

Grants Awarded in 2005

The National MPS Society has awarded \$320,000 in new grants for 2005-2006. Two grants for MPS III research were awarded \$60,000 each, \$30,000 for each of the 2 years of funding. This year a special grant category was created for research specific to Bone and Joint research, and those researchers will receive \$50,000 for each of the 2 years of funding, for a total per grant of \$100,000. The MPS II research grant offered this year will not be funded due to the lack of acceptable proposals received.

**Dr. Matthew Ellinwood, Iowa State University, Department of Animal Science Ames, IA .
"Therapy for MPS IIIB: Naglu Targeting to the CNS"**

The blood brain barrier is the single greatest hurdle to bringing therapies for neuropathic lysosomal storage diseases to clinical application. One approach to overcome this barrier involves direct injection into the central nervous system of either recombinant enzyme, gene therapy vectors, stem cells, or gene therapy treated stem cells. An alternate method is to genetically engineer the missing enzyme of a particular lysosomal storage disease, so that it is able to cross the blood brain barrier by an active process of uptake from the blood and delivery across the blood brain barrier. This would allow for treatment of the central nervous system either by intravenous delivery of the engineered recombinant enzyme, or alternatively, treatment with a liver directed gene therapy vector designed to deliver the gene for the engineered enzyme. We propose to evaluate this latter method to develop a treatment for mucopolysaccharidosis type IIIB (MPS IIIB). Using parts of proteins that bind the LDL receptor we propose to genetically engineer the Naglu enzyme so that it will cross the blood brain barrier via this receptor system. We will evaluate this method in a cell culture system, and successfully engineered enzyme will then be evaluated clinically using liver directed gene transfer into the murine and canine models of MPS IIIB.

**Dr. Donald Anson, Department of Genetic Medicine, North Adelaide, South Australia, Australia
"Lentiviral-mediated Gene therapy for MPS IIIA"**

Gene therapy is an attractive option for the treatment of the mucopolysaccharidosis (MPS) including MPS III. The development of a new generation of technologies for the delivery of genes has meant that gene therapy now looks a more realistic option than ever before. However, the safety and efficacy of these technologies need to be carefully evaluated before clinical application can be considered, and even with these new vectors, treatment of brain pathology is challenging. This project aims to use a small animal (mouse) model of one of the most common MPS, MPS III A, to evaluate a new gene delivery technology for its ability to treat brain pathology. Two methods for delivering the vector to the brain will be evaluated. The level and

distribution of gene delivery that results, its persistence, and its effect on disease pathology, will be assessed using a variety of enzymatic, molecular and biological techniques.

Dr. Calogera Simonaro, Mt. Sinai School of Medicine, New York, NY 10029
"Pathogenesis and Treatment of Bone and Joint Disease in the Mucopolysaccharidoses"

The overall goal of our work is to use animal models of the mucopolysaccharidoses (MPS) to investigate the underlying causes(s) of cartilage and bone disease in these disorders. We have already shown that the cells in MPS cartilage are prone to death, and that in response to this primary damage, a series of biochemical changes occur (known as ?inflammation?), exacerbating the disease. We will continue to evaluate the causes(s) of MPS bone and joint disease, and based on these results, identify new ways to monitor disease severity, progression and treatment response. We will also evaluate new approaches to therapy. We will extend our findings in the cartilage of MPS animals to other joint tissues and bone. We will assess cell death and ?inflammation? in these tissues, as well as study bone growth. We will also utilize findings from these studies to identify specific ?biomarkers? that might predict disease severity and treatment response. For this we will study these markers in the blood and joints of MPS VI animals treated by enzyme replacement and gene therapy. Lastly, we will use MPS animal models to evaluate new approaches to treat these disorders, including using inhibitors of ?inflammation? and/or cell death, as well as enzyme replacement therapy using a new form of the recombinant enzyme that can penetrate cartilage more efficiently.

