Cleveland Clinic

Center for ALS and Related Disorders

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The following is description of the ongoing and developing projects in the laboratory of Erik P. Pioro, M.D., Ph.D., Director of the Cleveland Clinic Center for ALS and Related Disorders, and the costs associated with them. Funds will be directed to support the continuation of these studies and to accelerate the developing projects, all of which have high impact for novel therapeutic development in ALS.

A. Ongoing ALS projects in the laboratory of Erik P. Pioro, M.D., Ph.D. in the Cleveland Clinic Center for ALS and Related Disorders, Lerner Research Institute, which require the continued support of his lab personnel (two research fellows and one technician):

1. ***Discovery that the slow wallerian degeneration (Wld^S) gene exerts significant neuroprotection in the wobbler mouse model of ALS.*** We find evidence of preserved motor neuron number, muscle mass, reduction of neuronal vacuolization, and regeneration of nerves.

   - **Relevance to ALS**: Use of the Wld^S gene or its mechanisms of action could be a treatment to retard or arrest ALS progression and possibly rescue lost motor function in patients with ALS.
   - **Ongoing and future projects are aimed at**:
     (1) Identifying gene pathways which are activated (leading to neuroprotection) and suppressed (preventing neurodegeneration) to characterize the mechanism of Wld^S action. This is the first step to finding novel therapies which mimic or enhance its action;
     (2) Developing a technique to deliver the Wld^S gene or its chemical equivalent to muscle and/or CNS initially in tissue culture and then to the intact mouse; this is a necessary prelude to developing a gene therapy for patients with ALS

2. ***Discovery that protein aggregates form in many wobbler mouse spinal cord neurons, as they do in ALS, where they have been thought to be harmful.*** However, we find evidence that these inclusions may be neuroprotective at least initially, by sequestering harmful proteins from the rest of the cell.

   - **Relevance to ALS**: Characterizing the contents of these inclusions will identify the harmful proteins which in turn may reveal the underlying cause of ALS. This could lead to developing a cure for ALS. Because the inclusions have been thought to be harmful, some novel treatments designed to reduce their formation may, in fact, be detrimental to the neuron.

- **Ongoing and future projects are aimed at**:
  (1) Purifying the aggregate contents by using sophisticated techniques (e.g., mass spectrophotometry) to identify the protein contents and cellular pathways involved in neurodegeneration and neuroprotection
  (2) Assessing function of the enzyme system which degrades these abnormal proteins and enhancing it with medications, if suboptimal.

3. ***Discovery that microglia, rather than being helpful to motor neurons in the brain and spinal cord, may contribute to their demise when they become overly activated in ALS.*** This occurs when the function of CX3CR1, a receptor found only on microglia, is removed partially or completely. We observe this microglial neurotoxicity in two extensively studied mouse models of ALS, the transgenic $SOD1^{G93A}$ mutant and the wobbler mouse.

   - **Relevance to ALS**: Microglia have been suspected to be harmful to motor neurons in ALS but this has never been demonstrated in a living animal until now. About 30% of humans are estimated to possess microglia with suboptimally functioning CX3CR1, potentially allowing them to become over activated. Medications that suppress microglial activation may be effective therapies in such individuals to suppress or arrest the progression of ALS.

   - **Ongoing and future projects are aimed at**:
     (1) Develop *in vitro* (tissue culture) model of the CX3CR1 spinal cord in co-culture with embryonic stem-cell motor neurons (in collaboration with researcher at Johns Hopkins University) to identify chemicals involved in neurotoxicity and neuroprotection. This will lead to experiments which block the toxic compounds and enhance the protective compounds to develop novel therapies targeting the neuroinflammatory cascade we identified.
     (2) Perform gene array studies of purified microglia from the affected mice to determine the regulators of neurotoxicity and possibly neuroprotection.
     (3) Determining whether the more severe effect of microglial activation in male mice is related to hormonal or genetic causes. (Male patients with ALS are also known to develop ALS more frequently and more severely than females).

B. Developing ALS projects which will require the support of two additional research fellows and one technician. These studies will be done in outside collaborations with established experts in the fields of genetics and stem cell biology, respectively:

1. ***Identifying human genes responsible for causing hereditary ALS.***
   To date, the SOD1 gene mutation is essentially the only known cause of hereditary ALS, although it accounts for only about 20% of familial cases. This means about 80% of hereditary ALS has no known explanation. Plans are underway to test novel experimental therapies to block the damaging effects of mutated SOD1 in patients with this mutation. It is crucial to identify genes responsible for the other cases of hereditary ALS so similar treatments can be developed.
   - Blood is being routinely collected from all Cleveland Clinic patients with non-SOD1 hereditary ALS and family members to extract DNA. Cutting edge high sensitivity techniques in use by collaborators at the National Institutes of Health will be applied to these samples to identify

abnormal genetic regions. This will provide novel directions to identify other genes responsible for ALS.

2. ***Using adult derived stem cells to replace and repair atrophied muscle in ALS***.
Although replacing motor neurons is important in restoring function, without healthy muscle to perform the function, the rescue of motor neurons will be ineffective.
   - Stem cells from adult muscle will be isolated and purified for injection into the denervated muscle of the ALS mice listed above to determine their ability to integrate and restore function. Wobbler mice with preserved motor neuron number and enhanced regenerative potential because of the Wld^S gene will be used to further enhance the muscle bulk and strength.
   - Bone marrow-derived stem cells from an adult mouse which are labeled fluorescent green will be administered to mice with ALS (wobbler and $SOD1^{G93A}$ mutant) and tracked to determine their ability to target muscle (as well as brain and spinal cord), and their ability to rescue the disease phenotype. This will explore the feasibility of enhancing the capability of endogenous stem cells to restore neurologic function in ALS patients.

Both sets of experiments will assess the potential for using adult-derived stem cells from muscle and bone marrow to restore muscle function (and possibly brain/spinal cord function) in patients with ALS.

C. Annual salary support for laboratory personnel performing the aforementioned experiments and analyses (ongoing and future):

- Research fellows ~ $60,000 (including benefits): two presently employed = $120,000; two planned to be hired = $120,000, total = $240,000 annually.
- Lab technician ~ $40,000 (including benefits): one presently employed = $40,000; one planned to be hired = $40,000, total = $80,000 annually.

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D. Costs of breeding and maintaining experimental animals (mice) in the Biological Resources Unit (BRU) at the Cleveland Clinic, required for the experimental studies listed above.

- These costs presently average $2000.00 per month (~$12,000.00 per year) and are estimated to increase by approximately 20% ($400.00 per month) in the next 6 months because of rising BRU charges and addition of greater cage numbers for planned experiments.

E. Costs of purchasing equipment necessary to perform many of the aforementioned experiments and analyses, which have, to date, been borrowed temporarily from other laboratories:

- Microscope capable of brightfield and fluorescence photomicroscopy - $45,000.00
- Software for microscopic image analysis - $15,000.00
- Equipment for testing rodent strength (digital gauge and grip device) - $4000.00.