Research Presented

2006 Lysosomal Diseases and the Brain Conference

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--- Speaker Abstracts ---

CONDITIONAL KNOCKOUT OF THE MURINE GLUOCEREBROSIDASE LOCUS

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Total loss of function of the lysosomal enzyme glucocerebrosidase (GBA) leads to a perinatal lethal phenotype in the mouse, similar to that seen for severe type II Gaucher patients inheriting two null alleles. As it is clear that some residual activity is required for viability in the mouse as well as humans, a conditional approach was used to selectively target GBA function. Using an engineered murine GBA locus with flanking loxP sites and a Cre mouse strain expressing the recombinase in endothelial and hematopoietic cells (Tie2Cre) we have produced a mouse with decreased GBA activity in the liver, spleen, bone marrow and peripheral white blood cells. These mice store glucocerebroside in the liver and spleen and have significant pathology by 26 weeks of age with obvious rafts of lipid-engorged macrophages (Gaucher cells) in the spleen. Interestingly, liver pathology is somewhat attenuated in comparison and no bone marrow involvement can be seen at 26 weeks. This work represents the first conditional murine model of Gaucher disease. Although significant visceral pathology is produced by targeting hematopoietic cells alone, other Cre-expression approaches can be used to address the various symptoms seen across the Gaucher spectrum, including neurological manifestations. The flexibility of this conditional approach to vary both the timing and distribution of Cre expression will allow for extensive phenotypic modulation and the production of a complete set of models for investigating the pathophysiology of Gaucher disease and testing novel therapeutic approaches.

Gene Therapy for the CNS Manifestations of Batten Disease

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Late infantile neuronal ceroid lipofuscinosis (LINCL), a pediatric autosomal recessive lysosomal storage disorder, manifests with blindness, progressive neurodegeneration, and death by age 8 to 12. The disease results from mutations in the CLN2 gene and deficiency in its product tripeptidyl peptidase (TPP-I), resulting in progressive loss of pigmented retinal epithelium and neurons. We have demonstrated that direct central nervous system gene transfer of AAV2CUhCLN2 (a serotype 2, adeno-associated gene transfer expressing the human CLN2 cDNA) in rats and non-human primates mediates long term TPP-I expression in the brain. On the basis of this efficacy data we carried out pre-clinical toxicology assessment of AAV2CUhCLN2 in rats and non-human primates at doses scalable to humans. There were no biologically significant differences between control and vector groups for all toxicology parameters. Taken together, the long term gene expression following gene transfer and the safety data in multiple animal models supported the initiation of clinical trials to assess the safety of AAV2CUhCLN2 administration to the CNS of children with LINCL. The clinical studies, now ongoing, includes children with severe and moderate LINCL phenotype. Following administration with 3.6x1012 particle units of the AAV2CUhCLN2 vector to the brain at 12 locations through 6 burr holes, the children are being following with a LINCL neurologic rating scale and CNS nuclear magnetic resonance and magnetic resonance spectroscopy. In parallel, we have developed a 2nd generation strategy using a non-human primate-derived AAV vector (AAVrh.10hCLN2) that is capable of significantly improving the abnormal phenotype of a homozygous LINCL knockout mouse. Should the toxicity studies with AAVrh.10hCLN2 be consistent with moving this vector to the clinic, studies will be planned using moderate and mild cases of LINCL.

G_{M1}-ganglioside as mediator of ER-stress- and mitochondria-elicited neuronal apoptosis

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G_{M1}-gangliosidosis is a neurodegenerative disorder of glycosphingolipid metabolism caused by deficiency of lysosomal β-galactosidase. The hallmark of the disease is the massive and progressive accumulation of G_{M1}-ganglioside (GM1) in the CNS. Mice carrying a null mutation at the *β-gal* locus display a generalized CNS condition that results in loss of coordination, tremor, ataxia, and ultimately paralysis of the hind limbs. We have demonstrated that neuronal apoptosis in the mutant mice occurs via GM1-mediated activation of an unfolded protein response (UPR), via depletion of calcium stores from the ER. However, because of the interplay between ER and mitochondria in the control of intracellular calcium levels, we hypothesized that the mitochondrial branch of the apoptotic pathway is also implicated in GM1-mediated neurodegeneration. We have now experimental evidence that in *β-gal-/-* and GM1-loaded *β-gal+/+* MEFs and neurospheres the calcium discharged from the ER is preferentially taken up by mitochondria and in turn induces mitochondrial membrane permeabilization (MMP). This event is accompanied by the release from the mitochondria of apoptogenic factors, such as cytochrome c and apoptosis-inducing factor (AIF), that are normally confined to the inter-membrane space. Consequently, changes in mitochondrial transmembrane potential and uncoupling of the respiratory chain, lead to increased production of reactive oxygen species (ROS). These findings suggest a primary role of GM1 in neuronal apoptosis via multiple events that hinge on the calcium-mediated crosstalk between ER and mitochondria cell death pathways. (Supported by NIH grants DK52025; CA21765 and ALSAC).

ADVANCES IN TREATING THE CNS DEFICITS OF LYSOSOMAL STORAGE DISEASES

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Lysosomal storage diseases (LSDs) represent a significant portion of inborn

metabolic disorders. More than 60% of LSDs have CNS involvement. LSD therapies for systemic diseases have been developed, but efficacy does not extend to the CNS. In this study, we tested whether adeno-associated virus type 4 (AAV4) vectors could mediate global functional and pathological improvements in a murine model of mucopolysaccharidosis type VII (MPS VII) caused by ß-glucuronidase deficiency. Recombinant AAV4 vectors encoding ß-glucuronidase were injected unilaterally into the lateral ventricle of MPS VII mice with established disease. Transduced ependyma expressed high levels of recombinant enzyme, with secreted enzyme penetrating cerebral and cerebellar structures, as well as the brainstem. Immunohistochemical studies revealed close association of recombinant enzyme and brain microvasculature, indicating that ß-glucuronidase reached brain parenchyma via the perivascular spaces lining blood vessels. Aversive associative learning was tested by context fear conditioning. Compared with age-matched heterozygous controls, affected mice showed impaired conditional fear response and context discrimination. This behavioral deficit was reversed 6 weeks after gene transfer in AAV4 ßglucuronidase-treated MPS VII mice. Our data show that ependymal cells can serve as a source of enzyme secretion into the surrounding brain parenchyma and CSF. Secreted enzymes subsequently spread via various routes to reach structures throughout the brain and mediated pathological and functional disease correction. Together, our proof-of-principal experiments suggest a unique and efficient manner for treating the global CNS deficits in LSD patients. Abstract from G Liu, I Martins, JA Wemmie, JA Chiorini, BL Davidson. Functional correction of CNS phenotypes in a lysosomal storage disease model using adenoassociated virus type 4 vectors. J Neurosci 25(4):9321-9327, 2005.

