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[Experimental Studies]

Modulation of Macrophage and Microglial Responses to Axonal Injury in the Peripheral and Central Nervous Systems

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Abstract

OBJECTIVE: After axonal injury, macrophages rapidly infiltrate and become activated in the mammalian peripheral nervous system (PNS) but not the central nervous system (CNS). We used the dorsal root pathway to study factors that modulate the response of macrophages to degenerating axons in both the PNS and the CNS.

METHODS: Lewis rats underwent transection of dorsal roots (Group I), stab within the spinal cord (Group II), crush at the dorsal root entry zone (Group III), transection of dorsal roots combined with a CNS lesion (Group IV), or systemic administration of a known activator of macrophages, lipopolysaccharide, alone (Group V) or combined with transection of dorsal roots (Group VI). ED-1 antibody stained for macrophages and activated microglia at 7, 14, and 42 days postinjury.

RESULTS: At early time points, Group I demonstrated ED-1 cells in the PNS but not the CNS portion of the degenerating dorsal roots. Group II revealed ED-1 cells near the stab lesion. Group III demonstrated ED-1 cells adjacent to the dorsal root entry zone crush site. Group IV revealed ED-1 cells along both the PNS and the CNS portions of the degenerating dorsal roots when the CNS lesion was placed near the transected roots. Group V demonstrated few ED-1 cells in the PNS and the CNS, whereas Group VI revealed a marked ED-1 cellular response along both the PNS and the CNS portions of the transected dorsal roots.

CONCLUSION: Local CNS trauma and systemic administration of lipopolysaccharide can "prime" macrophages/microglia, resulting in an enhanced response to degenerating axons in the CNS. Such priming might prove useful in promoting axonal regeneration.

The response of infiltrating monocytes to Wallerian degeneration of axons is dramatically different in the adult mammalian peripheral nervous system (PNS) and central nervous system (CNS) (2, 11, 14, 24, 26, 34, 39, 42, 45, 46, 48, 50, 55, 58). After axonal injury in the PNS, circulating monocytes rapidly infiltrate the site of injury and, after a delay of approximately 24 hours, the distal degenerating segment of nerve (3, 5, 6, 18, 25, 29, 39, 43, 44, 46, 56). In contrast, the response of circulating monocytes and resident microglia to degenerating axons in the CNS is greatly delayed (19, 35, 45, 46, 52, 57, 58). For example, George and Griffin (19) did not observe a significant macrophage/microglial response to degenerating axons in the CNS until 3 weeks postinjury. This delayed response in the CNS results in a much slower breakdown and removal of axonal and myelin debris, the latter of which contains molecules shown to be inhibitory to axonal regeneration (7, 11, 13, 19, 32, 44, 51, 62).

Several studies have attempted to identify factors in both the PNS and the CNS that account for this differential cellular response to axonal injury (26, 35, 47). A confounding factor in most of these studies was that comparisons between the PNS and CNS were conducted on different axonal pathways, such as the sciatic nerve in the PNS and the optic nerve in the CNS. To eliminate this variable, we characterized the macrophage/microglial response to injury along a single axonal pathway, the dorsal sensory root, which traverses both the PNS and CNS (19). Parasagittal sections of this pathway provided us with the unique opportunity to directly compare the macrophage/microglial response to degenerating axons after transection of a dorsal root in the PNS and CNS simultaneously. We then attempted to enhance and accelerate the inflammatory response along the CNS portion of this axonal pathway using two experimental strategies. The first strategy involved making a CNS lesion adjacent to the dorsal root entry zone (DREZ) of the transected dorsal root. The DREZ represents the interface between the PNS and the CNS and in the rat occurs very close to the surface of the spinal cord. The second strategy involved systemically administering a known macrophage activator, lipopolysaccharide (LPS), at the time of dorsal root transection (1, 27, 28, 40, 41, 59). Both of these strategies accelerated and enhanced the CNS macrophage/microglial response to degenerating dorsal root axons.

MATERIALS AND METHODS

Animal surgeries

Adult male Lewis rats, 200 to 300 g, obtained from Charles River Breeding Laboratories (Raleigh, NC), were anesthetized by intraperitoneal (i.p.) administration of pentobarbital (50 mg/kg). This initial dose was supplemented with additional doses as needed. Once the animals were deeply anesthetized, the dorsal spine was shaved and prepared with alcohol and povidone iodine, and a skin incision was made in the thoracolumbar region. Bilateral laminectomies were performed under microscopic magnification, exposing the dura mater. An initial dural opening was made, and the following experiments were performed: in Group I animals (n = 18), the L5-L6 dorsal roots were transected 5 mm from their entry point into the spinal cord. In Group II animals (n = 4), a stab lesion was made in the right dorsal column of the spinal cord using a Hamilton 10-μL gas-tight syringe with a 33-gauge needle (VWR Scientific Products, Brisbane, CA) at the level of the L5-L6 DREZ. In Group III animals (n = 4), a crush injury was performed in which the right L5-L6 roots were crushed at the DREZ three times for 10-second periods with fine forceps to ensure adequate destruction of axons. In Group IV animals (n = 4), transection of the right L5-L6 roots was combined with a contralateral DREZ crush injury performed either far from (C4-C5, n = 2) or near to (L5-L6, n = 2) the transected roots on the right. The contralateral DREZ crush was made far from the transected dorsal roots on the right by extending the skin incision to the cervical vertebral level and performing a left hemilaminectomy followed by a crush of the C4-C5 dorsal roots at the DREZ. Group V uninjured control animals (n = 6) received daily i.p. injections of 1 mg LPS (*Escherichia coli*) over 4 consecutive days. In Group VI animals (n = 6), transection of the right L5-L6 dorsal roots was performed, followed by administration of daily i.p. injections of 1 mg LPS over 4 consecutive days, with the first

injection administered on the day of the operation.

In earlier experiments, animals received daily i.p. injections of 1 mg LPS on the 4 consecutive days before surgery. This resulted in an unacceptably high intraoperative mortality rate from hemorrhaging and edema, however, in LPS-pretreated animals. This protocol was therefore abandoned in favor of postoperative LPS administration.

