Behavioral Consequences of Bone Marrow Transplantation in the Treatment of Murine Mucopolysaccharidosis Type VII

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Abstract

The *gus*^{mps}/*gus*^{mps} mouse is a model of the human lysosomal storage disease mucopolysaccharidosis type VII caused by deficient β-glucuronidase activity. Bone marrow transplantation has been shown to correct some of their biochemical and pathological abnormalities but its efficacy in correcting their neurological functional deficits is unknown. We transplanted the neonatal *gus*^{mps}/*gus*^{mps} mice and their normal controls and evaluated their central nervous system function with two behavioral tests: the grooming test, a developmentally regulated and genetically based activity, and a Morris water maze test which assessed spatial learning abilities. The two transplanted groups groomed less than the normals, were unable to remember the location of an invisible platform from day to day, and were severely impaired at developing strategies to locate the platform in unfamiliar locations. The performance of both normal and mutant transplanted groups was clearly inferior to the untreated normals and, in some instances, close to or worse than the untreated mutants, even though the enzyme abnormalities of the mutants have been partially corrected. Hence, the behavioral deficits in the mutant mice were not restored to normal while similarly treated normal mice showed significant functional deterioration, indicating the detrimental consequence of this therapy in the neonatal period. (*J. Clin. Invest.* 1994. 94:1180–1186.) Key words: Sly syndrome • lysosomes • central nervous system diseases • behavioral sciences • β-glucuronidase

Introduction

Deficiency in β-glucuronidase activity (EC 3.2.1.31) leads to the lysosomal storage disease mucopolysaccharidosis type VII (MPS VII,[1] Sly disease) not only in humans (1) but also in a recently discovered murine mutant, the *gus*^{mps}/*gus*^{mps} mouse (2). Clinical and pathologic abnormalities common to the human and the mouse phenotypes include shortened life span, dwarfism, dysmorphic facial features, skeletal deformities, corneal clouding and abnormal lysosomal storage material in the brain, peripheral organs and macrophages (1–7). Although only the more severely affected human patients demonstrate mental retardation, the mutant mice have clearly defined neurological deficits which result in cognitive, memory, and central nervous system (CNS)–mediated functional deficiencies (8). The mutation of the *gus*^{mps}/*gus*^{mps} mouse is due to a single base pair deletion in the β-glucuronidase gene, resulting in a premature stop codon within the open reading frame and a complete absence of β-glucuronidase messenger RNA, thus accounting for the total enzyme deficiency (9).

Because the *gus*^{mps}/*gus*^{mps} mice have been extensively characterized in their genetics, biochemistry, pathology, and behavioral abnormalities, they provide an excellent model system to study the pathobiology of lysosomal storage diseases and to evaluate the efficacy of different therapies. Various therapeutic strategies such as bone marrow transplantation (BMT)[1] with either syngeneic normal donors (10, 11) or retrovirally transfected mutant stem cells (12), infection with viral vectors (13), and somatic cell therapy with genetically modified skin fibroblasts (14) have been performed on these *gus*^{mps}/*gus*^{mps} mice. These treatments resulted in variable histochemical, biochemical, and pathological improvement of the peripheral organs, while the restoration of β-glucuronidase activity in the CNS has been minimal. It is not clear if any improvement in CNS function has resulted from these therapeutic interventions.

The accumulation of storage material in the CNS of the mutant mice is consistent with the mental retardation observed in severely affected MPS VII patients. We have recently demonstrated that the generalized pathology and neurological involvement of the disease in the mutant *gus*^{mps}/*gus*^{mps} mice result in CNS functional deficits that can be measured with behavioral tests (8). One behavioral test is to monitor the amount of time spent in grooming, an activity that follows a complex and stereotypical set of movements (15, 16) characteristic of the species, strains, and developmental stages of the animals (17). It was suggested that the widespread occurrence of cytoplasmic vacuolation in neurons, glia, and mesenchymal cells in the brains of *gus*^{mps}/*gus*^{mps} mice (7) contributed to levels of grooming activity that are significantly lower than normal (8). Another behavioral test, the Morris water maze (18), measures the ability of rodents for spatial mapping, reference memory, and discrimination reversal. The pathological presentation of the *gus*^{mps}/*gus*^{mps} mice includes lysosomal distention in the hippocampus and the neocortex (7), structures recognized as critical for spatial learning (19–23). This further supports a specific neurological basis for the deficits in cognition and memory demonstrated by the mutant mice during the water maze tests (8).

Of all the experimental therapeutic interventions performed on the mutant mice, only BMT has been tried in humans (24).

