Original paper

Folia Neuropathologica

Cognitive impairment of MRL mice is related to NMDA receptor-mediated inflammatory response and production of adhesion molecules in MRL/lpr mice-derived micro-vascular endothelial cells

Xiaoyan Guan, Jingyuan Wang

Department of Rheumatology and Immunology, Xiang' an Hospital of Xiamen University, Xiang' an District, Xiamen City, Fujian Province, China

Folia Neuropathol 2023; 61: 1-12 *DOI: https://doi.org/10.5114/fn.2023.125903*

Abstract

Systemic lupus erythematosus (SLE) is a chronic recurrent autoimmune disease affecting almost all organs. This study was conducted to investigate cognitive impairment of SLE mice (MRL/lpr mice), and explore associated pathological mechanism. Behavior tests (open-field test, elevated plus-maze test, forced swimming test, sucrose preference test, and Morris water maze test) were conducted in MRL/MPJ and MRL/lpr mice. ELISA test was performed to determine levels of antibodies (anti-dsDNA, anti-RPA, anti-ACA, and anti-NR2a/b) and inflammatory factors [tumour necrosis factor α (TNF-α), interleukin (IL)-6, IL-8, and IL-10]. Micro-vascular endothelial cells (MVECs) were isolated, identified, and divided into MVECs (NC), anti-NR2a/2b, memantine, glycine, dexamethasone, and IL-1β groups. Cell proliferation was measured using cell counting kit-8 (CCK-8) assay, and Western blotting was applied to evaluate ELAM-1, VCAM-1, ICAM-1, IKBα, p-IKBα expression. MRL/lpr mice demonstrated lower locomotion/exploration ability, higher anxiety, obvious depression symptoms, lower learning/memory capability compared with MRL/MPJ mice. MRL/lpr mice demonstrated high levels of anti-NR2a/b antibody and auto-antibodies. NMDA receptor antagonist (memantine) significantly increased, and NMDA receptor agonist (glycine) significantly decreased MVECs proliferation compared with NC group ($p < 0.05$). Memantine significantly reduced and glycine predominantly enhanced TNF-α, IL-6, IL-8, and IL-10 levels compared with NC group ($p < 0.05$). NMDA receptor antagonist and agonist modulated adhesion molecules expression in MVECs. ELAM-1, VCAM-1, and ICAM-1 expressions were significantly down-modulated in memantine group, and remarkably up-modulated in glycine group compared with NC group ($p < 0.05$). NMDA receptor antagonist and agonist regulated phosphorylation of p-IKBα. The above effects of memantine evenly equaled to dexamethasone, and glycine evenly equaled to IL-1β. In conclusion, cognitive impairment of MRL mice might be associated with NMDA receptor-mediated inflammatory response and production of adhesion molecules in MRL/lpr mice-derived MVECs.

Key words: *systemic lupus erythematosus, MRL/lpr mice, cognitive impairment, NMDA receptor.*

Introduction

Clinically, systemic lupus erythematosus (SLE), as a chronic recurrent autoimmune disease, often involves many organs, including lung, heart, skin, kidneys, etc. [13,41]. SLE is commonly characterized by production of a variety of auto-antibodies, such as anti-double stranded-DNA (dsDNA), anti-ribosomal P protein (anti-RPA), and anti-cardiolipin antibody (anti-ACA) [24]. In addition to the above-mentioned dysfunction of peripheral organs, SLE can also lead to some neuropsychiatric disorders, such as cognitive impairment, headache, and psychiatric disorders, including depression and anxiety disorder, in up to 75% of SLE patients [6,13].

Communicating author:

Jingyuan Wang, Department of Rheumatology and Immunology, Xiang' an Hospital of Xiamen University, 2000 Xiang' an East Road, Xiang' an District, Xiamen City, Fujian Province, China, e-mail: maiquan0000@163.com

Among all SLE disorders, neuropsychiatric SLE (NPSLE) is considered to be particularly severe type [5]. Among the various neuropsychiatric symptoms of SLE, the most common is cognitive impairment, involving memory impairment, anxiety, and/or mood diseases (ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature, 1999). According to a former investigation [2], production of auto-antibodies in brain tissue and integrity of blood-brain barriers are the main reasons affecting neuropsychiatric symptoms of NPSLE. Moreover, a previous study [27] also reported that matrix metalloproteinase-9 (MMP-9), anti-N-methyl-D-aspartate receptor sub-unit 2a/b (anti-NR2a/b) antibodies, and pro-inflammatory cytokines may be involved in the pathogenesis of NPSLE. However, despite the above conclusions of previous studies, there is no consistent view on the pathogenesis of cognitive impairment in NPSLE patients.

At present, although some new methods and strategies have been applied in the clinical treatment of NPSLE and have potential application prospects, its' treatment usually focuses on symptoms' relief and systemic immunosuppression [17,26]. Glucocorticoid is the main drug for the treatment of NPSLE [4], and dexamethasone (DEX) can treat cognitive impairment in rats caused by bacterial meningitis through inhibiting neuronal apoptosis and inflammatory response [3,34]. Clinically, injection of methylprednisolone and/or prednisolone in patients with NPSLE can obviously alleviate neuropsychiatric symptoms [28], but the specific mechanism is not clear. Therefore, finding the exact pathogenic mechanism of NPSLE and developing corresponding drugs are the key to the treatment of cognitive impairment in NPSLE patients.

MRL/Tnfrsf6$^{lpr/lpr}$ (MRL/lpr) mouse, as commonly used animal SLE model, can show many characteristics of SLE, especially cognitive impairment (depression-like behavior and memory/ learning deficits) of NPSLE [13,25]. However, as a homologous control for MRL/lpr mouse and MRL/Tnfrsf6 (MRL/MPJ) mouse does not show obvious neuropsychiatric disorders [23]. Therefore, in the present study, we applied the specific SLE animal model (MRL/lpr strain) as the research object to find the targeted molecules involved in cognitive impairment of MRL/lpr mouse model, in order to obtain an effective method to treat cognitive impairment in NPSLE patients in the future.

Material and methods

Animals

Female MRL/MPJ mice and MRL/lpr mice, aging 6-8 weeks, were purchased from Slac Laboratory Animal Co., Ltd. (Shanghai, China), and housed at 23-25°C temperature, with a 12 h/12 h of light/dark cycle. All experimental mice were allowed food and water freely.

All animal protocols have been approved by the Ethical Committee of Xiang' an Hospital of Xiamen University, China. All procedures or protocols were conducted in line with NIH guide for care and use of laboratory animals.

Behavioral evaluations

Behavioral tests were carried out for evaluating behavior of MRL/lpr mice and MRL/MPJ mice, as described by former published studies [15,20,21,26,37]. In this study, behavioral tests mainly included open-field test (MRL/lpr, n = 10; MRL/MPJ, n = 10) to evaluate locomotion and exploration; elevated plus-maze test (MRL/lpr, n = 10; MRL/MPJ, n = 10) to evaluate anxiety; forced swimming test (MRL/lpr, n = 10; MRL/MPJ, n = 10) to evaluate depression-like behavior; sucrose preference test (MRL/lpr, n = 10; MRL/MPJ, n = 10) to evaluate anhedonia; and Morris water maze test (MRL/lpr, n = 10; MRL/MPJ, n = 10) to evaluate learning and memory. Briefly, prior to the above tests, MRL/lpr and MRL/MPJ mice were exposed to testing room with low incandescent light for 30 min. All the above tests were recorded and observed with viewer tracking software, whereas, the manually scored tests (forced swimming test and Morris water maze test) were validated by a blinded investigator.

