RESEARCH ARTICLE

# TNFα mediates stress-induced depression by upregulating indoleamine 2,3-dioxygenase in a mouse model of unpredictable chronic mild stress

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**ABSTRACT. Depression is often preceded by exposure to stressful life events. Chronic stress causes perturbations in the immune system, and up-regulates production of proinflammatory cytokines, which has been proposed to be associated with the pathogenesis of clinical depression. However, the potential mechanisms by which stress-induced proinflammatory cytokines lead to the development of depression are not well understood. Here, we sought to screen the main proinflammatory cytokines and the potential mechanisms linking inflammation to depression-like behavior during unpredictable, chronic, mild stress (UCMS), *in vivo*. Mice were allocated into four groups in each separate experiment: saline-control, saline-UCMS, drug-control and drug-UCMS. Development of depression-like behavior was reflected as a reduction in sucrose preference, and increased immobility in both the forced swim and tail suspension tests. The following drugs were administered intraperitoneally: the pan-anti-inflammatory tetracycline derivative, minocycline (30 mg/kg, daily), the tumor necrosis factor (TNF)α monoclonal antibody, infliximab (10 mg/kg, twice weekly), and the indoleamine 2, 3-dioxygenase (IDO) inhibitor, 1-methyltryptophan (1-MT, 10 mg/mouse, daily). Plasma TNFα, IL-1β and IL-18 increased significantly after the four-week UCMS exposure. Pretreatment of mice with minocycline completely blocked any upregulation. Concurrent with development of depression-like behaviors, the concentration of TNFα in plasma and the cerebral cortex increased remarkably. The tryptophan-degrading enzyme IDO was up-regulated in the cortex following UCMS exposure. Treatment of mice with minocycline, infliximab or 1-MT prevented the development of depression-like behaviors. Furthermore, blockade of TNFα inhibited expression of IDO and protected cortical neurons from UCMS-induced damage. These results suggest that TNFα plays a critical role in mediating UCMS-induced depression through up-regulation of IDO and subsequent damage of cortical neurons.**

**Key words:** unpredictable chronic mild stress, depression-like behavior, TNFα, minocycline, indoleamine 2,3-dioxygenase

Depression is a multi-factorial disease. Despite advances in our understanding of the pathophysiology of depression, side-effects of antidepressants, and drug resistance are still significant problems. Clinical trial data suggest that at least one-third of depressed patients are non-responsive or resistant to all existing antidepressants [1, 2]. This suggests the presence of additional biological mechanisms involved in the pathogenesis of depression, and indicates the urgent need for new types of antidepressant.

Various hypotheses have been proposed concerning the pathophysiology of depression, including the cytokine hypothesis that was proposed in 1991 and constitutes a relatively recent account of depression [3, 4]. The main idea behind the cytokine hypothesis is that activation of the inflammatory immune system, particularly the release of proinflammatory cytokines, provokes numerous neuroendocrine and neurochemical changes that contribute to depression. This has been corroborated by results from animal models and clinical trials during the last few years [5-8]. Furthermore, antidepressants also have certain anti-inflammatory effects [9].

As a fundamental factor in the provocation of depression, chronic stress is associated with dysregulated immunity and subsequent low-grade inflammation [10]. Studies carried out with some stress protocols (physical, psychological or mixed) show a proinflammatory response, mainly characterized by release of inflammatory mediators, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)α, and cyclooxygenase-2 [11-13]. Thus, chronic stress facilitates the development of depression by promoting the expression of proinflammatory cytokines [14].

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TNFα is one of the proinflammatory cytokines that plays a significant role in mood regulation. Increased TNFα has been reported in clinical depression [15], and this association has been confirmed by meta-analysis [16]. Selective serotonergic reuptake inhibitors (SSRIs), a widely used type of antidepressant medication, can significantly inhibit the production of both TNFα and nitric oxide in cultured microglia treated with LPS [9]; however, the target of the SSRIs and the specific effects of microglia-derived TNFα on the brain serotonin microenvironment are unclear. Clinical studies have also shown that anti-TNFα treatment alleviated the symptoms of depression and anxiety of patients with chronic inflammatory diseases [17, 18]. However, the mechanism that underlies this observation remains unknown.

To explore further the link between inflammation and depression, recent studies have focused on the relationship between inflammation and tryptophan metabolism. A reduction in the bioavailability of tryptophan could affect serotoninergic neurotransmission and might play a synergistic role in the induction of depressive symptoms. Activation of the tryptophan-degrading enzyme, indoleamine 2,3-dioxygenase (IDO), catalyzes the initial rate-limiting step in tryptophan catabolism along the kynurenine pathway, leading to the depletion of tryptophan and the formation of tryptophan catabolites such as kynurenine, kynurenic acid, xanthurenic acid, and quinolinic acid. Some catabolites, such as kynurenine and quinolinic acid, are anxiogenic and depressogenic, and induce neurotoxic effects [19, 20]. Enhanced activation of IDO has been demonstrated in clinically depressed patients, as well as in experimental animals exhibiting depression-like behavior [21, 22]. IDO is induced by proinflammatory cytokines, but mainly by interferon-gamma (IFNγ) [23] and TNFα [24, 25].

In the present study, an animal model of stress-induced depression was established using a four-week, unpredictable, chronic mild stress (UCMS) paradigm. Firstly, proinflammatory cytokine expression levels in the peripheral and central nervous systems were analyzed, in the presence and absence of the anti-inflammatory tetracycline derivative, minocycline. Subsequently, IDO was measured in order to explore its possible role in the pathophysiology of depression. To confirm this hypothesis, the cytokine monoclonal antibody, infliximab, and the IDO competitive inhibitor, 1-methyltryptophan (1-MT), were used to detect the changes in depression-like behaviors induced by stress. Additionally, immunohistochemistry was performed to evaluate any neuronal damage, to further explore the mechanisms underlying certain cytokine-mediated depressive behaviors. This study points to a close link between peripheral inflammation and depression-like behaviors, which may point to a promising inflammatory biomarker, and its involvement in stress-induced depression.