After each procedure, the dura was reapproximated using interrupted 10-0 sutures (Ethicon, Inc., Somerville, NJ) or covered with Gelfilm (Pharmacia & Upjohn, Bridgewater, NJ). The paraspinal muscles were reapproximated with interrupted 4-0 sutures (Ethicon, Inc.), and the skin was closed with autoclips. Throughout the operative procedures, sterile technique was maintained.

Histological processing

The animals were killed with an overdose of i.p. pentobarbital (250 mg/kg) on the following postoperative days: Group I on postoperative Day 7 (n = 6), 14 (n = 6), or 42 (n = 6), Group II on postoperative Day 14 (n = 4), Group III on postoperative Day 14 (n = 4), Group IV on postoperative Day 14 (n = 4), and Group VI on postoperative Day 7 (n = 6). Group V animals (n = 6) were killed 7 days after receiving the initial i.p. injection of LPS. After the animals were killed, they were immediately perfused transcardially with 60 ml of 0.1 mol/L phosphate-buffered saline (PBS) (pH 7.4) followed by 300 ml of 4% paraformaldehyde. The spinal cords and attached dorsal roots were then dissected *en bloc*. The sciatic nerves were also harvested bilaterally from all animals. All tissue was then placed in 10% neutral phosphate-buffered formalin solution for 24 hours, embedded in paraffin, cut in the parasagittal plane (8-µm-thick sections), heat-mounted onto 3-aminopropyltriethoxysilane-coated glass slides, and stained with hematoxylin and eosin for cell architecture and Luxol fast blue for myelin.

Immunohistochemistry

Mounted sections of spinal cord and attached dorsal roots or sciatic nerves were immunohistochemically stained using the avidin-biotin-peroxidase complex method (33). Sections were deparaffinized in xylene for two changes of 10 minutes each, rinsed in absolute ethanol, and then immersed in a solution of 30% hydrogen peroxide in anhydrous methanol for 10 minutes to block endogenous peroxidase. Sections were rehydrated with a graded series of decreasing ethanol concentrations for 2 minutes each and finally placed in two changes of 0.1 mol/L PBS for 10 minutes each. After preincubation with serum block (2% rat and 2% horse serum in 0.1 mol/L PBS), the sections were incubated with the primary monoclonal ED-1 antibody (Serotec, Oxford, UK) overnight at 4°C followed by three washes in PBS for 10 minutes each. Sections were then incubated for 2 hours at room temperature with the secondary antibody (rat-absorbed, biotinylated horse anti-mouse immunoglobulin G antibody; Serotec) and then washed three times in 0.1 mol/L PBS for 10 minutes each. Horseradish peroxidase-streptavidin (Zymed Laboratories, South San Francisco, CA), diluted 1:800 with 0.1 mol/L PBS, was subsequently added to each slide, and the slides were incubated for 90 minutes. The sections were washed two times with 0.1 mol/L PBS for 10 minutes each, followed by a wash in 0.05 mol/L Tris (pH 7.4) for 10 minutes. Visualization of the immunoreactive sites was achieved using 3,3'-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) as the chromogen. Sections were then dehydrated in a graded series of ascending ethanol concentrations and finally coverslipped with Permount (Carl Zeiss, Inc., Thornwood, NY).

RESULTS

Peripheral dorsal root transection

Parasagittal sections of dorsal roots entering the spinal cord allowed us to directly compare the macrophage/microglial response to degenerating axons in both the PNS and the CNS. A schematic representation of the dorsal root transection experiment (Group I) is depicted in Figure 1A. After peripheral transection of dorsal roots, ED-1-positive cells are seen in the PNS portion of the injured root but not in the CNS portion at both 7 days (Fig. 5A) and 14 days (Fig. 1B) postinjury. However, by 42 days after dorsal root transection, a robust ED-1-positive cellular response is seen in both the PNS and the CNS portions of the injured pathway (Fig. 1C). Many of the ED-1-positive cells are large, elongated, and multivacuolated, consistent with the morphology of activated macrophages.

