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Final Narrative Report

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**Original Aims of the Project**

The goal of this project was to study the use of the cell adhesion molecule L1 as a potential therapy following spinal cord injury. Previous studies indicated that at 10 weeks following injury the control rats were barely standing while rats treated with mouse L1 were walking although not well. We proposed to address three critical parameters to optimize the administration and effects of human L1 following spinal cord injury: 1) The form of L1 to be administered. 2) The time course of treatment was to be lengthened. 3) Different doses of L1 to be administered.

**Project Progress and Challenges**

We found that human L1 and a fragment containing the 6 Ig domains were equipotent in promoting neurite outgrowth in culture. However, the potency of the human protein was not confirmed in initial experiments using the rat in vivo model of spinal cord contusion injury. Therefore, in the first year of this project, we re-evaluated all the data and came to the realization that human L1 may not be suitable for in vivo study in the rat because it may be rejected immunologically as a foreign protein despite the use of cyclosporin for immunosuppression. However, two independent animal studies performed at NYU Medical Center utilizing mouse L1 showed significant efficacy. In addition, in vitro studies have consistently shown efficacy for the human L1 as well as the mouse L1 but immune complications are not expected in the short-term culture assays in vitro. In an initial test of the human L1 protein in the spinal cord, there was some evidence for immune response of increased macrophages associated with the site of application of the human L1 protein in the rat spinal cord. When one considers that rat and mouse L1 proteins are 98.5% identical at the amino acid level while rat and human L1s are only 91% identical, it reasonable to propose that human L1 may be rejected in rat while mouse L1 would not. This difference led us to re-evaluate our proposed experiments and to consider the possibility that it was important to test the mouse protein rather than the human protein when working in the rat spinal cord injury model that is so useful for preclinical testing.

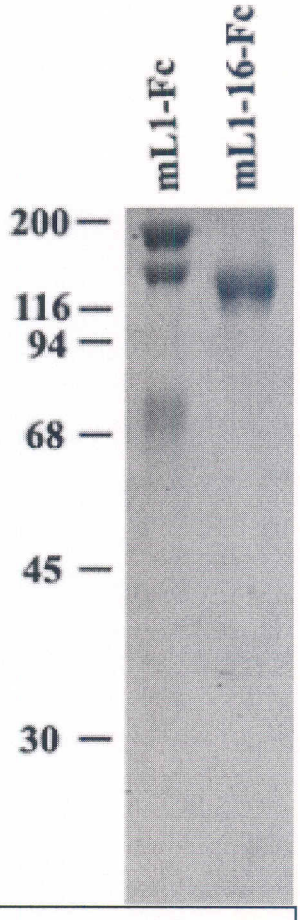



Figure 1. SDS-PAGE of L1 Proteins

Towards this end, we postponed the proposed plan to first confirm that the mouse protein and its 6 Ig domains are indeed effective in promoting recovery following spinal cord injury. Given the many improvements in the design of the protein expression and purification systems that we have implemented for the human L1 protein, it was straightforward to apply these systems to the mouse protein. At the same time that we prepared a full-length construct for the extracellular region of mouse L1, we prepared the 6 Ig domains that we have found to be most potent for the human protein (figure 1). During the same period, we also tested a variant of the protein lacking exon 2 and found that it was actually less effective than the protein with this exon, indicating the importance of exon 2, which is expressed in L1 found in the nervous

system. This result underscored the importance of using L1 containing exon 2. These results have been published (Jacob et al., 2002).

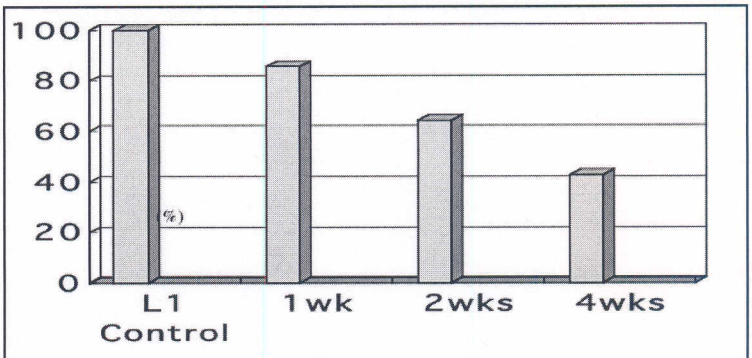



Figure 2. Measurement of intact L1 after incubation in vitro at 37°C for the times indicated. Quantitation was done following immunoblotting with anti-L1 antibodies.

Comparisons of the activity of mouse L1 and human L1 in vitro, indicated that they are equipotent; both promoted neurite outgrowth when used at 10-30 µg/ml. An important issue that had not been investigated previously was the stability of L1 in vitro and in vivo. L1-Fc was incubated for 1-4 wks at 37°C and then analyzed by separation on denaturing gels and the amount of the intact protein was quantitated. The results indicate that more than half of the protein was intact even after 2 weeks and as much as 40% still persisted out to 4 weeks (figure 2). Functional analysis confirmed that the activity of the protein to promote neurite outgrowth in culture paralleled these results, suggesting that it may be practical to apply L1 in vivo for more than 2 weeks, the time of treatment that had been tested previously.

