RESEARCH IN THE WORKS 2006:
Muscular Dystrophy Canada's Neuromuscular Research Partnership Grants

The Neuromuscular Research Partnership (NRP) is a result of an agreement between the ALS Society of Canada, Muscular Dystrophy Canada (MDC) and the Canadian Institutes of Health Research (CIHR). Collectively, the partners will contribute over $2,500,000 towards funding research that will ultimately result in discovering the causes, treatments and an eventual cure for neuromuscular disorders. The Partners are pleased to announce the results of the seventh NRP competition for 2006.

Twenty five operating grant applications were received by the March 1, 2006 deadline, of which 11 were approved for funding.

Thank you to all of our donors whose generosity has made this possible.

RESEARCH

1). Dystroglycan function and dysfunction in the fly CNS
Period: one year
Principal Investigator(s): CARBONETTO, Salvatore T.
Co-Investigators: RAO, Yong
Institution: McGill University, Department: Neurology

A number of different proteins interact with each other to form larger protein complexes that are critical in muscle function. Previous research has examined the role played by the proteins alpha dystroglycan and beta dystroglycan (DG) in forming these larger dystrophin complexes and has shown that muscle deficient in DG undergoes severe deterioration. Recent research has also shown that DG plays an essential role in the organization of neurons during early brain development, as well as playing an ongoing role in the transmission of signals between neurons. Defects in DG function contribute to the mental retardation that often accompanies Duchenne muscular dystrophy as well as several other forms of muscular dystrophy. Research examining DG function in the neural development of photoreceptors in the eye of the fly has shown that it is critical to normal development. The fly eye provides a useful model for further study of the role played by DG in the development and operation of the central nervous system. Understanding how DG operates in the nervous system will be important in developing strategies to help mitigate the impact of mutations that affect dystroglycan function in the brain, leading to mental retardation associated with dystroglycan mediated muscle diseases.

2. Analysis of transmitter release mechanisms
Period: one year
Principal Investigator(s): Charlton, Milton P.
Co-Investigators:
Institution: University of Toronto

Botulin toxin is a protein produced by the bacterium *Clostridium botulinum.* Affecting the nervous system, it is the most poisonous naturally occurring substance in the world. The toxic protein is an enzyme that attacks proteins at the nerve terminals of the neuromuscular junction, preventing the vesicles from releasing the neurotransmitter acetylcholine. By preventing acetylcholine release, the toxin interferes with nerve impulses causing paralysis of muscle. This foreign toxin protein can exist as an active enzyme in the nerve terminal for several months and is responsible for the lengthy time course of botulinum paralysis which can last up to nine months. Although it is highly toxic, it is used in medicine in minute doses, both to treat painful muscle spasms, and as a cosmetic treatment (Botox). This proposal examines the mechanisms of the longevity of botulism and botulinum toxins. Normally, the cell has a series of processes that can identify and "digest" abnormal or foreign proteins it finds, including a chemical process called ubiquitination and the subsequent deconstruction of the toxin by the proteosome complex or other cellular mechanisms. The hypothesis of the current research is that the toxin forms a protein complex that protects it from undergoing this normal digestion. The researchers have previously identified a small chemical (a peptide) that attaches itself onto the toxin. This study will test whether this peptide appears in motor neurons and neuromuscular junctions and whether it can block ubiquitination of the toxin, preventing the cell from digesting it. The research will also show whether the toxin is modified by other processes in the cell. If the peptide under examination does protect the toxin from degradation, researchers will seek interfering peptides that could be used to prevent the peptide from binding to the toxin thereby providing a treatment for botulism.

This research also deals with mechanisms that differentiate synapses into strong and weak types. The research focuses on the presynaptic terminal where the chemical transmitters are released. Researchers will try to determine why and how some presynaptic terminals release transmitter with high probability while others release with low probability. Studies into the mechanisms of this presynaptic differentiation may allow future development of therapeutics that can be selectively aimed at synaptic enhancement, thereby allowing for faster recovery from injury to muscles.

