Human stem cell derived oligodendrocytes for treatment of stroke and MS

Reporting Period: Year 2

Over the last year we have succeeded in generating nearly pure cultures of human ES cell derived oligodendrocyte precursors from two different human ES cell lines. We are now also testing whether manipulation of transcription factors or morphogenic signaling pathways regulates the ability of these cells to differentiate into oligodendrocytes that produce myelin. We are testing these cells in a rodent stroke model to determine if they survive in the region of the stroke. If they survive, we will test whether they help to treat the strokes. We are also testing cells in transplantation into a developmental ischemia model and a model for genetic failure to produce myelin.

Reporting Period: Year 3

Our proposal centers on developing novel effective methods to generate oligodendrocytes from human ES cells. We focus on identifying signaling pathways (using studies in rodent neural stem cells) that can be adapted to human ES cells and used to regulate the efficiency of oligodendrocyte specification and differentiation from human ES cells. We then hope to use these human ES cell derived oligodendrocytes to determine whether transplantation of these cells is feasible in well characterized animal models associated with damage to oligodendrocytes. Over the last year we have made major progress toward these goals. First, we have completed and submitted for publication two studies identifying the roles of Wnts and Sox10 in regulating the development of oligodendrocytes both during brain development and during stem cell differentiation in vitro. One of these papers is in the final stages of consideration after revision and the other is submitted awaiting reviews. Second, we have developed a novel method for culturing human ES cell derived oligodendrocyte precursors. This is based on modifications of published methods but leads to greatly enhanced purity of final oligodendrocytes in our cultures (about 80% oligodendrocytes and 20% astrocytes). We have used this culture approach to address the role of sonic hedgehog in the differentiation of oligodendrocytes from human oligodendrocyte progenitors and have identified sonic hedgehog as a major regulator of oligodendrocyte differentiation and myelin production. This is quite distinct from rodent neural cells where sonic hedgehog doesn't appear to have this function. This will provide a novel therapeutic target to affect oligodendrocyte maturation and regeneration in disease models and will be of great utility for studying the function of mature human oligodendrocytes. This work is in preparation for submission. Third, we have made some significant progress in our transplantation studies. We completed studies transplanting human ES derived oligodendrocyte progenitors into a rodent model of focal stroke and found that at 1 week post stroke and 2 weeks post stroke the survival of oligodendrocytes from these transplants is very minimal. Thus, we have discontinued this work because of this feasibility issue. We have moved on to examine studies of transplantation into newborn rodents with hypoxic injury and with dysmyelination because of the shiverer mutation. The progress here is good. The hypoxia model we are using is a chronic (up to 1 week) exposure to low oxygen tension of P2 mice, which is known to cause oligodendrocyte injury. We are initially characterizing the injury to oligodendrocytes at various durations of hypoxic exposure so that we can identify the best time point to transplant our cells into the brains. We are using immunodeficient mice to decrease the chances of rejection of the transplanted cells. In addition, we are generating a mouse colony with the shiverer allele combined with an immunodeficiency allele in order to be able to transplant cells in this model. In the meantime, we are determining the survival of transplanted cells into newborn mice to identify technical factors that will need to be overcome to allow efficient transplantation and to determine if our human cells participate in differentiation in these mice. Preliminarily we have found good survival of oligodendrocyte lineage cells after transplantation into P2 mice and the expression of myelin antigens after an appropriate period of development in vivo. This is very encouraging.

Reporting Period: Year 4

In the last year we have continued our efforts to transplant oligodendrocyte progenitors obtained by differentiation of human ES cells. Our progress in this area has been mixed because of substantial technical hurdles in consistent production of the oligodendrocyte progenitors from frozen stocks of cells. This will necessitate a no-cost extension for a small portion of the work to
allow completion of the analysis of already transplanted animals. We have made substantial progress as well in showing that these cells are capable of myelinating axons effectively in vitro. In addition, we've found that the human ES derived oligodendrocytes are capable of myelinating artificial nanofibers in vitro as well. This may serve as a useful platform in the future for drug discovery or other high throughput studies. We have also identified an important novel molecular regulator of oligodendrocyte number and development and this work will continue into the future.