## METHODS AND MATERIALS

### *Reagents*

Minocycline hydrochloride (cat# M9511) was purchased from Sigma Aldrich (Sigma, USA). Infliximab produced by Cilag AG (Schaffhausen, Swiss) was obtained from the Shanghai Hospital (Shanghai, China). 1-methyltryptophan (1-MT) (cat# 21339-55-9) was obtained from Seebio Biotech (Shanghai, China). Quantscript cDNA RT kit (cat# KR-103), reverse transcriptase kit and TRIzol reagent were purchased from Tiangen Biotech (Beijing, China). RT-PCR primers for IDO (cat# M301147, M301148) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; cat# M301155, M301156) were all obtained from Sangon Biotech (Shanghai, China). Other reagents and chemicals were obtained from Sigma Aldrich (Sigma, USA).

### *Mice*

Experiments were performed on nine-week-old, male BALB/c mice obtained from the Animal Centre (Second Military Medical University, Shanghai, China), whose body weights were 22-25 g. All procedures were approved by the local animal care committees in accordance with related regulations and laws. Before the start of the experiments, two weeks were allowed for the mice to adapt to their new circumstances and to the sucrose solution provided. All animals were maintained under a controlled environment (temperature: $22 \pm 1^{\circ}$C; humidity: $52 \pm 2\%$; 12 h day/night rhythm), and received food and water *ad libitum*, except for during the sucrose preference test. Animals were housed in individual cages and randomly assigned to different groups in three separate experiments. For experiment one, mice were allocated to the control group, stress group, minocycline group and stress + minocycline group. For experiment two, mice were allocated to the control group, stress group, infliximab group and stress + infliximab group. For experiment three, mice were allocated to the control group, stress group, 1-MT group and stress + 1-MT group.

#### *Unpredictable, chronic, mild stress*

The mice in the stressed groups were subjected to various stressors, once daily according to the UCMS regimen described previously [26]. Briefly, the mice were subjected to various stressors in a chronic, inevitable, and unpredictable way, according to a random schedule, for four consecutive weeks. The stressors were: damp bedding for 12 h; 45°cage tilting for 18 h; continuous lighting for 24 h; water deprivation for 18 h; food deprivation for 18 h; shaking for 10 minutes; 4°C ice-cold swimming for five minutes; 45°C oven for five minutes; confinement in a tube for two h. Random stressors were applied deliberately, at random times, both night and day, in order for there to be complete unpredictability. The same stress was not applied successively so that the mice could not anticipate the type of stress. Immediately after cessation of each stress session, the mice were returned to their home cages and maintained under standard conditions until the next stress session.

### *Treatment administration*

Treatment or vehicle administrations started on day 1 and were stopped the day after the end of UCMS (day 28). On the day of injection, fresh solutions were prepared by dissolving compounds in sterile, endotoxin-free, isotonic saline and administered intraperitoneally (i.p.). Infliximab is a monoclonal antibody (mAb) that neutralizes the biological activity of TNFα. Infliximab was administered twice weekly at a dose of 10 mg/kg according

to a previous report [27]. 1-MT, the IDO inhibitor, was given at a dose of 10 mg/mouse daily [28]. Minocycline, an anti-inflammatory, tetracycline derivative which blocks acute increases in several proinflammatory cytokines, was injected at 30 mg/kg, once daily [29]. These levels of infliximab, 1-MT and minocycline were confirmed to reduce significantly TNFα, IDO and inflammatory cytokines levels respectively. The control group received the same volume of saline solution as that used for the treatments.

### *Behavioral experiments*

All behavioral experiments were performed during the dark phase (19:00 pm-21:00 pm) on different days, in the following order (1) sucrose preference test (2) forced swim test (3) tail suspension test. Tests were performed on consecutive days. The experimenters scoring behavior were blind to treatment groups.

### *Sucrose preference test*

The sucrose preference test is used to validate animal models of depression, especially UCMS. Sucrose preference is measured by a preference for a sucrose solution over water, using a two-bottle, free-choice method [30]. Blunted sucrose intake is thought to reflect impaired sensitivity to reward, and to model anhedonia, a core symptom of major depression. Briefly, each mouse was pre-exposed to 1% of sucrose solution for two weeks to habituate them to the test. Mice were denied food and water for 16 h before the test. On the test day, mice were given two h of free choice between two bottles of either 1% sucrose or standard drinking water. At the end of the period, the bottles were weighed and the consumption was calculated. Percentage preference for sucrose is calculated using the following formula: sucrose preference = volume of sucrose consumption/(volume of sucrose consumption + volume of water consumption) × 100%. The sucrose preference test was performed weekly during baseline and chronic stress periods.

### *Forced swim and tail suspension tests*

The forced swim test (FST) and tail suspension test (TST) are the two most commonly used animal behavioral tests for antidepressant screening [31]. Both the FST and TST are based on the measurement of the time that mice spend in an immobile position. Increased immobility in these two tests is claimed to reflect a helpless or resignation-like state. In the FST, mice were placed individually in cylinders (height, 28 cm; inside diameter, 12 cm) containing 12 cm of water (25 ± 1°C) for a period of six min. A mouse was judged immobile when it floated in an upright position, and could only move slowly to keep its head above water. Mice were dried immediately and returned to their home cages after the swimming test. In the TST, the duration of immobility was detected automatically using Tail Suspension SOF-821 (Med Associates Inc.). Briefly, the mice were suspended by the tail using adhesive tape attached to a hook for six minutes. A load cell was connected to the hook to measure activity. When a mouse struggled, the load cell would capture and record the change in load and represent the activity as a voltage output. When the mouse was immobile, the voltage output was taken as the lower threshold. Following escape-oriented struggling during the first minute, the mice showed increasing periods of immobility. The duration of the voltage output lower than the lower threshold during the final 5 min was recorded and regarded as the mice's immobility duration.

### *Sample collection*

After the behavioral tests, three mice, randomly chosen from each group, were deeply anesthetized and transcardially perfused with 4% paraformaldehyde for subsequent Nissl staining. The other animals were anesthetized and sacrificed. Blood was collected and brains removed. Blood, collected over EDTA (1.5%), was centrifuged at 12,000 rpm for 10 min, and the supernatant collected. The brain cortex tissue was dissected and immediately flash-frozen in liquid nitrogen, and stored at -80°C for further analysis.

