Int J Clin Exp Med 2015;8(8):13408-13412
www.ijcem.com /ISSN:1940-5901/IJCEM0011815

Original Article

Regional tissue immune responses after sciatic nerve injury in rats

Yu-Ming Chen[1], Ruo-Wu Shen[2], Bei Zhang[3], Wei-Ning Zhang[4]

[1]Department of Neurology, First People's Hospital of Qingdao Economic and Technological Development Zone, Qingdao 266555, Shandong, China; [2]Department of Anatomy, Medical College of Qingdao University, Qingdao 266071, Shandong, China; [3]Department of Immunology, Medical College of Qingdao University, Qingdao 266071, Shandong, China; [4]Department of Gastroenterology, First People's Hospital of Qingdao Economic and Technological Development Zone, Qingdao 266555, Shandong, China

Received June 23, 2015; Accepted August 8, 2015; Epub August 15, 2015; Published August 30, 2015

Abstract: Inflammatory cells play a critical role during nerve regeneration following peripheral nerve injury. In this study, we investigated immune responses in rat sciatic nerve after injury. Wistar rats were randomly divided into the sciatic nerve injury (model) group and control group. The right sciatic nerve of rats in the model group was transected and sutured end-to-end. Our results showed that rats in the model group functionally recovered following sciatic nerve injury. We detected inflammatory cell infiltration in the remaining sciatic nerves following injury. In addition, expression of interferon-γ (INF-γ), interleukin-10 (IL-10), and the INF-γ/IL-10 ratio was significantly elevated one week following nerve injury, but gradually decreased thereafter. Our findings demonstrate that immune responses and inflammatory cell activation are involved during recovery from sciatic nerve injury.

Keywords: Peripheral nerve injury, INF-γ, IL-10, recovery

Introduction

Peripheral nerve injury, most commonly resulting from trauma, is a serious problem that affects a patient's quality of life [1, 2]. Peripheral nerve axotomy disturbs axonal continuity, and is followed by Wallerian degeneration, which is characterized by a typical dying-back pattern (degeneration occurs in the distal segment and progresses toward the proximal region) [3]. Although injured nerves can regenerate, the regeneration rate is relatively slow and functional recovery is typically poor [4]. The precise mechanisms underlying nerve tissue recovery following injury have not yet been fully illustrated. However, local inflammation is a known consequence of peripheral nerve injury [3, 5].

Recent studies highlight the participation of cytokine pathways after peripheral nerve injury [6]. Known cytokines in the regeneration process include interleukin-1 (IL-1), tumor necrosis factor (TNF), IL-6-like, transforming growth factor-β (TGF-β), IL-10, and interferon-γ (IFN-γ) [6]. These cytokines can be released by T helper 1 (Th1) and T helper 2 (Th2) cell subgroups and are therefore known as Th1/Th2 cytokines. Th1/Th2 cytokines are in dynamic equilibrium under physiological conditions, and the interaction between these cytokines is extremely important for the body to maintain normal immune function. Th1 or Th2 bias, or Th1/Th2 disequilibrium, may cause various diseases [7]. Th1 cells primarily secrete IL-2, IFN-γ, and TNF-α. IFN-γ is produced by activated T cells and can promote expression of class II major histocompatibility complex (MHC) molecules, which activates macrophages, improves their function, induces Th0 cell differentiation into Th1 cells, and inhibits Th2 cell activation. IFN-γalso has the ability to induce expressions of other cytokines, and assist with cellular immunologic responses. Th2 cells primarily secrete IL-4, IL-5, and IL-10. Of these, IL-4 and IL-10 can induce the differentiation of Th0 cells into Th2 cells, promote B cell proliferation and antibody production, inhibit Th1 cell activation, and assist with the humoral immune response [5, 7].

