

Original Research

Danggui-Shaoyao-San Promotes Amyloid- β Clearance through Regulating Microglia Polarization via Trem2 in BV2 Cells

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Abstract

Background: Alzheimer's disease (AD) is a chronic neurodegenerative brain disorder currently without satisfactory therapeutic treatments. Triggering receptors expressed on a myeloid cells-2 (*Trem2*) gene mutation has been reported as a powerful AD risk factor that induces *Trem2* gene deletion aggravated microglia disfunction and Amyloid- β ($A\beta$) aggregation in the brain. The traditional Chinese medicine (TCM) formula Danggui-Shaoyao-San (DSS) has shown therapeutic effect on alleviating the symptoms of AD. However, the neuroprotective effect and underlying mechanism of DSS against AD is still far from fully understood. **Methods:** Double-label immunofluorescence and Western blotting were employed to evaluate the different polarization states of mouse BV2 microglial (BV2) cells after lipopolysaccharide (LPS) or interleukin (IL)-4 treatment. *Trem2* over-expression lentiviral vector and *Trem2* siRNA were used respectively to evaluate the effect of *Trem2* on microglia polarization via detecting the proteins expression of *iNOS* and arginase1 (*Arg1*) by Western blotting while the $A\beta$ -scavenging capacity of BV2 cells was assessed by flow cytometry. Cell counting kit-8 (CCK8) assay was performed to assess the effect of DSS on the viability of BV2 cells. Flow cytometry was used to investigate the effect of DSS on the $A\beta$ -scavenging capacity of BV2 cells treated with corresponding concentration of DSS-containing serum. Protein of *Trem2* and the gene expression of the M1 or M2 phenotype in BV2 cells treated with DSS after *Trem2* over-expression or silence were detected by Western blot and RT-qPCR, respectively. **Results:** *In vitro* experiments. DSS exhibited anti-inflammatory and neuroprotective functions. It was found that *Trem2* had an effect on inducing a shift of M1 microglia towards the M2 phenotype and enhanced the $A\beta$ -scavenging capacity of BV2 cells, further that DSS administration relieved inflammation by engulfing $A\beta$ through the activities of *Trem2*. Importantly, DSS treatment effectively increased the $A\beta$ -scavenging capacity of BV2 cells through accelerating the shift of M1 microglia towards an M2 phenotype via increasing *Trem2* expression. **Conclusions:** Results demonstrated that DSS promoted the clearance of $A\beta$ through the regulation of microglia polarization via increased expression of *Trem2* in BV2 cells.

Keywords: Danggui-Shaoyao-San; triggering receptor expressed on myeloid cells-2; microglia polarization; Amyloid- β deposition; Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative brain disorder currently without satisfactory therapeutic alternatives [1,2]. With an increasingly aged population, the incidence of AD is increasing annually and the number of AD patients is predicted to reach 130 million worldwide by 2050 [3,4]. Amyloid- β ($A\beta$) protein is a fragment comprising between 39 and 43 residues of amyloid precursor protein found in the brains of AD patients [5]. The excessive aggregation of $A\beta$ is hypothesized to play a dominant role in triggering the pathological processes of AD [6]. $A\beta$ deposition causes oxidative stress, inflammation, and neuronal apoptosis in areas of the brain related to learning and memory, eventually leading to AD [7,8]. Therefore, $A\beta$ has been widely viewed as a principal therapeutic target of AD.

Microglia are resident immune cells of the central nervous system (CNS) and serve the role of immune surveillance [9]. In pathological situations, microglia are activated by toxic $A\beta$ protein as well as cytotoxic substances derived from degenerative neurons [10]. Activated microglia have typical M1 and alternative M2 phenotypes. M1 microglia produce destructive pro-inflammatory mediators such as tumor necrosis factor α (*TNF- α*) and interleukin 1 β (*IL-1 β*), which contribute to amplifying a neuroinflammatory response and $A\beta$ aggregation [11]. Alternatively, M2 microglia secrete a spectrum of anti-inflammatory factors such as interleukin 10 (*IL-10*) and interleukin 4 (*IL-4*) and promote microglia to phagocytose $A\beta$, which eventually prevents the development of AD [12]. It has been reported that the switching from the M1 to the M2 microglia state



contributes to the termination or reverse of AD progression [13,14]. Consequently, interventions targeting the promotion of M2 polarization of microglia may be a valuable treatment for AD.

A triggering receptor expressed on myeloid cells-2 (*Trem2*) is a cell surface receptor protein of the immunoglobulin receptor superfamily that is predominantly expressed on brain microglia [15]. Recent studies have revealed that *Trem2* gene mutation is a powerful AD risk factor that slows down the clearance of cell debris and toxic A β protein [16]. In an AD mouse model, *Trem2* gene deletion aggravated microglia dysfunction and A β aggregation in the brain [17]. In contrast, *Trem2* over-expression increased M2 microglia polarization markers *Arg1*, *IL-10*, and *IL-4* expression levels, while decreased the expression of M1 microglia biomarkers inducible nitric oxide synthase (*iNOS*) and *TNF- α* [18]. These data strongly suggest that up-regulating *Trem2* expression might enhance the A β -scavenging capacity in AD via regulating the M1/M2 phenotypic polarization of microglia.

Danggui-Shaoyao-San (DSS) is a traditional Chinese medicine formula, first recorded in Synopsis of Prescriptions of Golden Chamber written by Zhang Zhong-Jing during the Eastern Han Dynasty. DSS was originally employed to treat gynecological diseases and was then found to have effects on forgetfulness. More recently, medical records and clinical studies have suggested that DSS may significantly improve the clinical symptoms of patients with dementia [19–21]. Experimental animal studies have also demonstrated that DSS alleviates cognitive impairment in AD models [22–24]. Furthermore, DSS may also increase the expression of the M2 microglia polarization marker *IL-4* and reduce the level of the M1 microglia biomarker *TNF- α* [25]. Based those findings, the current authors have hypothesized that DSS might attenuate the progression of AD through modulating microglia polarization via *Trem2*. Therefore, in this study, the effects and underlying mechanism of DSS against AD were investigated.

2. Materials and Methods

2.1 Materials

Six herbs of Danggui-Shaoyao San, *Angelica sinensis* (Oliv.) Diels (Umbelliferae), *Paeonia lactiflora* Pall. (Paeoniaceae), *Poria cocos* (Schw.) Wolf (Polyporaceae), *Atractylodes macrocephala* Koidz. (Compositae), *Ligusticum chuanxiong* Hort. (Umbelliferae) and *Alisma orientalis* (Sam.) Juzep. (Alismataceae) were purchased from the Pharmacy Room of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (Guangzhou, China). LPS (lipopolysaccharide) (Cat No. L2630, sigma, St. Louis, MO, USA), murine *IL-4* (Cat No. 96-214-14-20, PeproTech, Suzhou, China), *Arg1* (Cat No. 16001-1-AP, Proteintech Group, Wuhan, China), and antibodies against OX42 (Cat No. ab1211), *Trem2* (Cat No. ab86491), *iNOS* (Cat No. ab15323), *Arg1* and *GAPDH* (Cat No. ab8245)

were purchased from Abcam (Cambridge, UK).