### *Cytokine analysis*

Frozen cortex tissue was lysed with RIPA lysis buffer and homogenized on ice. The homogenized tissue was kept at 4°C for 30 min and the supernatants collected by centrifugation. The total protein concentration of the supernatants was determined using a BCA assay kit. The expression levels of cytokines were analyzed using a Bio-Plex Pro Mouse Cytokine 6-Plex panel in combination with the Bio-Plex Suspension Array System (Bio-Rad Laboratories Inc., Hercules, CA, USA). This assay is a bead-based suspension array system where microsphere sets (5.6 ml beads) are internally dyed with different ratios of fluorophores conjugated to different capture probes (cytokine-specific antibodies), which have been validated in blood, brain and CSF [32, 33]. This assay was performed by the Miao Tong Biotec Inc. (Shanghai, China). Plasma and brain tissue lysates were thawed, centrifuged at 4,500 rpm for three min at 4°C. Micro-beads labeled with anti-cytokine antibodies were vortexed for 30 s and diluted. Then, 50 μL of the bead solution was added to each well of a 96-well plate. After a washing step, 50 μL of diluted plasma (1:3 dilution) or a standard sample were mixed into each well. The plate was covered and vortexed at 1,100 rpm for 30 s on a plate shaker, and then incubated with shaking for 30 min, in the dark, at room temperature. The plate was then washed, and 25 μL of biotinylated detection antibody was added to each well. The plate was incubated on a shaker for 10 min. After a washing step, the beads were then incubated with 50 μL of streptavidin-phycoerythrin for 10 min. After this, he plate was washed and placed in the array reader for determination of the respective concentration of target cytokines.

Total RNA was extracted from the brain tissues using TRIzol reagent. Total mRNA (1 μg) was reverse transcribed using Quantscript cDNA RT Kits according to the manufacture's manual. A total of 2 μL of the first-strand cDNA solution was used in combination with the SYBR® Premix Ex Taq™ solution for real-time RT-PCR. [26]. The real-time RTPCR was run on Applied Biosystems 7500 (Life Technologies Corporation., Carlsbad, CA, USA) with initial activation for 30 second at 95°C, followed by 40 cycles of denaturation (95°C, 5 second), annealing (60°C, 34 second), and extension (72°C, 30 s). GAPDH was used as the endogenous housekeeping

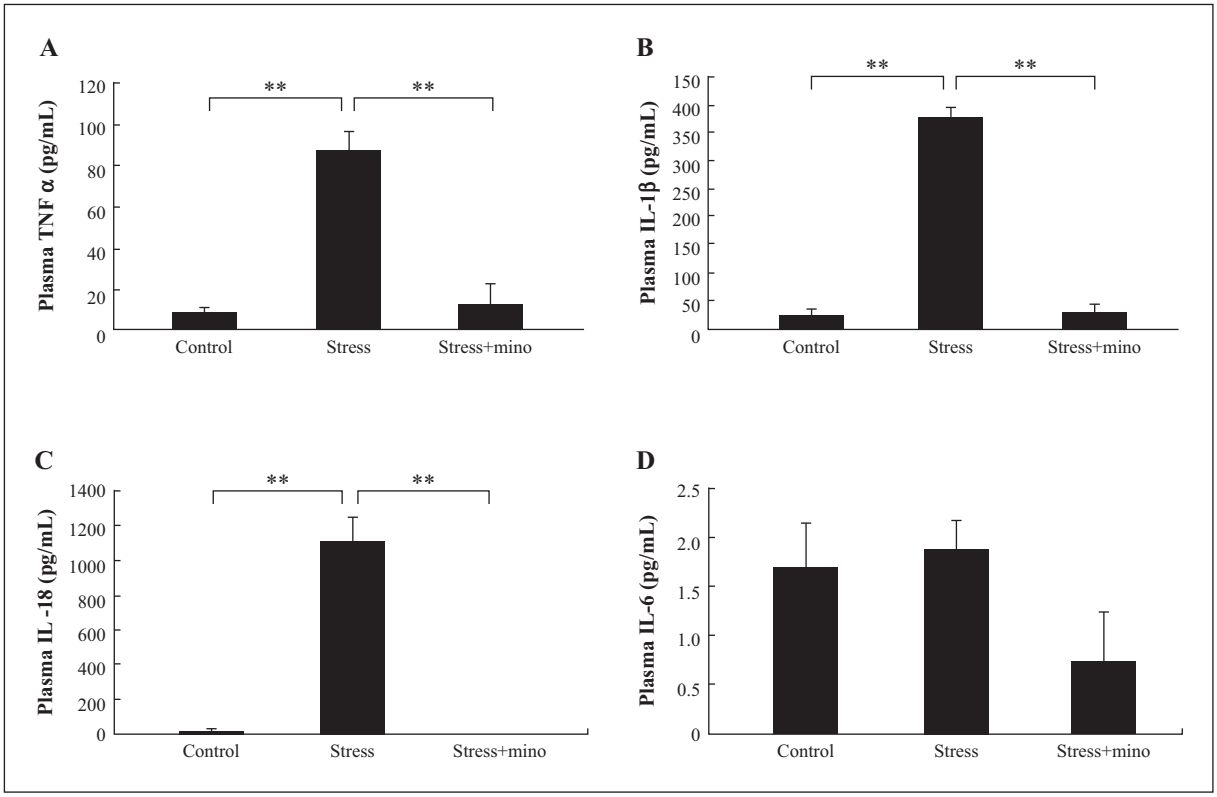


**Figure 1**

Minocycline blocks the upregulation of circulating proinflammatory cytokines induced by UCMS. Plasma cytokines were assayed with a Bio-Plex Cytokine panel after a four-week UCMS in the presence or absence of minocycline. Minocycline (30 mg/kg daily i.p) blocked the upregulation of TNFα, IL-1β and IL-18. Data are represented as mean ± SEM (n = 3-10 mice per group). $^{*}P<0.05$, $^{**}P<0.01$ for each comparison.

control to normalize gene expression data. The following oligonucleotides were used as primers: IDO (forward, 5'-CACTGAGCACGGACGGACTGAGA-3'; reverse, 5'-TCCAATGCTTTCAGGTCTTGACGC-3') GAPDH (forward, 5'-TCCCTCAAGATTGTCAGCAA-3'; reverse, 5'-AGATCCACAACGGATACATT-3').