Enzyme-linked immunosorbent assay

Auto-antibodies in serum of mice, including anti-double stranded DNA (anti-dsDNA) antibody [35], anti-ribosomal P protein (anti-RPA) antibody [25], anti-cardiolipin (anti-ACA) antibody [22], and anti-N-methyl-D-aspartate receptor sub-unit 2a/b (anti-NR2a/b) antibody [37], were measured using enzyme-linked immunosorbent assay (ELISA) method, as described by previous studies. Antibody against NR2a/b was measured with ELISA using synthetic DWEYSVWLSN (DWEYS peptide, GL Biochem (Shanghai) Ltd., Shanghai, China), according to a previous study [38]. Anti-dsDNA antibody, anti-RPA antibody, and anti-ACA antibody were measured with mouse anti-dsDNA antibody ELISA kit (Jingmei Biotechnology, Guangzhou, China), mouse anti-RPA antibody with ELISA kit (Meimian Biotechnology, Wuhan, China), and mouse anti-ACA antibody with ELISA kit (Jingmei Biotechnology, Guangzhou, China), according to protocols of the manufacturers.

Moreover, endothelial cell-related inflammatory factors in micro-vascular endothelial cells (MVECs), including tumour necrosis factor α (TNF-α), interleukin (IL)-6, IL-8, and IL-10, were also measured using ELISA kits. TNF-α, IL-6, IL-8, and IL-10 were detected using mouse

TNF-α detection ELISA kit (Lkcx Biotechnology, Beijing, China), mouse IL-6 was detected with ELISA kit (Lkcx Biotechnology, Beijing, China), mouse IL-8 was detected with ELISA kit (Meimian Biotechnology, Wuhan, China), and mouse IL-10 was detected with ELISA kit (Lkcx Biotechnology), as instructed by protocols of the manufacturers.

Micro-vascular endothelial cells isolation and identification

Primary MVECs were isolated and identified according to previous study [19]. In brief, the cerebral cortex of MRL/MPJ mice was dissected and cut into 2.5 cm tissue fragments, and then digested using DMEM medium (Gibco BRL, Gaithersbuerg, MD, USA), supplemented with 0.1% trypsin (Beyotime Biotechnology, Inc., Shanghai, China) and 0.1% EDTA (Beyotime Biotechnology, Inc.), in PBS for 30 min at 37°C . Then, the tissues were further homogenized and centrifuged at 1,000 r/min for 5 min to obtain the pellets. The pellets were then suspended in 20% BSA solution, centrifuged at 1,000 r/min for 5 min, and the obtained pellets were digested using 0.25% trypsin and centrifuged at 1,000 r/min for 5 min. The pellets were then re-suspended in DMEM to obtain MVECs. The cells were subsequently cultured in DMEM containing 15% fetal bovine serum (FBS, Gibco BRL), 1 ng/ml bFGF (PeproTech Ltd., Rocky Hill, NJ, USA), 100 U/ml penicillin (Beyotime Biotechnology, Inc.), and 100 µg/ml streptomycin (Beyotime Biotechnology, Inc.), at 4°C .

The isolated MVECs were identified by factor VIII immunofluorescence staining with rabbit anti-mouse factor VIII antibody (Novus Biologicals, Littleton, CO, USA) at 4°C overnight, followed by staining with Cy3-conjugated goat anti-rabbit IgG (Beyotime Biotechnology Inc.), at 37°C for 60 min. Finally, MVECs were stained using DAPI for 5 min in dark, and observed under a laser confocal fluorescence microscope (Leica, Frankfurt, Germany).

In vitro micro-vascular endothelial cells treatment and trial grouping

The isolated MVECs were divided into 6 groups, including normal control MVECs group (NC group), anti-NR2a/2b group, memantine group, glycine group, dexamethasone group, and IL-1β group. In anti-NR2a/2b group, MVECs were treated with anti-NR2a/2b at dosage of 20 µg/ml. In memantine group, glycine group, and dexamethasone group, MVECs were treated with 20 µg/ml memantine, 1 µg/ml glycine, and 10 µmol/l dexamethasone, respectively. In IL-1β group, MVECs were treated with IL-1β at dosage of 10 ng/ml.

Cell proliferation assay

Cell proliferation was detected using cell counting kit-8 assay (CCK-8). MVECs were seeded into 96-well plates at density of 3×10^4 cells/well, and then cultured for 24 hours. Then, MVECs were treated with 10 µl CCK-8 solution (Beyotime Biotechnology Inc.) per well for 3 hours, as described by the manufacturer.

Western blotting assay

Micro-vascular endothelial cells were treated with ice-cold lysis buffer containing 10 mmol/l PMSF (pH, 7.5). The lysates were then subjected to SDS-PAGE and electro-transferred onto polyvinylidene difluoride (PVDF) membranes. PVDF membranes were incubated overnight at 4°C with one of the following primary antibodies, including rabbit anti-mouse ELAM-1 antibody, VCAM-1 antibody, ICAM-1 antibody, IKBα antibody, p-IKBα antibody, and GAPDH antibody (all antibodies were purchased from Abcam Biotechnology, Cambridge, Massachusetts, USA). Subsequently, the PVDF membranes were incubated with HRP-conjugated goat anti-rabbit IgG (Beyotime Biotechnology Inc.), at room temperature for 1 hour. Bound antibodies in the above PVDF membranes were developed with chemiluminescent substrate (ECL, Beyotime Biotechnology Inc.), according to the instruction of manufacturer. The immunoreactive bands were imaged and scanned with gel imaging system (Tannon-4200, Tannon, Shang, China).

Statistical analysis

Data were displayed as mean ± standard deviation (SD) and analyzed using SPSS software 19.0 (IBM Corp., Armonk, NY, USA). Differences between two groups were calculated using Student's *t* test. All of the experiments were conducted at least for six repeats in this study. Significance was defined as *p*-value less than 0.05.

Results

MRL/lpr mice showed lower locomotion/exploration ability and higher anxiety

The results indicated that the movement distance was significantly shorter, the times of line crossings and stand were remarkably less, and the residence time in central area was predominantly less in MRL/lpr mice compared with those of MRL/MPJ mice (Fig. 1A, all $p < 0.05$). Therefore, MRL/lpr mice showed remarkably lower locomotion/ exploration ability. According to the elevated plus-maze test findings, MRL/lpr mice demonstrated higher anxiety characteristics, including lower values of open-arms entries (OE), open-arms time (OT),

