Progress Report

For 6/1/2008-5/31/13

Stimulating CNS Regeneration After Traumatic Brain Injury

Grant #08-3207-BRE-E-1

Program Director: Steven W. Levison, PhD
Department of Neurology and Neurosciences
UMDNJ-New Jersey Medical School
UH-Cancer Center, H1226
205 South Orange Ave,
Newark, NJ 07103
Tel: 973-972-5162
Fax: 973-972-26668

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BODY OF REPORT

1. Original aims of the project.

The **overall goal** of this multi-investigator proposal was to identify strategies to enhance regeneration of brain cells and to promote recovery of function after traumatic brain injuries (TBI). The proposed studies used a well-established, clinically relevant rodent model of TBI to assess recovery in pediatric, juvenile and adult animals, and to assess the roles of stem cells and microglia in promoting or inhibiting regeneration.

2. Project successes.

Significant data were generated by each of the 3 supported individual projects and by the Animal Core. We forged a productive, interactive team of investigators comprised of clinical and basic neuroscientists that offered an integrated, interdisciplinary approach to this complex problem.

Project I made significant progress on transplanting neural stem cells and progenitors into the brains of adult rats during acute and delayed recovery from TBI. We found that subacute transplantations were more effective than acute transplantations and so proceeded to only transplant cells at 7 days of recovery. In evaluating SVZ vs. VZ neural precursors, we found that the VZ cells were more primitive and thus produced a larger range of neuronal subtypes. Moreover, the VZ cells engrafted more successfully. A manuscript is in preparation describing these data. Given the greater success in using VZ cells we focused on the VZ cells. However, we found that the did not replace cells within the core of the CCI injury. Therefore, we collaborated with Dr. Cheul Cho at NJIT to produce a multi-functional scaffold to serve as a delivery vehicle for neural precursors into the lesion site. These studies took the project into a new direction. To date we have published one manuscript describing the manufacture and optimization of the scaffold and a second manuscript is under it's second review. A third manuscript is in preparation.

Project II focused on the responses of the endogenous neural precursors of the SVZ. We evaluated both immature rats and mice and compared the regenerative responses across ages. Furthermore, we evaluated which neural precursors responded to the injury and asked whether they replaced any of the neurons that had died as a result of the injury. These data are contained in rich manuscript that we have submitted to the Journal of Neurotrauma.

Project III focused on the effects of activated microglia on VZ and SVZ precursor differentiation into neurons. This project compared the responses of neurospheres from embryonic day 14 (E14) cortices with those generated from the SVZ of postnatal day 6 pups. Based on our previous findings in the embryonic basal forebrain, the hypothesis was formulated that medium harvested from microglial cultures would promote neuronal differentiation from the undifferentiated precursor cells in these neuropheres. Therefore, the numbers of TuJ1 positive cells that emerged from 4 different treatments of the cultures was quantified: defined medium alone (N2), N2 medium containing 1% fetal calf serum (FCS), medium harvested from microglia stimulated with lipopolysaccharide (LPS-conditioned medium) or LPS-CM +1% FCS. Several differences were observed between the two cell sources. First, P6 neurospheres reached their peak of neuronal differentiation at the second passage while E14 neurospheres required two additional passages. Contrary to our expectations, LPS-CM inhibited the number of neurons or neuronal precursors that were produced. Cell death was not assessed, but may play a significant role. Moreover, while LPS-CM produced the fewest neurons in both cultures, the addition of 1% FCS boosted neuronal differentiation in the P6 cultures while making only a modest difference in the embryonic cultures. While numbers of TuJ1 cells varied among treatments, additional measurements revealed unique differences in the morphology of those Tuj1 cells. At the first passage 20 cells were assessed for axon length, number of dendrites, length of dendrites, the number of secondary brances from dendrites and the length of those secondary branches. These data are listed in the table below.

Treatment	Axon length	# dendrites	Dendrite length	# of secondary branches	Length of secondary branches
N2	56.5 ± 8.7	2.3 ± 0.29	16.8 ± 1.84*	1.4 ± 0.30	11.1 ± 1.11†
1% FCS	41.7 ± 4.5	3.7 ± 0.31*	42.6 ± 4.65	1.5 ± 0.21	15.2 ± 1.84†
LPS-CM	121.6 ± 37.0*	2.6 ± 0.35	40.3 ± 6.29	2.0 ± 0.30	42.7 ± 6.64
CM+1%FCS	61.0 ± 8.37	2.4 ± 0.24	41.9 ± 6.08	2.6 ± 0.41	31.8 ± 4.25

A one-way ANOVA was done for each measurement with a post-hoc Student-Newman-Keuls test for significance at the 95% confidence level. An asterisk (*) indicates a difference from all other groups; a cross (†) indicates that each of these two treatments was significantly different from the other two. While neuronal survival and/or differentiation of neuronal precursors into neurons are undoubtedly essential elements of neuroregeneration, we find these differences in neuronal morphology to be of significance since they may also indicate additional capacity for attaining neurite outgrowth and connectivity. These data are being prepared for publication.

