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Endogenous Repair after Spinal Cord Contusion Injuries in the Rat

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Contusion injuries of the rat thoracic spinal cord were made using a standardized device developed for the Multicenter Animal Spinal Cord Injury Study (MASCIS). Lesions of different severity were studied for signs of endogenous repair at times up to 6 weeks following injury. Contusion injuries produced a typical picture of secondary damage resulting in the destruction of the cord center and the chronic sparing of a peripheral rim of fibers which varied in amount depending upon the injury magnitude. It was noted that the cavities often developed a dense cellular matrix that became partially filled with nerve fibers and associated Schwann cells. The amount of fiber and Schwann cell ingrowth was inversely related to the severity of injury and amount of peripheral fiber sparing. The source of the ingrowing fibers was not determined, but many of them clearly originated in the dorsal roots. In addition to signs of regeneration, we noted evidence for the proliferation of cells located in the ependymal zone surrounding the central canal at early times following contusion injuries. These cells may contribute to the development of cellular trabeculae that provide a scaffolding within the lesion cavity that provides the substrates for cellular infiltration and regeneration of axons. Together, these observations suggest that the endogenous reparative response to spinal contusion injury is substantial. Understanding the regulation and restrictions on the repair processes might lead to better ways in which to encourage spontaneous recovery after CNS injury.

INTRODUCTION

It has been generally thought that there is little or no useful regeneration or repair after spinal cord lesions in adult mammals (42), and much effort has been put forth in attempts to promote regrowth and reconnections of lost pathways. However, there are reports of apparent spontaneous repair, at least after some kinds of CNS injury, and it is clear that many CNS axons do retain the ability to sprout and regenerate if given the appropriate environment. For example, peripheral nerve grafts (21, 24) or implanted Schwann cells (e.g., 39, 45, 46) can promote growth across considerable distances after complete CNS tract section. There is also evidence of repair in some models of SCI. Bunge and colleagues (17) reported that Schwann cells could be seen myelinating axons at the edge of the lesion cavity after photochemical thrombosis lesions in rat. Schwann cells have also been seen myelinating axons after contusion spinal injuries in the monkey (15), and large numbers of Schwann cells and axons have been noted by Bunge and colleagues in long-term spinal injury sites in human pathological studies (18). The lack of emphasis on such findings in the literature might be due in part to the widespread use of transection and hemisection models of SCI in animals. Recent advances have been made in the reproducibility and reliability of contusion injury models meant to mimic fracture-dislocation injuries in man. In addition to providing a basis for evaluating potential therapies, such models have provided much material for examining the detailed histopathology of contusion lesions (e.g., 23, 38) and for studying their biology.

A standardized model of contusion spinal cord injury in rats has been developed in conjunction with a consortium of investigators seeking to provide coordinated preclinical examination of spinal cord injury therapies (the Multicenter Animal Spinal Cord Injury Study or MASCIS). This model uses an impact device designed by Gruner and Young at New York University (NYU) (22, 30). The protocol produces lesions that spare a small rim of fibers, and severity of injury can be varied and controlled (see 2, 3). We have recently shown that these lesions exhibit progressive secondary expansion and produce long-term expression of cell death by

[1] The following members of the Multicenter Animal Spinal Cord Injury Study (MASCIS) Principal Investigators Group contributed to protocol design and contributed animals to this study: D. K. Anderson (University of Florida, VAMC), A. Faden (Georgetown University), C. Hsu (Washington University), L. K. Noble (University of California, San Francisco), S. K. Salzman (DuPont Institute), MSB, JCB, and WY.

