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Spinal Cord Regeneration: Reactive Gliosis and Cell Death After Traumatic Injury

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Traumatic injury to the spinal cord initiates a complex cascade of degenerative events which results in functional impairment. Several factors are responsible for limiting functionally effective regeneration in injured spinal cord. These include (i) the formation of an astroglial scar which is believed to be an impediment to successful axonal regeneration and (ii) neuronal and glial apoptosis following spinal cord injury. Our laboratory has used a multidisciplinary approach to investigate: (i) the mechanisms underlying the development of reactive astrogliosis in vitro and following spinal cord injury, (ii) the signaling transduction pathways for TNF--initiated neuroal and glial apoptosis after spinal cord injury.

We demonstrated in cultured astrocytes that acidic pH initiates a rapid increase in immunoreactivity for GFAP (GFAP-IR), a hallmark of reactive gliosis (2; Oh et al., *Glia* **13:** 319-322, 1995). We extended these studies by investigating the effects of certain treatments on reactive gliosis developing *in situ* in a rat spinal cord injury model (Fig. 1). A significant reactive gliosis was observed within two days of cord lesion in untreated crush or vehicle-treated, crush control animals as evidenced by increased GFAP-IR. By contrast, infusion of PIPES buffer (pH 7.4) into the lesion site significantly reduced this increase. The increased GFAP-IR appeared to be linked to Ca^{2+} influx since infusion of a blocker of L-type calcium channels, nifedipine, reduced the ensuing reactive gliosis significantly. While Ca^{2+} modulates many signaling pathways within cells, its effect on reactive gliosis appeared to result from an activation of calpain I. Calpain inhibitor I, a selective inhibitor of m-calpain, also significantly reduced reactive gliosis. However, calpain inhibitor II, a close structural analog which blocks m-calpain, had no salutary effect. We suggest, therefore, that the initial reactive gliosis seen *in vivo* may result from the activation of a neutral, Ca2+-dependent protease, calpain I, through calcium influx.

We previously showed (Lee et al., Exp. Neurol., in press) that after traumatic spinal cord injury (SCI), tumor necrosis factor-α (TNF-α) may serve

as an external signal initiating apoptosis in neurons and oligodendrocytes (Fig. 2). To further characterize the apoptotic cascade initiated by TNF-α after SCI, we examined the expression of TNF-α, nuclear factor-κB (NF-κB), inducible nitric oxide (NO) synthase (iNOS) and the level of NO. Immunocytochemical, western blot and reverse transcription polymerase chain reaction (RT-PCR) analyses showed an early upregulation of TNF-α and NF-κB after injury (Fig. 3). Peak expressions of TNF-α and NF-κB were observed by 1 hr after injury. By 4 hr after injury, the expression of iNOS and the level of NO were markedly increased in the injured spinal cord. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling (TUNEL)-positive cells were also first observed in the lesioned area 4 hr after SCI (Fig. 4). The largest number of TUNEL positive cells was observed between 24-48 hr after injury. Injecting a neutralizing antibody against TNF-α into the lesion site after injury significantly reduced the expression of such downstream molecules as NF-κB and iNOS, reduced the level of NO, and reduced the number of apoptotic cells in the injured spinal cord. The injection into the lesion site of two NOS inhibitors, NG-monomethyl-L-arginine monoacetate (L-NMMA) and S-methylisothiourea sulfate (SMT), and an NO scavenger, carboxy PTIO, also significantly reduced the level of NO and the number of apoptotic cells in the injured spinal cord. These data suggest that apoptosis initiated by TNF-α after SCI is mediated in part by a feedback loop via the NO produced in response to enzymatic synthesis by NOS which enzyme is itself expressed by induction via TNF-α.