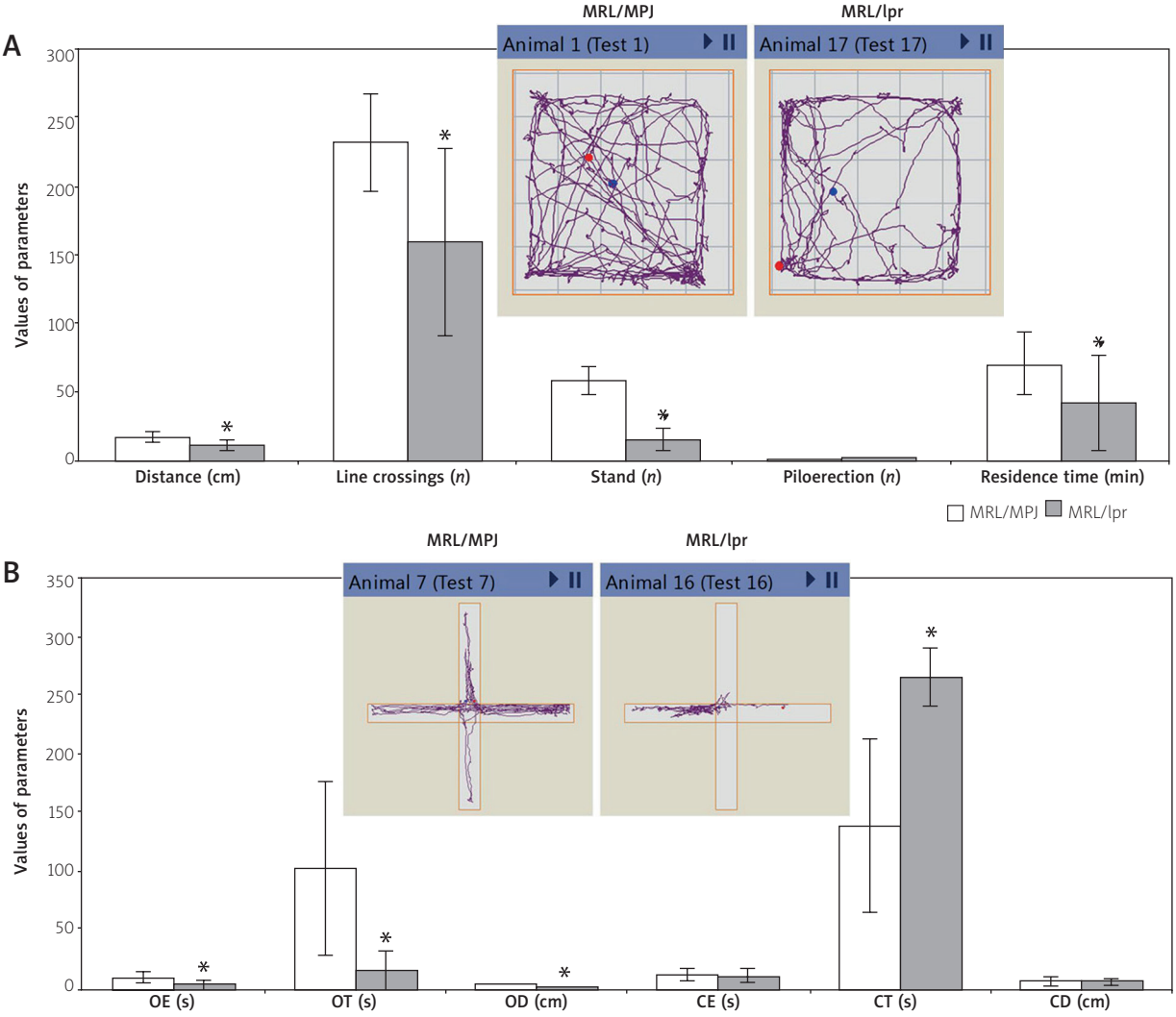

Fig. 1. Evaluation for the locomotion and exploration ability and anxiety in MRL/lpr mice. **A**) Locomotion and exploration ability of mice (by evaluating distance, line crossings, stand, piloerection, and residence time). **B**) Anxiety of MRL/lpr mice (by evaluating OE, OT, OD, CE, CE, and CD). OE – open-arms entries, OT – open-arms time, OD – open-arms distance, CE – close-arms entries, CT – close-arms time, CD – close-arms distance; $^{*}p < 0.05$ vs. MRL/MPJ mice.

and open-arms distance (OD), and higher values of close-arms time (CT), when compared with those of MRL/MPJ mice (Fig. 1B, all $p < 0.05$). Therefore, MRL/lpr mice showed obviously higher anxiety.

MRL/lpr mice showed obvious depression

The sucrose preference test findings indicated that sugar preference rate in MRL/lpr mice was significantly lower compared with that of MRL/MPJ mice (Fig. 2A, $p < 0.05$), suggesting that MRL/lpr mice demonstrated clear anhedonia and depression. The forced swimming test results showed that resting time of MRL/lpr mice was markedly longer compared with that of MRL/MPJ mice (Fig. 2B, $p < 0.05$), suggesting that MRL/lpr mice presented depression tendency.

MRL/lpr mice appeared lower learning and memory

Morris water maze test indicated that crossing entries of MRL/lpr mice were significantly less, and crossing time was significantly shorter than those of MRL/MPJ mice (Fig. 2C, all $p < 0.05$). Meanwhile, the movement distance of MRL/lpr mice was significantly shorter than that that of MRL/MPJ mice (Fig. 2C, $p < 0.05$). These results clearly suggest that MRL/lpr mice demonstrated obviously lower learning and memory capability.

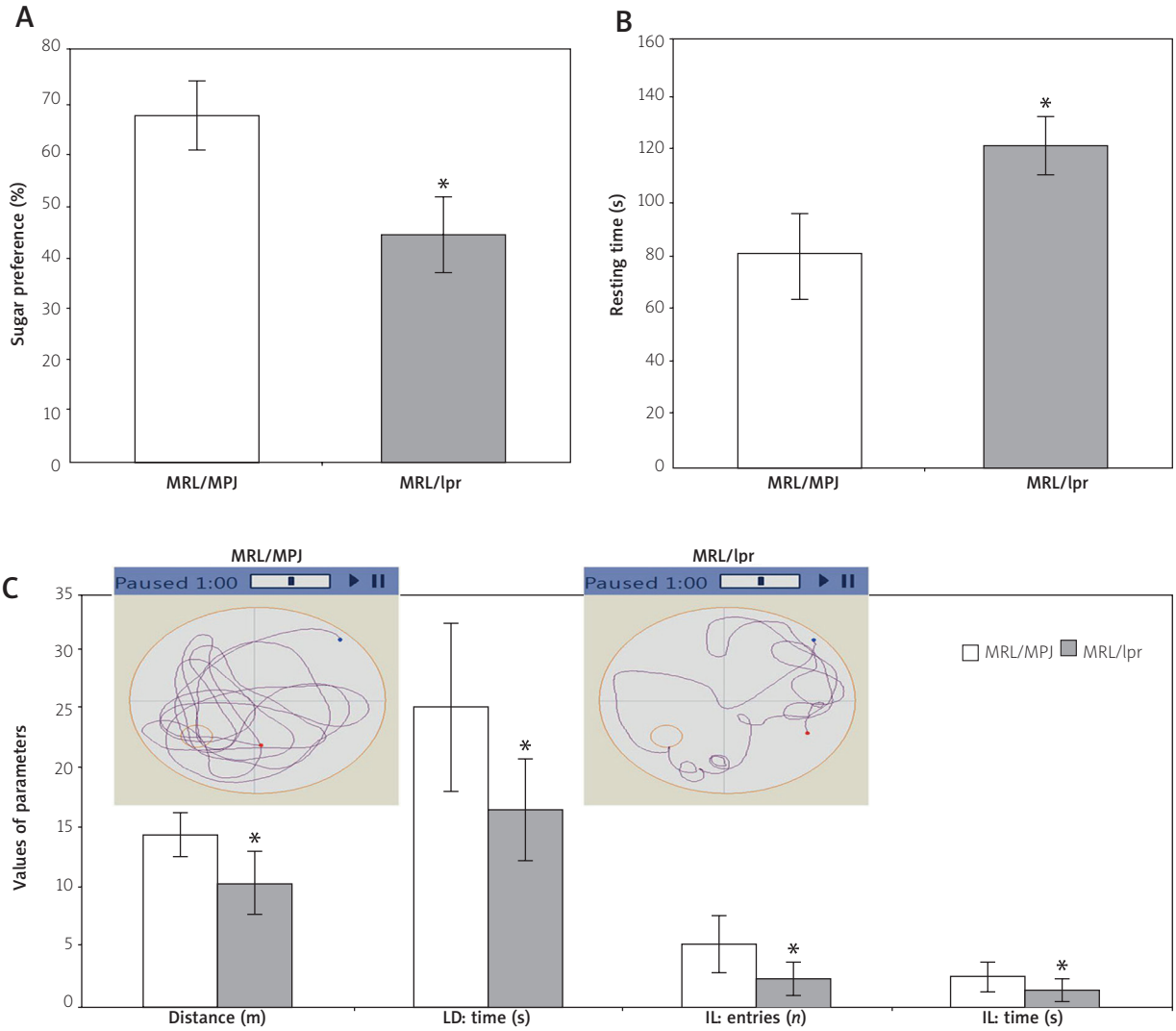

Fig. 2. Determination for depression, and learning and memory in MRL/lpr and MRL/MPJ mice. **A**) Sugar preference of MRL/lpr and MRL/MPJ mice determined by sucrose preference test. **B**) Depression symptoms of MRL/lpr and MRL/MPJ mice determined by forced swimming test. **C**) Learning and memory of MRL/lpr and MRL/MPJ mice determined by Morris water maze test. LD – left down quadrant of water maze (target quadrant), LD time – target quadrant stay time, IL entries – number of crossing platform, IL time – platform study time; $^{*}p < 0.05$ vs. MRL/MPJ mice.

