Monophosphoryl Lipid A Tolerance Against Chronic Stress-Induced Depression-Like Behaviors in Mice

Fu Li, Xu Lu, Yaoying Ma, Yue Gu, Ting Ye, Chao Huang

Department of Pharmacy, Changzhou Geriatric Hospital Affiliated to Soochow University, Changzhou No.7 People’s Hospital, Changzhou, Jiangsu, China (Dr Li); Department of Pharmacology, School of Pharmacy, Nantong University, Nantong, Jiangsu, China (Drs Lu, Ma, Gu, Ye, and Huang).

F.L., X.L., Y.M., and Y.G. contributed equally to this work.

Correspondence: Chao Huang, Department of Pharmacology, School of Pharmacy, Nantong University, #19 Qixiu Road, Nantong 226001, Jiangsu Province, China (huachao@ntu.edu.cn).

Abstract

Backgrounds: Our recent studies reported that a single injection with lipopolysaccharide (LPS) before stress exposure prevents depression-like behaviors in stressed mice. Monophosphoryl lipid A (MPL) is a derivative of LPS that lacks the undesirable properties of LPS. We hypothesize that MPL can exert a prophylactic effect on depression.

Methods: The experimental mice were pre-injected with MPL before stress exposure. Depression in mice was induced through chronic social defeat stress (CSDS). Behavioral tests were conducted to identify depression-like behaviors. Real-time polymerase chain reaction and biochemical assays were performed to examine the gene and protein expression levels of pro-inflammatory cytokines.

Results: A single MPL injection 1 day before stress exposure at the dosages of 400, 800, and 1600 μg/kg but not 200 μg/kg prevented CSDS-induced depression-like behaviors in mice. This effect of MPL, however, vanished with the extension of the interval time between drug injection and stress exposure from 1 day or 5 days to 10 days, which was rescued by a second MPL injection 10 days after the first MPL injection or by a 4× MPL injection 10 days before stress exposure. A single MPL injection (800 μg/kg) before stress exposure prevented CSDS-induced increases in the gene expression levels of pro-inflammatory cytokines in the hippocampus and prefrontal cortex. Pre-inhibiting the innate immune stimulation by minocycline pretreatment (40 mg/kg) abrogated the preventive effect of MPL on CSDS-induced depression-like behaviors and neuroinflammatory responses in animal brains.

Conclusions: MPL, through innate immune stimulation, prevents stress-induced depression-like behaviors in mice by preventing neuroinflammatory responses.

Keywords: Innate immunization, monophosphoryl lipid A, neuroinflammatory response, prevention

Introduction

Depression is a common neuropsychological disorder with a high rate of morbidity across the world, which is associated with severe social and economic burdens (Smith and Mazure, 2021). Nowadays, most studies are focusing on the development of drugs that can ameliorate the already-developed disease symptoms, and most clinically available antidepressants in their practical use display several undesirable effects, including insomnia, sexual dysfunction, and even increased suicide ratio (Möller et al., 2008; Brietzke et al., 2019; Luft et al., 2021). An alternative strategy proposed by previous studies (Gu et al., 2021; Ji et al., 2021) suggests the prevention of the occurrence of depression from the source. This strategy may be of great
We previously reported that pre-injection with lipopolysaccharides (LPS) can prevent depression-like behaviors in chronically stressed mice by preventing neuroinflammation. We found that MPL pre-injection could prevent depression in chronically stressed mice by preventing neuroinflammation. We expect that this finding would promote the development of innate immune stimulation-based strategies for the prevention of depression and other such neuropsychiatric disorders.

**Significance Statement**

We previously reported that pre-injection with lipopolysaccharides (LPS) can prevent depression-like behaviors in chronically stressed mice, suggesting that innate immune pre-stimulation is a potential strategy for the prevention of depression. Monophosphoryl lipid A (MPL) is a chemically detoxified lipid A moiety that possesses unique immunomodulatory properties at nonpyrogenic doses. Moreover, it lacks the potential undesirable effects of LPS and provides a significant therapeutic window. We found that MPL pre-injection could prevent depression in chronically stressed mice by preventing neuroinflammation. We expect that this finding would promote the development of innate immune stimulation-based strategies for the prevention of depression and other such neuropsychiatric disorders.

Monophosphoryl lipid A (MPL) is a chemically detoxified lipid A moiety derived from Salmonella Minnesota R595 LPS with unique immunomodulatory properties at nonpyrogenic doses (Hesam et al., 2018). MPL lacks the potential undesirable effects of LPS and offers a significant therapeutic relative to doses (Hesam et al., 2018). MPL lacks the potential undesirable effects of LPS and provides a significant therapeutic window.

**METHODS**

**Animals**

Six-week-old male C57BL/6J mice and 8-week-old male and female CD1 mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Mice were housed 5 per cage at conditions of 12-hour-light/-dark cycle, lights on from 7:00 AM to 7:00 PM, 23°C±1°C ambient temperature, 55%±10% relative humidity, and free access to food and water. The female CD1 mice were used to induce aggressive behaviors in the male CD1 mice. The male CD1 mice would attack the intruder mice when a male C57BL/6J intruder came in and a female CD1 sexual partner was removed (Goyens and Noirot, 1975). Animal experiments were approved by the University Animal Ethics Committee of Nantong University (permit no. 2110836) and conducted in accordance with internationally accepted guidelines for the use of animals in toxicology as adopted by the Society of Toxicology in 1999.

**Materials**

Both MPL and Hoescht 33258 are the products of Sigma (St Louis, MO, USA). Minocycline was purchased from Selleck (Shanghai, China). The anti-iba-1 antibody was from Abcam (Cambridge, MA, USA). The MPL was dissolved in dimethyl sulfoxide (DMSO) as a stock solution and was diluted to a final concentration.
100 μg/mL using Ringer’s solution. The minocycline was dissolved in di-H₂O as a stock solution.

