MACROPHAGE DEPLETION ALTERS THE BLOOD NERVE BARRIER WITHOUT AFFECTING SCHWANN CELL FUNCTION AFTER NEURAL INJURY

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INTRODUCTION: Until recently, there has been a limited understanding of the cellular and molecular pathogenesis of chronic nerve compression (CNC) injuries (1-3). Previous work has revealed that during the early phases of CNC injury, axonal pathology or degradation is morphologically absent while Schwann cells undergo a dramatic process of proliferation and apoptosis. Furthermore, there is a slow, gradual macrophage recruitment, which contrasts with the immediate sharp increase in macrophage number after acute neural injuries. Within the region of CNC injury, there is increased vascular permeability and neural vascularity. It remains unknown what the triggers are that induce the alterations in vascularity and Schwann cell number as a result of CNC injury. As macrophages have been shown to produce cytokines that may alter neural vascularity and affect Schwann cell response to injury, we sought to explore the role of macrophages in CNC injury by selectively depleting the population of hematogenously-derived macrophages in nerves undergoing CNC injury and evaluating both the integrity of the blood-nerve barrier and Schwann cell function.

METHODS:

Surgical Technique. A previously described model of nerve compression was applied to one-month old Sprague rats (1-3). A dorsal gluteal-splitting approach was used to expose both sciatic nerves of each animal. The right sciatic nerves were mobilized and a sterile one-inch biologically inert tube (I.D. of 1.3 mm) wasatraumatically placed around each nerve with the mobilized left sciatic nerves as a control. Electrodiagnostic studies were performed at the time of specimen harvest and nerve samples were harvested at both two weeks one month after surgery. Nerve segments at the site of, 5 mm distal to, and 5 mm proximal to compression were harvested for these experiments. IRB approval was obtained for animal use from the University’s IACUC.

Macrodepletion via Injection of Clodronate Liposomes. As previously described (4), 86 mg phosphatidylcholine and 8 mg cholesterol (Sigma, St. Louis, MO) at a molar ratio of 6:1 (140 μmol of total lipid) were dissolved in 10 ml chloroform in a 500-mL round-bottom flask then dried in vacuo on a rotary evaporator to form a film. The film was dispersed into liposomes after the addition of 2.5 g dichloromethylene bisphosphonate (Cl2MBP or clodronate; a gift from Boehringer Mannheim GmbH, Mannheim, Germany) to 10 ml PBS. The preparations were kept at room temperature for 2 h, sonicated for 3 min at 20°C (50 Hz), and then kept at room temperature for an additional 2 h. The liposomes were centrifuged at 100,000 × g for 30 min and resuspended in 4 ml PBS. Injections (2 ml/injection) of Cl2MBP-liposomes were made at days 1, 3, and 6 post-injury into the tail vein. Evaluation of Blood-Nerve Barrier. Integrity of the blood-nerve barrier (BNB) was evaluated using Evans Blue Albumin (EBA) which was prepared by mixing 1% Evans Blue dye with 5% bovine albumin in distilled water. Under anesthesia, 3 ml of EBA was injected into the right femoral vein with a16-gauge needle. EBA was allowed to circulate for 15 minutes before sciatic nerves were harvested and preserved in 4% PFA at 4°C. Nerves were then frozen embedded in OCT compound, and cut into 10-mm-thick sections. Frozen sections were placed on microscope slides, mounted and counterstained with DAPI, and then visualized using a fluorescent microscope. The functionality of the blood-nerve barrier was evaluated by comparing the fluorescence of EBA within the endoneurial microvessels compared to the surrounding endoneurium. Immunostaining for ED-1 for Macrophage Identification. Frozen sections of sciatic nerves were fixed in acetone at 4°C for 10 minutes for ED-1 labeling. After rinses in with PBS, slides were immersed in 4% normal goat serum/PBS blocking solution for 1 hour. The sections were then incubated with anti-rat macrophage monoclonal antibody clone ED-1 (1:300, Chemicon) overnight at 4°C. Following PBS washes, slides were incubated in FITC-labeled goat anti-mouse IgG (1:200, Chemicon) for 1 hour. After PBS washes, slides were counterstained and mounted with DAPI and viewed under a fluorescent microscope.

RESULTS: At the two week time point, there were dramatically fewer macrophages present in macrophage-depleted nerves compared to non-depleted nerves (Figure 1). The efficacy of the clodronate-liposomes injections to remove hematogenously-derived macrophages was confirmed by IHC staining of the spleen.

Two weeks of CNC injury, there was a notable increase in EBA fluorescence present in the endoneurium of proximal, distal, and compressed nerve sections compared to normal, uninjured nerve. Leakage of EBA into the endoneurium was most obvious in compressed nerve sections, followed by distal sections, with proximal sections having almost normal BNB integrity. The blood vessels of normal nerve sections had discrete boundaries while blood vessels within CNC nerves did not remain distinct, indicating a less functional BNB. At one month, the integrity of the BNB was further altered within the endoneurial vasculature. Macrophage depletion attenuated the disintegration of the blood-nerve barrier at all observed time points. Evaluation of Schwann cell nuclei in CNC nerves indicated that the total number of cell nuclei between compressed, proximal, and distal sections of macrophage-depleted and non-depleted animals do not differ quantitatively. This indicates that the Schwann cell response to CNC injury is likely independent of macrophages.

DISCUSSION: Our data reveals that macrophages are largely responsible for the increased vascular permeability associated with CNC injury. Macrophage recruitment has been shown to closely mirror an up-regulation in inducible nitric oxide synthase in response to CNC injury (2), which may be one mechanism of the observed breakdown of the BNB. However, as the BNB remains partially compromised after CNC injury even in the absence of macrophages, alternative mechanisms must be present that may involve other cell lines. As Schwann cells have been shown to up-regulate the vasoactive peptide-vascular endothelial growth factor (3), they may be partially responsible for the failure of the BNB after CNC injury. In conclusion, as Schwann cells proliferate after CNC injury in the absence of macrophages, it is likely that the Schwann cell response to CNC injury is not derived from macrophage-derived mitogenic signals, but rather must be mediated via alternative mechanisms. Further experimentation is required to determine the stimuli for Schwann cell proliferation secondary to CNC injury.

REFERENCES

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