Dr. Sharon Byers, Department of Genetic Medicine, North Adelaide, South Australia, Australia
"The Pathogenesis of Cartilage Degradation in MPS VI"

Cartilage/joint disease presents a significant challenge to the clinical care of patients with mucopolysaccharidosis (MPS). With the advent of enzyme replacement therapy (ERT), the injection of enzyme into the circulation and into the localized joint space has the potential to significantly alter the progression of cartilage disease in MPS. However, to maximize the benefit of ERT it must be applied prior to the start of clinical symptoms. The benefit of ERT to patients with established symptoms is less clear cut. The pathogenesis of cartilage destruction in MPS I is poorly understood although it appears to resemble that observed in osteoarthritis (OA). In particular the turnover of the glycosaminoglycan (GAG) component of MPS cartilage and the relationship between GAG storage and disease progression is unclear. The focus of this proposal is to understand the mechanism of cartilage destruction in MPS, using the MPS VI cat model. The degradation of cartilage proteoglycans and the subsequent uptake and storage of their GAG component will be investigated. This information will be used to develop supplemental therapies targeted to joint disease in MPS.

1st Year Research Reviews

The pathogenesis of cartilage degradation in MPS VI.

Dr. S. Byers

The immediate aims of this proposal are to (i) characterise the breakdown products of cartilage proteoglycan in MPS VI with specific reference to the fragments generated by aggrecanase or matrix metalloproteinase activity (MMP) and to (ii) assess proteoglycan turnover in MPS VI cartilage cells. The long-term goal of the proposal is to determine if cartilage degradation in MPS VI follows a pathway similar to that observed in osteoarthritis (OA) or rheumatoid arthritis (RA). If this correlation can be made it will open the way to apply OA or RA therapies to MPS VI as a supplement to systemic treatments such as ERT in an effort to specifically target joint disease.

We have made significant progress towards aim (i) with the identification of an MMP driven cartilage turnover process. Normal and MPS VI cartilage from 2, 6 and 11 month old cats was extracted into 4M guanidine HCl buffer and digested with chondroitinase ABC prior to electrophoresis on SDS-PAGE. Western blot analysis was carried out using a panel of antibodies directed towards the carbohydrate or protein components of intact proteoglycan or towards neo-epitopes generated by aggrecanase or MMP cleavage of proteoglycan. Total proteoglycan analysis demonstrated predominantly aggrecan with lesser amounts of decorin/biglycan that did not significantly alter between normal and MPS VI or with age. The presence of low levels of an ITEGE neo-epitope indicative of aggrecanase digestion of aggrecan was observed in both normal and MPS VI cartilage that did not alter with age. In contrast, a DIPEN neo-epitope indicative of an MMP directed digestion of aggrecan accumulated with age. Gelatin zymography demonstrated the presence of detectable levels of MMP-9 in cartilage from 2 month old MPS VI cats. MMP-9 did not become apparent in normal cartilage until 6 months of age or later. MMP-2 levels were also increased in MPS VI although not to the same extent. MMP-3 and MMP-7 were not detected in either normal or MPS VI cat cartilage.

In degenerative joint diseases such as OA or RA, MMPs degrade both the proteoglycan and collagen component of cartilage leading to irreversible tissue damage. Our initial results suggest that the same mechanism may be involved in MPS VI joint disease and that MMPs would constitute a potential intervention point.

Lentiviral-Mediated Gene Therapy for MPS III-A

Chief investigators: Assoc Prof Donald S. Anson and Dr Sharon Byers

Staff: Ms Lann Tay, summer student and Ms Chantelle McIntyre, PhD student

Lentiviral vectors represent highly modified viruses that can be used to introduce gene sequences into cells. In this project a lentiviral vector will be used to introduce a normal copy of the sulphamidase gene, which is defective in MPS III-A, into the liver and the central nervous system of MPS III-A mice. The effect of gene transfer on the course of the disease will then be monitored. This will include assessment of brain function as well as histopathology and biochemical analyses of tissue samples.

Progress to date

Ms Lann Tay, a third year student from the University of South Australia, was funded from the grant. Ms Tay worked on finalising a lentiviral vector construct that is to be used in the study. We hope to attract Ms Tay back as an Hons student in 2007.

In order to maximise the return from the funding received from the MPS Society, and to have a full time person work on the project, we have established a postgraduate scholarship award in conjunction with the University of Adelaide. Ms Chantelle McIntyre was awarded this scholarship and started work on the project in January of this year. Ms McIntyre is a very able and focussed student and she has quickly established the project and the work is now moving rapidly forward.