**Reporting Period:** Year 5 NCE

In this NCE period we were completing studies with animals that had received neonatal ischemic injury and were implanted with human ES cell derived cells of the oligodendrocyte lineage. These experiments showed that the cells survive and have oligodendrocyte lineage markers for three weeks post injection. Longer survival experiments are still ongoing.

Human stem cell derived oligodendrocytes for treatment of stroke and MS

**Grant Type:** Comprehensive Grant

**Grant Number:** RC1-00135

**Investigator:**

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<tr>
<th>Name</th>
<th>Samuel Pleasure</th>
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<tr>
<td>Institution</td>
<td>University of California, San Francisco</td>
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**Disease Focus:** Immune Disease, Multiple Sclerosis, Neurological Disorders, Stroke

**Human Stem Cell Use:** Adult Stem Cell, Embryonic Stem Cell

**Award Value:** $2,459,235

**Status:** Closed

**Application Title:** Human stem cell derived oligodendrocytes for treatment of stroke and MS
Public Abstract:

Strokes that affect the nerves cells, i.e., "gray matter", consistently receive the most attention. However, the kind of strokes that affecting the “wiring” of the brain, i.e., “white matter”, cause nearly as much disability. The most severe disability is caused when the stroke is in the wiring (axons) that connect the brain and spinal cord; as many as 150,000 patients are disabled per year in the US from this type of stroke. Although oligodendrocytes (“oligos”) are the white matter cells that produce the lipid rich axonal insulator called myelin) are preferentially damaged during these events, stem cell-derived oligos have not been tested for their efficacy in preclinical (animal) trials. These same white matter tracts (located underneath the gray matter, called subcortical) are also the primary sites of injury in MS, where multifocal inflammatory attack is responsible for stripping the insulating myelin sheaths from axons resulting in axonal dysfunction and degeneration. Attempts to treat MS-like lesions in animals using undifferentiated stem cell transplants are promising, but most evidence suggests that these approaches work by changing the inflammation response (immunomodulation) rather than myelin regeneration. While immunomodulation is unlikely to be sufficient to treat the disease completely, MS may not be amenable to localized oligo transplantation since it is such a multifocal process. This has led to new emphasis on approaches designed to maximize the response of endogenous oligo precursors that may be able to regenerate myelin if stimulated. We hypothesize that by exploiting novel features of oligo differentiation in vitro (that we have discovered and that are described in our preliminary data) that we will be able to improve our ability to generate oligo lineage cells from human embryonic stem cells and neural stem cells for transplantation, and also to develop approaches to maximize oligo development from endogenous precursors at the site of injury in the brain. This proposal will build on our recent successes in driving oligo precursor production from multipotential mouse neural stem cells by expressing regulatory transcription factors, and apply this approach to human embryonic and neural stem cells to produce cells that will be tested for their ability to ameliorate brain damage in rodent models of human stroke. Furthermore, we hope to develop approaches that may facilitate endogenous recruitment of oligo precursors to produce mature oligos, which may prove a viable regenerative approach to treat a variety of white matter diseases including MS and stroke.

Statement of Benefit to California:

Diseases associated with disruption of oligodendrocyte function and integrity (such as subcortical ischemic stroke and multiple sclerosis) are major causes of morbidity and mortality. Stroke is the third leading cause of death and the leading cause of permanent disability in the United States, costing over $50 billion dollars annually, as approximately 150,000 chronic stroke patients survive the acute event and are left with permanent, severe motor and/or sensory deficits. While much less common, multiple sclerosis (MS) is the primary non-traumatic cause of neurologic disability in young adults. Most patients are diagnosed in their 20s-40s and live for many decades after diagnosis with increasing needs for expensive services, medications and ultimately long-term care. Existing strategies for stem cell based therapies include both strategies to replace lost cells and to augment regeneration after injury, but most of these efforts have emphasized the role of undifferentiated stem cells in treatment despite the realization that the main nexus of injury in both diseases is frequently a differentiated cell type – the oligodendrocyte. This project will use new insights into the development of oligodendrocytes from the laboratories of the investigators to find ways to improve production of oligodendrocytes from human ES cells and human neural stem cells, test whether these cells can improve the clinical outcome in rodent models of stroke and MS after transplantation and search for new molecular treatments that would augment the regeneration of oligodendrocytes from resident brain stem cells after injury. This is the first step to translating the basic fundamental understanding of oligodendrocyte development into viable therapies for important human diseases that are major burdens on the citizens of California.