Original Paper

The Effects of Interleukin-17 (IL-17)-Related Inflammatory Cytokines and A20 Regulatory Proteins on Astrocytes in Spinal Cord Cultured *In Vitro*

Shaohui Zong^{a,d} Keke Li^a Gaofeng Zeng^b Ye Fang^a Jingmin Zhao^{c,d}

^aDepartment of Spine Osteopathia, the First Affiliated Hospital of Guangxi Medical University, Nanning,

^bCollege of Public Hygiene of Guangxi Medical University, Nanning, ^cDepartment of Osteopathia, the First Affiliated Hospital of Guangxi Medical University, Nanning, ^dResearch Center for Regenerative Medicine, Guangxi Medical University, Nanning, China

Key Words

Interleukin-17 • Astrocyte • Inflammatory cytokines • Chemokines • A20

Abstract

Background/Aims: This study focused on investigating the expression of several inflammatory cytokines and chemokines, including regulatory proteins in the astrocytes of mice stimulated with IL-17. **Materials and Methods:** The cultured astrocytes from the spinal cords of mice were stimulated with IL-17. The expression of interleukin-6 (IL-6), tumor necrosis factor (TNF), monocyte chemoattractant protein-1/5 (MCP-1/5) and macrophage inflammatory protein-2 (MIP-2) stimulated with IL-17 (50 ng/ml) at different time points (3 h, 6 h, 12 h, 24 h and 48 h) were determined using real-time PCR and ELISA. The expressions of A20 (tumor necrosis factor α inducible protein 3, TNFAIP3) and NF- κ B were examined using real-time PCR and western blotting. **Results:** Compared with the control group, the mRNA expression levels of IL-6, TNF, MCP-1/5 and MIP-2 increased significantly at 6 h after IL-17 stimulation, while the protein expression levels also increased significantly and peaked at 12 h. The mRNA expression level of NF- κ B increased and peaked at 6 h before gradually declining, while the expression of A20 decreased. The protein expression level of NF- κ B increased and peaked at 12 h, while the expression A20 had an opposite response. **Conclusion:** The study showed that NF- κ B may have an effect on the cytokines secreted by astrocytes after IL-17 stimulation. Moreover, both A20 and NF- κ B could regulate the expression and secretion of inflammatory mediators.

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S. Zong and K. Li contributed equally to this work and should be considered as co-first authors.

Shaohui Zong and Gaofeng Zeng

Department of Osteopathia, the First Affiliated Hospital of Guangxi Medical University, No. 6 Shuangyong Road, Nanning, Guangxi, (China), Research Center for Regenerative Medicine, Guangxi Medical University, Nanning, Guangxi, (China); and College of Public Hygiene of Guangxi Medical University, No. 22 Shuangyong Road, Nanning, Guangxi, (China); Tel. +86-0771-5356577, E-Mail xiaohui3008@126.com

Introduction

IL-17 (also called IL-17A) is a cytokine subtype that is mainly secreted by CD4⁺ and CD8⁺ T cells and NK/T cells, which plays a role in bridging the acquired immune inflammatory response and the natural immune inflammatory response through the induction of inflammatory cytokines (e.g., IL-1 β , IL-6 and TNF) and chemokines [1]. In the central nervous system (CNS), neurons, astrocytes and microglia are innate immune response cells that can promote the secretion of inflammatory cytokines. Astrocytes are the most widely distributed glial cells in the CNS; they not only have insulation, nutrition, protection and support roles in neurons but also have important roles in the damage and repair process of the CNS [2, 3].

IL-17 stimulates astrocytes to release chemokines that cause peripheral neutrophils to infiltrate the disease-afflicted CNS [4]. During the inflammation process in the CNS, the interactions among IL-17, IL-23 and other proinflammatory cytokines produced by astrocytes have negative effects on the treatment and recovery of spinal cord injury (SCI) [5]. Compared with the histology and behavior between IL-17-gene knockout mice models and normal mice after SCI, Hill et al. found that IL-17 had significant roles in the functional recovery and glial scar formation in mice after SCI; in addition, the lack of IL-17 expression could help improve the movement of the mouse and reduce lesion areas, decreasing secondary damage after SCI [6]. As glia cells were not involved in the damage response in this process, there was little effect on the formation of glial scars. The authors also found that a lack of IL-17 could inhibit the expression of inflammatory factors, such as IL-1, TNF, and IL-6, thereby reducing the inflammatory response and leukocytic aggregation and reducing damage to the surrounding normal tissue and improving the function of IL-17-gene knockout mice after SCI [6], which indicated that IL-17 was a key factor during the inflammatory response after SCI.

