Activated autologous macrophage implantation in a large-animal model of spinal cord injury

Laboratory investigation

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Object. Axonal regeneration may be hindered following spinal cord injury (SCI) by a limited immune response and insufficient macrophage recruitment. This limitation has been partially surmounted in small-mammal models of SCI by implanting activated autologous macrophages (AAMs). The authors sought to replicate these results in a canine model of partial SCI.

Methods. Six dogs underwent left T-13 spinal cord hemisection. The AAMs were implanted at both ends of the lesion in 4 dogs, and 2 other dogs received sham implantations of cell media. Cortical motor evoked potentials (MEPs) were used to assess electrophysiological recovery. Functional motor recovery was assessed with a modified Tarlov Scale. After 9 months, animals were injected with wheat germ agglutinin–horseradish peroxidase at L-2 and killed for histological assessment.

Results. Three of the 4 dogs that received AAM implants and 1 of the 2 negative control dogs showed clear recovery of MEP response. Behavioral assessment showed no difference in motor function between the AAM-treated and control groups. Histological investigation with an axonal retrograde tracer showed neither local fiber crossing nor significant uptake in the contralateral red nucleus in both implanted and negative control groups.

Conclusions. In a large-animal model of partial SCI treated with implanted AAMs, the authors saw no morphological or histological evidence of axonal regeneration. Although they observed partial electrophysiological and functional motor recovery in all dogs, this recovery was not enhanced in animals treated with implanted AAMs. Furthermore, there was no morphological or histological evidence of axonal regeneration in animals with implants that accounted for the observed recovery. The explanation for this finding is probably multifactorial, but the authors believe that the AAM implantation does not produce axonal regeneration, and therefore is a technology that requires further investigation before it can be clinically relied on to ameliorate SCI. (DOI: 10.3171/FOC.2008.25.11.E3)

Key Words • activated autologous macrophage • axonal regeneration • dog • macrophage implantation • spinal cord injury

Spinal cord injury is a devastating clinical condition, with immense costs to both the individual and society. Current treatments for SCI are of limited therapeutic efficacy, which may be due to the poor capacity of axons in the mammalian CNS to regenerate. Over the past 2 decades, novel experimental therapeutic approaches have been developed to promote axonal regeneration and, by extension, functional recovery in animal models of SCI. Several of these strategies have used intraspinal cellular transplantation techniques in an attempt to overcome the unfavorable posttraumatic environment of the injured spinal cord, long thought to be inhibitory to axonal regrowth. Implantation of fetal cells, Schwann cells, neural stem cells, and olfactory ensheathing glia has been attempted, with varying degrees of preclinical success.

Some investigators believe that the immune-privileged status of the CNS results in a restricted inflammatory response when it is injured, which in turn contributes to its poor regenerative capacity relative to the PNS. In particular, it is thought that decreased entry of activated macrophages into the injured spinal cord is a significant impediment to axonal regrowth and repair. Activated macrophages are believed to play a major role in the clearance of myelin debris—which may release specific inhibitors of axonal growth—in addition to serving as a source of cytokines and growth factors important

Abbreviations used in this paper: AAM = activated autologous macrophage; CNS = central nervous system; EMG = electromyography; IACUC = Institutional Animal Care and Use Committee; MEP = motor evoked potential; PNS = peripheral nervous system; SCI = spinal cord injury; WGA-HRP = wheat germ agglutinin–horseradish peroxidase.
in regeneration. As a result, recent studies in rats have examined the impact of implanting autologous macrophages, activated ex vivo by coincubation with either segments of injured peripheral nerve or skin, into injured areas within the spinal cord. Notably, Rapalino et al. demonstrated partial recovery of motor function in rats with complete SCI when PNS-stimulated AAMs were implanted immediately after injury. They also reported continuity of labeled axons across the transected site. On the basis of these promising results in a single species, an open-label nonrandomized phase I human clinical trial of AAM implantation in complete SCI has recently been completed, and phase II trials are currently ongoing.

