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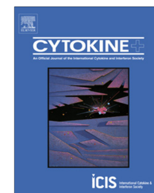

Interleukin-37 suppresses the inflammatory response to protect cardiac function in old endotoxemic mice

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ABSTRACT

Myocardial inflammatory responses to endotoxemia are enhanced in old mice, which results in worse cardiac dysfunction. Anti-inflammatory cytokine interleukin (IL)-37 has a broad effect on innate immunoresponses. We hypothesized that IL-37 suppresses myocardial inflammatory responses to protect cardiac function during endotoxemia in old mice. Old (20–24 month) wild-type (WT), and IL-37 transgenic (IL-37tg) mice were treated with lipopolysaccharide (LPS, 0.5 mg/kg, iv) or normal saline (0.1 ml/mouse, iv). Six hours later, left ventricle (LV) function was assessed using a pressure-volume microcatheter. Levels of monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 in plasma and myocardial tissue, as well as mononuclear cell density in the myocardium, were examined. Cardiac microvascular endothelial cells isolated from WT and IL-37tg mice were treated with LPS (0.2 μg/ml) for 0.5–24 h. Nuclear factor-kappa B (NF-κB) p65 phosphorylation was examined by immunoblotting, and MCP-1 levels in cell culture supernatant was determined using enzyme-linked immunosorbent assay. LV dysfunction in old WT endotoxemic mice was accompanied by up-regulated MCP-1, myocardial accumulation of mononuclear cells and production of TNF-α, IL-1β and IL-6. Expression of IL-37 suppressed myocardial inflammatory responses to endotoxemia in old mice, resulting in improved LV function. Treatment of old WT endotoxemic mice with recombinant IL-37 also improved LV function. *In vitro* experiments revealed that cardiac microvascular endothelial cells from IL-37tg mice had attenuated NF-κB activation and MCP-1 production following LPS stimulation. In conclusion, IL-37 is potent to suppress myocardial inflammation and protects against cardiac dysfunction during endotoxemia in old mice.

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1. Introduction

Major surgery and trauma can cause endotoxemia [1]. Excessive production of pro-inflammatory mediators caused by bacterial lipopolysaccharide (LPS) frequently leads to cardiac dysfunction. We and others have observed that LPS induces cardiac contractile depression through up-regulation of myocardial production of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 [2–4]. Importantly, morbidity and mortality due to the systemic inflammatory response syndrome associated with trauma or sepsis are significantly increased in the elderly [5,6].

Old animals are found to display enhanced systemic and myocardial inflammatory responses to LPS [4,7]. We previously reported that enhanced monocyte chemoattractant protein-1 (MCP-1) production in old endotoxemic mice has a critical role in mononuclear cell accumulation and cytokine production in the myocardium and contributes to the mechanism of exaggerated cardiac contractile depression. It appears that the MCP-1-mediated myocardial inflammation, i.e. mononuclear cell infiltration and production of cardiodepressant cytokines, plays an important role in cardiac contractile depression in old endotoxemic animals, and suppression of MCP-1 production and/or the

Abbreviations: IL, interleukin; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; LPS, lipopolysaccharide; NF-κB, nuclear factor-kappa B; TLR, toll-like receptor; MIP, macrophage inflammatory protein.

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MCP-1-mediated myocardial inflammation may have therapeutic potential for preservation of cardiac function in the elderly during endotoxemia.

Nuclear factor-kappa B (NF-κB) is a master transcription factor that mediates cellular inflammatory responses, including the expression of chemokines and cytokines [8]. Toll-like receptor (TLR) 4 on cell surfaces is activated in response to LPS and interacts with intracellular adaptors to activate NF-κB. Activated NF-κB translocates to the nucleus to initiate the transcription of pro-inflammatory genes [9]. Thus, NF-κB is critical in mediating LPS-induced cytokine production and cardiac dysfunction [10–12].

IL-37 is a member of the IL-1 family of cytokines. It is expressed in humans, and its expression has been identified in most cell types, including monocytes, dendritic cells, endothelial cells and epithelial cells, and acts as a natural regulator of the inflammatory responses [13]. IL-37 has repressive effects on LPS-stimulated cells, such as macrophages and endothelial cells, which implies that it may interfere with the TLR4 signaling pathway. *In vitro* studies show that expression of IL-37 in macrophages or epithelial cells dampens constitutive or induced production of pro-inflammatory cytokines, such as IL-1α, IL-1β, TNF-α, and macrophage inflammatory protein (MIP)-2 [14]. *In vivo* studies indicate that expression IL-37 in mice protects against chemical-induced colitis [15], and metabolic syndrome [16]. Further, mice that express IL-37 transgenic gene exhibit reduced lung, kidney and liver injury during endotoxemic shock [14]. However, it remains unclear whether IL-37 suppresses myocardial inflammatory responses in old animals subjected to endotoxemia. In addition, the effect of IL-37 on cardiac dysfunction caused by endotoxemia has not been determined.