Chemokines are small molecules that can secrete micromolecular proteins and have selective roles in engaging monocytes, neutrophils and macrophages [7]. The chemokine CC subfamily is the best known chemokine family, including MCPs (monocyte chemotactic proteins), while MIP-2 (macrophage inflammatory protein-2) belongs to the chemokine CXC family [8, 9]. In the process of acute inflammation, MIP-2 is the main chemokine, and longer expressions of MIP-2 lead to more severe inflammation reactions [10]. IL-17 can increase the expression of MCP-1 in endothelial cells, which causes the chemotaxis of peripheral T lymphocytes into the CNS [11]. At present, some proinflammatory factors, including TNF, IL-1 β and IFN- γ , can induce astrocytes to express MCP-1, which might be connected with the activation of NF- κ B, and Ping et al. demonstrated that TNF can induce mouse monocytes to express MCP-1 through the NF- κ B pathway [12].

Astrocytes secreted TNF in the spinal cord and promoted the activation of NF- κ B (nuclear factor-kappa B) when combined with TNF receptors, resulting in the production of inflammatory mediators, such as oxygen free radicals, which cause serious damage [13-15]. As the induced expression protein of TNF in endothelial cells, A20 (tumor necrosis factor α inducible protein 3, TNFAIP3) was first identified as an anti-apoptotic molecule and was subsequently found to be a negative regulator of NF- κ B, a function that is even more important [16]. Deletions of the A20 gene increased the activity of inhibitor NF- κ B kinase (IKK), ultimately enhancing the activity of NF- κ B [17]. Therefore, in this study astrocytes of spinal cord in mice were stimulated with IL-17 *in vitro* to investigate the expression of several inflammatory cytokines and chemokines, including regulatory proteins in the response to early inflammatory injury.

Materials and Methods

Ethics statement

This project fully considered and protected the rights and interests of the study objects. It meets the criteria of Ethical Review Committee. The Medical Ethics Committee of First Affiliated Hospital of Guangxi Medical University has approved the protocol (Permission numbers: NO. 2015, KY-E-018).

Materials

Fetal bovine serum (FBS) and DMEM/F12 medium were purchased from Hyclone (Logan, UT, USA). Recombinant Murine IL-17 was purchased from Pepro Tech China (PeproTech, Inc., USA). Trypsin was purchased from Sigma-Aldrich (St. Louis, MO, USA). TNF, IL-6, MCP-1, MCP-5 and MIP-2 ELISA kits were obtained from the Wuhan Cusabio Biotech Company (China). A20 (TNFAIP3) antibody and p-NF-κB/p65 (Ser536) antibody were purchased from Origene (OriGene Technologies, Inc., USA); GAPDH rabbit polyclonal antibody was purchased from Bioss, Inc. (China).

Isolating and culturing mouse astrocytes

Three-day-old BALB/C mice were used for this experiment. All of the animals were purchased from the Guangxi Medical College Animal Experimentation Center. The suffering of these animals was minimized. The mice were in a CO₂ chamber for anesthesia and sacrificed in a quick motion. The isolation of mouse astrocytes in the spinal cord was undertaken using the procedures described by Kerstetter AE [18]. The astrocytes isolated from the spinal cord were placed in an incubator at 37°C (5% CO₂). Every three days, the media was replaced and the non-adherent cells were discarded. When it was confluent by 80% - 90%, oligodendrocytes and microglial cells were discarded using orbital shaker (200r/min) at 37°C for 6 - 8h. Then the cells were split at a ratio of 1:2. The third passage of the cells was used in the following experiment. The morphological characteristics of the astrocytes were observed using an inverted microscope. Few irregular shaped cells were observed after 7 days of culturing. After 14 days of culturing, the cells were rich in cytoplasm with many branches and the nuclei were elliptical on one side of the cell body.

The stimulation of astrocytes by IL-17

The third passage of the astrocytes cultured *in vitro* was used for this experiment. These cells were randomly divided into groups stimulated with IL-17. Each subgroup of astrocytes was stimulated with five concentrations of IL-17, ranging from 10 to 200ng/ml (10, 20, 50, 100, and 200 ng/ml). In addition, the IL-17 stimulation time ranged from 3 h to 48 h (3 h, 6 h, 12 h, 24 h, and 48 h).