Finally, the **Animal Core** that we assembled to serve our projects was not only essential to enable us to produce reproducible injuries for all projects on this grant, but it also enabled us to assist other investigators at Rutgers and UMDNJ. To date, the animal core has assisted Dr. John Pintar at RWJMS and Dr. Wilma Friedman at Rutgers, Newark.

3. Project challenges.

Several issues arose during the first six months after this grant was approved by the New Jersey Brain Commission. For example, although we received a letter indicating that our grant was approved for funding by the Commission in April 2008, we did not receive a letter of award from the State until August 27, 2008. Without the funds from the State we were delayed in fully initiating experiments. Furthermore, the experiments outlined in our proposal required permission from the Institutional IACUC committees. Although we initiated the paperwork to receive permission to perform the studies outlined in our grant prior to receiving the award letter, our protocols required several rounds of revision before they were approved and the IACUC committee only meets once per month. The experiments described in our proposals involved survival surgery of animals at multiple ages, transplantation experiments, and since the experiments were collaborative, there was some confusion amongst the members of the review committee about where work would be performed that slowed down the approval process. Thus, the animal protocols for Projects 1 and 2 were not approved until July 28th, 2008 (Project 2) and November 21, 2008 (project 1). This delay, especially for project 1, contributed greatly to the inability to conduct experiments towards completing more of the experiments described in the aims of these projects. Finally, there was a delay in obtaining the EGFP transgenic rats for use in project 1 from the commercial source, thus, the first litter of EGFP pups was not born until February, 2009. Subsequent to these challenges, all of the projects proceeded forward at a good pace.

4. Implications for future research and/or clinical treatment

We have made significant discoveries that will continue to shape future research programs on regeneration after TBI. As a consequence of funding from the NJCBIR, there is now a strong cohort of investigators at all professional levels who will continue the research began under the auspices of this multi-PI grant.
In addition, we submitted a provisional patent on a new technology that we developed. This patent details a novel method to propagate stem cells. To date all of our data supporting the utility of this product have been generated using neural stem cells. We are in the process of performing additional pilot studies using a variety of other types of human stem cells. Once those studies have been completed we plan to establish a company to market our novel tissue culture product that would be used to culture a variety of stem cells suitable for clinical applications. It is entirely conceivable that products would be ready to sell within 2 years.

5. Plans to continue the research, including applications submitted to other sources for ongoing support.

The studies that we initiated under this multi-investigator award are being continued. Dr. Levison has obtained a new NCBIR award to fund a research project that arose from research conducted under this award. He has discussed his research with a program officer at the NIH, and after obtaining some additional preliminary data based upon this individual's recommendations, will submit an NIH R01 to support future studies.

Dr. Frances Calderon, who was brought in to assist Dr. Gandhi with the work on his project, now has her own grant and is continuing studies that she began under the auspices of our multi-PI grant. She will be submitting an R01 to the NIH in the fall of 2013.

Dr. Cheul Cho, who collaborated with us on work related to project 1 has submitted an R15 grant to the NIH 1R15NS087501, entitled "Engineering Multifunctional Microspheres for Brain Injury Repair".

We continue to look for RFAs from the DOD and the army, but they have yet to issue a program announcement that is in line with our research projects.

Below we list a provisional patent that we submitted earlier this year. We are planning to submit a full patent that is based entirely upon research supported by the NJCBIR.

6. Explain how you have leveraged NJCBIR funding to obtain additional federal or other support for brain injury research and list the appropriate funding organizations.

The following are grants that have been obtained to continue to support our research.

NJ Commission on Brain Injury Research CBIR13IRG017 (Levison, PI)
"Enhancing Cell Replacement After Traumatic Brain Injury"
Dates 6/1/2013-5/31/2016 6.25% Effort
Direct Costs $150,000/yr
Total Costs: $450,000

The goal of this project is to test the hypothesis that intranasal administration of leukemia inhibitory factor (LIF) after traumatic brain injury will reduce the extent of damage and increase the production of new neurons and glia from precursors of the subventricular zone.

Business Development Grant (Levison, PI)
Rutgers University Office of Technology Transfer and FNJH
"Improved Growth Matrices For Stem Cell Propagation"
Supported: 5/1/2013-10/30/2013 3% effort

The goal of this grant is to develop a multifunctional growth matrix for propagating adherent stem cells.

NJ Commission on Brain Injury Research (Skop, PI; Levison, Sponsor)
"Delivering Neural Stem Cells Using a Multifunctional Microsphere Scaffold for Traumatic Brain Injury Repair"
Dates 6/1/2012-5/31/2015 3% Effort
Direct Costs $33,500/yr
Total Costs: $100,500

The goal of this project is to produce multifunctional microspheres to serve as a delivery vehicle to support neural stem cell derived regeneration of the neocortex after traumatic brain injury.

