Research paper

Individual differences in the brain are associated with resilience versus susceptibility to lipopolysaccharide-induced memory impairment

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ABSTRACT

Sepsis impairs learning and memory function, yet marked interindividual variability exists in the degree to which sepsis compromises learning and memory function. Thus, testing resilience versus susceptibility to systemic inflammation induced-memory impairment and the underlying mechanism is needed. In the present study, we firstly used lipopolysaccharide (LPS) to induce memory impairment, and then evaluated cognitive function on days 4–7 after the first LPS challenge. Subjects' scores on both behavioral measures were subjected to a hierarchical cluster analysis, identifying two clusters that differed notably on the Y-maze and fear conditioning tests. This analysis divided these subjects into two groups, one cluster (13 of 34 subjects) displayed impaired working and associative memory, named "Susceptive". The remaining cluster (21 of 34 subjects) showed normal memory, named "Resilient". We have also included another group receiving normal saline to serve as the control group. The three groups underwent a battery of biochemical detections. In addition, we investigated whether the individual differences would disappear between the "Resilient" and "Susceptive" groups by using microglia inhibitor minocycline. We showed that as compared with the "Resilient" or control group, the "Susceptive" group was accompanied by increased tumor necrosis factor-alpha, interleukin-1beta (IL-1β), IL-6, and biomarkers of microglia activation ionized calcium binding adaptor molecule-1 and cluster of differentiation 68. Notably, after decreasing the activation of microglia, the differences in cognitive function between the "Resilient" and "Susceptive" groups disappeared. Collectively, our study suggests that individual differences in the brain are associated with resilience versus susceptibility to LPS-induced memory impairment.

1. Introduction

Sepsis-associated encephalopathy (SAE) is a severe central nervous system (CNS) complication in response to sepsis secondary to systemic infection without direct clinical or laboratory evidence of CNS infection [1]. SAE is considered as the most common encephalopathy in intensive care units, and the prevalence can vary from 9% to 71% among various studies [1,2]. The manifestation of SAE can range from delirium to coma, which is characterized by the alteration of behavior, cognition, wakefulness and consciousness, and quite a proportion of these patients suffer from long-term cognitive dysfunction [3,4]. SAE seriously harms human's physical and mental state, reduces patients' quality of life, increases their mortality, and brings an extremely heavy burden to families and the society [5–7]. However, one interesting phenomenon is that sepsis does not impact all people equally. Some individuals show cognitive resilience to the effects of sepsis, whereas others display striking vulnerability, the reasons for which remain largely to be elucidated.

Although the mechanism of SAE remains unclear, several potential mechanisms, including inflammation, oxidative stress, neurotransmission disturbance, cell death and mitochondrial dysfunction, have been proposed to be related to the pathogenesis of SAE [8–10]. Among them, inflammation plays a key role in the development of cognitive impairment associated with SAE. The inflammatory response seems to be evoked by the release of bacterial lipopolysaccharide (LPS) or other microbial matters into the circulatory system and lymphatic [3]. At the early stage of SAE, the inflammatory response mainly presents as the activation of microglia and sickness behavior during the immune challenge, which are mediated by elevation in brain proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-6 and IL-1β [8,11–13].

In light of these findings, the primary aim of this study was to categorize memory within general sepsis animals using LPS. Furthermore, we investigated whether the individual differences between the "Resilient" and "Susceptive" groups would disappear by using microglia inhibitor minocycline. In the present study, we hypothesized that

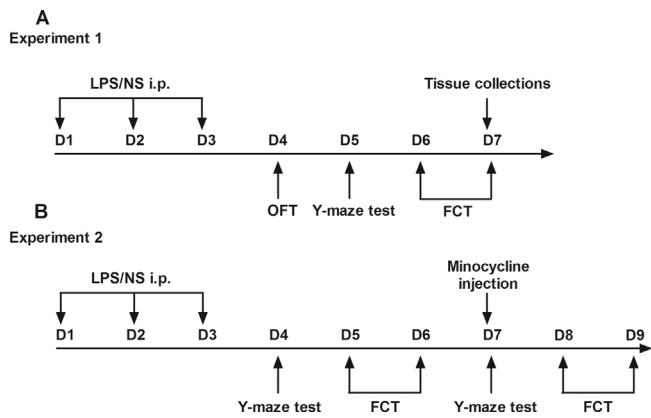* Corresponding author.
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Fig. 1. The experimental design of treatment and behavioral tests. LPS, Lipopolysaccharide; NS, Normal saline; i.p., Intraperitoneally; OFT, Open field test; FCT, Fear conditioning test.

individual differences in response to systemic inflammation in the brain are associated with resilience versus susceptibility to LPS-induced memory impairment.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (2–4 months, 23–26 g) were purchased from the Chang Zhou Cavens Laboratory Animal Ltd, Jiangsu, China. The animals were housed under a 12:12 light: dark cycle in a room at $21 \pm 1\,°C$ and humidity was $50 \pm 10\%$ for 2 weeks prior to LPS administration, and were free to food and water access. The protocol of study was approved by the Ethics Committee of Jinling Hospital, Nanjing University, and all procedures were performed in accordance with the Guideline for the Care and Use of Laboratory Animals from the National Institutes of Health, USA.