2.2 DSS Preparation

The extraction of DSS was performed in the Laboratory of Science and Technology Innovation Center, Guangzhou University of Chinese Medicine. *Angelica sinensis* (Oliv.), *Paeonia lactiflora* Pall, *Poria cocos* (Schw.) Wolf, *Atractylodes macrocephala* Koidz., *Ligusticum chuanxiong* Hort. and *Alisma orientalis* (Sam.) Juzep. were mixed with a ratio of 3:16:4:4:8:8. The herbs were soaked in distilled water (1:8, w/v) for 1 h and extracted for 1 h. After the filtrate was collected, distilled water was added (1:6, w/v) for a second extraction. The two filtrates were then mixed and concentrated to 1.28 g/mL DSS extract at 60 °C.

Analysis of the main active components of the DSS water extract was performed using HPLC-MS/MS in a previous study [26]. It was found that the extract included 78 main constituents. The concentration of albiflorin, paeoniflorin, benzoic acid, gallic acid, ferulic acid, *Chuanxiong* lactone I, *Chuanxiong* lactone A were 1.76, 12.15, 0.68, 1.17, 0.29, 0.46 and 0.19 mg per gram extractum, and the highest content was paeoniflorin.

2.3 Serum Containing Preparation

A total of 20 male C57BL/6 mice (seven months old) were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China) and randomly divided into four groups (five mice/group), (1) normal group (0.9% saline, 10 mL/kg/day, i.g.), (2) high-dose DSS group (6.4 g/kg/day, i.g.), (3) mid-dose DSS group (4.8 g/kg/day, i.g.), (4) low-dose DSS group (3.2 g/kg/day, i.g.). The drug intervention period was continued for seven days. Serum samples were collected from the eyeball blood of mice 1 h after the last treatment administration. The serum samples were heat inactivated at 56 °C for 30 min, then divided and stored at –20 °C until use. The experiment was carried out in accordance with guidelines established by the National Institutes of Health of the United States for the care and use of laboratory animals and was approved by the Laboratory Animal Ethics Committee of the Guangzhou University of Chinese Medicine (Guangzhou, China).

2.4 Cell Culture

BV2 microglia cells were obtained from the Science and Technology Innovation Center of Guangzhou University of Chinese Medicine. The cell line has been authenticated based on morphology and functional expression, and mycoplasma testing has been done. Cells were cultured at 37 °C in an atmosphere of 5% CO₂ in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were seeded onto a 96-well plate at a density of 5 × 10³ cells per well. For cell subculture, BV2 cells were detached after reaching 90% confluency by using 0.25% Trypsin-EDTA. The BV2 cells underwent various analyses

Table 1. Primer sequences designed for RT-qPCR.

Gene	Forward sequence	Reverse sequence	Size
<i>IL-1β</i>	TCAGGCAGGCAGTATCACTC	AGTCATATGGGTCCGACAG	250 bp
<i>TNF-α</i>	AGCACAGAAAGCATGATCCG	CTGATGAGAGGGAGGCCATT	212 bp
<i>IL-6</i>	CACAGAGGATACCCTCCCAACAGA	ACAATCAGAATTGCCATTGCACAAC	124 bp
<i>Arg1</i>	ATCGTGTACATTGGCTTGCG	CGTCGACATCAAAGCTCAGG	184 bp
<i>IL-10</i>	GCTGGACAACATACTGCTAACCG	CACAGGGGAGAAATCGATGACAG	218 bp
<i>iNOS</i>	TTGGCTCCAGCATGTACCCT	TCCTGCCCACTGAGTTTCGTC	121 bp
<i>GAPDH</i>	ATGGGTGTGAACCACGAGA	CAGGGATGATGTTCTGGGCA	229 bp

after three generations of passaging.

2.5 Double-Label Immunofluorescence Assay

Antigen repair was performed with 0.01 M citrate buffer at 90 °C for 30 min. Subsequently, cells were saturated and permeabilized by 0.1% Triton X-100 (Solarbio, Beijing, China) and blocked in goat serum at room temperature. Samples were then incubated overnight at 4 °C with primary antibodies of anti-OX42, anti-*iNOS*, and anti-*Arg1*. They were then incubated with goat anti-mouse or goat anti-rabbit IgG at 37 °C for twenty minutes. Finally, the samples were stained with Alexa Fluor 488 anti-rabbit IgG or Alexa Fluor 594 anti-rabbit IgG at room temperature away from light for 1 h. Confocal microscopy (Serial No. 538623, Nikon 80i, Tokyo, Japan) was utilized to capture images. Positive cells were identified from three different perspectives in each sample.

2.6 Cell Viability Assay

The effect of DSS on the viability of BV2 cells was assessed by cell counting kit-8 (CCK8). The cells were plated into 96-well plates at a density of 2×10^5 cells per well and cultured in medium overnight. Cells were then treated with 10% by volume with different doses of DSS drug-containing serum (0, 3.2, 4.8, and 6.4 g/kg/d) for 12, 24 or 48 h. Subsequently, 20 μ L CCK8 solution ((Lot No. A311-01, Vazyme Biotech Co., Ltd., Nanjing, China) was added into the medium and it was incubated for 45 min. Absorbance was measured at 450 nm using a microplate reader (Serial No. 255927, BioTek, Winooski, VT, USA).

2.7 Flow Cytometry

The capacity of BV2 cells to phagocytose A β was assessed using flow cytometry. BV2 cells were treated with different concentrations of drug for 24 h. Then, the cells were incubated with 500 nM fluorescently labelled FAM-A β for 2 h. After washing three times with cold PBS, samples were assessed by flow cytometry software (FlowJo, v10.6.2, Tree Star, Inc., Ashland, OR, USA).

2.8 Transfection of *Trem2* Over-Expression Lentivirus and *Trem2* siRNA

BV2 cells were plated in six-well plates with fresh medium. *Trem2* over-expression lentivirus, *Trem2* siRNA, and non-targeting siRNA (control vector) transfection

were performed respectively according to the manufacturer's instructions by Lipofectamine 2000 reagent (Lot No. 2744064, Invitrogen, Carlsbad, CA, USA). BV2 cells were cultured in serum-free medium for 8 h then treated with different concentrations of DSS drug-containing serum (0, 3.2, 4.8, and 6.4 g/Kg/d) for 24 h.

2.9 Western Blot Analysis

The protein of BV2 cells was obtained by lysis with RIPA buffer. Bicinchoninic acid (BCA) assay was employed to quantify the protein concentration. Samples were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and blocked by 5% BSA for 1.5 h at room temperature. The membranes were then incubated overnight at 4 °C with the *Trem2*, *Arg1*, and *iNOS* antibodies specific for target proteins, and then incubated with the secondary antibodies for 1 h at 37 °C. Detection was performed using the Gel Imaging System (Serial No. 721BR19506, Bio-Rad, Hercules, CA, USA) and quantified by Image Lab 5.2.1 software (Bio-Rad, Hercules, CA, USA).