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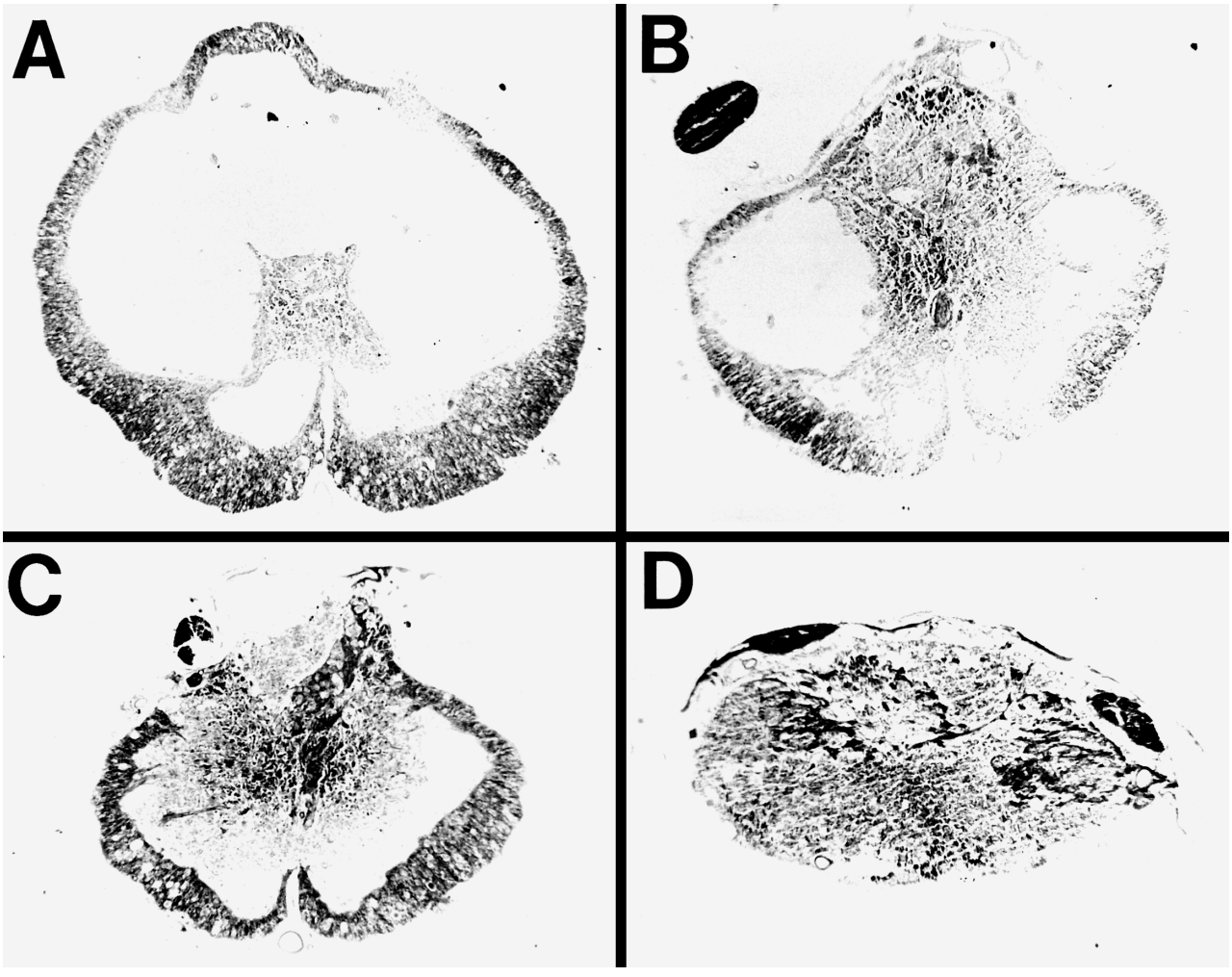
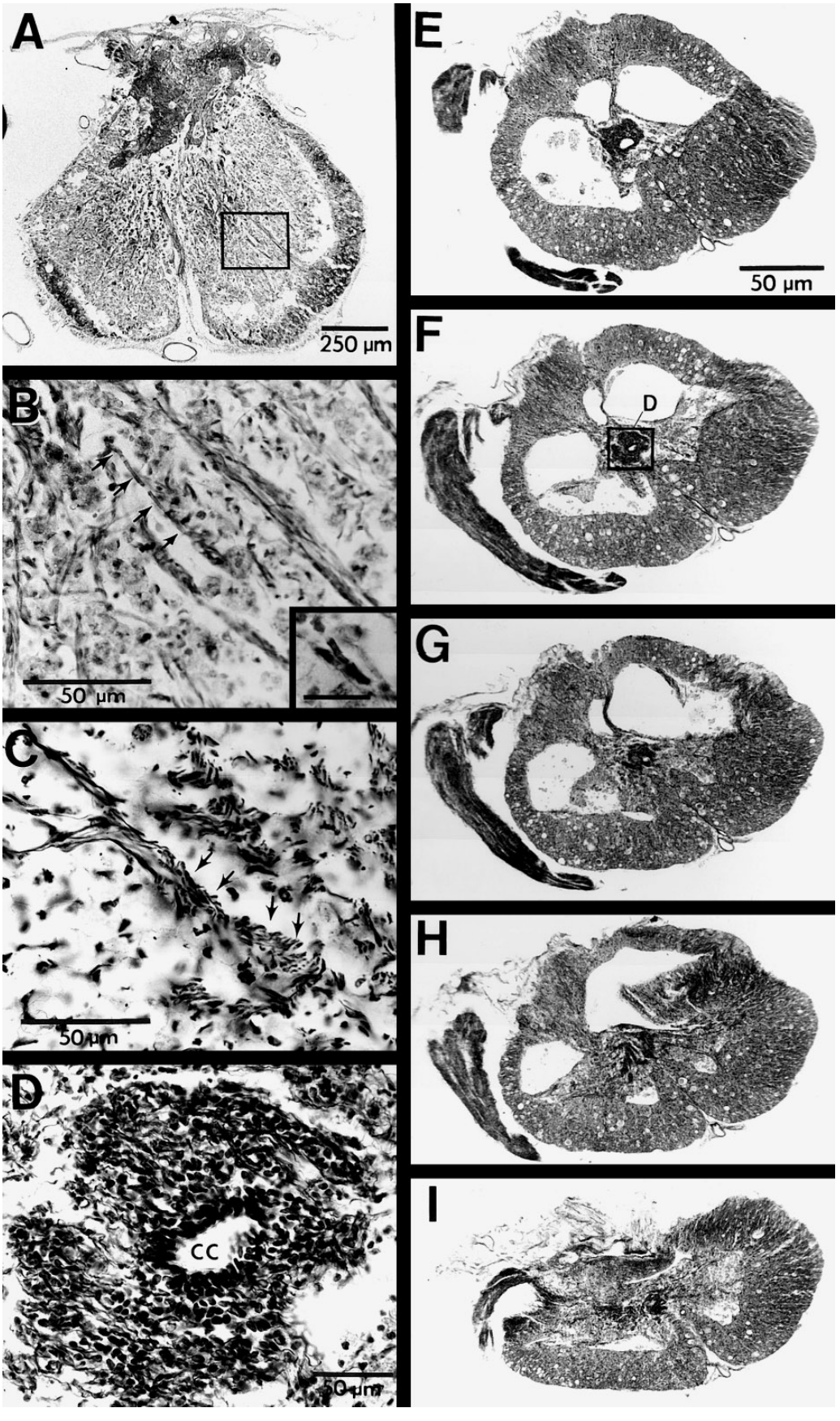


FIG. 1. Examples of chronic contusion lesions with varying amounts of fiber growth into the lesion cavity 6 weeks after injury. These sections were at the maximal extent of the lesion. A, a 12.5-mm MASCIS lesion; the fiber ingrowth was rated as "1." B, a 12.5-mm MASCIS lesion with fiber ingrowth rated as "2." C, a 25-mm MASCIS lesion with fiber ingrowth rated as "3." D, a 50-mm MASCIS lesion with fiber ingrowth rated as "4." (Luxol fast blue-stained 20-μm paraffin sections.)

apoptosis in the oligodendrocytes of long tracts undergoing Wallerian degeneration (23). In this report, in contrast, we provide a description of apparent attempts at repair after damaging the cord using the NYU/MASCIS injury model.

A large number of rats with different levels of injury severity were studied, and it was found that many exhibited large numbers of axons and Schwann cells within large lesion cavities at the center of the damaged area and, further, that there is an inverse relationship between the injury severity and the amount of such fiber ingrowth. Further studies using a variety of postoperative survival times were aimed at describing the patterns of degeneration and potential regeneration after contusion injuries. A rather surprising finding was the presence of proliferating ependymal cells at early postinjury times, which later may have contributed to the expansion of the ependymal zone and the formation of cellular trabeculae within the lesion cavity. Together these findings, while mostly descriptive,

FIG. 2. A chronic contusion lesion cavity from a 25-mm injured cord containing fascicles of myelinated fibers at low (A) and higher (B) magnification; internodal segments of myelin can be easily discerned (small arrows and inset, B). In an adjacent section (C), many silver stained fibers can be seen. A series of silver-stained sections through the distal part of a lesion cavity in which the central canal has hypertrophied is shown in E–I; the central canal area in F is shown at higher magnification in D. The trabeculae dividing the cavities contain fascicles of axons. A piece of a root is located on the left side of the sections; it can be seen entering the cord in I where the cavities appear to be partially collapsed.


A
250 µm
B
50 µm
C
50 µm
D
CC
50 µm
E
50 µm
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direct attention to phenomena of endogenous repair in this lesion type and may be important for understanding ways in which to enhance natural healing processes in the adult CNS.

METHODS

Subjects. A total of 610 rats (Long-Evans, Simonsen Laboratories) were used in these studies. All cord contusion injuries were made using the device designed by Gruner and Young (22, 30). Five-hundred sixty-five rats from the 1995 MASCIS database were used to provide information on the relationship between fiber tract sparing and the presence and amount of fibers invading the lesion cavity at 6 weeks postinjury. Equal numbers of males and females were used, and they were divided among three different injury severities (see below). These animals were included in a randomized trial of YM-14673 (a TRH analogue, see Ref. 8) vs saline. In that study, one group, which received 1 mg/kg YM-14673 at 3 h postinjury at one injury level (12.5 mm) showed significant improvement in locomotor outcome compared to the saline control groups (MASCIS group, unpublished data). In the present study, these data were not used in order to evaluate drug effects, but rather to expand upon a hypothesis generated by looking at a subgroup of those animals that received only saline ($n = 14$), in which a clear relationship between lesion severity and the presence of fibers within the lesion cavity was apparent.