High levels of anti-NR2a/b antibody and auto-antibodies produced in MRL/lpr mice

In this study, the anti-NR2a/b antibody level in MRL/lpr mice was remarkably higher compared with that of MRL/MPJ mice (Fig. 3A). Furthermore, the auto-antibodies, including anti-ACA-IgG (Fig. 3B), anti-RPA (Fig. 3C), and anti-dsDNA (Fig. 3D) in MRL/lpr mice were predominantly higher compared with those of MRL/MPJ mice (all $p < 0.05$).

Effects of NMDA receptor antagonist and agonist on MVECs proliferation

Micro-vascular endothelial cells were successfully isolated (Fig. 4A) and identified by staining factor VIII molecule (Fig. 4B). The results showed that NMDA receptor antagonist (memantine) increased proliferation of MVECs compared with that of NC group (Fig. 4C). Especially for 20 μg/ml memantine, which even showed significantly increased proliferation (Fig. 4C, $p < 0.05$). However, NMDA receptor agonist (glycine) decreased

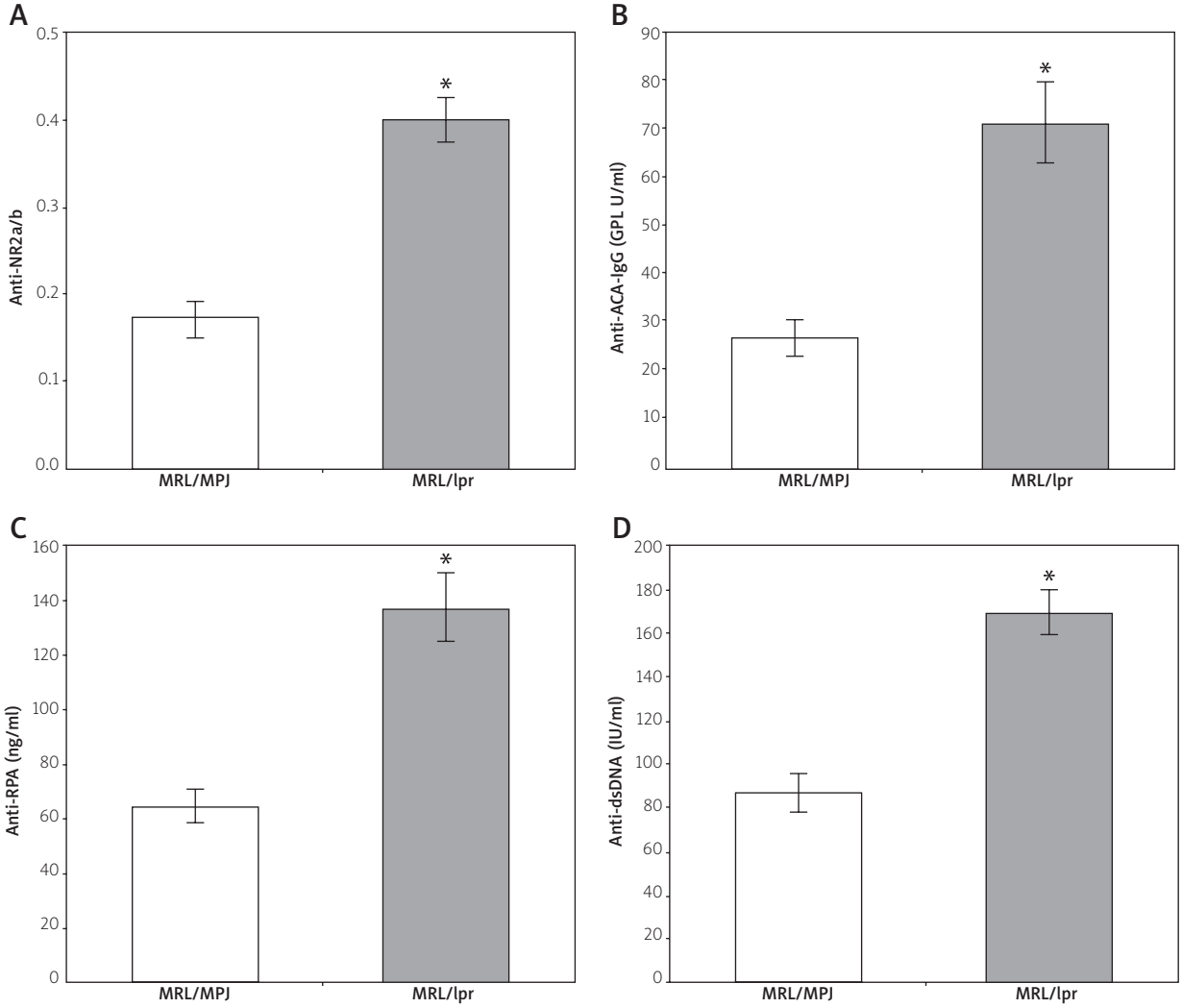

Fig. 3. Evaluation for anti-NR2a/b antibody and auto-antibodies levels in MRL/lpr and MRL/MPJ mice. **A**) Determination of anti-NR2a/b antibody levels. **B**) Determination of anti-ACA-IgG levels. **C**) Determination of anti-RPA levels. **D**) Determination of anti-dsDNA levels. $^{*}p < 0.05$ vs. MRL/MPJ mice.

proliferation of MVECs compared with that of NC group (Fig. 4D). Particularly for 0.5 µg/ml and 1 µg/ml glycine, which even showed remarkably decreased proliferation (Fig. 4D, both $p < 0.05$). Moreover, 5 µmol/l and 10 µmol/l dexamethasone also significantly decreased MVECs proliferation compared with those of NC group (Fig. 4E, both $p < 0.05$). Therefore, we discovered equal effects of glycine and dexamethasone on proliferation of MVECs.

NMDA receptor antagonist and agonist associated with MVECs-produced inflammatory factors

The results showed that NMDA receptor antagonist (memantine) significantly reduced levels of TNF-α (Fig. 5A), IL-6 (Fig. 5B), IL-8 (Fig. 5C), and IL-10 (Fig. 5D) compared with those of NC group (all $p < 0.05$). Moreover, the memantine demonstrated the same effects as the dexamethasone treatment. However, anti-NR2a/2b treatment predominantly increased TNF-α (Fig. 5A), IL-6 (Fig. 5B), IL-8 (Fig. 5C), and IL-10 (Fig. 5D) levels compared with those of NC group (all $p < 0.05$). Furthermore, NMDA receptor agonist (glycine) predominantly enhanced levels of TNF-α (Fig. 5A), IL-6 (Fig. 5B), IL-8 (Fig. 5C), and IL-10 (Fig. 5D) compared with those in NC group (all $p < 0.05$). Additionally, glycine evenly illustrated the equal effects with IL-1β.