Pharmacological Treatment and Behavioral Procedures

In dose-dependent analyses, a single MPL was administered i.p. at doses of 200, 400, 800, and 1600 μg/kg 1 day before stress exposure (Fig. 1A). In the time-interval experiment, a second MPL injection (800 μg/kg) was administered 10 days after the first MPL injection or with a 4× MPL injection 10 days before stress exposure at the dose of 800 μg/kg, as shown in Fig. 2-4A. The mice were allocated into the vehicle, MPL, vehicle + chronic social defeat stress (CSDS), and MPL + CSDS groups to evaluate the effect of a single MPL injection (800 μg/kg) on CSDS-induced neuroinflammatory response (n=8, in each group; Fig. 5A). The dose range of MPL was selected in accordance with previous suggestions (Watts et al., 2019, 2020). To evaluate the role of the innate immune stimulation on the preventive effect of MPL on mouse behavior and neuroinflammatory response, the mice were allocated into the vehicle, vehicle + CSDS, MPL + CSDS, minocycline + CSDS, and minocycline + MPL + CSDS groups, and, in the present experiment, the mice received 2 days of minocycline treatment (40 mg/kg) before MPL injection, which was followed by another 2 days of post-treatment (Fig. 6D). The social interaction test (SIT), tail suspension test (TST), and forced swim test (FST), were conducted 1, 2, and 3 days after the discontinuation of defeat stress, respectively. The brain tissues, which were prepared for the detection of mRNA and Iba-1-positive microglia, were collected immediately after the discontinuation of the behavioral assays. To investigate whether minocycline pretreatment indeed inhibited the immune activity, the experimental mice were pre-treated with 2 days of minocycline (40 mg/kg) (Fig. 6A), and the blood was collected 5 hours after MPL injection by the enzyme linked immunosorbent assay. The herein-used dose of minocycline could efficiently inhibit the innate immune cells (Ye et al., 2020; Gu et al., 2021). All behavioral tests were performed during the light phase. As DMSO may have immune-modifying effects (Huang et al., 2020) and as

Figure 1. Dose-dependent effect of MPL pre-injection on CSDS-induced depression-like behaviors. (A) A schematic diagram showing the experimental arrangement for the evaluation of the effect of MPL pre-injection at different dosages on CSDS-induced depression-like behaviors in mice. (B, C) Quantitative analysis showing the effect of MPL pre-injection (200, 400, 800, or 1600 μg/kg) 1 day before stress exposure on CSDS-induced changes in the time spent in the interaction zone in the SIT with target absence (B) or presence (C) (n=10; **P< .01 vs vehicle; #P< .05 or ##P< .01 vs vehicle + CSDS). (D, E) Quantitative analysis showing the effect of MPL pre-injection (200, 400, 800, or 1600 μg/kg) 1 day before stress exposure on CSDS-induced increases in immobility time in the TST (D) and FST (E) (n=10; **P< .01 vs vehicle, #P< .05 or ##P< .01 vs vehicle + CSDS). Data are shown as mean ± SEM. CSDS, chronic social defeat stress; FST, forced swimming test; MPL, monophosphoryl lipid A; SEM, standard error of mean; SIT, social interaction test; TST, tail suspension test.
its concentrations in different MPL doses may vary widely, we selected the diluted Ringer’s solution, which contained the DMSO that was used to dissolve the highest dose of MPL (1600 μg/kg) as a vehicle control. Additional experimental procedures are available online in the supplementary Materials.

Statistical Analysis

Statistical analyses were performed using Graphpad Prism 8 (Graphpad Software, Inc., La Jolla, CA, USA). Differences between the mean values of data were evaluated by a 2-way ANOVA.
RESULTS

Dose-Dependent Effect of MPL Pretreatment on Depression-Like Behaviors in CSDS Mice

We first evaluated the dose-dependent effect of MPL pretreatment (200, 400, 800, and 1600 μg/kg) on CSDS-induced depression-like behaviors. A 2-way ANOVA was applied for the time spent in the interaction zone when the target was absent in the SIT, which showed no significant effects for CSDS exposure (F(1,36) = 2.65, P = .11), MPL treatment (F(4,36) = .58, P = .68), and the CSDS × MPL interaction (F(4,36) = .99, P = .42) (Fig. 1B). On the other hand, when the target was present, the 2-way ANOVA for the same index demonstrated significant effects for CSDS exposure (F(1,36) = 9.17, P < .01), MPL pretreatment (F(4,36) = 3.00, P < .05), and the CSDS × MPL interaction (F(4,36) = 5.82, P < .001) (Fig. 1C). In the TST, the 2-way ANOVA showed significant effects for CSDS exposure (F(1,36) = 37.01, P < .001), MPL pretreatment (F(4,36) = 10.04, P < .001), and the CSDS × MPL interaction (F(4,36) = 12.09, P < .001) (Fig. 1D), while, in the FST, the application of 2-way ANOVA revealed significant effects for CSDS exposure (F(1,36) = 33.22, P < .001), MPL treatment (F(4,36) = 5.49, P < .001), and the CSDS × MPL interaction (F(4,36) = 7.13, P < .001) (Fig. 1E). Post-hoc analysis showed that a single MPL injection at the dose of 200 μg/kg did not affect CSDS-induced decreases in the time spent in the interaction zone in the SIT (Fig. 1B–C) as well as the increases in the immobility time in the TST (Fig. 1D) and FST (Fig. 1E). At the doses of 400, 800, or 1600 μg/kg, MPL pretreatment prevented the decreased time spent in the interaction zone in the SIT (Fig. 1B–C) and the increased immobility time in the TST (Fig. 1D) and FST (Fig. 1E) in CSDS mice. The effect at 800 μg/kg was found to be like that observed at 1600 μg/kg; thus, the 800-μg/kg dosage was used in further studies. Under stress-naïve conditions, a single MPL injection at any dose did not affect the mouse behaviors in the SIT (Fig. 1B–C), TST (Fig. 1D), and FST (Fig. 1E) groups.

Effect of Time Interval on MPL-Induced Prevention of Depression-Like Behaviors in CSDS Mice

According to the above-mentioned dose-dependent observations, we assumed that the time interval between MPL injection and stress exposure may affect the preventive effect of MPL pretreatment on CSDS-induced depression-like behaviors (Fig. 2A). As shown in Fig. 2B–C, a 2-way ANOVA involving a 1-day interval experiment in the SIT for the time spent in the interaction zone when the target was absent indicated no significant effects for CSDS exposure (F(1,36) = .07, P = .96), MPL treatment (F(4,36) = .02, P = .89), and the CSDS × MPL interaction (F(4,36) = .34, P = .56). On the other hand, in the presence of the target, the 2-way ANOVA for the same index indicated significant effects for CSDS exposure (F(1,36) = 20.59, P < .001), MPL treatment (F(4,36) = 12.54, P < .01), and the CSDS × MPL interaction (F(4,36) = 15.04, P < .001). The 2-way ANOVA involving the 1-day interval experiment in the TST revealed significant effects for CSDS exposure (F(1,36) = 8.81, P < .01), MPL treatment (F(4,36) = 5.66, P < .05), and the CSDS × MPL interaction (F(4,36) = 11.66, P < .01) (Fig. 2D). In addition, in the FST, the 2-way ANOVA for the 1-day interval experiment showed significant effects for CSDS exposure (F(1,36) = 5.00, P < .05), MPL treatment (F(4,36) = 16.00, P < .001), and the CSDS × MPL interaction (F(4,36) = 8.98, P < .01) (Fig. 2E). Post-hoc analysis revealed that a single MPL injection at the dose of 800 μg/kg 1 day before stress exposure prevented the decreased time spent in the interaction zone in the SIT (Fig. 2B–C) and the increased immobility time in the TST (Fig. 2D) and FST (Fig. 2E) in the CSDS mice.