Therapy and pathogenesis of metachromatic leukodystrophy: Studies in a mouse model.

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Metachromatic leukodystrophy is a severe lysosomal storage disorder caused by the deficiency of the sulfatide degrading enzyme arylsulfatase A (ASA). In the nervous system this leads to sulfatide storage in oligodendrocytes/Schwann cells, subsets of neurons and astrocytes. Patients develop a wide variety of neurologic symptoms, which are due to progressive demyelination. In ASA deficient mice the

sulfatide storage pattern is similar to that in humans. The mice do, however, not develop the widespread demyelination observed in patients.

To assess the therapeutic potential of enzyme replacement therapy (ERT), ASA knockout mice were treated by intravenous injection of recombinant human ASA. The uptake of injected enzyme was high into liver, moderate into peripheral nervous system (PNS) and kidney and very low into brain. A single injection led to a time- and dose-dependent decline of the excess sulfatide in PNS and kidney by up to 70%, but no reduction was seen in brain. Four weekly injections with 20 mg/kg body weight not only reduced storage in peripheral tissues progressively, but surprisingly also reduced sulfatide storage in brain and spinal cord. Reduction of storage in brain, however, was limited to macrophages, no reduction was seen in neuronal or glial cells. Improved neuromotor coordination capabilities and normalized peripheral compound motor action potential demonstrate the benefits of ERT on the nervous system function.

Since ASA deficient mice lack the pathological hallmark of MLD the demyelination we attempted to aggravate the phenotype of ASA deficient mice. For that purpose we generated transgenic mice, which overexpress the sulfatide synthesizing enzyme galactosylceramide-sulfotransferase (CST) under the control of the PLP promoter in oligodendrocytes or the UDP-galactose:ceramide galactosyltransferase (CGT) under the control of the Thy1.2 promoter in neurons. The transgenic mice were crossed with ASA deficient mice. The rationale behind this approach is that the transgenes should cause an enhanced synthesis of sulfatide, which on the background of ASA deficient mice should enhance sulfatide storage and thus the phenotype.

PLP- CST transgenic/ASA deficient mice store about twice as much sulfatide as ASA deficient mice. In contrast to the ASA deficient mice, they also develop severe motor coordination deficits at the age of 6-8 months. Histological examination demonstrates demyelination in the peripheral and central nervous system. This shows that the enhancement of sulfatide synthesis by the transgene causes an aggravation of the myelin pathology compared to the mice which are ASA deficient only. Thy1.2-CGT transgenic/ASA deficient mice showed an enhanced accumulation of C18:0 fatty acid containing sulfatide in the cortex and storage material could be detected histochemically in in neurons of spinal cord and forebrain. Animals develop severe motor deficits at the age of 2 months and there life span is reduced to 6 to 11 months. Ultrastructural examination revealed axonal degeneration. This suggests, that neuronal storage contributes to the neurologic deficits occuring in metachromatic leukodystrophy.

Cell-Fusion as a Tool for Generating a Neurological Disease Model

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Glucocerebrosidase mutations are thought to be a risk factor of parkinsonism and Parkinson disease. Patients with Gaucher disease and parkinsonism may have α–synuclein positive Lewy bodies in a Gaucher-specific hippocampal distribution. We hypothesized that the mutated glucocerebrosidase is degraded by the proteasomal pathway and causes neuronal toxicity. We found that proteasome inhibitors (ALLN) increased the amount of cellular glucocerebrosidase and had greater toxicity to fibroblasts of Gaucher patients and to neuroblatosma M17-Gaucher fibroblast fusion cell product. Over expression of wild type and mutated α-synuclein further increased the sensitivity to the proteasome inhibition particularly in cells of patients with the chronic neuronopathic form of the disease and familial parkinsonism. An active-site chemical chaperone partly reversed the sensitivity to proteasome inhibition in Gaucher fibroblasts with the N370S mutation. These findings support a mechanism by which the mutated glucocerebrosidase may cause Lewy body-related disease and describe a neuronal-fibroblasts cellular hybrid model to further study the Gaucher parkinsonism link.

Novel mouse models for type 1 and type 2 Gaucher disease for development of new therapies.

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Gaucher disease is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme glucocerebrosidase, resulting in accumulation of its substrate glucosylceramide, mostly in visceral tissues. The current treatment for this disease is enzyme replacement therapy. However, since this treatment is non-curative and expensive, other treatment alternatives need to be developed. Previous mouse models with glucocerebrosidase deficiency have either been lethal during the first day of life or viable but without clinical features of Gaucher disease. We have recently created a novel mouse model for type 1 Gaucher disease by inducing enzyme deficiency after birth, thereby circumventing the lethal developmental defects that ensue in animals born with total defieciency of glucocerebrosidase. Our mouse model is viable and has characteristic features of type 1 Gaucher disease, including severly reduced enzyme activity, 150-300 fold increase in glucocerebroside substrate and massive infiltration of Gaucher cells in bone marrow, liver and spleen. Clinical symptoms include hepatosplenomegaly and anemia, but the CNS is unaffected as in type 1 Gaucher disease. Bone marrow cells from the type 1 Gaucher mouse were transduced by coculture with retroviral vector producer cells. The vector had the SFFV LTR driving the expression of the glucocerebrosidase gene followed by an IRES and the GFP marker gene. The initial transduction efficiency was 20-30% as measured by FACS two days following transduction. Five months after transplantation, the mice that received glucocerebrosidase-transduced marrow showed a marked improvement in enzyme activity in spleen and BM compared to GFP transplanted controls. In addition the infiltration of Gaucher cells was practically eliminated in spleen and liver in the glucocerebrosidase treated mice. The findings show that transduction efficiency of less than 50% is sufficient to cure type 1 Gaucher mice. Therefore, our novel mouse model for type 1 Gaucher disease serves as an excellent tool for the development of novel therapeutic approaches for type 1 Gaucher disease, including preclinical studies that are essential in the development of a clinical gene therapy protocol for the disease.