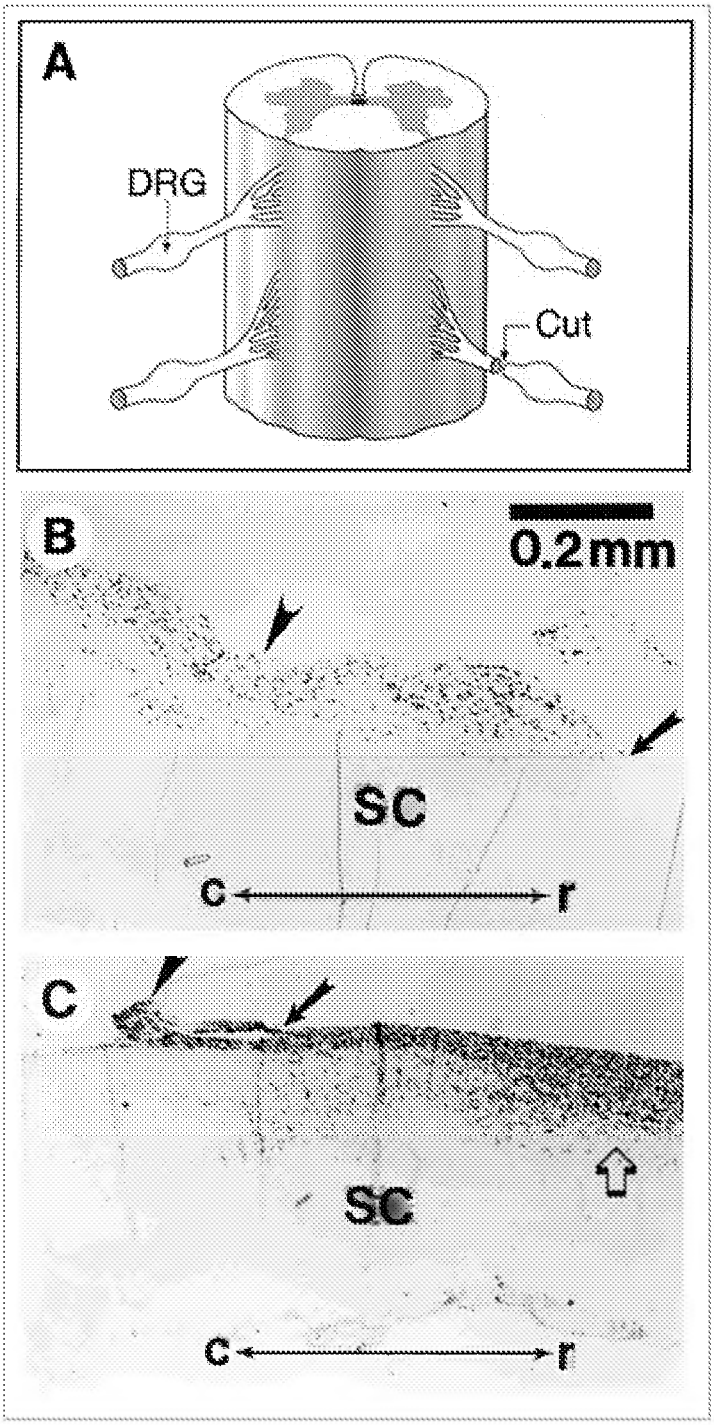

FIGURE 1. *A*, schematic representation of the dorsal root transection experiment, depicting the spinal cord and the entering dorsal roots and indicating the site of transection between the dorsal root ganglion (*DRG*) and its entry into

the dorsal spinal cord (*bent arrow*). *B*, photomicrograph of a parasagittal section through the spinal cord (*SC*) showing an entering dorsal root (*arrowhead*) stained with ED-1, 14 days after transection. *c*, caudal; *r*, rostral. Note that the stained macrophages are confined to the PNS portion of the degenerating dorsal root (*arrowhead*) and do not extend beyond its entry point into the CNS (*arrow*). *C*, photomicrograph of a parasagittal section stained with ED-1, 42 days after dorsal root transection. The ED-1-positive cells in the degenerating dorsal root (*arrowhead*) are seen past the entry point of the dorsal root into the CNS (*closed arrow*) and extend rostrally in the dorsal column of the spinal cord (*open arrow*). The 0.2-mm magnification bar in *B* also applies to *C*.

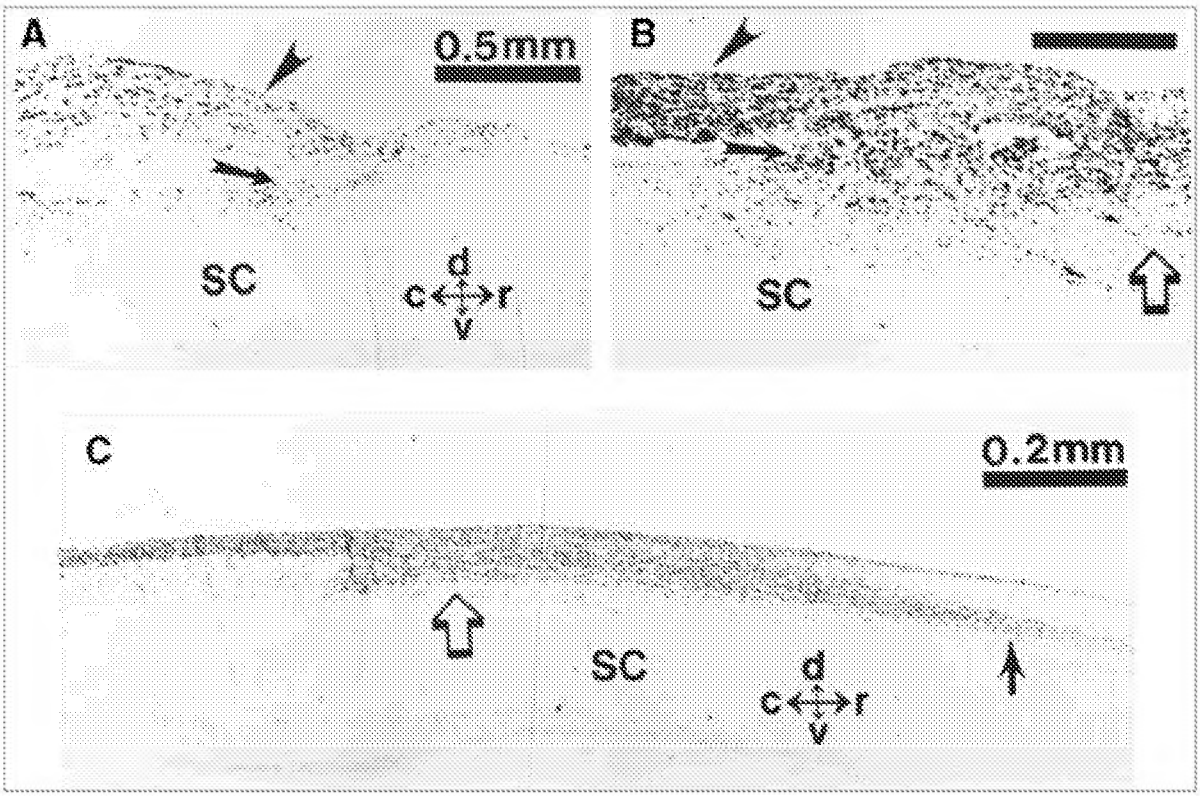

FIGURE 5. Photomicrographs of parasagittal sections through the spinal cord (*SC*), showing cut dorsal roots (*arrowheads*) entering the spinal cord (*closed arrows*) stained with ED-1, 7 days after dorsal root transection. *c*, caudal; *r*, rostral; *d*, dorsal; *v*, ventral. *A*, ED-1-stained cells confined to the PNS portion (*arrowhead*) of a transected degenerating dorsal root in a control animal not exposed to LPS. The 0.5-mm magnification bar in *A* also applies to *B*. *B*, ED-1-stained cells in both the PNS (*arrowhead*) and CNS (*open arrow*) portions of the degenerating dorsal root in an animal injected with LPS after dorsal root transection. Note the medium-sized ameboid and round morphology of many of the cells in both the PNS and CNS, indicating active phagocytosis. *C*, lower-power parasagittal photomicrograph showing ED-1-stained cells extending rostrally in the dorsal column (*open arrow*) of the spinal cord in an animal injected with LPS after dorsal root transection. Note how the ED-1-positive cell column is displaced ventrally (*closed arrow*) by entering intact dorsal root axons as it extends rostrally, following the course of the degenerating axons.