Additional animals not included in the MASCIS study ($n = 45$) were given 25-mm injuries (see below) and then sacrificed at several intervals from 30 min to 21 days after lesioning in order to examine the time course of axonal invasion and cellular changes. Some of these rats were used in a study of cell death after spinal cord injury (23), which also contains details of the methods.

Surgeries. Contusion lesions were made at three different severities by dropping the NYU device rod (10 g) from distances of 12.5, 25, or 50 mm onto the intact dura exposed with a laminectomy at the T9–T10 spinal level as described in detail in previous publications (2, 3). During surgery, rats were anesthetized with sodium pentobarbital (40–60 mg/kg depending upon sex), an external jugular catheter and tail artery catheter were used to monitor blood pressure and blood gases and to administer drugs. Cephalosporin antibiotic was given iv shortly after injury and continued sc twice daily for 1 week. Rats recovered from surgery in temperature and humidity controlled incubation chambers. They were then transferred to their home cage, and bladder evacuation was accomplished using the method of Crede until bladder function returned. This was usually within 2 to 3 weeks depending upon the level of injury.

Histology. Rats from the MASCIS protocol were sacrificed 42–44 days after injury by intracardiac perfusion with 10% buffered formalin under deep pentobarbital anesthesia. The fixed cords were embedded in paraffin and sectioned at 20 μm; every third, fourth, and fifth section was mounted sequentially on three sets of slides. For the analysis here, the sections were stained with luxol fast blue (LFB). Some were counterstained with cresyl violet. Alternate sets were prepared with the silver staining method of Bodian (43) to demonstrate nerve fibers. Fiber sparing was scored according to the method published previously (2, 16). Sparing was expressed as the percentage of the cross-sectional area of the cord that contained relatively intact fiber tracts as judged from the LFB myelin stain in the section with the largest lesion extent. Data are presented as the mean ± SEM. This measure has previously been shown to correlate highly with functional, locomotor outcome at 6 weeks postinjury (2, 8, 9, 16). The amount of fiber ingrowth into the lesion cavity was rated on a scale of 0–4 with 0 indicating no fibers in the lesion cavity to 4 indicating large numbers of fibers packing the lesion center (see Figs. 1 and 2A–2C).

Two of the cases were reconstructed in three dimensions by digitizing structures drawn using a camera lucida in every 10th section. Structures were entered into a Silicon Graphics computer as a series of contours using a digitizing tablet. The contours were stacked and edited using in-house software, lofted by constructing a polygonal wire frame surface and then rendered as surface models using LightWave.

The other rats were perfused with 4% paraformaldehyde and sampled as described in Crowe *et al.* (23).

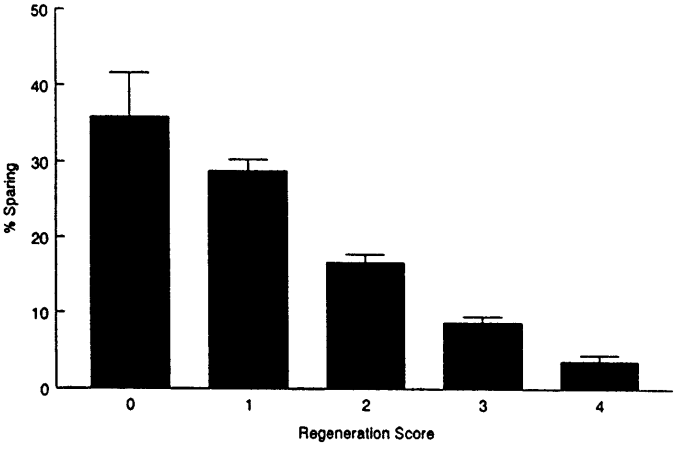

FIG. 3. The relationship of tissue sparing to the density of fiber ingrowth is shown for a large series of injured rats ($n = 565$). Tissue sparing at the lesion epicenter is expressed as the percentage of area occupied by surviving tissue over the total section area; density of ingrowth was subjectively rated in the same cases at the lesion center, as ranging from "none" (0), to "packed" (4). Examples of the various density ratings are shown in Fig. 1. A significant inverse relationship ($P < 0.0001$) was noted.

Briefly, 1-mm-thick blocks at the lesion center and at 5 and 10 mm rostral and caudal to the lesion center were embedded in plastic (Maraglas, Electron Microscopy Inc.). Blocks were sectioned at 1 μm on an ultramicrotome (LKB IV) and stained on the slides with toluidine blue.

RESULTS

The appearance of contusion lesions at 6 weeks after injury. The appearance of contusion lesions at 6 weeks after injury is shown in Fig. 1. As shown in previous studies (2, 7, 8, 9, 16, 23, 38), a rim of spared fibers remains at the lesion center which is larger in milder injuries than in more severe injuries. The percentage of area spared in the section exhibiting the most severe damage averaged 4.5 ± 0.6% for the 50-mm injuries, 12.1 ± 1.0% for the 25-mm injuries, and 28.7 ± 1.1% for the 12.5-mm injuries. Corresponding locomotor abilities, as measured by the scale developed by Basso *et al.* (1) in prior studies (2) have averaged 8, 10.6, and 11.4, respectively (of 21 possible points). In many of these cases, the lesion cavity (Fig. 1) was partially or completely filled by a cellular matrix in which large num-

