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Decreased peripheral nerve damage after ischemia–reperfusion injury in mice lacking TNF-alpha

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Abstract

We sought to explore the role of tumor necrosis factor-alpha (TNF-α) in the pathogenesis of peripheral nerve ischemia–reperfusion (IR) injury. We established an ischemia–reperfusion model in wild type (WT) and TNF-α knockout (KO) mice. Electrophysiology, behavioral score and morphological indices (edema and ischemic fiber degeneration [IFD]) were examined to determine the influence of TNF-α on peripheral nerve structure and function following ischemia followed by reperfusion. TNF-α and nuclear factor-kappa B (NF-κB) expression were evaluated using immunohistochemistry. TNF-α KO mice, compared to WT had, in sciatic nerve, marked improvement in nerve pathology. This is a region subject to moderate ischemia–reperfusion injury. There was also a significant improvement in electrophysiological and some behavioral indices. TNF-α and NF-κB expression were abundant in sciatic–tibial nerves of WT mice subjected to IR, but there was less, or complete lack of, expression in ischemic nerve of TNF-α KO mice. We conclude that TNF-α plays an essential role in the pathogenesis of peripheral nerve ischemia–reperfusion injury, possibly partly through the activation of NF-κB.
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Keywords: Ischemia–reperfusion (IR) injury; Peripheral nerve; Tumor necrosis factor-alpha (TNF-α); Nuclear factor-kappa B (NF-κB)

1. Introduction

Ischemia to nerve results in ischemic fiber degeneration (IFD) [1]. Reperfusion following ischemia (IR) results in additional fiber degeneration, and mechanisms of reperfusion injury have been postulated to be due to the additional insult of inflammatory response [2] and oxidative injury [3]. The pro-inflammatory cytokines, especially tumor necrosis factor-alpha (TNF-α), is a major component of the inflammatory response following IR injury and is rapidly upregulated from mRNA level to protein level [4–7]. TNF-α regulates inflammation, immune modulation, and apoptosis by activating nuclear factor-kappa B (NF-κB) and the caspase pathway [8,9]. Studies support a key role of TNF-α in IR injury to the central nervous system [10]. The role of TNF-α in the pathogenesis of peripheral nerve damage is less well understood and the molecular mechanism of TNF-α in IR injury is still unclear.

In this study, we explore the role of TNF-α in IR-induced peripheral nerve injury using TNF-α knock-out (KO) mice. Using the IR model, we evaluate the role of TNF-α on peripheral nerve electrophysiology, structure and function. Using immunohistochemistry, we investigate the signal pathway of TNF-α in IR injury to peripheral nerve.

2. Materials and methods

2.1. Animals

Thirty-four male mice at 6 to 10 weeks of age were used in this experiment. B6,129SF2/J and TNF-α-deficient [TNF (−/−)] mice of strain B6,129S-*Tnf*^{tm1Gkl} (Jackson Laboratory, Bar Harbor, ME) were used as wild type (WT) control (n=20) and TNF-α KO control (n=14), respectively. The experimental protocol was approved by the Mayo Clinic

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Institutional Animal Care and Use Committee and conformed to NIH guidelines for the use of animals in research.

2.2. Ischemia–reperfusion model

The model was produced by ligating and then releasing 6–0 silk suture ties placed around the supplying arteries to the right sciatic–tibial nerve, as previously described [11]. After 2 h of ischemia, while maintaining the right hind limb at 28 °C, the ties were released using a slipknot technique ideal for ready release and rapid reperfusion. The limb temperature was monitored with an intramuscular thermistor probe (Bailey Instruments, Saddle Brook, NJ) and was maintained at 28 °C during ischemia by using an infrared lamp. Animals were studied 7 days after reperfusion.

2.3. Electrophysiology

We used techniques that are standard for our laboratory [12]. The conduction velocity (CV) and amplitude of sensory nerve action potential (SNAP) of digital nerve and compound muscle action potential (CMAP) of sciatic–tibial nerves were measured using fine stainless steel near-nerve stimulating and recording electrodes in this study. SNAP was recorded from the ankle while stimulating the digital nerve at the tip of the digit. CMAP was recorded from the dorsum of the hind paw while stimulating at the level of the sciatic notch. Recordings were done at 35.0 °C, amplified 1000-fold and stored on computer disk, and analyzed offline using Nicolet digital oscilloscope (Nicolet Instruments, Madison, WI).