NJ Commission on Brain Injury Research (Calderon, PI)
"Enhancement of Neural Stem Cell Survival and Transplantation Efficacy by
Docosahexaenoic Acid and its derivative NPD1 in Traumatic Brain Injury"
Dates 6/1/2013-5/31/2016 5% Effort
Direct Costs $150,000/yr

Total Costs: $450,000

The goal of this grant is to evaluate the utility of dietary supplementation in reducing inflammation and promoting the retention of transplanted neural precursors in traumatic brain injury.

7. List and include a copy of all publications emerging from this research, including those in preparation.

Project 1.

Abstracts:

1. Siriwardane M, He W, Levision S, Crawford A, Skop N, Maniker A, Gandhi CD: Evaulation of Acute and Subacute Neural Stem Cell Delivery for Traumatic Brain Injury. Neurotrauma Society Symposium, 2010.
2. Calderon F, Siriwardane M, Skop N, Jiang Y, Bajaj V, Levison S, Gandhi CD: Controlled Cortical Impact Trauma and Evaluation of Acute and Subacute Neural Stem Cell Delivery. Neurotrauma Society Symposium, 2011.
3. Nolan S, Siriwardane M, Calderon F, Jiang Y, Cho C, Levison S, Gandhi CD: Engineering a Microscaffold to Improve Neural Stem Cell Transplantation for TBI Repair. Neurotrauma Society Symposium, 2011.
4. Gandhi CD, Siriwardane M, Maniker A, Skop N, Levison S: Acute and Subacute Neural Stem Cell Delivery for TBI. CNS, 2011.
5. N Skop, C Cho, CD Gandhi and SW Levison (2011) "Optimizing a Tissue Engineered Scaffold to Promote Regeneration Using Neural Stem Cells After Traumatic Brain Injury" *American Society for Neurochemistry Meeting*
6. M Siriwardane, N Skop, SW Levison, F Calderon and CD Gandhi (2011) Characterization of Controlled Cortical Impact Trauma and Evaluation of Acute and Sub-acute Neural Stem Cell Delivery. *Presented at Neurotrauma Meeting*
7. N Skop, M Siriwardane, F Calderon, Y Jiang, C Cho, SW Levison and CD Gandhi (2012) Multifunctional Microsphere Scaffold to Improve Neural Stem Cell Transplantation for Traumatic Brain Injury Repair. Presented at the 44th Annual Meeting of the American Society for Neurochemistry, Baltimore, MD March 4-7th, 2012.

Publications

1. Skop NB, Calderon F, Levison SW, Gandhi CD, Cho CH: Heparin Cross-Linked Chitosan Microspheres for the Delivery of Neural Stem Cells and Growth Factors for Central Nervous System Repair, *Acta Biomaterialia,* 9: 6834-6843, 2013.

2. Nolan B. Skop, Frances Calderon, Cheul H. Cho, Chirag D. Gandhi, and Steven W. Levison (2013) Optimizing Chitosan-Heparin Scaffolds as a Stem Cell Delivery Vehicle (Resubmitted to *J. Tissue Engineering and Regenerative Medicine* 6/13/13).

Project 2

Abstracts:

1. MT. Goodus, A. Guzman, Y. Jiang, FC Calderon and SW Levison (2012) Proliferative Response of Neural Stem/Progenitor Cells after Traumatic Brain Injury. Presented at the 44th Annual Meeting of the American Society for Neurochemistry, Baltimore, MD March 4-7th, 2012.
2. MT Goodus and SW Levison (2013) Neural Stem Cell Proliferation and Cytokine Production in the Developing Subventricular Zone Following Pediatric Traumatic Brain Injury. Submitted to ISN/ASN Joint Meeting, April 20th, 2013, Cancun, Mexico.

Publications

1. Matthew V Covey, Yuhui Jiang, Vamsi V. Alli, Zengang Yang and Steven W. Levison (2010) "Defining the critical period for neocortical neurogenesis after pediatric brain injury" *Developmental Neuroscience* 32:488-498 (DOI:10.1159/000321607). PMID: 21160158.

2. Matthew T. Goodus, Alanna M. Guzman, Frances Calderon, Yuhui Jiang and Steven W. Levison. Regenerative Reponses of the Subventricular Zone Following Pediatric Traumatic Brain Injury. (Submitted to J. Neurotrauma, 7/31/2013).

Project 3:

Publications

1. Acevedo, G., Padala, N.K., Li, N., and Jonakait, G.M. (2013) Astrocytes inhibit microglial surface expression of dendritic cell-related co-stimulatory molecules through a contact-mediated process. J. Neurochem.125: 575-587.

Patents

1. Provisional Patent: NJMS 12-56 "Improved growth matrices for stem cell propagation in vitro and in tissue regeneration". Submitted 1/28/2013.

8. Financial summary.

Given our slow start to this project, we did not spend all the funds allocated to our work during the 3 year award period. Accordingly, we requested one and then a second no-cost extension to allow us to complete our stated goals. At the end of our second no-cost extension, we have spent all but #3319.90 of the funds awarded to us. Our grants and contracts office is providing a separate closeout document.