### *Nissl staining*

Following the behavioral tests, some mice were perfused transcardially through the left ventricle with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain tissues were removed and post-fixed immediately in the same fixative solution at 4°C for 48 h. The tissues were dehydrated, immersed in paraffin, and cut into sections for subsequent Nissl staining as described previously [26]. Photographs of immunohistochemical slides were taken using a digital camera connected to microscope at 100× and 400× magnifications. Neurons with a round cell body, a visible nucleolus and abundant Nissl bodies were considered undamaged. Nissl-positive cells, with dark-staining in which the nucleolus was not discernable, were considered damaged.

### *Statistical analysis*

Data were analyzed using a one-way ANOVA, followed by a *post hoc*, pairwise, multiple comparison using Fisher's least significant difference test if the interaction was significant. Statistical significance was determined as $P<0.05$. All data are presented as the mean ± SEM.

## RESULTS

### *Minocycline blocks the upregulation of pro-inflammatory cytokines induced by UCMS*

The semi-synthetic tetracycline derivative, minocycline, has potent anti-inflammatory effects in addition to its microbicidal properties. As shown in *figures 1A-C*, plasma TNFα ($P<0.01$), IL-1β ($P<0.01$) and IL-18 ($P<0.01$) increased significantly after the four-week UCMS exposure. There was no difference in IL-6 levels (*figure 1D*) between the control and the stress groups. Pretreatment of mice with minocycline completely blocked the upregulation of plasma proinflammatory cytokines induced by UCMS, including TNFα ($F_{(2,14)} = 34.83$, $P<0.01$), IL-1β ($F_{(2,17)} = 108.48$, $P<0.01$) and IL-18 ($F_{(2,9)} = 39.23$, $P<0.01$).

The effects of stress on the hippocampus are well established. Functional alterations and a reduction in the volume of the hippocampus have been confirmed in major depressive disorder [34]. However, in the cortex, morphological plasticity and underlying mechanisms have been less intensively studied than in the hippocampus. We selected the cortex in our present study to investigate its potential importance in stress-related depression. A marked increase in TNFα ($F_{(2,11)} = 5.162$, $P<0.05$) (*figure 2*) was observed in the cerebral cortex tissues of UCMS-exposed mice, which was blocked by pretreatment with minocycline.

### *Minocycline attenuates UCMS-induced, depression-like behavior*

To evaluate the role of inflammation in the pathophysiology of stress-related depression, depressive behavioral

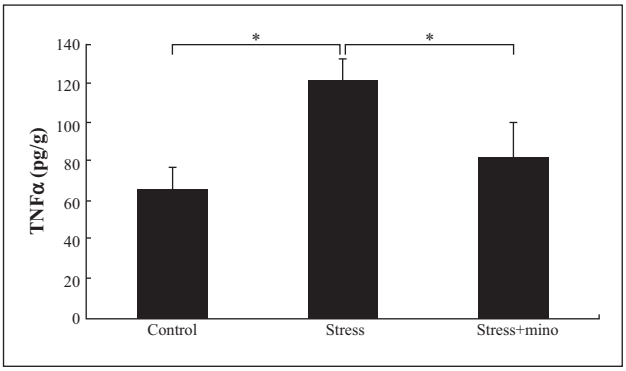


**Figure 2**
Effects of minocycline on cortex TNFα post-UCMS. Cytokines were assayed with a Bio-Plex Cytokine panel after a four-week UCMS in the presence or absence of minocycline (30 mg/kg daily i.p). Data are represented as mean ± SEM (n = 4-6 mice per group). $^{*}P<0.05$.

tests were performed in the presence or absence of minocycline. Mice were randomly allocated into four groups: control, stress, minocycline, and stress + minocycline groups respectively. During the first four weeks, minocycline was administered daily in the last two groups. From the fifth to the sixth week, the minocycline administration in the stress plus minocycline group was stopped, and only the stress aspect was retained (*figure 3A*). This "new" group was labeled as stress+mino*, to distinguish it from the previous stress+mino group. Mice in the simple stress group continued to be subjected to the previous stress conditions. As illustrated in *figure 3*, pretreatment with minocycline completely abrogated the UCMS-induced depression-like behaviors, including the decreased sucrose preference ($F_{(3,51)} = 8.76$, $P<0.01$) (*figure 3B*) in the sucrose preference test, and the increased immobility in the FST ($F_{(3,51)} = 8.38$, $P<0.01$) (*figure 3C*) and TST ($F_{(3,48)} = 4.24$, $P<0.01$) (*figure 3D*). Minocycline treatment alone had no significant effect on these behaviors. Two weeks after withdrawal of minocycline from stress plus minocycline group, exposure to stress again induced depression-like behaviors, including decreased sucrose preference ($F_{(2,14)} = 10.76$, $P<0.01$) (*figure 3E*) and increased immobility time in the FST ($F_{(2,15)} = 5.97$, $P<0.05$) (*figure 3F*) and TST ($F_{(2,13)} = 8.09$, $P<0.05$) (*figure 3G*). As expected, mice in the simple stress group consistently displayed depression-like behaviors ($P<0.01$) compared with the control group.

### *Upregulation of TNFα and depression-like behavior induced by UCMS are modulated by the anti-TNFα mAb, infliximab*

Our data show that four weeks of UCMS significantly upregulated TNFα in plasma and the cortex. Therefore, we hypothesize that TNFα is a key biomarker in the process of UCMS-induced depression. To confirm this hypothesis, the level of TNFα and the extent of depression-like behaviors in UCMS-exposed mice were analyzed in the presence or absence of the anti-TNFα mAb, infliximab. As illustrated in *figure 4A*, pretreatment of mice with infliximab abrogated any increase in TNFα in stressed mice ($F_{(2,14)} = 76.2$, $P<0.01$). Accompanying the downregulation of TNFα, infliximab pretreatment abrogated the decrease in sucrose preference ($F_{(3,24)} = 8.16$, $P<0.01$) (*figure 4B*), and the increase in immobility time ($F_{(3,23)} = 27.15$, $P<0.01$) (*figure 4C*) induced by UCMS. Infliximab treatment alone had no significant effect on the above depressive behaviors. Thus, the blockade of TNFα by infliximab abrogated the depression-like behaviors induced by UCMS.