In the 5-day-interval experiment for SIT, a 2-way ANOVA performed for the time spent in the interaction zone in the absence of the target revealed no significant effects for CSDS exposure (F(1,36) = .15, P = .70), MPL treatment (F(4,36) = 5.3, P = .47), and the CSDS × MPL interaction (F(4,36) = .66, P = .80). On the other hand, in the presence of the target, the 2-way ANOVA for the same index revealed significant effects for CSDS exposure (F(1,36) = 21.35, P < .001), MPL treatment (F(4,36) = 6.53, P < .05), and the CSDS × MPL interaction (F(4,36) = 7.03, P < .05) (Fig. 2F–G). In the TST for the 5-day-interval experiment, the 2-way ANOVA revealed significant effects for CSDS exposure (F(1,36) = 4.66, P < .05), MPL treatment (F(4,36) = 10.54, P < .01), and the CSDS × MPL interaction (F(4,36) = 17.70, P < .001) (Fig. 2H). In the FST for the 5-day-interval experiment, the 2-way ANOVA revealed significant effects for CSDS exposure (F(1,36) = 13.75, P < .001), MPL treatment (F(4,36) = 7.09, P < .05), and the CSDS × MPL interaction (F(4,36) = 5.91, P < .05) (Fig. 2I). Post-hoc analysis revealed that a single MPL injection (800 μg/kg) 5 days before the stress exposure could prevent the decreased time spent in the interaction zone in the SIT (Fig. 2F–G) and the increased immobility time in the TST (Fig. 2H) and FST (Fig. 2I) in the CSDS mice.

In the 10-day-interval experiment for SIT, a 2-way ANOVA performed for the time spent in the interaction zone in the absence of the target showed no significant effects for CSDS exposure (F(1,36) = .70, P = .41), MPL treatment (F(4,36) = .004, P = .95), and the CSDS × MPL interaction (F(4,36) = .35, P = .56). In the presence of the target, 2-way ANOVA for the time spent in the interaction zone revealed a significant effect for CSDS exposure (F(1,36) = 35.22, P < .001), but not for MPL treatment (F(4,36) = .39, P = .53) and the CSDS × MPL interaction (F(4,36) = .07, P = .79) (Fig. 2J–K). For the TST, the 2-way ANOVA revealed a significant effect for CSDS exposure (F(4,36) = 26.21, P < .001), but not for MPL treatment (F(4,36) = .04, P = .84) and the CSDS × MPL interaction (F(4,36) = .12, P = .74) (Fig. 2L). For the FST, the 2-way ANOVA revealed a significant effect for CSDS exposure (F(1,36) = 14.04, P < .001), but not for MPL treatment (F(4,36) = .01, P = .97) and the CSDS × MPL interaction (F(4,36) = .36, P = .7) (Fig. 2M). Post-hoc analysis revealed that a single MPL injection (800 μg/kg) administered 10 days before stress exposure failed to prevent a CSDS-induced decrease in time spent in the interaction zone in the SIT (Fig. 2J–K) and increase in the immobility time in the TST (Fig. 2L) and FST (Fig. 2M).

Preventive Effect of a Second MPL Injection on Depression-Like Behaviors in CSDS Mice

As in the 10-day-interval experiment the preventive effect of MPL vanished at a long-term time point after a single MPL injection, we speculated whether a repeat injection of MPL 10 days after the first MPL injection still could induce a preventive effect (Fig. 3A). In the SIT, a 2-way ANOVA for the time spent in the interaction zone in the absence of the target indicated no significant effects for CSDS exposure (F(1,36) = .94, P = .34), MPL treatment (F(4,36) = .004, P = .95), and the CSDS × MPL interaction (F(4,36) = .19, P = .66). In the presence of the target, the 2-way ANOVA for the same index revealed significant effects for CSDS exposure (F(1,36) = 16.79, P < .001), MPL treatment (F(4,36) = 6.19, P < .05), and the CSDS × MPL interaction (F(4,36) = 9.30, P < .01) (Fig. 3B–C). For the TST, the 2-way ANOVA revealed significant effects for CSDS exposure (F(1,36) = 13.55, P < .001), MPL treatment (F(4,36) = 5.23, P < .05), and the CSDS × MPL interaction (F(4,36) = 8.09, P < .01) (Fig. 3D). For the FST, the 2-way ANOVA demonstrated significant effects for CSDS exposure (F(1,36) = 10.75, P < .001), MPL treatment (F(4,36) = 5.73,
Interaction (F2,54 = 9.04, 2-way ANOVA showed significant effects for CSDS exposure (800 μg/kg, 1 day before stress exposure) 10 days after the first MPL injection on CSDS-induced decrease in the time spent in the interaction zone in the SIT (Fig. 3B–C) and CSDS-induced increases in the immobility time in the TST (D) and FST (E). Post-hoc analysis showed that a second MPL injection in a short-term period could prolong the persistence of the preventive effect of MPL. We compared the effect of a single MPL injection (post-hoc analysis), the 4× MPL injection (800 μg/kg) 10 days after the first MPL injection still prevented CSDS-induced depression-like behaviors. (A) A schematic diagram showing the differential effect of the 1× and 4× MPL injection ten days before stress exposure on CSDS-induced depression-like behaviors. (B–E) Quantitative analysis showing the differential effect of the 1× and 4× MPL injection (800 μg/kg) 10 days before stress exposure on CSDS-induced decrease in the time spent in the interaction zone in the SIT with target absence (B) or presence (C) and CSDS-induced increases in the immobility time in the TST (D) and FST (E) in mice (n = 10, **P < .01 vs vehicle, ###P < .01 vs vehicle + CSDS). Data are shown as mean ± SEM. CSDS, chronic social defeat stress; FST, forced swimming test; MPL, monophosphoryl lipid A; SEM, standard error of mean; SIT, social interaction test; TST, tail suspension test.

**Effect of Repeated MPL Injections 10 Days Before Stress Exposure on Depression-Like Behaviors in CSDS Mice**

The preventive effect of MPL increased in parallel with an increase in the MPL dosage, which suggested that a repeated injection in a short-term period could prolong the persistence time for the preventive effect of MPL. We compared the effect between the 1× and 4× MPL injections in the herein-used depression model (Fig. 4A). A 2-way ANOVA for the time spent in the interaction zone in the absence of a target in the SIT revealed no significant effects for CSDS exposure (F1,36 = 8.77, P < .01) (Fig. 3E). Post-hoc analysis showed that a second MPL injection (800 μg/kg) 10 days after the first MPL injection still prevented CSDS-induced decrease in time spent in the interaction zone in the SIT (Fig. 3B–C) and CSDS-induced increases in the immobility time in the TST (Fig. 3D) and FST (Fig. 3E).

