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The Immune Response to Intrathecal Enzyme Replacement Therapy in Mucopolysaccharidosis I Patients

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Abstract

BACKGROUND—Intrathecal (IT) enzyme replacement therapy with recombinant human α-L-iduronidase (rhIDU) has been studied to treat glycosaminoglycan storage in the central nervous system of mucopolysaccharidosis (MPS) I dogs and is currently being studied in MPS I patients.

METHODS—We studied the immune response to IT rhIDU in MPS I subjects with spinal cord compression who had been previously treated with intravenous rhIDU. We measured concentrations of specific antibodies and cytokines in serum and cerebrospinal fluid collected prior to monthly IT rhIDU infusions and compared serologic findings to clinical adverse event reports to establish temporal correlations with clinical symptoms.

RESULTS—Five MPS I subjects participating in IT rhIDU trials were studied. One subject with symptomatic spinal cord compression had evidence of an inflammatory response with cerebrospinal fluid leukocytosis, elevated IL-5 and elevated IgG. This subject also complained of lower back pain and buttock parasthesias temporally correlated with serologic abnormalities. Clinical symptoms were managed with oral medication and serologic abnormalities resolved though this subject withdrew from the trial to have spinal decompressive surgery.

CONCLUSION—IT rhIDU was generally well tolerated in the subjects studied though one subject had moderate to severe clinical symptoms and serologic abnormalities consistent with an immune response.

INTRODUCTION

Mucopolysaccharidosis I (MPS I) is a lysosomal storage disease caused by deficiency of the enzyme α-L-iduronidase (EC 3.2.1.76) leading to accumulation of glycosaminoglycans

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DISCLOSURES The Los Angeles Biomedical Research Institute at Harbor-UCLA and the Department of Pediatrics at Harbor-UCLA have a financial interest in rhIDU (formulated as laronidase). Paul Harmatz has provided consulting services to BioMarin Pharmaceutical Inc., and has also received research grants, participated in advisory boards, and received speakers honoraria and travel support from BioMarin.

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(GAG) throughout the body and resulting in multi-system dysfunction (1,2). Intravenous enzyme replacement therapy with recombinant human α-L-iduronidase (rhIDU) has been available for the treatment of MPS I patients since 2003 (3). When administered as a weekly infusion, intravenous (IV) rhIDU has been shown to reduce lysosomal GAG storage and improve many, though not all, of the clinical symptoms (3,4). An important shortcoming of IV rhIDU is the inability to effectively treat neurologic manifestations of central nervous system (CNS) GAG storage in MPS I patients (5). These include intellectual disability in the severe (Hurler) form of the disease, spinal cord compression owing to storage in the cervical meninges of patients with attenuated disease (Hurler-Scheie and Scheie), and communicating hydrocephalus owing to obstructed cerebrospinal fluid (CSF) reabsorption in all forms of MPS I (6,7). Treatment of the latter neurologic manifestations of CNS GAG storage in MPS I patients often requires cervical laminectomy with removal of thickened meninges to decompress the spinal cord and ventricular-peritoneal shunt implantation to relieve CSF pressure, respectively (6–9).

Intrathecal (IT) enzyme replacement therapy has been successfully used for treatment of lysosomal storage diseases in several animal models (10–15). When IT rhIDU is administered to MPS I dogs the enzyme diffuses into the brain, spinal cord and meninges and reduces GAG storage in these structures (15,16). Some of these dogs developed a CNS inflammatory response to IT rhIDU with mild-moderate aseptic meningitis (15,16). An immune response has also been observed in other animals when enzyme is administered directly into the CSF (12,13,17), and the CSF anti-rhIDU IgG titer in MPS I dogs correlates with diminished enzyme penetration into the brain and diminished efficacy in reducing GAG storage (17). The development of serum IgG antibodies against the recombinant protein is a common complication of intravenous enzyme replacement therapy and MPS I patients receiving IV rhIDU often develop serum anti-rhIDU IgG antibodies (18). These patients generally do not suffer from clinically significant immunologic adverse events (18) though the immune response to IV rhIDU has been shown to reduce therapeutic efficacy in MPS I dogs (19). Currently, IT rhIDU is being studied in clinical trials as a potential treatment for central nervous system (CNS) disease in human MPS I patients. The immune response to this therapy in humans has not been studied thus far.

In this study we characterized the rhIDU-specific immune response in CSF and serum collected from MPS I subjects who had previously been treated with IV rhIDU and who were enrolled in clinical trials of IT rhIDU. We measured absolute concentrations of anti-rhIDU antibodies and T_H1 and T_H2 cytokines (20). We also reviewed case report forms for evidence of clinical adverse events that were thought to be at least possibly related to IT rhIDU treatments and correlated these with laboratory findings. We found no serologic or clinical evidence of an anaphylactoid reaction in any subject. Most subjects had only mild to moderate clinical symptoms during the course of intrathecal infusions and were able to complete the treatments. One subject with ongoing symptomatic spinal cord compression complained of intermittent lower back pain, buttock pain and parasthesias following IT rhIDU infusions and developed a transiently elevated CSF white blood cell count and anti-rhIDU IgG titer. The symptoms were managed with oral medications and the CSF abnormalities resolved over two to three months though the subject ultimately withdrew from the trial for spinal decompressive surgery.