NMDA receptor antagonist and agonist-modulated adhesion molecules expression in MVECs

Expressions of adhesion molecules, including ELAM-1, VCAM-1, and ICAM-1, were evaluated using Western blotting assay (Fig. 6A). The findings verified

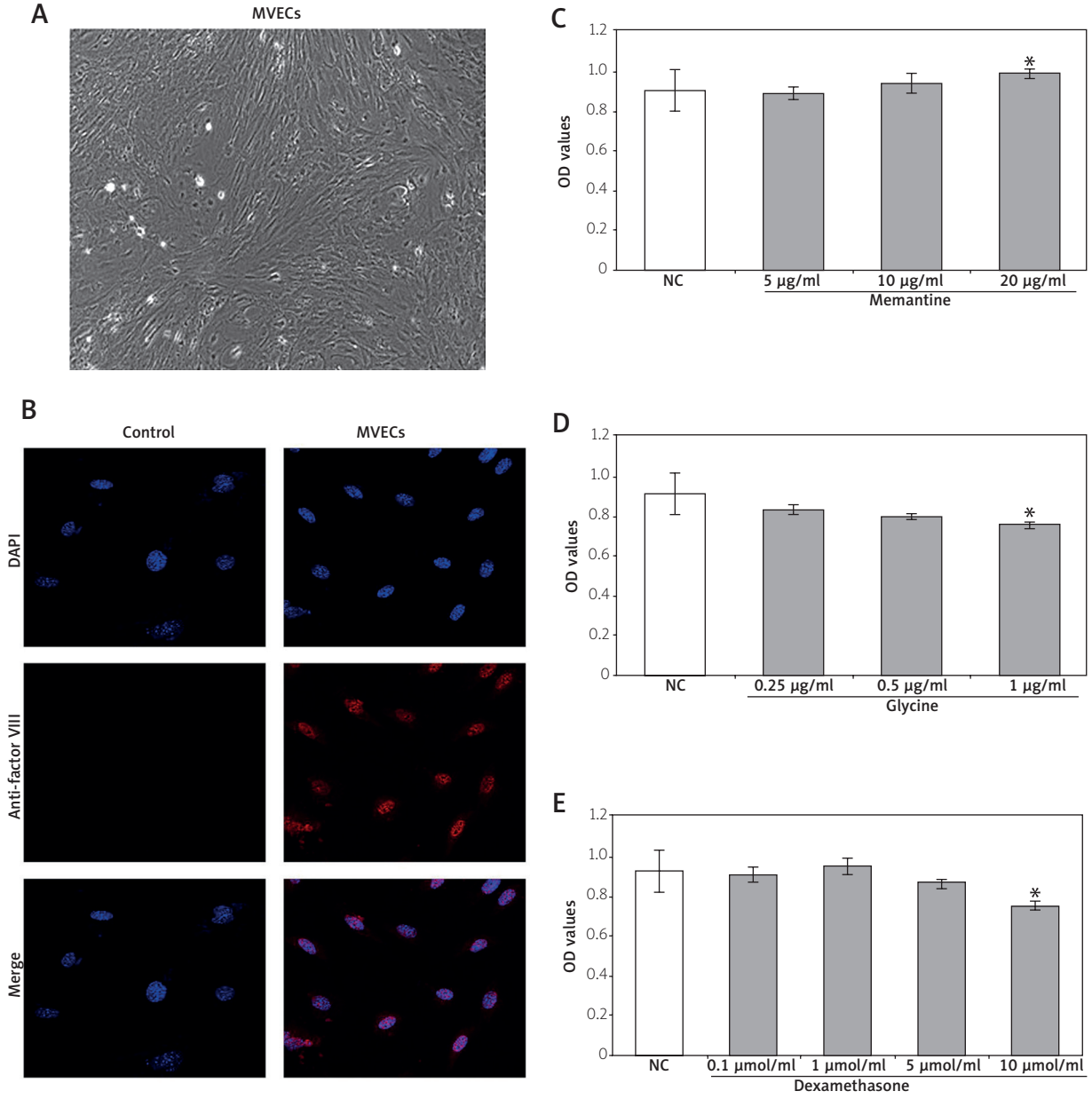

Fig. 4. Effects of NMDA receptor antagonist and agonist on the proliferation of MVECs. **A**) Isolation for the MVECs. **B**) Identification for the MVECs by staining factor VIII. **C**) Effect of NMDA receptor antagonist (memantine) on proliferation of MVECs. **D**) Effect of NMDA receptor agonist (glycine) on proliferation of MVECs. **E**) Effect of dexamethasone on proliferation of MVECs; $^{*}p < 0.05$ vs. NC group.

that ELAM-1 (Fig. 6B), VCAM-1 (Fig. 6C), and ICAM-1 (Fig. 6D) expressions were significantly down-modulated in NMDA receptor antagonist (memantine) treatment groups, when comparing with those in NC group ($p < 0.05$). However, NMDA receptor agonist (glycine) markedly increased ELAM-1 (Fig. 6B), VCAM-1 (Fig. 6C), and ICAM-1 (Fig. 6D) expressions, when comparing with those in NC group ($p < 0.05$). Interestingly, effects of meantime evenly equaled to dexamethasone, and glycine evenly equaled to IL-1β (Figs. 6B-D). Furthermore, anti-NR2a/2b significantly up-regulated ELAM-1 (Fig. 6B), VCAM-1 (Fig. 6C), and ICAM-1 (Fig. 6D) expressions compared with those in NC group (all $p < 0.05$).

NMDA receptor antagonist and agonist-regulated phosphorylation of p-IKBα

Expressions of p-IKBα and IKBα were also evaluated with Western blotting analysis (Fig. 7A). When

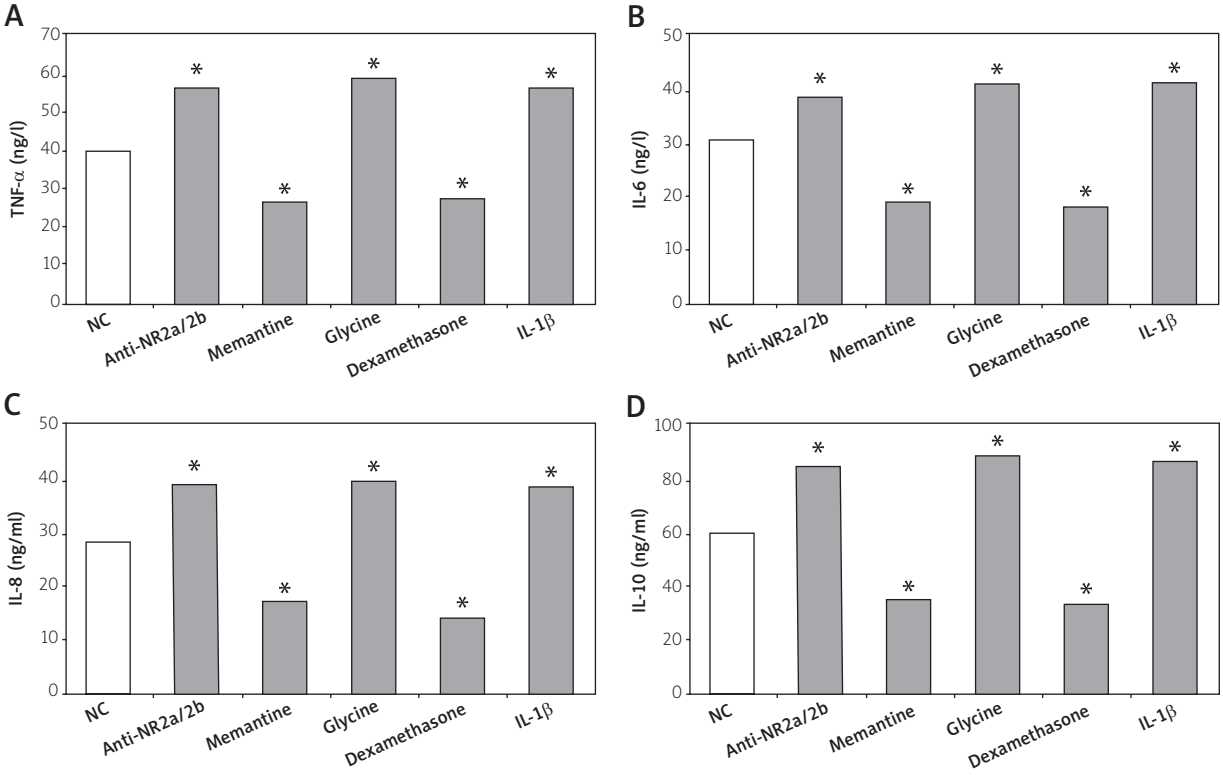

Fig. 5. Regulatory effects of NMDA receptor antagonist and agonist on production of inflammatory factors TNF-α (**A**), IL-6 (**B**), IL-8 (**C**), and IL-10 (**D**) in MVECs, as determined by ELISA kits. $^{*}p < 0.05$ vs. NC group.

comparing with NC group, an increased ratio of p-IKBα/IKBα triggered by NMDA receptor antagonist (memantine) (Fig. 7B, $p < 0.05$) was found, which was evenly similar to effects of anti-NR2a/2b and dexamethasone treatment. While, when comparing with NC group, a decreased ratio of p-IKBα/IKBα was induced by treatment of NMDA receptor agonist (glycine) (Fig. 7B, $p < 0.05$), which was evenly similar to IL-1β treatment.

