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MD2 Mediates Inflammation and Nerve Repair in Sciatic Nerve Injury

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Abstract

When the peripheral nerve is physically injured, activated Schwann cells and recruited macrophages release a variety of inflammatory cytokines, such as TNFα, IL-1β, IL-6. Myeloid Differentiation protein 2 (MD2) is a secreted protein composed of 160 amino acids, it is a valuable anti-inflammatory target and can be utilized in a number of inflammatory contexts. However, the role of MD2 in peripheral nerves is unclear. In this experiment, our aim was to study whether MD2 is involved in the inflammatory response of peripheral nerve injury and its role in the repair of peripheral nerve injury. We established a model of sciatic nerve injury, real-time quantitative PCR, immunoblotting and immunofluorescence staining showed that MD2 was upregulated after sciatic nerve injury and the inflammatory reaction in MD2 Knockout (MD2-KO) mice against C57BL/6 background was lighter than that in C57BL/6 mice. The results of immunoblotting and immunohistochemical staining indicated that Growth Associated Protein 43 (GAP43) and p75 Neurotrophic Factor Receptor (p75NTR) were highly expressed in MD2-KO mice after sciatic nerve injury. Enhanced sciatic function index in MD2-KO mice suggested that knockout of MD2 was beneficial to the repair of sciatic nerve injury. In summary, we conclude that MD2 participates in the inflammatory reaction of peripheral nerve injury and plays an important role in the repair of peripheral nerve injury.

Keywords: Nerve repair; Inflammatory response; MD2

Introduction

The peripheral nerves refer to all the nerves outside the brain and spinal cord and most peripheral nerves contain both sensory and motor nerves [1,2]. The peripheral nerve is formed by bundles of nerve fibers, each of which is surrounded by Schwann cells and produces several layers of lipid insulation called myelin sheath [3,4]. With the rapid development of economy and society, the number of patients with peripheral nerve injury caused by mechanical impact or sharp cutting continues to increase [5]. Peripheral nerve injury brings great pain to patients, although traditional surgical treatment and combined treatment of cell engineering, gene engineering can restore the continuity of the nerve, it does not mean the complete recovery of the nerve function [6-8]. Therefore, it is of great significance to regulate and assist the recovery of nerve function from the point of view of immunology.

Studies suggest that the recovery of nerve function is closely related to inflammatory response [9]. When the peripheral nerve is injured, the periphery of the injury will be filled with damage associated molecular patterns [10]. Schwann cells that can feel these damage associated molecular patterns produce inflammatory response and release a variety of inflammatory cytokines, such as TNFα, IL-1β, IL-6, which participate in the recruitment of macrophages [11-15]. Once macrophages are recruited to the injured site, they are also activated to cause inflammation [16-19].

MD2 is an attractive pharmacological target for the development of effective anti-inflammatory drugs [20]. MD2 is composed of 160 amino acids with a molecular weight of 25-30 kDa, it has been identified and named because of its high homology with MD1. Its N-terminal has a signal peptide composed of 16 amino acid residues, which makes MD2 secretory and can be secreted directly through the outer membrane. Studies have found that MD2 may contain two relatively independent functional domains, which can bind not only to toll-like receptor 4 and toll-like receptor 2, but also to a variety of ligands. MD2 can sense changes in the external environment and activated MD2 can further activate downstream signal pathways and trigger inflammatory response.

It is very important to regulate inflammatory response in the process of peripheral nerve injury repair, but the role of MD2 in peripheral nerve injury is not clear. In order to verify the role of MD2, a model of sciatic nerve injury in C57BL/6 mice was established to explore the role of MD2 in inflammatory response and post-injury repair of sciatic nerve injury, so as to open up a new idea for clinical treatment of peripheral nerve injury.

Materials and Methods

Animal care

All animals were humanely cared for in accordance with the standards of the National Laboratory for the Care and Use of Laboratory Animals (NIH Publications 86-23, Revised 1985), which were formulated by the National Academy of Sciences and published by the National Institutes of Health. All animal procedures were approved by Qingdao University Laboratory Animal Welfare Ethics Committee.

