THE STATE UNIVERSITY OF NEW JERSEY
RUTGERS

Final Narrative Report
New Jersey State Commission on Spinal Cord Research

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Vaccination Therapies of Rat Spinal Cord Injury
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A. Original Aims: Lisa McKerracher and Samuel David's laboratories in 1999 reported that inoculating mice with spinal cord homogenates promotes spinal cord regeneration (summarized in Figure 1). They further showed that the beneficial effects could be passively transferred through sera of inoculated mice, suggesting that spinal cord homogenate vaccination stimulated production of antibodies that promoted regeneration. Schwartz, et al (2001) found that vaccinations with myelin basic protein or glatiramer acetate (Copaxone or copolymer-1) are neuroprotective by activating lymphocytes that promote repair in contused rat spinal cords. However, it was not clear whether such inoculations are beneficial after spinal cord contusions and whether the vaccine would be effective for chronic spinal cord injury. We therefore proposed to carry out following experiments over two years.

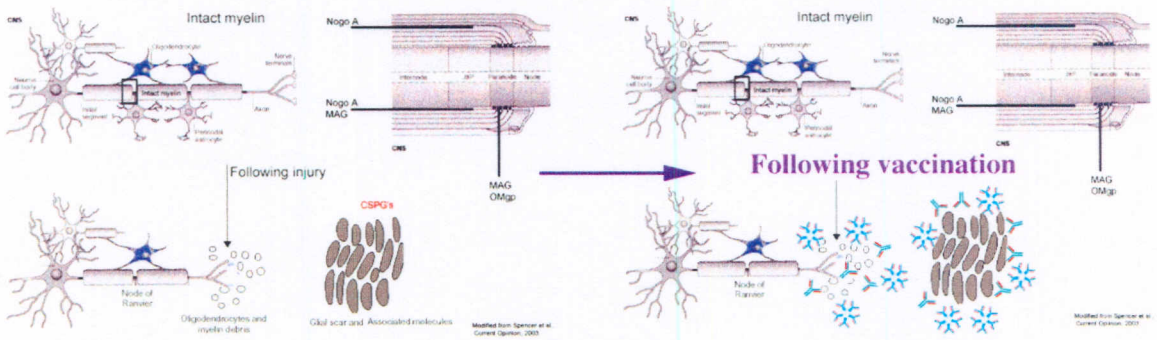


Figure 1. Following injury, injured or cut axons face several inhibitors that prevent regeneration in the CNS environment. A regenerating axon will encounter many myelin-associated molecules expressed on the membrane face or released after damage that can inhibit axonal extension. These molecules are believed to provide the primary block to regeneration prior to glial scar formation and it associated release of inhibitory molecules. Regimens of multiple injections of myelin-associated proteins were utilized to stimulate (IgM, IgG isotypes) polyreactive antibody production, neutralizing the inhibitory effects of myelin molecules thus promoting CNS regeneration (Huang et al., 1999 and Ellezam et al., 2003). Picture modified from Spencer et al., Current Opinion, 2003.

1. Confirm that a SCI therapeutic vaccination approach developed in mouse hemi-section experiments improves regeneration in the rat contusion model. The experiments were designed to extend the previous work by ascertaining whether vaccination is effective when given for consecutive weeks:
    a. prior to injury for three weeks and continuing three weeks into the injury
    b. three weeks of vaccination beginning immediately after injury
    c. starting 6 weeks after injury
2. Confirm that the beneficial effects of spinal cord homogenate vaccination can be passively transferred through sera of inoculated rats:
    a. shortly after injury
    b. three weeks after injury
    c. 6 weeks after injury

3. Compare the effects of spinal cord homogenate with two other vaccination approaches on antibody production, spinal cord regeneration, and locomotor recovery after severe spinal cord contusion.
   a. Myelin Basic Protein
   b. Glatiramer Acetate

The goal of this grant was to confirm that spinal cord homogenate vaccination promotes regeneration and improves recovery in our rat contusion model and particularly whether the vaccines work when started shortly or 6 weeks after injury. We also wanted to compare regenerative effects of spinal cord homogenate to that of glatiramer acetate, a safer non-demyelinating form of myelin basic protein. We hoped to validate and extend Sam David and Lisa McKerracher's work thus indicating the usefulness of vaccination therapies in human spinal cord injury.

B. Project successes: We successfully completed Aim 1. Our goal was to determine whether therapeutic vaccinations using spinal cord homogenate or liver homogenate, started pre plus post-injury or post-injury in a standardized spinal cord contusion, promotes axonal regeneration, prevents tissue loss and functional recovery in rats.

Adult Long-Evan's hooded rats (77±3days) were injured using the MASCIS standard weight drop Impactor (10g, 25mm).