3). The role of protein chaperones in regulating neurofilament dynamics in health and neuromuscular disease.
Period: one year
Principal Investigator(s): Durham, Heather, D.
Co-Investigators:
Institution: McGill University

This research is based on discoveries involving two central biological features: first, there is a series of complex protein filaments making up internal "skeletons" and forming complex networks of fibers (fibrillar networks) within the cell. The axons of neurons contain high concentrations of these neurofilaments. Genetic mutations of the filaments, or of associated proteins, or as a result of other injury, lead to abnormal organization of neurofilaments, a feature commonly observed in neuromuscular disease. Mutations of a filament called the neurofilament light subunit (NFL) have been implicated in causing Charcot-Marie-Tooth (CMT) disease.

Second, abnormal protein displays a propensity to become insoluble – the normal mechanisms of the cell can no longer digest and remove these abnormal proteins and they aggregate into inclusions, essentially, lumps of abnormal protein that clog the cell. These mutated proteins and inclusions are associated with amyotrophic lateral sclerosis (ALS) and some forms of Charcot-Marie-Tooth (CMT) disease among other neuromuscular degenerative diseases. Normally, the cell uses small proteins called chaperones and other mechanisms (the ubiquitin/proteasome systems) to manage and dispose of abnormal proteins. Mutations in one of these chaperone proteins, the small heat shock protein number 27 (HSP27) have been implicated in causing CMT.

Mutations in the neurofilament (NFL) and in heat shock protein (HSP 27) not only both cause CMT but lead to similar alterations in neurofilament organization. This discovery has encouraged the researchers to examine the role of protein chaperones in the neurofilament structure and transportation systems in the cell and to try to discover how these basic mechanisms are disrupted in CMT. Therapies designed to improve chaperone function and prevent abnormal protein aggregation and motor neuron death have been studied in a model of amyotrophic lateral sclerosis (ALS). This research will evaluate the effectiveness of these approaches in experimental models of CMT. This model primarily uses cells derived from the spinal cord and nerves. The hypothesis is that the protein chaperoning and ubiquitin/proteasome systems play an important role in regulating the assembly, and maintaining the stability, of neurofilament networks. If HSPs are important in the integrity of these neurofilament networks, then an increase in the activity (upregulation) of HSPs may protect neurons from disease mechanisms.

4). Mechanism of sensory protection of denervated muscle
Period: one year
Principal Investigator(s): Fahnestock, Margaret
Co-Investigators: Bain, James, Batt, Jane
Institution: McMaster University

In many neuromuscular disorders, the flow of nerve signals to skeletal muscle is lost (denervation) and many muscle fibers shrink (experience atrophy) and die. It has been discovered that even if regeneration and reinnervation by the original motor nerve or a collateral nerve occurs, the muscle still can not regain its receptivity to signals. Research has consistently shown that prolonged denervation of a muscle leads to poor functional recovery, primarily because of the inability of the muscle to recover from muscle fiber death, due to the development of fibers tissue (fibrosis) and /or exhaustion of the regenerative capacity of muscle's satellite cells. Many clinical approaches use therapeutic regimens to attempt to maintain healthy muscle during periods of denervation by maintaining stimulation of muscle activity (via physiotherapy, electrical stimulation, etc). Unfortunately, the loss of the normal cellular nutritional (trophic) supports supplied by the motor axon is partially responsible for these irreversible changes and these are not addressed by muscle stimulation. Poor functional recovery of muscle and nerve after nerve damage or disease is a serious problem in patients. Delayed muscle reinnervation following injury is a key factor leading to poor functional recovery. Even if nerve

regeneration is successful, by the time the regenerating motor nerve reaches the muscle, it has often suffered profound and irreversible damage. The research team has previously shown that a substitute sensory nerve attached onto the end of the nerve stump or muscle during prolonged denervation, significantly protects the structure of the skeletal muscle and increases functional recovery by supplying some of these cellular supports. This technique of sensory protection has been used to protect denervated rat muscle for up to six months. The presence of the sensory nerve results in substantial nutritional support to the muscle, even in the complete absence of any electro-stimulatory activity.