Similarly, we have generated mice that contain a mutation in the glucocerebrosidase gene that leads to glucocerebrosidase deficiency during development. These mice are born alive but develop a neurodegenerative disorder in infancy, which is characterized by abnormal movements, muscle spasms,

seizures and difficulties in breathing. This clinical condition is reminiscent of type 2 Gaucher disease in humans and leads to death within 2-3 weeks. Specimens from brains of these mice have greatly increased accumulation of glucosyl ceramide and pathological examination of brain tissue shows abnormal neurons in large numbers distributed throughout the brain. The neuronal abnormalities include vacuoles in the cytoplasm as indicators of neuronal toxicity. Although the pathology is seen throughout the CNS, the pathological changes are most prominent in the brainstem. TUNEL staining shows greatly increased number of apoptotic cells throughout the brain and astrogliosis is evident by increased staining for glial fibrillary associated protein (GFAP). Staining for microglial cells shows very large microglial cells distributed throughout the brain. These pathological and clinical features are consistent with neuronopathic type 2 Gaucher disease. These mice can be used to investigate the pathogenic mechanism of type 2 Gaucher disease and may eventually be used to develop effective therapeutic approaches for this lethal neurodegenerative disorder

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Therapeutic Strategies Against Gain and Loss of Function Misfolding Diseases

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The misfolding and/or misassembly of what is typically one protein inside the cell leads to gain of toxic function diseases such as Huntington's and Parkinson's disease, whereas extracellular misfolding and/or misassembly appears to cause analogous diseases known as the amyloidoses, including Alzheimer's disease and numerous rarer familial diseases including familial amyloid polyneuropathy. Mutations can also predispose a protein to misfold in the endoplasmic reticulum (ER), culminating in protein degradion by the proteasome–resulting in a loss of function disease. The most common mutations in proteins that cause Gaucher Disease and Cystic fibrosis have sufficient activity in their destination environment (the lysosome and the plasma membrane, respectively), that they could function there–if they were able to fold in the unique environment of the ER and exit this organelle. The seminar will start with an overview of the mechanistic insight that we, and others, have acquired from studying these diseases and then outline how

this information has been utilized to develop small molecule therapeutic strategies to intervene in maladies that often lead to death within ten years of onset.

Gene expression profiling and neuronal survival in the brain of a mouse model of Gaucher disease

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Gaucher disease is a lysosomal storage disorder, resulting from an inborn deficiency of glucocerebrosidase. The neuronopathic forms of this disease are associated with neuronal loss and neurodegeneration. However, the pathophysiological mechanisms leading to prenatal and neonatal death remain uncharacterized. To investigate the genes responsible for the neuronal symptoms of Gaucher disease, gene expression profiles were analyzed in brains of the Gaucher disease mouse model using a cDNA microarray, and found that the bcl-2 gene is down-regulated. Immunoblotting and apoptosis assay were performed to study the relationship between the decreased expression of Bcl-2 and neuronal death on the brains of Gaucher mice fetuses at embryonic day 17.5 (E17.5) and E19.5. Decreased expression of Bcl-2 was observed in the brain stem and cerebellum but not in cortex by immunoblotting. In situ labeling of DNA fragmentation using TUNEL assay confirmed that apoptosis occured in brain stem and cerebellum. More apoptotic cells were detected in the brains of Gaucher mice fetuses at E19.5 than at E17.5. We also studied the effects of neurotrophic factors during development in Gaucher mice. The expression of brain-derived neurotrophic factor and nerve growth factor was reduced in the cerebral cortex, brainstem, and cerebellum of Gaucher mice, compared with that in wild-type mice. ERK 1/2 expression was downregulated in neurons from Gaucher mice and correlated with a decreased number of neurons. These results suggest that the accumulation of either glucocerebroside or glucosylsphingosine, as a result of glucocerebrosidase deficiency, affects gene expression and could be responsible for neuronal cell death.

TRP-ML channels and the function of intracellular organelles

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Mutations in TRP-ML channels are associated with disease states or sever hearing and neurological phenotype. Mutations in the founding member the TRP-ML sub-family, TRP-ML1 cause the lysosomal storage disease Mucolipidosis type IV (MLIV), while mutations in TRP-ML3 results in the varitint-waddler phenotype. How TRP-ML1 controls lysosomal function and lipid metabolism and why mutations in TRP-ML3 lead to the varitint-waddler phenotype is unknown. To begin to address these questions we analyzed the channel and cellular functions of these channels. We found that TRP-ML1 is a dual function lysosomal H+ selective channel that regulates lipid trafficking, hydrolysis and metabolism. TRP-ML1 regulates lysosomal acidification by providing a H+ leak pathway. Recombinant and native TRP-ML1 are cleaved by Cathepsin B (CatB), although the cleaved N and C terminal halves of the channel remain associated. The cleaved form of the channel is predominant in native cells. The cleavage of TRP-ML1 inactivates channel function and lipid trafficking. Accordingly, inhibition of CatB decreased TRP-ML1 cleavage and increased channel activity, but reduced lipid metabolism. Lipid analysis revealed reduced lipid hydrolase activity in four TRP-ML1-/- cell lines with different mutations in the TRP-ML1 gene. These findings suggest a model in which the H+ channel function of TRP-ML1 controls lysosomal pH by providing a H+ leak pathway. Attenuation of lysosomal acidification is required for lipid trafficking along the endocytotic pathway. In the lysosomes TRP-ML1 is cleaved by CatB to inactivate the channel function and activate enzymes that control lipid metabolism. Interruption of any of the functions of TRP-ML1 leads to aberrant lipid trafficking and/or metabolism and MLIV.

TRP-ML3 turned to be a channel very different than TRP-ML1. TRP-ML3 functions as a selective Ca2+ channel that is regulated by monovalent ions and pH. TRP-ML3 can be found at the plasma membrane and early endosomes. When at the plasma membrane its activity in regulated by extracellular Na+ that inhibits channel function. When TRP-ML3 is in endosomes, its activity is regulated by a narrow pH range. It is fully active at pH of 6.5 and fully inhibited at pH of 6. Cytoplasmic pH has no effect on channel activity. TRP-ML3 appears to be important for the regulated and constitutive endocytosis since in preliminary experiments we found that deletion of TRP-ML3 by siRNA resulted in enhanced

endocytosis. The mechanism by which TRP-ML3 regulates endocytosis is under investigation.

"Pathogenic mechanisms that underlie Juvenile Batten Disease"

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Juvenile neuronal ceroid lipofuscinosis (JNCL) is an autosomal recessive disorder of childhood caused by mutations in CLN3. The onset of the disease typically occurs around 4-7 years of age with progressive visual loss that culminates in total blindness. The disease progresses to include seizures, memory loss, and motor deficits followed by premature death before or around the third decade of life. Using a Cln3 knockout (Cln3-/-) mouse we have tracked the time course of neurological disease and have discovered alterations at the cellular, molecular and biochemical level that underlie the progressive loss of vision and deteriorating motor function.

Examination of retino-recipient regions revealed a decreased number of neurons within the dorsal lateral geniculate nucleus (LGNd). Furthermore, we demonstrate decreased transport of amino acids from the retina to the LGN, suggesting an impediment in communication between the retina and projection nuclei. Collectively, this suggests that the cause of visual deterioration in JNCL may originate in the LGNd.