Ms McIntyres work has initially focussed on finalising initial studies on somatic lentiviral-mediated gene therapy in the MPS IIIA mouse. This work had two clear aims

- 1) To analyse samples from mice treated with a lentiviral construct expressing murine sulphamidase from the phosphoglycerate kinase gene promoter (LV-pgk-msulp) that was delivered via intravenous injection.
- 2) To assess several different promoters for their ability to drive high levels of sulphamidase expression after lentiviral-mediated gene delivery.

The results of (2) have shown that the elongation factor 1 α gene promoter is significantly better (approximately 3-fold higher expression) than the pgk promoter originally used. Accordingly this promoter will be used for all further studies. As it has been widely established that the efficacy of gene therapy for the storage diseases (by whatever route) correlates with the level of

enzyme expression we are also exploring other means of enhancing sulphamidase expression from our vectors including codon optimisation of the sulphamidase coding sequence and the use of RNA transport elements in the sulphamidase transcript.

The further analysis of samples from the MPS IIIA mice treated with the LV-pgk-msulp construct has confirmed the efficacy of intravenously delivered vector for treating much of the somatic disease. More interestingly, our results suggest that this approach may also be efficacious for treating central nervous system pathology. We have found that the elevated level of β -hexosaminidase expression seen in MPS IIIA central nervous system tissue is significantly reduced in the treated mice. β -hexosaminidase levels are widely accepted as a measure of pathology in the storage disorders. Unfortunately, sulphamidase levels could not be measured directly in central nervous system tissue from treated mice due to the limited sensitivity of the sulphamidase enzyme assay. However, our result is not without precedent, AAV-mediated liver targeted gene therapy (1) and high dose enzyme replacement therapy studies (2) in the MPS VII mouse have both indicated that enzyme transport across the blood brain barrier does occur in adult mice, albeit at low levels. These results suggest that high level expression of sulphamidase in somatic tissues such as the liver has the potential to be efficacious in treating the central nervous system as well as poorly vascularised somatic tissues/cells such as chondrocytes. We are currently trying to confirm these results by direct measurement of storage material in the central nervous system using mass spectrometry. We feel this is an exciting result as it raises the possibility of a relatively non-invasive gene therapy approach for MPS IIIA central nervous system disease. This study is currently being prepared for publication and will be presented at the 2006 International MPS Meeting in Venice by Dr Sharon Byers.

We have also established the stereotaxic injection equipment necessary for the direct injection of virus into the CNS. We are currently preparing virus for our first experiments and anticipate that these will take place in the next 4-6 weeks.

In addition, we have imported the MPS IIIA strain from Jackson labs (Sgshmps3a/PstJ) in order to establish a colony that has been backcrossed onto on a pure genetic background (C57BL/6J) that is more suitable for our research purposes (removing genetic variation) than our previous colony (the original "New York" strain) that was on a mixed (129SvJ, C57BL/6, SJL, CD1) genetic background. These mice have now cleared quarantine.

The CIB, Dr Sharon Byers, has now established that a modified water maze, the T maze, offers the most robust way of assessing neurological function, accordingly, we will be using this test in all our future studies in the MPS IIIA mice.

Our priorities for the second year of finding are to

- 1) Establish the direct delivery of our vector to the CNS by stereotaxic injection.
- 2) Study vector distribution after stereotaxic injection using a vector carrying the LacZ marker gene

- 3) Assess the effect of delivery of the sulphamidase gene to MPS IIIA mice via stereotaxic injection.
- 4) Repeat our somatic gene delivery experiment with a more efficient vector design
- 5) Incorporate the T maze behavioural analysis into our experimental design.

References

1. Sferra TJ, Backstrom K, Wang CS, Rennard R, Miller M, Hu Y. Widespread correction of lysosomal storage following intrahepatic injection of a recombinant adeno-associated virus in the adult MPS VII mouse. *Mol Ther* 2004; 10: 478-91.
2. Vogler C, Levy B, Grubb JH, Galvin N, Tan Y, Kakkis E, Pavloff N, Sly WS. Overcoming the blood-brain barrier with high-dose enzyme replacement therapy in murine mucopolysaccharidosis VII. *Proc Natl Acad Sci (U S A.)* 2005; 102: 14777-82.