### *Infliximab protects cortical neurons from damage induced by UCMS*

Clinical and experimental evidence suggests that the cortex is pathophysiologically involved in depression. However, morphological plasticity and the mechanisms involved in stressed conditions have been less intensively studied in the cortex than in the hippocampus, and for this reason we selected the cortex in our present study to investigate its potential importance in stress-related depression.

We used the cortex for morphological assessment of the effects of pretreatment with infliximab on cortical neurons. Normal neurons have a round cell body, a visible nucleolus and abundant Nissl bodies. In damaged neurons the nucleolus is not discernible. As shown in *figure 5*, normal neurons were observed in the control group and the stress plus infliximab group, while the number of damaged neurons in the stress group was very obviously increased.

### *Downregulation of TNFα blocks the upregulation of IDO induced by UCMS; 1-MT abrogates the UCMS-induced depression-like behavior*

TNFα is a potent inducer of IDO expression [24, 25]. To explore the molecular mechanisms underlying the involvement of TNFα in stress-induced depression, IDO expression in the cortex was measured in the presence or absence of infliximab. As shown in *figure 6A*, the upregulation of cortical IDO induced by UCMS was completely blocked by pretreatment with infliximab ($F_{(2,8)} = 63.51$, $P<0.01$). Furthermore, the effects of the IDO antagonist 1-MT on IDO expression and UCMS-induced depressive behaviors were assessed. The results showed that 1-MT blocked the enhancement of IDO transcript induced by UCMS ($F_{(2,7)} = 41.2$, $P<0.01$) (*figure 6B*). Accompanying the downregulation of IDO, UCMS-induced depression-like behaviors were significantly attenuated by pretreatment with 1-MT, including decreased sucrose preference ($F_{(2,20)} = 7.23$, $P<0.01$) (*figure 6C*) and increased immobility time ($F_{(2,13)} = 7.95$, $P<0.01$) (*figure 6D*) in the FST.

## DISCUSSION

In the present study, it has been shown that exposure to UCMS induces a significant upregulation of TNFα, both in the periphery and the cerebral cortex, as well as an activation of IDO, which is associated with depression-like behavioral syndromes. The results suggest that stress-induced, depression-like behavior may be mediated by TNFα through the upregulation of IDO and subsequent damage to neurons, suggesting that TNFα might be an important biomarker, and a potential target for future antidepressant therapy.

An important role for dysregulation of the immune system in the pathogenesis of depression has been established, with a dramatic paradigm shift over the past 20 years. This shift is based on the increased prevalence of depressive disorders in patients with chronic inflammatory conditions [4]. As previously reported, chronic stress increases circulating proinflammatory cytokines and induces depression