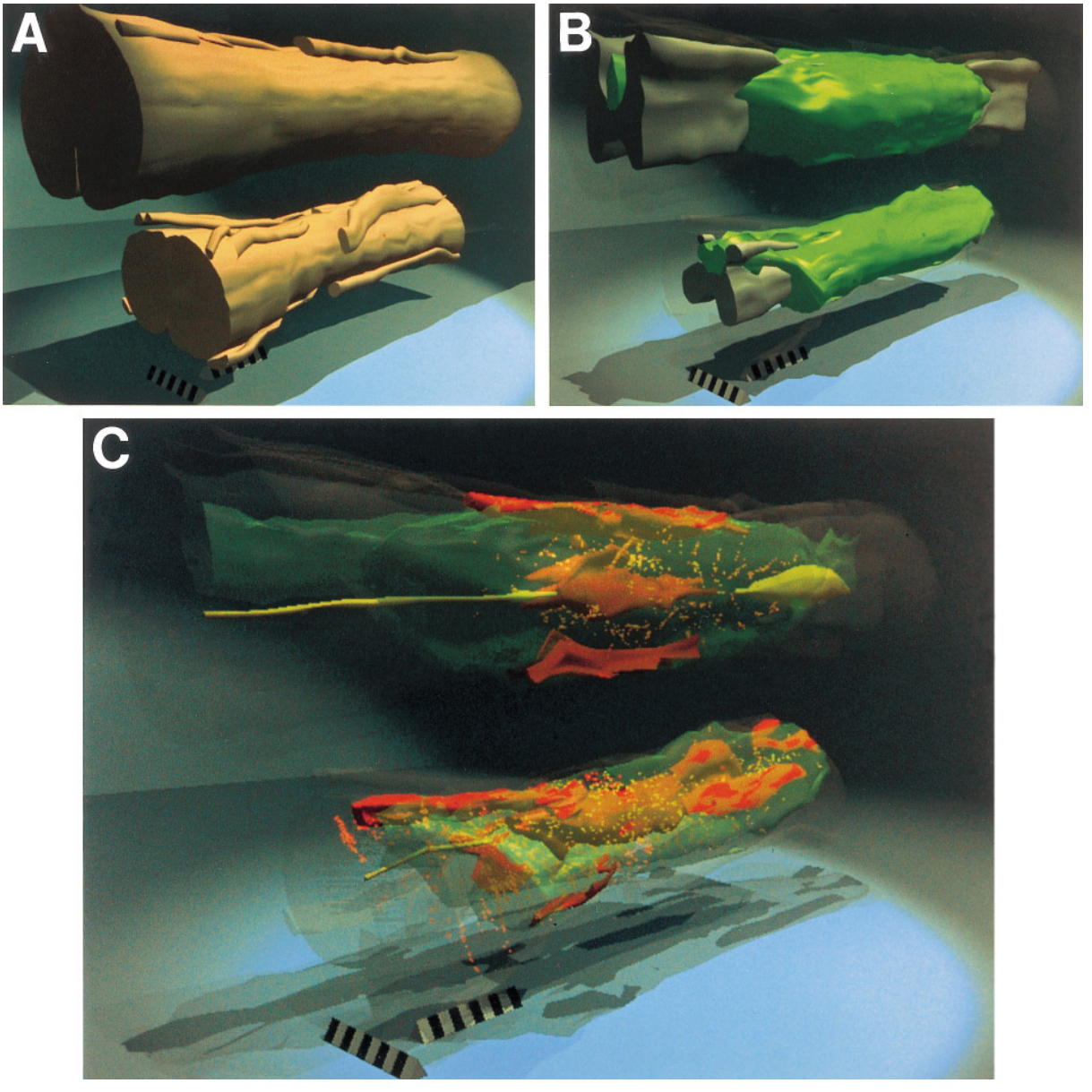

FIG. 4. Three-dimensional reconstructions of a 12.5 (top) and a 25 (bottom)-mm injury in which the areas of dense fiber ingrowth (red/orange), fiber fascicles (yellow/orange), and the central canal (yellow) are illustrated inside of the lesion cavity (green). The outside of the cord and the relationship of the lesion to the surviving gray matter are illustrated in A and B for orientation.

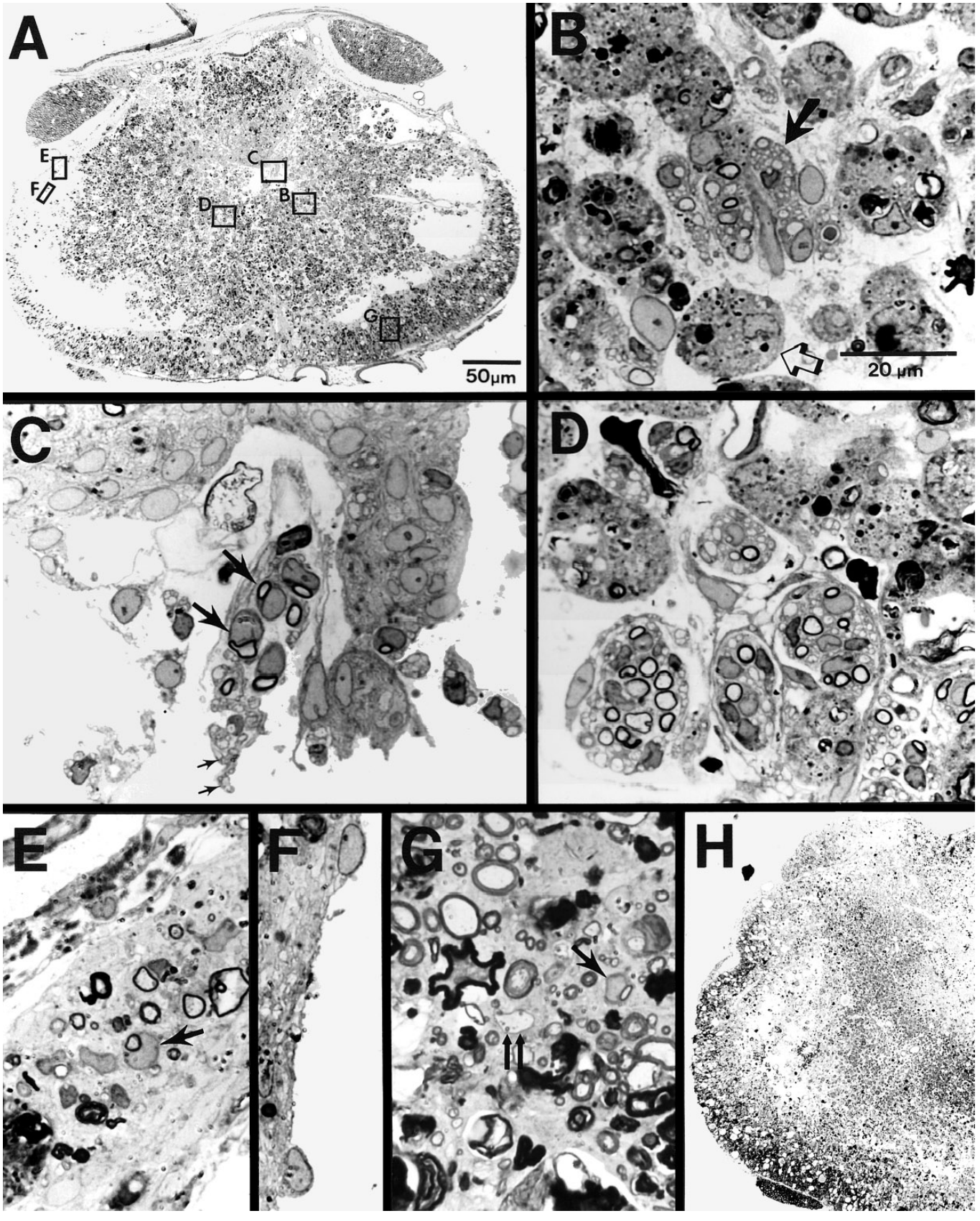

FIG. 5. One-micrometer-thick toluidine blue-stained sections through the lesion center in a 25-mm injured cord after 3 weeks (A–G) and 5 days (H). The lesion center is occupied both early and late by large numbers of macrophages (A, B, D, and H), invading cells, many of which are Schwann cells (B, C, D, E, and G), and proliferating astrocytes (C, F). Large numbers of axon fascicles are present (A–D) and interdigitate