Group A: 20 rats were vaccinated twice weekly beginning 3 weeks before contusion and continuing for 3 weeks after injury with spinal cord homogenate (SCH 100 μg, n=10) or liver homogenate (LH 100 μg, n=10).
Group B: 20 rats were vaccinated starting the day of injury and continued for 3 weeks with SCH (100 μg, n=10) or LH (100 μg, n=10).

Since Huang, et al. (1999) demonstrated that adult mice immunized in a similar manner showed regeneration of corticospinal tracts, we predicted that SCH vaccination in Long Evans rats would create antibodies against myelin-associated growth inhibitors that would therefore stimulate regeneration and locomotor recovery. Alternatively, vaccines made from liver homogenate that did not induce a significant antibody response against myelin-associated growth inhibitors including myelin basic protein (Ellezam et al., 2003) would therefore not stimulate regeneration.

Two primary outcome measures were used:

1) The BBB locomotor scale was used to track the recovery of the rats for 3 months after injury (Figure 2)
2) Anterogradely labeled axons were traced using biotinylated dextran amine to detect the regenerating corticospinal tracts (Figure 3).

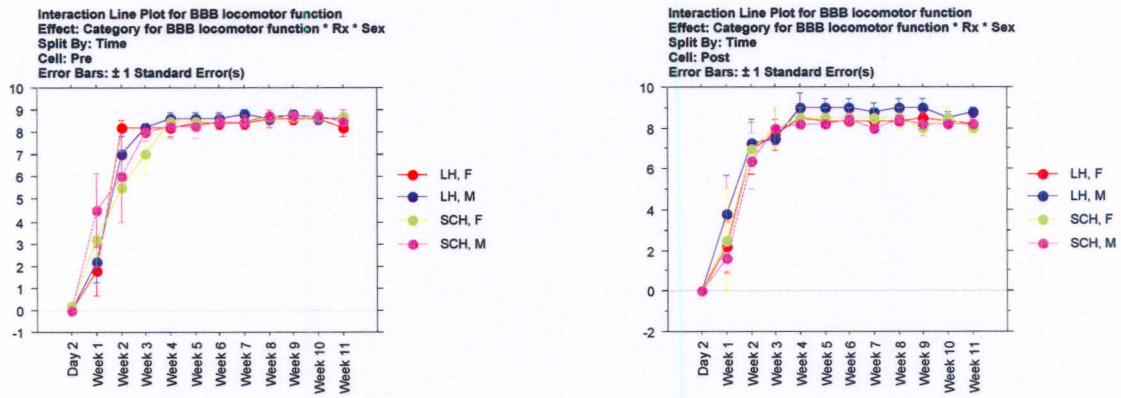


Figure 2. Vaccination with homogenate of spinal cord in rat did not increase functional recovery following a 25mm drop weight contusion injury. BBB (Basso et al., 1995; Basso et al., 1996) scoring represents open-field scores of locomotor function. Two observers assigned the scores every week. Rats vaccinated 3 weeks pre-plus-post injury (left panel) as well as rats vaccinated post-injury injury (right panel), regardless of treatment, had little or no hindlimb movements until 1-week post injury, strongly suggesting that the contusion had produced consistent spinal cord damage. Two-way analysis of variance (ANOVA) revealed no significant differences of BBB scores between spinal cord homogenate and liver homogenate vaccinated male and female rats after the third week (n = 36).