Taskinen et al. reported that the mRNA levels of IFN-γ and IL-10 are upregulated after rat sciatic nerve transection [5]. IFN-γ [8] and IL-10 [9, 10]

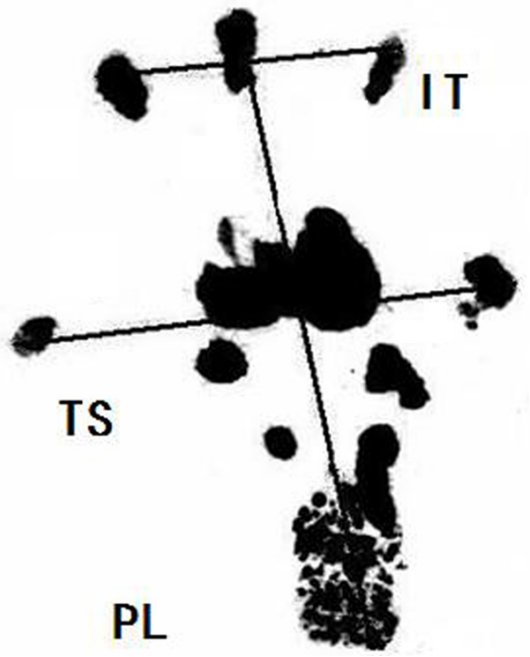

Figure 1. Sketch map of SFI scores.

have been implicated to play predominant roles in immune responses. Nevertheless, the potential involvement of IFN-γ and IL-10 in nerve recovery after peripheral nerve injury remains poorly understood.

In our present study, we established a rat sciatic nerve injury model and investigated local histological changes in injured nerve tissues following injury. In addition, we evaluated the expression of IFN-γ and IL-10 in local injury sites. Our findings provide valuable insights into understanding the molecular mechanisms of functional recovery following peripheral nerve injury.

Materials and methods

Animals

Forty male Wistar rats (aged 2 months) weighing 220-240 g were purchased from the Animal Center of Shandong Lukang Pharmaceuticals Company (Permission No. scxk Lu 20080002). Animals were randomly divided into the experimental group (n=20) or the control group (n=20).

Establishment of a rat model of sciatic nerve injury

In order to establish a rodent model of sciatic nerve injury, rats were anesthetized via intraperitoneal injection of 10% chloral hydrate (300 mg/kg). Animals were fixed in a prone position. A longitudinal incision was made on the posterior side of the femur of the unilateral lower limb. The right sciatic nerve was exposed and was sharply transected 0.8 cm below the piriform muscle. An end-to-end anastomosis of the epineurium was performed using 8-0 atraumatic sutures. After surgery, the incision was closed. The sham operation group was used as control.

Evaluation of peripheral nerve injury

Peripheral nerve injury was analyzed using the sciatic functional index (SFI), which is a standard method used to evaluate the degree of functional loss (or recovery) [11]. High SFI scores correlate with increased functional recovery.

Two footprint parameters, including toe spread (TS), the distance between the first and fifth toes, and paw length (PL), the distance between the tip of the third toe and the most posterior part of the foot in contact with the ground, were measured. SFI scores were determined using the following equation:

$$SFI=-38.3(EPL-NPL)/NPL+109.5(ETS-NTS)/NTS+13.3(EIT-NIT)/NIT-8.8$$

where SFI is the sciatic nerve function index, EPL is the experimental paw length, NPL is the normal paw length, ETS is the experimental toe spread, NTS is the normal toe spread, and IT is the intermediary spread. Walking patterns were recorded using a video camera when the rat walked through the glass runway. Ten frames were used to analyze foot placing. Images were loaded into a computer using a frame grabber as described previously. The sketch map is shown in **Figure 1**.

Hematoxylin-eosin (HE) staining and immunohistochemistry

Rats were intracardially perfused with 4% paraformaldehyde 1, 2, 4, 8, or 12 weeks after the operation. At each time point, the right sciatic nerve near the transection site was removed and fixed in 4% formalin. After 24 hours, formalin-fixed nerves were embedded in paraffin, sectioned, and stained with HE. IFN-γ, IL-4, and IL-10 expression was detected using immunohistochemistry. Samples were deparaffinized and rehydrated, and endogenous peroxidases

Table 1. Sciatic functional index (SFI) values. Data are presented as the mean ± standard deviation (SD)

	Control	One week	Two weeks	Four weeks
SFI	0	-97.84±1.50#	-91.97±3.07#,▲	-86.52±3.61#,▲,※

#P<0.01 compared with control group; ▲P<0.05 compared with 1-week group; ※P<0.05 compared with 2-weeks group. F=53.46, q=3.05~21.18.