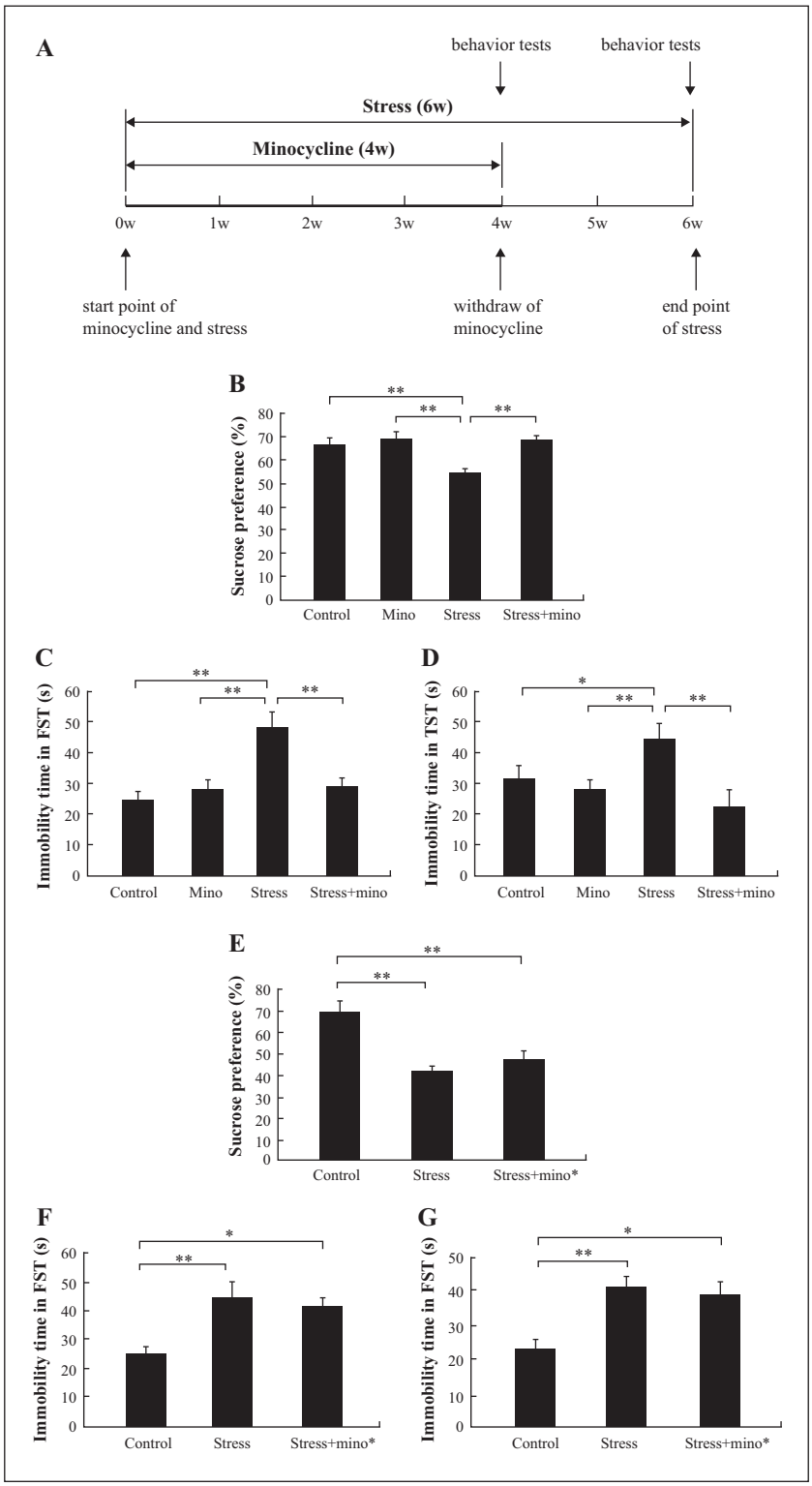


**Figure 3**

Minocycline attenuates UCMS-induced depression-like behaviors. **A)** Schematic representation of UCMS treatment and minocycline administration; **B-D)** Depression-like behaviors post-four-week UCMS. **B)** Sucrose preference, **C)** Immobility time in the FST, **D)** Immobility time in the TST. **E-G)** Depression-like behaviors post-two-week minocycline cessation. **E)** Sucrose preference, **F**) Immobility time in the FST, **G)** Immobility time in the TST. Data are represented as mean ± SEM (n = 4-15 mice per group). $^{*}P<0.05$, $^{**}P<0.01$ for each comparison.

[35]. Animal models of depression are widely used to test the antidepressant efficacy of compounds, and to investigate the pathophysiological mechanisms that underlie depression. Thus, UCMS represents a suitable model with which to study the depression-like behavioral implications of chronic inflammation.

In the present UCMS model, depression-like behaviors (*figure 3*) were seen to accompany the upregulation of proinflammatory cytokines (*figures 1-2*). This means that the effects of inflammation exist not only at the biochemical level, but also at the behavioral level. This is consistent with the cytokine hypothesis of depression [3] and several other reports [16]. To further confirm the relationship between inflammation and depression in the UCMS model, the effects of treatment with minocycline on stress-induced depressive behaviors were observed. Minocycline is an

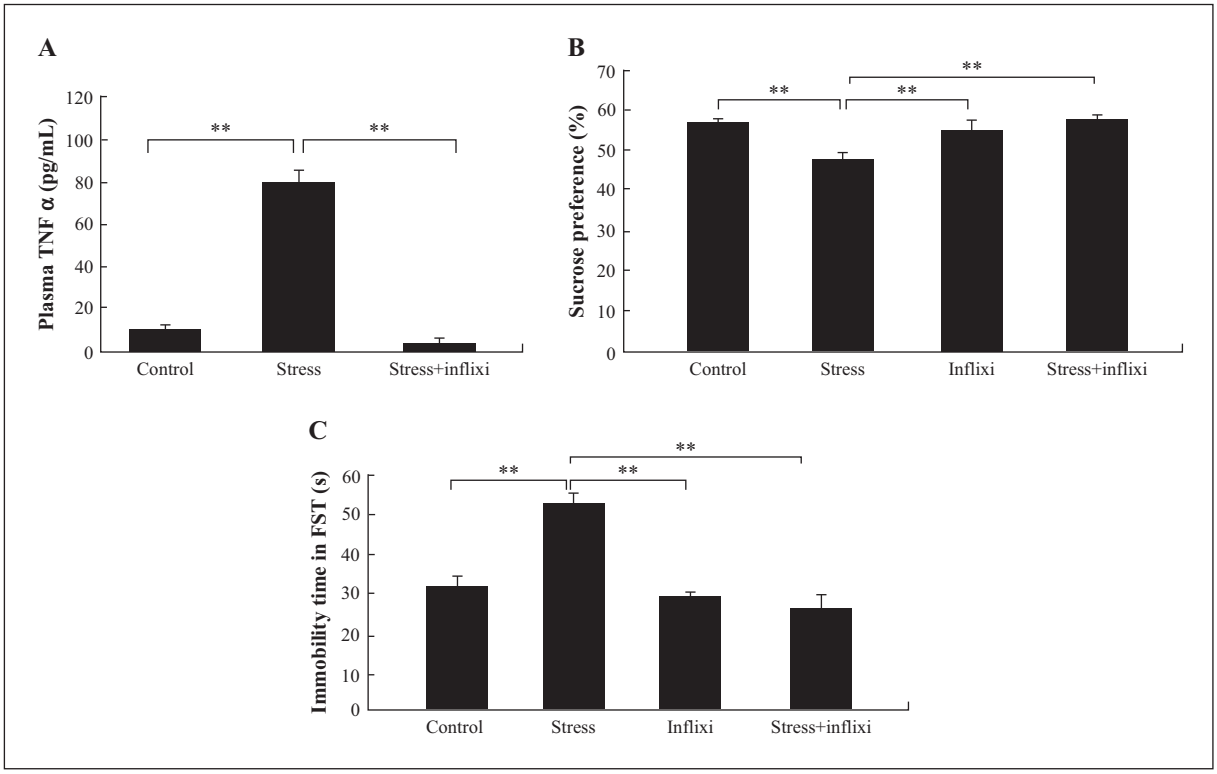


**Figure 4**

Infliximab attenuates depression-like behaviors induced by UCMS. Mice are subjected to UCMS in the presence or absence of infliximab for four weeks. TNFα protein was assayed with Bio-Plex Cytokine panel. Data are represented as mean ±SEM (n = 3-8 mice per group). $^{**}P<0.01$ for each comparison.

anti-inflammatory tetracycline derivative which blocks acute increases in several proinflammatory cytokines, with excellent penetration into the CNS when administered peripherally [36]. Minocycline is also an inhibitor of microglial activation [37]. Minocycline has been demonstrated to confer therapeutic benefits in many CNS disease models [38, 39]. Given these findings, peripheral administration of minocycline is a pharmacological strategy for the inhibition of proinflammatory mediator production. As expected, minocycline completely blocked the upregulation of proinflammatory cytokines and depression-like behaviors induced by UCMS (*figures 1-3*).