METHODS

This study involved de-identifed patient samples and case report forms. The study was reviewed and approved by the John Wolf Human Subjects Committee of the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, and a waiver of informed consent was granted.

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Recombinant human α-L-iduronidase (Aldurazyme, BioMarin Pharmaceutical Inc., Novato, CA) is approved for IV use in humans by the U.S. Food and Drug Administration and was administered via the IT route under an Investigational New Drug authorization (IND) from the FDA and the European Union Drug Regulating Authorities (EUDRA). Patient samples and case report forms from three multicenter, multinational safety studies were included.

In a pilot study (NCT 00215527), MPS I subjects with spinal cord compression who were at least 8 years old and who had received IV rhIDU treatments for at least six months were treated monthly with 1.74mg IT rhIDU for a total of four doses ("pilot study"). This dose was chosen for human trials based on the lowest intrathecal dose required to achieve increased enzyme levels in the brain and meninges and brain GAG reduction in MPS I dogs (15). In an extension study (NCT 00786968), MPS I subjects who completed the pilot study with good response to IT rhIDU, defined by improvement in any outcome measure and no safety concerns, received up to 12 months of additional therapy at 30 to 90 day intervals ("extension study"). In a "cognition study" (NCT 00852358), MPS I subjects with cognitive impairment or decline who were at least 6 years old and who had received IV rhIDU treatments for at least six months were enrolled in a 1-year randomized controlled trial followed by 1-year open label phase of 1.74mg IT rhIDU given at 30 to 90 day intervals ("cognition study").

One subject (001-01-03, Table 1) received off-study, off-label IT rhIDU treatments before enrollment in the extension study. Samples and clinical information from the off-study period were included.

In each study rhIDU (0.58mg/mL) was diluted with 6–8mL of Elliotts B Solution (Ben Venue Laboratories, Inc., Bedford, Ohio), a buffered intrathecal injection diluent with pH, electrolytes, glucose and osmolarity roughly similar to human cerebrospinal fluid. The diluted enzyme was administered via lumbar puncture and infused slowly over 2–3 minutes. Subjects were pretreated with acetaminophen and an antihistamine on an individual basis prior to infusions. Each subject was monitored closely during infusions for any adverse clinical reactions.

Safety was assessed throughout each subject's study participation with periodic clinical and laboratory evaluations. A history and physical examination was performed at each visit during the studies and any clinically significant worsening from baseline medical status was documented as an adverse event. Laboratory assessments included: CSF opening pressure; CSF and serum chemistry and cell counts, GAG quantitation, cytokine quantitation, and ELISA for α-L-iduronidase specific antibodies.

CSF and Serum Collection

Samples were collected at baseline and immediately prior to each intrathecal enzyme infusion. Approximately 6–10mL of CSF was withdrawn at each lumbar puncture and 2.5–5mL of whole blood was collected in a serum separator tube. Samples were aliquoted and stored at −80°C then later thawed for subsequent antibody and cytokine assays.

CSF and Serum ELISA for α-L-iduronidase Specific Antibodies

Anti-rhIDU antibodies were measured by an enzyme-linked immunosorbent assay (ELISA) method. In brief, CSF and serum samples collected from each subject at each time point were first diluted 1:100 then subsequent 1:3 serial dilutions were incubated on 96-well assay plates (Immulon 1B U-bottom microtiter plates, Dynex Technologies, Chantilly, VA) containing wells with adsorbed rhIDU (BioMarin Pharmaceutical Inc.). Specific binding of CSF and serum anti-rhIDU antibodies to the coated wells was detected using alkaline phosphate conjugated goat anti-human IgG (Southern Biotechnology Associates, Inc.,

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Birmingham, AL), IgM or IgE (Bethyl Laboratories, Montgomery, TX) secondary antibodies. Absorbance at 405nm was measured as optical density (OD) values using a BioTek Synergy 2 Microplate Reader (BioTek Instruments, Inc., Winooski, VT), converted to OD units per μL based on the dilution, and then subsequently converted to protein concentration in ng/mL by comparison with a human IgG standard curve, using linear regression to calculate the conversion factor.