**Effect of MPL on Enhanced Neuroinflammatory Response in CSDS Mice**

Based on our previous reports, innate immune stimulants can prevent chronic stress-induced depression-like behaviors by preventing neuroinflammatory response (Gu et al., 2021), and the MPL may produce similar effects (Fig. 5A). We collected brain tissues from mice treated without or with MPL and/or CSDS immediately after the discontinuation of stress exposure. Regarding the levels of IL-1β mRNA in the hippocampus and prefrontal cortex, 2-way ANOVA showed significant effects for CSDS exposure (hippocampus: F1,36 = 83.90, P < .001; cortex: F1,36 = 54.53, P < .001), MPL injection (hippocampus: F1,36 = 59.01, P < .001; cortex: F1,36 = 44.58, P < .001), and the CSDS × MPL interaction (F1,36 = 6.00, P < .05), 4× MPL injections (F1,36 = 11.75, P < .001), and the CSDS × MPL interaction (F1,36 = 16.96, P < .001) (Fig. 4D). For the FST, the 2-way ANOVA showed significant effects for CSDS exposure (F1,36 = 7.00, P < .05), 4× MPL injections (F1,36 = 11.22, P < .001), and the CSDS × MPL interaction (F1,36 = 18.06, P < .001) (Fig. 4E). Unlike the effect of a single MPL injection (post-hoc analysis), the 4× MPL injections (on each day for 4 consecutive days at the dose of 800 μg/kg) prevented the decreased time spent in the interaction zone in the SIT (Fig. 4B–C) and the increased immobility time in the TST (Fig. 4D) and FST (Fig. 4E) in CSDS mice.
Regarding the levels of TNF-α mRNA in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for CSDS exposure (hippocampus: $F_{1,16}=40.09$, $P<.001$; cortex: $F_{1,16}=9.90$, $P<.01$), MPL injection (hippocampus: $F_{1,16}=24.29$, $P<.001$; cortex: $F_{1,16}=11.53$, $P<.01$), and the CSDS × MPL interaction (hippocampus: $F_{1,16}=12.18$, $P<.01$; cortex: $F_{1,16}=0.71$, $P=.42$).

(Fig. 5B, E).
interaction (hippocampus: \( F_{1,36} = 25.69, P < .001 \); cortex: \( F_{1,36} = 13.66, P < .001 \) \( \text{Fig. 5C, F} \). As for the levels of IL-6 mRNA in the hippocampus and prefrontal cortex, the results of 2-way ANOVA demonstrated significant effects for CSDS exposure (hippocampus: \( F_{1,36} = 11.90, P < .01 \); cortex: \( F_{1,36} = 74.53, P < .001 \), MPL injection (hippocampus: \( F_{1,36} = 4.84, P < .05 \); cortex: \( F_{1,36} = 50.19, P < .001 \), and the CSDS × MPL interaction (hippocampus: \( F_{1,36} = 7.75, P < .01 \); cortex: \( F_{1,36} = 54.93, P < .001 \) \( \text{Fig. 5D, G} \). Post-hoc analysis suggested that a single MPL pretreatment 1 day before stress exposure at the dose of 800 \( \mu \)g/kg could prevent CSDS-induced increases in the levels of IL-1\( \beta \), TNF-\( \alpha \), and IL-6 mRNA in the hippocampus (\( \text{Fig. 5B–D} \) and prefrontal cortex (\( \text{Fig. 5E–G} \)).

Considering that microglial activation can mediate the progression of neuroinflammatory response, we then examined the activation state of microglia by measuring the length of microglial process and the gene expression levels of ionized calcium binding adaptor molecule-1 (Iba-1) in the brain in mice treated with or without CSDS and/or MPL. Regarding the length of microglial process in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for CSDS exposure (hippocampus: \( F_{1,28} = 4.84, P < .05 \); cortex: \( F_{1,56} = 9.39, P < .01 \), and the CSDS × MPL interaction (hippocampus: \( F_{1,56} = 7.14, P < .01 \); cortex: \( F_{1,56} = 9.69, P < .01 \) \( \text{Fig. 5H, I, K} \). Regarding the gene expression levels of Iba-1 in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for CSDS exposure (hippocampus: \( F_{1,36} = 11.34, P < .01 \); cortex: \( F_{1,36} = 20.24, P < .001 \), MPL injection (hippocampus: \( F_{1,28} = 11.21, P < .01 \); cortex: \( F_{1,56} = 12.45, P < .01 \), and the CSDS × MPL interaction (hippocampus: \( F_{1,56} = 15.86, P < .001 \); cortex: \( F_{1,56} = 11.11, P < .01 \) \( \text{Fig. 5J, L} \). Post-hoc analysis showed that a single MPL pretreatment 1 day before stress exposure at the dose of 800 \( \mu \)g/kg prevented CSDS-induced decrease in microglial process length (\( \text{Fig. 5H, I, K} \) and CSDS-induced increase in the expression levels of Iba-1 mRNA (\( \text{Fig. 5J, L} \)). The results demonstrated that MPL pretreatment prevents CSDS-induced neuroinflammatory responses in the brain.

**Innate Immunization Mediates the Preventive Effect of MPL on Depression**

Considering that the MPL is known to stimulate the innate immune system, we examined whether the innate immunization mediates the preventive effect of MPL on depression (\( \text{Fig. 6A,D} \)). The results listed in Fig. B–C indicated that minocycline pretreatment (40 mg/kg) prevented a single MPL injection (800 \( \mu \)g/kg)-induced increase in the blood IL-1\( \beta \) (2-way ANOVA: significant effects for MPL injection \( F_{1,36} = 143.00, P < .001 \), minocycline pretreatment \( F_{1,36} = 88.82, P < .001 \), and the MPL × minocycline interaction \( F_{1,36} = 81.88, P < .001 \)) \( \text{Fig. 6B} \) and IL-6 (2-way ANOVA: significant effects for MPL injection \( F_{1,36} = 115.40, P < .001 \), minocycline pretreatment \( F_{1,36} = 60.86, P < .001 \), and the MPL × minocycline interaction \( F_{1,36} = 59.53, P < .001 \)) \( \text{Fig. 6C} \) levels, suggesting that the herein-used minocycline was effective in the suppression of the endogenous inflammatory response. For behavioral assays in the SIT, 2-way ANOVA for the time in the interaction zone in the SIT revealed no significant effects for the target revealed no significant effects for MPL injection \( F_{1,36} = 1.80, P = .19 \), minocycline pretreatment \( F_{1,36} = 1.04, P = .31 \), and the MPL × minocycline interaction \( F_{1,36} = .002, P = .97 \) \( \text{Fig. 6E} \). On the other hand, in the presence of the target, the 2-way ANOVA revealed significant effects for MPL injection \( F_{1,36} = 4.13, P < .05 \), minocycline pretreatment \( F_{1,36} = 11.83, P < .01 \), and the MPL × minocycline interaction \( F_{1,36} = 16.43, P < .001 \) \( \text{Fig. 6F} \).