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1. *Abbreviations used in this paper:* BMT, bone marrow transplantation; MPS VII, mucopolysaccharidosis type VII.

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Many patients with various lysosomal storage diseases have been treated worldwide with variable degrees of success (25). Because of the inherent difficulties in human studies and the phenotypic variability of lysosomal storage diseases, it has been difficult to evaluate the efficacy of BMT, particularly in lysosomal storage disorders which involve neurodegeneration. Since the neurological deficits of the gus^{mps}/gus^{mps} mice can be demonstrated with behavioral tests, we propose to evaluate the efficacy of BMT in the prevention of neurological deficits in the mutant mice.

Methods

Animals. Male mutants (gus^{mps}/gus^{mps}) and their normal littermates (+/+ and $+/gus^{mps}$) obtained from the mutant strain B6.C-H-2*bml*/ByBir-gus^{mps}/+, were supplied at the Jackson Laboratory (2). The 47 mice were divided into 4 groups. 11 normal (+/?) and 9 mutant littermates were treated with BMT performed on day 1 after birth. In brief, the mice received 2 Gy and then were transplanted with bone marrow cells from normal (+/+) syngeneic females obtained from the B6.C-H-2*bml*/ByJ strain (11). 17 normal and 10 mutants did not receive any treatment. Mice were maintained on 4.5% Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and housed on a 12:12 light/dark cycle. Behavioral testing was always conducted during the animal colony's light phase.

Assessment of engraftment. Peripheral blood smears were prepared and stained with Wright's stain as previously described (2). Donor-derived granulocytes were differentiated from those of the host by the absence of dense granules in the cytoplasm.

Grooming tests. Each mouse received two test trials on the same day, under baseline conditions with no external stimulus and stimulated conditions with the mouse lightly moistened by a water mister. The mouse was videotaped close up for 10 min under each condition while enclosed within a cylindrical wall 10 cm in diameter and 15 cm high. The videotapes were reviewed at one-fifth the actual speed and the total time spent in face grooming, in body grooming, and in other movements was determined. Actions that qualified as face grooming included any movement of the forelimbs around the face and head such as: flailing of the forelimbs below the face; licking of the forepaws; overhand, parallel, or single strokes of the forelimbs overtop of the head, side of snout, or face; and shimmying (16, 17). Any grooming that did not include the face and head was categorized as body grooming. This consisted of scratching the body with the hind legs and licking the abdomen, genitals, and flanks. Other movements were activities that did not contribute to grooming, such as digging, rearing, locomotion, and standing still (17). Only the time spent in face and body grooming were analyzed in detail.

Morris water maze. The procedure for this test was modified from R. Morris (18). A pool 182 cm in diameter was filled 30 cm deep with water at 21°C. Four cardinal points around the circumference of the pool were designated as North, South, East, and West, thus dividing the pool into four quadrants (SW, NW, NE, SE). A transparent circular plexiglass platform 10 cm in diameter was placed 40 cm from the wall, its top surface 1 cm below the surface of the water. On all trials, mice were released into the water facing the wall of the pool. Four trials were made for each mouse on each day, using each cardinal point once as the starting point. The sequence of start positions was randomly selected for the day, but all mice followed the same order.

The behavioral testing was conducted on 13 consecutive days. On the first day, the animals were habituated to the water by letting them swim in the pool, without the platform, for 30 s per trial. On days 2–6, the platform was located in the SW quadrant and the time each mouse needed to reach the platform was recorded. If the mouse found the platform within 120 s, it was allowed to remain on the platform for 30 s. If it did not find the platform during the allocated time, it was guided by the experimenter to the platform and allowed to remain there for 30 s. On day 7, the platform was removed and the amount of time each mouse spent in any one quadrant was recorded. On days 8 to 11, the quadrant was placed back in the SW quadrant and the procedures of days 2 to 6 were repeated. On days 12 and 13, the platform was moved to the NE quadrant, and again the procedures of the previous days were repeated. No less than 90 s and no more than 180 s elapsed between two trials, during which the mouse was dried with a towel and placed under a heating lamp. Each trial was recorded with an overhead camera. No attempt was made to obstruct the mice from the view of the room. All the cues in the room were held constant throughout the testing (e.g., experimenter, work bench, rack of cages, TV monitor, light fixtures, etc.).