2.2. Experimental design and drug treatments

Experiment 1 (Fig. 1A): Mice were given intraperitoneally with LPS (E. coli serotype 0111:B4, Sigma, USA, 1 mg/kg) or the same volume of normal saline (NS) once daily for three consecutive days. We performed the open field test (OFT), Y-maze test, and fear conditioning test (FCT) from days 4–7 after LPS injection. Then the brain tissues were harvested and the prefrontal cortex (PFC) and hippocampus tissues were dissected.

Experiment 2 (Fig. 1B): Mice were injected with LPS as experiment 1 and underwent Y-maze test and FCT from days 4–6. A single dose of minocycline (M-9511, Sigma, USA, 50 mg/kg) [14] or the same volume of saline was injected on day 7. Thereafter, we investigated whether the individual differences between the "Resilient" and "Susceptive" groups would disappear by using microglia inhibitor minocycline four hours before the Y-maze test and FCT.

2.3. Behavioral experiments

Each animal underwent no more than three tests, with less stressful tests (OFT, Y-maze test) preceding more stressful tests (FCT).

2.3.1. OFT

A white plastic chamber (40 cm × 40 cm × 40 cm) was required for the test. The mice were gently put facing the wall into the chamber for 5 min. An automatic video tracking system (XR-XZ301) was placed right above it. The total distance moved was recorded. After each test, the subface was cleaned thoroughly using 75% alcohol to wipe off the presence of olfactory cues.

2.3.2. Y-maze test

The Y-maze test was performed as described previously [15]. The test was begun at 24 h after the last LPS or NS administration. The maze consisted of three arms, and each arm was 40 cm long, 10 cm wide and 12 cm high. Each mouse was placed into the terminal of one same arm and allowed to move freely across the apparatus for 5 min. The total number and sequence of arm entries were recorded. The mouse entering into each of the three arms continuously was defined as one successful alternation. The number of alternations was recorded. The alternation rate (%) was computed as [number of alternations/(total number–2) × 100].

2.3.3. FCT

To assess the associative memory, we used the fear conditioning chamber (50 cm high × 26 cm long × 26 cm wide). Briefly, the test consisted of two phases, the training phase consisted of a simplex exposure to a novel context for 180 s, then followed by a tone for 30 s (70 dB, 3 kHz) and a electric foot shock for 2 s (0.75 mA), then rest for 30 s in the chamber. Twenty-four hours later, the mice were placing back to the same chamber for the contextual fear conditioning phase for 5 min. Two hours later, each mouse was placed to a novel chamber that was different in color, shape, and smell for 6 min. A training tone (70 dB, 3 kHz) was delivered for 3 min to assess the tone fear conditioning. The freezing behavior was defined as an absence of all visible movement except the movement necessitated by respiration of the body and was recorded by a video tracking system automatically. At the end of test, we used 75% alcohol to clean the chamber avoiding the presence of olfactory cues.

2.4. Enzyme-linked immunosorbent assay

The mice were killed by decollation, and the brain tissues were isolated immediately and washed with ice-cold normal saline to remove the surface blood. The PFC and hippocampus were separated, weighed, and put into a homogenizer. The tissue was homogenized with 1 ml ice-cold normal saline per 100 mg brain tissue. Hypothermal centrifugation was performed at $10,000 \times g$ for 10 min, and the supernatant was obtained. Standard curves for all cytokines (in duplicates) were generated using the reference cytokine concentrations supplied. The specific enzyme linked immunosorbent assay kits was used for TNF-α, IL-6, IL-1β and IL-10 quantifying for mice according to the protocol provided by the manufacturer (Jiancheng, China).

2.5. Western blot analysis

The mice were killed by decollation, and the brains were quickly removed for determination of the Iba-1 and CD68 levels at the specified time points. Briefly, the PFC and hippocampus were grossly dissected out and homogenized on ice. The lysates were collected and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blot analysis. The following dilutions were incubated with rabbit anti-Iba-1 (1:800; Abcam, Cambridge, UK), mouse anti-CD68 (1:1000; Abcam, Cambridge, UK) and mouse anti-tubulin (1:100; Santa Cruz Biotechnology, Dallas, TX, USA)overnight at 4 °C, followed by secondary antibody (goat anti-mouse and goat anti-rabbit, Bioworld Technology) diluted in 1:5000 for 2 h at room temperature. Enhanced chemiluminescence was used for detecting the protein bands and the quantitation of bands was handled using the Image J software (NIH Image, Bethesda, MD, USA).

2.6. Immunofluorescence

The animals were anesthetized with 2% sodium pentobarbital (60 mg/kg, Sigma Chemical Co., St. Louis, MO) in saline and perfused with phosphate-buffered saline (PBS), followed by paraformaldehyde (4%) in PBS (pH = 7.4) (PFA). The brains were immediately dissected