Therapy for MPS IIIB: Naglu Targeting to the CNS

Dr. Matthew Ellinwood, Iowa State University, Dept. of Animal Science, Ames, IA

The object of this research proposal is to generate a fusion of the enzyme Naglu, that will be able to cross the blood brain barrier by active transcytosis, leading to a CNS treatment of MPS IIIB. Such an enzyme fusion could be used for either gene therapy, or intravenous enzyme replacement therapy. The strategy we propose is to create fusions between Naglu and the ligand domains of the LDL receptor ligands Apolipoprotein B and E (ApoB and ApoE). The grant has 3 specific aims.

Aim 1 is to construct and evaluate the enzyme fusions in vitro.

Aim 2 is to evaluate the behavior of the fusions in vivo using mice and an AAV2/8 vector.

Aim 3 is to evaluate efficacious vectors from Aim two in the canine model.

To date we have constructed three Naglu enzyme fusions, one with the ApoE at the beginning of the enzyme (amino terminus fusion), and one each with ApoB and ApoE at the end of the enzyme (carboxy terminus fusions). Our results indicate that, compared to the normal human Naglu enzyme, the amino-terminus ApoB-Naglu fusion protein expressed in human embryonic kidney cells (293 cells) produce enzymatic activity of 60% whereas the carboxy-terminus Naglu-ApoB and Naglu-ApoE fusions produce about 30% of activity. Moreover, the Naglu activity was also detected in the media of the transfected cells suggesting that the ApoB and ApoE fused Naglu proteins are secreted in the cell culture media. The expression of the

recombinant ApoB and ApoE Naglu fusions was confirmed by western blot using an anti-human Naglu specific antibody and an antibody which recognized a tag sequence of the fused domain. We also performed immunofluorescence cell staining to confirm that our recombinant constructs yield intact enzyme fusions able to localize in the lysosomes. Immunofluorescent cell staining with confocal microscopy demonstrates that the myc signal (a tag element in the fusions) in transfected primary normal dog fibroblast co-localize with both an anti-human Naglu antibody and a lysosome specific anti-Lamp1 antibody. These results suggest that significant Naglu activity is still present if fused with the ApoB ligand domain at its amino-terminal extremity and that the Naglu activity is higher with an amino-terminus fusion than with a carboxy fusion.

Further work underway at this time includes the purification of enzyme fusions to measure the specific activity of the enzyme fusions relative to normal human Naglu. Additionally, AAV2/8 vector constructs are being designed to express these fusions and will be evaluated using a liver expression/gene therapy approach in the MPS IIIB knockout mouse. These studies will form the preliminary study for a gene therapy evaluation of this approach using the MPS IIIB dog.

Work supported by this grant was presented this April (2006) in Alexandria, Virginia at the NINDS Workshop: Glycosphingolipids in Health & Disease, and at the 9th International Symposium of Mucopolysaccharide and Related Disorders, in Venice Italy, June, 2006.

The entire amount of this award (\$30,000) has gone toward the salary and benefits support of Dr. Rafi Awedikian (~80% supported on MPS Society funds).

Pathogenesis & Treatment of Bone & Joint Disease in the Mucopolysaccharidoses

Calogera M. Simonaro, PI: Mount Sinai School of Medicine

The major goal of our research is to gain a better understanding of bone and joint disease in the MPS disorders, and, based on these findings, to develop improved therapies for these organs. We are also attempting to identify improved biomarkers that can be used to predict the severity of bone and joint disease in individual MPS patients, as well as their response to therapy. During the past year we have demonstrated an important connection between the immune and skeletal systems in the MPS disorders. In addition to our previous results showing that cells in the joints and bones of MPS animals undergo premature cell death due to the accumulation of glycosaminoglycans (GAGs), our new studies suggest that an inflammatory disease is occurring in these animals similar to rheumatoid arthritis.

In MPS and arthritis, the stimulation of inflammatory cytokines (IL-1 β and TNF- α), proteins that stimulate or inhibit the proliferation or function of immune cells, leads to the secretion of

several matrix metalloproteinases (MMPs), which in turn lead to cartilage degradation and, ultimately, to bone destruction. The activation of cells of the synovial membrane, which lines the cavities of joints, as well as specialized immune cells, or T cells, have a positive effect on osteoclast (bone absorbing cells) formation by stimulating certain blood cells (macrophages) to secrete additional proinflammatory cytokines into the joint space. The production of these cytokines leads to the differentiation and activation of osteoclasts, resulting in bone loss and joint destruction. These findings were not restricted to one particular MPS disorder or species, and appear to define a common, broad mechanism of bone and joint destruction in these disorders. The use of anti-inflammatory agents to block the secretion of proinflammatory cytokines at various stages in the inflammatory cascade is presently being investigated in our lab. This therapeutic strategy might be used as a combination therapy with ERT to alleviate the bone and joint disease in MPS.