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Specific-pathogen-free male 8-week-old C57BL/6 mice and age-matched male MD2-KO mice on C57BL/6 background were obtained from Beijing Charles River Experimental Animal Technology. In the animal laboratory of Qingdao University, mice were housed in the animal facility with a 12 h light/dark cycle. The relative humidity of the environment was 60%. The temperature was 22 ± 2°C. The experiment began one week after they adapted to the environment.

Experimental groupings and surgical procedures

The experiment consisted of three groups. C57BL/6 mice were randomly divided into control group and injured group, MD2-KO mice were treated as MD2-KO group. The model of sciatic nerve injury was constructed in the injured group and MD2-KO group. We establish sciatic nerve injury model as follow: After anaesthesia of mice, the thigh skin and its surrounding areas were shaved with a razor, followed by disinfecting with 75% alcohol. Skin layers and muscle were separated through surgery using sterile operational conditions. After exposing the sciatic nerve, the sciatic nerve was clamped vertically with hemostat for three buckles for 60 second, then the sciatic nerve was put back to the original position, the wound was washed with saline and the skin was sutured with 6-0 no damage thread layer by layer. Control group exposed sciatic nerve for 1 minute without injury.

Luxol Fast Blue (LFB) staining

Sciatic nerves in different treatment groups were removed at 24 hours (h) after surgery and fixed in 4% paraformaldehyde for 48 h. After paraffin embedding, sciatic nerves were cut longitudinally. The slides were incubated with 0.1% LFB in acidified 95% ethanol at 60°C overnight, then they were differentiated in 0.05% Li_2CO_3 and 70% ethanol for 30 seconds, dehydrated in different concentrations of ethanol successively. The stained sections were observed under an optical microscope (Olympus, Japan).

Real-Time quantitative PCR (RT-qPCR)

Total RNA was isolated from nerve tissues using RNAisoPlus reagent (Takara, Dalian, China) at 6 h, 12 h, 24 h and 48 h post nerve injury. The concentration and purity of RNA were determined by spectrophotometer (Eppendorf, Hamburg, GER). DNase treatment and cDNA synthesis were performed by using a PrimeScript RT-PCR kit (Takara, Dalian, China). cDNA was subsequently amplified by CFX96 real-time PCR detection system (Bio-Rad, USA) with specific primers. Primers for genes were obtained from Takara. The primers used were as follows: MD2: 5' TGACTGAATCTGAGAAGCA--3'(forward) and 5'-TTCGGCAACTTTGGAATG-3'(reverse); TNFα: 5'-GCCAGGAGGGAGAACAGAAACTC-3'(forward) and 5'-GGCCAGTGAGTGAAAGGGACA-3'(reverse); IL-1β: 5'-TCGCAGCAGCACATCAACAAGAG-3'(forward) and 5'-TGCTCATGTCCTCATCCTGGAAGG-3'(reverse); IL-6: 5'-CCACTTCACAAGTCGGAGGCTTA-3'(forward) and 5'-TGCAAGTGCATCATCGTTGTTC-3'(reverse); GAPDH: 5'-TCTTGGGCTACACTGAGGAG-3'(forward) and 5'-CATACCAGGAAATGAGCTTGA-3'(reverse). Relative quantification was determined by normalization to GAPDH. The RT-qPCR assays were performed in triplicate and the relative expression levels were calculated based on the $2^{-\Delta\Delta Ct}$ method.

Western blot

The sciatic nerves of mice were removed to extract the protein at the appropriate time. Nerves were homogenized on ice in RIPA buffer (50 mmol/L Tris-HCl; 150 mmol/L NaCl; 1% Nonidet; 0.5% deoxycholate; 1 mmol/L EDTA) mixed with protease inhibitors (Solarbio, Beijing, China). The protein concentration was determined using a BCA kit (Beyotime, Shanghai, China). Next, equal concentrations of protein lysates were loaded to the 10% or 12.5% SDS-PAGE gels and run for proper time, separated proteins in the gel were then transferred to polyvinylidene fluoride membrane (GE Healthcare, Freiburg, DE). The membrane was blocked with 5% skim milk for 1 h at room temperature and subsequently incubated at 4°C overnight with the primary antibodies. The antibodies used were rabbit anti-MD2 antibody (Bioss, 1:1000), rabbit anti-TNFα antibody (Abcam, 1:1000), rabbit anti-IL-1β antibody (Bioss, 1:2000), mouse anti-IL-6 antibody (Huaan, 1:2000) (HUABIO), rabbit anti-p75NTR antibody (Huaan, 1:2000), rabbit anti-GAP43 antibody (Huaan, 1:1000), beta actin antibody (HRP Conjugated) (Abways, 1:5,000). The membranes were washed 3 times with TBST (Tris-buffered saline with 0.1% Tween 20) and incubated with the corresponding horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Then membranes were washed with TBST for another three times and incubated with Sparkjade ECL super (Sparkjade Science Co., Ltd.) to visualize. The band densities were analyzed by Image J software (NIH, USA).