TNFα is suggested to be a key contributor to chronic glial activation and is involved in the modulation of neuronal activity/viability, cognition, behavioral and neurodegenerative processes [40, 41]. Protective effects of minocycline on neurons have been observed in both TNFα knockout mice and wild-type mice[42]. Increased TNFα has been found in experimental animals and stressed humans [12, 13, 23, 43]. In the present study, TNFα was significantly increased both in the periphery and the cortex (*figures 1-2*), and this can be related pathophysiologically to depression. This is in agreement with a recent report from Couch and colleagues. They reported the upregulation of TNFα transcript in the pre-frontal area of mice susceptible to stress-induced anhedonia [44]. They also showed that an increased number of Iba-1-positive microglial cells were present in the prefrontal area of susceptible animals compared to resilient animals. In our study, we observed a similar activation of microglia in stressed animals (data not shown). TNFα is thought to be expressed principally by microglia in the CNS. Therefore, in our study, minocycline is acting not only as an anti-inflammatory drug, but also as a type of microglial inhibitor. Our data provide further confirmation of the role of TNFα in stress-induced depression at the protein level. Additionally, we also noticed a decrease in the presence of TNFα protein in the hippocampus, which is in contrast to what has been observed in the cortex. These results corroborate a recently published paper reporting that chronic stress leads to the apoptosis of microglia and a reduction in the presence of these cells within the hippocampus, but not in other brain regions [45]. This observation, is of interest, and requires further investigation for confirmation of the differential expression of TNFα in the central nervous system.

Furthermore, our results show that pretreatment with infliximab blocked the increase in TNFα and depression-like behaviors induced by UCMS (*figure 4*). In addition to this behavorial improvement, infliximab abrogated neuronal damage caused by UCMS (*figure 5*). This protection by infliximab might be related to a limiting of the neuronal functional impairment and structural damage caused by excessive TNFα. It has been reported that stress could cause the loss of Nissl bodies in cerebral cortex neurons [26]. Inflammatory responses in the brain contribute to cellular damage associated with stress-induced, neuropsychiatric diseases. Clinically, a limited number of anti-TNFα interventions has been tried to improve both skin and psychiatric pathology in patients co-morbid for psoriasis and certain psychiatric diseases (affective disorders). Infliximab or etanercept (an anti-TNFα receptor) treatment are known to relieve the fatigue and symptoms of depression associated with this chronic disease [17, 18]. In a recently published study, it was also reported that continued administration of infliximab decreased depression

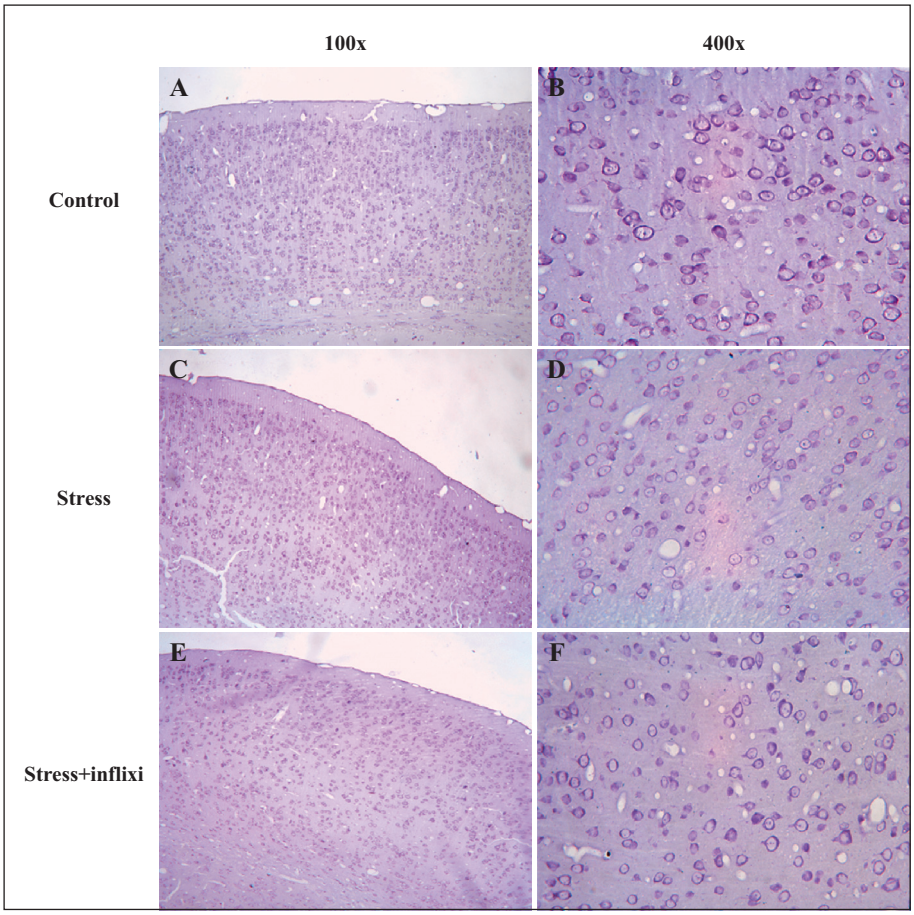


**Figure 5**

Infliximab protects cortical neurons from damage induced by UCMS. Mice are subjected to UCMS in the presence or absence of infliximab for four weeks. Undamaged neurons in the cerebral cortex were measured by Nissl staining. Representative slides of Nissl staining at two different magnifications (A, C and E: ×100; B, D and F: ×400).

and anxiety-like behavior in a rat model of chronic, mild stress [30], although the potential mechanisms were not explored.

To explore the molecular mechanisms of TNFα-mediated depression, the effects of an anti-TNFα mAb on IDO were also assessed. As we mentioned earlier, IDO is the first rate-limiting, tryptophan-degrading and inflammatory inducible enzyme in the kynurenine pathway, and has attracted much attention in inflammation-related depression [44]. TNFα has been suggested to be one of the most important cytokines for the induction of IDO. Fujigaki *et al.* reported the contribution of TNFα to LPS-induced IDO activation, but the connection between this IDO activation and depression-like behaviors was not referred to [25]. Our results demonstrate that infliximab abrogated the upregulation of IDO in the cortex induced by UCMS (*figure 6A*). Furthermore, the competitive inhibitor of IDO, 1-MT had effects similar to those of infliximab, abrogating the increased expression of IDO and the depression-like behavior induced by UCMS (*figure 6B-D*). These results suggest that UCMS-induced depression might be mainly mediated by TNFα through subsequent IDO activation. Microglia are the main source of brain IDO [21]. Our previous work has confirmed that TNFα could induce IDO expression in cultured microglia [21]. We noticed that Couch and colleagues reported the activation of microglia and up-regulation of TNFα transcript, but not IDO transcript in stress-susceptible mice [46]. This discrepancy may result from the model used. Their model involves 10-day, subacute stress while ours includes 28-day chronic stress.