Table 2. Average absorbance of IFN-γ and IL-10 in sciatic nerves. Data are presented as the mean ± standard deviation (SD)

	n	Control	1 week	2 weeks	4 weeks
IFN-γ	20	0	0.443±0.078#,*	0.219±0.060#,*	0*
IL-10	20	0	0.524±0.068#,▲	0.287±0.038#,▲	0.133±0.039#,▲
IFN-γ/IL-10	20	0	0.9374±0.263※	0.5241±0.1464※	0※

#P<0.01 compared with the control group; *F=64.50, q=8.06~17.78, P<0.01, paired comparison with IFN-γ in model rats; ▲F=64.73, q=2.90~19.59, P<0.05, paired comparison with IL-10 in model rats; ※F=26.75, q=4.75~10.33, P<0.01, paired comparison with IFN-γ/IL-10 ratio in model rats.

were inactivated by incubating samples in 3% hydrogen peroxide. Sections were then heated in citrate buffer (0.01 M, pH 6.0) and treated with 3% hydrogen peroxide for 5 min at room temperature. Nonspecific reactivity was blocked by incubating samples with 5% bovine serum albumin for 20 min at room temperature. Next, samples were probed overnight at 4°C with the following primary antibodies: monoclonal rabbit anti-mouse IFN-γ (diluted 1:100), IL-4 (diluted 1:100), or IL-10 (diluted 1:100) (Wuhan Boshide Biotech Co. Ltd, China). Sections were exposed to the sheep anti-rabbit IgG secondary antibody for 20 min at 37°C, treated with streptavidin-biotin complex (SABC) (Wuhan Boshide Biotech Co. Ltd, China) for 20 min at room temperature, and stained with 3, 3'-diaminobenzidine tetrahydrochloride (DAB) for 5-30 min. Nuclei were counterstained with hematoxylin. Negative control slides were treated as described above but without the primary antibody incubation. Samples were examined using light microscopy (Olympus BX-50, Tokyo, Japan). The average absorbance was analyzed using an image analysis system (Sample PCI, the US). Five fields were randomly chosen from each slide and the average number of positive cells was counted and recorded.

Statistical analyses

Data were analyzed using SPSS 13.0 and PPMS 1.5 software. Results are shown as the mean ± standard deviation (SD). Comparisons between two groups were conducted using *t*-tests. Univariate analyses of variance and Newman-Keuls tests (q) were used to compare the results of different experimental groups at different time points. P<0.05 was considered statistically significant.

Results

Functional recovery following sciatic nerve injury

Recovery after sciatic nerve injury was determined using the sciatic functional index (SFI). As shown in **Table 1**, SFI values decreased gradually with increased time duration following nerve injury. Significantly reduced SFI scores were detected one week following injury (P<0.01). Compared with other time points, the lowest SFI values were noted 4 weeks after injury (P<0.05). These data suggest that functional recovery gradually increased following sciatic nerve injury.

Histological examinations

No inflammatory cell infiltration was observed in normal control samples. One week after nerve injury, inflammatory cells, including neutrophils, T lymphocytes, and macrophages, were noted in nervous tissues from animals in the model group. We also found notable mesenchymal cell and Schwann cell proliferation in the model group. Two weeks following injury, the number of inflammatory cells increased markedly and we detected increased Schwann cell and fibrous tissue proliferation. Moreover, the proliferated Schwann cells were irregularly arranged. Four weeks following nerve injury, the number of inflammatory cells decreased, whereas irregularly arranged Schwann cells were still observed.

IFN-γ expression in sciatic nerves

In order to understand the role of IFN-γ during recovery from sciatic nerve injury, we analyzed IFN-γ expression using immunohistochemistry. IFN-γ-positive cells were not observed in sciatic

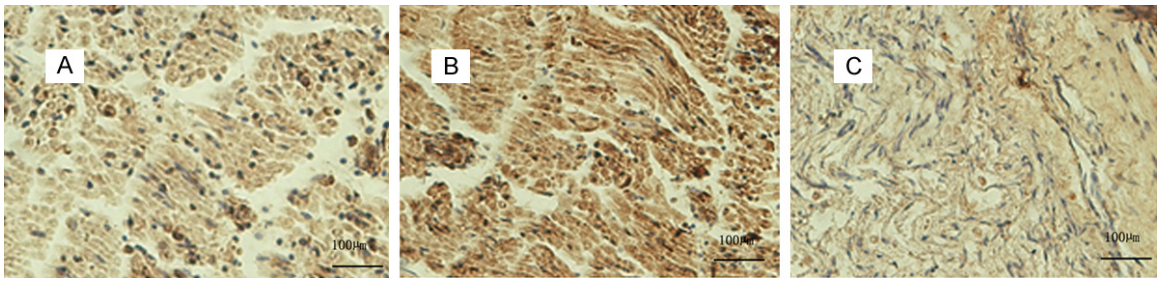

Figure 2. The expression of INF-γ ×200. A. One week after injury, B. Two week after injury, C. Four weeks after injury.