The detection of inflammatory factors using ELISA

The expression levels of TNF, IL-6, MCP-1, MCP-5 and MIP-2 secreted into the culture medium after stimulation with IL-17 were determined using ELISA kits according to the manufacturer's instructions. The cells in each subgroup had a density of 1×10^7 per well, which were measured using a cell counting chamber. The medium supernatant from each subgroup was collected and used to evaluate the levels of TNF, IL-6, MCP-1, MCP-5 and MIP-2. First, 100 µl of the cell media from each group were placed in a 96-well microtiter plate at 37°C. The plates were then covered with a plastic film and incubated at room temperature for 2 h. The liquid was removed from each well without washing, 100 µl of biotin antibody was added to each well, and the plates were then incubated for an additional 1 h at 37°C. After washing, the unbound antibodies were washed off, followed by the addition of avidin-HRP (1:1000 dilutions). The plates were incubated for 1 h at 37°C. Finally, the color reaction was developed by incubation with a TMB substrate and the enzyme reaction was terminated with 50 µl of Stop Solution. The OD was read at 450 nm using an ELISA plate reader (Bio-Rad). All of the samples were assayed in duplicate.

The detection of inflammatory factors using real-time PCR

Total RNA was extracted from the astrocytes using TRIZOL reagent (Invitrogen, USA) and was used to synthesize cDNA with an RT kit (Ferma, USA). According to the manufacturer's protocol, reverse-transcription PCR (RT-PCR) was performed with first-strand cDNA synthesized with 1 µg of total RNA and oligo d(T) 18 primers. The primers for the real time-PCR assays for TNF, IL-6, MCP-1, MCP-5 and MIP-2 were designed using Primer Premier 5.0 (Table 1). GAPDH was used to normalize each sample and each gene. All of the samples were assayed in duplicate.

The detection of A20 and NF-κB by real-time PCR

The mRNA levels of A20 and NF-κB were determined using real-time PCR. Each subgroup was stimulated by IL-17 at a concentration of 50 ng/ml for different times (3 h, 6 h, 12 h, 24 h, and 48 h). The primers for the real time-PCR assays for A20 and NF-κB were designed using Primer Premier 5.0 (Table 1).

Western blot analysis

After culturing for different times (3 h, 6 h, 12 h, 24 h, and 48 h), the cells were washed with PBS 3 times and were lysed at 4°C with lysis buffer (RIPA lysate containing 1% PMSF) for 30 min. The total protein from the cells was extracted following the manufacturer's protocols (Beyotime Technology, Inc., Jiangsu, China). Then, the protein concentration in the supernatant was determined using a BCA Protein Assay Reagent Kit (Beyotime Technology, Inc.). An amount equivalent to 40 µg of total protein sample were dissolved in 20 µl of 1×sodium dodecyl sulfate loading dye and boiled for 5 minutes. After an equivalent amount of the sample was added, the proteins were separated by 8 and 12% SDS-PAGE gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were blocked for 2 h at room temperature with a blocking solution (5% nonfat milk in Tris buffered saline with Tween 20 (TBST) and incubated overnight at 4°C with rabbit polyclonal antibody anti-A20 (1:1000), rabbit polyclonal antibody anti-p-NF-κB (1:1000) and anti-beta actin (1:2500). After washing 3 times for 10

min with TBST, the membranes were incubated with secondary antibodies (1:2500, Beyotime Technology, Inc.) for 1 h at room temperature. Then, the membranes were washed with TBST (3 times×10 min). Finally, the immunoreactive proteins were visualized using an ECL detection kit according to the manufacturer's instructions and were exposed to a radiograph film. The results of the graphs were analyzed by the Digital Gel Imaging Analyst (Nikon 990-Doc 1000, USA). GAPDH was used as a loading control for comparisons between samples.

Statistical analyses

All data are presented as the means ± SD. Statistical analyses were performed using SPSS16.0 (SPSS Company, USA). One-way ANOVA followed by the LSD multiple-range test was used to analyze differences between groups. Values of $p < 0.05$ (two-tailed) were considered statistically significant.