CNS lesions

After a direct needle stab into the dorsal column of the spinal cord (Group II; Fig. 2A), a robust, highly localized ED-1-positive cellular response is seen at 14 days postinjury (Fig. 2B). There is no extension of the ED-1-positive cells rostrally along the injured and degenerating axons. Figure 2C shows a diagram of a crush injury made at the DREZ (Group III) that occurs very close to where the dorsal root enters the spinal cord in the rat, thereby directly and simultaneously injuring both the PNS and the CNS portions of the dorsal root pathway. In contrast, cut lesions were performed more distally and directly injured only the PNS portion of the dorsal root, as illustrated in Figure 1A. After a crush injury, the ED-1-positive cellular response at 14 days is most dense at the DREZ, the site of the crush, and tapers off both peripherally in the PNS and centrally in the CNS (Fig. 2D).

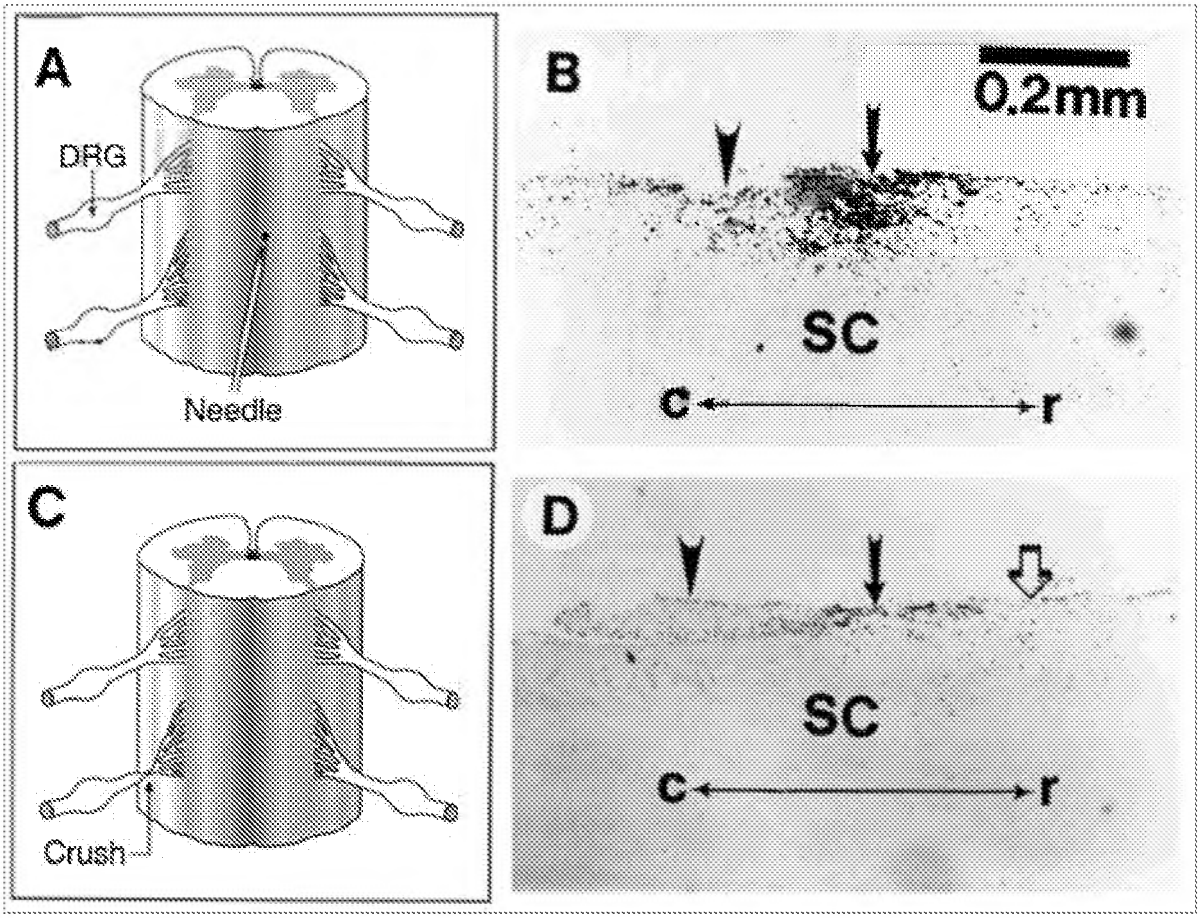

FIGURE 2. *A*, schematic representation of a direct stab injury to the CNS depicting the spinal cord, the entering dorsal roots, and the dorsal root ganglion (*DRG*). *B*, photomicrograph of a parasagittal section through the spinal cord (*SC*) stained with ED-1, 14 days after a direct stab injury. *c*, caudal; *r*, rostral. The ED-1-positive cells (*arrow*) are seen adjacent to the area of the stab (*arrowhead*) and do not extend rostrally in the dorsal column. *C*, schematic representation of a crush injury made at the DREZ (*bent arrow*), depicting the spinal cord and entering dorsal roots. *D*, photomicrograph of a parasagittal section through the spinal cord (*SC*) and entering dorsal root stained with ED-1, 14 days after the DREZ crush injury. *c*, caudal; *r*, rostral. Most ED-1-positive cells are seen at the site of the crush injury (*closed arrow*), with a few cells located proximally in the dorsal root (*arrowhead*) and rostrally within the dorsal column of the spinal cord (*open arrow*). The 0.2-mm magnification bar in *B* also applies to *D*.

Combined dorsal root transection and DREZ crush lesions

Schematic representations of experiments in which a dorsal root transection was combined with a contralateral DREZ crush lesion are shown in Figure 3, A and C (Group IV). When the DREZ crush lesion is placed far away from the entry zone of the transected dorsal root (Fig. 3A), ED-1-positive cells are seen in the PNS but not the CNS portion of the transected dorsal root at 14 days postinjury (Fig. 3B). When the DREZ crush lesion is performed close to the entry zone of the transected dorsal root, a robust ED-1-positive cellular response is seen in both the PNS and the CNS portions of the transected root at 14 days postinjury (Fig. 3D) and extends rostrally in the dorsal column.

