2004 Research Grants

Gene Therapy for MPS II

Dr. Maria Pia Cosma, Telethon Institute of Genetics and Medicine, Naples, Italy

Eight human genetic diseases are caused by a deficiency in the enzymatic activity of sulfatases. Among these are five different types of mucopolysaccharidoses (MPS types II, IIIA, IIID, IVA, and VI). The final goal of this project is to develop a gene therapy approach to treat the MPS II (Hunter syndrome) which is due to the deficit of the Iduronate sulfatase (IDS). To this end, we will use a mouse model of MPS II which exhibits many of the characteristics of the human disease. IDS, as well as all the other sulfatases, need to be modified in the cell after they are synthesized in order to be active. We have recently identified SUMF1 gene (Sulfatase Modifying Factor 1), which is responsible for this modification. SUMF1 has a striking enhancing effect on the activity of all sulfatases in cultured cells. To increase our chances of having active IDS in vivo we will deliver both IDS and SUMF1 genes in the MPS II mouse model. The simultaneous delivery of SUMF1 and IDS genes should result in a more effective treatment of MPS II. Ultimately, this protocol could set the stepping stone for the treatment of other MPS due to sulfatase deficiencies.

General MPS / ML Grant

?Characterization of the Systemic Inflammatory Response to Lysosomal Storage?
Dr. Mark Sands, Washington University School of Medicine, St. Louis, MO

Our laboratory is studying several mouse models of lysosomal storage disease that have characteristics similar to the human disease. While we were studying the MPS I mouse, we discovered that the mice have anemia and have difficulty putting on body weight as fat. Our curiosity about this lack of fat led to a variety of new studies. The MPS I mice eat the same and absorb the same amount of nutrients as unaffected mice. MPS I mice also have the same respiration and activity levels as normal mice, thus they have similar metabolic rate. Both the anemia and fat loss could be caused by abnormal inflammatory or immune responses. We therefore looked for inflammation markers in the blood and found an increase in the amount of inflammatory proteins in the affected mice. We are currently determining if the anemia, fat loss, and inflammation are common to other lysosomal storage diseases. Our ultimate goal is to determine what role the inflammatory proteins are playing in lysosomal storage diseases.

1st Year Research Reviews

Research Updates

In 2004 the National MPS Society awarded grants to Dr Maia Pia Cosma for her work, ?Gene Therapy for MPS II? and to Dr. Mark Sands for his work ?Characterization of the Systemic Inflammatory Response to Lysosomal Storage?. Below are the reviews they submitted, summarizing the first year of research.

Dr. Maria Pia Cosma

TIGEM, Naples, Italy

?Gene Therapy for MPS II?

Mucopolysaccharidosis type II (Hunter syndrome) is due to the deficiency of the enzyme iduronate sulfatase (IDS). The IDS enzyme promotes the degradation of complex molecules named glycosoamminoglycans (GAGs). In particular IDS promotes the degradation of dermatan sulfate and heparan sulfate, which once degraded can be eliminated from the cells. In Hunter Syndrome these compounds accumulate as un-degraded molecules in the cells of the patients. The cells, overloaded with these molecules within particular organelles, the lysosomes, initiate a systemic degeneration of all of the tissues of the body. To date, there is no effective treatment for MPSII and gene therapy is an attractive approach to cure the MPSII syndrome. To this aim we used the Hunter mouse model that exhibits many of the characteristics of MPSII, including skeleton abnormalities such as coarse faces, macrodactlya, and elevated accumulation of GAGs in the urine and many organs. We generated a viral vector carrying the human IDS that produces the active enzyme exclusively in the liver. The viral particles were administrated intravenously (IV) to group of adult MPS II mice and mainly targeted the liver. When the IDS enzyme was produced from the liver it was secreted into the bloodstream and taken up by the other organs leading to the correction of the systemic defects. We analyzed the correction of the disease phenotype in treated mice after one and seven months. In both groups, upon the gene therapy treatment, the plasma levels of IDS enzyme were increased in all treated mice and the accumulation of GAGs in the urines was reduced. We also carried out behavioral tests on the treated animals that performed as well as the normal wild-type control mice. Furthermore, even the skeletal abnormalities, such as coarse faces and macrodactlya, resulted completely corrected. The tissues of treated mice were also analyzed and showed a rescue of the enzymatic activity of IDS and complete GAGs clearance. Finally, microscopically, there was a marked reduction in vacuolization in all of the organs examined.
We also characterized the CNS defects of the MPSII mice and found degeneration of Purkinje cells in the cerebellum and GAGs accumulation in the choroid plexus. The described gene therapy approach has the caveat that it does not correct the central nervous system defects. The IDS secreted from the liver into the bloodstream does not cross the brain blood barrier and therefore does not reach the brain. To rescue the central nervous system phenotype, we are performing experiments based on the delivery of the IDS adeno-associated vector in the

cerebrospinal fluid and direct injection in the cerebellum. Preliminary results showed a partial clearance of the accumulation of GAGs in the choroid plexus. The results achieved so far demonstrate possibility and the efficacy of gene therapy by AAV carrying the active IDS to treat mucopolysaccharidosis type II.?