Results

The expression of inflammatory factors

The stimulation dose of IL-17 used in the study was determined by ELISA. We used different concentrations of IL-17 (10, 20, 50, 100, and 200 ng/ml) to stimulate cells in each subgroup and detected the expression levels of inflammatory cytokines at 12 h. As shown in Fig. 1, the expression levels of IL-6, TNF, MCP-1, MCP-5 and MIP-2 increased with the increasing dosage of IL-17. Compared with the control group, the expression levels of IL-6, TNF, MCP-1, MCP-5 and MIP-2 significantly increased. However, this trend was not significant compared with different concentrations of IL-17 (10, 20, 50, 100, and 200 ng/ml). Thus, the concentration of IL-17 that we used in our next experiment was 50 ng/ml. The expression levels of inflammatory factors were detected at different time points. As shown in Fig. 2, the mRNA expression levels of IL-6, TNF, MCP-1, MCP-5 and MIP-2 increased and peaked at 6 h ($p < 0.05$). In addition, as shown in Fig. 3, compared with the control, the protein expression levels of IL-6, TNF, MCP-1, MCP-5 and MIP-2 were increased ($p < 0.05$). As time went on, the trend gradually increased and peaked at 12 h ($p < 0.05$), after which it declined gradually. Moreover, the increasing trends of these inflammatory factors at different time points varied.

Table 1. Sequences of primers for PCR

Molecule	Sequences(5'-3')
IL-6	forward 5'-CCACGGCCTTCCTAC-3'
	reverse 5'-AAGTGCATCATCGTTGT-3'
TNF	forward 5'-CATCTTCTCAAATTCGAGTGACAA-3'
	reverse 5'-TGGGAGTAGACAAGGTACAACCC-3'
MCP-1	forward 5'-CAGCCAGATGCAGTTAACGC-3'
	reverse 5'-GCCTACTCATTTGGGATCATCTTG-3'
MCP-5	forward 5'-GGAAGCTGAAGAGCTACAGGAGAA-3'
	reverse 5'-GAAGGTTCAAGGATGAAGGTTTGA-3'
MIP-2	forward 5'-GCCCCCTCCACCTGCCGGCTGC-3'
	reverse 5'-CTGAACCAGGGGGCTTCAGGG-3'
A20	forward 5'-TGGGAGCAGTGTTAAAGGCA-3'
	reverse 5'-TCGGAAGTGTGGGCAAAACT-3'
NF-κB	forward 5'-GAGGTCTCTGGGGTACCAT-3'
	reverse 5'-AAGGCTGCCTGGATCACTTC-3'
GAPDH	forward 5'-CCTTCATTGACCTCAACTAC-3'
	reverse 5'-GGAAGGCCATGCCAGTGAGC-3'

Fig. 1. The determination of IL-17 concentrations. Cells were stimulated with different concentrations of IL-17 (10, 20, 50, 100, and 200 ng/ml). The relative protein levels of IL-6, TNF, MCP-1, MCP-5 and MIP-2 were determined at 12 h. The results are expressed as the means $\pm$ SD. * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with IL-17 (10 ng/ml) group.

Fig. 2. The relative mRNA expression levels of inflammatory cytokines. Cells were stimulated with IL-17 (50 ng/ml), and the relative mRNA expression levels of IL-6, TNF, MCP-1, MCP-5 and MIP-2 were detected at different time points (3 h, 6 h, 12 h, 24 h, and 48 h). The results are expressed as the means $\pm$ SD. * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with IL-17 (stimulated for 6 h) group.

Fig. 3. The relative protein expression levels of inflammatory cytokines. Cells were stimulated with IL-17 (50 ng/ml), and the relative protein levels of IL-6, TNF, MCP-1, MCP-5 and MIP-2 were detected at different time points (3 h, 6 h, 12 h, 24 h, and 48 h). The results are expressed as the means $\pm$ SD. * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with IL-17 (stimulated for 12 h) group.

Fig. 4. The relative mRNA expression levels of A20 and NF- κ B. Cells were stimulated with IL-17 (50 ng/ml), and the relative mRNA expression levels of A20 and NF- κ B were detected at different time points (3 h, 6 h, 12 h, 24 h, and 48 h). The results are expressed as the means $\pm$ SD. * $p < 0.05$ compared with the control group; # $p < 0.05$ compared with IL-17 (stimulated for 6 h) group.

The effect of IL-17-related regulatory proteins A20 and NF- κ B

In this study, real-time PCR was used to detect the mRNA expression levels of A20 and NF- κ B. As shown in Fig. 4, the mRNA expression level of NF- κ B increased and peaked at 6 h ($p < 0.05$) and then declined gradually. However, the expression of A20 decreased, thus presenting an opposite trend. Western blotting analyses of A20 and p-NF- κ B were performed to detect protein expression. As shown in Fig. 5, the protein expression level of NF- κ B increased and peaked at 12 h ($p < 0.05$), while A20 had an opposite trend.