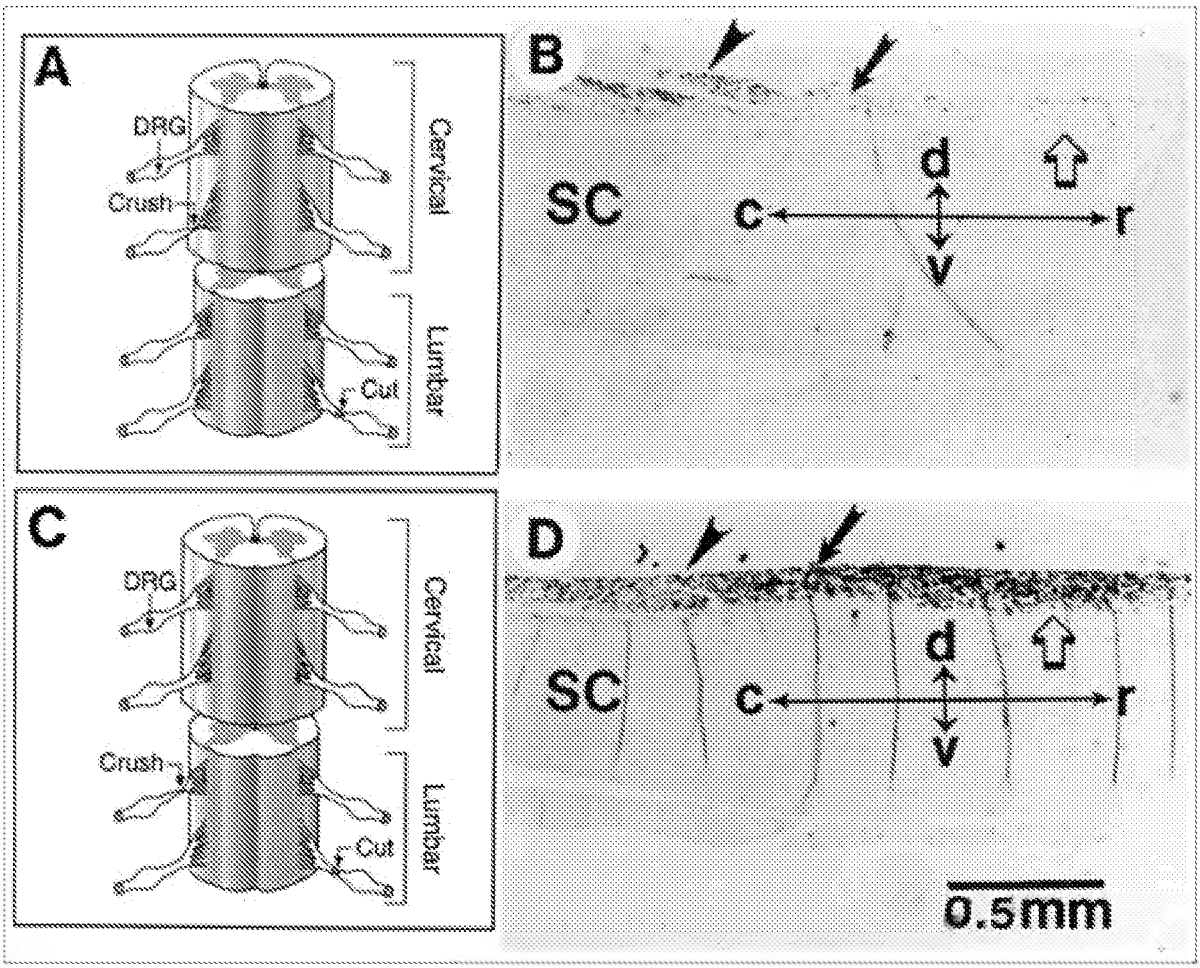

FIGURE 3. *A*, schematic representation depicting the spinal cord and entering dorsal roots in an experiment in which a dorsal root transection (*Cut*) was combined with a contralateral DREZ crush injury (*Crush*); the break in the spinal cord indicates that the contralateral DREZ crush injury was made in the cervical portion of the spinal cord far rostral to the transected dorsal root entering in the lumbar region. *DRG*, dorsal root ganglion. *B*, photomicrograph of a parasagittal section through the spinal cord (*SC*), showing cut dorsal rootlets (*arrowhead*) stained with ED-1, 14 days after the cut/crush injuries depicted in *A*. *c*, caudal; *r*, rostral; *d*, dorsal; *v*, ventral. ED-1-stained cells are confined to the PNS portion of the cut, degenerating dorsal root and do not extend beyond its entry point (**closed arrow**) into the dorsal column of the spinal cord (*open arrow*). *C*, schematic representation depicting the spinal cord and entering dorsal roots in an experiment in which a dorsal root transection (*Cut*) was combined with a contralateral DREZ crush injury (*Crush*); the contralateral DREZ crush injury was made in the lumbar portion of the spinal cord near the entry point of the transected dorsal root. *D*, photomicrograph of a parasagittal section through the spinal cord (*SC*), showing the cut root stained with ED-1, 14 days after the cut/crush injuries depicted in *C*. ED-1-stained cells are seen in both the PNS (*arrowhead*) and CNS portions (*open arrow*) of the degenerating dorsal root pathway. *Closed arrow* indicates the DREZ of the cut root. The 0.5-mm magnification bar in *D* also applies to *B*.

LPS injections

A parasagittal ED-1-stained section of a dorsal root entering the spinal cord from an uninjured control animal is shown in Figure 4A. No ED-1-positive cells are seen in either the PNS or CNS portions of the dorsal root. When uninjured animals received i.p. injections of LPS (Group V), a few ED-1-positive cells are seen in both the dorsal root and spinal cord 7 days after the initial injection (Fig. 4, B-D). More ED-1 cells are seen in the PNS portion of the root than the CNS portion (Fig. 4B). High-power photomicrographs of the spinal cord from an LPS-injected animal demonstrate the slender and ramified morphology of LPS-activated macrophages/microglia in both the PNS and the CNS (Fig. 4, C and D).