Enzyme Uptake Inhibition Assay

We measured the in vitro inhibition of exogenous rhIDU uptake by anti-rhIDU antibodies in the serum and CSF of study subjects using a previously described method (19). In brief, rhIDU (BioMarin Pharmaceutical Inc.) was diluted to a concentration of 18 Units/mL in Minimal Essential Medium, without FBS, plus 1% L-glutamine (Hyclone Classical Liquid Media MEM EBSS, Thermo Fisher Scientific Inc., Waltham, MA) and then incubated for 1 hour at room temperature in the absence or presence of either subject serum or CSF added at 1:50 dilution. Skin fibroblasts from an MPS I Hurler individual (GM 1391; National Institute of General Medical Sciences, Human Genetic Cell Repository, Coriell Institute for Medical Research, Camden, NJ) were passed to a confluence of 85%–95% in 6-well cell culture cluster plates and the diluted serum or CSF plus rhIDU mixture or rhIDU alone was added in 2mL aliquots to triplicate wells containing these confluent cells. Cells were incubated for 1 hour at 37°C and 5% CO_2, rinsed, harvested by trypsinization and pelleted by centrifugation. The pellet was washed and resuspended, disrupted by sonication, and centrifuged. Enzyme activity in the supernatant was measured in triplicate assays by detecting cleavage of 4-methylumbelliferyl (19,21) from 4-methylumbelliferyl α-L-iduronide (4-MUI, Calbiochem, San Diego, CA) by rhIDU using a spectrofluorophotometer at 365nm excitation and 440nm emission (Shimadzu RF-1501, Shimadzu Corp., Tokyo, Japan). One unit of activity was equivalent to 1 nmol converted substrate per hour.

CSF and Serum Cytokine Assay

Cytokine levels in CSF and serum samples collected from each subject at each time point were quantified using a multiplex bead based immunoassay (Invitrogen Human Cytokine 10-Plex Panel, Life Technologies, Grand Island, NY). Briefly, the wells of a 96-well filter plate (1.2-micron Durapore membrane 96-well plate, EMD Millipore, Billerica, MA) were pre-wet with working wash solution provided by the manufacturer (Wash Solution Concentrate, Life Technologies) and polystyrene beads individually coated with antibodies specific to the human cytokines interleukins (IL) 1β, 2, 4, 5, 6, 8, 10, tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ) or granulocyte macrophage colony stimulating factor (GM-CSF) were added (Human Cytokine 10-Plex Antibody Bead Concentrate, Life Technologies). Incubation buffer and assay diluent provided by the manufacturer (Life Technologies) along with 50μL of either CSF or serum samples were added and the plate was incubated on a shaker for 2 hours at room temperature. Serial dilutions of a standard solution of the above ten cytokines provided by the manufacturer were also plated in duplicate (Human 16-Plex Standard, Life Technologies). After the incubation 100μL of biotinylated antibodies specific to each assayed cytokine (Human Cytokine 10-Plex Biotinylated Antibody Concentrate, Life Technologies) was added to each well and the plate was incubated on a shaker for 1 hour at room temperature. Streptavidin conjugated with R-phycoerythrin (Streptavidin-RPE Concentrate, Life Technologies) was added to each well followed by a 30-minute incubation on a shaker at room temperature. After aspiration and washing, the beads were resuspended in working wash solution and the fluorescent intensity of streptavidin-RPE tagged cytokine specific beads was read with a Luminex-200 dual laser detection system (Luminex Corp., Austin, TX). Standard curves were generated by the Luminex xMAP analysis software package (Luminex Corp.) for each cytokine and were

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used to convert measured fluorescent intensity to absolute concentration in pg/mL. All samples were assayed in duplicate.

Adverse Event Review

Case report forms labeled with subject number and initials were used to determine total adverse events (AE) based on history, physical examination, and neurologic examination. An adverse event was defined as any undesirable physical, psychological or behavioral effect experienced by a subject during his or her participation in the study including: subjective or objective symptoms reported by the subject and/or observed by the medical staff; clinically significant laboratory anomalies; and exacerbation of pre-existing disease related symptoms or laboratory abnormalities. These were characterized by type, severity, onset and duration, and rated by the study investigator as definitely, probably or possibly related to the IT rhIDU infusion. The subset of neurologic AE was identified for each subject and the recorded dates were compared to the dates of sample collections and IT rhIDU infusions.

RESULTS

We analyzed samples from a total of five subjects who gave signed, written informed consent and enrolled in the clinical trials described (Table 1). One additional subject who enrolled in the pilot study died before any samples could be collected and so was not included in this analysis. A full discussion of the outcomes of the clinical trials is beyond the scope of this manuscript. Data from the completed pilot and extension studies will be detailed in a forthcoming report (Dickson et al, manuscript in preparation), while the cognition study remains ongoing.

At baseline prior to the initiation of IT rhIDU infusions all subjects had measurable serum levels of anti-rhIDU IgM and IgG (Figure 1), an expected immune response with previous exposure to intravenous rhIDU therapy. Most subjects had higher serum levels of anti-rhIDU IgM than IgG. There was no detectable level of anti-rhIDU IgE in the serum of any subject studied. Serum anti-rhIDU IgD was also checked for consistency to rule out primary immunodeficiency (data not shown). None of the subjects had detectable levels of any anti-rhIDU antibody in the CSF at baseline (data not shown).