In the TST, the 2-way ANOVA for the immobility time revealed significant effects for MPL injection \( F_{1,36} = 9.29, P < .01 \), minocycline pretreatment \( F_{1,36} = 6.46, P < .05 \), and the MPL × minocycline interaction \( F_{1,36} = 4.58, P < .05 \) \( \text{Fig. 6G} \). In the FST, the 2-way ANOVA for
the immobility time revealed significant effects for MPL injection \((F_{1,36} = 5.91, P < .05)\), minocycline pretreatment \((F_{1,28} = 4.27, P < .05)\), and the MPL \(\times\) minocycline interaction \((F_{1,28} = 9.32, P < .01)\) (Fig. 6H).

Post-hoc analysis showed that under minocycline pretreatment (400 mg/kg), the single MPL injection (800 μg/kg) failed to prevent CSDS-induced decrease in the time spent in the interaction zone in the SIT (Fig. 6I) and increases in the immobility time in the TST (Fig. 6G) and FTS (Fig. 6H) in the experimental mice. Furthermore, treatment with minocycline alone tended to induce an increase in the time spent in the interaction zone in the SIT (Fig. 6I, P = .69) as well as a decrease in the immobility time in TST (Fig. 6G, P = .79) and FST (Fig. 6H, P = .49) in CSDS mice, although these data did not show a statistical significance.

**Innate Immunization Mediates the Preventive Effect of MPL on Neuroinflammatory Response in CSDS Mice**

Considering that the prevention of depression through MPL pretreatment is accompanied by a decrease in the neuroinflammatory response in CSDS mice. As shown in Fig. 7A and D, a 2-way ANOVA for the IL-1β mRNA expression levels in the hippocampus and prefrontal cortex revealed significantly affected MPL injection (hippocampus: \(F_{1,18} = 13.52, P < .001\); cortex: \(F_{1,18} = 15.41, P < .001\)), minocycline pretreatment (hippocampus: \(F_{1,18} = 5.12, P < .05\); cortex: \(F_{1,18} = 10.44, P < .01\)), and the MPL \(\times\) minocycline interaction (hippocampus: \(F_{1,18} = 11.26, P < .01\); cortex: \(F_{1,18} = 23.92, P < .001\)). For the expression levels of TNF-α mRNA in the hippocampus and prefrontal cortex (Fig. 7B,E), the 2-way ANOVA revealed significant effects for MPL injection (hippocampus: \(F_{1,18} = 10.71, P < .01\); cortex: \(F_{1,18} = 9.88, P < .01\)), minocycline pretreatment (hippocampus: \(F_{1,18} = 7.53, P < .05\); cortex: \(F_{1,18} = 7.89, P < .01\)), and the MPL \(\times\) minocycline interaction (hippocampus: \(F_{1,18} = 13.54, P < .001\); cortex: \(F_{1,18} = 6.49, P < .05\)). For the expression levels of IL-6 mRNA in the hippocampus and prefrontal cortex (Fig. 7C,F), the 2-way ANOVA revealed significant effects for MPL injection (hippocampus: \(F_{1,18} = 17.11, P < .001\); cortex: \(F_{1,18} = 8.89, P < .01\)), minocycline pretreatment (hippocampus: \(F_{1,18} = 6.03, P < .05\); cortex: \(F_{1,18} = 4.74, P < .05\)), and the MPL \(\times\) minocycline interaction (hippocampus: \(F_{1,18} = 6.88, P < .05\); cortex: \(F_{1,18} = 5.14, P < .05\)). Post-hoc analysis revealed that, under minocycline pretreatment (400 mg/kg), a single MPL injection (800 μg/kg) failed to reverse CSDS-induced increases in the expression levels of IL-1β (hippocampus: Fig. 7A; cortex: Fig. 7D), TNF-α (hippocampus: Fig. 7B; cortex: Fig. 7E), and IL-6 (hippocampus: Fig. 7C; cortex: Fig. 7F) mRNA in the hippocampus and prefrontal cortex. Furthermore, treatment minocycline alone appeared to affect the CSDS-induced changes in the levels of IL-1β (hippocampus: Fig. 7A, P = .53; cortex: Fig. 7D, P = .29), TNF-α (hippocampus: Fig. 7B, P = .57; cortex: Fig. 7E, P = .88), and IL-6 (hippocampus: Fig. 7C, P = .91; cortex: Fig. 7F, P = .95) mRNA in the hippocampus and prefrontal cortex, although no statistical significance was recorded.

We also evaluated the abrogation effect of minocycline on the preventive effect of MPL pre-injection on CSDS-induced neuroinflammatory responses by evaluating the activation state of microglia. Regarding the length of microglial process in the hippocampus and prefrontal cortex, the 2-way ANOVA showed significant effects for MPL injection (hippocampus: \(F_{1,18} = 9.07, P < .01\); cortex: \(F_{1,18} = 26.10, P < .001\)), minocycline pretreatment (hippocampus: \(F_{1,18} = 4.60, P < .05\); cortex: \(F_{1,18} = 6.63, P < .05\)), and the MPL \(\times\) minocycline interaction (hippocampus: \(F_{1,18} = 6.79, P < .05\); cortex: \(F_{1,18} = 8.25, P < .01\) (Fig. 7G, H, J)). Regarding the expression levels of iba-1 mRNA in the brain, the 2-way ANOVA showed significant effects for MPL injection (hippocampus: \(F_{1,18} = 7.83, P < .01\); cortex: \(F_{1,18} = 16.40, P < .001\)), minocycline pretreatment (hippocampus: \(F_{1,18} = 9.33, P < .01\)), and the MPL \(\times\) minocycline interaction (hippocampus: \(F_{1,18} = 5.94, P < .05\); cortex: \(F_{1,18} = 9.06, P < .01\)) (Fig. 7I, K). Post-hoc analysis revealed that, under minocycline pretreatment (40 mg/kg), the single MPL injection (800 μg/kg) did not prevent CSDS-induced reductions in microglial process length (Fig. 7G, H, J) and CSDS-induced increases in the expression levels of iba-1 mRNA (Fig. 7I, K) in the hippocampus and prefrontal cortex. These results demonstrated that minocycline pretreatment can abrogate the preventive effect of MPL pre-injection on CSDS-induced neuroinflammatory responses in the brain.