Immunofluorescence staining

The sciatic nerve was taken out after 24 h of surgery and fixed in 4% paraformaldehyde for 48 h. After paraffin embedding, sciatic nerve was cut longitudinally. Antigens were retrieved, then sections were blocked with 5% bovine serum albumin at room temperature for 1 h and incubated with rabbit anti-MD2 antibody (Bioss, 1:200) or rabbit anti-CD68 antibody (Servicebio, 1:1000) overnight at 4°C. After three times of washing with phosphate-buffered saline, the sections were further incubated with CY3-labeled goat anti-rabbit IgG (Servicebio, 1:500) for 2 h. Nuclei were stained with DAPI. Images were obtained by 3D HISTECH (Hungary).

Haematoxylin and Eosin (HE) staining

At 12 h and 24 h after sciatic nerve injury, the mice were killed. The sciatic nerve was fixed in 4% paraformaldehyde for 48 h, then dehydrated in a series of graded alcohols. The specimens were embedded in paraffin blocks after treatment with xylene. The sciatic nerve was cut longitudinally and then stained with HE according to standard procedures.

Immunohistochemical staining

Immunohistochemical staining was used to detect the expression of p75NTR and GAP43. Specimens were incubated with 3% hydrogen peroxide solution at room temperature for 25 minutes. After washing three times with phosphate buffered saline, 3% bovine serum albumin was used to incubate the specimens at room temperature for 30 minutes. After pouring out the liquid, the specimens were incubated with rabbit anti-p75NTR antibody (Huaan, 1:200) or rabbit anti-GAP43 antibody (Huaan, 1:200) at 4°C overnight, then they were washed three times with phosphate buffered saline, each for 5 minutes. Goat anti-rabbit secondary antibody (Servicebio, 1:200) was added to the specimens and incubated for 50 minutes at room temperature, the

specimens were then washed again three times with phosphate buffered saline, each for 5 minutes. All images were obtained by optical microscopy.

Sciatic Function Index (SFI)

The recovery of sciatic nerve function was evaluated by self-made mice footprint walking box (about 50 cm in length, about 10 cm in width and about 15 cm in height). Cover the bottom of the box with white paper. Before the measurement, the mice' feet were stained with ink brush so that the footprints can be clearly recorded. There are three variables in the footprint measurement: Footprint Length (PL), which is the distance from the back heel to the front toe; Toe width (TS), which is the distance from the first toe to the fifth toe; middle toe distance (IT), distance from the second toe to the fourth toe. Calculate the SFI. The SFI score of normal mice is 0 and the complete loss of sciatic nerve function is –100.

Statistical analysis

The data were presented as means ± S.E.M. Differences were considered to be significant at $P<0.05$. Statistical analysis was performed by the Student's t-test or one-way analysis of variance and Bonferroni correction was used to examine the difference between groups using Graph pad Prism 7.0 software (USA).

Results

Model of sciatic nerve injury

After sciatic nerve clamp injury, the mice regained consciousness. We observed that affected hind limbs of the mice were weak and dragged forward by the healthy limbs. After LFB staining, the myelin sheath was specifically stained blue, the axons were not stained. Results showed that the myelin sheath structure of control group was clear and complete and the arrangement was regular, while light staining and disordered distribution of the myelin sheath was seen in the injured group, suggesting an obvious demyelination state (Figure 1). The model of sciatic nerve injury was established successfully.