After the initiation of monthly IT rhIDU infusions none of the subjects developed a measurable anti-rhIDU IgM level in the CSF (Figure 2b) while all subjects had relatively low and constant anti-rhIDU IgM levels in the serum (Figure 2a). One subject, 001-01-04, had an elevated and fluctuating level of anti-rhIDU IgG in the serum while all others had much lower relatively constant levels over time (Figure 2c). Only one subject, 001-01-03, developed a CSF anti-rhIDU IgG level (Figure 2d), peaking on day 90, prior to the fourth IT rhIDU infusion, and resolving over the following two months. The serum and CSF anti-rhIDU IgG antibodies in this subject did not inhibit rhIDU uptake in vitro (data not shown). No subject had appreciable levels of anti-rhIDU IgE in the serum or CSF (Figure 2e,f).

At baseline prior to the initiation of IT rhIDU infusions there was no consistent pattern of abnormality across T_H1 (cellular immune response) or T_H2 (humoral immune response) cytokines in the serum or CSF (Figure 3). All subjects had elevated IL-8 levels compared to the other cytokines studied and this elevation was greater in the CSF than the serum in most subjects (Figure 3a,b). Individual subjects also displayed much lower but still detectable levels of TNFα, IFNγ and IL-6 in the serum (Figure 3a) and IL-6 in the CSF as well (Figure 3b).

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After the initiation of monthly IT rhIDU infusions all subjects were found to have a persistently elevated IL-8 level in the CSF that was relatively constant over a four to five month period (Figure 4b,d,f,h,j). In contrast, most subjects had fluctuating IL-8 levels in the serum (Figure 4a,c,e,g,i). No subject had a pattern of cytokine elevations consistent with a T_H1 or T_H2 immune response in the serum or CSF. Subject 001-01-03 had a transiently elevated TNFα level in the serum (Figure 4a), and an elevated IL-5 level in the CSF that peaked on day 90 just prior to the fourth IT rhIDU infusion (Figure 4b).

At the time of enrollment subject 001-01-03 was 13 years old with a history of hydrocephalus, for which a ventriculoperitoneal shunt was placed at age 6 years, and spinal cord compression diagnosed on magnetic resonance imaging at age 11 years. She received six off-label off-study IT rhIDU treatments every one to two months prior to being approved for enrollment in the extension study during which she received five IT rhIDU treatments at 90-day intervals (Table 1). During the initial course of treatments she developed an elevated CSF white blood cell (WBC) count that peaked on day 90 prior to the fourth infusion and mirrored the elevations in CSF anti-rhIDU IgG (Figure 5a) and IL-5 (Figure 5c). The differential cell count in the CSF was predominantly lymphocytic (data not shown). B and T cell subsets of this lymphocyte count were not measured. This subject also had a markedly elevated CSF protein level at baseline (Figure 5c), attributed to spinal cord compression. She was treated with a 7-day course of oral prednisone after the fourth IT rhIDU infusion on day 90 (Figure 5a, arrow) based on her complaints of back and buttock pain and history of spinal cord compression. Subsequently the CSF WBC count, anti-rhIDU IgG, IL-5 and protein levels dropped (Figure 5a,c), though the CSF protein level increased again after one month (Figure 5c).

This subject was pretreated with oral prednisone prior to subsequent IT rhIDU infusions for pain and CSF pleocytosis. During the extension study her CSF WBC count normalized and the CSF anti-rhIDU IgG level became undetectable (Figure 5b). In contrast, while the IL-5 level became undetectable in the CSF the total protein and IL-8 levels increased further compared to baseline during the extension study (Figure 5d). The CSF glucose level remained within the normal range during the entire evaluation period (Figure 5c,d).

Subject 001-02-01, a 27 year old female who completed the pilot study, also received 8 additional IT rhIDU treatments during the extension study (Table 1). Throughout the course of her participation, the CSF WBC count, protein and glucose remained within normal limits, and all CSF anti-rhIDU antibody levels remained undetectable (data not shown). This subject had an elevated CSF IL-8 level during the pilot study (Figure 4h) that persisted without marked variation during the extension study (data not shown).

Subject 001-01-03 also experienced neurologic adverse events that coincided with the CSF findings and were thought to be related to IT rhIDU infusions (Table 2). She complained of buttock pain immediately following, or within 1–2 days of, IT rhIDU infusions on several occasions (Figure 5a, arrow heads), including the fourth injection on day 90. The pain was bilateral, lasted 2–4 days and was relieved by oral hydrocodone. She also had a history of frequent migraine headaches prior to enrollment and experienced one episode of severe headache and facial flushing following infusion number 7 for which she was hospitalized and observed according to study protocol (Figure 5b, arrow head). Finally, subject 001-01-03 developed symptoms consistent with worsening spinal cord compression during the extension study. She complained of leg pain, foot parasthesias, and hand weakness, and then developed urinary urgency and incontinence. She was evaluated by her neurosurgeon, underwent spine imaging, and withdrew from the trial for spinal decompressive surgery that was performed two months following the last IT rhIDU infusion shown in Figure 5b and 5d. No serologic or adverse event data were collected after withdrawal from the trial, therefore

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we cannot evaluate the effect of decompressive surgery on the CSF abnormalities. The subject's symptoms were not attributed to IT rhIDU, and the compression symptoms resolved following surgery.