2.10 Real-Time Fluorescence Quantitative Polymerase Chain Reaction (RT-qPCR)

TRIzol reagent (Lot No. 93289, Sigma, St. Louis, MO, USA) was applied to extract total RNA from BV2 cells with different treatments in each group according to the instruction manual. The ratio of OD260 to OD280 of total RNA was measured by microspectrophotometer and the purity and concentration of RNA were calculated. Samples with a ratio between 1.8–2.0 met experimental requirements. cDNA templates were prepared by performing reverse transcription reactions according to the reverse transcription protocol of PrimeScript™ RT Master Mix (Lot No. RR036B, Takara, Beijing, China). Following $\times 8$ dilution, samples were amplified by RT-qPCR (Serial No. 275007042, ABI, CA, USA). Each sample was repeated three times and the average value employed to calculate the relative content of the product. *GAPDH* was used as the housekeeping gene and the primer list is given in Table 1. The relative mRNA concentrations were measured by $E = 2^{-\Delta\Delta C_t}$ and the key threshold cycle (CT) value of each reaction was examined.

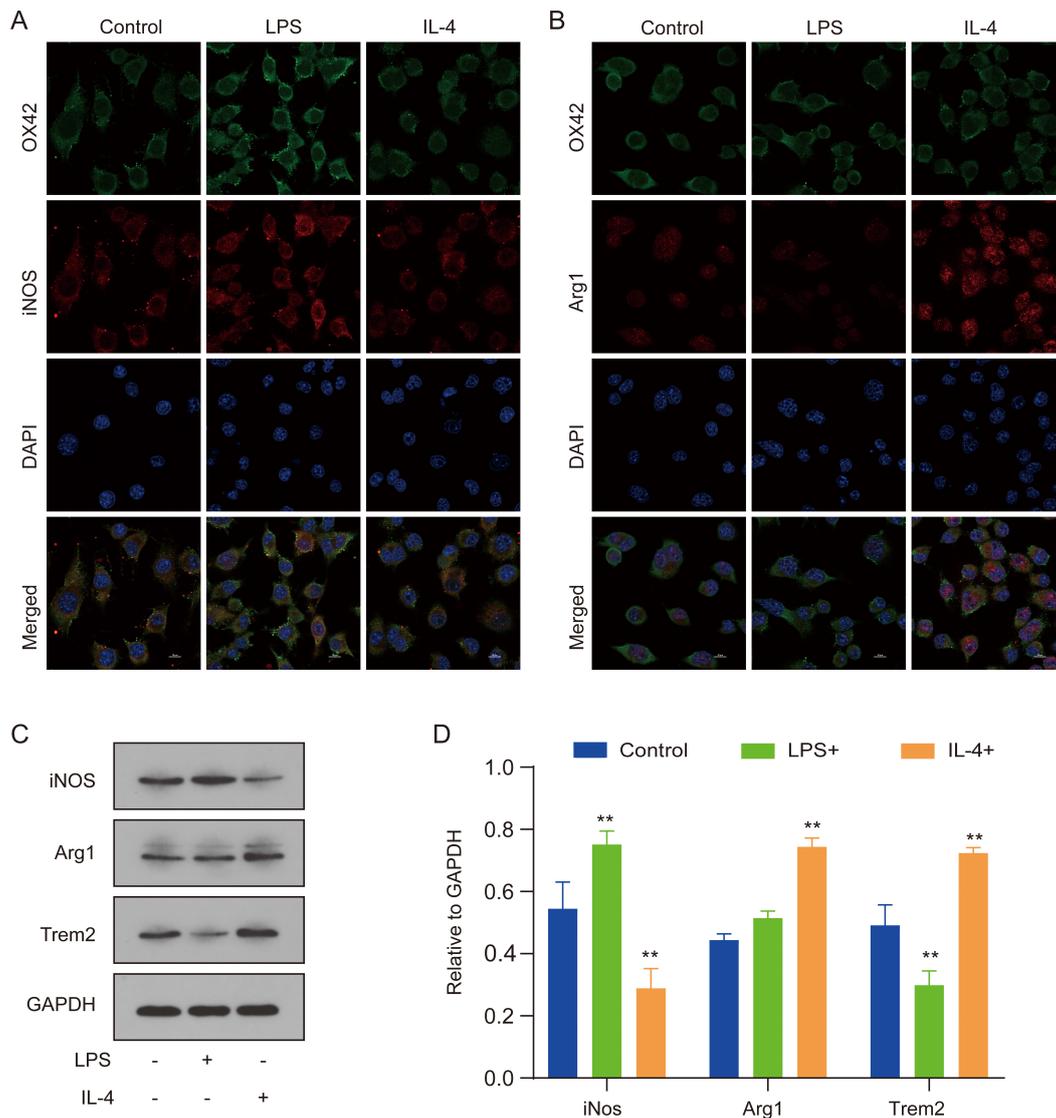


Fig. 1. *Trem2* expression in different polarization states of BV2 cells. (A,B) Cell morphology changes and immunofluorescence of *iNOS* (red)/*Arg1* (red) and double immunofluorescence labeling with OX-42 (green). (C,D) Western blot analysis of *iNOS*, *Arg1*, and *Trem2* protein expression in control, LPS (100 ng/mL), and the *IL-4* (20 ng/mL) group. *GAPDH* served as the loading control. Scale bar: 10 μ m, ** p < 0.01 compared with the control group.

2.11 Statistical Analysis

All data were analyzed by the software package Statistic Package for Social Science (SPSS, v.26.0, Armonk, NY, USA) and data were expressed as mean \pm standard error. Comparison between two groups was analyzed by *t*-tests, while multiple group comparisons were performed by one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. A *p*-value < 0.05 was considered to indicate a significant difference.

3. Results

3.1 *Trem2* Expression in Different Polarization States of BV2 Cells

BV2 cells were treated either with LPS at a concentration of 100 ng/mL or *IL-4* at a concentration of 20 ng/mL in

DMEM for 24 h to induce either the M1 or M2 phenotype. As shown in Fig. 1A, the results of immunofluorescence double-labeling revealed that activated BV2 microglia cells with large and round cellular bodies, disappeared cellular projections and amoeboid shape were observed after either LPS or *IL-4* treatment. LPS up-regulated the M1 microglia biomarker *iNOS*, which indicated an alteration of microglia polarization toward M1 status. While *IL-4* up-regulated the M2 microglia polarization marker *Arg1*, which promoted microglia polarization toward a M2 phenotype (Fig. 1B). Moreover, LPS enhanced the protein expression of *iNOS* and decreased the expression of *Trem2* in the M1 polarization state. In contrast, *IL-4* remarkably increased the protein expression of *Arg1* and *Trem2* in the M2 polarization state (Fig. 1C,D). Taken together, *Trem2* was highly expressed in