Motor deterioration in JNCL can include decreased coordination, gait abnormalities, myoclonic jerks, an inability to initiate movements, and spasticity. First focusing on the cerebellar motor system of the Cln3 mouse, we demonstrate that the coordination deficits are, in part, due to a unique form of cerebellar astrogliosis proximal to regions devoid of Purkinje cells. Additional anatomical changes, including loss of output neurons in the medial deep cerebellar nuclei and a reduction in the cerebellar volume were also noted. Interestingly, we reveal that anatomical and molecular changes occur within Cln3-/- mice cerebella during early stages of postnatal development. Deficits in coordination are detected by two weeks postnatal in Cln3-/- mice and by the first two weeks postnatal, deficits in the

cyto-architecture of the cerebellum are apparent and notable differences in the proliferation rate of granule neuron progenitor cells and molecules cues regulating their proliferation were seen.

Further exploring deficits in movement associated with JNCL, we examined the striatal motor system. Later in Cln3-/- mouse pathology we demonstrate an imbalance in the catabolism of dopamine, specifically within the striatum, that leads to oxidative damage, decreased post-synaptic D1α receptors, inflammatory changes, and cell loss in CNS regions that form connections with the striatum. As this cell death progresses, it ultimately reaches a critical level at which point deficits in general locomotion become apparent in the Cln3-/- mice.
In summary our characterization of the Cln3-/- mouse has proven this model to be valuable for elucidating the pathogenic mechanisms that underlie the sequelae of neurological insults associated to JNCL.

Impact of systemic inflammation on chronic neurodegenerative disease

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We have all at one time or another experienced being ill or sick during a systemic infection. At the site of an infection, cells of the immune system generate inflammatory cytokines and mediators, and it is these molecules that communicate with the brain to generate a fever and the spectrum of behavioural changes known as “sickness behaviour”. The communication from the blood to the brain involves generation of cytokines in the brain and this includes interleukin-1 (IL-1) and interleukin-6 (IL-6). This signalling process is not a pathological process but part of our normal physiology.

In chronic neurodegenerative disease of the CNS there is a highly atypical inflammatory response. This inflammatory response is characterised by the presence of large numbers of activated microglia and perivascular cells. The role of the activated microglia in disease pathogenesis has been investigated in diverse animal models, including models of Alzheimer’s disease, lysosomal storage diseases and prion disease. In our own studies of murine prion disease the activated microglia appear to play a relatively minor role since deletion of cytokine

genes or treatment with anti-inflammatory drugs has little impact on disease progression. However, the microglia appear to be “primed” either by the presence of the protease resistant protein PrPsc or ongoing neuronal degeneration. Following a systemic challenge with a low dose of endotoxin (LPS) to mimic a systemic infection, the mice exhibit exaggerated sickness behaviour and a switch in cytokine synthesis in the brain, from a profile dominated by transforming growth factor-β1(TGF-β1) and PGE2 to enhanced synthesis of IL-1, IL-6 and TNF-α. This switch in cytokine profile is likely reflected in a switch in microglia phenotype. Associated with this cytokine switch was an acute increase in the number of neurons undergoing apoptosis. These data suggest that systemic inflammation generated by infection or injury may impact on the diseased brain to increase the rate of neuronal degeneration in chronic neurodegenerative diseases, including perhaps lysosomal storage diseases.

Mutant Glucocerebrosidase and the Synucleinopathies

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Recent studies implicate an association between parkinsonism and mutations in glucocerebrosidase (GBA), the lysosomal enzyme deficient in Gaucher disease. Parkinsonian manifestations have been described in some patients with Gaucher disease and in heterozygote relatives. GBA mutations are encountered with increased frequency in different populations of subjects with parkinsonism as well, suggesting their role as a risk factor. To further explore this association, GBA was sequenced in 75 autopsied brain samples from U Penn. Postmortem diagnosis identified 35 cases with diffuse Lewy body dementia (DLB), 29 with Parkinson disease (PD), and 12 with multiple system atrophy (MSA). Of the 75 subjects, 9 (12%) were heterozygous for GBA mutations (23% of DLB subjects and 4% of PD), expanding the spectrum of synucleinopathies associated with GBA. Immunofluorescence studies and confocal microscopy of brain samples from 5 subjects with synucleinopathies who carried GBA mutations demonstrated that mutant glucocerebrosidase was present in a-synuclein-positive inclusions in both GBA heterozygotes and homozygotes with parkinsonism. Mutant glucocerebrosidase was found in ubiquitinated and non-ubiquitinated aggregates, co-localizing with lysosomal markers. In 4 control samples from parkinsonian

subjects without GBA mutations, synuclein-positive aggregates did not show immunoreactivity to glucocerebrosidase. We suggest that GBA mutations may enhance synuclein aggregation by a toxic gain-of-function mechanism or may interfere with the lysosomal clearance of toxic a-synuclein oligomers. Unraveling the relationship between these proteins may advance our understanding of the etiology, genetics, and pathogenesis of the synucleinopathies and may explain some of atypical phenotypes encountered among patients with Gaucher disease.

THERAPEUTIC HORIZONS: IS THERE A ROLE FOR NEURAL STEM CELLS (NSCs) AGAINST LYSOSOMAL STORAGE DISEASES?

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The biology of NSCs endows them with capabilities that might permit them to complement other therapeutic approaches to neurogenetically-based CNS lesions. Our experience with NSCs (mouse and human: derived directly from the neuroectoderm as well as from embryonic stem cells) in various mouse models of lysosomal storage diseases (including both neuronopathies & leukodystrophies) highlights some of these properties. They include: (1) *Genetic manipulability*; NSCs are easily transduced *ex vivo* by most viral & nonviral gene transfer methods. (2) *Facile engraftability* following simple & safe implantation into germinal zones, unimpeded by the blood-brain barrier. (3) *Sustained, immediate, direct gene expression* throughout the CNS, from fetus to adult. (4) *Potential for reintegration into host cytoarchitecture & differentiation along all lineages*; important if neurons & glia are both affected & might also allow regulated protein delivery; while many vectors depend on relaying new genetic information through old neural circuits -- which may, in fact, have degenerated -- NSCs may participate in pathway reconstitution. (5) *Ability to migrate* enabling replacement of genes & cells not only in discrete sites (where homogeneous distribution of molecules is insured) but also to disseminated lesions. (6) *Plasticity;* accommodation to the region of engraftment often obviates the necessity for donor cells from many specific regions, or precise targeting during reimplantation, or the need for tissue-specific promoters. (7) *Compensatory of transgene non-expression* due to intrinsic expression of many gene products by NSCs & an ability to integrate multiple transgene copies into its genome. (8) *Minimization of side-effects;* distribution of

gene products is restricted to the CNS or to specific CNS regions; conditioning regimes not required (as in bone marrow transplants). (9) *May amplify distribution of viral-mediated genes*. (10) *Tropism for & trophism within regions of CNS degeneration;* and inflammation, i.e., altered migration to regions of pathology & differentiation towards specific cell replacement. (11) *Immunotolerance* (12) *Inherent expression by NSCs of neuroprotective molecules & trophic factors*. (13) *Ability to exert anti-inflammatory and anti-scarring actions*. Many of these properties seem to be conserved in human NSCs, possibly accelerating progress towards human applications. Furthermore, NSC-mediated interventions appear to work synergistically with other approaches, e.g., oral substrate reduction therapy.