The technique of sensory protection offers potential therapeutic benefits for patients with focal muscle denervation such as injuries to the peripheral nerve (sensory to motor nerve transfers). It also provides a powerful experimental research model to investigate the molecular changes in muscle that occur due to inactivity versus those that occur due to the loss of nutritional supports. The research proposal is designed to investigate the cellular and molecular changes in muscle and nerve accompanying sensory protection. The objective of the research is to learn more about the molecular and cellular mechanisms involved in the recovery of muscle function during sensory protection. This knowledge may lead to novel pharmacological treatments for muscle that is chronically denervated as a result of neuromuscular disorders thus protecting muscle structure and increasing the possibility of future functional recovery.

The researchers utilize a rat model, involving denervation of the rat gastrocnemius, soleus and plantaris muscles by cutting the tibial nerve. Sensory protection consists of attaching the saphenous nerve, a pure sensory nerve, to the end of the tibial nerve stump. The animals, along with suitable control groups, are allowed to remain in the colony for up to six months, sufficient time for severe muscle atrophy to occur in non-sensory-protected animals. This model has led to improved understanding of the cellular and molecular changes accompanying long-term denervation and reinnervation of muscle. The technique of sensory protection provides a powerful experimental research model to identify and investigate those factors involved in nerve-muscle communication and contributed to or modulated by, the sensory nerve, as opposed to those coupled to muscle depolarization. Such factors are potential targets for therapeutic intervention. This work also will advance the clinical use of sensory protection for the treatment of prolonged muscle denervation due to injury or disease.

5). Development of an integrating adenoviral vector for gene therapy of Duchenne Muscular Dystrophy
Period: three years
Principal Investigator(s): Gilbert, Renald
Co-Investigators: Massie, Bernard
Institution: McGill University

Duchenne muscular dystrophy (DMD) is a progressive disease of skeletal and cardiac muscle leading to muscle weakness and premature death. There is currently no effective treatment available. The damage and destruction of skeletal and cardiac muscle that characterizes DMD is due to a deficiency of an essential protein called dystrophin. One

potential approach to treat this disease is to re-introduce dystrophin production into the dystrophin starved (dystrophic) muscle. This could be performed by directly transferring the DNA coding sequence for dystrophin directly into the muscle tissue (gene therapy). To accomplish this, the dystrophin genetic code is attached to a virus (called a vector) and the virus enters the cells, carrying the code along with it. These viral vectors are of critical importance in introducing gene sequences into cells.

Another method to introduce dystrophin would be to transplant muscle cells (myoblasts or myogenic stem cells) that will form new muscles (cell therapy). The likelihood of success of cell therapy is considerably improved if the cells utilized for transplantation are derived from the patient (autologous cell transfer), as this essentially eliminates an immune response and the problems of tissue rejection.

The goal of this research is to develop and test a novel vector that will enable the integration and long-tern expression of full-length dystrophin DNA code at specific muscle sites. This vector is derived from an improved generation of adenoviral vectors, known as gutless or helper-dependent adenovirus (HDAd). These adenoviruses are safer because they do not carry any viral genes. The safety and efficacy of this improved vector will be studied in the mouse model of DMD (mdx mice) and pre-clinical applications for both cell and gene therapy will be evaluated.

There are two potential therapeutic applications for this new vector:
A) Cell therapy treatment of DMD. At the present time no efficient vector exists that can stably introduce the full-length dystrophin into the cells needed for cell therapy. Once fully developed, this vector should permit stable integration of the full-length dystrophin coding sequence into laboratory pools (cultures) of myoblasts and myogenic stem cells previously isolated from the patient's dystrophin deficient muscles. Autologous cell transplantation using these genetically modified cells should regenerate and repair the dystrophic muscle without major immune reactions.
B) Gene therapy. Direct injection of this vector between the muscles, or dissemination through the blood circulation, should restore normal dystrophin expression in muscle. This research will explore ways to improve the integrating capacity of dystrophin expression leading to permanent expression, thus sparing the treated muscle from further damage.