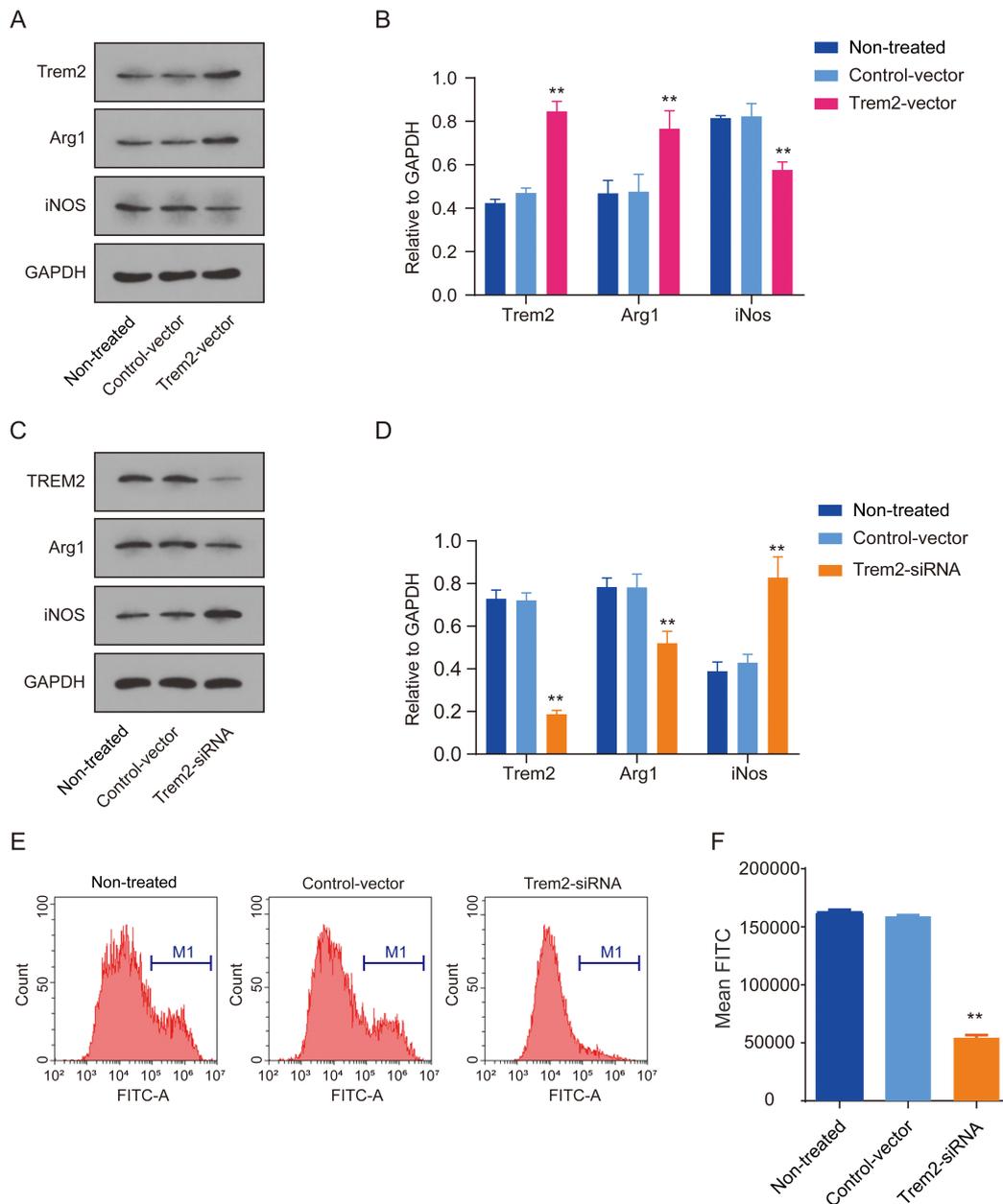


Fig. 2. Effect of *Trem2* on microglia polarization and A β -scavenging capacity of BV2 cells. (A,B) Western blot analysis of *Trem2*, *Arg1*, and iNOS in *Trem2* over-expression. *GAPDH* served as loading control. (C,D) Western blot analysis of *Trem2*, *Arg1*, and iNOS in *Trem2*-siRNA. (E) Flow cytometry was used to determine the phagocytosis capacity of A β by BV2 microglia. The M1 range representative FITC positive event. (F) Comparison of mean FITC fluorescence intensity among the different groups. ** $p < 0.01$, compared with the control-vector group.

the M2 polarization phenotype and poorly expressed in the M1 polarization phenotype.

3.2 Effect of *Trem2* on Microglia Polarization and A β -Scavenging Capacity of BV2 Cells

To further confirm the effect of *Trem2* on BV2 microglia activation, the *Trem2* over-expression lentiviral vector and *Trem2* siRNA were used to transfect BV2 cells. The expression of microglia activation marker proteins was then evaluated by Western blot. Results showed that *Trem2*

over-expression significantly reduced the protein expression level of iNOS, whereas increased expression of *Arg1* in BV2 cells (Fig. 2A,B). In contrast, *Trem2* silencing obviously enhanced iNOS expression and inhibited *Arg1* production. Results further revealed that *Trem2* induced a shift of M1 microglia towards the M2 phenotype (Fig. 2C,D). The effect of *Trem2* on the A β scavenging capacity of BV2 cells was then assessed by flow cytometry. This showed that *Trem2* silencing remarkably suppressed A β clearance in BV2 cells, which further confirmed that *Trem2* enhanced

the A β -scavenging capacity of BV2 cells (Fig. 2E,F).

3.3 Effect of DSS on BV2 Cell Viability

To investigate the effect of DSS on BV2 cell viability, BV2 cells were cultured with different concentrations of DSS-containing serum (3.2 g/kg/d, 4.8 g/kg/d, and 6.4 g/kg/d) for different durations (12 h, 24 h and 48 h). As shown in Fig. 3A, compared with the normal group, cell viability significantly increased in the low, medium, and high dose DSS-containing serum groups. These findings showed that DSS-containing serum increased BV2 cell viability in a dose- and time-dependent manner.

3.4 Effect of DSS on *Trem2* Expression and Microglia Polarization in BV2 Cells

To verify the effect of DSS on *Trem2* expression in BV2 cells, *Trem2* protein expression levels were examined in BV2 cells treated with different concentrations of DSS-containing serum for 12 h, 24 h, and 48 h. As shown in Fig. 3B,C, the results of Western blot showed that DSS enhanced *Trem2* expression in BV2 cells and these effects were dose and time dependent. Next, at the 48 h time point, the effect of DSS on the M2-associated mRNA expression levels of *Arg1* and *IL-10* were determined and it was found that DSS-containing serum dramatically increased the expression of *Arg1* and *IL-10* mRNA (Fig. 3D,E). Alternatively, it was observed that DSS-containing serum significantly reduced the M1-associated mRNA levels of *iNOS* and *TNF- α* (Fig. 4D,E). These findings showed that DSS inhibited the alteration of microglia polarization toward the M1 phenotype and induced microglia into the M2 anti-inflammatory phenotype via increasing the expression of *Trem2*.