bers of randomly oriented myelinated axons appeared to be growing. The fibers were covered by bright LFB-staining myelin (Fig. 2B, inset) which had a different appearance than the darkly stained, compacted myelin in the spared rim of axons. These fibers frequently coursed in fascicles (Figs. 2A and 2B) and were usually embedded in the lesion matrix which sometimes formed trabeculae separating open cavitated areas. Alternate sections stained using the Bodian silver method showed that the same areas contained large numbers of axons (Fig. 2C). Histologic examples showing the range of cavity filling at the lesion center are presented in Fig. 1. Since the lesion center was characterized by total destruction of axons and gray matter at earlier times following the contusion injuries (see below), these fibers were interpreted as having grown into the cavity after injury rather than as sparing or sprouting of spared axons. It was interesting that an inverse relationship (Spearman rho $= -0.58$, $P < 0.0001$) was observed between the amount of spared fibers and the amount of apparent ingrowth. The mean tissue sparing score for each category of "regeneration" score is depicted in Fig. 3. In order to understand how these areas of dense fiber ingrowth were organized in three dimensions, reconstructions were made of two cases which exhibited considerable ingrowth. The results are shown in Fig. 4. The outside of the cord is shown in yellow ochre, the gray matter is shown in gray, the lesion is indicated in green, areas of dense fiber ingrowth are shown in red–orange, individual fascicles of fibers are indicated in yellow–orange, and the central canal is indicated in yellow. A case with a milder injury (12.5 mm; 20.78% sparing at the lesion epicenter) is shown on the top and a heavier injury (25 mm; 0.99% sparing) is shown on the bottom. The case with the heavier injury is the most shrunken (Fig. 3A), but has the largest areas of dense fiber ingrowth. Both cords have areas of dense fiber ingrowth connected to the entry zones of the dorsal roots, which likely provide the main source for axons and Schwann cells which provide most of the myelin. Interestingly, in the top case especially, there is a region supporting dense ingrowth which appears to be related to the central canal. The cells around the central canal can be observed to be proliferating at the edges of the lesioned zone (as shown in Figs. 2D and 2E–2I). These zones are frequently associated with the lesion matrix trabeculae which separate the open cavities and may serve to guide fibers from the CNS (like the corticospinal tract, see Ref. 35) into the center of the lesion. At the rostral end of the smaller lesion, the central canal (yellow) is expanded due to proliferation of the surrounding ependymal cells. Additional evidence to support this contention is presented below.

Early degeneration and the beginnings of repair. Early degeneration and the beginning at repair were studied in 1-μm-thick toluidine blue-stained sections (Figs. 5 and 6). By 48 h, there were no apparent axons or spared cells within the central core of the developing lesion cavity. Rather, the tissue was filled with blood elements, and the neuropil had a granular appearance with no clear cellular structure. The white matter surrounding the core was degenerating with many collapsed axons, swollen axons, puffy myelin, unravelling myelin, and empty myelin sheaths. Macrophages were infrequent at this time point but increased radically in number by 5 days (Figs. 5H and 6D) and were observed to contain myelin fragments in addition to numerous small granules (Fig. 6D). The central cavitated region was filled with these cells. At the root entry zone, Schwann cells appeared to be infiltrating the lesion edge at 5 days. By 1 week, fascicles of axons start appearing in the lesion cavity especially dorsally and by 3 weeks, these fascicles were observed in the center of the lesion cavity (Figs. 5A–5D), which still also contained large numbers of macrophages. At this later time, few granules were observed in the macrophages, but advanced digestion of membrane was evident in the vacuoles (Figs. 5B and 5D). Schwann cells accompany the ingrowing fascicles (see especially Fig. 5C, Figs. 5B and D, and Fig. 6C) and numerous unmyelinated axons were evident at the advancing tissue edge (small arrows, Fig. 5C). The cellular infiltrate into the lesion cavity appeared to contain many Schwann cells; astrocytes appeared to border the cavity wall (Figs. 5C and 5F). The peripheral rim of spared fibers exhibited significant myelin loss (Figs. 5E and 5G; double arrows in Fig. 5G) and occasional remyelination by Schwann cells (arrows, Figs. 5E and 5G).

Evidence for ependymal cell proliferation. Evidence for ependymal cell proliferation was observed mainly at the rostral and caudal parts of the lesion rather than in the center. In many cases, there was an enlargement of the central canal (Figs. 2E–2I, 4C, and 6A and 6B), the cellular components of which blended imperceptibly into the matrix trabeculae which divided the lesion cavity into compartments. The central canal ependymal cell proliferation was present in the rostral and caudal areas of the principle lesion site (e.g., Figs. 6A

between macrophages; both unmyelinated (B; C, small arrows; D) and myelinated axons can be seen. Schwann cells most frequently ensheath the axons in these fascicles (e.g., larger arrows, C). The spared rim of fibers is thicker ventrally (A, G) than dorsally (A, E). The residual fibers often appear to have reduced myelin sheaths (e.g., G, double arrows) and are occasionally ensheathed by Schwann cells (arrow, E and G). Many collapsed axons and axons with disrupted myelin sheaths are evident in the spared white matter (E and G). (The micrometer indicator in A applies to H; the bar in B applies to C–G.)