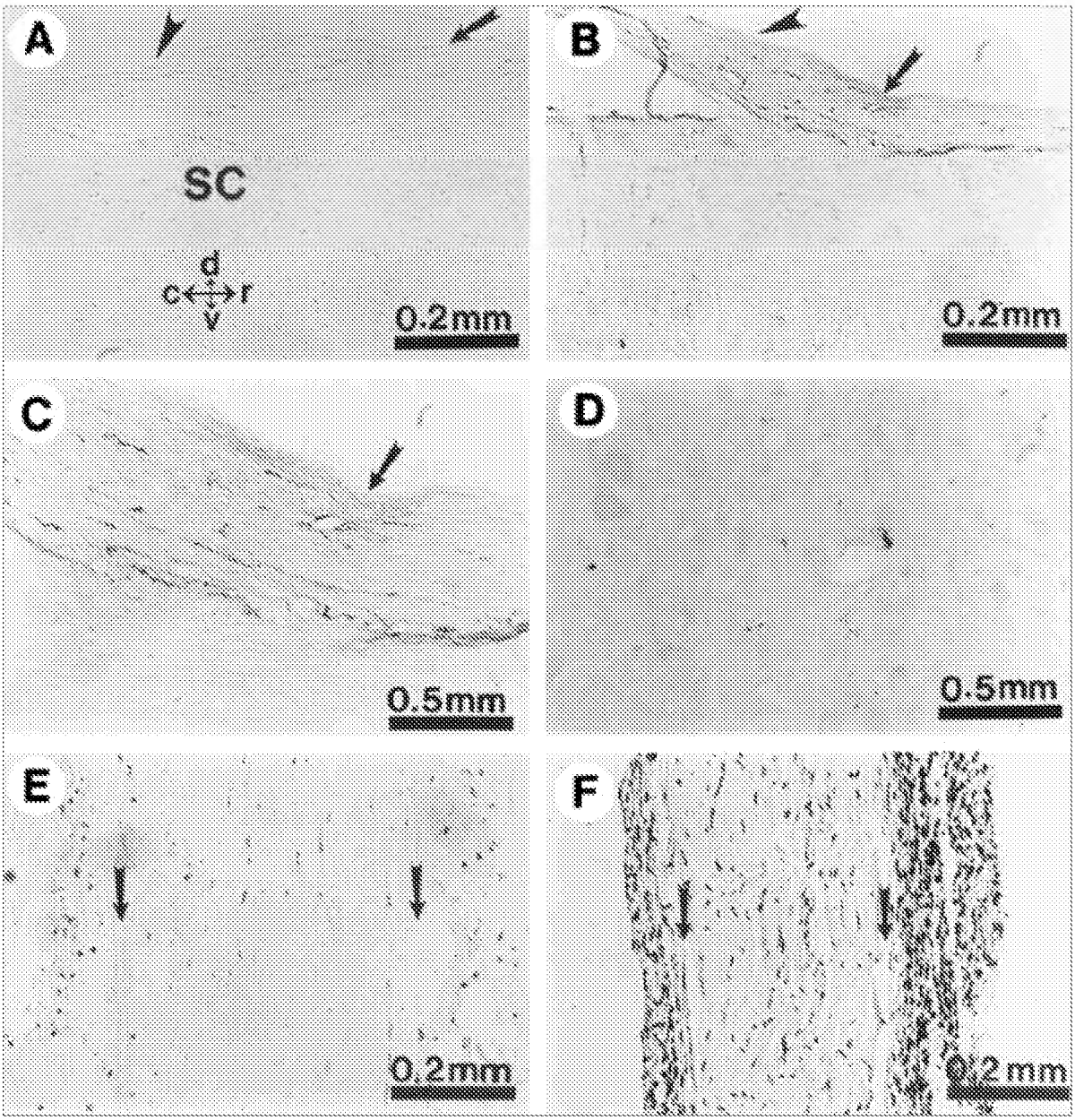

FIGURE 4. Photomicrographs of parasagittal sections through the spinal cord (*SC*), showing dorsal roots (*arrowheads*) entering the spinal cord (*arrows*) stained with ED-1 (*A-D*). *A*, a dorsal root (*arrowhead*) entering the spinal cord (*arrow*) from an uninjured control animal not exposed to LPS. No ED-1-positive cells are seen in either the dorsal root or spinal cord. *c*, caudal; *r*, rostral; *d*, dorsal; *v*, ventral. *B*, a dorsal root entering the spinal cord from an uninjured animal killed 7 days after receiving its initial injection of LPS. A few ED-1-positive cells are seen in both the PNS (*arrowhead*) and the CNS (below *arrow*). *C*, high-power photomicrograph of the dorsal root in *B*, showing the thin and ramified morphology of the ED-1-stained cells. *D*, high-power photomicrograph of the spinal cord in *B*. Note the sparse staining of ramified microglia within the CNS. *E* and *F*, photomicrographs of longitudinal sections through uninjured sciatic nerves stained with ED-1. The interface is demarcated between the epineurium (outside *arrows*) and the intraneural components of the nerves (between *arrows*). *E*, a normal sciatic nerve from a control animal not exposed to LPS, demonstrating ED-1-stained macrophages both intraneurally and in the epineurium. *F*, a sciatic nerve from an animal killed 7 days after receiving its initial injection of LPS, demonstrating a marked increase in the number of ED-1-stained cells both intraneurally and in the epineurium.

Administration of i.p. injections of LPS (Group V) dramatically increased the number of ED-1-positive macrophages in peripheral nerves such as the sciatic nerve. Figure 4E shows a sciatic nerve stained with ED-1 from an uninjured control animal. A moderate number of ED-1-positive cells scan be seen intraneurally, with greater numbers

present in the epineurium. Figure 4F reveals a markedly increased macrophage response in the sciatic nerve of an uninjured LPS-injected animal 7 days after the first injection. Again, a larger number of macrophages are seen in the epineurium. In both the control and the LPS-injected animals, the ED-1-labeled macrophages reveal a slender, ramified morphology (Fig. 4, E and F).

Dorsal root transection in LPS-injected animals

Figure 5A shows an ED-1-positive cellular response limited to the PNS portion of a transected dorsal root 7 days postinjury in a control animal not exposed to LPS (Group I). Many of the macrophages in the PNS portion of the root are large and multivacuolated. Figure 5B shows a robust ED-1-positive cellular response in both the PNS and the CNS portions of a transected dorsal root 7 days postinjury in an animal that received i.p. injections of LPS (Group VI). Figure 5C shows the ED-1-positive cells extending rostrally in the dorsal column of another LPS-injected animal 7 days after dorsal root transection. Note how the ED-1-positive column of cells rostrally is displaced ventrally by intact dorsal root axons entering the cord (Fig. 5C). Many of the ED-1-positive cells are large and multivacuolated in both the PNS and the CNS portions of the degenerating dorsal roots.