3.5 Effects of DSS on A β -Scavenging Capacity of BV2 Cells

The effect of DSS on the A β -scavenging capacity of BV2 cells treated with a corresponding concentration of DSS-containing serum for 48 h was analyzed by flow cytometry. Results showed that DSS increased the A β -scavenging capacity of BV2 cells in a dose-dependent manner (Fig. 4A,B). Furthermore, it was found that DSS remarkably decreased mRNA expression of *IL-1 β* and *IL-6* (Fig. 4E,F). This indicated that DSS administration significantly decreased the amount of A β deposits and mRNA expression of A β -mediated inflammatory factors in BV2 cells.

3.6 *Trem2* Silencing Inhibited DSS-Mediated Microglia Polarization toward M2 Phenotype and A β -Scavenging Capacity of BV2 Cells

Different concentrations of DSS-containing serum were used to treat BV2 cells transfected with siRNA-*Trem2* and it was found that DSS increases the A β -scavenging capacity when the BV2 cells were transfected with siRNA-*Trem2* (Fig. 5A,B). Importantly, it was found that DSS up-

regulated the expression levels of *Trem2* (Fig. 6A,B). Moreover, it was observed that high doses of DSS-containing serum significantly increases *Arg1* and *IL-10* levels in BV2 cells transfected with siRNA-*Trem2* (Fig. 6C,D). These results strongly suggested that *Trem2* silencing inhibited DSS-mediated microglia polarization toward a M2 phenotype and A β -scavenging capacity of BV2 cells.

4. Discussion

Microglia are the main endogenous immune cells of the CNS. Microglia not only exert a classic role as “scavengers” for the maintenance and restoration of the CNS, but also play a key role in neuron functioning such as cell migration, apoptosis, survival, and synaptic plasticity. The effects of microglia in various neurodegenerative diseases including AD have recently drawn increasing attention [27,28]. *Trem2* is a transmembrane protein selectively expressed on microglia in the brain, which enables microglial responses including proliferation, survival, clustering, and phagocytosis through sustaining cellular energetic and biosynthetic metabolism during AD [28–30]. An analysis of genetic variability revealed that genetic variants of *Trem2* were associated with a threefold increase in the risk of developing AD and that the complete loss of *Trem2* resulted in an early-onset dementia [31,32]. Furthermore, recent studies in AD mouse models have confirmed that the loss of *Trem2* function caused classic AD pathology and suggested an important role of *Trem2* in microglia function [33–35]. In the current study, it was found that *Trem2* had positive effects on improving a shift of M1 microglia towards an M2 phenotype. Moreover, it was shown that *Trem2* enhanced the A β -scavenging capacity of BV2 microglia cells. Such findings indicate that *Trem2* displays a positive effect on anti-inflammatory M2 polarization of microglia and A β clearance (Fig. 7).

DSS is a classical Chinese complex prescription for the treatment of AD. Recent studies have drawn attention to the fact that DSS exhibits anti-inflammatory and antioxidant activities and prevented cell apoptosis in the hippocampus [36–38]. Furthermore, DSS administration was shown to ameliorate a scopolamine-induced decrease in the levels of acetylcholine in AD model mice [39]. Importantly, DSS treatment has been reported to inhibit neuroinflammation in LPS-induced BV-2 microglia cells [40]. However, the specific mechanisms of action of DSS on microglia in AD have not yet been completely elucidated. In this study, it was observed that DSS elevated the viability of BV2 cells and *Trem2* protein expression in a dose and time dependent manner. Furthermore, DSS remarkably enhanced the expression of M2 microglia phenotype markers *Arg1* and *IL-10* mRNA, while reducing the expression levels of M1 microglia phenotype markers *iNOS* and *TNF- α* mRNA. These results indicate that DSS treatment accelerates a shift of M1 microglia towards a M2 phenotype via increasing *Trem2* expression *in vitro*.