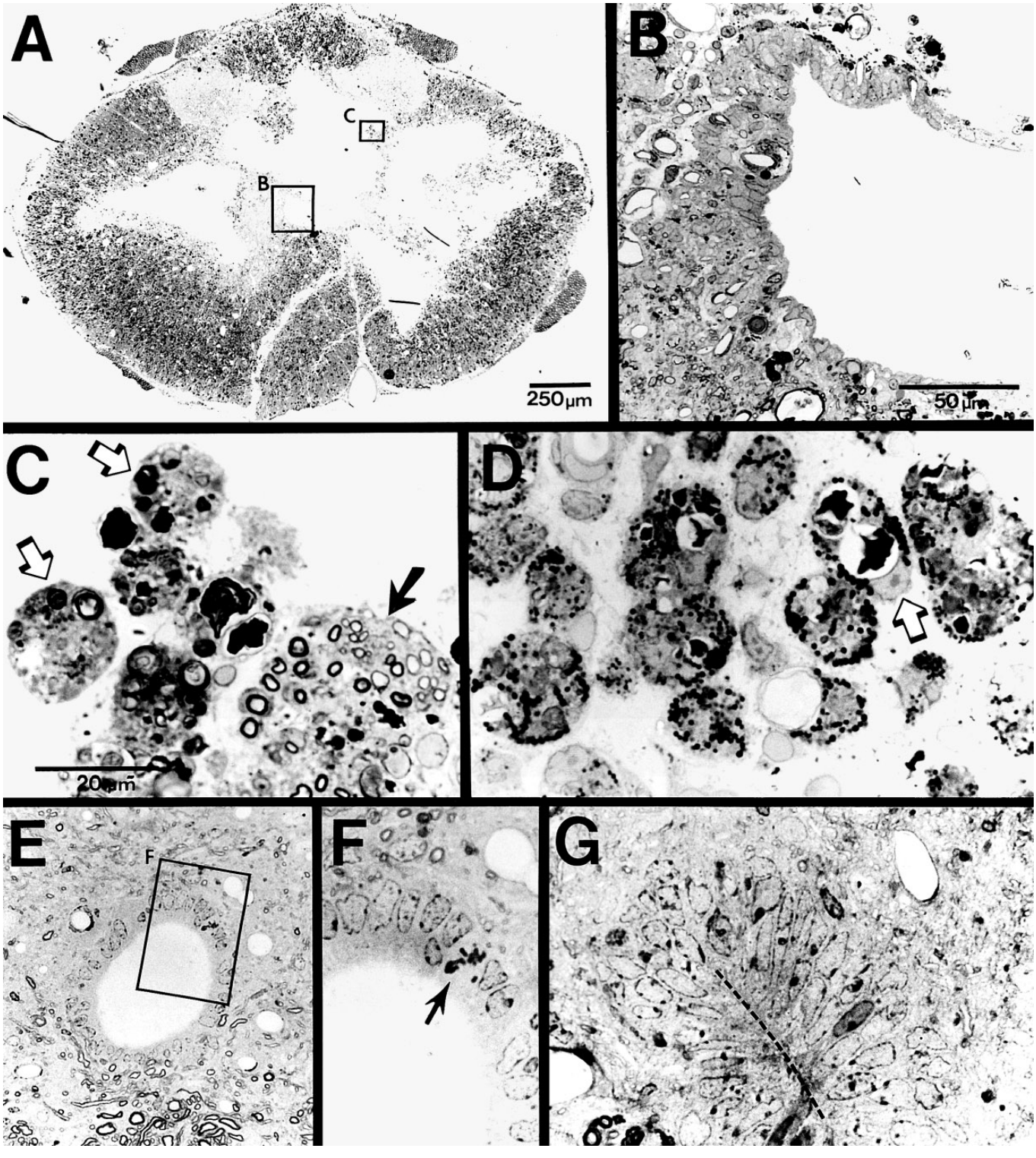

FIG. 6. One-micrometer-thick toluidine blue-stained sections from a region 5 mm rostral to the center of a 3-week 25-mm injury (similar to the region shown in Fig. 2E at 6 weeks). The central canal is expanded and the ependymal cells appear to have proliferated (B) and are connected to cellular bridges or trabeculae dividing cavitated areas. A fascicle of axons (block arrow, C) is next to several macrophages (open block arrows, C). The macrophages in D are from the lesion center at 5 days and contain many more dense granules than those from the 3-week lesion center shown in C. Ependymal cells around the central canal at the edges of the lesion are undergoing mitosis (E, F). Sections with multiple layers of cells around the central canal can be observed (G). (In G, the canal is occluded and is marked with a dashed line.) (10 mm rostral to the lesion center.) (Bar in B applies to E; bar in C applies to D, F, and G.)

and 6B), but was also evident in regions where the lesion is restricted to a small zone, e.g., in the dorsal funiculus (Figs. 6E–6G; Fig. 4C). Dividing cells were observed (Figs. 6E and 6F), and the result of such division, canals with several cell layers (Fig. 6G), could frequently be found. Profile counts of the nuclei located immediately around the central canal in such sections indicates that the number of profiles are increased at 24 and 48 h after injury. At 6 weeks, the density of ependymal zone cells is increased around the central canal at the point where the central canal opens into the lesion cavity (data not shown).

DISCUSSION

The contusion lesions produced in this, and similar models, are characterized by an early hemorrhagic central necrosis that spreads radially and rostrocaudally over time to produce a classic "football"-shaped cystic cavity or cell-filled injury site (14–16, 31, 38). While there is still a lack of detailed data on human SCI pathology, the reports available suggest that this type of lesion is similar to a large number of human SCIs (6, 18, 20, 34). An important feature of the model lesion is that even in severe injuries, a small peripheral rim of spared tissue and axons remains (14–16, 31, 38). This has been emphasized as well in human SCI pathology, where even neurologically "complete" patients usually have some tissue sparing (6, 18, 20, 34). This spared tissue is the target for both acute and chronic treatments; acute treatments attempt to delay or retard the secondary injury, thus leaving more residual fibers and function. Chronic treatments could attempt either to add to these spared fibers or to use rehabilitative strategies to enhance their residual function. We have shown that the kinds of contusion lesions used here in rats respond to early pharmacological intervention, just as do human cord injuries (8, 10, 11).

Regeneration and tissue repair after SCI. Regeneration and tissue repair after SCI have been studied by many laboratories, although the importance of secondary injury processes in the acute and subacute phase have often overshadowed the reparative events that no doubt occur after cord injury. While complete cord transections yield complete and permanent paralysis, even very severe contusion lesions are followed in rats, and humans, by some recovery of function (2). That recovery is lost, at least temporarily, after transection of the remaining spared fibers. The relationship between the amount of spared fibers and locomotor recovery has been well documented (1, 2, 9, 16, 38). Nevertheless, rats with essentially no visible spared fiber tracts can show better hindlimb movement than rats with complete cord transections (2). While these findings have been used to emphasize the small number of fibers needed for minimal recovery (2), it is possible that regenerating fibers entering the lesion cavity in these severe injuries may play a role. The data in the present study show that "MASCIS" contusion lesions are sometimes filled with axons that must have regenerated, since at earlier postinjury time points there are no survivors. While the huge numbers of these fibers attracted our attention, in retrospect, it seems that lesser severity lesions like those we have studied using the OSU contusion device may contain smaller complements of fibers. In addition, prior studies of contusion lesions have suggested that regeneration might occur and have noted that Schwann cells migrate into the CNS after injury (15, 17). These findings are in contrast to those reported after adult and even neonatal hemisection lesions in which no regeneration is thought to occur except after transplantation or the blockade of endogenous inhibitors of axonal growth (39, 45, 36, 41, 42). Many studies indicate that much of the lack of regeneration after cord lesions is the result of a lack of suitable substrate for axonal elongation rather than a lack of constitutive axonal growth ability. The presence of substantial numbers of regenerating axons within the lesion matrix after severe contusion injuries strongly suggests that under some conditions, the tissue repair response in the adult rat is capable of providing a substrate for growth, much of which is provided by invading Schwann cells. Whether this growth includes a substantial number of CNS axons, and whether these might contribute to recovery, remains to be seen. However, corticospinal tract axons have been shown to regenerate into spinal cord lesions in the rat (35, and unpublished data from the laboratory of W. Young). This reaffirms the strategy of using exogenous Schwann cells and other cellular implants to provide a bridge across the lesioned CNS since such bridges may be a natural feature of the spontaneous recovery process in some kinds of spinal cord lesions.