Combined therapy for the treatment of globoid cell leukodystrophy

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Galactocerebrosidase (GALC) is the enzyme responsible for the lysosomal catabolism of several important galactolipids including galactosylceramide and psychosine. The deficiency of GALC activity results in the severe autosomal recessive disorder called globoid cell leukodystrophy (GLD) or Krabbe disease. The accumulation of psychosine in the CNS and PNS results in the apoptotic death of oligodendrocytes and Schwann cells. Other mechanisms including activation of resident microglial cells, recruitment of blood macrophages, expression of pro-inflammatory cytokines and major histocompatibility complex molecules also play important roles in the pathogenesis of this disease. The only treatment currently available for some human patients is hematopoietic stem cell transplantation (HSCT). While this treatment results in significant maintenance of cognitive abilities, motor function continues to be a problem. There are several mouse models with low GALC activity available for study. Bone marrow transplantation (BMT) of young affected mice can prolong their lives to over one year from 40-60 days in untreated mice. The brains of long-lived mice with successful engraftment had psychosine levels near normal, low, but significant GALC, activity and evidence for significant remyelination. The PNS still shows significant pathology that will require additional treatment. In addition, we have cloned mouse GALC cDNA in pZAC2.1, a type-2 adeno-associated viral vector (AAV), packaged in AAV-1, AAV-2 and AAV-5 serotypes. In vitro transduction of mouse neural cells and skin fibroblasts with these vectors showed an increase in GALC activity.

Subsequently, these viral vectors were injected intra-ventricularly or intra-cranially into the brains of newborn affected mice. Frozen sections were stained with anti-GALC antibody, and transduced cells were detected by their bright perinuclear staining. Injected mice lived only up to 80 days, despite the high expression of GALC activity in brain. There was improved myelination and a reduction in the expected psychosine concentration. Of course, this treatment did not attempt to correct the PNS. It seems apparent that more than one approach may be needed to prevent and correct the pathology seen in both the CNS and PNS in this disease.

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--- Poster Session Abstracts ---

Development of an orally administered macrophage mediated gene therapy for Gaucher Disease.

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An orally administered macrophage delivered gene therapy is being developed for treatment of Gaucher disease. Current enzyme replacement therapy (ERT) improves blood counts and reverses hepatosplenomegaly. However, ERT is intravenously administered, costly, and has not significantly addressed bone or neurological complications. DNA encoding human glucocerebrosidase (huGBA) has been formulated inside 2-4 micron yeast cell wall particles (YCWP). YCWP-huGBA formulations were used to introduce huGBA DNA into J774 murine macrophages in culture and into long-lived Gaucher mice generated by gene targeting. As observed in human patients, the reduced GBA activity in these Gaucher mice results in glucocerebroside storage and Gaucher cells in tissues. The clinical manifestations in these mice can be accelerated by short courses of

conduritol- β-epoxide. Following oral intake, YCWP-huGBA formulations are taken up through intestinal Peyers Patches where they are phagocytosed by macrophages that migrate to the reticuloendothelial system. Within the macrophage endosome the huGBA DNA is released at acid pH and is expressed to produce normal huGBA. This results in huGBA expression in J774 murine macrophages in-vitro, and in tissues of Gaucher mice in-vivo. Preliminary findings demonstrate that this therapy sufficiently corrects tissue GBA activity to ameliorate symptoms in treated, compared to untreated, severely affected Gaucher mice. As a consequence of improved delivery of huGBA, we expect that this approach will achieve significant reversal of tissue pathology, including that in bone. If macrophages containing huGBA migrate into brain, then resulting increased GBA levels could also provide clinical benefit for neurologic manifestations of the disease. The successful development of this therapy should provide a safer, more efficient and cost effective treatment for patients with Gaucher disease, and serve as a prototype for therapy for other lysosomal diseases.

APOPTOSIS AND NEURODEGENERATION IN A MURINE MODEL OF NIEMANN-PICK TYPE C DISEASE

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Niemann Pick Type-C (NP-C) is an autosomal recessive neurodegenerative disease caused by mutations in NPC1 (95%) or NPC2 (5%), resulting in accumulation of unesterified cholesterol and glycolipids in lysosomes. Neurosteroids, synthesized in a developmental- and regional-specific fashion in the neurous system, affect growth and differentiation of neurons. The NIH mouse model of NP-C has a mutation in the NPC1 gene, and exhibits several pathological features of the most severe NP-C patients. In this mouse, we showed that there is a substantial reduction in the synthesis of the neurosteroid allopregnanolone (Allo) at birth, which may result in abnormal neural development. Treatment of mice with a single injection of Allo at postnatal day 7 resulted in increased survival, delayed neurological deficits, and increased Purkinje and granule cell survival. In the present study, we studied the mechanism of Neurodegeneration, by determine whether apoptosis plays a role in this process, we analyzed the pathological

manifestation of apoptosis and Neurodegeneration in several brain regions of NP-C mice at 30 days, before a neurodegenerative phenotype is apparent, and at 63 days, the end of the life. Brain sections from control, untreated NP-C, and Allo-treated NP-C mice were analyzed for apoptosis by TUNEL assays and analyzed for Neurodegeneration using a marker for degenerating neurons. We found apoptosis of neurons throughout the brain in the same regions in which there was substantial Neurodegeneration, but the number of apoptotic neurons was less 20% of the number of degenerating neurons. In Allo-treated mice, the abundance of apoptotic neurons decreased by demonstrates that presymptomatic Allo treatment of NP-C mice decreases subsequent apoptosis and extensive Neurodegeneration. The results further suggest that apoptosis accounts for only a small portion of neuronal loss in the NP-C mouse.