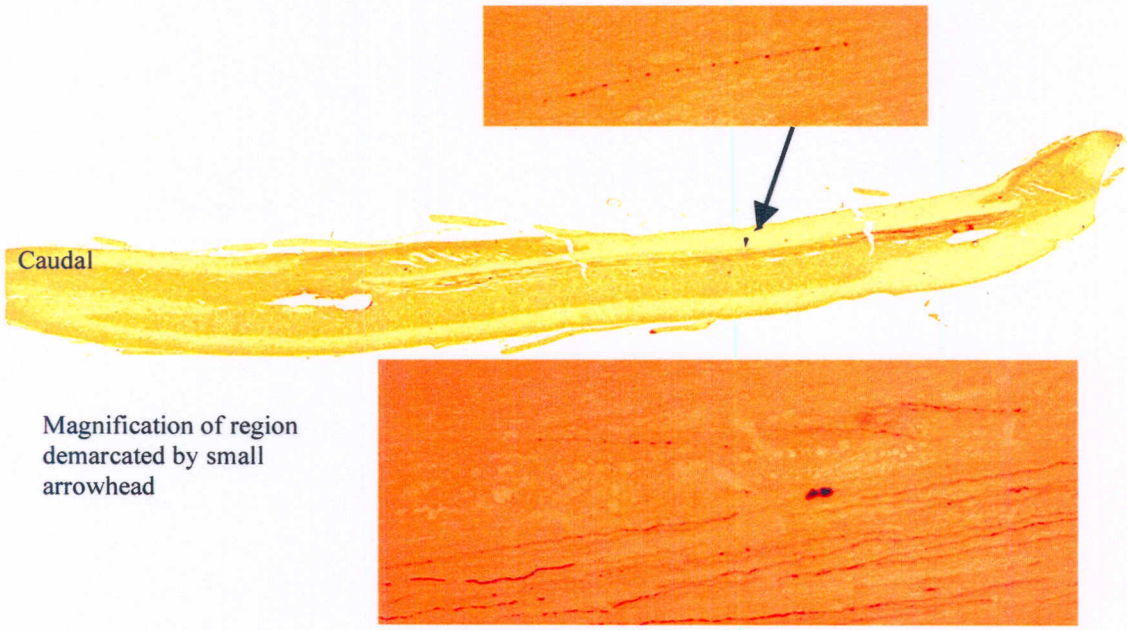
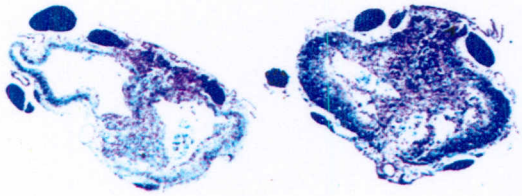
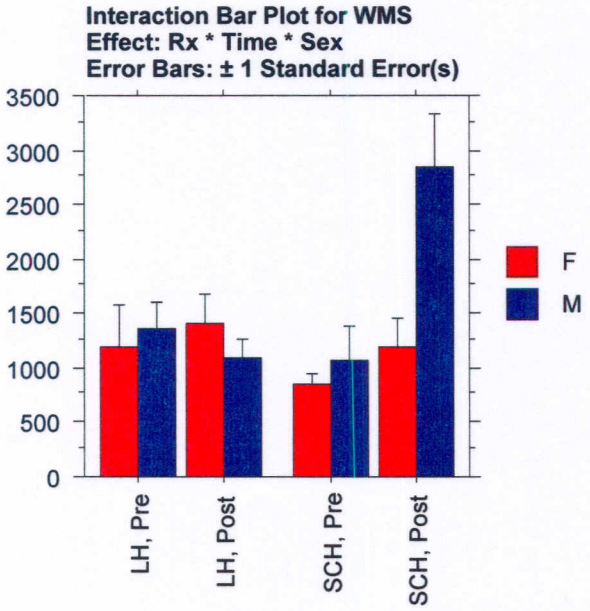


Figure 3. BDA labeled corticospinal tract fibers in proximal regions of injured spinal cord 12 weeks post SCI. Two weeks before sacrifice, we injected BDA into the motor cortices bilaterally. After sacrifice, the rats were perfused with formaldehyde. The fixed spinal cords were embedded in paraffin and stained to visualize BDA-labeled corticospinal axons. We systematically searched for anterogradely labeled axons on serially sectioned paraffin embedded horizontal sections through the whole dorsoventral extent of the spinal cord. Every 10^{th} section was stained with diaminobenzidine tetrahydrochloride (DAB) chromagen, approximately 40 μm apart. The presence of BDA-labeled CST fibers was noted at different distances from the lesion epicenter. We did not observe any regenerating axons within or distal to the contusion site although significant axonal sprouting was present in proximal thoracic spinal cords of rats vaccinated 3 weeks pre-plus-post injury, $p = .0261$.

We also quantified the area of spared white matter at the lesion epicenter to determine whether spinal cord homogenate vaccination was neuroprotective. Male rats vaccinated with spinal cord homogenate post-injury had significantly greater white matter area compared to rats treated with liver homogenate post-injury, as well as animals vaccinated with spinal cord homogenate or liver homogenate pre plus post-injury.





Bonferroni/Dunn for WMS
Effect: Rx
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
LH, SCH	-178.611	442.664	.4144	

Bonferroni/Dunn for WMS
Effect: Time
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Pre, Post	-594.371	448.943	.0114	S