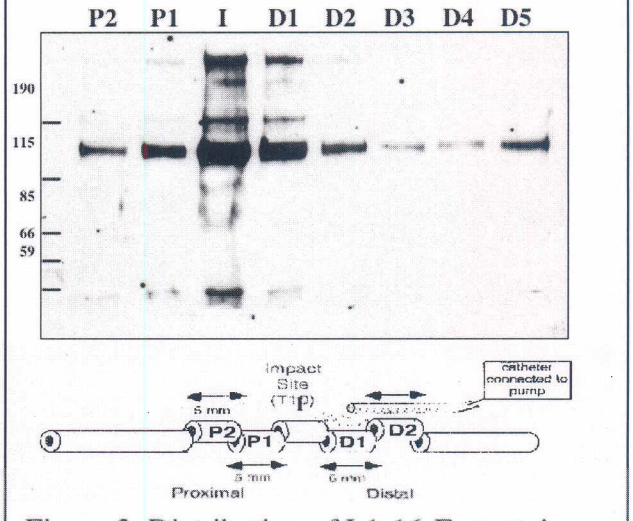



Figure 3. Distribution of L1-16-Fc protein in 5 mm segments of spinal cord 2 weeks following contusion and minipump implantation. Diagram below shows the injury site (I), distal (D), and proximal (P) segments.

Considering that L1 as an Fc fusion protein had a relatively long half-life at 37°C in vitro, it was of interest to determine its distribution in vivo following application with a minipump. At 2 weeks following pump implantation into the injured spinal cord, we analyzed the L1 protein. The results indicated that the L1-16-Fc protein was present at highest levels in the injury site near the point of delivery of the catheter tube as well as in the D1 segment that was taken 5 mm distally (figure 3). Lower levels of protein were also detected with increasing distance from the site of application as expected. These results confirmed that intact L1-16-Fc protein was still present in vivo at 2 weeks following implantation of the minipump.

After producing the mouse L1-16-Fc protein, verifying its activity in vitro and its relative stability, we tested it in vivo following spinal cord contusion with the MASCIS impactor model. The BBB scores suggested a slight

improvement over controls that may be more pronounced with the 4 week vs. the 2 week application (Figure 4). A more dramatic difference was observed in histological analysis where BDA was applied to the motor cortex in the brain, and axons

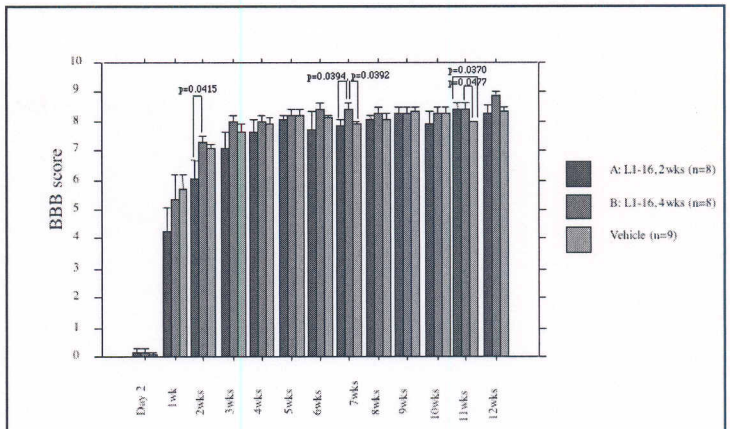
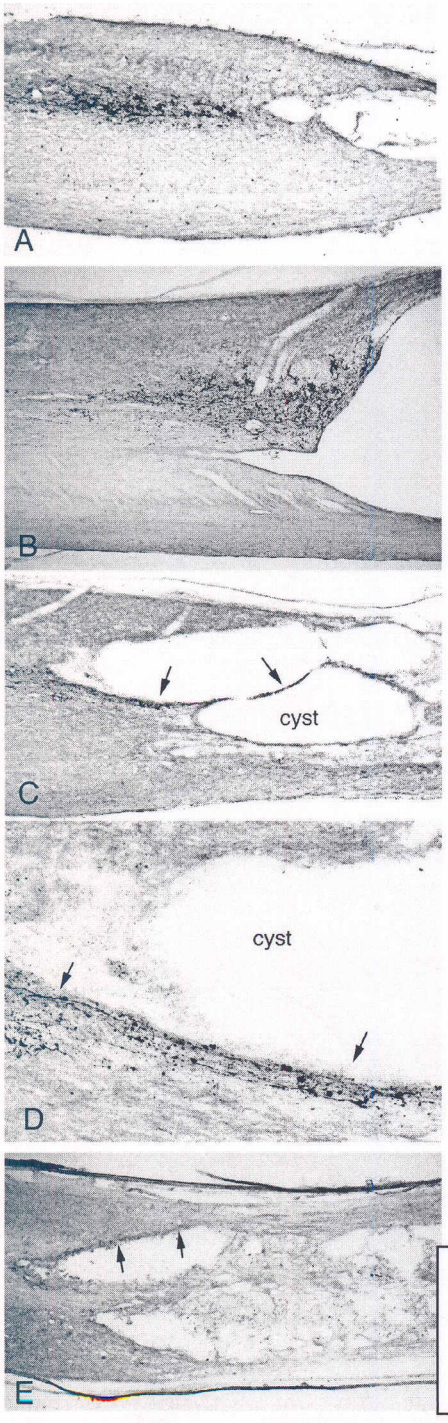



