Biochemistry and Biophysics Reports 15 (2018) 19–24

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

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

Maternal exercise training attenuates endotoxin-induced sepsis in mice offspring

Mami Yamada, Chihiro Hokazono, Mitsuharu Okutsu*

Graduate School of Natural Sciences, Nagoya City University, 1 Yamanohata, Mizuho-cho, Mizuho-ku, Nagoya, Aichi 467-8501, Japan

ARTICLE INFO

Keywords:
LPS-induced sepsis
Inflammatory response
Maternal exercise
Offspring

ABSTRACT

Regular exercise during pregnancy can prevent offspring from several diseases, such as cardiovascular diseases, obesity, and type II diabetes during adulthood. However, little information is available about whether maternal exercises during pregnancy protect the offspring from infectious diseases, such as sepsis and multiple organ dysfunction syndrome (MODS). This study aimed to investigate whether maternal exercise training protects the offspring from endotoxin-induced septic shock in mice. Female C57BL/6 mice performed voluntary wheel exercises during pregnancy. All dams and offspring were fed normal chow with sedentary activity during lactation and after weaning. At 10-week-old, mice were intraperitoneally injected a lethal (30 mg/kg) or nonlethal (15 mg/kg) dose of lipopolysaccharide (LPS), following which the survival of mice that were administered a lethal dose was monitored for 60 h. Plasma, lung, and liver samples were collected 18 h after the injection to evaluate the cytokine concentration or mRNA expression from those administered a nonlethal dose. Although maternal exercise training could not prevent lethality during an LPS-induced septic shock, it significantly inhibited the LPS-induced loss of body weight in female offspring. Regular maternal exercise significantly inhibited the mRNA expression of the LPS-induced inflammatory cytokines, such as interleukin-1β (IL-1β) and interferon-γ (IFN-γ), in the plasma and liver. Thus, maternal exercise inhibited the LPS-induced inflammatory response in female offspring, suggesting that regular exercise during pregnancy could be a potential candidate of the onset of sepsis and MODS in offspring.

1. Introduction

Maternal behavior during pregnancy affects the embryonic environment, which in turn, affects the prenatal development of offspring and leads to their predisposition to various chronic diseases in adulthood, such as cardiovascular diseases, hypertension, obesity, and type II diabetes [1,2]. This nongenetic impact has been obtained from the developmental programming hypothesis, which proposes that fetal and early neonatal environmental stimuli acting during the critical windows of development, such as fetal and/or early postnatal periods, can permanently alter the cell/tissue structure and function [3].

Regular exercise is a potent stimulus to enhance mammalian health. In addition, recent research has highlighted that regular exercise during pregnancy contributes to offspring health in adulthood. Maternal exercise during pregnancy has been reported to improve the metabolism via an increase in the lean mass and a decrease in the fat mass percentage in male offspring [4]. Another recent study has reported that maternal exercise during pregnancy decreases the endothelium-independent vascular function in adult swine offspring [5]. Furthermore, we have recently reported that regular exercise during pregnancy prevents the maternal high-fat diet–induced hypermethylation of the peroxisome proliferator-activated receptor-γ coactivator-1α (pgc-1α) gene and the age-dependent metabolic dysfunction in offspring [6]. These findings suggest that regular exercise during pregnancy is a determining stimulus for the predisposition of offspring to prevent cardiovascular and metabolic diseases. However, whether maternal exercise affects the offspring inflammatory response in adulthood remains unknown.

Inflammation can be categorized into two types: acute and chronic. Acute inflammation is the initial response to harmful stimuli that are attained by the activation of immunological cells and the induction of inflammatory cytokines. Notably, a dysregulated inflammatory response leads to persistent tissue damage, various pathophysiological disorders, or death [7]. Sepsis is characterized by the dysregulation of an inflammatory response primarily following a bacterial infection [8]. In the US, the incidence of sepsis is reported to be > 1.5 million cases per year [9]. The sepsis-induced mortality rate is estimated to be 30%, which increases with age from 10% in children to 40% in the elderly [10]. However, the exact reason for an uncontrolled inflammation and

* Corresponding author.
E-mail address: okutsu@nsc.nagoya-cu.ac.jp (M. Okutsu).

https://doi.org/10.1016/j.bbrep.2018.06.001
Received 22 March 2018; Received in revised form 11 May 2018; Accepted 2 June 2018

death in some patients with sepsis remains unclear.

Lipopolysaccharide (LPS), which induces acute inflammation and sepsis-like conditions, accelerates the release of various humoral mediators, particularly inflammatory cytokines that play major roles in the induction of systemic inflammation and the development of sepsis [8]. Among the inflammatory cytokines, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-1β (IL-1β) are detected in the blood of patients with sepsis and induce septic shock-like conditions when administered in animals in vivo, suggesting their critical pathogenic roles in sepsis [11].

Exercise training is considered to be associated with inflammatory response. Regular exercise attenuates vital organ dysfunction and damages inflicted by LPS-induced sepsis [12]. We have recently reported that extracellular superoxide dismutase, which is increased by exercise training, protects against endotoxemia-induced multiple organ dysfunction syndrome (MODS) in mice [13]. However, the impact of exercise on the prenatal regulation of inflammatory response, particularly in LPS-induced sepsis, remains unclear.

Here, we used voluntary wheel running during pregnancy to test in mice the hypothesis that maternal exercise training reduces inflammatory responses to endotoxin in adult offspring.