Despite the rapid progression of AAM implantation therapy for SCI from the laboratory to evaluation in the clinical setting, accepted pharmacological practice suggests that human trials should be based on reproducible animal experiments that demonstrate safety in at least 2 different species, and, ideally, efficacy in a nonrodent species. Consequently, we sought to replicate the results obtained by Rapalino et al. by using a similar protocol of AAM implantation in a canine model of SCI. Ethical and medical support concerns at our institution pertaining to induction of paraplegia in large animals necessitated a hemisection as opposed to a complete transection model. Nevertheless, this protocol presented a unique opportunity to assess the efficacy of AAM implantation in partial SCI, where normally some natural recovery is expected to occur.

**Methods**

**Ethics Review**

This study was conducted at the Neurosurgery Research Laboratories in the Division of Neurological Surgery of the Barrow Neurological Institute and St. Joseph’s Hospital and Medical Center, with experimental approval from the St. Joseph’s Hospital and Medical Center’s IA-CUC. As stated earlier, to comply with IACUC review, the original experimental protocol, which called for complete spinal cord transection, had to be modified to a hemisection model.

**Nerve Harvesting**

In 7 healthy adult female beagles (Dogs D1–7), each weighing ~20 kg, general anesthesia was induced using a 1:1 mixture of propofol and 2.5% pentothal for induction, and 3% inhaled isoflurane for maintenance. A linear skin incision overlaying the sural nerve was made in each animal, and a 4-cm segment of the caudal cutaneous sural nerve was harvested. The wound was closed in a multilayered fashion, and antibiotic ointment was applied postoperatively.

**Preparation of AAMs**

In accordance with the methodology of Rapalino et al., we isolated fractions of blood enriched with autologous macrophages by the process of density-gradient centrifugation, which separates cell types on the basis of size and density. In contrast to Rapalino et al., who used Percoll as the density-gradient medium, we chose to use the commercially available Opti-Prep solution (Nycomed Pharma AS) as described in detail in the monocyte isolation method of Graziani-Bowering et al. This method is quick, relatively cheap, obtains high-purity monocyte yields, and avoids the problem of constituent colloidal silica particles in Percoll medium being ingested by monocytes, which can lead to their premature activation. Furthermore, it is not subject to lymphocyte contamination of isolated monocyte fractions, which may occur when using Percoll. Briefly, 50 ml of blood was withdrawn from each dog by venipuncture. Blood was centrifuged at 550 G for 20 minutes at room temperature and the buffy coat was isolated. Ten milliliters of buffy coat was mixed with 4 ml of Opti-Prep, then overlaid with 7.5 ml of a 1.078-g/ml lymphocyte-specific density layer, which was in turn overlaid with 20 ml of 1.068-g/ml solution and 0.5 ml of HEPES buffered saline. The solution was centrifuged at 600 G for 25 minutes at room temperature. The top monocyte-rich fraction was then isolated, washed with phosphate-buffered saline, and suspended in medium. Isolated autologous monocytes were activated by coincubation with harvested sural nerve segments at 37°C for 24 hours. Nerve segments were then removed, the cells were washed again, and resuspended in medium...
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TABLE 2: Modified Tarlov Scale*

<table>
<thead>
<tr>
<th>Grade</th>
<th>Hindlimb Motor Function</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>no voluntary movement</td>
</tr>
<tr>
<td>1</td>
<td>barely perceptible movement</td>
</tr>
<tr>
<td>2</td>
<td>brisk movement of limb but no coordination w/ uninjured side; no weight support</td>
</tr>
<tr>
<td>3</td>
<td>alternate stepping &amp; propulsive movement; no weight support</td>
</tr>
<tr>
<td>4</td>
<td>weight support</td>
</tr>
<tr>
<td>5</td>
<td>ambulation w/ mild deficit</td>
</tr>
<tr>
<td>6</td>
<td>normal ambulation</td>
</tr>
</tbody>
</table>

* Adapted from Nacimiento et al.

for subsequent implantation. Light microscopy of cultured cells confirmed macrophage morphological features (that is, large granulated cells with pseudopodia and horseshoe nuclei). Table 1 shows the AAM yields obtained in each animal.