Dr. Mark S. Sands
Washington University, St. Louis, MO
?Characterization of the Systemic Inflammatory Response to Lysosomal Storage?

Specific Aim 1: The goal of this Specific Aim was to determine if the abnormal clinical signs observed in the MPS I mouse (adipose storage deficiency, anemia, muscle wasting) were common to other lysosomal storage diseases.

As presented in the preliminary data section of the proposal every mouse model of lysosomal storage disease had a significant decrease in adiposity (15-57% decrease compared to normal control animals). With respect to anemia, the models of MPS (I, IIIB and VII) all had anemia as presented in the preliminary data. However, neither the Neimann-Pick AB (NPAB) nor the infantile neuronal ceroid lipofuscinosis (INCL) models had significantly decreased hematocrits. We are currently sectioning quadriceps muscles from all of the models to determine if there is significant muscle wasting.

Specific Aim 2: The goal of this Specific Aim was to determine if the abnormal levels of pro-inflammatory molecules seen in the MPS I model were common to other models of lysosomal storage disease.

Our preliminary data showed that both MPS I and NPAB mice had significantly elevated levels of several cytokines and chemokines. Interestingly, the biggest changes were in the levels of circulating chemokines and soluble VCAM. We have completed our analyses of the MPSIIIB, MPS VII and INCL models. The levels of pro-inflammatory molecules varied among the various models but there was evidence of systemic inflammation in all of the models. MPS IIIB mice had elevated levels of MCP-1, MCP-3, MIP-1a, VCAM and IL-1b. MPS VII mice had elevated levels of MCP-1, MCP-3 and VCAM. INCL mice had elevated levels of soluble VCAM. Therefore, inflammation appears to be a common clinical feature of lysosomal storage diseases. Interestingly, MCP-1 and/or VCAM are elevated in every model of lysosomal storage disease.

Specific Aim 3: The goal of this Specific Aim was to test the hypothesis that systemic inflammation contributed to the disease progression in murine models of lysosomal storage diseases.

We proposed several different approaches to test this hypothesis. Since MCP-1 was significantly elevated in nearly every model of lysosomal storage disease, we obtained the MCP-1-null mouse from the Jackson Laboratory and have moved both the MPS I and NPAB mutations onto that genetic background. We currently have colonies of MCP-1- and NPAB-

deficient mice that carry both the disease mutation and the MCP-1 mutation and are generating double mutant animals to be tested for adiposity, anemia and muscle wasting.
We have also created an HIV-based gene transfer vector encoding the M3 protein from g-Herpes virus. M3 is a secreted protein that binds most chemokines with high affinity and inactivates the pro-inflammatory function. In this way we can determine the effects of selectively inhibiting the function of the elevated chemokines present in the murine models. We currently have several MPS I and MPS IIIB mice that have been injected with the HIV-M3 virus and are generating additional animals to complete those experimental groups.
In a similar fashion, we have obtained a transgenic mouse model that expresses M3 from an inducible promoter in the presence of the antibiotic doxycycline. We have moved the transgenes onto the MPS I background and currently have mice that are expressing high levels of M3 systemically. All of the animals will be analyzed at 5 or 7 months of age to determine the effects of inhibiting a host of different chemokines (HIV-M3 and transgenic M3) or just MCP-1's actions (MCP-1 knockout).

2nd Year Research Reviews

Research Grant Reviews 2004

In 2004 the National MPS Society awarded grants to Dr. Maria Pia Cosma for her work "Gene Therapy for MPS II" and to Dr. Mark Sands for his work, "Systemic inflammation associated with lysosomal storage diseases". Below are the reviews they submitted summarizing the second year of their research.