2. Materials and methods

2.1. Animals

Eight-week male (n = 3) and female (n = 13) C57BL/6 J mice were purchased from Japan SLC (Shizuoka, Japan). At the time of mating, a sedentary male mouse was placed in sedentary female cages overnight, and pregnancy was confirmed by a vaginal plug. Mice with plug were assigned into the following two groups: sedentary (Sed; n = 6) and exercise training (Ex; n = 7). Ex mice were individually housed in cages equipped with running wheels until delivery. The running activity was monitored using a wireless running wheel (Med Associates, Inc., Fairfax, VT) continually during exercise training periods. The running wheels were removed from cages within 12 h after delivery. All dams and offspring were fed normal chow with sedentary activity during lactation and after weaning (at 21 days). At 10 weeks of age, the offspring were assigned into the following four groups: Sed-saline, Sed-LPS, Ex-saline, and Ex-LPS. The offspring in the Sed-LPS and Ex-LPS groups were intraperitoneally (i.p.) injected a lethal (30 mg/kg; male: Sed = 7, Ex = 8; female: Sed = 9, Ex = 7) or nonlethal (15 mg/kg; male: Sed = 7, Ex = 7; female: Sed = 8, Ex = 9) dose of LPS (Sigma-Aldrich, St. Louis, MO) (Fig. 1A). The offspring in the Sed-saline and Ex-saline groups were i.p. injected with a saline (male: Sed = 6, Ex = 6; female: Sed = 7, Ex = 7). Notably, each group comprised offspring from > 5 different dams. All experimental procedures in this study were performed under the approval of the Ethics Committee of Nagoya City University.

2.2. Endotoxin exposure

The Sed-LPS and Ex-LPS mice were i.p. injected with a lethal (30 mg/kg) or nonlethal (15 mg/kg) dose of LPS at 10 weeks. The mice injected a lethal dose of LPS were monitored for 60 h to record survival. The body weights of mice that were injected with a nonlethal dose of LPS were measured at 18 h. The plasma, lung, and liver were harvested from the mice after sacrificing them by cervical dislocation under anesthesia. Both the Sed-saline and Ex-saline groups received an equivalent volume of the vehicle.

2.3. Cytokine antibody analysis

To determine the volume of multiple cytokine proteins in the blood, mixed plasma samples from all mice in each group were analyzed using cytokine antibody array. Pooled plasma was diluted and subjected to cytokine profiling using the proteome profiler mouse cytokine array (R &D Systems, Minneapolis, MN) according to the manufacturer's instructions. Briefly, membranes were blocked with a blocking reagent, and then 2 ml of pooled plasma samples from each group were individually added and incubated at 4 °C overnight. Membranes were washed and incubated by streptavidin-HRP at room temperature for 30 min. The membranes were incubated with Chemi Reagent Mix and imaged using an ImageQuant LAS 500 (GE Healthcare, Little Chalfont). The images were quantified using Image J software.

2.4. Semiquantitative RT-PCR

To assess the inflammatory cytokine mRNA expression, total RNA was isolated from the lung and liver using TRIzol (Invitrogen, Madison, WI) according to the manufacturer's instructions. Reverse transcription was performed with 2 μg of the total RNA using the SuperScript II First-Strand Synthesis System for RT-PCR (Life Technologies, Carlsbad, CA). Semiquantitative RT-PCR analysis was performed to measure *IL-1β*, *IFN-γ*, *Toll-like receptor-4 (TLR-4)*, and *GAPDH* mRNAs. The following PCR primers were used: *IL-1β*: 5′-TGCCACCTTTTGACAGTGATG-3′ and 5′-GGTATTTTGTCGTTGCTTGGTTCT-3′; *IFN-γ*: 5′-AGGAACTGGCAAA AGGATGGT-3′ and 5′-AACCCCGCAATCACAGTCTT-3′; *TLR-4*: 5′-TCCC TGCATAGAGGTAGTTCCTA-3′ and 5′-CCCTGAAAGGCTTGGTCT TGA-3′; and *GAPDH*: 5′-TGAAGTCGCAGGAGACAACC-3′ and 5′-TGAA GTCGCAGGAGACAACC-3′. The template denaturation was performed at 94 °C for 5 min followed by 30 (*IL-1β*), 32 (*IFN-γ*), 29 (*TLR-4*), and 30 (*GAPDH*) cycles consisting of 30 s at 94 °C, 30 s at 60 °C, and 40 s at 72 °C. The PCR products were separated by electrophoresis on 2%

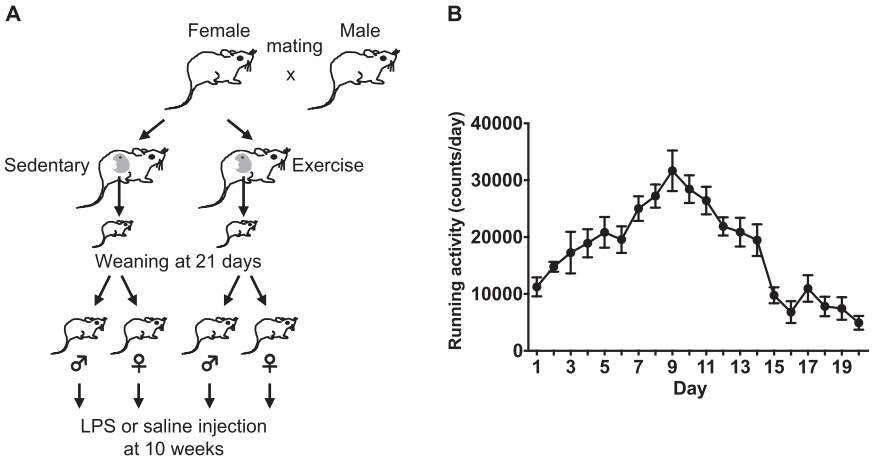

Fig. 1. Study design and running activity during pregnancy. A) Study design; B) Running activity during pregnancy.