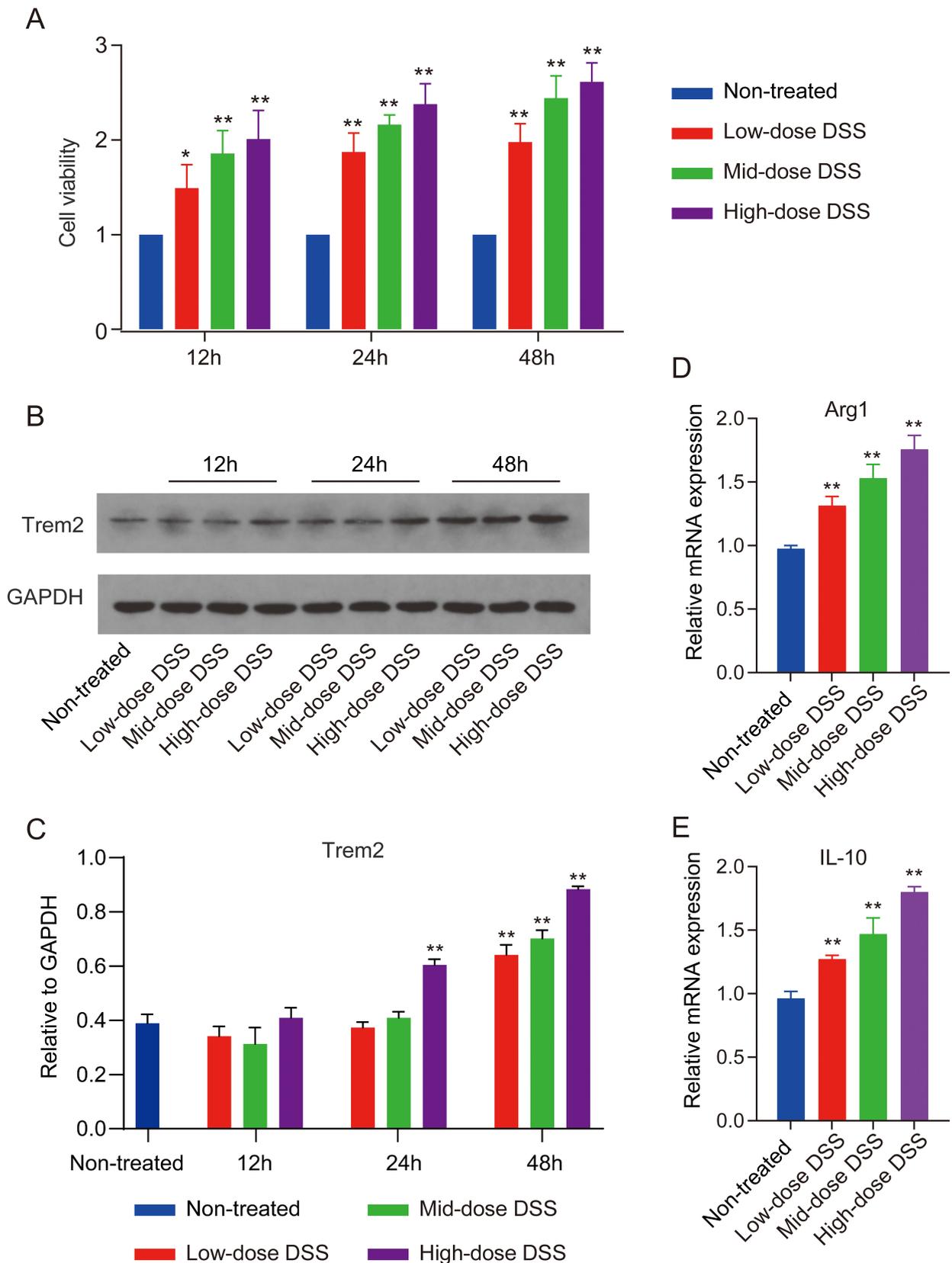


Fig. 3. DSS enhanced cell viability in a dose-dependent manner and increased *Trem2* expression, which promoted the conversion of microglial cells to the M2 state. (A) Cell viability was detected by CCK8 Assay. (B,C) Western blot analysis of *Trem2* in control and DSS treated groups. *GAPDH* served as control. (D,E) mRNA level of the M2 biomarker *Arg1* and *IL-10* treated in four different groups for 48 h using RT-qPCR technology. * $p < 0.05$, ** $p < 0.01$, compared with the normal group.

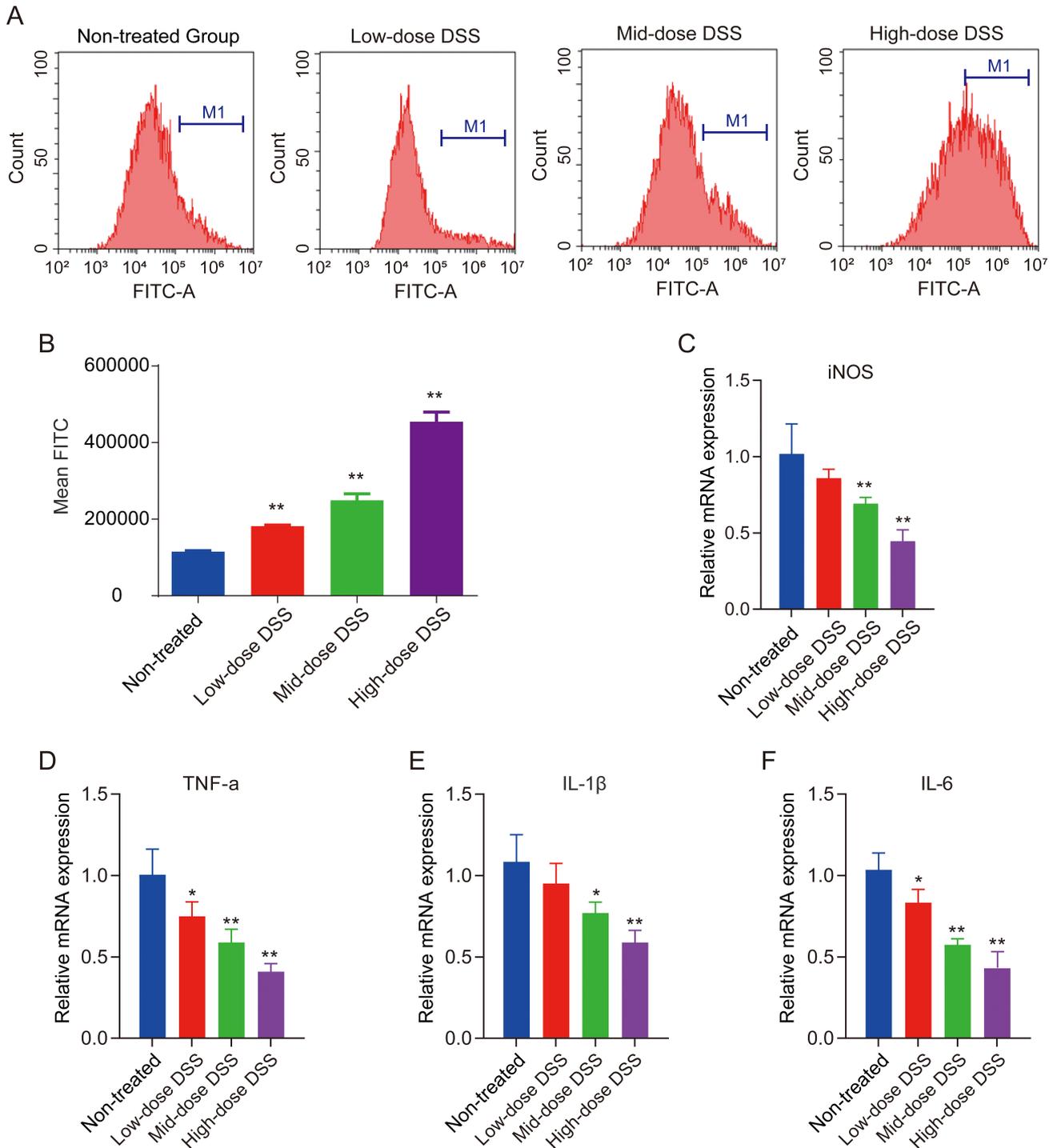


Fig. 4. DSS enhances the phagocytosis capacity of microglial cells in a dose-dependent manner. (A) Fluorescence intensity of cells was measured by flow cytometry to determine the phagocytosis capacity of A β by BV2 microglia treated in normal group and low/middle/high DSS concentration groups. The M1 range representative FITC positive event. (B) Comparison of mean FITC fluorescence intensity among the above four groups. (C–F) mRNA level of *iNOS* and pro-inflammatory cytokines *IL-1 β* , *TNF- α* , and *IL-6* using RT-qPCR technology in different groups. * $p < 0.05$, ** $p < 0.01$, compared with the normal group.

AD is the most prevalent neurodegenerative disorder leading to severe cognitive and functional deterioration in an aged population [41]. The presence of A β peptide depositing in the hippocampal and cortical regions is the major hallmark of AD pathology [42,43]. A β deposition forms

cytotoxic soluble oligomers and amyloid fibrils, which are thought to cause oxidative damage, inflammation, neural dysfunction, and eventually lead to AD [44,45]. M2 microglia activation is essential for boosting the clearance of excess toxic A β [46,47]. The beneficial properties of DSS