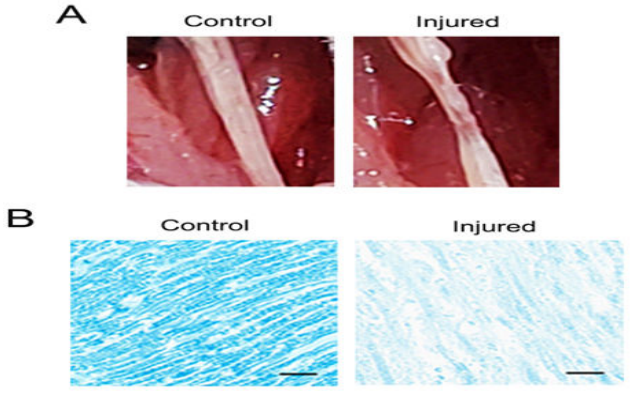

Figure 1: Anatomical images and LFB staining of sciatic nerve. (A) Establishment of sciatic nerve injury model. Images show the normal nerve (left), injured nerve (right). (B) The nerves of mice in the control group and injured group were stained with LFB at 24 h after operation. Shown are representative images of three independent experiments (n=3 per group; Scale bar=20 μm).

MD2 expression was increased after sciatic nerve injury

As a potential therapeutic target for a variety of diseases, it is of great significance to study whether MD2 is involved in peripheral nerve injury. Real-time qPCR assay was used to examine the mRNA expression of MD2. The results suggested that the mRNA level of MD2 increased after sciatic nerve injury. At 24 h after surgery, the mRNA level of MD2 in the injured group was significantly higher than that in the control group (Figure 2A). In addition, the increased protein level of MD2 in injured group was also confirmed by western blot (Figure 2B). We used immunofluorescence to visualize the activation of MD2. The nerve in the control group and injured group was transected longitudinally. Immunofluorescence staining results showed that the expression of MD2 was obviously increased after sciatic nerve injury (Figure 2C). These data indicated that increased MD2 expression was linked to sciatic nerve injury, implicating that MD2 play an important role in sciatic nerve injury.

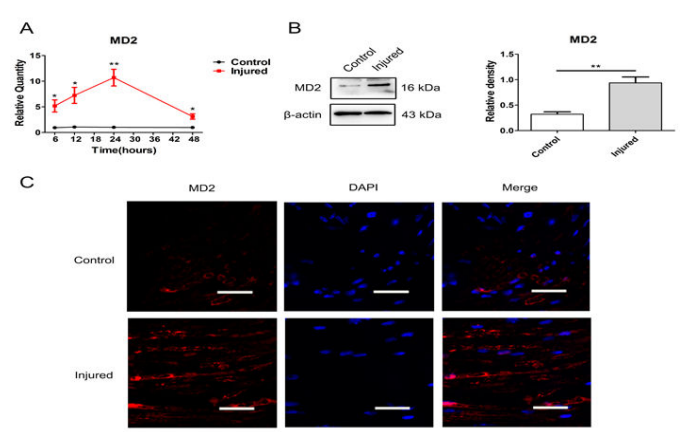

Figure 2: MD2 expression was upregulated after sciatic nerve injury. (A) The mRNA level of MD2 in the injured group was significantly increased compared with the control group at 6 h, 12 h, 24 h and 48 h post sciatic nerve injury, (B) Comparison of protein level of MD2 in the control group and injured group, (C) The longitudinal sections of the sciatic nerve from control group mice and injured group mice were stained with anti-MD2 antibody (red) and DAPI (nuclei, blue). (Scale bar=50 μm). Data are from three independent experiments (n=3 per group; $^{*}P<0.05$, $^{**}P<0.01$).

The inflammatory response was enhanced after sciatic nerve injury

TNFα, IL-1β, and IL-6 are important inflammatory cytokines secreted by cells after sciatic nerve injury. RT-qPCR experiments were performed in the control group and injured group at 6 h, 12 h, 24 h and 48 h after surgery. The results suggested that compared with those in the control group, the mRNA levels of TNFα, IL-1β and IL-6 were upregulated in the injured group at 6 h, 12 h, 24 h after surgery. However, the expression of IL-6 did not change at 48 h after sciatic nerve injury (Figure 3A). Western blotting results showed that the protein levels of TNFα, IL-1β and IL-6 were increased at 24 h after sciatic nerve injury (Figure 3B). Our findings indicated that inflammatory cytokine production was significantly increased post sciatic nerve injury.