Spinal Cord Hemisection

Within 5 days of nerve harvest, all 7 dogs underwent thoracolumbar surgery after general anesthesia was induced using the drug combination described earlier. The T13–L1 vertebrae were exposed through a midline incision over the shaved and prepared thoracolumbar region. Preoperative and intraoperative radiography was used to confirm the correct spinal cord level. After performing a 2-level laminectomy with a high-speed drill, complete hemostasis was achieved, and the operating microscope was brought to the surgical field. In all but one dog (D1, positive control), a left lateral hemisection of the spinal cord was performed using a sharp scalpel blade. The cut ends were separated to create a 5-mm gap, then reinspected at high power under direct vision for any remaining intact fibers, which were subsequently sectioned. Baseline hindlimb MEPs were recorded before and after spinal cord hemisection (see below for further details). After implantation of AAMs or sham cell media, the 2-level laminectomy segment was reattached using miniplates and screws, and the wound was closed in a multilayered fashion. Antibiotic ointment was applied to the wound at the completion of the procedure. Surgeons were blinded to the assigned treatment group of each dog, except D1, which did not undergo implantation of AAMs or sham cell media.

Implantation of AAMs or Sham Cell Media

Using a 30-gauge blunt needle attached to a 100-µl Hamilton syringe (Hamilton Co.), 20 µl of media containing AAMs were implanted in 4 dogs (D2, D3, D4, and D7, hereafter identified as “implanted”) immediately following spinal cord hemisection. The exact number of AAMs received by each animal is listed in Table 1. To ensure immediate and easy accessibility of AAMs to the injured area, we injected them as described previously, directly into the injury site and into the distal stump. Care was taken to deliver the inoculum slowly, over 10–15 minutes, with the syringe immobilized to avoid any unnecessary leakage or additional tissue damage. The syringe was also withdrawn over a period of 5 minutes. Two additional dogs (D5 and D6, hereafter identified as “negative control”) received 20 µl of sham cell media in a similar manner. The positive control dog (D1) did not undergo any injection. All injections were made in a blinded fashion by the operating surgeon.

Postoperative Care

The Foley catheter that was placed during surgery was removed 72 hours postoperatively and each dog’s bladder was expressed every 4–6 hours until a neurogenic-type bladder developed (normally 2 weeks postoperatively). All dogs were placed on a multilayered padding system that was changed as needed. Dogs were moved every 4–6 hours and allowed out of their cages 1–2 times daily. Daily inspection of all dogs was done to look for pressure sores, urinary tract infection, and any other signs of systemic disease. Proper medical care was delivered whenever it was deemed necessary. Dogs were allowed to survive for 9 months under continuous observation and assessment.

Functional Motor Assessment

We used a modified Tarlov Scale (Table 2) to evaluate functional motor recovery. Dogs were evaluated monthly by an observer who was blinded to treatment group. During assessment, dogs moved freely in an open field or on a treadmill for an observation period of 10 minutes, and were rated Grade 0–6 on the basis of ability to ambulate and evidence of spontaneous or voluntary hindlimb stepping.

Electrophysiological Protocol

Stimuli for generating MEPs were provided by a “Mag-Stim” transcutaneous magnetic stimulator placed over the region of the motor cortex. Following motor cortex stimulation, MEP recordings were made using bipolar electrodes in the vastus lateralis of both hindlimbs in each dog. Recordings were made before hemisection, immediately after hemisection, and at monthly intervals during the recovery period prior to termination. The EMG activity was also measured in the forelimbs as a baseline to control for anesthetic and technical factors. A stimulus intensity of 70% maximal was used in all cases. The MEP amplitudes were calculated using only the primary evoked potential (at 20–30 msec) as an average of 5 stimuli 30 seconds apart. Amplitudes were normalized to background EMG activity by scaling to the baseline EMG activity 150 msec from stimulus onset for each averaged trial.