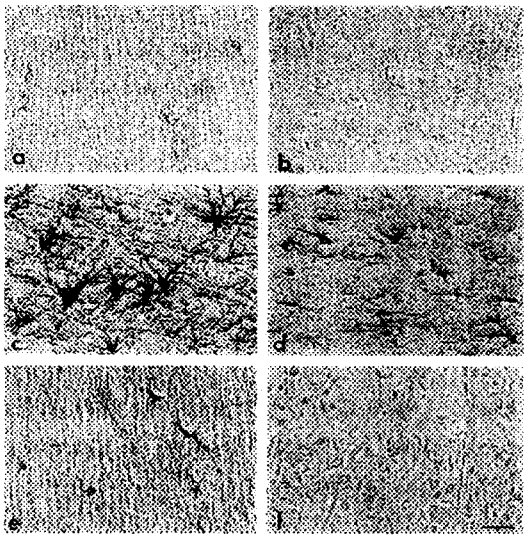

Figure 1. Effect of buffer treatment on development of reactive gliosis following a crush lesion to rat spinal cord. Two days after lesioning, spinal cords were isolated and processed for GFAP-IR. a: White matter from unoperated controls. b: Gray matter from unoperated controls. c: White matter of vehicle crush control (saline 1 2% DMSO). d: Gray matter of vehicle crush control. e: White matter of crushed spinal cord treated with buffer, PIPES (pH 7.4). f: Gray matter of crushed spinal cord treated with PIPES (pH 7.4).

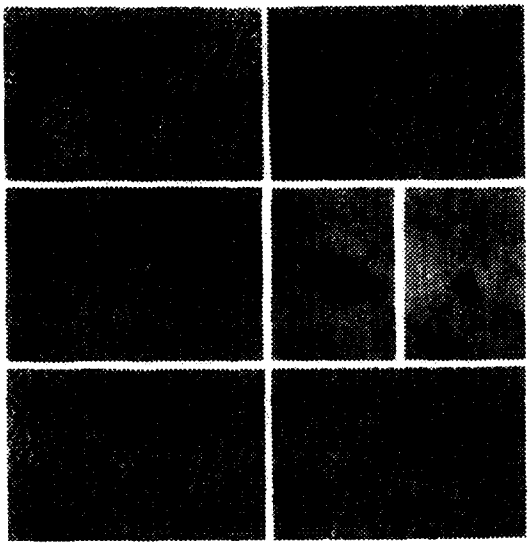

Figure 2. Tunel labeling and TNF-α immunoreactivity in rat spinal cord after injury. A: TUNEL-positive cells in the gray matter. B: TUNEL-positive glial cells in the white matter 24hr after injury. C: TUNEL-positive cells 24hr after injury treated with an antibody that neutralizes TNF-α. D: Double-labeling of TUNEL-positive cells with NSE. E: Double-labeling of TUNEL-positive cells with APC. F: TUNEL-positive cells 24 hr after injury treated with the inhibitor to NOS, L-NMMA. G: TNF-α immunoreactivity 1 hr after injury.

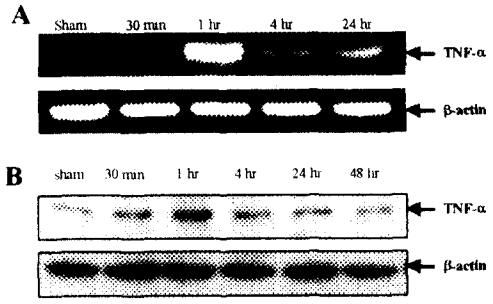

Figure 3. Temporal expression of TNF-α after spinal cord injury. Tissues were obtained at the each time after spinal cord injury. A: The result of RT-PCR analysis of TNF-α mRNA. B. The result of Western blot analysis of TNF-α protein.

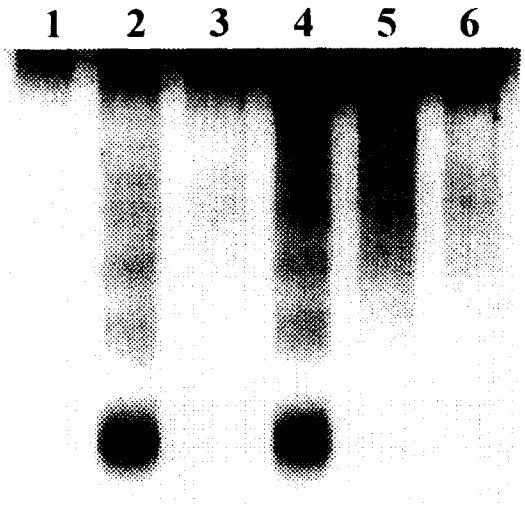

Figure 4. DNA laddering after spinal cord injury following treatment of pharmacological agents. Each pharmacological agent was injected into lesion site immediately after SCI. Spinal cord samples were harvested 24 hr postinjury. Genomic DNA was isolated and labeled with $p^{32}dCT$. Lane 1, Sham-operated; lane 2, Vehicle (non-immunized serum, 4 l); lane 3, Neutraling Ab to TNF- (4 l); lane 4, Vehicle (DMSO, 2 l); lane 5, SMT (2 l); lane 6, Carboxy-PTIO (2 l)