Discussion

MRL/lpr mice are suitable animal models for systemic lupus erythematosus [10]. MRL/lpr mice can spontaneously develop immune complex mediated lupus-like disease symptoms in various organs, such as kidney, skin, and nervous system, and a variety of auto-antibodies can be detected [16,33]. In fact, MRL/lpr mice have been shown to have cognitive impairment and other behavior impairments before complete involvement of other organs [11]. These behavior impairments are important pathogenic factors of MRL/lpr mice. Therefore, this study aimed to explore the pathogenesis of cognitive impairment in MRL/lpr mice. Here, the open-field test, elevated plus-maze test, forced swimming test, sucrose preference test, and Morris water maze test [11] were used to evaluate locomotion/exploration, anxiety, depression-like behavior, anhedonia, and learning/memory of MRL/lpr mice, respectively. The above findings suggest that MRL/lpr mice present spontaneous behavior disorders, which is consistent with previous results of other research [26,33]. Therefore, this study elucidated the pathological mechanisms of behavior disorders.

In MRL/lpr mice, the inflammation and systemic auto-immunity are accompanied by a series of brain functional defects, such as behavior abnormalities, which are comparable to those found in SLE patients [13,16]. The brain reactive auto-antibodies associated with NPSLE and cytokines in patients' cerebrospinal fluid (or serum) have been proven to be the key factors of central nervous system injury [16,39]. Therefore, auto-antibodies and pro-inflammatory factors are critical for the diagnosis of neuropsychiatric manifestations [9,16,20]. Moreover, previous studies [12,16,18] have shown that the occurrence and progression of cognitive impairment or other behavior disorders are associated with auto-antibodies, including anti-ACA-IgG, anti-RPA, and anti-dsDNA as well as NMDA-glutamate receptor. The findings of the current study showed that the anti-NR2a/b antibody and auto-antibodies of MRL/lpr mice

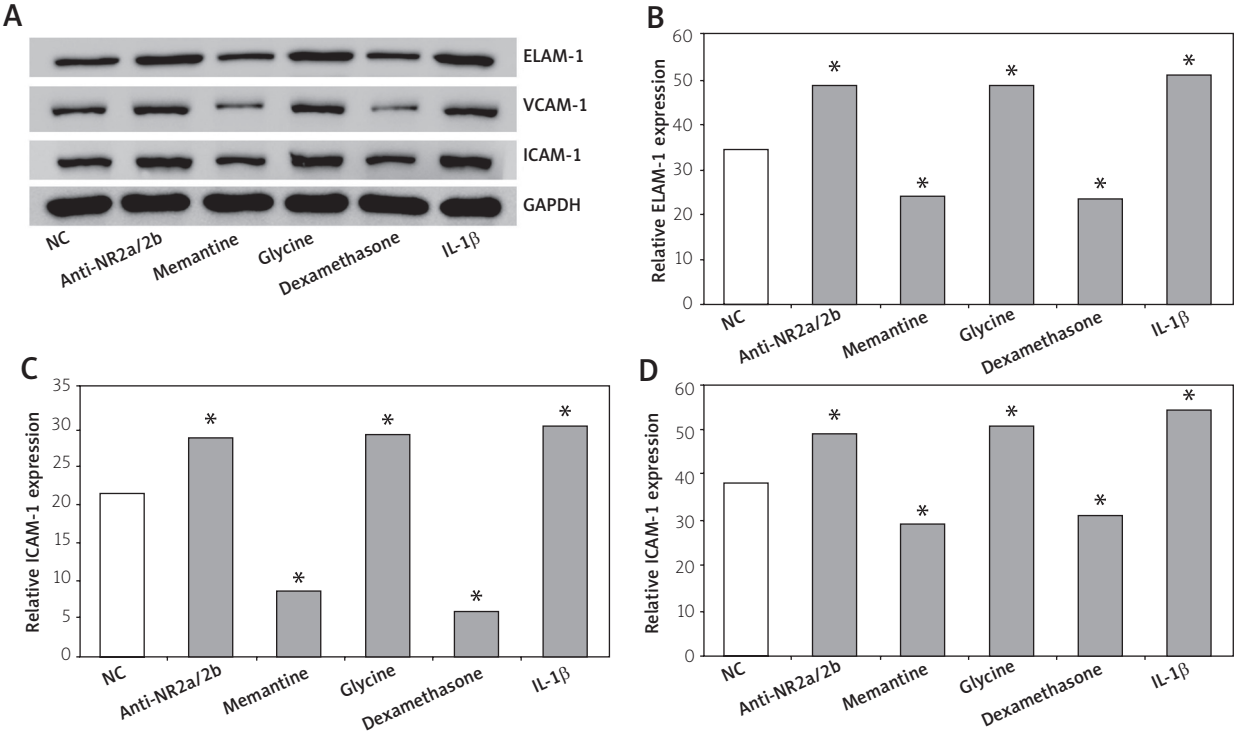

Fig. 6. Effects of NMDA receptor antagonist and agonist on expression of adhesion molecules in MVECs, determined by Western blotting assay. **A**) Western blotting images. **B**) Effects of NMDA receptor antagonist and agonist on ELAM-1 expression. **C**) NMDA receptor antagonist and agonist on VCAM-1 expression. **D**) NMDA receptor antagonist and agonist on ICAM-1 expression. $^{*}p < 0.05$ vs. NC group.

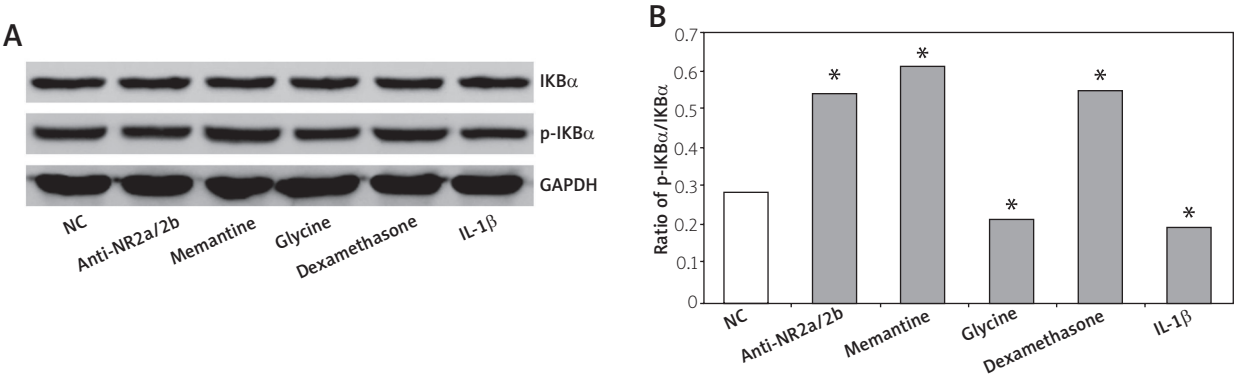

Fig. 7. Changes of IKBα phosphorylation in MVECs undergoing NMDA receptor antagonist and agonist treatment. **A**) Western blotting images. **B**) Statistical analysis for the ratio of p-IKBα/IKBα in MVECs. $^{*}p < 0.05$ vs. NC group.

were higher compared with those of MRL/MPJ mice (control group). Therefore, this study further confirmed that anti-NR2a/b antibody and auto-antibodies play a key role in the cognitive impairment of MRL/lpr mice. However, the differences in brain morphology between MRL/lpr mice and MRL/MPJ mice were not compared in the present study. Currently, there is no literature suggesting differences in size, appearance, and morphology of the brain between these two kinds of mice. The description of growth and size difference in phenotypic description of MRL/MPJ mice in Jackson laboratory only mentioned an enlargement of spleen, and the rest were not mentioned.