DISCUSSION

Previous studies have shown that after injury to dorsal roots, very few monocytes are initially recruited from the circulation into the CNS portion of the injured axonal pathway and that resident microglia mount a delayed and reduced response (3, 10, 19, 46). Not until 14 to 18 days after axonal injury are a significant number of macrophages/microglia visible in the CNS portion of the injured pathway within the dorsal column (3, 19). Our experiments confirmed these findings (Figs. 1B and 5A) and revealed that by 42 days postinjury, a robust macrophage/microglial response occurs in the CNS portion of the degenerating axonal pathway (Fig. 1C) (19). In contrast, macrophages in the PNS portion of injured dorsal roots mount an early and robust response similar to that seen in degenerating peripheral nerves, with a marked increase in ED-1-stained cells occurring by 3 days postinjury and continuing to increase over a 14-day period (Figs. 1B and 5A) (3).

Macrophage/microglial response to direct CNS trauma

To characterize the macrophage/microglial response to direct CNS injury, stab lesions were performed in the dorsal column of experimental animals. Focal trauma to the CNS resulted in a highly localized ED-1-positive cellular response confined to the site of injury at early time points (Fig. 2B). Similarly, a crush injury at the DREZ, causing direct injury to both the PNS and CNS portions of the dorsal root simultaneously, resulted in an ED-1-positive cellular response limited to the site of injury (Fig. 2D). Whether the ED-1-stained cells seen at the sites of CNS injury are blood-borne monocytes or activated resident microglia is unknown, as ED-1 stains both cell populations (16). One hypothesis is that the ED-1-stained cells are monocytes that infiltrate the CNS locally at the site of vascular damage caused by the direct trauma. Another possibility is that blood-borne microglial activating factors, such as complement, infiltrate the CNS at the site of blood-brain barrier compromise (13, 38, 54). These factors activate resident microglia but are diluted at sites distant from the trauma, resulting in a highly localized, ED-1-positive cellular response. A third hypothesis is that direct CNS injury causes resident glia to secrete diffusible factors, such as cytokines, that serve to "prime" microglia and enable them to become activated in the presence of degenerating axons. These priming factors act in a paracrine fashion such that only those microglia near the site of the direct trauma are primed and respond to degenerating axons. It has been shown that interleukin-1, tumor necrosis factor [alpha], monocyte-macrophage colony-stimulating factor, and nerve growth factor, all of which are produced by resident glia, can activate macrophages/microglia (3, 8, 14, 20-22, 30, 36, 49, 60).

To distinguish these possibilities, we attempted to elicit an early, robust macrophage/microglial response to degenerating axons in the CNS by combining peripheral transection of a dorsal root with focal trauma to the CNS in the form of a contralateral DREZ crush lesion (Fig. 3, A and C). We hypothesized that if resident microglia were activated by diffusible blood-borne factors such as complement, or were primed by cytokines secreted in response to the contralateral DREZ crush, they would be able to respond to the degenerating dorsal root axons within the CNS. In either case, ED-1-positive cells would be seen in both the PNS and the CNS portions of the peripherally transected dorsal root pathway at early time points. In contrast, if the DREZ crush injury resulted only in the infiltration of blood-borne monocytes, these ED-1-positive cells would be clustered most densely near the site of the DREZ crush, with few if any ED-1 cells present along degenerating axons in the contralateral dorsal column. The results of our experiments demonstrated that when the contralateral DREZ crush was made close to the entry point of the peripherally transected dorsal root, numerous ED-1-positive cells were seen along both PNS and the CNS portions of the peripherally transected root at early time points (Fig. 3, C and D). At the site of the contralateral DREZ crush, a localized ED-1-positive cellular response was found. In contrast, when the contralateral DREZ crush was performed far from the entry point of the peripherally transected dorsal root, activated macrophages/microglia were not found in the CNS portion of the degenerating peripherally transected root (Fig. 3, A and B). These experiments suggest that direct CNS trauma can confer on nearby resident microglia the capacity to respond to degenerating axons in the CNS at early time points. This capacity is most likely a result of either diffusion of blood-borne microglial activating factors into the CNS or secretion of microglial priming factors by resident glia in response to local trauma.

LPS-primed macrophage/microglial response to dorsal root transection

Next, we sought to determine whether an early and robust microglial response to degenerating axons in the CNS could be elicited without direct trauma to the CNS. To accomplish this goal, we systemically administered LPS immediately after dorsal root transection (Group VI). It was hypothesized that LPS, a bacterial endotoxin known to activate macrophages, would prime resident CNS microglia in the same manner as local CNS trauma (1, 27, 28, 40, 41, 59). We found that LPS, in the absence of trauma, caused a marked increase in the number of densely stained ED-1-positive cells in peripheral sciatic nerves (Fig. 4, E and F) but only a slight increase in the number of ED-1-positive cells in the CNS (Fig. 4, B-D). In LPS-treated animals that underwent peripheral transection of dorsal roots, however, a robust ED-1-positive cellular response was seen in both the PNS and the CNS portions of the injured dorsal root pathway as early as 7 days postinjury (Fig. 5, B and C). The ED-1-positive cells in the CNS were found only along degenerating axons in the dorsal column (Fig. 5C). These findings indicate that high levels of LPS do not activate microglia nonspecifically, but rather they prime microglia to become activated only in the presence of degenerating axons. LPS may act directly on resident microglia or act indirectly via the secretion of cytokines, such as tumor necrosis factor [alpha], which are known to activate macrophages/microglia. Another possibility is that LPS acts by altering the properties of the blood-brain barrier to enhance the transmigration of circulating monocytes into the CNS (49, 60). Further studies to elucidate the effect of LPS on the integrity of the blood-brain barrier are required to distinguish between these possibilities.

