

# Macrophage related cytokine expressions in depression mouse model induced by chronic unpredictable mild stress

Shoulian Zhu<sup>1</sup>, Lejing Li<sup>2</sup>, Ying Li<sup>1</sup> and Wenyuan Cao<sup>1\*</sup>

<sup>1</sup>Department of Neurology, Linzi District People's Hospital, Zibo, Shandong Province, China

<sup>2</sup>Department of Acupuncture and Moxibustion, Linzi District People's Hospital, Zibo, Shandong Province, China

**Abstract:** Depression is characterized by mental retardation, interest blank, hypoactivity, anxiety, appetite loss, sexual dysfunction, sleep disorders and other symptoms. The incidence of depressed patients has demonstrated an upward trend in recent years. Antidepressant drugs are commonly used in modern medicine, but they have the side effect of drug resistance. This study aims to explore the effect of acupuncture stimulation on expressions of macrophage-related cytokines in mice with depression induced by chronic unpredictable mild stress (CUMS), and to explore the underlying immunological mechanism. The CUMS model was successfully developed. The secretion of nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B) and interleukin (IL)-8 increased significantly in the modeling group compared with the blank control group ( $P < 0.05$ ). However, no significant difference of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) concentration was found ( $P > 0.05$ ). After acupuncture treatment, the behavior indicator was improved and meanwhile, the levels of TNF- $\alpha$ , NF- $\kappa$ B, IL-8 decreased significantly compared with the sham group ( $P < 0.05$ ). Depression mice were given treatment of acupuncture, and the effect of behavior change was observed and the content of macrophage cytokine production was measured, respectively. These findings suggested that inflammatory cytokines secreted by peritoneal macrophages increased significantly in mild depression mice, which can be improved by stimulation with acupuncture.

**Keywords:** Depression, macrophage, interleukins, acupuncture.

## INTRODUCTION

Depression is one of the most common neuropsychiatric disorders, with a lifetime prevalence above 17% (Merikangas *et al.*, 2009). Depression is related to nervous system, endocrine and immune system in pathogenesis (Mlyniec *et al.*, 2016). Research into the neurobiological underpinnings of mood disorders has progressed rapidly over decades, and major strides have been made in understanding the neurochemical, neuroanatomical, and genetic bases for depression (Cerullo *et al.*, 2014; Hamazaki *et al.*, 2015; Won and Kim, 2016). However, the exact pathogenesis and mechanism are still unclear. Recently, there are evidences showing that macrophages are related to depression, including depression-macrophage hypothesis and cytokine hypothesis (Roman *et al.*, 2013), nevertheless, it is still unclear how the macrophages and cytokines participate in the incidence and development of depression. It rarely works administering some drug alone in some certain link due to the complicated etiology and pathogenesis. Acupuncture, used clinically for more than 2500 years in East Asia, has been reported as an effective treatment approach in various kinds of neurological diseases including various diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD) and movement disorders (Peeraully *et al.*, 2013; Wang *et al.*, 2012; Yeo *et al.*, 2012). It is reported that acupuncture is effective on depression treatment (Chan *et al.*, 2015). The mechanism

cannot be fully explained though. This study observed the relationship of acupuncture and macrophage related cytokines and investigate the effects of immune mechanism of acupuncture on depression.

## MATERIALS AND METHODS

### Animals

A total of 20 BALB/c, 20-23g, male mice were purchased from the National Rodent Laboratory Animal Resources, Shanghai Branch (China) and maintained in a pathogen-free animal facility (Certification number: 2014-0013). All animals were cared for in accordance with the institutional guidelines for the care and use of experimental animals.

### Reagents and instruments

Premium newborn bovine serum was obtained from Sciencell bio-technology Co., Ltd. Batch NO: 20130801; LPS (Sigma, USA); MTT (Amresco, USA); RPMI Medium 1640 (Gibco, USA); Mouse NF- $\kappa$ B, IL-8, IL-1 TNF $\alpha$ , ELISA Kit were purchased from Cusabio Biotech Co., LTD, USA. All other chemicals of reagent grades were obtained from Sigma unless otherwise noted. Open box (homemade 50 cm $\times$ 50 cm $\times$ 50cm); the CO<sub>2</sub> incubator, NU-4750, NuAire Inc, USA.

