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Interleukin-27 is elevated in sepsis-induced myocardial dysfunction and mediates inflammation

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ABSTRACT

Introduction: Interleukin (IL)-27 is an important cytokine involved in many human inflammatory diseases. In this study, we investigated its role in the pathogenesis of sepsis-induced myocardial dysfunction (SIMD).
Methods: Twenty patients with SIMD and 24 healthy donors were prospectively enrolled. Expression of IL-27 was detected in serum from SIMD patients by ELISA. Cardiac dysfunction was induced by administration of *Escherichia coli* lipopolysaccharide (LPS) to C57BL/6 (wild type) or IL-27R$^{-/-}$ mice. IL-27 mRNA in the myocardium was measured by RT-PCR. Cytokine levels in serum were determined by ELISA.
Results: Expression of IL-27 in the serum was markedly increased in patients with SIMD compared with that in controls. Serum IL-27 levels and cardiac IL-27 mRNA expression were significantly increased after LPS injection compared with control specimens. Compared with wild-type mice, IL-27R$^{-/-}$ mice had higher expression of brain natriuretic peptide, cardiac troponin I, IL-6, IL-12, tumor necrosis factor-α and transforming growth factor-β.
Conclusions: IL-27 is an important protective mediator of SIMD.

1. Introduction

Sepsis is the leading cause of death in critically ill patients. The annual incidence of severe sepsis is increasing and has recently been reported as 132 per 100,000 population, with a mortality approaching 50% [1]. Care of patients with sepsis costs as much as US$50,000 per patient [2], resulting in an economic burden of nearly US$17 billion annually in the United States alone [3]. SIMD is one of the major predictors of morbidity and mortality of sepsis. It is present in >40% of cases of sepsis and its appearance can increase the mortality rate up to 70% [4]. The concept of SIMD emerged from the study of Parker and coworkers in 1984 [5]. However, nearly 30 years of research on SIMD have not been sufficient to improve the results substantially, and there are controversies about its pathophysiology and treatment strategies [6]. Many pathophysiological mechanisms are found in SIMD, such as cytokines, Calcium transport, NO, mitochondrial dysfunction. Accumulating evidence indicates that cytokines are important mediators of SIMD. Levels of tumor necrosis factor (TNF)-α, IL-1β, IL-6 and complement anaphylatoxin C5a are elevated in the circulation during sepsis, and directly repress myocardial contractility *in vitro* [7]. Therefore, understanding the role of cytokines in SIMD may result in therapeutic approaches.

IL-27 was identified in 2002 by Pfanz and colleagues as a novel bioactive cytokine [8]. Similar to IL-12 and IL-23, IL-27 is a heterodimeric cytokine consisting of EBI3 (an IL12 p40-related polypeptide denoted EBV-induced gene 3) and p28 an IL-6 and p35

Abbreviations: SIMD, sepsis-induced myocardial dysfunction; IL, interleukin; LPS, lipopolysaccharide; WT, wild type; WBC, white blood cell; TNF, tumor necrosis factor; TGF, transforming growth factor ; EBI3, Epstein–Barr-virus-induced gene 3; APC, antigen-presenting cells; MIP, macrophage inflammatory protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; PiCCO, pulse indicator continuous; SVV, stroke volume variation; PPV, pulse pressure variation; CVP, central venous pressure; GEDI, global end-diastolic volume index; dpmx, maximum rate of the increase in pressure; CFI, cardiac functional index; CPI, cardiac power index; GEF, global ejection fraction; MAP, mean arterial pressure; CI, cardiac index; SVI, stroke volume index; SVRI, systemic vascular resistance index; EVLWI, extravascular lung water index; PCT, procalcitonin; cTn, cardiac troponin; CK-MB, creatine kinase-MB; NT-pro-BNP, N-terminal precursor of brain natriuretic peptide; APACHE, Acute physiology and chronic health evaluation.