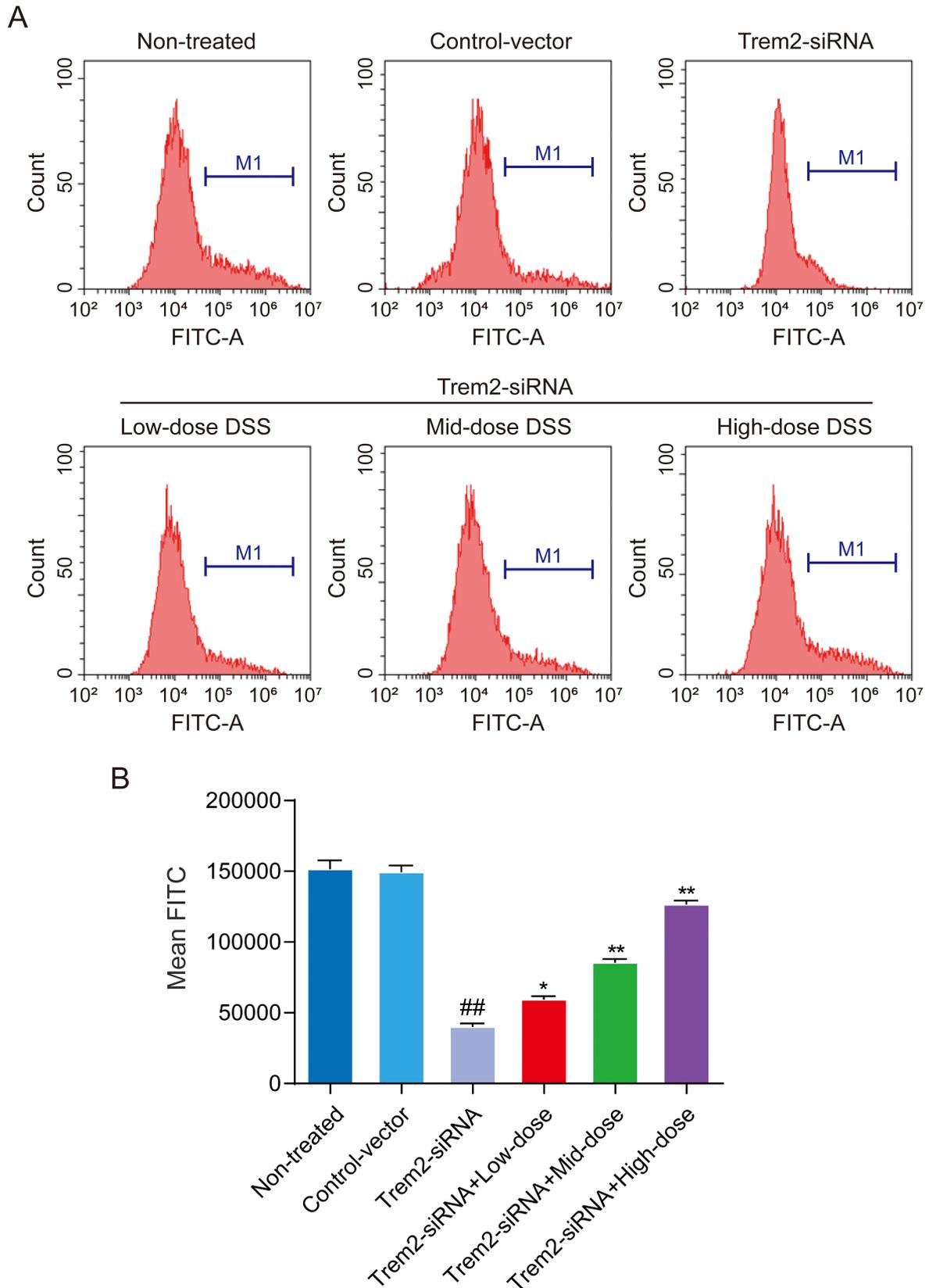


Fig. 5. DSS did not recover $A\beta$ -scavenging capacity in BV2 cells after silencing gene *Trem2*. (A) Different concentrations of DSS serum were used in normal BV2 cells and BV2 cells to silence *Trem2* by siRNA and flow cytometry was used to detect the phagocytic function on $A\beta$. The M1 range representative of a FITC positive event. (B) Comparison of mean FITC fluorescence intensity among the above six groups. ## $p < 0.01$, compared with control-vector group, * $p < 0.05$, ** $p < 0.01$, compared with the *Trem2*-siRNA group.

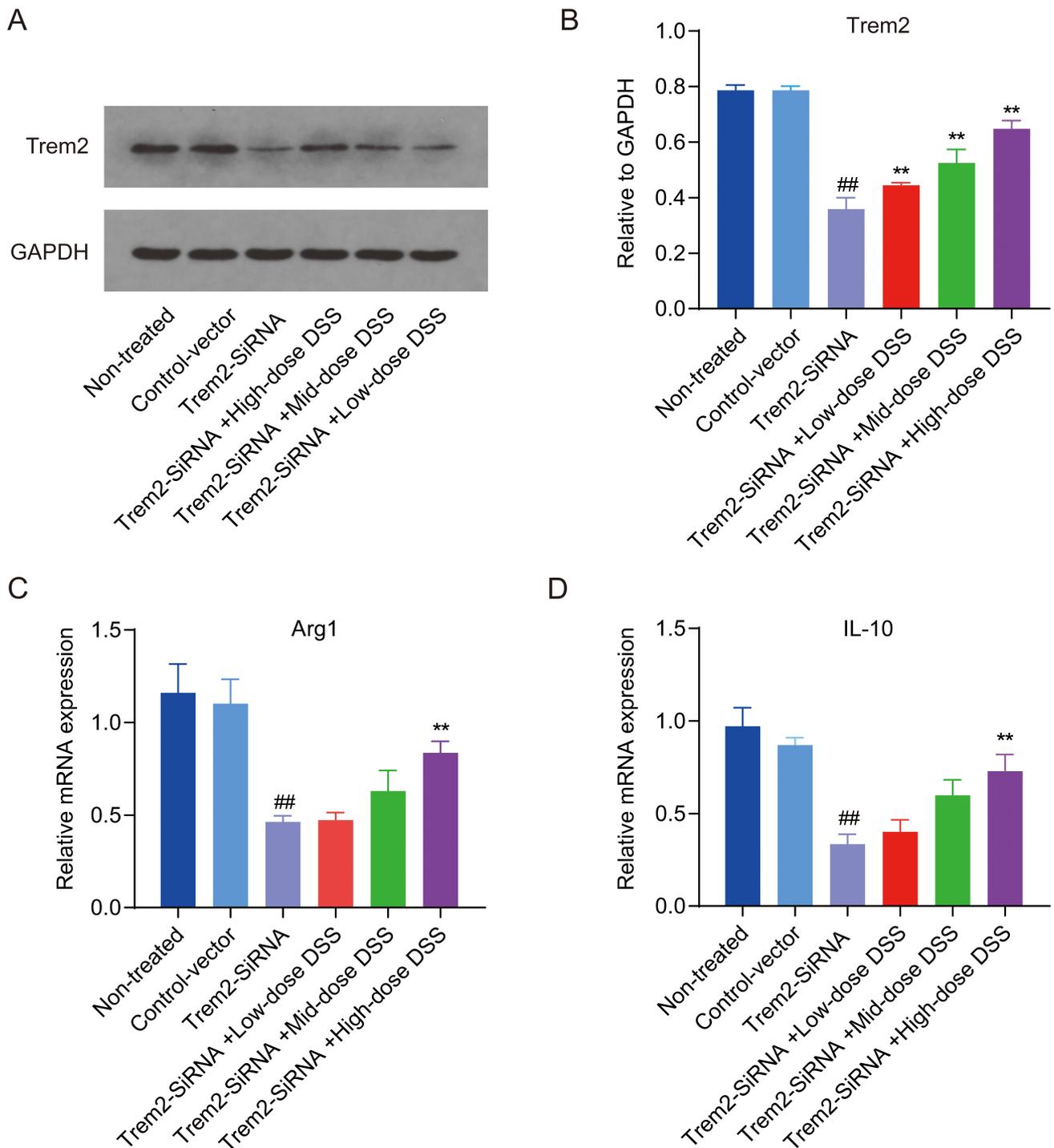


Fig. 6. *Trem2* silencing inhibited DSS-mediated microglia polarization toward M2 phenotype and $A\beta$ -scavenging capacity of BV2 cells. (A,B) Western blot analysis of *Trem2* in BV2 cells treated with different concentrations of DSS-containing serum after transfection with siRNA-*Trem2*. (C,D) mRNA level of the M2 phenotype biomarker *Arg1* and *IL-10* of the different groups using RT-qPCR. ## $p < 0.01$, compared with control-vector group, ** $p < 0.01$, compared with the *Trem2*-siRNA group.