According to previous studies [33,40], IL-6 and IL-1β in serum and cerebrospinal fluid of NPSLE patients were significantly higher compared with those of SLE patients

without neuropsychiatric symptoms and healthy subjects. This suggests that inflammatory factors and cytokines may play an important role in the pathogenesis of NPSLE. Therefore, we determined the inflammatory cytokines in the isolated MVECs. In this study, only the whole cortex was used to isolate MVECs instead of hippocampus and amygdala, which are closely related to cognition and emotion. Actually, this study mainly focused on the expressions or levels of factors related to inflammation, and the research objects were MVECs. The number of viable cells obtained from primary culture of MVECs is small, while there are more mouse brain MVECs in the cortical micro-vascular segment. Moreover, the operation of cortical tissue is simpler than that of hippocampus and amygdala in separating excess brain's white matter and tissue. Therefore, it is easier to obtain more MVECs by isolating cells from the cortex. The findings indicated that NMDA receptor antagonist (memantine) increased the proliferation; however, NMDA receptor agonist (glycine) decreased the proliferation of MVECs. Therefore, the proliferation of MVECs may be associated with the state of NMDA receptor. Furthermore, this study evaluated the effects of memantine and glycine on the levels of inflammatory cytokines.

Tumour necrosis factor α in hippocampus binding to TNF type 1 receptor on the surface of astrocytes leads to a signal cascade reaction between astrocytes and neurons, resulting in cognitive impairment [14]. Previous studies [7,8,33] confirmed that the levels of IL-6, IL-8, and IL-10 in the hippocampus of MRL/lpr mice were significantly increased, but their role in cognitive function has not been fully explained. In this study, we found that NMDA receptor antagonist and agonist was associated with inflammatory factors produced by MVECs, as the results of memantine-caused reduction and glycine-caused enhance of TNF-α, IL-6, IL-8, and IL-10. Interestingly, the effects of memantine demonstrated the same effects on inflammatory factors production with dexamethasone, which could be defined as positive control. Furthermore, the effects of glycine on inflammatory factors production illustrated equal effects in IL-1β, which could be defined as positive control. NMDA receptor antagonist and agonist play a critical role in modulating the production of inflammatory cytokines, such as TNF-α, IL-6, IL-8, and IL-10 in MVECs. Therefore, potential drugs may affect the cognitive impairment of MRL/lpr mice through regulating the above-mentioned inflammatory cytokines.

Earlier studies [31,32] have shown that adhesion molecules play an important role in transmembrane signal transduction, and participate in tissue injury in MRL/lpr mice. In lupus-like symptoms of MRL/lpr mice, the inter-cellular adhesion molecules are closely related to the severity of lupus nephritis [30]. Based on the association between NMDA glutamate receptor and the pathology of MRL/lpr mice, we explored the effects of NMDA receptor antagonist and agonist on levels of adhesion molecules in MVECs. The findings confirmed that NMDA receptor antagonist (memantine) down-regulated, and NMDA receptor agonist (glycine) up-regulated ELAM-1, VCAM-1, and ICAM-1 expressions in MVECs. It is worth noting that the effect of memantine was evenly equivalent to dexamethasone, whereas glycine was equivalent to IL-1β. Therefore, NMDA receptor antagonist and agonist can regulate the expression of adhesion molecules in MVECs, which may be of great significance to improve cognitive impairment or other pathological symptoms in MRL/lpr mice.

Secretion of inflammatory cytokines, including IL-6 and TNF-α, is regulated by NF-κB signaling pathway [36]. When NF-κB inhibitor (Iκb) is activated (or phosphorylated), which can inhibit translocation of NF-κB from the cytoplasm to nucleus, inhibit its' binding to target genes, thereby inhibiting the secretion of inflammatory cytokines [1,29]. NMDA receptor antagonist (memantine) triggered an increased ratio of p-IKBα/IKBα in MVECs, which was evenly similar to the dexamethasone treatment. At the same time, the treatment of NMDA receptor agonist (glycine) resulted in a decreased ratio of p-IKBα/IKBα, which was evenly similar to the IL-1β treatment. Consequently, the NF-κB signaling pathway was activated in MVECs, resulting in an increased secretion of inflammatory cytokines, i.e., TNF-α, IL-6, IL-8, and IL-10.

In conclusion, MRL/lpr mice showed obvious cognitive impairments. NMDA receptor antagonist and agonist can regulate the proliferation of MVECs, the production of inflammatory factors, and the levels of adhesion molecules in MVECs. Moreover, the production of inflammatory cytokines in MVECs may be mediated by NF-kβ-associated signaling pathway. Therefore, the cognitive impairment of MRL/lpr mice may be related to NMDA receptor-mediated inflammatory response and the secretion of adhesion molecules in MRL/lpr mice-derived MVECs.

Funding

This study was granted by the Xiamen Medical and Health Science and Technology Project (grant No. 3502Z20194041), and Research Foundation of Xiang' an Hospital of Xiamen University (grant No. PM201809170017).

Disclosure

The authors report no conflict of interest.