Intrathecal delivery of Aldurazyme®(laronidase) in normal canines: A comparison of enzyme elevation in CNS tissue resulting from chronic or intermittent bolus delivery

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Aldurazyme®(laronidase) is a recombinant form of alpha-L-iduronidase used for enzyme replacement therapy (ERT) in mucopolysaccharidosis type I (MPS I) patients. Unfortunately, the enzyme does not cross the blood-brain barrier, so ERT does not treat the neurological aspects of the disease. It has previously been shown in dogs that intrathecal delivery of Aldurazyme results in elevated iduronidase activity in brain tissue. However, frequent bolus delivery of Aldurazyme in patients by spinal puncture would probably not be clinically acceptable due to inconvenience and risks. In contrast, delivery of Aldurazyme to the CNS using an implanted drug delivery system may be a clinically practical alternative. In this study using twelve normal, purpose-bred dogs, we compared continuous, chronic infusion of Aldurazyme into the cerebrospinal fluid (CSF) via implanted intrathecal catheter to once-weekly bolus injections into the cisterna magna. The goal was to determine whether chronic infusion of Aldurazyme into the CSF would

result in elevation of alpha-L-iduronidase enzyme levels in the brain and spinal cord tissue of the dogs above endogenous levels.

Four groups of three dogs were studied. The "bolus" group received four weekly 1-mg injections of Aldurazyme (spanning 21 days) into the cisterna magna. The "low-dose pump" group received the same cumulative dose of Aldurazyme over 21 days delivered at the rate of 56 ul / hr (8 ug / hr) via an implanted catheter positioned in the intrathecal space of the high cervical spine. The "high-dose pump" and "buffer only pump" groups received the same catheter implant and rate of delivery (56 ul/hr), but received 32 ug / hr (16 mg cumulative dose) or formulation buffer only (no enzyme), respectively. On day 23, the dogs were euthanized and the brains rapidly harvested and flash frozen. Blinded assay of alpha-L-iduronidase activity in systematically sampled brain and spinal cord tissues from "superficial" and "deep" regions (where "deep" refers to tissue more than 3 mm from the pial surface in the brain, and the inner-most, meninges-free 2 to 3 mm of tissue in the spinal cord) showed significant elevation of enzyme levels above those of the buffer only controls in all treatment groups. Both the greatest elevation and most extensive anatomical distribution was obtained in the "bolus" group. As expected with recombinant human protein delivery into a non-immuno-suppressed canine host, proliferative and/or inflammatory reactions were observed in the CNS tissues of most of the dogs. These changes ranged from mild in tissues from the "bolus" groups, to severe in the spinal cord tissues of the "high dose pump" group.

The results suggest that sufficient elevation of enzyme activity in CNS tissue may result from delivery of Aldurazyme into the CSF for this mode of treatment to be clinically useful. Intermittent bolus delivery of the enzyme (e.g., via a programmable implanted pump) is likely to be a preferred mode of delivery over chronic infusion, for better enzyme uptake and distribution and reduced risk of adverse reaction.

Notes: The Aldurazyme®(laronidase) and formulation buffer used in this study were graciously provided by BioMarin/Genzyme LLC via a Material Transfer Agreement with Medtronic, Inc. Thanks to Patricia Dickson, M.D., Harbor-UCLA Medical Center, for her review and comments regarding this study.

A New Substrate for Measurement of Lysosomal β-Glucosidase in Intact Cells

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Previously, we reported the development of a new series of substrates for lysosomal enzymes based on 2,3 dicyanohydroquinone (DCH), the first of which was shown to be specific substrate for acid β-galactosidase (Kaneski, et al., 1994, J. of Lipid Res. 35:1441). We now report that the second member of the series, 4-octyl-2,3-dicyano-1,4-hydroquinonyl-β-D-glucopyranoside (C8-DCH-β-glu), can be used as a specific substrate for lysosomal β-glucosidase in both intact cells and cell homogenates. In cultured fibroblasts, the compound is hydrolyzed by living cells to a highly fluorescent product (C8-DCH) that can be detected at standard wavelengths (excitation: 360, emission: 460) using a microplate reader. However, unlike substrates based on 4-methylumbelliferone, the fluorescence intensity of product is relatively independent of pH in physiological ranges (pH 4-7). This substrate allows repeated, real-time measurements of lysosomal beta-glucosidase activity in living cells, and can be used to screen new treatments that may enhance activity of this enzyme in its native environment within the cell.

PharmAcological chaperone Isofagomine has potential to treat neuropathic forms of Gaucher disease

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Gaucher disease is a lysosomal storage disorder caused by deficiency in acid beta-glucocerebrosidase (GCase) activity. This disease has been broadly classified into three forms on the basis of CNS involvement; a chronic non-neuronopathic form (Type I) and the more severe infantile and juvenile neuronopathic forms (Types II and III, respectively). Though enzyme replacement (ERT) and substrate reduction

therapies are currently available, small molecule pharmacological chaperones that enhance the activity of misfolded mutant enzymes represent a promising new therapeutic approach to treat Gaucher disease. Importantly, small molecules have advantages over ERT due to potential oral bioavailability and penetration into the central nervous system (CNS). Here, we show that a potent inhibitor of GCase, isofagomine (IFG), acts as a pharmacological chaperone, increasing GCase activity in normal human fibroblasts, in Type I patient fibroblasts homozygous for the N370S missense mutation and in a neuronopathic Type II patient lymphoblast cell line. IFG also facilitates trafficking of wild type and N370S GCase protein to the lysosomes of cultured fibroblasts, as measured by immunofluorescence/co-localization. When dosed orally to rats at 100 mg/kg, IFG was detected in plasma and brain tissue, indicating penetration into the CNS. Furthermore, oral administration of IFG to wild type C57BL6 mice resulted in a dose-dependent increase in brain GCase activity after 4 weeks of treatment. Taken together, these data indicate that IFG is orally available, crosses the blood-brain barrier and can enhance the activity of wild type as well as Types I and II mutant GCase in vitro and wild type brain GCase in vivo. Thus, the small molecule chaperone, IFG, represents a promising therapeutic approach for the treatment of neuronopathic forms of Gaucher disease.

Neuropsychological profile of adult patients with Niemann-Pick C1 (NPC1) mutations.

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Niemann-Pick disease type C (NPC), a fatal disease, is due to mutations in the gene coding for the cholesterol trafficking protein NPC1. Currently, miglustat a small iminosugar molecule which reversibly inhibits glucosylceramide synthase, is being tested in a clinical trial in patients with NPC. The aim of this investigation is to evaluate a battery of tests that might be useful for documenting cognitive deficits from early to advanced stages of the disease. Eight men and two women, (age range 19 to 40 y) were tested with a battery of tests consisting of the MMSE,

Trail making test A & B, subtests of CERAD (verbal fluency), as well as the clock-drawing test, the mosaic test, the Corsi-block tapping test and the Grooved Pegboard test. External staging criterion was a five-step clinical scale. Results show that Trail making tests A & B and verbal fluency are sensitive even in early stages of NPC disease. Corsi-Block Tapping, Mini Mental, Find Similarities and clock drawing are useful for patients with advanced disease, whereas Grooved pegboard, Trail making and Mosaic test are not useful due to impaired fine motor skills in advanced disease. The visual-spatial working memory seems to be less affected by the neurodegenerative process than the verbal working memory. The series of test used here could be supplemented by the severe impairment battery and the Raven matrices for patients with advanced disease because in contrast to the Mosaic test the focus is more on visuospatial abstraction ability. This study contributes towards a better understanding of the testing parameters that could be used to determine drug efficacy in the clinic.