Discussion

The space between neurons and neurites in the CNS is almost entirely filled with astrocytes, which provide structural support for and separate connections between neurons,

Fig. 5. Western blot analysis of A20 and p-NF- κ B. (A) Representative photographs of A20 and p-NF- κ B protein levels. (B) A bar graph quantifying the A20 protein level. (C) A bar graph quantifying the p-NF- κ B protein level. The results are expressed as the means $\pm$ SD. * $p < 0.05$ compared with the control group. GAPDH was used as the internal control; # $p < 0.05$ compared with IL-17 (stimulated for 12 h) group.

and thus have the protective function of isolation [19]. Moreover, astrocytes could provide nutrients and support neuron metabolism in the spinal cord, maintain the stability of the intracellular environment and the dynamic pH balance, and produce a series of soluble molecules in the formation of the blood brain barrier (BBB) with endothelial cells [20].

The invasion of inflammatory cells and the release of cytokines and chemokines were caused by a series of cell damage after SCI; these rapid changes affect the internal homeostasis of neurons, oligodendrocytes and astrocytes, and at the same time, the astrocytes were mostly activated as reactive astrocytes [21]. Additionally, these activated astrocytes, referred to as reactive astrocytes, secrete inflammatory factors, such as TNF, IL-6 and others, which in return further promote the activation of inflammatory factors and form a vicious circle [22]. Study found that both CLA (conjugated linoleic acid)-isomers increased TNF and IL-6 mRNA in primary astrocyte cultures indicate that astrocytes may contribute to neuronal inflammation [23]. It also showed that IL-6 could attenuate cell proliferation and anti-apoptotic effects of MDZ in astrocytes [24]. Thus, in this experiment we used IL-17 to stimulate the astrocytes cultured *in vitro* and to investigate the relationship of several inflammatory cytokines and chemokines, including regulatory proteins, which might provide a theoretical basis for anti-inflammatory therapy of SCI in clinic.

The inflammatory response is the major factor which affects the pathophysiology of SCI. Recently, study has found that IL-17 is a pro-inflammatory cytokine, which plays a key role in the CNS and is related to the innate immune responses as a secondary immune response that affects the secretion of neurotransmitters and the neuron excitability [1], which could influence the functional outcome and the neuroinflammatory response. For example, the expression and secretion of IL-1 β and IL-6 can be induced by IL-17 [25], which are the main cytokines that promote the differentiation of CD4⁺ T cells into Th17 cells. Therefore, the continuously increased expression of IL-17 and its receptor may be a key factor in the progression of the inflammatory response.

Our experimental results showed the mRNA and protein levels of IL-6 and TNF of astrocytes stimulated with IL-17 increased to a peak level in 12 hours, indicating both IL-6 and TNF played an important role in the early inflammatory response. Therefore, it is the key to effectively control and reduce the intensity and scope of inflammatory reaction after SCI at the early stage.

MCP-1 is a key chemokine in migration regulation and in the infiltration of monocytes and macrophages, while MIP-2 is an important chemokine among neutrophils [26, 27]. Moreover, MCP-1 is mainly expressed by astrocytes, microglial cells and neurons, and it has a chemotactic and activated effect on monocytes. Gene expression and the regulation of MCP-1

occur in the distal gene control region and can be combined with NF- κ B and regulated TNF [28]. In this experiment, the increased mRNA and protein levels of MIP-1/5 and MIP-2 of astrocytes stimulated with IL-17 showed a similar tendency, reaching up to a peak level at 6h and 12h respectively and then gradually decreased, which implied that IL-17 had a close relation with inflammatory cytokines and chemokines.

In the CNS, astrocytes serve as a bridge between the CNS and the immune system [29]. Aberrant expression of cytokines and chemokines in astrocytes often accompanies CNS disorders, such as multiple sclerosis (MS), Alzheimer disease (AD) [29, 30]. MS is an inflammatory/autoimmune and demyelinating disease mediated mainly by T cells, which causes neurological disorders [31]. The current therapy of MS focuses on a modulation or suppression of immune functions, of which sphingosine-1-phosphate (S1P) receptors on astrocytes involved in demyelination processes and subsequent axonal degeneration are important features [32]. Besides, Tzartos et al. [33] found that in pathological conditions, MS closely related to IL-17 produced by astrocytes in CNS. Our data in this experiment showed that after stimulated with IL-17, expression of cytokines and chemokines (IL-6, TNF, MCP-1/5, and MIP-2) in astrocytes increased rapidly at early stages (mRNAs in 6h, proteins in 12h). Thus, it demonstrated that in the inflammatory response of CNS, IL-17 both involved in the production and participation of inflammatory diseases (e.g. MS, SCI).