### Chronic unpredictable mild stress (CUMS) induced depression model

BALB/c mice were all fed separately in three weeks with certain randomly stimulations each day: Fasting solids and liquids 24h; tilted feeding for 24h; horizontal

\*Corresponding author: e-mail: girlsflower@163.com

vibration 40 min (200r/min); wet culture 20h; The animals were maintained in a controlled environment at room temperature under a 12/12-h light/ dark cycle; tail clamp for 5 min (1cm from the tail head); cold stimulation 4°C for 40 min and thermal stimulation 45°C for 20 min; noise stimulation (80 dB) 1.5h. The behavior changes were detected during the process of model building and post-treatment at a different time.

#### **Open field test**

The open field box was standard as 50cm×50cm×50cm and the bottom was divided into 25 squares in 10cm×10cm. The experimental room was kept dark and quiet during the test. Each animal was located in the central square and observed and recorded the behavior changes in 3min. Central square retention time recorded since the mice was placed in the central square to all the limbs were out of the center; horizontal movements recorded as the times of all the limbs passing through the lines; vertical movements recorded the times of mice erecting and fore limbs climbing; cleaning schedule recorded the frequency of mice facial cleaning; fecal grains recorded the fecal grains during the test.

#### **Sucrose preference test**

Two bottles of 100ml 1% sucrose at the 1<sup>st</sup> day and one bottle of normal drinking water 100ml, 100ml 1% sucrose at the 2<sup>nd</sup> day was administered to the mice for 24h. The mice were fasting on the 3<sup>rd</sup> day. In the 4<sup>th</sup> day, the mice were administered 50ml drinking water and 50ml 1% sucrose water for 1h. The volume of the residual drinking water and sucrose water after 1hour was measured to calculate the humoral consumption and sucrose preference. Humoral consumption = (50-remains of normal drinking water) + (50-remains of sucrose water); Sucrose preference = (50-remains of sucrose water) / [(50-remains of normal drinking water) + (50-remains of sucrose water)] x 100%.

#### **Separation and purification of macrophages**

The mice were placed into 75% ethanol for 5 mins after cervical dislocation executing and the limbs were fixed on an anatomical plate with needles. PBS was used to do peritoneal lavage aseptically. Lavage fluid was collected, centrifugalizing 1000r/min for 5min and abandoned the supernatant. Cells were cultured in a cell culture plate at 37°C and placed in 5% CO<sub>2</sub> incubator for 2h after PBS washing 2 times and 10% calf serum RPMI-1640 culture solution to regulate the cell concentration to 2 x 10<sup>6</sup> mL<sup>-1</sup>. The supernatant was abandoned and washed with non-calf serum RPMI-1640 culture for 2 times. Then RPMI-1640 culture medium (containing 10% FBS, 100U/ml penicillin and 100mg/L streptomycin) was put into cell culture box to cultivate continuously. The adherent cells are the peritoneal macrophage.

#### **Cytokine assay**

The mice celiac macrophages were obtained during model building and treatment, which was stimulated with

10ug/mL LPS and cultured in 5% CO<sub>2</sub> incubator for 36 h at 37°C. Cytokines were detected by ELISA method in 100uL cell supernatant. Cell density and quantity were detected by MTT method to calculate the cytokines produced by a unit cell.

#### **Treatment protocols**

The mice with depression were randomly divided into four groups, including blank group, model group, sham group and acupuncture group. There were no significant differences of behavior changes, sucrose preference, food intake and other indicators among the four groups. The acupuncture group received electric acupuncture once a day; sham group received electric acupuncture on non-effective acupuncture points for continuous 14 days. The acupuncture points were according to “experimental acupuncture” and the mice acupoints were Baihui point and Shenting point. Huatuo 0.25mm X 25mm acupuncture pin produced by Suzhou medical supplies were used. Oblique insertion towards the tail in Baihui and Shenting points in 2mm deep for 2mins at 1mA.

#### **STATISTICAL ANALYSIS**

All statistical analysis on the data was performed in SPSS 16.0 software. Data were analyzed using t-test and presented as mean ± SEM. P<0.05 was statistically significant.