**Discussion**

In our past studies, the innate immune stimulants such as LPS and M-CSF reportedly prevented the development of depression-like behaviors in chronically stressed mice (Gu et al., 2021; Ji et al., 2021), which suggested that drugs with innate immune-stimulating activities may possess the potential to prevent the occurrence of depression. One of the major contributions of the present study was the confirmation of a preventive effect of MPL—a chemically detoxified lipid A moiety with less undesirable actions and a greater therapeutic window compared with LPS (Elliott, 1998; Chilton et al., 2013)—on chronic stress-induced depression-like behaviors in mice. As MPL is already commercialized as a vaccine adjuvant (Didierlaurent et al., 2009), our findings indicate the possibility of the use of MPL as a vaccine-like drug for the prevention of depression, which may contribute to the reduction of morbidity from depression.

Similar to past findings hinting at the involvement of depression prevention by LPS or M-CSF (Gu et al., 2021; Ji et al., 2021), the preventive effect of MPL was also found to be dependent on its using dosage. No effect of a relatively low dose of 200 μg/kg MPL on CSDS-induced decrease in social interaction and increase in immobility time in the TST and FST was noted in dose-dependent analyses, whereas a relatively high dose of MPL pre-injection (400, 800, or 1600 μg/kg) displayed a preventive effect on CSDS-induced depression-like behaviors. The preventive effect of a single MPL pretreatment on depression in CSDS mice vanished with the extension of the observation time after administering MPL. If the time interval between drug treatment and stress exposure was 10 days, a single MPL injection did not prevent CSDS-induced depression-like behaviors in the mice. These findings present with 2 opinions: first, sufficient dosage of MPL that likely induces a proper activation of the innate immune system, which is required for the preventive effect of MPL on depression; second, the innate immunization effect of a single MPL injection cannot persist to induce a long-term preventive effect; and the disappearance of the preventive effect of MPL on depression suggests limited plasticity for the innate immune system against stress stimulation. These viewpoints are supported by several multiple injection experiments, wherein consecutive 4× MPL injections 10 days before stress exposure were found to successfully prevent decreased social interaction in the SIT and the increased immobility time in the TST and FST in CSDS mice. The 4× injections enabled the MPL to reach a level that could induce a stronger activation of the innate immune system and thereby prolonged the time interval with protective effect between the drug treatment and stress exposure. In other words, after multiple MPL injections, the body may acquire a relatively long-term ability and immune plasticity to protect the brain against stress-induced depression. Future studies should
be conducted to identify the cellular and molecular mechanisms underlying the induction of persistent ability through repeated MPL injections to protect the brain against stress stimulation.

Immune preconditioning is an old but still new concept. It describes a phenomenon in which a brief and moderate innate immune stimulation confers protection against subsequent stress response.

**Figure 7.** Effect of minocycline on the preventive effect of MPL on CSDS-induced neuroinflammatory response. (A–F) Quantitative analysis showing the abrogation effect of minocycline on the preventive effect of pre-injection (800 μg/kg) on CSDS-induced increases in the levels of IL-1β, TNF-α, and IL-6 mRNA in the hippocampus (A: IL-1β, B: TNF-α, C: IL-6) and prefrontal cortex (D: IL-1β, E: TNF-α, F: IL-6) (n = 8, **P < .01 vs vehicle; ###P < .01 vs vehicle + CSDS; &&&P < .01 vs MPL + CSDS). (G) Representative images showing the abrogation effect of minocycline on the preventive effect of pre-injection (800 μg/kg) on CSDS-induced shortening of microglial process in the hippocampus and prefrontal cortex. Iba-1: microglia; Hoechst 33258: nucleus; scale bars: 8 or 75 μm. (H–K) Quantitative analysis showing the abrogation effect of minocycline on the preventive effect of pre-injection (800 μg/kg) on CSDS-induced shortening of microglial process (hippocampus: H; cortex: J; n = 15) and CSDS-induced increases in the expression levels of Iba-1 mRNA (hippocampus: I; cortex: K; n = 10) (**P < .01 vs vehicle + CSDS; ###P < .01 vs MPL + CSDS) in the hippocampus and prefrontal cortex. Data are shown as mean ± SEM. CSDS, chronic social defeat stress; Iba-1, ionized calcium binding adaptor molecule-1; IL-1β, interleukin-1β; MPL, monophosphoryl lipid A; SEM, standard error of mean; TNF-α, tumor necrosis factor-α.
stimuli (Larochelle et al., 2015; McDonough and Weinstein, 2020). No preventive effect of MPL on CSDS-induced depression-like behaviors was, however, observed in mice receiving minocycline (an inhibitor of the innate immune system) pretreatment, which suggests that adequate innate immunization is necessary to facilitate the preventive effect of MPL on depression. The innate immune activation is mediated by several different types of immune cells in the body, such as macrophages, microglia, and T cells (Martins-Ferreira et al., 2021; Morvan et al., 2021; Shamaei et al., 2021). The contributions of these cells to the protective effect of innate immune stimulants in different types of pathological models have been revealed in past studies. For example, macrophage preconditioning with a synthetic malaria pigment has been demonstrated to prevent pro-inflammatory cytokine production (Taramelli et al., 2000). Microglial activation has also been confirmed to be essential for the neuroprotective effect of a low dose of LPS preconditioning in traumatic brain injury models and epilepsy models (Mirrone et al., 2010; Chen et al., 2014). Reber et al. (2016) reported that the suppression of the T-cell function could abrogate the behavioral improvement effect of a heat-killed preparation of Mycobacterium vaccae in a psychiatric model induced by social defeat stress. In future studies, we should consider the cellular basis behind the preventive effect of MPL on depression.