References

1. Al-Rasheed NM, Al-Rasheed NM, Bassiouni YA, Hasan IH, Al-Amin MA, Al-Ajmi HN, Mohamad RA. Vitamin D attenuates pro-inflammatory TNF-α cytokine expression by inhibiting NF-κB/p65 signaling in hypertrophied rat hearts. J Physiol Biochem 2015; 71: 289-299.
2. Ballok DA. Neuroimmunopathology in a murine model of neuropsychiatric lupus. Brain Res Rev 2007; 54: 67-79.
3. Barichello T, Santos AL, Silvestre C, Generoso JS, Cipriano AL, Petronilho F, Dal-Pizzol F, Comim CM, Quevedo J. Dexamethasone treatment reverses cognitive impairment but increases brain oxidative stress in rats submitted to pneumococcal meningitis. Oxid Med Cell Longev 2011; 2011: 173035.
4. Bazsó A, Szappanos Á, Rásonyi R, Nagy E, Farkas A, Várnai B, Patócs A, Kiss E, Poór G. Polymorphisms of human glucocorticoid receptor gene in systemic lupus erythematosus: a single-centre result. Clin Rheumatol 2019; 38: 1979-1984.
5. Bertsias GK, Boumpas DT. Pathogenesis, diagnosis and management of neuropsychiatric SLE manifestations. Nat Rev Rheumatol 2010; 6: 358-367.
6. Brey RL, Holliday SL, Saklad AR. Neuropsychiatric syndromes in lupus: prevalence using standardized definitions. Neurology 2002; 58: 1214-1220.
7. Cash H, Relle M, Menke J, Brochhausen C, Jones SA, Topley N, Galle PR, Schwarting A. Interleukin 6 (IL-6) deficiency delays lupus nephritis in MRL-Faslpr mice: the IL-6 pathway as a new therapeutic target in treatment of autoimmune kidney disease in systemic lupus erythematosus. J Rheumatol 2010; 37: 60-70.
8. Choi EW, Lee M, Song JW, Kim K, Lee J, Yang J, Lee SH, Kim IY, Choi JH, Seong JK. Fas mutation reduces obesity by increasing IL-4 and Il-10 expression and promoting white adipose tissue browning. Sci Rep 2020; 10: 12001.
9. Choi MY, Fritzler MJ. Autoantibodies in SLE: prediction and the p value matrix. Lupus 2019; 28: 1285-1293.
10. Crampton SP, Morawski PA, Bolland S. Linking susceptibility genes and pathogenesis mechanisms using mouse models of systemic lupus erythematosus. Dis Model Mech 2014; 7: 1033-1046.
11. Gao HX, Campbell SR, Cui MH. Depression is an early disease manifestation in lupus-prone MRL/lpr mice. J Neuroimmunol 2009; 207: 45-56.
12. Gerosa M, Poletti B, Pregnolato F, Castellino G, Lafronza A, Silani V, Riboldi P, Meroni PL, Merrill JT. Antiglutamate receptor antibodies and cognitive impairment in primary antiphospholipid syndrome and systemic lupus erythematosus. Front Immunol 2016; 7: 5.
13. Gulinello M, Putterman C. The MRL/lpr mouse strain as a model for neuropsychiatric systemic lupus erythematosus. J Biomed Biotechnol 2011; 2011: 207504.
14. Habbas S, Santello M, Becker D. Neuroinflammatory TNF-alpha impairs memory via astrocyte signaling. Cell 2015; 163: 1730-1741.
15. Huang MW, Stock AD, Mike EV, Herlitz L, Kolbeck R, Putterman C. Anti-IFNAR treatment does not reverse neuropsychiatric disease in MRL/lpr lupus mice. Lupus 2019; 28: 1510-1523.
16. Jeltsch-David H, Muller S. Neuropsychiatric systemic lupus erythematosus and cognitive dysfunction: the MRL-lpr mouse strain as a model. Autoimmun Rev 2014; 13: 963-973.
17. Kamal A, Khamashta M. The efficacy of novel B cell biologics as the future of SLE treatment: a review. Autoimmun Rev 2014; 13: 1094-1101.
18. Kowal C, Degiorgio LA, Lee JY, Edgar MA, Huerta PT, Volpe BT, Diamond B. Human lupus autoantibodies against NMDA receptors mediate cognitive impairment. Proc Natl Acad Sci U S A 2006; 103: 19854-19859.
19. Lou J, Gasche Y, Zheng L, Critico B, Monso-Hinard C, Juillard P, Morel P, Buurman WA, Grau GE. Differential reactivity of brain microvascular endothelial cells to TNF reflects the genetic susceptibility to cerebral malaria. Eur J Immunol 1998; 28: 3989-4000.
20. Mike EV, Makinde HM, Der E, Stock A, Gulinello M, Gadhvi GT, Winter DR, Cuda CM, Putterman C. Neuropsychiatric systemic lupus erythematosus is dependent on sphingosine-1-phosphate signaling. Front Immunol 2018; 9: 2189.
21. Nielsen DM, Crinic LS. Elevated plus maze behariour, auditory startle response, and shock sensitivity in predisease and in early stage autoimmune disease MRL/lpr mice. Brain Behav Immun 2002; 16: 46-61.
22. Oaks Z, Winans T, Caza T, Fernandez D, Liu Y, Landas SK, Banki K, Perl A. Mitochondrial dysfunction in the liver and antiphospholipid antibody production precede disease onset and respond to rapamycin in lupus-prone mice. Arthritis Rheumatol 2016; 68: 2728-2739.
23. Otani Y, Ichii O, Otsuka-Kanazawa S, Chihara M, Nakamura T, Kon Y. MRL/MpJ-Fas (lpr) mice show abnormalities in ovarian function and morphology with the progression of autoimmune disease. Autoimmunity 2015; 48: 402-411.
24. Rohraff DM, He Y, Farkash EA, Schonfeld M, Tsou PS, Sawalha AH. Inhibition of EZH2 ameliorates lupus-like disease in MRL/lpr mice. Arthritis Rheumatol 2019; 71: 1681-1691.
25. Sato H, Onozuka M, Hagiya A, Hoshino S, Narita I, Uchiumi T. Characterization of anti-P monoclonal antibodies directed against the ribosomal protein-RNA complex antigen and produced using Murphy Roths large autoimmune-prone mice. Clin Exp Immunol 2015; 2015: 236-244.
26. Stock AD, Wen J, Doerner J, Herlitz LC, Gulinello M, Putterman C. Neuropsychiatric systemic lupus erythematosus persists despite attenuation of systemic disease in MRL/lpr mice. J Neuroinflammation 2015; 12: 205.
27. Tay SH, Mak A. Anti-NR2A/B antibodies and other major molecular mechanisms in the pathogenesis of cognitive dysfunction in systemic lupus erythematosus. Int J Mol Sci 2015; 16: 10281-10300.
28. Tsuchiya H, Iwasaki Y, Shoda H, Takahashi Y, Fujio K. Limbic encephalitis in a patient with systemic lupus erythematosus successfully treated with high-dose glucocorticoids and intravenous cyclophosphamide therapy: the potential pathogenicity of anti-glutamate receptor antibodies. Mod Rheumatol Case Rep 2021; 5: 250-253.
29. van Delft MA, Huitema LF, Tas SW. The contribution of NF-kB signalling to immune regulation and tolerance. Eur J Invest 2015; 45: 529-539.
30. Wang W, Cao L, Wang X, Fan Y. Radix paeoniae rubra ameliorates lupus nephritis in lupus-like symptoms of Mrl mice by reducing intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and platelet endothelial cell adhesion molecule-1 expression. Comb Chem High Throughput Screen 2020; 23: 675-683.
31. Wang W. Functional studies of adhesion molecules on CD4-CD8-double negative T cells of autoimmune MRL/Mp-lpr/mice. Hokkaido IgakuZasshi 1993; 68: 755-766.
32. Wang X, Hisha H, Cui W, Song C, Mizokami T, Okazaki S, Li Q, Feng W, Kato J, Jiang S, Fan H, Ikehara S. The characteristics

of hematopoietic stem cells from autoimmune-prone mice and the role of neural cell adhesion molecules in abnormal proliferation of these cells in MRL/lpr mice. Haematologica 2007; 92: 300-307.
33. Wang Y, Tang J, Shen L, Li J, Zha C, Wang R, Hu K, Xi J, Chang J, Xie C. Effects of dexamethasone on MRL/lpr mice with systemic lupus erythematosus complicated with cognitive dysfunction. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2017; 42: 251-256.
34. Weisfelt M, Hoogman M, van de Beek D, de Gans J, Dreschler WA, Schmand BA. Dexamethasone and long-term outcome in adults with bacterial meningitis. Ann Neurol 2006; 60: 456-468.
35. Wen J, Xia Y, Stock A, Michaelson JS, Burkly LC, Gulinello M, Putterman C. Neuropsychiatric disease in murine lupus is dependent on the TWEAK/Fn14 pathway. J Autoimmun 2013; 43: 44-54.
36. Xu B, He X, Sui Y, Wang X, Wang X, Ren L, Zhai YX. Ginkgetin aglycone attenuates neuroinflammation and neuronal injury in the rats with ischemic stroke by modulating STAT3/JAK2/SIRT1. Folia Neuropathol 2019; 57: 16-23.
37. Yan L, Wu P, Gao DM, Hu J, Wang Q, Chen NF, Tong SQ, Rao L, Liu J. The impact of vitamin D on cognitive dysfunction in mice with systemic lupus erythematosus. Med Sci Monit 2019; 25: 4716-4722.
38. Yang Y, Yuan C, Shen SQ, Wang XE, Mei QH, Jiang WQ, Huang Q. Autoantibodies to NR2A peptide of the glutamate/NMDA receptor in patients with seizure disorders in neuropsychiatric systemic lupus erythematosus. Mediators Inflamm 2017; 2017: 5047898.
39. Yokoyama T, Fujii T, Kondo-Ishikawa S, Yamakawa N, Nakano M, Yukawa N, Yoshifuji H, Ohmura K, Mimori T. Association between anti-U1 ribonucleoprotein antibodies and inflammatory mediators in cerebrospinal fluid of patients with neuropsychiatric systemic lupus erythematosus. Lupus 2014; 23: 635-642.
40. Yoshio T, Okamoto H, Kurasawa K. IL-6, IL-8, IP-10, MCP-1 and G-CSF are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus. Lupus 2016; 25: 997-1003.
41. Zhang Q, Liang Y, Yuan H, Li S, Wang JB, Li XM, Tao JH, Pan HF, Ye DQ. Integrated analysis of lncRNA, miRNA and mRNA expression profiling in patients with systemic lupus erythematosus. Arch Med Sci 2019; 15: 872-879.