#### **RESULTS**

##### ***Behavioral changes of mice depression model induced by CUMS***

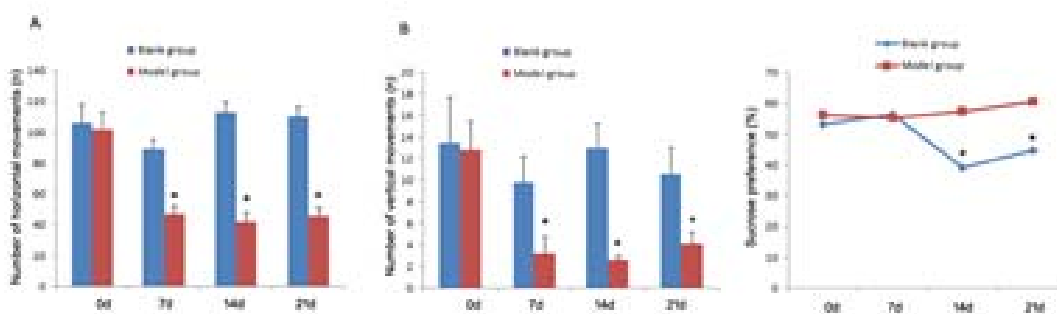
The mice in the model group were raised separately and received all types of unpredictable stimulus for 3 weeks. The mice in control group were grouped as 5 in each cage for 3 weeks and received behavioral test and sucrose preference test on the 7<sup>th</sup> day, 14<sup>th</sup> day and 21<sup>st</sup> day. The results showed that comparing with the control group, the horizontal movements of the model group was decreased significantly; the vertical movements began to decline since the 7<sup>th</sup> day and the sucrose preference decreased from the 14<sup>th</sup> day to 21<sup>st</sup> day, which was shown in fig. 1.

##### ***Changes of TNF- $\alpha$ , NF- $\kappa$ B, IL-8 secreted by macrophages induced by CUMS***

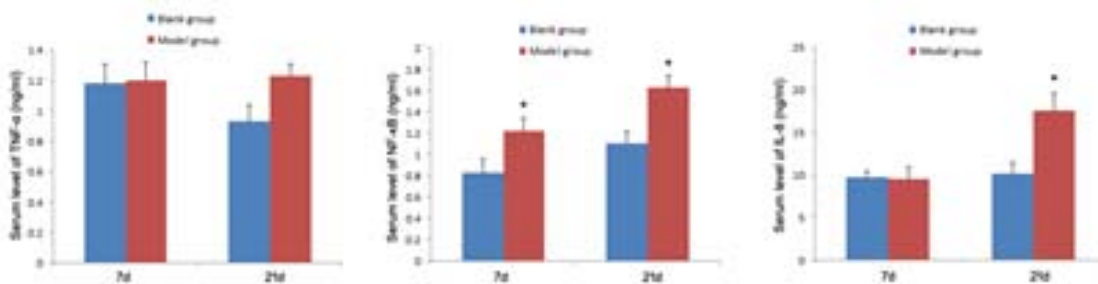
The macrophages were separated on the 7<sup>th</sup> day and 21<sup>st</sup> day in the chronic unpredictable depression model. The results illustrated that the ability of secreting NF- $\kappa$ B increased significantly in the middle-late stages of model and the IL-8 increased in the late stage (21st day). There was a rise in trend of TNF - $\alpha$ , however, the difference was not significant, which could be seen in fig. 2.

##### ***The effects of acupuncture on behavior and macrophages cytokine of depressive mice***

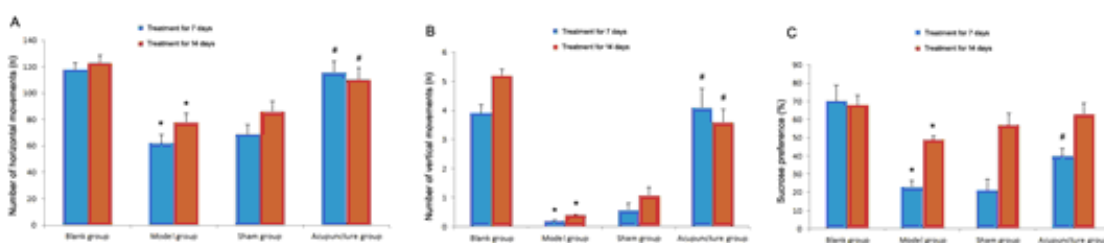
The results showed that acupuncture could benefit the horizontal movements, sucrose preference and other



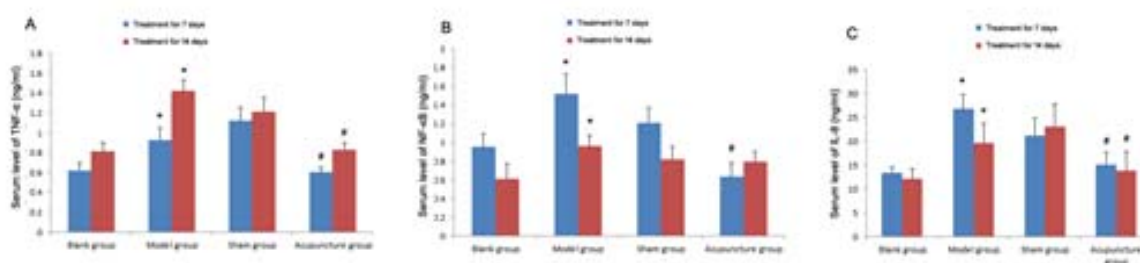
**Fig. 1:** Behavioral changes of chronic mild unpredictable depressed model mice ( $\bar{x}\pm s$ , n=10). Note: compare with the control group, \* p<0.05



