicine. The objective of this study was to investigate serial cytokine changes in an experimental model of sepsis using ELISA and real-time PCR.

2. Materials and methods

2.1. Animal preparation

Ten healthy adult beagles (12–24 months of age, 5 female and 5 male) weighing 6–8 kg each were used. The dogs were hospitalized and fasted for 12 h before the study. All dogs were housed separately in cages with a 12 h light and 12 h dark cycle. Beginning at LPS administration, the dogs were fed a commercial diet (ProPlan; Purina Korea, Seoul, Korea) and provided tap water for 12 h. This study was approved by the Committee on Bioethics of Chonbuk National University (CBU 2011-0005).

2.2. Experimental set-up

Seven dogs received an IV bolus of high dose (1 mg/kg) lipopolysaccharide (LPS) (*E. coli* serotype O111:B4; Sigma, St. Louis, MO, USA) solution to induce sepsis. The other three dogs were used as the control group. Body temperature, pulse rate, respiratory rate, and blood pressure were measured before starting and 1, 3, 6, 12, 24, and 48 h after administering LPS.

2.3. Sample collection

Blood samples were collected from the jugular vein before and 1, 3, 6, 12, 24 and 48 h after administering LPS solution. Six milliliters of blood were collected in potassium EDTA-treated tubes (Becton Dickinson, Franklin Lakes, NJ, USA), and five milliliters of blood were collected in plastic vacuum-filled tubes. Serum samples were immediately separated by centrifugation at 3500 rpm for 5 min and frozen at −70 °C until processing.

2.4. Laboratory analysis

A CBC was evaluated at baseline, 1, 3, 6, 12, 24, and 48 h using an automatic impedance cell counter (Vet ABC blood counter ABX Diagnostics, Montpellier, France). The CBC included a white blood cell (WBC) count (with differential count of monocytes, lymphocytes, and granulocytes).

2.5. Measurement of serum cytokine

Serum IL-6 and TNF-α concentrations were determined by sandwich ELISA (DuoSet ELISA Development kit, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. The plates were read immediately on a microplate spectrophotometer (Epoch™, BioTek® Instruments, Inc., Vermont, USA) set to 450 nm. Wavelength was corrected by subtracting readings at 540 nm from readings at 450 nm. Sample concentrations were calculated using Gen 5 software (ver. 1.10. BioTek® Instruments, Inc., Vermont, USA).

2.6. Isolation of canine PBMC

To isolate peripheral blood mononuclear cells (PBMC), blood in 6 ml potassium-EDTA treated tubes was immediately centrifuged at 700 × *g* for 30 min through Histopaque® 1119 and 1077 (Sigma–Aldrich, St. Louis, MO, USA) The PBMC layer was harvested. To remove residual red blood cells (RBC), 5 ml of lysis buffer (83% ammonium chloride solution, pH 7.2) was added for 5 min and centrifuged at 200 × *g* for 10 min. PBMCs without RBCs were washed twice with PBS by centrifugation at 200 × *g* for 10 min. Cell counts were assessed by VET ABC impedance cell counter. Cell purity was determined by a conventional diff-quick method.

2.7. RNA extraction and cDNA-synthesis

Isolated PBMCs were immediately placed in Buffer RLT (Qiagen, Germany) and stored at −70 °C. Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's recommendations. cDNA was synthesized with the ImProm-II™ Reverse Transcription System (Promega, Madison, WI, USA), using the manufacturer's protocol. The resulting cDNAs were stored at −20 °C until use.

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