Figure 4. Functional analysis using BBB scoring.




extending to the injury site were observed to be labeled 2 weeks later. By comparison to controls where the labeled axons stopped before or at the site of injury, L1 treatment produced growth into the vicinity of the injury and many fibers coursed around the edges of the cysts that formed (Figure 5). Moreover, BDA+ fibers were observed in tissue bridges that remained as the walls of some cysts (arrows in Figure 5C). Immunnostaining to determine where the applied L1-Fc was localized indicated accumulations in the walls of the cysts (Figure 5E). Thus, the L1-Fc was detected in the very same places where the BDA+ fibers were observed, suggesting that the presence of the fibers along the walls of the cysts may be due to the action of L1 at these sites where it accumulates.

The experiments described above and those done previously with L1 all relate to acute injury. We next addressed the possibility that L1 may also have affects in chronic injury. Preliminary experiments indicated that L1 stability in the injury site was very poor at 2 weeks following injury and was actually better when applied at 4 weeks following injury. These experiments were quite challenging and involved the modification of the surgical design in order to introduce the minipump and catheter at 4 weeks following contusive injury. This optimization was performed by Dr. Young and his colleagues and we then applied a modified surgical protocol to perform our chronic

Figure 5. BDA labeling of fibers following acute injury in control (A), and L1-Fc treated (B-D) rats. Note that the staining with anti-Fc recognizes the walls of the cyst and the labeled fibers are enriched in the same vicinity (B-D).

injury experiments. The design of these experiments were to contuse the spinal cord at week 0, implant the minipumps at 4 weeks (chronic), inject the BDA label at 12 weeks and sacrifice the animals at 14 weeks for histological analyses. As of this date, we only have preliminary analyses of this experiment but the results, so far, show that in contrast to the controls where most of the fibers are arrested before they reach the cysts, the L1 treated animals show robust growth past this position and around the cysts (Figure 6). Additional studies are needed to quantitate this data.

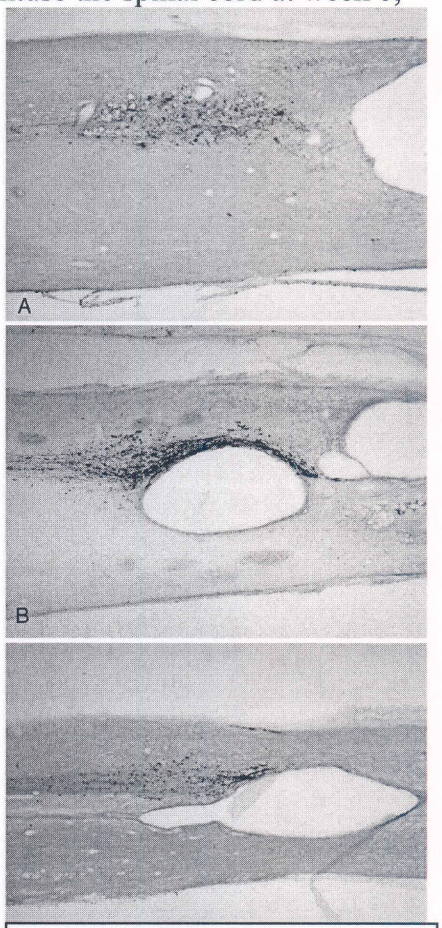



Figure 6. BDA labeling of fibers following chronic injury in control (A), L1-Fc (B) and L1-16-Fc (C) treated rats.

In summary, both in acute and chronic spinal cord injury, we have observed regenerating or spared fibers growing further into the site of injury than in controls. While data on functional recovery is only modest, at best, L1 may be useful in combination therapies. Future investigations may take advantage of L1 either as a soluble molecule or as a cell surface protein on cells that can bridge spinal cord lesions to promote fiber regrowth. It is unclear why more extensive functional recovery was not achieved in recent experiments by comparison to the initial studies performed at NYU Medical Center. However, it is difficult to resolve which of the many factors might contribute including differences in cohorts of animals used, the animal facilities and personnel, and the methods of protein production and purification. Nevertheless, the consistent and robust promotion of fiber growth observed with both full length L1-Fc and the shorter L1-16-Fc, suggests that even smaller fragments of L1 or peptides thereof may help to promote axonal regeneration. It may be advantageous to use smaller molecules that are more permeable in the spinal cord. We hope to complete a quantitative analysis of the histological samples and this may provide a basis for future investigations of L1 alone or in combinations with other treatments.

Publication:

Jacob, J., Haspel, J., Kane-Goldsmith, N. and Grumet, M. L1 mediated homophilic binding and neurite outgrowth are modulated by alternative splicing of exon 2. J. Neurobiol. (in press) 2002.