In the present study, we tested the hypothesis that IL-37 suppresses the inflammatory responses in old endotoxemic mice to protect cardiac function against endotoxemic depression. We examined: (1) whether old IL-37 transgenic (IL-37tg) mice have better cardiac function during endotoxemia, (2) whether expression of IL-37 results in reduced MCP-1 production in old mice during endotoxemia and attenuated myocardial mononuclear cell accumulation and cytokine production, (3) whether IL-37 suppresses LPS-induced NF-κB activation and (4) whether recombinant IL-37 can protect old mice against endotoxemic cardiac dysfunction.

2. Materials and methods

2.1. Animals and treatment

Adult (3–4 month old) male C57BL/6 (wild-type, WT) mice were obtained from Jackson Laboratory (Bar Harbor, Maine, USA). Male WT mice of 20–24 months old were obtained from the National Institute on Aging (Bethesda, Maryland, USA). Old (20–24 month) male IL-37tg mice were from our colonies at University of Colorado Denver Anschutz Medical Campus. IL-37tg mice are C57BL/6 background [14]. They are fertile and display normal growth, behavior and lifespan. No any unique abnormality in organ/tissue has been observed in old IL-37tg mice.

All animals in this study were acclimated for at least 14 days before the experiments in an animal facility with a 12:12-h light-dark cycle and free access to water and regular chow diet. This study was approved by the Animal Care and Research Committee of the University of Colorado Denver, and it conforms to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996).

LPS (0.5 mg/kg; from *Escherichia coli*, serotype 0127:B8; Sigma Chemical Co, Saint Louis, Missouri, USA) or sterile normal saline (0.1 ml/mouse, iv) was administered to old WT and IL-37tg mice through a tail vein. An additional group of old WT mice were treated with recombinant IL-37 (rIL-37, 0.05 mg/kg, iv: R&D System, Minneapolis, Minnesota, USA) 30 min after injection of LPS. Six hours after injection of LPS or saline, cardiac function was assessed. Then, blood were collected for analysis of cytokines. Myocardial tissue specimens were prepared for evaluation of mononuclear cell accumulation and cytokine levels.

2.2. Measurement of cardiac function

Left ventricle (LV) hemodynamics was analyzed at 6 h after LPS treatment as described previously [17]. Briefly, mice were anesthetized with pentobarbital sodium (50 mg/kg, ip; Vortech Pharmaceuticals, Dearborn, Michigan, USA) and anticoagulated using heparin (1000 units/kg, ip; Elkins-Sinn, Cherry Hill, New Jersey, USA). Animals were laid supine on a heating pad and the core body temperature was kept at 37 ± 0.5 °C. A microcatheter (1F; Millar Instruments, Houston, Texas, USA) was introduced into the LV through the right common carotid artery. LV pressure-volume loops were recorded using the MPVS-400 system. LV pressure, LV volume, heart rates, and related function parameters were analyzed with the aid of PVAN software (Millar Instruments, Houston, Texas, USA).

2.3. Isolation and culture of cardiac microvascular endothelial cells

Microvascular endothelial cells were isolated from hearts of adult (3–4 month old) mice as described previously [18]. Briefly, epicardial mesothelial cells and endocardial endothelial cells in heart tissue were devitalized by immersing beating hearts in ice-cold calcium-free phosphate-buffered saline (PBS), and then dipping them into 70% ethanol. Ventricular tissue was cut into fine pieces, and digested in nominally calcium-free Hank's balanced salt solution (HBSS) supplemented with collagenase II (1.0 mg/ml), glucose (2.0 mg/ml), taurine (2.5 mg/ml), bovine serum albumin (BSA, 0.1%), and $MgCl_2$ (1.4 mM). Then, the tissue was digested in HBSS containing 0.125% trypsin, 0.1 mM EDTA and 2.0 mg/ml glucose. Spinning the solution at 500 rpm for 5 min to remove the tissue debris and remaining myocytes. Endothelial cells were collected by centrifuging the supernatant at 1200 rpm (4 °C) for 8 min. Cells were seeded in 24-well plates and cultured at 37 °C for 2 h in endothelial cell growth medium (EBM-2 from Lonza, Walkersville, Maryland, USA). Non-attached cells were removed. When culture became 90% confluence, LPS was added to the medium (final concentrations 0.2 μg/ml).

2.4. Immunoblotting

For analysis of myocardial TLR4 levels, myocardial homogenate was mixed with a sample buffer (10% glycerol, 2% SDS, 100 mmol/L Tris-HCl, pH 6.8, 0.02% bromophenol blue; Bio-Rad Laboratories Inc, Hercules, California, USA). For analysis of intercellular adhesion molecule (ICAM)-1 levels and NF-κB p65 phosphorylation in cardiac microvascular endothelial cells, the cells were lysed with the same sample buffer. The following primary antibodies were used to detect the different proteins on Western blots: TLR4 (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA), phospho-NF-κB p65 (Cell Signaling Inc., Beverly, Massachusetts, USA), total NF-κB p65 (Cell Signaling Inc., Beverly, Massachusetts, USA), ICAM-1 (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA), or GAPDH (Cell Signaling, Inc. Beverly, Massachusetts, USA). Membranes were exposed on X-ray films. NIH Image J software was used to analyze the area and density of protein bands.

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