Retrograde Labeling and Histological Assessment

Retrograde tracing and histological assessments were performed on all dogs 9 months after spinal cord hemisection and AAM implantation. The dogs were anesthetized and a laminectomy, as described earlier, was performed at L-2, 2 levels below the site of the initial surgery. Micropipette electrode recordings were completed to confirm the position of the injection pipette within spinal cord gray...
showed similar recovery until the end of the survival period. D: The of response. Three of 4 implanted and 1 of 2 negative control dogs hindlimb at 35 days posthemisection, with a slightly prolonged latency Dog D3 showing partial recovery of MEP response in the affected left depression in the contralateral hindlimb. C: The MEP recordings from D3. Note complete loss of response on the hemisected side and a slight ed Dog D3. B: Immediate posthemisection MEP recordings from Dog misection. A: Baseline MEPs obtained before hemisection in implant- hindlimbs of experimental animals before and after spinal cord he -cally evoked MEP responses recorded in the vastus lateralis of both 65-mm deep to the surface of the spinal cord, and WGA- HRP injections were made at depths within this range based on optimal recordings. Three injections of 16 nl of a 10% solution of WGA-HRP were made 2 cm distal to the injury and 2 mm apart symmetrically on both sides of the spinal cord, for a total of 6 injections. After 48 hours, the dogs were killed and perfused with graded sucrose solutions and 4% paraformaldehyde. Axial sections of the midbrain 50 µm thick were obtained through the region of the red nucleus. After tetramethylbenzidine reaction and counterstaining with thionine Nissl stain, a manual, quantitative count of the number of cells in each red nucleus (left or right) demonstrating WGA-HRP uptake was performed in each dog. The mean number of labeled cells in the left red nucleus of implanted animals

<table>
<thead>
<tr>
<th>Dog ID No.</th>
<th>Treatment Group</th>
<th>Preop Motor Score</th>
<th>Postop Motor Score</th>
<th>Pretension Motor Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>pos control</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>D2</td>
<td>implanted</td>
<td>6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>D3</td>
<td>implanted</td>
<td>6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>D4</td>
<td>implanted</td>
<td>6</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>D5</td>
<td>neg control</td>
<td>6</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>D6</td>
<td>neg control</td>
<td>6</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>D7</td>
<td>implanted</td>
<td>6</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

matter. Neuronal firing was usually encountered 2.5–3.5 mm deep to the surface of the spinal cord, and WGA-HRP injections were made at depths within this range based on optimal recordings. Three injections of 16 nl of a 10% solution of WGA-HRP were made 2 cm distal to the injury and 2 mm apart symmetrically on both sides of the spinal cord, for a total of 6 injections. After 48 hours, the dogs were killed and perfused with graded sucrose solutions and 4% paraformaldehyde. Axial sections of the midbrain 50 µm thick were obtained through the region of the red nucleus. After tetramethylbenzidine reaction and counterstaining with thionine Nissl stain, a manual, quantitative count of the number of cells in each red nucleus (left or right) demonstrating WGA-HRP uptake was performed in each dog. The mean number of labeled cells in the left red nucleus of implanted animals was compared with the number of labeled cells in control dog D1 by using the one-sample t-test. In addition, horizontal sections at the level of the ventral funiculus that were cut through the site of the spinal cord hemisection were obtained in all hemisected animals, and examined for signs of fiber continuity or reorganization.

Results
Treatment With AAMs Does not Produce Functional Motor Recovery Beyond Control Levels

Postoperatively, all hemisected dogs (D2–D7) lost all voluntary movement in the left hind limb before experiencing some degree of recovery over the next 9 months. At the end of the 9-month observation period, negative control Dog D5 showed stepping ability of the left hindlimb without weight bearing (Motor Score 3), while D6 showed some weight-bearing capacity (Motor Score 4). In the implanted group, Dogs D2 and D3 demonstrated weight support (Motor Score 4) by the end of the experiment, but D4 and D7 only had positive stepping without any weight bearing (Motor Score 3). Overall there was no difference in the motor functional outcome between the AAM-treated group and the control group at the end of 9 months. These results are summarized in Table 3.