Role of macrophages/microglia in axonal regeneration

Several lines of evidence suggest that macrophages/microglia play an important role in axonal regeneration. In the PNS, a reduction in the macrophage response after peripheral nerve injury has been associated with reduced axonal regeneration (4, 9, 12, 13). In the CNS, activated macrophages/microglia seem to create a favorable environment for regeneration by degrading specific inhibitory molecules on adult mammalian central myelin and astrocytes, which prevents axonal growth and reactive neurite spouting (3, 11, 14, 15, 19, 53, 57, 62). Macrophages/microglia also up-regulate molecules that promote axonal growth such as nerve growth factor (3, 9, 30, 31). Recent work has shown that activated macrophages implanted into transected adult rat spinal cords promote axonal regeneration and behavioral recovery (51). In adult mammals, an early robust macrophage response to axonal

injury occurs in the PNS, where regeneration occurs, but not in the CNS, in which long distance axonal regeneration is far more limited (3, 19, 43, 50, 61). Interestingly, in certain fish and amphibia, where axonal regeneration occurs in the CNS, a significant macrophage/microglial response to injury is seen in both the PNS and the CNS at early time points (17, 23, 37). Thus, inducing an early, robust macrophage/microglial response to degenerating axons in the mammalian CNS, similar to what is normally seen in the mammalian PNS, may be important in creating a favorable environment for nerve regeneration. A recent study has shown that direct trauma to the CNS causes an up-regulation of certain molecules, such as proteoglycans, which inhibits the axonal growth of transplanted adult mammalian peripheral neurons in the CNS (15). It is conceivable that modulating the macrophage/microglial response after trauma to the CNS could be helpful to either prevent or degrade depositions of these factors that inhibit regeneration.

CONCLUSION

The macrophage/microglial response was studied during Wallerian degeneration of adult mammalian sensory dorsal root nerves. We have shown that direct CNS trauma and systemically administered LPS accelerate and enhance the macrophage/microglial response in the CNS to degenerating dorsal root axons. We suggest that both direct trauma and exposure to LPS result in "priming" of resident microglia, which enables them to respond to degenerating axons in the CNS in an early, robust manner such as that normally seen in the PNS. The effect that this enhanced response has on axonal regeneration in the CNS must be examined.

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COMMENTS

It has been well documented that injury to the spinal cord causes a sequential series of events characterized as "secondary injury." It has been assumed that the infiltration of inflammatory cells at the site of injury is part of this process and is an impediment to recovery. Lazar et al. present some interesting data to suggest that macrophages and microglial infiltration may have an important role in facilitating recovery by "cleaning up" the injured tissue and creating the substrate for recovery. Clearly, lipopolysaccharide administered close to the time of injury can enhance the number of mononuclear cells. As stated by the authors, it will be important now to determine how this changes axonal regeneration.

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Lazar et al. have studied the effects of combinations of injuries to the spinal cord and dorsal root entry zone in rats on the response of macrophage infiltration at the site of degenerating axons. Their data show that local central nervous system (CNS) trauma in conjunction with administration of lipopolysaccharide, a known activator of macrophages, led to the most brisk response in terms of macrophage activation.

Previous studies suggest that macrophage infiltration at the site of peripheral nerve injury promotes axonal regeneration. It is indeed interesting that the CNS typically does not demonstrate a robust macrophage response after injury to the peripheral nerve system. Undoubtedly, this is but one key observation that continues to set regeneration capacities in both systems apart. The authors' hypothesis is that priming the CNS to axonal injury by stimulation of a macrophage response will lead to a more favorable environment for regenerative processes within the CNS. In future studies, they will determine whether targeted stimulation of CNS macrophage infiltration will be of benefit in central axonal regeneration.

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Toronto, Ontario, Canada

Axon; Lipopolysaccharide; Macrophage; Microglia; Regeneration; Wallerian degeneration

IMAGE GALLERY

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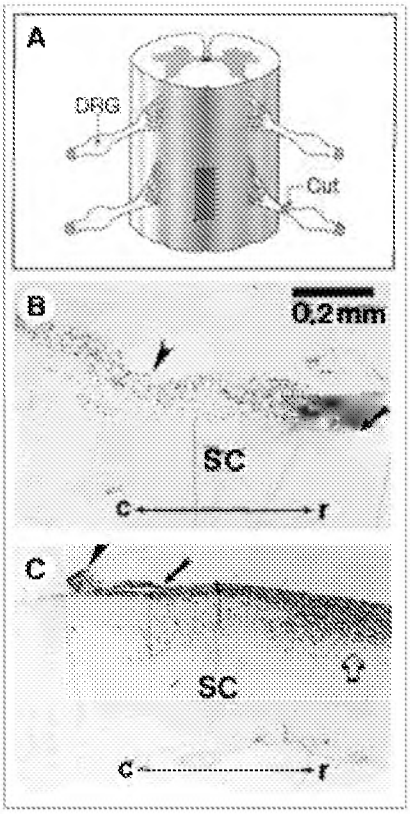
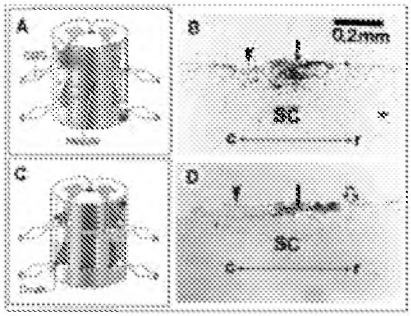


Figure 1



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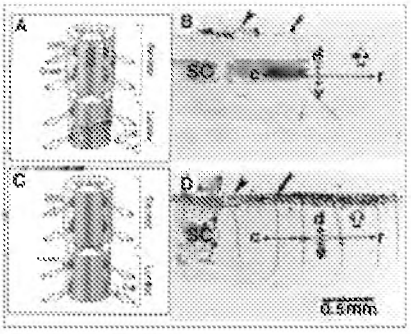

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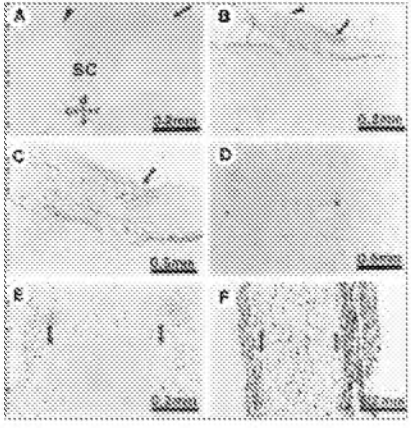

Figure 4

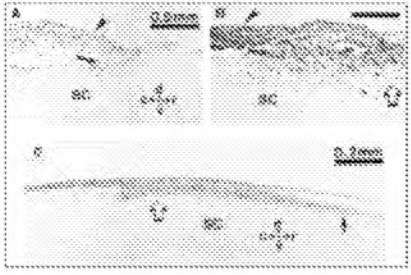

Figure 5

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