on *Trem2* expression and microglia activation impel further investigation of the effect of DSS on $A\beta$ clearance and inflammation in BV2 cells. The results reported here reveal that DSS treatment significantly improved the clearance of $A\beta$ in BV2 cells and suppressed the expression of inflammation-related mRNA of *IL-1 β* and *TNF- α* , which,

at least in part, accounts for the cytoprotective effect on AD. To further explore whether DSS administration improved the shift of M1 microglia towards a M2 phenotype and assist in $A\beta$ clearance through acting on *Trem2*, BV2 cells were transfected with siRNA-*Trem2*. The result showed that the effect of DSS on microglia activation and $A\beta$ clearance

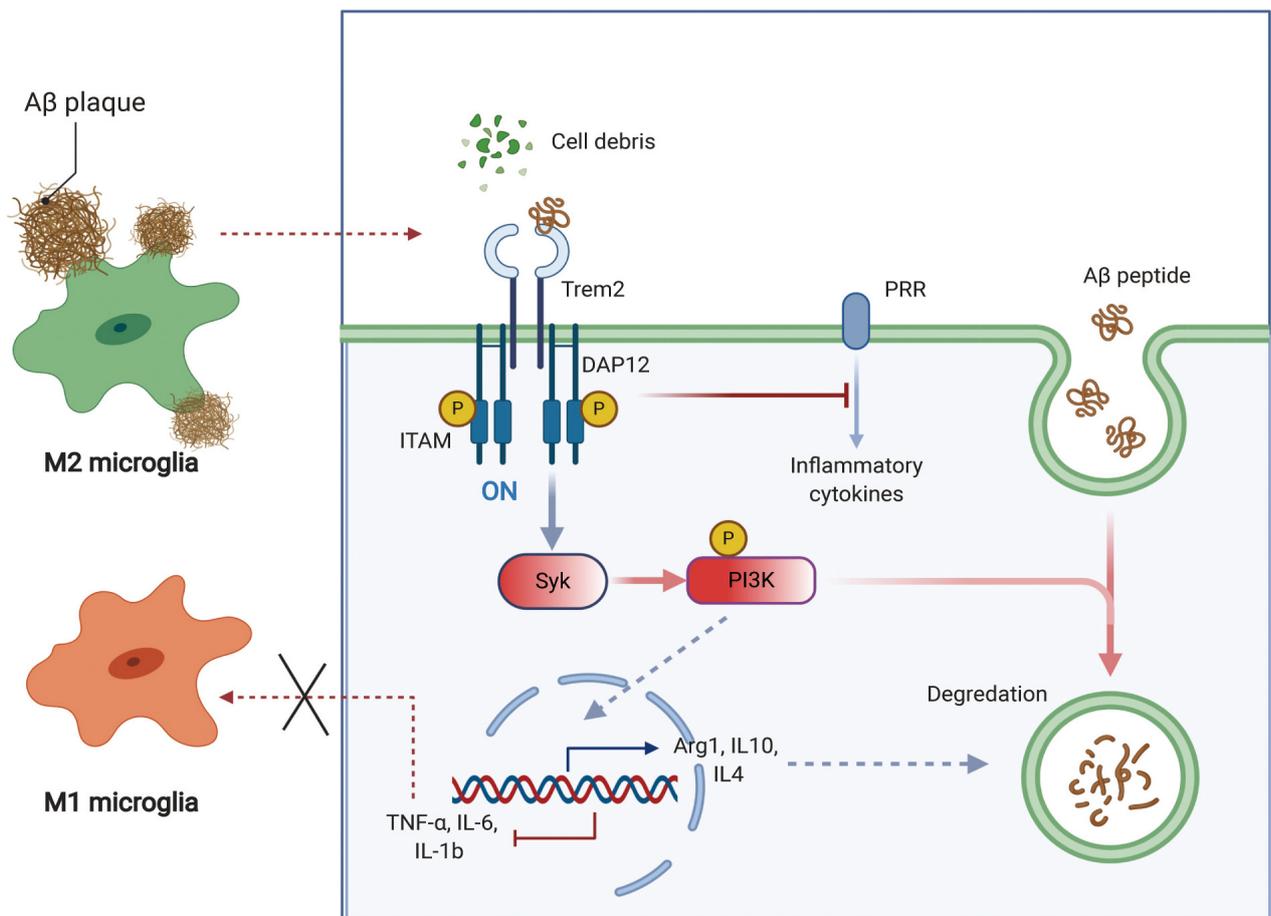


Fig. 7. The underlying molecular mechanisms of *Trem2* displayed a positive effect on anti-inflammatory M2 polarization of microglia and $A\beta$ clearance.

clearly reduced after *Trem2* silencing, which strongly indicates that the neuroprotective effect of DSS on BV2 cells was largely dependent on the activation of *Trem2*.

However, this study had some limitations. First, DSS prevented the development of $A\beta$ accumulation through a variety of mechanisms, but only its role in $A\beta$ clearance and microglia activation was explored. Additionally, whether DSS intervened in the functions of other signaling molecules and whether *Trem2* is the direct target of DSS requires further research. It is proposed that the molecular mechanisms related to *Trem2* pathway *in vivo* should be further explored, as should the molecular basis of DSS and the cross-talk between the multiple mechanisms for treating AD.

5. Conclusions

In conclusion, this study confirmed that DSS administration promotes the clearance of $A\beta$ through the regulation of microglia polarization via increasing the expression of *Trem2* in BV2 cells. DSS appears to be a potential therapy against the development of AD.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

QW, XC and CY designed, supervised the study, and supported funding acquisition. GCC, MMH, YC, and CY performed the experiment. YLL, ML and LW analyzed the data. GCC wrote the manuscript, CW, YXH and JHN acquired the data and revised the manuscript. YSM and LY constructed the figures and revised the manuscript. LY, XC, and CY finalized the paper. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The experiment was approved by the Laboratory Animal Ethics Committee of Guangzhou University of Chinese Medicine (approval ID: 20200721005) and performed in accordance with guiding principles of the United States National Institutes of Health for the care and use of laboratory animals.

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Conflict of Interest

The authors declare no conflict of interest.

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