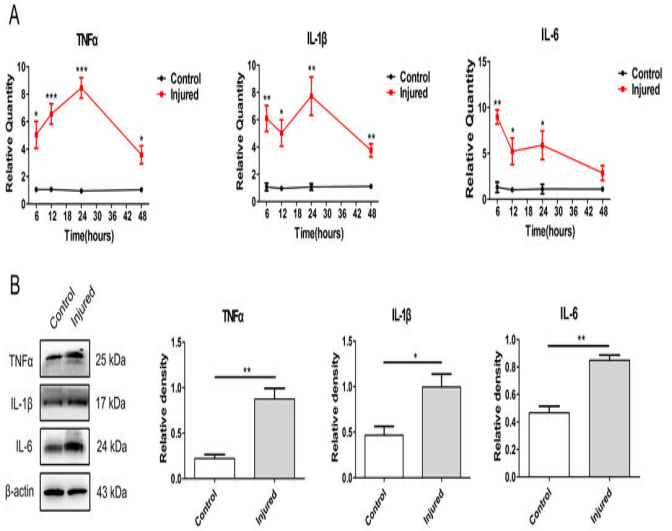

Figure 3: The levels of TNFα, IL-1β and IL-6 at different time after surgery. (A) Changes in mRNA expression levels of TNFα, IL-1β and IL-6 at 6 h, 12 h, 24 h and 48 h after surgery, (B) Compared with the control group, the protein levels of TNFα, IL-1β and IL-6 in injured group were significantly increased at 24 h post sciatic nerve injury. Data are from three independent experiments (n=3 per group; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

Histological changes after sciatic nerve injury

The sciatic nerves of mice from different treatment groups were removed at 12 h, 24 h after surgery. The longitudinal section of the nerve performed HE staining. In the control group, we observed that the fibrous structures on the longitudinal section of sciatic nerve were parallel and ordered, with uniform thickness and shape at both 12 h and 24 h and there were no inflammatory cells. In the injured group, the nerve fibres were disordered and the axons of the mice were completely broken. Moreover, there was infiltration of a large number of inflammatory cells. Compared with 12 h, the inflammatory reaction was more serious at 24 h after nerve injury (Figure 4A). Through HE staining in the injured group and MD2-KO group, we found that after 12 h and 24 h of nerve injury, the inflammatory cells have also been observed in MD2-KO group, but the amount of infiltrated inflammatory cells in MD2-KO group was fewer than that in injured group. In addition, we stained CD68 macrophages with immunofluorescence. We found that the infiltration of macrophages increased after sciatic nerve injury, however, compared with the injured group, the infiltration of macrophages decreased in the MD2-KO group (Figure 4B).