Treatment With AAMs Does not Produce Electrophysiologi-cal Recovery Beyond Control Levels

Cortically evoked MEP baseline responses recorded prior to spinal cord hemisection from the vastus lateralis of both hindlimbs in all dogs showed a latency of 20–30 msec and amplitude of 10–20 µV (Fig. 1A). Two hours posthemisection, no MEP response was detected from the transected side in any of the implanted or negative control dogs (Fig. 1B). At 35 days posthemisection, and for the remainder of the survival period, 3 of 4 implanted dogs and 1 of 2 negative control dogs showed clear recovery of MEP response, albeit with lower amplitude and slightly prolonged latency (Fig. 1C). The remaining dogs, 1 implanted and 1 negative control, showed only subtle signs of MEP response recovery up to 9 months posthemisection (Fig. 1D). The baseline MEP response was maintained throughout the experiment in the positive control dog (D1).

Retrograde Tracing and Histological Assessment Does not Show Long-Tract Regeneration in Animals Treated With AAMs

Results from cell counting performed to assess WGA-HRP uptake in the red nuclei of experimental dogs are summarized in Table 4. Significant WGA-HRP uptake was identified in the right and left red nuclei of the positive control dog (D1), implying intact rubrospinal tracts bilaterally; quantitative cell counts in this dog demonstrated 225 cells showing uptake in the left red nucleus and 262 in the right. Taking all hemisected dogs (D2–D7) together, the mean number of cells showing uptake in the left red nucleus was 261 ± 30. This was not statistically different from the cell count in the left red nucleus of Dog D1 (p > 0.05), confirming that the rubrospinal

| Fig. 1. Representative tracings (average of 5 sweeps) of cortically evoked MEP responses recorded in the vastus lateralis of both hindlimbs of experimental animals before and after spinal cord hemisection. A: Baseline MEPs obtained before hemisection in implanted Dog D3. B: Immediate posthemisection MEP recordings from Dog D3. Note complete loss of response on the hemisected side and a slight depression in the contralateral hindlimb. C: The MEP recordings from Dog D3 showing partial recovery of MEP response in the affected left hindlimb at 35 days posthemisection, with a slightly prolonged latency of response. Three of 4 implanted and 1 of 2 negative control dogs showed similar recovery until the end of the survival period. D: The MEP recordings obtained at 9 months posthemisection in implanted Dog D4. This was the only dog among those implanted with activated macrophages that did not show obvious MEP recovery at 9 months posthemisection. |
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Table 4: Cell counts in the red nucleus

<table>
<thead>
<tr>
<th>Dog ID No.</th>
<th>Treatment Group</th>
<th>Lt</th>
<th>Rt</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>pos control</td>
<td>225</td>
<td>262</td>
</tr>
<tr>
<td>D2</td>
<td>implanted</td>
<td>195</td>
<td>1</td>
</tr>
<tr>
<td>D3</td>
<td>implanted</td>
<td>188</td>
<td>0</td>
</tr>
<tr>
<td>D4</td>
<td>implanted</td>
<td>367</td>
<td>5</td>
</tr>
<tr>
<td>D5</td>
<td>neg control</td>
<td>291</td>
<td>2</td>
</tr>
<tr>
<td>D6</td>
<td>neg control</td>
<td>364</td>
<td>5</td>
</tr>
<tr>
<td>D7</td>
<td>implanted</td>
<td>200</td>
<td>5</td>
</tr>
</tbody>
</table>

tract originating in the left red nucleus and crossing to the right lateral funiculus of the spinal cord was intact in all of these dogs. By contrast, in the right red nucleus, no more than 5 cells showing uptake were detected in any of the hemisected dogs, and there was no noticeable difference between negative control and AAM-treated dogs. This shows that, at the end of the 9-month observation period, AAM treatment did not produce regeneration of fibers within rubrospinal tract damaged by the original hemisection. The very small numbers of cells counted in the right red nuclei in hemisected animals is consistent with a small ipsilateral projection from the ipsilateral magnocellular red nucleus. Representative axial sections cut through the red nuclei are shown for negative control animal D6 in Fig. 2, and for AAM-treated animal D7 in Fig. 3. On examination of horizontal sections that were cut through the site of spinal cord hemisection and AAM implantation in Dogs D2–D7, we also found no sign of rubrospinal tract fiber continuity suggestive of axonal regeneration (Fig. 4). Again there was no difference between negative control and AAM-treated dogs.