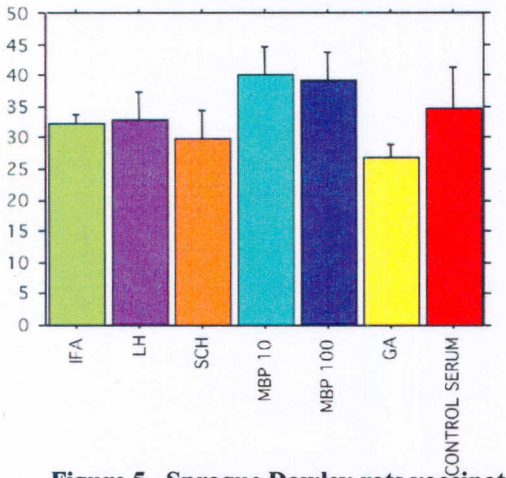
Bonferroni/Dunn for WMS
Effect: Sex
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
F, M	-463.118	441.897	.0407	S

Figure 4. Male rats that received spinal cord homogenate vaccination after injury appear to have greater white matter cross-sectional areas at the lesion epicenter. However, the white matter areas did not correlate with improved locomotor recovery. This is surprising since it has been previously shown that white matter sparing linearly predicts BBB scores in our model (Basso et al., 1996b; Beattie et al., 1997). Because white matter area at 12 weeks after injury may reflect both spared and regenerated axons, it is possible that the post-injury spinal cord homogenate vaccination stimulated growth of axons other than corticospinal tract, since the increased white matter area was not associated with improved locomotor recovery (photomicrograph shows typical "rim" white matter staining using luxol fast blue, counterstained with cresyl violet nissl satin).

We could not confirm the findings of Huang et al. of locomotor improvement and regeneration in our contusion model using Long-Evans hooded rats. Rats that received spinal cord homogenate vaccination pre plus post-injury or post-injury alone did not show axonal regeneration across the injury site or significant locomotor improvement compared to their respective liver homogenate controls.

C. Project challenges: This project was designed to take a novel and promising therapy a step closer to clinical trial. We however could not reproduce the regenerative effects reported in mouse hemisection (Huang et al., 1999) nor in rat retinal ganglion studies (Ellezam et al., 2003). Interestingly, we found that animals in our studies did not respond similarly to vaccination protocols as reported in the previous two studies. Using the identical protocol as reported in the Huang et al., studies, we in fact reduced plasma immunoglobulins (IgG and IgM as well as immunoglobulins against spinal cord homogenate itself) while their studies showed increases in immunoglobulins. We initially attributed this to difference in immune response to vaccination between species and strains of animals (BALB/c mouse and Sprague Dawley vs. Long Evan's rats), however our latest immunization data indicate that strain differences do not indeed account for differences we observe in response to vaccination therapy (Figure 5). We conclude from this data that injury severity may dictate overall immune response to CNS injuries and therefore believe that alternative approaches to antibody-mediated therapies may be more reproducible and therefore better therapies.



ANOVA Table for IgG 3 Weeks

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Rx	6	686.432	114.405	1.007	.4381	6.042	.333
Residual	32	3635.541	113.611				

Figure 5. Sprague Dawley rats vaccinated using various antigens show no significant changes in serum IgG titers 3 weeks following 25-mm contusion spinal cord injury. 6 rats per treatment group were given twice weekly vaccinations using homogenates of spinal cord (100 μg) or liver (100 μg), myelin basic protein (10 μg and 100 μg, glatiramer acetate (100 μg) mixed in equal parts of IFA and compared to serum from normal rats (n =3).

D. Implications for future research and/or clinical treatment: It had long been assumed that the presence of the inflammatory response inevitably leads to a detrimental outcome; however, more recent work has established that careful and controlled manipulation of this inflammatory response may, in fact, be beneficial. Additionally, modulation of the immune system to maximize the beneficial effects and to reduce the deleterious effects was the focus of studies reporting that vaccination with spinal cord or myelin proteins can promote tissue repair and functional recovery in spinal cord injury. Our findings to date implicate many factors that may influence the effectiveness of vaccination therapy in clinical use including but not limited to injury severity and highlight the importance of gaining greater understanding of immune therapies before any clinical applications can be proposed.

E. Plans to continue this research: Although vaccination therapies as regenerative treatments remain controversial, the ability to "turn off" those inhibitory factors that impede regeneration after CNS injury is still and intriguing therapy. While a "vaccination" approach may not be the most practical, data from these studies has fostered the idea of "disinhibiting" those inhibitory factors that stop regeneration in the CNS following injury. The advent of small interfering RNA technologies may be a more practical means of regulating myelin-associated inhibitors following spinal cord injury. Recent publications including Ahmed et al., 2005 and 2006 are exploring the use of siRNA disinhibition of axonal growth in the CNS with promising results both in vivo and in vitro. We have been actively investigating this avenue in collaboration with a biotechnology company and plan a large-scale study before the end of 2006.

F. Related publications: Adamson, C.L., Vargese, R. and Young, W. 2002. Vaccination therapy in rat spinal cord injury. The Journal of Neurotrauma, 19 (10), 1354.