Proliferation of ependymal/subependymal cells after injury. Work on the frog in our laboratory and others (5, 26, 37) has shown that during metamorphosis, the site of a spinal transection is reconstituted, apparently by ependymal cell proliferation. Robust regeneration of descending fibers through this reconstituted cord has been documented (5, 26). In mammals, very early injury to the cord may allow for cord reconstitution and substantial regeneration, as has recently been reported in the rat (33). Additional data from a recent study in the opossum (4, 44) have shown that complete cord section prior to postnatal day 5 in the neonatal opossum results in almost complete cord reconstitution and sparing of locomotor function. These data, and recent studies showing the presence of subependymal progenitor or stem cells in the adult rat brain and spinal cord (28, 29, 36), led us in the present study to examine the region of the central canal in normal rats and rats with

contusion lesions. We found that at several time points after injury, there appeared to be swelling and proliferation of cells in the region of the central canal at the rostral and caudal edges of the expanding secondary injury. Bunge and colleagues have also described "ependymal buds" at the rostral and caudal extents of photochemical lesions of the rat spinal cord (17). A recent account by Frisen *et al.* (27) reports that both nestin and vimentin appear to be induced in cells near the central canal after a knife-cut injury of the dorsal columns in rats, as well as in long tracts undergoing degeneration. Further, some of these cells appear to be astrocytes. These data support the hypothesis that the lesion matrix is formed in part through the development of cellular trabeculae emerging from the region of the central canal at the ends of the expanding lesion cavity. While pluripotent cells may exist and be stimulated to proliferate after injury, it is clear that they are not able to reconstitute the cord in the adult mammal the way they appear to do in amphibians and fetal mammals. It may be that the proliferation and differentiation of these cells is regulated by factors expressed in the injured adult nervous system. Such regulation could provide the means to enhance the reparative response using manipulation of endogenous precursors.

In summary, these data provide evidence that, in the MASCIS model, considerable axonal sprouting and regeneration occur into the developing lesion cavity. The amount of regeneration into the lesion cavity is related to both the severity of injury and the amount of Schwann cell invasion. Both are compatible with the notion that greater injury severity may induce a more robust immune response that induces secondary injury early on, but initiates "downstream" events in the reparative processes that enhance axonal growth, as suggested by the work of Schwartz and colleagues (32). This presents somewhat of a dilemma since sparing of tissue by reducing the secondary injury is a major strategy for enhancing recovery of function. Finally, it seems that contusion lesions in the adult rat spinal cord can initiate proliferation of ependymal zone cells, similar to the process seen in amphibians, and presumed to occur in fetal mammals. It is now well established that adult neural stem cells are under the control of growth factors and probably inhibitors (36). Thus, it is possible that such factors could be used to encourage and modulate tissue repair conducive to regeneration. Together, these results suggest that continued attention to the endogenous processes mediating partial repair after SCI may be rewarded with new strategies that could be combined with exogenous tissue bridges or other treatments to enhance recovery after spinal cord injury.

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REFERENCES

1. Basso, D. M., M. S. Beattie, and J. C. Bresnahan. 1995. A sensitive and reliable locomotor rating scale for open field testing in rats. *J. Neurotrauma* **12:** 1–22.
2. Basso, D. M., M. S. Beattie, and J. C. Bresnahan. 1996. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp. Neurol.* **139:** 244–256.
3. Basso, D. M., M. S. Beattie, J. C. Bresnahan, D. K. Anderson, A. I. Faden *et al.* 1996. MASCIS evaluation of open field locomotor scores: Effects of experience and teamwork on reliability. *J. Neurotrauma* **13:** 343–359.
4. Basso, D. M., J. R. Terman, X. M. Wang, G. F. Martin, and J. C. Bresnahan. 1996. Opossums develop some stepping after spinal cord transection (TX) on postnatal day (PD) 8 but develop normal locomotion after TX on PD 5. *Soc. Neurosci. Abstr.* **22:** 1489.
5. Beattie, M. S., J. C. Bresnahan, and G. Lopate. 1990. Metamorphosis alters the response to spinal cord transection in *Xenopus laevis* frogs. *J. Neurobiol.* **21:** 1108–1122.
6. Becerra, J. L., W. R. Puckett, E. D. Hiester, R. M. Quencer, A. E. Marcillo, M. J. Post, and R. P. Bunge. 1995. MR-pathologic comparisons of Wallerian degeneration in spinal cord injury. *Am. J. Neuroradiol.* **16:** 125–133.
7. Behrmann, D. L., J. C. Bresnahan, and M. S. Beattie. 1993. A comparison of YM-14673, U-50488H, and Nalmefene after spinal cord injury in the rat. *Exp. Neurol.* **119:** 258–267.
8. Behrmann, D. L., J. C. Bresnahan, and M. S. Beattie. 1994. Modeling of acute spinal cord injury in the rat: Neuroprotection and enhanced recovery with methylprednisolone, U-74006F and YM-14673. *Exp. Neurol.* **126:** 1–16.
9. Behrmann, D. L., J. C. Bresnahan, M. S. Beattie, and B. R. Shah. 1992. Spinal cord injury produced by consistent mechanical displacement of the cord in rats: Behavioral and histologic analysis. *J. Neurotrauma* **9:** 197–217.
10. Bracken, M. B., and T. R. Holford. 1993. Effects of timing of methylprednisolone or naloxone administration on recovery of segmental and long-tract neurological function in NASCIS 2. *J. Neurosurg.* **79:** 500–507.
11. Bracken, M. B., M. J. Shepard, W. F. Collins, Jr., T. R. Holford, D. S. Baskin, H. M. Eisenberg, E. Flamm, L. Leo-Summers, J. C. Maroon, L. F. Marshall, P. L. Perot, Jr., J. Piepmeier, V. K. H. Sonntag, F. C. Wagner, Jr., J. L. Wilberger, H. R. Winn, and W. Young. 1992. Methylprednisolone or naloxone treatment after acute spinal cord injury: 1-year follow-up data—Results of the second National Acute Spinal Cord Injury Study. *J. Neurosurg.* **76:** 23–31.
12. Bregman, B. S., E. Kunkel-Bagden, P. J. Reier, H. N. Dai, M. McAtee, and D. Gao. 1993. Recovery of function after spinal cord injury: Mechanisms underlying transplant-mediated recovery of function differ after spinal cord injury in newborn and adult rats. *Exp. Neurol.* **123:** 3–16.