Fig. 2. Photomicrograph of a representative axial section obtained 9 months posthemisection that was cut at the level of the red nuclei in negative control Dog D6. Note the marked staining of cell bodies within the left red nucleus following injection of retrograde WGA-HRP tracer. As expected, there is no staining in the right red nucleus, implying complete disruption of the rubrospinal tract on the hemisected side.

Fig. 3. Photomicrograph of a representative axial section obtained 9 months posthemisection that was cut at the level of the red nuclei in implanted Dog D7. Note the marked staining of cell bodies within the left red nucleus (representative cell body shown by single yellow arrow) following injection of retrograde WGA-HRP tracer. There is no similar staining in the right red nucleus, implying persistent complete disruption of the rubrospinal tract on the hemisected side without any evidence of axonal regeneration. This finding was consistent across all implanted animals.

Premature Termination of Protocol

Because we did not observe convincing evidence of increased functional motor recovery or axonal regeneration among the animals treated with AAMs in this pilot study of 7 animals, a joint decision was made by the IACUC and the principal investigators to terminate the experimental protocol.

Discussion

In this study we implanted AAMs, activated by coinubcation with segments of autologus sural nerve, in a canine model of hemisection SCI. The AAMs were implanted immediately after the creation of the SCI. In dogs receiving AAMs, we did not find evidence of functional and electrophysiological recovery beyond that shown by dogs receiving sham cell media implantation. More significantly, we did not find any morphological or histological evidence of ipsilateral long-tract axonal regeneration in implanted dogs. This result contradicts reports of axonal regeneration in studies of AAM implantation in rodent models of SCI, which have mainly come from a single group. Although our sample size was admittedly small in this pilot study, our negative findings nevertheless raise concerns regarding the efficacy of AAM therapy across different types of SCI, and point to important methodological considerations that may be encountered when attempting to translate AAM implantation to human patients.

To date, work involving AAM implantation has been done exclusively in models of complete SCI, usually transection or severe contusion with confirmed complete loss of motor function. In particular, the re-
cently published phase I human trial\textsuperscript{15} has focused on complete SCI (that is, American Spinal Injury Association Grade A) only. In one study, Prewitt et al.\textsuperscript{26} used a dorsal quadrant aspiration model of injury, which caused only sensory loss, but that study was fundamentally different from ours in that microglia rather than activated peripheral monocytes were coimplanted with fetal spinal cord tissue into the area of the SCI by using a nitrocellulose membrane.

In our study we used a hemisection model of SCI, which may have impacted our observed findings. We used a hemisection model because of the IACUC’s request. The care of a dog that has a complete spinal cord transection is complicated, just as it is in a patient; perhaps even more so. We did not think it reasonable in a pilot project to assess a first round of AAM implantation under complete cord transection conditions. If we had seen positive results, we were prepared to move into a second stage of treatment after complete cord transection with proper care.