In mammals, the NF- κ B family has five sub-units, two of which are the most important: p50 and p65. Nuclear transcription factor κ B/p65 (NF- κ B/p65), a wide spread cell nuclear transcription factor, can regulate the transcription ability of many genes in tumors and thus regulates tumor cell proliferation and apoptosis [34]. In the central nervous system, activated NF- κ B can promote the activation of astrocytes and regulate the expression of proinflammatory cytokines; however, in most cases, the activation of NF- κ B is transient and occurs in cycles. As a result, the regulation of this process is specifically dependent on negative feedback, such as regulation by A20 [35]. Therefore, we know that the main function of A20 is to play a role in negatively regulating NF- κ B.

A20 was originally identified as a TNF α -induced protein 3 (TNFAIP3), a key regulator of inflammation signaling pathways, as well as a NF- κ B inhibitor, which plays a critical role in the regulation of innate and adoptive immunity [36]. Recently, A20 has also been proposed to function as a tumor suppressor. It was found that the inactivation of A20 is associated with the occurrence of B cell tumors, suggesting that it inhibits tumors [37]. Moreover, A20 are essential molecules and terminate the activation of NF- κ B [38], which is mediated by the innate receptors of the immune response, including TNF receptor (TNFR), Toll-like receptor (TLR) and nucleotide-binding oligomerization domain protein 2 (NOD2).

Regulatory A20 protein not only regulates the activation of NF- κ B but also is the key suppressor in the signaling pathways of TNFR1, myD88 and NOD2 and regulates some pro-inflammatory cytokines, including TNF, IL- β and IL-6 [39]. Studies have also demonstrated that A20-deficient mice spontaneously develop inflammation reactions in multiple organ systems and die within two weeks [40]. This suggests that A20 has vital anti-inflammatory effects.

In our experiment, we found that the protein level of A20 in the astrocytes decreased at 6 h after IL-17 stimulation, while the mRNA and protein levels of IL-6, TNF, MIP-2, and MCP1/5 increased significantly. After a period of time, the protein level of A20 gradually increased, while the mRNA and protein levels of the cytokines decreased. Thus, we inferred that there may be a relationship between A20 and these cytokines.

Studies have found that IL-17 could activate signaling pathways, such as JAK (Janus kinase) and PI3K, when combined with IL-17R, and this in turn could result in the activation of NF- κ B [41]. In our study, the protein levels of p-NF- κ B increased at 3 h and peaked at 6 h, while the mRNA and protein levels of IL-6, TNF, MIP-2, MCP-1/5 increased significantly. Thus, we believe that NF- κ B may have an effect on these cytokines secreted by astrocytes after IL-17 stimulation.

In this study, we confirmed that IL-17 could up-regulate the expression of NF- κ B in astrocytes. In addition, our study has shown that both A20 and NF- κ B not only affect cellular

proliferation and growth but also regulate the expression and secretion of inflammatory mediators. Ultimately, inflammatory cytokines, such as TNF, IL-6, MCP1/5 and MIP-2, have a dual role in CNS disease: on the one hand, they are excreted by pathologic tissues to attract monocytes/macrophages to the damaged areas to participate in tissue repair, which is an important component of organism defense. On the other hand, the massive amount of inflammatory cytokines produced in the damaged areas could also cause an exaggerated inflammatory response, which in turn aggravates tissue damage. As IL-17 has a close relationship with other cytokines which could mediate multiple inflammatory responses, it is vital to determine how IL-17 effects on the expression levels of other pro-inflammatory cytokines.

More importantly, our study may provide an interesting approach to identifying whether anti-IL-17 therapy could effectively promote the recovery of immune inflammatory response diseases in CNS such as SCI. However, this experiment using cells *in vitro* is not enough; it still needs further studies using animal models *in vivo*. As a result, we still need investigations of the molecular structure, biological function, gene mapping and signal transduction mechanism of inflammatory cytokines after immune-activation in the CNS both *in vitro* and *in vivo*. The regulatory functions of inflammatory cytokines on other cytokines and inflammatory cells require further investigation to explore how to enhance the beneficial functions while inhibiting the damaging effects in tissues to provide new ideas for the treatment of CNS disease.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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