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homolog [9]. IL-27 mediates its biological effects through a unique receptor subunit, IL-27RA (also known as WSX-1 or TCCR, T cell cytokine receptor), paired with glycoprotein gp130 for signaling highly expressed on T cells [10,11], and is mainly produced by antigen-presenting cells (APCs) such as macrophages and dendritic cells [12]. IL-27 is defined by the structural motifs that characterize its subunits and receptors, which highlight its structural relationship with IL-6 and IL-12, and help explain IL-12 family cytokines use of similar signaling pathways and overlapping activities [13]. These latter cytokines are critical determining factors in the development of T helper (Th)1 and Th17 cell responses, and represent major targets for drug development to manage inflammatory conditions. IL-27 is a member of this family of cytokines, and when it was first described, it was predicted that it would be a proinflammatory cytokine [8]. This prediction was strengthened by reports in mice that lacked the IL-27Rα and *in vitro* studies that emphasized the ability of IL-27 to promote natural killer and T cell proliferation and production of interferon (IFN)-γ [14]. Recent studies proved that IL-27 was able to stimulate monocytes, Langerhan's cells, mast cells, keratinocytes and epithelial cells to produce a variety of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, macrophage inflammatory protein (MIP)-1α, MIP-1β and β-defensin-2 [15,16]. IL-27 is a proinflammatory factor that is involved in the pathogenesis of many human inflammatory diseases such as infectious disease, colitis, asthma, psoriasis and arthritis [17–22]. In contrast, IL-27 plays an immunoregulatory role in suppressing the development of Th1, Th2 and Th17 cell subsets and driving the enlargement of inducible regulatory T (Treg) cells to produce anti-inflammatory cytokine IL-10 [23]. IL-27 can limit inflammation and there are reports that it antagonizes Treg cell development or conversion. Several studies have observed that IL-27 counteracts the ability of TGF-β and IL-2 to generate inducible Treg cells [24]. It is now generally accepted that IL-27 can limit many facets of T-cell-mediated pathology, and follow-up studies proved that it can promote early stages of Th1 cell generation [25,26]. IL-27 activates signal transducers and activators of transcription (STATs), depending on cell type and activation state. In resting lymphocytes, IL-27 activates STAT1, STAT3 and STAT5 and low levels of STAT4 [27]. STAT1 and STAT3 have many antagonistic functions but IL-27 activates both STAT1 and STAT3, and thus has the potential to induce both proinflammatory and anti-inflammatory effects [28,29]. Thus, considerable conflicting data still exist in the field and more research is required to delineate the role of IL-27 in inflammation.

However, current information on the levels of myocardial tissue IL-27 and its potential role in SIMD is still lacking. Regarding the regulatory role of IL-27 in inflammatory diseases, we hypothesized that IL-27 may participate in SIMD. The purpose of this study was to investigate the role of IL-27 in the pathogenesis of SIMD.

2. Methods

2.1. Study population

Information on 20 patients information was collected from The First Affiliated Hospital of Chongqing Medical University intensive care unit. The diagnosis of SIMD was based on one less invasive thermodilution-based technique consists of the pulse-induced cardiac output device clinical features and myocardial enzymes. Twenty-four control samples were obtained from healthy donors without medical problems. The study exclusion criteria were as follows: (1) patients with a history of heart disease; (2) acute heart disease such as acute coronary syndrome, acute heart failure or acute endocarditis; (3) patients who recently underwent cardiothoracic surgery or cardiopulmonary resuscitation; and (4) aged <45 or >80 years. The study protocol was approved by the Ethics Committee of Human Research of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China. All participants provided written informed consent.

2.2. Measurements

Before each therapeutic intervention, we performed a first set of hemodynamic measurements using the PiCCO system (PULSION Medical Systems, Munich Germany). Blood samples were taken from all patients for laboratory examinations. All enrolled patients and healthy volunteers's age, gender, temperature, pulse pressure, respiratory rate, acute physiology and chronic health evaluation (APACHE) II score, primary disease, reflecting organ function biochemical indicators, procalcitonin (PCT), hemodynamic parameters, myocardial enzymes, and serum IL-27 were recorded. After data collection, blood cultures were performed, antibiotics were administered, and fluid infusion was initiated.

2.3. Mice

Eight- to twelve-week-old male C57BL/6 mice weighing 20–25 g were obtained from and raised at Chongqing Medical University. IL-27R/WSX-1 knockout mice on a C57BL/6 background were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). The mice were bred and genotyped at the Laboratory Animal Center of Chongqing Medical University with a 12-h light/dark cycle and free access to rodent chow and tap water. All animal experiments were done in accordance with the Institutional Animal Care and Use Committee guidelines at the Chongqing Medical University. All housing conditions were accredited as specific pathogen free facilities. This housing facility is a barrier housing facility, and it has in keeping with national standard «Laboratory Animal-Requirements of Environment and Housing Facilities» (GB 14,925-2001). The care of laboratory animal and the animal experimental operation have conforming to «Chongqing Administration Rule of Laboratory Animal» (approval number: SYXK (YU) 2012-0001).

2.4. In vivo models

Intraperitoneal (i.p.) injection of lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 (Sigma–Aldrich, St. Louis, MO, USA) has been extensively used to model many of the clinical features of sepsis including elevated inflammation and cardiac dysfunction [30]. Wild-type and IL-27R$^{-/-}$ mice were injected with either 0.5 ml normal saline containing LPS (10 mg/kg bodyweight, i.p.) or 0.5 ml normal saline only. This dose of LPS was based on previous observation of overt myocardial dysfunction [31,32]. IL-27 neutralisation was performed by intravenously (i.v.) administration of 100 μg of anti-IL-27 antibodies (R&D systems) at 24 h before LPS infection. The release of cytokine in the serum of septic WT mice following IL-27 neutralisation with anti-IL-27 blocking antibodies at 4 h after LPS challenge. In parallel, WT mice injected IgG Isotype as control. We treated WT mice i.p. under light isoflurane anesthesia with 2 μg of recombinant mouse IL-27 (R&D systems) in 100 μl of phosphate buffered saline (PBS) at 24 h before LPS infection. In parallel, mice were injected solely with saline as control.

2.5. Morphological analysis

To examine cardiac tissues for histological alterations, the mice were killed at 24 h after LPS injection. Whole hearts were fixed in 10% formalin, embedded in paraffin, and sectioned onto slides. The