Among the remaining subjects there were a total of six serious adverse events reported: pneumonia (001-01-01, 001-02-01); hypoxia with respiratory distress (001-02-01), corneal transplantation (001-02-01); and buttock cramping, spinal cord compression, and prostate cancer (002-01-01). One instance of pneumonia was thought to be possibly related to IT rhIDU infusion (001-01-01), though none of the other serious adverse events described in any subject were thought to be related to the infusions. Most subjects complained of mild intermittent or isolated headaches following the first one or two IT rhIDU infusions; these were generally not recurrent and not temporally associated with any CSF or serum abnormality. Subject 001-01-04, a 24 year old female with cervical spinal stenosis, hydrocephalus and lumbo-peritoneal shunt, who had a history of chronic headaches, neck and upper back pain, complained of slightly worsened pain symptoms following the first three IT rhIDU infusions. She was also found to have a transiently elevated CSF opening pressure of 26 cm H_2O prior to the third injection. Her chronic pain symptoms were managed with oral steroids and analgesics during the trial.

DISCUSSION

We studied subjects enrolled in clinical trials to assess safety of IT rhIDU and functional improvement in MPS I patients with spinal cord compression or cognitive impairment. All subjects had previous exposure to IV rhIDU for at least six months, and in most cases several years, prior to study entry. All subjects experienced some form of neurological complaint or symptom, most commonly mild intermittent or isolated headaches.

Neurologic adverse reactions have been previously reported in patients receiving intrathecal injections of therapeutic proteins. Two patients treated with intrathecal rituximab for CNS B-cell lymphoma complained of painful parasthesias involving the buttocks, legs and feet immediately following lumbar puncture and injection (22). These symptoms were also reported in a patient treated after decreasing the protein concentration of the intrathecal injection, though after adding intrathecal dexamethasone to the protocol no additional treated patients complained of the same pain (22).

Similar neurologic symptoms can also be attributed to drug-induced aseptic meningitis, an uncommon though well known CNS inflammatory process characterized by CSF pleiocytosis with lymphocytosis, normal to low glucose, and elevated protein in the absence of infection (23,24). Possible mechanisms include a hypersensitivity reaction limited to the meninges, deposition of circulating immune complexes within the meninges, or direct chemical irritation of the meninges caused by intrathecal drug delivery (24).

In our study, subject 001-01-03 had laboratory evidence of an aseptic meningitis that developed during the course of IT rhIDU infusions. However, the concurrent development of an elevated anti-rhIDU IgG antibody level in the CSF suggests a specific immune response. Dogs receiving IT rhIDU developed a CSF pleocytosis and anti-rhIDU IgG antibodies (15), and histological evaluation of the meninges showed a plasma cell infiltrate in these dogs. While it is possible that aseptic meningitis could cause increased permeability of the blood brain barrier allowing leakage of lymphocytes into the CSF, the absence of correspondence between serum and CSF IL-5 and IL-8 levels in subject 001-01-03 (Figure 4a,4b) makes this mechanism unlikely as the sole explanation for the CSF anti-rhIDU IgG findings.

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Patients with all forms of MPS I have unmeasurable endogenous α-L-iduronidase activity though those with attenuated disease may synthesize trace amounts of endogenous enzyme depending on the underlying genetic mutation (1,25). This cross-reactive immunologic material (CRIM) is recognized as self protein by the host immune system and its presence is thought to mitigate development of serum antibodies to intravenous recombinant human enzyme in CRIM-positive as compared to CRIM-negative subjects (18). It is not clear how this concept extends to explain the development of anti-rhIDU IgG antibodies in the relatively immune privileged CNS. The subjects we studied all had an attenuated MPS I phenotype consistent with probable CRIM-positive status and all had varying concentrations of anti-rhIDU IgG in their sera while only one had an elevated level in the CSF. The subject with CSF anti-rhIDU IgG antibodies was found to have antibodies that did not inhibit rhIDU uptake or activity in vitro.

Froin's syndrome—elevated protein, xanthochromia, and hypercoagulability of the cerebrospinal fluid—is a well described phenomenon reported in patients with spinal canal obstruction caused by tumors, epidural abscess, or spinal meningitis (26,27) and accounts for some of the CSF findings in subject 001-01-03. However, this does not explain the observation that this subject's clinical symptoms and CSF protein level progressed even while all laboratory evidence of a specific CSF immune response and all serious adverse events related to IT rhIDU infusions resolved (Figure 5b,5d).