2.4. Behavioral score

Evaluation of limb neural function was performed using our standard behavioral scoring system [4]. The function of the limb was scored with the observer blinded to the status of the mice. Scores ranged from 0 (no function) to 20 (normal function). The score was based on gait (0 = no function to 3 = normal), paw position (0 = no flexion to 3 = normal), grasp (0 = no grasp to 3 = normal), pinch sensitivity (0 = absent to 2 = present), discoloration (0 = blackened to 3 = normal), inflammation (0 = marked to 3 = normal), and self-mutilation (0 = foot completely removed to 3 = normal). Increasing function was indicated by a larger score.

2.5. Neuropathology: edema and ischemia fiber degeneration (IFD)

After anesthesia with pentobarbital, mice underwent intracardiac perfusion with 4% paraformaldehyde. Following fixation, the entire lengths of sciatic–tibial nerves were harvested. The nerves were divided into four segments: proximal sciatic, distal sciatic, mid tibial, and distal tibial. The distal tibial and distal sciatic nerves were osmicated, dehydrated, infiltrated, and embedded in Spurr's resin. Transverse sections of 10 μm were stained with 1% toluidine blue. Under 400× magnification, these sections were graded for edema and IFD using previously described methods [13]. Fibers were considered to be undergoing IFD if axonal changes were visible. The axon may be swollen or shrunken, watery and light, or dark and shrunken. Secondary myelin changes were typically seen including attenuation, collapse, or breakdown. For each section the percent of fibers undergoing IFD was graded from 0 to 4 as follows: 0 ≤ 2%; 1 = 3–25%; 2 = 26–50%; 3 = 51–75%; 4 ≥ 75%. Edema was semiquantitatively graded from 0 to 4 as follows: 0 = normal; 1 = mild edema; 2 = moderate edema; 3 = severe edema; 4 = severe and global edema. No distinction was made as to endoneurial, perivascular, or subperineurial edema.

2.6. Immunohistochemistry

After intracardiac perfusion with 4% paraformaldehyde, the proximal sciatic and mid tibial nerves were harvested and post-fixed in 2% paraformaldehyde for 24 h, immersed in 30% sucrose for 24 h, covered with OCT compound, frozen with liquid nitrogen, and then stored at −80 °C. Frozen nerves were cut into 10 μm transverse sections in a freezing microtome (Microm Cryostat, HM 505 E, Carl Zeiss, Thornwood, NY) and thaw-mounted on gelatin-coated slides. Sections were stained with the following antibodies: rabbit anti-NF-κB antibody and goat anti-TNF-α antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). We used NF-κB p65 antibody, which binds to p65 subunit. A marked increase in staining occurs precisely because of NF-κB activation, such being the property of the antibody. Additionally, most of NF-κB staining was localized to Schwann cells, where staining was usually intense, suggesting a translocation of NF-κB p65 into nucleus in Schwann cell after ischemia/reperfusion injury. Filtered 3,3′-diaminobenzidine

Table 1
Behavioral score and electrophysiology in wild type and TNF-α knock out mice after ischemia–reperfusion injury

Group	Side	BS	SNAP		Sciatic CMAP	
			CV (m/s)	Amp (μV)	CV (m/s)	Amp (mV)
Wild	Left	20	27.42 ± 0.48	29.38 ± 2.76	19.00 ± 0.62	10.08 ± 0.23
	Right	11.3 ± 1.1	10.75 ± 3.33	6.89 ± 2.52	12.18 ± 1.86	1.31 ± 0.48
TNF-α −/−	Left	20	27.77 ± 0.84	22.82 ± 2.22	17.37 ± 0.52	9.92 ± 0.70
	Right	12.9 ± 1.1	12.56 ± 4.17	4.68 ± 1.76	16.92 ± 2.59*	1.89 ± 1.04

BS, Behavioral score; CV, Conduction velocity; Amp, Amplitude.
*$p < 0.05$ (WT vs. TNF-α KO).

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