"Systemic Inflammation Associated with Lysosomal Storage Diseases"

Dr. Mark S. Sands, Washington University, St. Louis, MO

The goal of this research was to determine the underlying cause of the anemia and adipose storage deficiency associated with MPS I and whether this phenotype was common to other lysosomal storage diseases (LSDs). We also proposed to determine if the systemic inflammation associated with MPS I was common to other LSDs and whether a reduction in the levels of certain pro-inflammatory molecules affected the disease progression. We initially showed that MPS I mice had a 41% decrease in body fat and an 18% decrease in hematocrit when compared to normal litter mates. The anemia was not a common finding among the other models of LSD. However, the inability to accumulate normal fat stores is common to other LSDs. Mouse models of MPS IIIB, MPS VII, Niemann-Pick AB (NPAB), and Infantile Neuronal Ceroid Lipofuscinosis (INCL) have 37, 51, 42, and 15% decreases in body fat, respectively, when compared to normal litter mates. We tested a number of simple hypotheses that might explain this decrease in fat stores. There was no significant difference in food intake, physical activity (open field test), metabolic rate (O2 consumption), fecal content (free fatty acids, triglycerides, cholesterol), serum content (free fatty acids, triglycerides, cholesterol), muscle triglycerides, or liver triglycerides between affected animals and normal controls. However, in the process of searching for the cause of the adipose storage deficiency, we discovered that the level of pro-inflammatory molecules (specifically chemokines) was significantly elevated in the MPS I mouse. We discovered similar changes in the other models of LSD. Interestingly, MCP-1, MCP-3 and soluble VCAM were elevated in the serum of every model we tested, and lymphotactin, M-CSF, MIP-1γ, and TIMP-1 were elevated in 3 out of 5 of the models. There is growing evidence that chemokines can have a direct effect on adipocytes and fat metabolism. We hypothesized that inhibition or genetic deletion of certain chemokines would affect, perhaps ameliorate the adipose deficiency associated with the models of LSD. We created a mouse model of MPS I that also expressed the pan chemokine-binding protein M3. M3 is a γ-herpes virus-encoded protein that binds and inactivates many chemokines. We also moved the MPS I and NPAB mutations onto a mouse model that is genetically deficient in MCP-1. We chose the knockout animal of MCP-1 since it is one of the chemokines that is elevated to the highest levels in every animal model of LSD. Neither the inhibition of the chemokines by M3 nor the genetic deletion of MCP-1 led to any increases in adiposity in either the MPS I or the NPAB mouse. Although our hypothesis that chronic inflammation associated with LSDs leads to adipose storage deficiency appears to be incorrect, these studies have enabled us to develop several additional hypotheses that could explain this phenotype. We are currently testing the

hypotheses that either malabsorption or a change in the gut biota result in the adipose deficiency that is common to the mouse models of LSD. We believe that the adipose storage deficiency is an important clinical finding and correction of this phenotype might improve the quality of life of the affected children.

"Gene Therapy for MPS II: Strengthening Iduronate Sulfatase Enzymatic Activity Through the Action of the Sulfatase Modifying Factor 1"

Dr. Maria Pia Cosma, TIGEM, Naples, Italy

Our results have been published in the following paper:
Cardone M, Polito VA, Pepe S, Mann L, D'Azzo A, Auricchio A, Ballabio A and Cosma MP.(2006). Correction of Hunter syndrome in the MPS II mouse model by AAV2/8-mediated gene delivery. Human Mol Genet, April,15(7), 1225-36

Mucopolysaccharidosis type II (MPS II; Hunter syndrome) is a lysosomal storage disorder caused by a deficiency in the enzyme iduronate 2-sulfatase (IDS). At present, the therapeutic approaches for MPS II are enzyme replacement therapy and bone marrow transplantation, although these therapies have some limitations. The availability of new AAV serotypes that display tissue-specific tropism and promote sustained expression of transgenes offers the possibility of AAV-mediated gene therapy for the systemic treatment of lysosomal diseases, including MPS II. We have characterized in detail the phenotype of IDS-deficient mice, a model of human MPS II. These mice display a progressive accumulation of glycosaminoglycans (GAGs) in many organs and excessive
excretion of these compounds in their urine. Furthermore, they develop skeleton deformities, particularly of the craniofacial bones, and alopecia, they perform poorly in open-field tests and they have a severely compromised walking pattern. In addition, they present neuropathological defects. We identified loss of Purkinje cells and cellular vacuolization in different regions of the brain: the hippocampus, thalamus, cerebellum and brainstem. We have designed an efficient gene therapy approach for the treatment of these MPS II mice. AAV2/8TBG-IDS viral particles were administrated intravenously to adult MPS II mice. The plasma and tissue IDS activities were completely restored in all of the treated mice. This rescue of the enzymatic activity resulted in the full clearance of the accumulated GAGs in all of the tissues analyzed, the normalization of the GAG
levels in the urine and the correction of the skeleton malformations and of the locomotor disabilities. Furthermore a partial clearance of the GAG accumulation was also evident within the choroid plexus in the treated mice. This was surprising given the presence of the hemato-encephalic barrier. We predict that due to the very high levels of IDS in the plasma, which ranged from 16 to 70 times higher than the normal wild-type values, a fractional amount of the enzyme crossed the barrier and corrected the defect. Overall, our findings suggest that this in vivo gene transfer approach has potential for the systemic treatment of patients with Hunter syndrome.