13. Bregman, B. S., E. Kunkel-Bagden, L. Schnell, H. N. Dai, D. Gao, and M. E. Schwab. 1995. Recovery from spinal cord injury mediated by antibodies to neurite growth inhibitors. *Nature* **378:** 498–501.
14. Bresnahan, J. C., J. S. King, G. F. Martin, and D. Yashon. 1976. A neuroanatomical analysis of spinal cord injury in the rhesus monkey (*Macaca mulatta*). *J. Neurol. Sci.* **28:** 521–542.
15. Bresnahan, J. C. 1978. An electron-microscopic analysis of axonal alterations following blunt contusion of the spinal cord of the rhesus monkey (*Macaca mulatta*). *J. Neurol. Sci.* **37:** 59–82.
16. Bresnahan, J. C., M. S. Beattie, F. D. Todd, and D. H. Noyes. 1987. A behavioral and anatomical analysis of spinal cord injury produced by a feedback-controlled impaction device. *Exp. Neurol.* **95:** 548–570.
17. Bunge, M. B., V. R. Holets, M. L. Bates, T. S. Clarke, and B. D. Watson. 1994. Characterization of photochemically induced spinal cord injury in the rat by light and electron microscopy. *Exp. Neurol.* **127:** 76–93.
18. Bunge, R. P. 1994. Clinical implications of recent advances in neurotrauma research. In *The Neurobiology of CNS Trauma* (S. A. Salzman and A. I. Faden, Eds.), pp. 329–339. Oxford Univ. Press, New York.
19. Bunge, R. P., M. B. Bunge, and E. R. Peterson. 1965. An electron microscope study of cultured rat spinal cord. *J. Cell Biol.* **24:** 163–191.
20. Bunge, R. P., W. R. Puckett, J. L. Becerra, A. Marcillo, and R. M. Quencer. 1993. Observations of the pathology of human spinal cord injury: A review and classification of 22 new cases with details from a case of cord compression with extensive focal demyelination. *Adv. Neurol.* **59:** 75–89.
21. Cheng, H., Y. Cao, and L. Olson. 1996. Spinal cord repair in adult paraplegic rats: Partial restoration of hindlimb function. *Science* **273:** 510–513.
22. Constantini, S., and W. Young. 1994. The effects of methylprednisolone and the ganglioside GM1 on acute spinal cord injury in rats. *J. Neurosurg.* **14:** 6446–6452.
23. Crowe, M. J., J. C. Bresnahan, S. L. Shuman, J. N. Masters, and M. S. Beattie. 1997. Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. *Nature Med.* **3:** 73–76.
24. David, S., and A. J. Aguayo. 1981. Axonal elongation into peripheral nervous system: "Bridges" after central nervous system injury in adult rats. *Science* **214:** 931–933.
25. Dusart, I., and M. E. Schwab. 1994. Secondary cell death and the inflammatory reaction after dorsal hemisection of the rat spinal cord. *Eur. J. Neurosci.* **6:** 712–724.
26. Forehand, C. J., and P. B. Farel. 1982. Anatomical and behavioral recovery from the effects of spinal cord transection: Dependence on metamorphosis in anuran larvae. *J. Neurosci.* **2:** 654–662.
27. Frisen, J., C. B. Johansson, C. Torok, and U. Lendahl. 1995. Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. *J. Cell Biol.* **131:** 453–464.
28. Gage, F. H., J. Ray, and L. J. Fisher. 1995. Isolation, characterization, and use of stem cells from the CNS. *Annu. Rev. Neurosci.* **18:** 159–192.
29. Gritti, A., E. A. Parati, L. Cova, P. Frolichsthal, R. Galli, E. Wanke, L. Faravelli, D. J. Morassutti, F. Roisen, D. D. Nickel, and A. L. Vescovi. 1996. Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. *J. Neurosci.* **16:** 1091–1100.
30. Gruner, J. A. 1992. A monitored contusion model of spinal cord injury in the rat. *J. Neurotrauma* **9:** 123–128.
31. Guizar-Sahagun, G., I. Grijalva, I. Madrazo, E. Oliva, and A. Zepeda. 1994. Development of post-traumatic cysts in the spinal cord of rats subjected to severe spinal cord contusion. *Surg. Neurol.* **41:** 241–249.
32. Hirschberg, D. L., E. Yoles, M. Belkin, and M. Schwartz. 1994. Inflammation after axonal injury has conflicting consequences for recovery of function: rescue of spared axons is impaired but regeneration supported. *J. Immunol.* **50:** 9–16.
33. Iwashita, Y., S. Kawaguchi, and M. Murata. 1994. Restoration of function by replacement of spinal cord segments in the rat. *Nature* **367:** 167–170.
34. Kakulas, B. A. 1984. Pathology of spinal injuries. *CNS Trauma* **1:** 117–129.
35. Li, Y., and G. Raisman. 1995. Sprouts from cut corticospinal axons persist in the presence of astrocytic scarring in long-term lesions of the adult rat spinal cord. *Exp. Neurol.* **134:** 102–111.
36. McKay, R. 1997. Stem cells in the central nervous system. *Science* **276:** 66–70.
37. Michel, M. E., and P. J. Reier. 1979. Axonal–ependymal associations during early regeneration of the transected spinal cord in *Xenopus laevis* tadpoles. *J. Neurocytol.* **8:** 529–548.
38. Noble, L. J., and J. R. Wrathall. 1989. Correlative analyses of lesion development and functional status after graded spinal cord contusive injuries in the rat. *Exp. Neurol.* **103:** 34–40.
39. Paino, C. L., C. Fernandez-Valle, M. L. Bates, and M. B. Bunge. 1994. Regrowth of axons in lesioned adult rat spinal cord: Promotion by implants of cultured Schwann cells. *J. Neurocytol.* **23:** 433–452.
40. Reddy, K. K. V., M. R. Del Bigio, and G. R. Sutherland. 1989. Ultrastructure of the human posttraumatic syrinx. *J. Neurosurg.* **71:** 239–243.
41. Schnell, L., and M. E. Schwab. 1990. Axonal regeneration in the rat spinal cord produced by an antibody against myelin-associated neurite growth inhibitors. *Nature* **343:** 269–272.
42. Schwab, M. E., and D. Bartholdi. 1996. Degeneration and regeneration of axons in the lesioned spinal cord. *Physiol. Rev.* **76:** 319–370.
43. Sheehan, D. C., and B. B. Hrapchak. 1980. *Theory and Practive of Histotechnology*, 2nd ed. Mosby, St. Louis.
44. Wang, X. M., D. M. Basso, J. C. Bresnahan, J. R. Terman, and G. F. Martin. 1996. Are supraspinal and propriospinal axons which grow through the lesion after transection of the thoracic cord in developing opossums maintained in the adult animal? *Soc. Neurosci. Abstr.* **22:** 1489.
45. Xu, X. M., V. Guénard, N. Kleitman, P. Aebischer, and M. B. Bunge. 1995. Combination of BDNF and NT-3 promotes supraspinal axonal regeneration into schwann cell grafts in adult rat thoracic spinal cord. *Exp. Neurol.* **134:** 261–272.
46. Xu, X. M., V. Guénard, N. Kleitman, and M. B. Bunge. 1995. Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. *J. Comp. Neurol.* **351:** 145–160.
47. Young, W. 1996. Spinal cord regeneration. *Science* **273:** 451.