The experiments should lead in the development of an invaluable vector that may be used for treating DMD using a cell and/or gene therapy approach. For both types of experiments (gene and cell therapy) duration of dystrophin expression and beneficial effects will be evaluated.

6). Folding and aggregation of ALS- associated mutants superoxide dismutases
Period: three years
Principal Investigator(s): Meiering, Elizabeth
Co-Investigators:
Institution: University of Waterloo

Research on amyotrophic lateral sclerosis (ALS) has discovered abnormal forms of the antioxidant enzyme, superoxide dismutase (SOD). These enzymes are destabilized in ALS and consequently have an increased tendency to form protein aggregates that appear to be toxic to neural cells. Previous research has clearly demonstrated an increase of toxic function for these abnormal SODS, however, the nature of this toxic function is not known. Just how these ALS-associated abnormal SODS contribute to ALS remains a mystery; however it is possible they represent a direct cause. There is mounting evidence that ALS may be another example of a growing group of protein conformational disorders, which includes various neurodegenerative diseases such as prion diseases, familial amyloid polyneuropathies and Alzheimer's disease. In these diseases, the normally formed protein becomes defective and misfolded, creating an abnormal shape that the cell can not digest, leading to the formation of toxic clumps or aggregates of protein. Mutant SODS have been found to form aggregates in patients with familial ALS, in transgenic mice, and in cell cultures. Furthermore, mutant SOD aggregates are observed in mice well before the onset of disease symptoms, implicating SOD aggregation as a possible direct cause of ALS.

Laboratory research conducted by this group to study the altered stability and aggregation of mutant SODs, beginning in October 2003, is yielding much data on SOD characteristics that have important implications for understanding the behaviour of this protein in ALS. Ultimately, these experiments may lead to urgently needed strategies for treating ALS by preventing or reversing toxic protein aggregation.

The hypothesis of this research is that mutant forms of superoxide dismutase (SOD) that cause familial amyotrophic lateral sclerosis (ALS) have decreased stability compared to normal SOD protein, display altered folding and dynamics, and have a greater propensity to form neurotoxic aggregates. This research will seek in-depth understanding of the notoriously complex and variable protein aggregation processes and their relation to protein stability and dynamics. Such knowledge is critical for understanding and ultimately intervening to ameliorate the effects of abnormal protein aggregation in ALS.

7). Peripherin abnormalities in amyotrophic lateral sclerosis
Period: three years
Principal Investigator(s): Robertson, Janis
Co-Investigators:
Institution: University of Toronto

Within their watery bodies, cells contain complex skeletons made up of filaments of protein. Several neurodegenerative diseases are now associated with the appearance of abnormal tangles of these filaments that subsequently form indigestible inclusions or aggregations of protein. The exact impact of these clumps of abnormal protein is unclear.

Peripherin is a protein intermediate filament (IF) (called a type III neuronal intermediate filament) found associated with the majority of pathological IF inclusions in the diseased motor neurons of patients with amyotrophic lateral sclerosis (ALS). Overproduction of peripherin in transgenic mice leads to the formation of abnormal protein aggregates in the

neurons that precedes massive age-related motor neuron degeneration, closely modeling ALS. This evidence suggests that peripherin may be an important factor contributing to motor neuron degeneration in ALS. The recent finding of peripherin gene mutations in a few ALS cases supports this finding. However, these mutations were only found in three ALS cases of several hundred tested and therefore cannot completely explain the widespread contribution of peripherin to filament pathology seen in both sporadic and familial forms of the disease.

Three known types of peripherin (called splice variants) exist in the mouse and these researchers have previously shown that there is differential expression of these different forms of peripherin in mouse models of ALS. These results suggest that uncontrolled (deregulated) peripherin formation may contribute to causing disease in ALS. The researchers have identified several splice variants of peripherin in human neuronal tissue and shown their deregulated expression in human ALS spinal cord tissue. This is the first evidence of peripherin splicing in humans and of the association of specific splicing events with ALS. This research will further characterize and describe peripherin forms and their creation as a means to understanding the mechanisms that cause ALS.