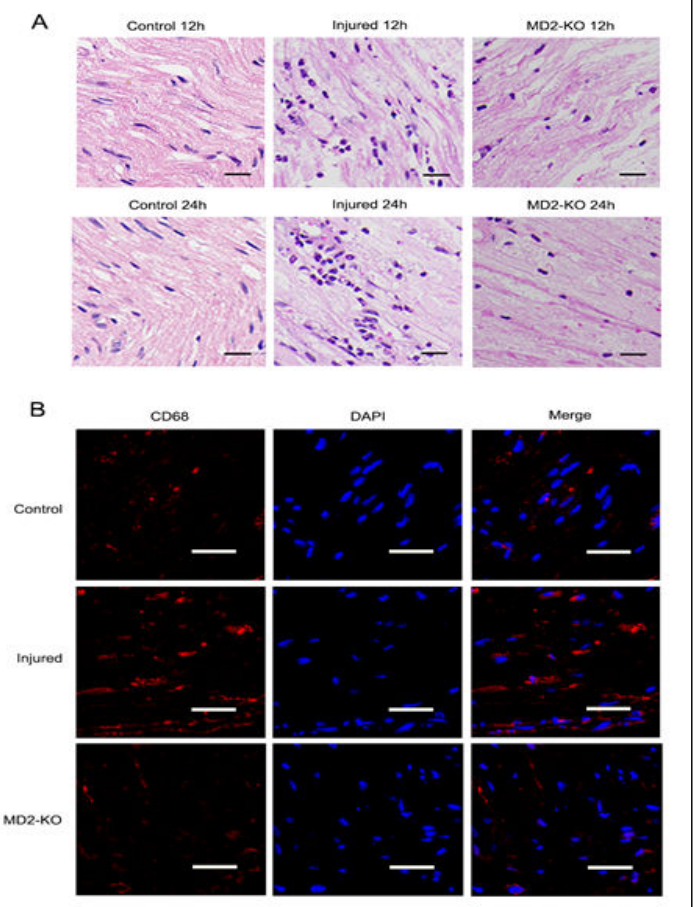

Figure 4: Histological staining images of sciatic nerve in different treatment groups. (A) Inflammatory cell infiltration was different in control group, injured group and MD2-KO group by HE staining. Compared with the control group, inflammatory cells increased after 12 h and 24 h of nerve injury in injured group. However, MD2-KO mice had lighter inflammation compared with C57BL/6 mice after sciatic nerve injury. Shown are representative images of three independent experiments (n=3 per group; Scale bar=20 μm), (B) Immunofluorescent staining for CD68 macrophages after 24 h of surgery in different treatment groups. Shown are representative images of three independent experiments (n=3 per group; Scale bar=50 μm).

MD2 regulated inflammatory responses in sciatic nerve injury

Since MD2 is activated after sciatic nerve injury, we want to explore the role of MD2 in inflammation after sciatic nerve injury at molecular level by using MD2 knockout mice. RT-qPCR showed that MD2 was not expressed in MD2-KO mice. Meanwhile, MD2 protein was not detected in MD2-KO mice. Compared with the injured group, the mRNA levels of inflammatory factors TNFα, IL-1β and IL-6 were significantly lower in the MD2-KO group at 6 h and 24 h after nerve injury. However, the expression of TNFα, IL-1β, and IL-6 did not change after 48 h of nerve injury in MD2-KO group (Figure 5A). The protein levels of TNFα, IL-1β and IL-6 were down-regulated in MD2-KO mice at 24 h post sciatic nerve injury (Figure 5B). Together, these results suggested that the production of inflammatory cytokines was regulated by MD2.

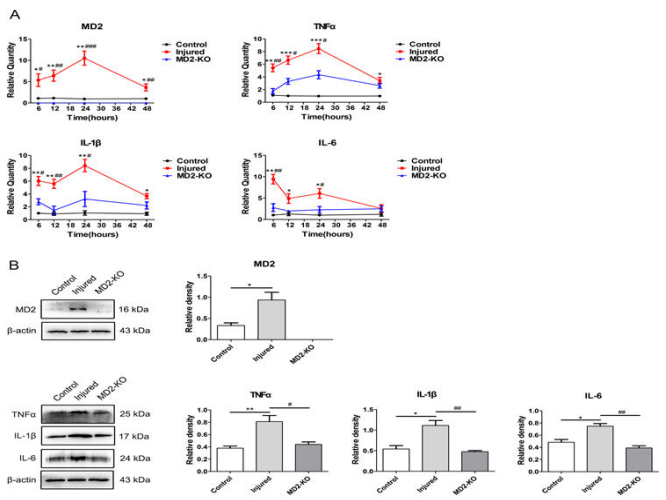

Figure 5: MD2 knockout reduced inflammatory response. (A) The mRNA levels of TNFα, IL-1β and IL-6 in the control group, injured group and MD2-KO group, (B) Comparison of protein levels of TNFα, IL-1β and IL-6 in injured group and MD2-KO group at 24 h after nerve injury. Data are from three independent experiments (n=3 per group; *P<0.05, **P<0.01, ***P<0.001, control *vs.* injured; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, injured *vs.* MD2-KO).