ENZYME REPLACEMENT THERAPY IN A MOUSE MODEL OF GLOBOID CELL LEUKODYSTROPHY

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Globoid cell leukodystrophy (GLD), also known as Krabbe's disease, is a devastating, degenerative neurological disorder. It is inherited as an autosomal recessive trait caused by loss-of-function mutations in the galactosylcerebrosidase (GALC) gene. Previously, we have shown that peripheral injection of recombinant GALC, administered every other day, results in a substantial improvement in early clinical phenotype in the twitcher mouse model of GLD. While we did detect active enzyme in the brain following peripheral administration, most of the administered enzyme was localized to the periphery. Given the substantial CNS involvement in this disease, we were interested in determining whether or not a single dose administration of the recombinant enzyme directly to the CNS would result in any substantial improvement. Following intracerebroventricular (icv) administration of GALC we noted a significant, 16.5%, reduction in the GALC

substrate psychosine, which is believed to play a pivotal role in the CNS pathology observed in this disease. Moreover, GALC was found not only in periventricular regions but also at sites distant to the injection such as the cerebral cortex and cerebellum. Most importantly, animals receiving a single icv dose of the enzyme at postnatal day 20 survived up to 51 days which compares favorably to the control twitcher animals, which normally only live to postnatal day 42. These results indicate that even a single dose administration of the recombinant enzyme can have significant clinical impact and suggests that other lysosomal storage disorders with significant CNS involvement may similarly benefit.

Sibling Cord Blood Banking: A Resource for Families and Investigators interested in Lysosomal Storage Disease

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A Sibling Donor Cord Blood Program was initiated at Children's Hospital Oakland Research Institute in 1998 as a resource to collect, characterize, and to release for transplantation cord blood units (CBU) from families affected by malignant and non-malignant disorders. Currently, 1686 CBUs from 1587 families have been collected among referrals from all 50 US States. The categories of participation include malignant disorders (50%), sickle cell disease (29%), thalassemia (6%), and other hereditary or rare hematological conditions (15%). Cord blood was collected for 15 children with metabolic diseases. CBT was used successfully in one child with Hurler's syndrome. The mean cell volume collected for the 1686 units was 102 ml (range, 31-284) with a mean total nucleated cell count (TNC) of 9.5 x 108 (range, 0.6-53.6) and mean CD 34+ cell count of 3.6 x 106 (range, 0.1-88.1). The post-thaw viability of CBU released for transplantation was 94.4 % (SD ±8.7%) and only 4.4% of CBUs were not processed due to having inadequate volume.

To date, 54 children have been treated by sibling donor cord blood transplantation (CBT), 38 using the cord blood unit as the sole source of stem cells. CBT recipients had hematological malignancies (N=20), thalassemia major (N=18), sickle cell anemia (N=8) or other non-malignant disorders (N=8), and all but 6

received HLA-identical allografts. The median total nucleated (TNC) and CD34+ cell doses were 3.1 x 107 TNC/kg and 0.7 x 105/kg recipient weight, respectively. The median time to ANC >500 and platelet >20,000/mm3 was 22 and 45 days, respectively. One of 52 (2%) evaluable patients had graft failure accompanied by autologous reconstitution. With a median follow-up of 9.6 (range, 0.1 - 94) months, 45 of 54 (83%) patients survive, and 9 patients (17%) died of relapse (N=6) or transplant-related causes (N=3) after CBT. Among the hemoglobinopathy patients, 23 of 26 (88%) survive, and 22 (85%) survive disease-free. Overall, the Kaplan-Meier probabilities of survival and event-free survival after sibling CBT are 79% and 72%, respectively.
Unrelated cord blood transplantation is an important therapeutic consideration for children born with certain types of lysosomal storage disease. The ability of cord blood cells to cross the blood-brain barrier and lead to enzyme replacement in critical tissues throughout the body has been reported. Families who have an affected child may consider prenatal diagnosis and even pre-implantation genetic diagnosis. We have assisted many families who have chosen this option and have decided to bank cord blood. Having a resource for sibling donor collections and banking in families who currently have a child with one of these diseases and are expecting a full sibling could become an important resource to the lysosomal storage disease community in advancing and developing new cell based therapies. The results of the CHORI Sibling Donor Cord Blood Program confirm the feasibility and success of remote site, directed donor cord blood collection and subsequent transplantation. As HLA matched sibling cord blood could become an important resource of cells for transplantation or gene therapy, a national resource to collect cord blood across the United States should be considered.

Mobility of Late Endosomal Tubules is Necessary for Clearance of Lysosomal Lipid Cargo in Lysosomal Storage Diseases

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Lysosomal storage diseases are characterized by intracellular accumulations of cholesterol and glycolipids in lysosomes. Fibroblasts from patients with Niemann

Pick C1 and 2 storage diseases accumulate LDL-derived cholesterol in lysosomes. Transfection of cultured normal and mutant fibroblasts with NPC1-GFP allowed us to visualize this membrane protein in living cells and revealed a late endosomal tubular (LET) trafficking pathway that is sensitive to accumulations of LDL-derived cholesterol. In contrast to the rapidly moving LETs in normal cells, those in cholesterol laden mutant NPC fibroblasts are immobile. Depletion of cholesterol in mutant cells by transfection with WT NPC1-GFP or extended incubation of cells in lipid depleted serum (LPDS) resulted in reestablishment of LET trafficking.
We report now that several other lysosomal storage diseases show commonality in the stasis of LET trafficking that results in lack of lipid transport from lysosomes. In Tay-Sachs fibroblasts the enzyme β-Hexosaminidase A is deficient and the monosialoganglioside, GM2 accumulates. The immobile structure of LETs in these living mutant cells is visualized either by uptake of fluorescent dextran or infection with NPC1-GFP. In infected cells, expression of NPC1-GFP over time results in LET mobility and clearance of accumulated lysosomal GM2 and cholesterol. Similar to Tay-Sachs fibroblasts, cells from other lysosomal lipid storage disorders show that reestablishment of late endosomal tubular mobility results in clearance of lysosomal glycolipids. We find also that depletion of cholesterol from mutant cells by growth in LPDS results in reestablishment of LET mobility with accompanying egress of lysosomal glycolipids and cholesterol. Our results show that stasis of late endosomal tubular trafficking is a pathological feature common to several lysosomal storage diseases. Immobility of late endosomal tubules, a result of increased membrane lipid content, prevents their communication with lysosomes and thus egress of lysosomal lipid cargo.