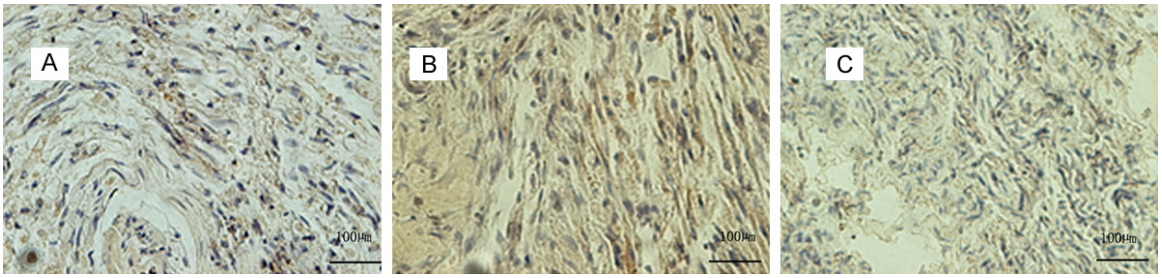

Figure 3. The expression of IL-10 ×200. A. One week after injury, B. Two week after injury, C. Four weeks after injury.

nerves from animals in the control group. We detected significantly increased numbers of IFN-γ-positive cells 1 week following nerve injury ($P<0.01$ compared with control) (**Table 2**; **Figure 2**). However, the number of IFN-γ-positive cells gradually decreased 1 week after injury (1 week vs. 2 weeks and 4 weeks, $P<0.01$; 2 weeks vs. 4 weeks, $P<0.01$).

IL-10 expression in sciatic nerves

Similarly, few IL-10-positive cells were observed in sciatic nerves from mice in the control group. However, IL-10 expression was significantly upregulated in sciatic nerves 1 week after injury and decreased gradually after that (1 week vs. control, $P<0.01$; 1 week vs. 2 weeks, $P<0.01$; 1 week vs. 4 weeks, $P<0.01$) (**Table 2**; **Figure 3**).

IFN-γ/IL-10 ratio

Finally, we calculated the IFN-γ/IL-10 ratio. As shown in **Table 2**, the IFN-γ/IL-10 ratio peaked 1 week following injury and the gradually decreased ($F=26.75$, $q=4.75\sim10.33$, $P<0.01$). Four weeks following injury, the IFN-γ/IL-10 ratio returned to baseline.

Discussion

Peripheral nerve injury is a prevalent condition but nerve regeneration is relatively difficult because of many restrictions. In this study, we examined the immune responses at the injured site following sciatic nerve injury in rats. Our results revealed that during sciatic nerve repair, IFN-γ and IL-10 expression initially increased 1 week following injury but gradually decreased to baseline levels after 4 weeks.

In order to establish a rat model of sciatic nerve injury, the right sciatic nerve of the rats was transected and sutured end-to-end. SFI values decreased as time progressed following injury, suggesting functional recovery following sciatic nerve injury. Histological examinations showed that the number of inflammatory cells increased following nerve injury, whereas inflammatory cell infiltration decreased 4 weeks after injury.