Interleukin-8, a chemokine also known as CXCL8, is produced by a variety of cells including monocytes, macrophages, fibroblasts and endothelial cells and functions as a chemoattractant for neutrophils and naïve T cells, stimulating neutrophil activation and degranulation (20). IL-5 is produced by T_H2 CD4 T cells and stimulates bone marrow production of eosinophils (20). The significance of the comparative CSF elevations of IL-8 and IL-5 in subject 001-01-03 is unclear owing to a lack of correspondence in CSF cell count findings and the lack of published normative data for comparison. Previously published studies of cytokine levels in the CSF focus on patients with infectious (28–31), autoimmune (32,33), or other neurologic disease (34–36). The variety of acute or chronic conditions studied and the inconsistency of methods used to quantify cytokine levels make comparison with our patients difficult. Immune tolerance to rhIDU, owing to the combination of immunosuppression by monthly steroid prophylaxis and antigen exposure, could theoretically explain the drop in specific CSF IgG and WBC count in subject 001-01-03, similar to the regimen shown to produce serum immune tolerance in MPS I dogs treated with intravenous rhIDU (37).

The small number of subjects we studied may bias our interpretation by limiting the variety of neurologic adverse events we were able to observe. The absence of a control group for comparison is also a limitation of our study that is inherent to enrolling a sufficient number of subjects with rare diseases in clinical trials. However, the potential benefits of this novel therapy for MPS I patients are significant—treating CNS disease including intellectual disability, spinal cord compression symptoms, and hydrocephalus—while the adverse events that we attribute to the immune response to IT rhIDU were tolerable and managed fairly readily with steroids and pain medication.

In summary, we studied laboratory and clinical data for five MPS I subjects with spinal cord compression and a history of prior IV rhIDU treatment enrolled in trials of IT rhIDU to assess for evidence of an immune response to the therapy. To our knowledge this study represents the first systematic characterization of the immune response to IT rhIDU in MPS I patients and the first measurement of absolute antibody and cytokine concentrations in the serum and CSF of MPS I patients. Four subjects had intermittent mild neurologic complaints including headache, back and neck pain that were managed with oral analgesics or steroids

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in one case. They had no laboratory evidence of an immune or inflammatory response to the therapy. One subject with symptomatic progressive spinal cord compression complained of back and buttock pain and parasthesias following IT rhIDU infusions and developed a transiently elevated CSF anti-rhIDU IgG level. She ultimately withdrew from the trial for spinal decompressive surgery. The remaining subjects completed their respective treatment courses without clinical or laboratory evidence of an enzyme specific immune response to IT rhIDU therapy.

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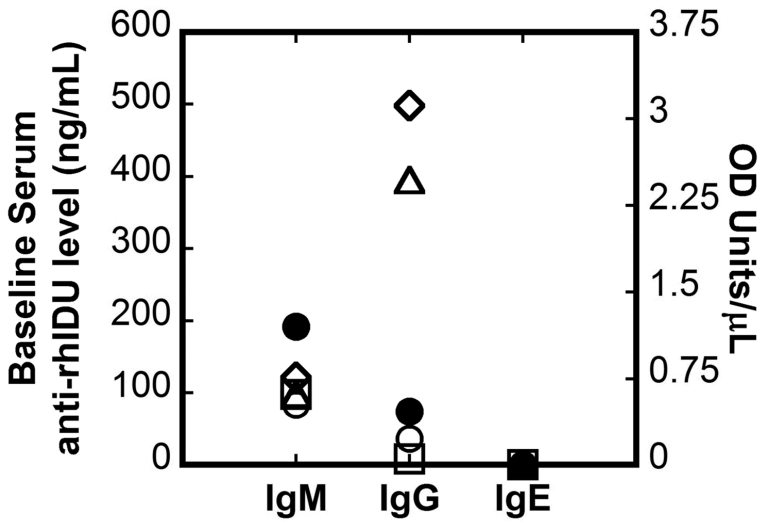

Figure 1.

Baseline anti-rhIDU IgM, IgG and IgE antibody concentrations in MPS I subject sera prior to starting IT rhIDU treatments. Right axis: measured OD Units/μL; left axis: converted antibody concentration in ng/mL. Subject 001-01-03 (filled circles), 001-01-01 (open diamonds), 001-01-04 (open triangles), 001-02-01 (open circles), 002-01-01 (open squares) (Table 1). There were no detectable levels of any anti-rhIDU antibody isotype in the CSF of any subject at baseline (data not shown).

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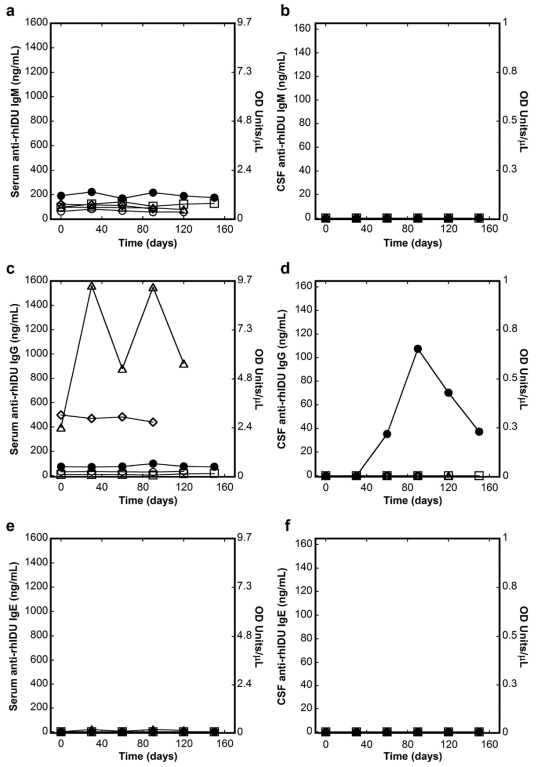

Figure 2.