8). Satellite stem cells from skeletal muscle for the treatment of neuromuscular disease
Period: five years
Principal Investigator(s): Rudnicki, Michael A.
Co-Investigators:
Institution: Ottawa Health Research Institute

Muscle satellite cells are believed to be responsible for the growth, repair and maintenance of skeletal muscle. In the laboratory, myoblasts derived from satellite cells contribute very poorly to regenerating muscle following transplantation. Important recent experiments have suggested that a small proportion of undefined, freshly isolated satellite cells, have the capacity to extensively contribute to regenerating muscle.
This research group has made the seminal discovery that 10% of muscle (sublaminar) satellite cells are *bona fide* stem cells and have developed the methodology to isolate the cells. This research will study the cellular and molecular properties of this novel population of muscle generating (myogenic) stem cells in mice and humans. Researchers will identify genes that are uniquely expressed in these stem cells, identify novel factors involved in regulating their function, as well as identify new markers to facilitate their purification. Researchers will investigate the potential of myogenic stem cells to repair muscle after transplantation. Researchers predict that these prospectively isolated human myogenic stem cells will exhibit a very high capacity to repopulate the satellite cell area and, as well, extensively contribute to muscle regeneration. This discovery provides a new paradigm that redefines satellite cell biology and opens new doors for therapeutic intervention for the treatment of devastating neuromuscular diseases such as Duchene Muscular Dystrophy. For example, isolated satellite stem cells may be of great utility for direct transplantation. Alternatively, this new knowledge will lead to identification of therapeutic interventions (biologics) or small drugs that specifically target the relevant pathways leading to satellite stem cell expansion. Such new drugs would greatly ameliorate the progression of diseases such as muscular dystrophy by stimulating the

endogenous regenerative capacity of skeletal muscle. This research should provide important new information into the biology of muscle regeneration and open new avenues for therapeutic intervention for the treatment of neuromuscular disease.

9). Development of immunological tolerance in monkeys for therapy is for muscular dystrophies based on cell transplantation
Period: three years
Principal Investigator(s): Tremblay, Jacques P.
Co-Investigators:
Institution: Centre hospitalier de l'Universite Laval (PQ)

This research group is developing treatments, primarily for Duchenne muscular dystrophy (DMD) based on the repair of the damaged muscle fibers and even their complete replacement by transplanting the cells (myoblasts) that form these muscle fibers. The cells used for transplanting are obtained from a normal donor. Their fusion with the remaining host muscle fibers or their fusion between themselves will permit the formation of muscle fibers, which produce dystrophin and which function normally. Moreover, as demonstrated in mouse and in DMD patients, the transplanted cells also form satellite cells that will also be able to participate in additional muscle repair. This research team has recently completed a Phase 1 clinical trial of myoblast transplantation to DMD patients and the expression of dystrophin was observed in 8 out of 9 patients who participated in the clinical trial.

One of the problems of this type of cellular therapy is that the myoblasts are obtained from a donor and are thus rejected by the patient unless the patient's immune system is suppressed by drugs. The sustained use an immunosuppressive drug induces adverse effects. This research is aimed at preventing the rejection of myoblasts transplanted into monkeys from different monkeys (allogeneic transplants) by developing a host specific immunological tolerance – to allow the immune system of the host to tolerate these genetically different cells. The proposed protocol could eventually be used in humans since it uses only two drugs (cyciophosphomide and treosulfon) which are already approved for human use. The main aim of this research is to develop immunological tolerance for allogeneic myoblast transplantation in monkeys using a protocol that is mild and that uses only two drugs, both already approved for human use. The experiments will also be done with fully genetically incompatible donors to verify whether the tolerance generating protocol permits the development of sufficient tolerance when donors are fully different (allogeneic); which may be the case in a clinical setting where patients are being treated. The protocol does not use any irradiation and all drugs used in this protocol are already clinically approved. Thus, the protocol could be eventually applicable for Duchenne Muscular Dystrophy patients. This protocol to induce immunological tolerance may also be applied to permit the successful transplantation of other types of cells and of organs.