Although the monoamine dysfunction remains a focus for explaining the pathogenesis of depression, a variety of newly proposed hypotheses in recent years have attracted increasing attention, which includes the popular neuroinflammation hypothesis (Turner et al., 2020). According to this hypothesis, the overproduction of pro-inflammatory cytokines can mediate the pathogenesis of depression and the suppression of the neuroinflammatory response progression, which is considered to be helpful in the prevention and/or treatment of depression (Yirmiya et al., 2015; Benatti et al., 2016). Our results revealed that (1) a single MPL pre-injection could prevent microglial process shortening (an activation phenotype of microglia) and an abnormal increase in the expression levels of pro-inflammatory cytokine and Iba-1 (a classical maker for microglia) mRNA in the hippocampus and the prefrontal cortex in CSDS mice; and (2) minocyline pretreatment abrogated the preventive effect of MPL on CSDS-induced depression-like behaviors, microglial process shortening, and increase in levels of pro-inflammatory cytokine and Iba-1 mRNA in the brain. These findings suggest a possibility that MPL pretreatment can modulate inflammatory damage after chronic social defeat exposure in mice, possibly by preventing microglial over-activation. This hypothesis is supported by numerous evidence. For example, past studies have shown that the pre-stimulation of the innate immune system by LPS can induce the microglia to transform into an epigenetically regulated immune-suppressive phenotype (Schaafsma et al., 2015). However, it is worth indicating, besides the microglia, peripheral monocytes and macrophages or the other nervous system cells such as astrocytes are also involved in this process as (1) stress stimulation, overactivated peripheral monocytes, and macrophages can migrate to the brain to induce behavioral abnormalities (Wohleb et al., 2013); (2) astrocyte dysfunction have been widely reported to mediate the pathogenesis of depression (Gómez-Galán et al., 2013; Zhang et al., 2020); and (3) adoptive transfer of monocytes isolated from LPS-preconditioned mice into naive mice after cerebral ischemia has been shown to reduce brain injury (Garcia-Bonilla et al., 2018). Collectively, our results suggest that a single MPL pretreatment renders mice tolerance against social defeat stress-induced depression-like behaviors by preventing microglial activation and neuroinflammation. This ability vanished at a 10-day interval between MPL treatment and stress exposure and could be rescued with a second MPL injection 10 days after the first MPL injection or by a repeated-MPL injection 1 day before stress exposure. As MPL is a potent innate immune stimulant with lesser toxicity than its parent molecule LPS (Ribi, 1984), it has been approved as a vaccine adjuvant for clinical use (Didierlaurent et al., 2009). Our findings thus provide a promising alternative for the development of drugs towards the prevention of depression, especially for patients with high risks of detrimental stress exposure.

Supplementary Materials

Supplementary data are available at International Journal of Neuropsychopharmacology (IJNPPY) online.

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References

Benatti C, Blom JM, Rigillo G, Alboni S, Zizzi F, Torta R, Brunello N, Tascedda F (2016) Disease-induced neuroinflammation and depression. CNS Neurol Disord Drug Targets 15:414–433

Breitze E, Vazquez GH, Kang MJY, Soares CN (2019) Pharmacological treatment for insomnia in patients with major depressive disorder. Expert Opin Pharmacother 20:1341–1349

Chen Z, Jalabi W, Hu W, Park HJ, Gale JT, Kidd GJ, Bernatowicz R, Gossman ZC, Chen JT, Dutta R, Trapp BD (2014) Microglial displacement of inhibitory synapses provides neuroprotection in the adult brain. Nat Commun 5:4486

Chilton PM, Hadel DM, To TT, Mitchell TC, Darveau RP (2013) Adjuvant activity of naturally occurring monophosphoryl lipopolysaccharide preparations from mucosa-associated bacteria. Infect Immun 81:3317–3325

Didierlaurent AM, Morel S, Lockman L, Giannini SL, Bisteanu M, Carlsen H, Kielland A, Vостерс Q, Vanderheyde N, Schiavetti F, Larocque D, Van Mechelen M, Garçon N (2009) AS04, an aluminum salt- and TLR4 agonist-based adjuvant system, induces a transient localized innate immune response leading to enhanced adaptive immunity. J Immunol 183:6186–6197

Elliott GT (1998) Monophosphoryl lipid A induces delayed preconditioning against cardiac ischemia-reperfusion injury. J Mol Cell Cardiol 30:3–17

Garcia-Bonilla I, Brea D, Benakis C, Lane DA, Murphy M, Moore J, Racchumi G, Jiang X, Iadecola C, Anrather J (2018) Endogenous protection from ischemic brain injury by preconditioned monocytes. J Neurosci 38:6722–6736

Gómez-Galán M, De Bundel D, Van Eeckhout A, Smolders I, Lindskog M (2013) Dysfunctional astrocytic regulation of glutamate transmission in a rat model of depression. Mol Psychiatry 18:582–594

Goyens J, Noiroit E (1975) Effects of cohabitation with females on aggressive behavior between male mice. Dev Psychobiol 8:79–84.
Griffiths M, Neal JW, Gasque P (2007) Innate immunity and protective neuroinflammation: new emphasis on the role of neuroimmune regulatory proteins. Int Rev Neurobiol 82:29–55.

Gu Y, Ye T, Tan P, Tong L, Ji J, Gu Y, Shen Z, Shen X, Lu X, Huang C (2021) Tolerance-inducing effect and properties of innate immune stimulation on chronic stress-induced behavioral abnormalities in mice. Brain Behav Immun 91:451–471.

Hesam S, Khoshkhollah-Sima B, Pourbadie HG, Babapour V, Zendedel M, Sayyah M (2018) Monophosphoryl lipid A and Pam3Cys prevent the increase in seizure susceptibility and epileptogenesis in rats undergoing traumatic brain injury. Neurochem Res 43:1978–1985.

Hosseini SM, Cholami Pourbadie H, Sayyah M, Zibaii M, Naderi N (2018) Neuropeptidergic effect of monophosphoryl lipid A, a detoxified lipid A derivative, in phototrophic model of unilateral selective hippocampal ischemia in rats. Behav Brain Res 347:26–36.

Hosseinzadeh M, Pourbadie HG, Khodagholi F, Daftari M, Naderi N, Motamedi F (2019) Preconditioning with toll-like receptor agonists attenuates seizure activity and neuronal hyperexcitability in the pilocarpine rat model of epilepsy. Neuroscience 408:388–399.

Huang SH, Wu CH, Chen SJ, Sytwu HK, Lin GJ (2020) Immunomodulatory effects and potential clinical applications of dimethyl sulfoxide. Immunobiology 225:151906.

Ji J, Xiang H, Lu X, Tan P, Yang R, Ye T, Chen Z, Chen D, He H, Chen J, Ma Y, Huang C (2021) A prophylactic effect of macrophage-colony stimulating factor on chronic stress-induced depression-like behaviors in mice. Neuropharmacology 193:108621.

Karki R, Kanneganti TD (2021) The ‘cytokine storm’: molecular mechanisms and therapeutic prospects. Trends Immunol 42:681–705.

Larcheille A, Bellavance MA, Rivest S (2015) Role of adaptor protein MyD88 in TLR-mediated preconditioning and neuroprotection after acute excitotoxicity. Brain Behav Immun 46:221–231.