The use of human neural stem cells to treat Neuronal Ceroid Lipofuscinoses

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Neural stem cell transplantation has therapeutic potential for treatment of neurodegenerative diseases or CNS injuries. We have established cell banks of prospectively isolated CD133+ human central nervous system stem cells (hCNS-SC) grown as neurospheres (hCNS-SCns) to generate these cell banks. Approximately 50 different hCNS-SCns cell lines have been transplanted into the

brains of NOD-Scid mice. Over 1000 individual mouse brains have been evaluated for engraftment, which has been found to be durable, reproducible and multipotential. The human cells showed extensive migration throughout the brain without any evidence of tumor formation. The progeny of human cells migrate into different regions of the brain and differentiate into neurons, astrocytes and oligodendrocytes in a site specific manner. hCNS-SCns can differentiate into myelinating oligodendroctyes when they are transplanted into myelin-basic protein (MBP) deficient shiverer-NOD-Scid mice. Transplantation of human neural stem cells resulted in locomotor recovery in spinal cord injured immunodeficient mice with remyelination provided by human cells. Therefore, hCNS-SCns may hold the prospect to replace the endogenous dysfunctional oligodendrocyte population in certain lysosomal storage disorders.

We have also tested the use of these hCNS-SC cell banks in a mouse model of neuronal ceroid lipofuscinosis (NCL), a progressive fatal neurodegenerative condition of affected infants and young children. Two sub-types, NCL1 and NCL2, result from genetic defects in independent lysosomal enzymes Palmitoyl Protein Thioesterase (PPT1) and Tripeptidyl Peptidase-I (TPP-I), respectively. Lack of either enzyme results in the accumulation of lipofuscin storage materials which can be characterized by pathological changes and neuronal cell loss in the brain. In vitro, hCNS-SCns produce and secrete PPT1 and TPP1, and these enzymes can be endocytosed by fibroblasts cultured from NCL1 and NCL2 patients through the 6-mannose phosphate receptor. Upon transplantation of hCNS-SCns into PPT1 -/- NOD-scid mouse (PPT1-/- mouse) brains, the human cells engraft and migrate widely into the cortex and the hippocampus, continue to produce PPT1 enzyme increasing the total enzyme level in the host brains tested 160-190 days post transplant. Transplanted recipients have reduced amounts of lipofuscin storage materials in the cortex, hippocampus, thalamus and cerebellum compared to age-matched non transplanted control group ($p<0.0001$).
NeuN defines a subset of host neurons. Quantitative analysis revealed that only 8% of the NeuN+ neurons in the CA1 area of the PPT1-/- hippocampus survive at the time point examined compared to PPT1+/+ animals. In transplanted animals as much as of 57% of NeuN+ host neurons were neuroprotected ($p<0.001$). Similarly, in other regions such as the CA2/3 of the hippocampus ($p<0.001$) and the cortex ($p<0.05$), significant survival of host neurons was found in transplanted animals.

These results demonstrate that transplantation of hCNS-SCns leads to global engraftment, provides PPT1 enzyme, reduces storage materials and increases the

survival of host neurons, indicating their potential for use as a therapeutic treatment for NCL1 and NCL2 patients.

Cell biology of sphingolipidoses

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Glycosphingolipid (GSL) storage disorders are inherited human metabolic disorders known to result from defects in the lysosomal enzymes involved in GSL degradation. Over the past few years our laboratory performed studies attempting to define the secondary and down-stream biochemical and cellular pathways affected in GSL storage disorders that are responsible for neuronal dysfunction, a characteristic of most of these disorders. It was previously demonstrated that upon GSL accumulation, cultured hippocampal neurons exhibit modified growth patterns, altered endoplasmic reticulum density, and altered calcium release from intracellular stores. One potential mechanism leading to pathological changes at the cellular level is impaired calcium homeostasis observed in a variety of the SL storage diseases, such as Tay-Sachs (Sandhoff), Niemann-Pick and Gaucher disease (GD). In a mouse model of Sandhoff disease, a dramatic reduction in the rate of Ca^{2+}-uptake via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) was observed. It was also observed that glucosylceramide (GlcCer), the GSL that accumulates in Gaucher disease, enhanced agonist-induced Ca^{2+}-release via the ryanodine receptor (RyaR), and RyaR-mediated Ca^{2+}-release was elevated in brain microsomes obtained post-mortem from Gaucher disease patients.
We also propose that the stored SL in GD (GlcCer) may affect phospholipid biosynthesis. Synthesis of phosphatidylcholine (PC), the major lipid of mammalian cell membranes, is increased in Gaucher models, implying involvement of CCT, the rate limiting step enzyme in PC synthesis.

In addition, we are working on structure-function relationships of glucocerebrosidase (Cerezyme®), the enzyme given to Gaucher disease patients in enzyme replacement therapy (ERT). The availability of these structures provides the possibility of engineering improved glucocerebrosidase for better enzyme replacement therapy, and of designing structure-based drugs aimed at restoring the activity of defective glucocerebrosidase in Gaucher disease.

Reduction of Sphingosine Kinase Activity Reduces Astrogliosis and Improves Neurological Function in Sandhoff Disease

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Sandhoff disease is a prototypical lysosomal storage disorder in which a heritable deficiency of a lysosomal enzyme, β-hexosaminidase, results in the storage of the enzyme's substrates GM2 and GA2 in lysosomes. As with many of the other more than 40 lysosomal storage disorders, neurodegeneration is involved as a prominent feature. Therapies for these diseases have generally been unsuccessful because of the lack of basic knowledge of the underlying neurodegenerative mechanisms. Glial-mediated neuronal dysfunctions have been invoked as neurodegenerative mechanisms in lysosomal storage diseases. However, the cellular and molecular mechanism(s) by which neuronal storage-induced glial activation leads to neuronal damage remain largely unexplained. Sphingosine-1-phosphate (S1P) is a bioactive lipid, produced by the action of sphingosine kinase, that mediates essential biological cellular functions including cell growth, differentiation, proliferation, apoptosis. In this study, we tested the hypothesis that S1P signaling is involved in glial cell mediated neuronal dysfunction in lysosomal storage diseases by establishing Sandhoff mice with the sphingosine kinase 1 (Sphk1) gene deleted. These mice, without Sphk1 expression, showed improved neurologic function and a longer life span. Deletion of Sphk1 expression resulted in a marked decrease the proliferation of immature glia and in a diminution of the severe astrocyte hyperplasia normally found in Sandhoff disease mice. The results indicate that the neuropathogenesis of Sandhoff disease involves Sphk1 activity, which may induce glial reactivity and proliferation of immature glia, resulting in gliosis.