In another study, it was reported that UCMS caused depression-like behavior, similar tothose found in the present study [14]. In contrast however, IL-1, rather than TNFα, was found to play a major role. It is of note that following the administration of low doses of LPS in humans, TNFα peaked at 3 h, whereas IL-1β was barely detectable, but peaked around 3 to 4.5 h [34]. Li *et al.* also showed thatfollowing LPS injection in guinea pigs, TNFα was not detectable in plasma until 30 min and IL-1β 60 min later [47]. Therefore, one possibility is that IL-1β acts downstream of TNFα.

Collectively, the present study suggests that TNFα, acting as one of the key inflammatory cytokines related to stress-induced depression, mediates UCMS-induced depressive behaviors through IDO activation and subsequent cortical neuronal damage. The investigation of inflammatory markers may provide insight into potential roles of psychoneuroimmunological processes in clinical depression. Moreover, inflammatory biomarkers may help identify depressed patients who are less likely to respond to conventional antidepressant treatment, and provide indicators of treatment response. Cases of depression, where there is increased inflammatory activity prior to treatment, have been reported to be less responsive to antidepressants [48,49]. Further studies on the specificity of TNFα and

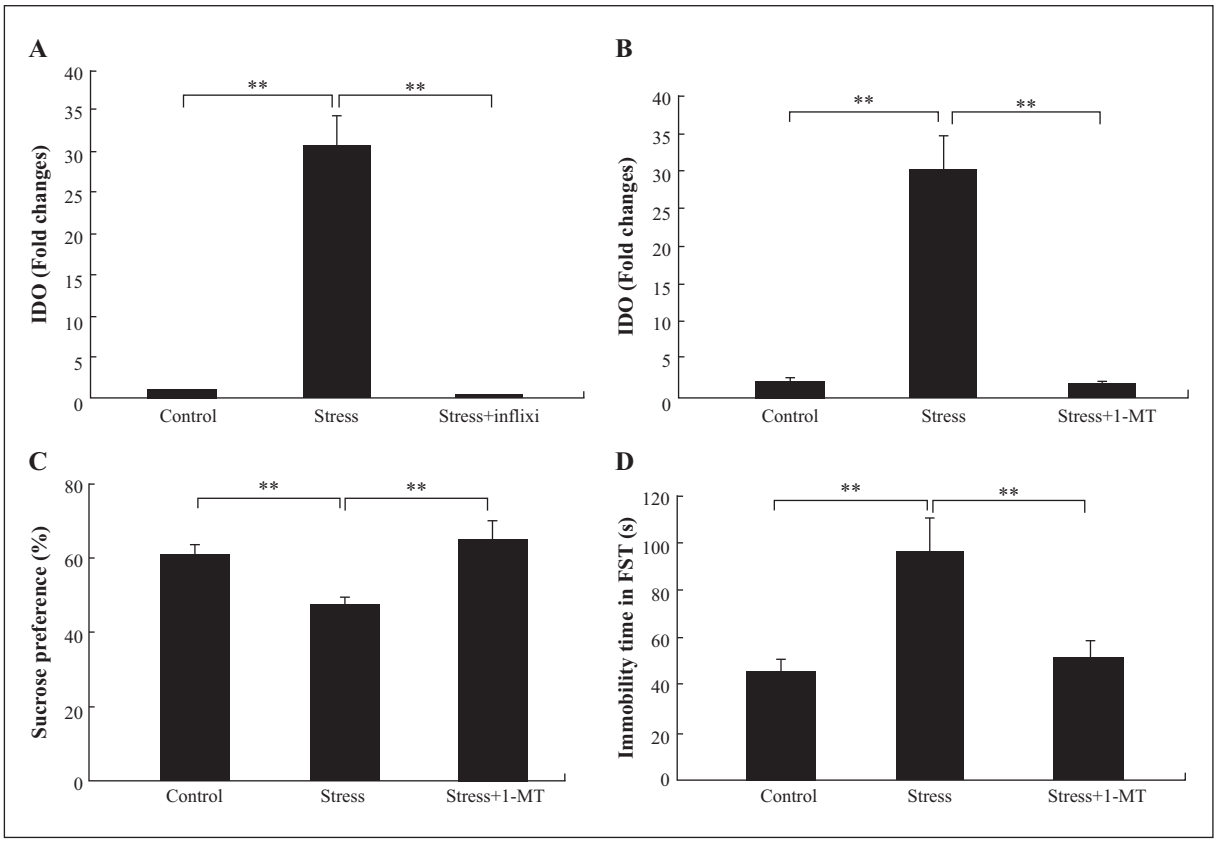


**Figure 6**

Infliximab blocks IDO expression, and the IDO antagonist 1-MT abrogated UCMS-induced depression-like behaviors. IDO transcript was assayed with RT-PCR. A. Infliximab blocked IDO expression in the cortex; B. 1-MT pretreatment blocked the upregulation of IDO transcript; C. Sucrose preference; D. Immobility time in the FST. Data are represented as mean ± SEM (n = 3-8 mice per group). $^{**}P<0.01$ for each comparison.

the molecular mechanisms involved in UCMS-induced depression-like behaviors are recommended, particularly the possible mediating role of corticosterone as glucocorticoids are the hormones that are released in response to stress, and which regulate metabolism and immunity. Increased secretion and reactivity of cortisol, together with an altered feedback inhibition are widely observed in depressed patients. In addition, thorough measurement of the changes in TNFα, IDO, and neuron damage in individual brain areas is also suggested, so that the key regions related to UCMS-induced depression linked to TNFα, might be identified and located.

## CONCLUSIONS

In conclusion, the present study supports the notion that TNFα may be a critical proinflammatory cytokine in mediating UCMS-induced, depression-like behaviors through upregulation of IDO and subsequent damage of cortical neurons. Inflammatory biomarkers may help to identify depressed patients who are less likely to respond to conventional antidepressant therapies, and could be used as indicators of therapeutic response to antidepressant medications.

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