The dynamic equilibrium of Th1/Th2 cytokines is proposed to be important for maintaining normal physiological conditions, and altered cytokine release may lead to pathogenesis. IFN-γ, secreted by CD4+ T lymphocytes, particularly Th1 cells, has an essential role in inflammation [8]. IL-10, a well-known potent anti-inflammatory cytokine, plays distinct roles in injured tissue by suppressing inflammatory cytokine release [10]. IL-10 is primarily secreted by secreted by the Th2 cell subgroup, and affects a variety of cell types in the immune system [9]. Here we found few cells that expressed IFN-γ and IL-10 under normal condi-

tions. However, IFN-γ-positive and IL-10-positive cells greatly increased 1 week after sciatic nerve injury, indicating that both cellular and humoral immunologic responses occur after injury. IFN-γ expression gradually decreased 2 weeks after injury, and IFN-γ levels returned to baseline 4 weeks following injury. These data indicate that the gradual reduction of cellular immunity may contribute to Wallerian degeneration and myelin fragment clearance by macrophages during late stages of nerve repair. The IFN-γ/IL-10 ratio peaked 1 week after injury, and then gradually decreased. In addition, the IFN-γ/IL-10 ratio was less than 1, indicating that Th2 cells and humoral immune responses may have a predominant function during the repair process.

In conclusion, our current data indicate that Th1 and Th2 cytokines undergo dynamic changes in nerve tissues after peripheral nerve injury. Both cellular and humoral immune responses were involved in this process. Cellular immunity may exist in the early stage of nerve repair, whereas humoral immunity contributes throughout the entire process. Immune responses following nerve injuries serve as a double-edged sword because they can promote or inhibit nerve regeneration. It has been reported that T lymphocytes and antigen-presenting cells, such as endothelial cells, promote nerve regeneration [12]. In contrast, local IgG deposition was found after peripheral nerve injuries, and the IgG deposition negatively correlated with nerve regeneration [13]. In future studies, we will continue to search for an efficient approach that improves nerve regeneration by interfering with local immune responses.

Disclosure of conflict of interest

None.

Address correspondence to: Ruo-Wu Shen, Department of Anatomy, Medical College of Qingdao University, No. 308 Ningxia Road, Qingdao 266071, Shandong, China. Tel: +86-18661785993; E-mail: shenruowu@aliyun.com; Yu-Ming Chen, Department of Neurology, First People's Hospital of Qingdao Economic and Technological Development Zone, No. 9 Huangpujiang Road, Qingdao 266555, Shandong, China. Tel: +86-18661676799; E-mail: lantuxiaoqu@126.com

References

[1] Abe N, Cavalli V. Nerve injury signaling. Curr Opin Neurobiol 2008; 18: 276-283.
[2] Robinson LR. Traumatic injury to peripheral nerves. Muscle Nerve 2000; 23: 863-873.
[3] Gaudet AD, Popovich PG, Ramer MS. Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. J Neuroinflammation 2011; 8: 110.
[4] Chan KM, Gordon T, Zochodne DW, Power HA. Improving peripheral nerve regeneration: From molecular mechanisms to potential therapeutic targets. Exp Neurol 2014; 261C: 826-835.
[5] Taskinen HS, Olsson T, Bucht A, Khademi M, Svelander L, Röyttä M. Peripheral nerve injury induces endoneurial expression of IFN-gamma, IL-10 and TNF-alpha mRNA. J Neuroimmunol 2000; 102: 17-25.
[6] Bastien D, Lacroix S. Cytokine pathways regulating glial and leukocyte function after spinal cord and peripheral nerve injury. Exp Neurol 2014; 258: 62-77.
[7] Romagnani S. The Th1/Th2 paradigm. Immunol Today 1997; 18: 263-266.
[8] Kraaij MD, Vereyken EJ, Leenen PJ, van den Bosch TP, Rezaee F, Betjes MG, Baan CC, Rowshani AT. Human monocytes produce interferon-gamma upon stimulation with LPS. Cytokine 2014; 67: 7-12.
[9] Acuner-Ozbabacan ES, Engin BH, Guven-Maiorov E, Kuzu G, Muratcioglu S, Baspinar A, Chen Z, Van Waes C, Gursoy A, Keskin O, Nussinov R. The structural network of Interleukin-10 and its implications in inflammation and cancer. BMC Genomics 2014; 15: S2.
[10] Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989; 170: 2081-2095.
[11] de Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol 1982; 77: 634-643.
[12] Lisak RP, Nedelkoska L, Studzinski D, Bealmear B, Xu W, Benjamins JA. Cytokines regulate neuronal gene expression: differential effects of Th1, Th2 and monocyte/macrophage cytokines. J Neuroimmunol 2011; 238: 19-33.
[13] Stoll G, Jander S, Schroeter M. Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. Adv Exp Med Biol 2002; 513: 87-113.