Anti-rhIDU antibody levels in MPS I subjects during the course of IT rhIDU treatments. Serum anti-rhIDU IgM (**a**), IgG (**c**) and IgE (**e**); CSF anti-rhIDU IgM (**b**), IgG (**d**), and IgE (**f**). Samples were drawn immediately prior to each monthly IT rhIDU infusion. Right axis: measured OD Units/μL; left axis: converted antibody concentration in ng/mL. Subject 001-01-03 (filled circles), 001-01-01 (open diamonds), 001-01-04 (open triangles), 001-02-01 (open circles), 002-01-01 (open squares) (Table 1). For subjects who received extended treatment courses (001-01-03, 001-02-01) only data from the initial treatment period are shown for comparison.

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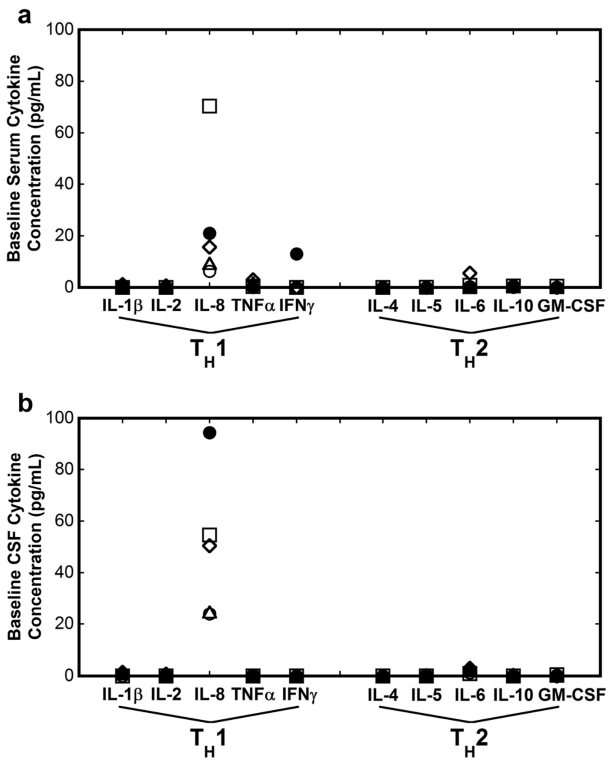

Figure 3.

Baseline absolute cytokine concentrations in the serum (**a**) and CSF (**b**) of MPS I subjects prior to starting IT rhIDU treatments. T_H1 (cellular immune response) cytokines: IL-1β, IL-2, IL-8, TNFα, IFNγ; T_H2 (humoral immune response) cytokines IL-4, IL-5, IL-6, IL-10, GM-CSF (20). Subject 001-01-03 (filled circles), 001-01-01 (open diamonds), 001-01-04 (open triangles), 001-02-01 (open circles), 002-01-01 (open squares) (Table 1).

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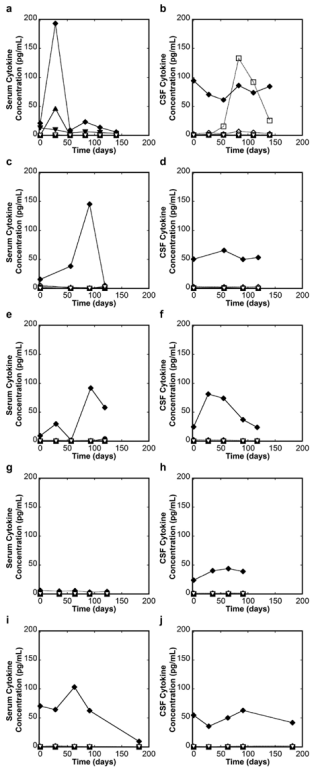

Figure 4.

Cytokine levels in MPS I subjects during the course of IT rhIDU treatments. Absolute concentrations of T_H1 (filled symbols: circles IL-1β; squares IL-2; diamonds IL-8; triangles TNFα; inverted triangles IFNγ) and T_H2 cytokines (open symbols: circles IL-4; squares IL-5; diamonds IL-6; triangles IL-10; inverted triangles GM-CSF) measured in samples drawn immediately prior to each monthly IT rhIDU infusion. Horizontal rows correspond to individual subjects: 001-01-03 serum (**a**) and CSF (**b**); 001-01-01 serum (**c**) and CSF (**d**); 001-01-04 serum (**e**) and CSF (**f**); 001-02-01 serum (**g**) and CSF (**h**); 002-01-01 serum (**i**) and CSF (**j**). For subjects who received extended treatment courses—001-01-03 (**a**,**b**), 001-02-01 (**g**,**h**)--only data from the initial treatment period are shown for comparison.