10). Enhancement of the specificity and efficiency of macrophage-fusion-mediated cell therapy of skeletal myopathies.
Period: two years
Principal Investigator(s): LONG, Michael (studentship under Dr. Fabio Rossi)
Co-Investigators:
Institution: Biomedical Research Center, University of British Columbia.

The goal of this research is to develop a method which allows blood cells to act as repair vehicles for damaged muscle. Damaged skeletal muscle is normally repaired by the production and subsequent fusion of muscle satellite stem cells with the damaged muscle cells. These satellite cells exhibit a number of undesirable properties for therapeutic applications, for example, they trigger an immune reaction, they are relatively rare and they cannot travel through the body via blood circulation. An attractive alternative are bone marrow derived (blood) cells that also go to the aid of damaged muscle in great numbers, however, they do not appear able to render much practical assistance as few of these cells are able to fuse with muscle cells. This research will attempt to increase the efficiency with which this fusion takes place. This would create an attractive alternative to satellite cells, namely, bone marrow derived (blood) cells for researchers to use in their therapeutic strategies to try to address muscle disorders. Two specific techniques will be examined in this research, the primary one involves using an experimentally modified measles virus to stimulate the blood cell's fusion mechanism to enhance fusion between these cells and muscle cells. It is thought that these modified donor cells will fuse with damaged muscle cells at a higher and more effective rate. Following fusion, functional levels of dystrophin protein should be increased and contribute to improved muscle function. This will be tested in a mouse model of Duchenne muscular dystrophy.

11). Isolation and analysis of embryonic stem cell derived skeletal muscle progenitors.
Period: two years
Principal Investigator(s): PRICE, Feodor (studentship and are Michael Rudnicki)
Co-Investigators:
Institution: Ottawa Health Research Institute

Embryonic stem cells create a number of intermediary cells that then develop further into skeletal muscle. These intermediary cells are known as progenitor cells. It is hoped that in the future, researchers will be able to take advantage of laboratory techniques using stem cells to create "batches" of muscle cells suitable for transplantation into patients with Duchenne muscular dystrophy. To achieve this goal, techniques will have to be developed to handle and better differentiate these progenitor muscle cells. These different types of cells exhibit subtle but clear differences in their chemical characteristics. Researchers can identify different types of cells and see what they are doing (active or inactive?) using these unique chemical identifiers. This research project will study how to manipulate stem cells to produce large numbers of muscle progenitor cells suitable for transplantation and will also assess their capacity to regenerate. This assessment will be done both in the laboratory and in the muscles of mice modeling Duchenne muscular dystrophy.

HOW WE FUND RESEARCH

For decades, Canadian researchers have been world leaders in the search for the causes, cures and treatment of muscular dystrophy and other neuromuscular disorders. Since its inception in 1954, Muscular Dystrophy Canada has provided support to these scientists and we are dedicated to continuing this mandate in the future.

Muscular Dystrophy Canada has three main research programs: the Neuromuscular Research Partnership (NRP), Fellowships, and the Young Investigators grants. From time to time Muscular Dystrophy Canada may take part in awarding other grants deemed to have a great impact on neuromuscular research.

The NRP is a partnership between CIHR (Canadian Institutes for Health Research), ALS Canada and Muscular Dystrophy Canada. It follows CIHR's "winter cycle" for awarding grants. The competition is announced in the fall and includes a request for proposals. Interested researchers apply throughout the winter and CIHR convenes a committee and ranks the proposals in April and May.

The CIHR ranks the proposals based on a set of strict and in-depth criteria. Muscular Dystrophy Canada convenes a committee to judge the relevance of the proposals to neuromuscular disorders and chooses to contribute on this basis.

Muscular Dystrophy Canada's committee consists of two scientists and a staff member from each of Muscular Dystrophy Canada and ALS.

All funds raised by Muscular Dystrophy Canada for research are given directly to the research institution (a university or hospital, for example) that holds the grant in trust for the researcher.

The NRP greatly reduces the administrative costs and time incurred for all parties and sets a high standard for the research conducted.