Several studies have shown that lateral hemisection of the spinal cord causes an initial paralysis of the ipsilateral limb, eventually followed by considerable recovery of motor function under normal circumstances.\textsuperscript{5,8,10,14,21} It has been suggested that collateral sprouting of dorsal root axons may occur caudal to the hemisected level, which is believed to enhance afferent input and promote restitution of motor function.\textsuperscript{5,14} Other authors have suggested that strengthening of latent synapses\textsuperscript{22} or the sprouting of axons in contralateral descending tracts, which reinnervates previously denervated targets,\textsuperscript{27,36} restores the necessary locomotion machinery and pattern for motor recovery. Our observation of increased latency of MEP responses in recovering animals suggests that such compensatory mechanisms may be at work, but also implies that recovery was not due to ipsilateral regeneration of severed long tracts, which was consistent with our histological findings. Admittedly, we did not look for anatomical evidence of collateral sprouting or examine changes at the synaptic level, because the focus of our study was to replicate earlier findings of axonal regeneration in SCI treated with AAM implantation. It is possible, therefore, that AAM therapy in our study may have produced changes in the levels of expression of various factors influencing synaptic formation and neuronal plasticity. Our results do suggest, however, that any changes in the levels of such factors did not add considerably to existing mechanisms of motor restitution in the hemisection model, and that their impact in this setting does not enhance functional recovery above predicted levels following partial SCI.

Mechanical factors inherent in the hemisection SCI model may offer an explanation for the absence of ipsilateral axonal regeneration. Zeev-Brann et al.\textsuperscript{37} showed that macrophage activity is initiated and upregulated by exposure to the environment of traumatized PNS tissue, but can also be suppressed on reexposure to the CNS environment. They stated that resuppression is due to the preponderance in the CNS of soluble inhibitors of phagocytic activity. Moreover, they demonstrated that phagocytic suppression was more pronounced in macrophages cultured with higher concentrations of CNS-conditioned medium. We speculate that the anatomy of the injury site in a hemisection model, which is bounded on 3 sides by intact spinal tissue, prevents the diffusion of soluble inhibitors of macrophage activity away from the lesion, thereby exacerbating the deleterious effect of these substances on phagocytosis by implanted AAMs. In the transection model, by contrast, diffusion of these soluble inhibitory substances is less restricted due to the gap-like anatomy of the lesion, with a resultant reduction in their ability to impact macrophage function at the site of injury. If axonal regeneration is indeed predicated on sustained macrophage function, and macrophage function is relatively more inhibited in a hemisection model of SCI, then this may account for our inability to find regenerating long-tract axons in this study. This phenomenon may be a consideration in future human trials examining the efficacy of AAM implantation as a therapy for partial SCI, which occurs more frequently than complete SCI.

Although macrophages may be important for myelin clearance and the creation of an environment favorable to axonal regeneration, recent studies have suggested that they may also contribute to the progression of post-traumatic secondary injury following initial SCI.\textsuperscript{9,25} Specifically, deleterious interleukin-16–mediated secondary damage by macrophages is thought to predominate right after spinal cord lesioning until 3 days postinjury,\textsuperscript{19} whereas the beneficial degradation and clearance of myelin products by these same macrophages occurs 1 to 2 weeks postinjury.\textsuperscript{50} In experimental protocols using implanted AAMs, then, the timing of their implantation following SCI may determine whether they contribute to cord repair or to further injury. In an attempt to replicate the protocol of Rapalino et al.,\textsuperscript{39} we implanted AAMs immediately after the creation of SCI. More recent studies from the same group, however, have suggested that delaying implantation improves functional and histolog-
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Conclusions

We observed partial electrophysiological and functional motor recovery in a large-animal model of partial SCI, but this recovery was not enhanced in animals implanted with AAMs and is in keeping with the partial restoration of motor function normally seen following spinal cord hemisection. Importantly, we did not see any morphological or histological evidence of axonal regeneration in implanted animals to account for the observed recovery. This failure may be due to the hemisection SCI model itself, timing of AAM implantation, the number of implanted AAMs, or the failure of this therapeutic approach altogether. However, in this instance we are highly suspicious, because of the quality of the surgical work, excellent care of the animals, and success of the laboratory producing the cells, that the AAM technology does not produce the desired effect of promoting axonal regeneration. We believe this therapy technology deserves significant further investigation before it can be relied on for clinical feasibility and benefit in SCI. Previous positive results in small-animal models do not explain mechanisms underlying AAM therapy for SCI.

Disclosure

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Drs. Assina and Sankar contributed equally to this paper.

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