The levels of p75NTR and GAP43 were higher in MD2-KO mice after sciatic nerve injury

Western blot was used to detect the levels of p75NTR and GAP43 at different time after sciatic nerve injury. It was found that the protein level of p75NTR was higher in MD2-KO mice than in C57BL/6 mice at 12 h and 24 h post sciatic nerve injury, while no difference was found for GAP43 (Figure 6A). Furthermore, we measured the level of GAP43 at 3 days, 1 week, 2 weeks and 3 weeks after sciatic nerve injury. Results showed that the protein level of GAP43 in MD2-KO mice was higher than that in C57BL/6 mice (Figure 6B). We monitored the long-term repair of the sciatic nerve by immunohistochemistry. Higher expression of p75NTR was shown in the MD2-KO group at 3 days, 1 week, 2 weeks and 3 weeks after sciatic nerve injury (Figure 6C). The expression of GAP43 was also increased in the MD2-KO group at 1 week and 2 weeks after sciatic nerve injury (Figure 6D). The above results suggested that there was a better recovery of the sciatic nerve in the MD2-KO mice.

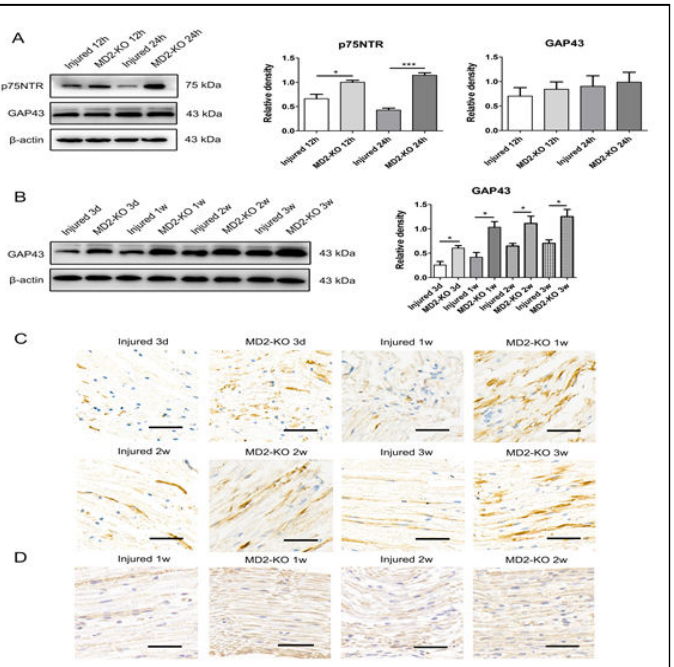

Figure 6: GAP43 and p75NTR were highly expressed in MD2-KO mice after sciatic nerve injury. (A) The protein levels of p75NTR and GAP43 in injured group and MD2-KO group at 12 h and 24 h after nerve injury, (B) Changes in protein level of GAP43 at 3 days, 1 week, 2 weeks and 3 weeks after nerve injury, (C) Immunohistochemical staining of p75NTR with nerve injury for 3 days, 1 week, 2 weeks and 3 weeks (Scale bar=50 μm), (D) Immunohistochemical staining of GAP43 of the sciatic nerve with injury for 1 week, 2 weeks (Scale bar=50 μm). Data are from three independent experiments (n=3 per group; *P<0.05, ***P<0.001).

Sciatic function index analysis

SFI analysis was performed at 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks after surgery to evaluate sciatic nerve recovery. It is a non-invasive, reliable parameter of walking behavior, which developed by Hruska, et al., and de Medinaceli, et al. The SFI score of mice decreased to nearly -100 in the injured group after 1 week of sciatic nerve injury, indicating that the sciatic nerve function of mice was extremely damaged. Compared with the injured group, the SFI score for the MD2-KO group was higher, showing that the MD2-KO mice had better recovery after sciatic nerve injury (Figure 7).

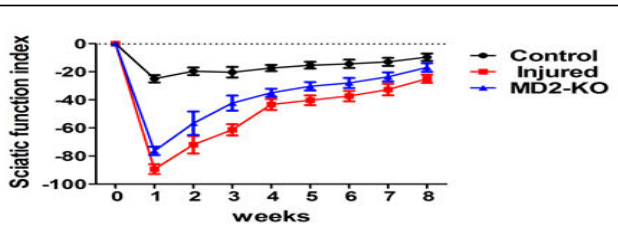

Figure 7: The functional recovery of mice in different groups. We used the SFI to reflect motor function recovery of mice after sciatic nerve injury. The score of a normal SFI is 0, and complete loss of neurological function is −100. SFI results showed that MD2-KO mice have better motor recovery after sciatic nerve injury. Data are from three independent experiments (n=3 per group).