Liu L, Sun B (2019) Neutrophil pyroptosis: new perspectives on sepsis. Cell Mol Life Sci 76:2031–2042.

Longhi L, Gesuete R, Perego C, Ortolano F, Sacchi N, Villa P, Stocchetti N, De Simoni MG (2011) Long-lasting protection in brain trauma by endotoxin preconditioning. J Cereb Blood Flow Metab 31:1919–1929.

Luft MJ, Dobson ET, Levine A, Croarkin PE, Strawn JR (2021) Pharmacologic interventions for antidepressant-induced sexual dysfunction: a systematic review and network meta-analysis of trials using the Arizona Sexual Experience Scale. CNS Spectr 12:1–10.

Martins-Ferreira R, Leal B, Costa PP, Ballestar E (2021) Microglial innate memory and epigenetic reprogramming in neurodegenerative disorders. Prog Neurobiol 200:101971.

Matsuwaki T, Shionoya K, Ihnatko R, Eskilsson A, Kakuta S, Dufour S, Schwanger M, Waisman A, Müller W, Pinteaux E, Engblom D, Blomqvist A (2017) Involvement of interleukin-1 type 1 receptors in lipopolysaccharide-induced sickness responses. Brain Behav Immun 66:165–176.

McDonough A, Weinstein JR (2020) The role of microglia in ischemic preconditioning. Glia 68:455–471.

Mirrione MM, Konomos DK, Gravanis I, Dewey SL, Aguzzi A, Heppner FL, Tsirka SE (2010) Microglial ablation and lipopolysaccharide preconditioning affects pilocarpine-induced seizures in mice. Neurobiol Dis 39:85–97.

Möller HJ, Baldwin DS, Goodwin G, Kasper S, Okasha A, Stein DJ, Tandon R, Versiani M; WPA Section on Pharmacopsychiatry (2008) Do SSRIs or antidepressants in general increase suicidality? WPA Section on Pharmacopsychiatry: consensus statement. Eur Arch Psychiatry Clin Neurosci 258(Suppl 3):3–23.

Morvan MG, Teque FC, Locher CF, Levy JA (2021) The CD8+ T Cell noncytotoxic antiviral responses. Microbiol Mol Biol Rev 85:e00155-20.

O’Neill E, Griffin ÉW, O’Sullivan R, Murray C, Ryan L, Yssel J, Harkin A, Cunningham C (2021) Acute neuroinflammation, sickness behavior and working memory responses to acute systemic LPS challenge following noradrenergic lesion in mice. Brain Behav Immun 94:357–368.

Place DE, Kanneganti TD (2020) The innate immune system and cell death in autoimmune and autoimmunity disease. Curr Opin Immunol 67:95–105.

Pourbadie HG, Sayyah M, Khoshkholgh-Sima B, Choopani S, Nategh M, Motamedi F, Shokrgozar MA (2018) Early minor stimulation of microglial TLR2 and TLR4 receptors attenuates Alzheimer’s disease-related cognitive deficit in rats: behavioral, molar, and electrophysiological evidence. Neurobiol Aging 70:203–216.

Reber SO, et al. (2016) Immunization with a heat-killed preparation of the environmental bacterium Mycobacterium vaccae promotes stress resilience in mice. Proc Natl Acad Sci U S A 113:E1310–E1319.

Ribi E (1984) Beneficial modification of the endotoxin molecule. J Biol Response Mod 3:1–9.

Schaafsma W, Zhang X, van Zomeren KC, Jacobs S, Georgieva PB, Wolf SA, Kettenmann H, Janova H, Saiepour N, Hanisch UK, Meerlo P, van den Elen PJ, Brouwer N, Boddeke HW, Egggen BJ (2015) Long-lasting pro-inflammatory suppression of microglia by LPS-preconditioning is mediated by RelB-dependent epigenetic silencing. Brain Behav Immun 48:205–221.

Shamaei M, Miraesedi M (2021) Nontuberculous mycobacteria, macrophages, and host innate immune response. Infect Immun 89:e0081220.

Smith MV, Mazure CM (2021) Mental health and wealth: depression, gender, poverty, and parenting. Annu Rev Clin Psychol 17:181–205.

Stevens SL, Leung PY, Vartanian KB, Weatherall PB, Simon RP, Stocks WS, Stenzel-Poore MP (2011) Multiple preconditioning paradigms converge on interferon regulatory factor-dependent signaling to promote tolerance to ischemic brain injury. J Neurosci 31:8456–8463.

Taramelli D, Recalcati S, Basilico N, Olliario P, Cairo G (2000) Macroage preconditioning with synthetic malaria pigment reduces cytokine production via heme iron-dependent oxidative stress. Lab Invest 80:1781–1788.

Turner AJ, Smyth N, Hall SJ, Torres SJ, Hussein M, Jayasinghe SU, Ball K, Clow AJ (2020) Psychological stress reactivity and future health and disease outcomes: a systematic review of prospective evidence. Psychoneuroendocrinology 114:104599.

Watts BA 3rd, Tamayo E, Sherwood ER, Good DW (2019) Monophosphoryl lipid A induces protection against LPS in medullary thick ascending limb through induction of Tollip and negative regulation of IRAK-1. Am J Physiol Renal Physiol 317:F705–F719.

Watts BA 3rd, Tamayo E, Sherwood ER, Good DW (2020) Monophosphoryl lipid A pretreatment suppresses sepsis and LPS-induced proinflammatory cytokine production
in the medullary thick ascending limb. Am J Physiol Renal Physiol 319:F8–F18.
Wohleb ES, Powell ND, Godbout JP, Sheridan JF (2013) Stress-induced recruitment of bone marrow-derived monocytes to the brain promotes anxiety-like behavior. J Neurosci 33:13820–13833.
Ye T, Wang D, Cai Z, Tong L, Chen Z, Lu J, Lu X, Huang C, Yuan X (2020) Antidepressive properties of macrophage-colony stimulating factor in a mouse model of depression induced by chronic unpredictable stress. Neuropharmacology 172:108132.

Yirmiya R, Rimmerman N, Reshef R (2015) Depression as a microglial disease. Trends Neurosci 38:637–658.
Yousefi N, Sotoodehnejadnematalahi F, Heshmati-Fakhr N, Sayyah M, Hoseini M, Ghassemi S, Aliakbari S, Pourbadie HG (2019) Prestimulation of microglia through TLR4 pathway promotes interferon beta expression in a rat model of Alzheimer’s disease. J Mol Neurosci 67:495–503.
Zhang J, Ning L, Wang J (2020) Dietary quercetin attenuates depressive-like behaviors by inhibiting astrocyte reactivation in response to stress. Biochem Biophys Res Commun 533:1338–1346.