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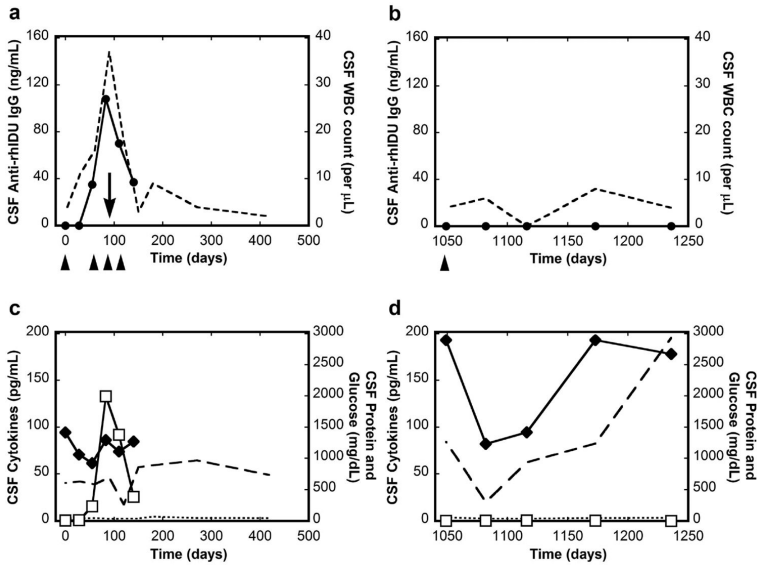

Figure 5.

Cerebrospinal fluid (CSF) findings in subject 001-01-03. CSF white blood cell (WBC) count (short dashed line: **a**,**b**), protein (long dashed line: **c**,**d**) and glucose (dotted line: **c**,**d**) were measured along with anti-rhIDU IgG antibody (filled circles with solid line: **a**,**b**), IL-8 (filled diamonds with solid line: **c**,**d**) and IL-5 (open squares with solid line: **c**,**d**) levels prior to each monthly IT rhIDU infusion. (**a,c**): initial off-study treatment period; (**b,d**): extension study. Subject complained of mild buttock pain (arrowheads, **a**) and moderate to severe headache with facial flushing (arrowhead, **b**) thought to be related to IT rhIDU. A 7-day course of oral prednisone was started following the fourth IT rhIDU infusion on day 90 (vertical arrow, **a**).

Normal values for CSF indices: WBC 0 – 7/μL; total protein 5 – 40 mg/dL; glucose 40 – 80 mg/dL (37).

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Table 1

Subject characteristics.

Subject ID	Gender (M/F)	MPS I Phenotype	Age at Diagnosis (years)	Age at IV rhIDU initiation (years)[e]	Age at Study Entry (years)	Number of IT rhIDU treatments
001-01-03[a]	F	Hurler-Scheie	1	7	13	11
001-01-01[b]	F	Hurler-Scheie	3	30	31	4
001-01-04[b]	F	Scheie	22	22	24	5
001-02-01[c]	F	Hurler-Scheie	3.5	22	27	12
002-01-01[d]	M	Hurler-Scheie	28	45	51	6

MPS I, mucopolysaccharidosis I; rhIDU, recombinant human α-L-iduronidase; IV rhIDU, intravenous rhIDU enzyme replacement therapy; IT rhIDU, intrathecal rhIDU enzyme replacement therapy.

[a] Received 6 IT rhIDU treatments off study and 5 treatments in extension study (NCT 00786968) before withdrawing to undergo spinal decompressive surgery for a history of ongoing spinal cord compression.

[b] Completed pilot study (NCT 00215527).

[c] Received 4 IT rhIDU treatments in pilot study (NCT 00215527) and 8 treatments in extension study (NCT 00786968).

[d] Enrolled in cognition study (NCT 00852358).

[e] All subjects had received IV rhIDU treatments for at least 6 months prior to enrollment in IT rhIDU trials.

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Table 2

Neurologic adverse events for subject 001-01-03.

Neurologic AE	IT Injection #	Timing following IT injection	Severity	Outcome
Buttock pain	1	Immediate	Mild	Resolved
	3	1 day	Mild	Resolved
	4	1 day	Mild	Resolved
Upper back pain	5	2 days	Mild	Resolved
	6	2 days	Mild	Resolved
Headache	1	23 days	Mild	Resolved
	2	24 days	Mild	Resolved
	5	20 days	Moderate	Resolved
	7	24 hours	Moderate/Severe[a]	Resolved
Elevated CSF opening pressure[b,c]				
26 cm H_2O	2	Immediately prior	Mild	Resolved
32 cm H_2O	8	Immediately prior	Mild	Resolved
Facial flushing	7	24 hours	Moderate/Severe[a]	Resolved

[a] Serious adverse events requiring concomitant medication administration and/or hospitalization.

[b] Normal range: 11.5 – 28 cm H_2O (37).

[c] Ventriculoperitoneal shunt function was verified prior to enrollment.