**Fig. 2:** Changes of macrophage cytokines of depressed model mice ( $\bar{x}\pm s$ , n=5). Note: compare with the control group, \* p<0.05



**Fig. 3:** The effects of acupuncture on behavior changes of depressed mice ( $\bar{x}\pm s$ , n=10). Note: compare with the control group, \*p<0.05; compare with the model group, #p<0.05



**Fig. 4:** Changes of macrophages cytokines after acupuncture treatment ( $\bar{x}\pm s$ , n=5). Note: compare with the control group, \*p<0.05; compare with the model group, #p<0.05

depressive behavioral at the 7<sup>th</sup> and 14<sup>th</sup> after treatment (fig. 3). The macrophage suspension was made after the last time of treatment. LPS was used to stimulate the macrophage for 36 h and detected the ability of cells secreting cytokine. The results illustrated that TNF- $\alpha$ , NF- $\kappa$ B and IL-8 decreased significantly of acupuncture group after treatment, which could be seen in fig. 4.

## DISCUSSION

Pro-inflammatory cytokines located in synovium, including IL-1, IL-6, IL-8 and TNF- $\alpha$ , have been known to play a key role in the progression of depression (Rethorst *et al.*, 2013). Inhibition of the expression of IL-8, TNF- $\alpha$  and NF- $\kappa$ B is a promising strategy for the

development of novel anti-depression therapies (Qi *et al.*, 2013). However, the molecular basis of depression remains to be elucidated. In this study, we established the chronic mild unpredictable depression model. The model group mice performed movements and exploratory behavior's ability decrease. Meanwhile, comparing with the control group, the sucrose preference ratio decreased significantly of depressive mice. The decrease of sucrose water consumption was a specific anhedonia, which was related to impaired brain rewards systematic neuronal structure (Son *et al.*, 2012). The model group mice were successfully established to have depressive behaviors under chronic mild unpredictable stimulations through the behavior evaluation and sucrose preference. We detected the different stages of depression model and macrophages cytokines changes based on the chronic mild unpredictability depression model. Our data showed that there were significant increases of macrophages cytokines NF- $\kappa$ B and IL-8. Though, there was not significant difference of TNF- $\alpha$ , it still increased. TNF- $\alpha$ , NF- $\kappa$ B and IL-8, as pro-inflammatory cytokines, pointed out that the formation of the depression was associated with inflammation, which was similar with previous studies (Davidson, 2010; Yang *et al.*, 2009). Bai found that the increase of peripheral and central NF- $\kappa$ B and the active of inflammatory reaction could lead to depression or depressive symptoms, regardless of psychogenic or physical depression (Bai *et al.*, 2014). However, previous reports showed that no elevation of cytokine levels was detected in depressive patients (Bai *et al.*, 2015; Li *et al.*, 2015). Our results showed that in the process depression, the related macrophages cytokines increase, which could not only active the patients' immune system, but related to the chronic stress reaction. IL-1 and 5-HT were highly linked and they could active indoleamine 2,3-dioxygenase (IDO), exhaust 5-HT precursor (TRP), enhance the activity of monoamine oxidase and decreased the level of 5-HT of synaptic cleft, impaired 5-HT receptor density and function (El-Haj *et al.*, 2002). Macrophage is an important participant of depression cytokine hypothesis and amine neurotransmitter hypothesis (Moon *et al.*, 2012). Acupuncture has significant effect on depression. However, the underlying mechanism is not clear.

## CONCLUSION

Taken together, we observed that acupuncture at Baihui and Shenting has a therapeutic effect on improving behaviors of depressive mice with the suppressive effect on the production of pro-inflammatory cytokines and NF- $\kappa$ B. It is illustrated that acupuncture can improve the inflammatory reaction in depressive mice.

## ACKNOWLEDGEMENTS

We thank Ding Zhang, Liu Mao, and Renhou Lai for manuscript preparation and technique supports for this study.

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