Discussion

According to the different parts connected to the center, the peripheral nerve is divided into cerebral nerve and spinal nerve. The sciatic nerve is a kind of spinal nerve, and it is the longest and thickest nerve in the human body. We selected the sciatic nerve for research and successfully constructed the model of sciatic nerve injury. LFB staining showed that myelin sheath was lightly stained, disorderly distributed and showed obvious demyelination after sciatic nerve injury.

As a regulatory molecule in innate immunity, MD2 plays a wide range of biological functions in pathological processes such as inflammation and infection, the regulation of MD2 may become an effective method for the treatment of diseases. Recently, it was found that hyperglycemia-induced extracellular advanced glycation end products directly combined with MD2 and initiated pro-inflammatory signaling pathway, leading to diabetic cardiomyopathy. It has also been reported that blocking MD2 can prevent inflammation and kidney disease caused by obesity. However, there are few studies on MD2 in peripheral nerve injury. Our study found that the mRNA level of MD2 began to increase after 6 h of nerve injury, immunoblotting and immunofluorescence proved that the level of MD2 protein increased after 24 h of nerve injury. These results suggested the activation of MD2 after sciatic nerve injury.

After peripheral nerve injury, the innate immune system immediately starts to participate in Wallerian degeneration, which is an inflammatory reaction of the nervous system [17]. Schwann cells and macrophages are activated and migrated to the injured site through the corresponding receptors, a large number of inflammatory factors are secreted in the early stage of peripheral nerve injury [19]. TNFα, IL-1β and IL-6 have obvious changes in the early stage of peripheral nerve injury, which are often used as inflammatory monitoring indicators of peripheral nerve injury. Therefore, TNFα, IL-1β and IL-6 were selected as inflammatory indicators to observe the inflammatory response after peripheral nerve injury. Whether MD2 plays an important role in initiating the early inflammatory outbreak and whether blocking MD2 can alleviate the inflammatory reaction, all of which need to be explored. In our experiment, the expressions of TNFα, IL-1β and IL-6 began to increase after 6 h of nerve injury, which was positively correlated with the expression of MD2. The transcription levels of inflammatory factors TNFα, IL-1β and IL-6 in MD2-KO mice were significantly lower than those in C57BL/6 mice at 6 h and 24 h after nerve injury. At 24 h after nerve injury, the protein levels of TNFα, IL-1β and IL-6 increased significantly, while the protein levels of inflammatory factors decreased in MD2-KO mice. These results indicated that blocking MD2 could alleviate the inflammatory reaction after sciatic nerve injury.

In order to explore whether MD2 plays an important role in the repair of sciatic nerve injury, we detected the relevant indexes. P75NTR and GAP43, as marker molecules of sciatic nerve repair and regeneration, are recognized as nerve growth promoting factors. P75NTR is a member of tumor necrosis factor receptor superfamily, which is expressed in Schwann cells in injured nerve. When nerve is injured, its expression level is significantly increased. GAP43 is widely distributed in nerve tissue, which can regulate nerve cell signal transduction, promote synaptic reconstruction and regeneration and play an important role in nerve injury repair. The increased expression of both suggests that nerve repair is better. At 12 h and 24 h after sciatic nerve injury, the level of p75NTR in MD2-KO mice was significantly higher than that in C57BL/6 mice. The expression level of GAP43 in MD2-KO mice was also higher than that in C57BL/6 mice by monitoring the changes of GAP43 in 3 days, 1 week, 2 weeks and 3 weeks after sciatic nerve injury.

These results indicated that after sciatic nerve injury, the inflammatory response of MD2-KO mice was weakened and the expression of related factors that promote nerve repair was increased, which was more conducive to the recovery of nerve function. It is of great significance to regulate and assist the repair of peripheral nerve injury.

Conclusion

The expression of MD2 was enhanced and a large number of inflammatory cytokines were released after sciatic nerve injury. The inflammatory response of MD2 knockout mice was weakened and the sciatic nerve repair and motor function recovery were better.

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Declaration of Conflicting Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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