In general, our preliminary results indicate that early in life, an abnormal cellular and molecular profile is seen in MPS bones and joints, with characteristic increases in cytokines, metalloproteinases (MMPs), and dead (apoptotic) cells, principally in joint and bone growth plate cells (chondrocytes), synovial membrane, and synovial fluid. There is an important stimulatory (chemotactic and pro-inflammatory) role for macrophage inflammatory proteins (MIPs) in the development of these lesions, and the formation of multinucleated osteoclast-like cells (MNCs). The production of the cytokine, TNF- α up-regulates an essential osteoclast differentiation/survival factor, the ligand of receptor activator NF- κ B (RANKL), and is a likely explanation for the appearance of the MNCs, which may result in clinical osteopenia (bone loss) in MPS. RANKL is produced by bone marrow stromal cells, osteoblasts, and synovial fibroblasts.

Normally, RANKL resides in cell membranes, and exerts its effect by direct interaction of bone cells (e.g., stromal cells/osteoblasts with osteoclast precursors), which express its receptor, RANK. RANKL is an essential factor for full differentiation and activation of osteoclasts. Thus, a consequence of having too much RANKL would be over-proliferation of osteoclasts, leading to bone destruction. Many of the cytokines known to stimulate bone resorption, such as IL-1 β and TNF- α , act through up-regulation of RANKL. In arthritis, RANKL mobilizes osteoclasts causing bone degradation. In the context of inflammation, TNF- α induces RANKL synthesis by marrow stromal cells, and prompts TNF- α expression by osteoclast precursors. Thus, RANKL is not only involved in osteoclast proliferation, but also pathological bone loss. In MPS bone marrow cultures, our preliminary results indicate there is an increase in osteoclasts. These results, combined with our previous findings, suggest that cooperation of TNF- α and RANKL plays an important role in inflammation and bone destruction. Therefore, it is important to evaluate treatment strategies aimed at mediating the effects of TNF- α .

MIP-1 α (macrophage inflammatory protein) is another pro-inflammatory molecule that binds to the cell surface CC chemokine (chemoattractant for white blood cells, or monocytes) receptors. MIP-1 proteins recruit pro-inflammatory cells (neutrophils, eosinophils, monocytes) to the site of injury, and are crucial for T-cell recruitment from the circulation to inflamed tissue. Therefore, MIP-1 proteins are key players in the pathogenesis of many inflammatory conditions, including asthma and arthritis. It has also been shown that this chemokine acts as a

recruitment factor for mature osteoclasts, as well as osteoclast precursors. RANKL-dependent proliferation of osteoclasts is up-regulated by MIP-1 α . In MPS animals and patients, up-regulation of these osteoclasts could occur by RANKL and MIP-1 α secretion in the area of bone destruction. We have detected MIP-1 α positive cells in the marrow cavity of MPS animals.

We have also evaluated the proteases, MMP-2, -9, and 13, in articular cartilage, growth plate cartilage, synovial fluid, and synovial membrane, gelatinase activity in synovial membrane, tartrate-resistant acid phosphatase activity for osteoclasts in bone marrow, and IL-1 β in growth plates from normal and MPS animals. All of these compounds are involved in bone and joint homeostasis and reactions to injury. The pattern of changes from normal in these parameters in MPS animals will provide insight into the pathogenesis of the bone and joint lesions, identify targets for therapy, and leads to biomarkers that can be used to determine the effects of treatment.

Summary: Overall, we have found that GAG accumulation in MPS animals stimulates a number of pathological processes. These include enhanced death (apoptosis) of bone and joint cells, as well as inflammation. The inflammatory response, in turn, leads to proliferation of bone absorbing cells (osteoclasts), which leads to bone destruction. In general, many of the pathologic processes in the MPS bones and joints have close similarities to those that occur in arthritis. Thus, some of the important biomarkers and therapeutic targets for arthritis should be considered for MPS. Indeed, we have recently found that several of these markers are abnormally expressed in MPS animals. These include TNF- α , MIP-1, MMPs, RANKL, and others.