Lysosomal enzyme assays. Dissected tissues were either stored at −70°C or immediately homogenized in 20 mM Tris·HCl, pH 7.5/140 mM NaCl/10 mM β-mercaptoethanol/0.25% Saponin homogenization buffer. Homogenates were sonicated on ice for 20 s and then centrifuged at 16,000 *g* for 30 min. Supernatants were assayed fluorometrically for lysosomal enzyme activities by using 4-methylumbelliferyl (4-MU) substrates (26). β-Glucuronidase, β-hexosaminidase, and α-galactosidase activities were assayed using the substrates 4-MU-β-glucuronide, 4-MU-*N*-acetyl-β-D-glucosaminide, and 4-MU-α-D-galactoside (Sigma Chemical Co., St. Louis, MO), respectively. Proteins were determined according to the method of Lowry (27).

Data analysis. All the behavioral tests and biochemical analyses were performed double-blind. The codes were broken when all test data had been collected. All statistical analysis was performed with the BMDP™ Statistical Software on the VAX. The designs for analysis of variance (ANOVA) with repeated measures, or one-way and two-way ANOVAs, were of either two groupings and two within factors, or two groupings and one within factor. Orthogonal components were included for analysis.

Results

The animals were divided into four groups based on their genotypes (normal or mutant) and whether they received neonatal BMT (treated or untreated). The time spent in different grooming activities under various conditions and their abilities to locate an invisible platform in the Morris water maze were assessed.

Grooming

Under baseline conditions, body grooming in the 14-wk-old untreated mutant mice was depressed to 10.5% of that of their normal littermates. In mutants treated with BMT, body grooming was depressed to 33% of normal. However, ANOVA indicated that there was a significant difference only between the normal and mutant genotypes ($F_{1,37} = 12.64$, $P < 0.01$) but no difference between animals as a result of the BMT treatment. Follow-up tests (Tukey) indicated that only the untreated mutants were significantly different from the normal controls ($P < 0.05$). At 19 wk of age, the amount of time spent in body grooming was again only significantly different between animals with different genotypes ($F_{1,33} = 6.70$, $P < 0.02$). No significant difference attributable to the BMT was observed between the experimental groups. Therefore, BMT was not associated with improved grooming activities in the mutants under baseline conditions at either 14 or 19 wk of age.

When the mice were stimulated with a mist of water sprayed on their bodies, the amount of time spent in body or face grooming increased (Fig. 1). The increase in body grooming was observed in all animals at both 14 and 19 wk of age and no significant difference was observed among the four groups (normal and mutant: treated and untreated). From ANOVA of face grooming, however, significant main effects of genotype ($F_{1,52}$

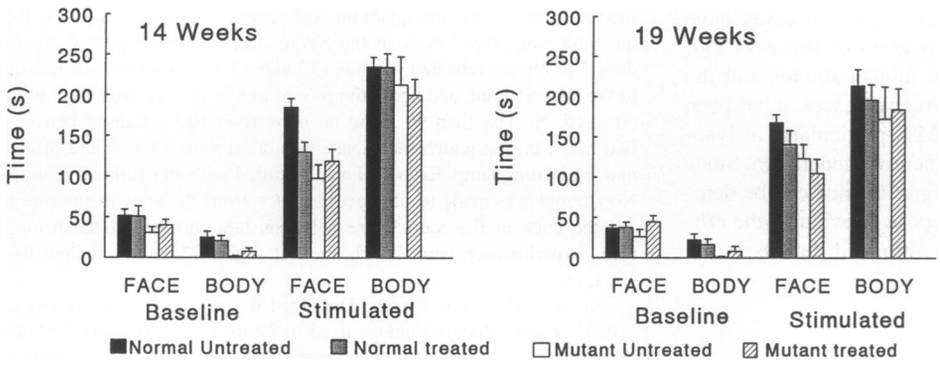

Figure 1. Grooming behavior of the normal and mutant mice after bone marrow transplantation. Each animal at both 14 and 19 wk of age was monitored for the amount of time spent in face and body grooming under normal conditions (baseline), as well as after stimulated with a spray of water mist (stimulated). Data are the mean±SEM.

= 12.03, $P < 0.002$) and genotype × treatment interaction ($F_{1,52} = 7.16$, $P < 0.01$) were found for mice at 14 wk of age. This indicated that the amount of time spent in face grooming was significantly different between animals of different genotypes and that BMT has exerted some effect. Follow-up tests (Tukey) clarified that compared with the normal untreated controls, face grooming was reduced in all three remaining groups (95% level of confidence in the treated normals and treated mutants; 99% level of confidence in the untreated mutants), but none of these three groups differed significantly from each other. Hence, BMT was associated with a lower level of performance when normal mice were treated, while no improved performance was obtained when the mutants were treated. At 19 wk of age, however, only significant main effects of genotype ($F_{1,42} = 7.24$, $P < 0.02$) were found. This time, face grooming was reduced only in treated mutants and their untreated controls (95% level of confidence). The normal treated animals may have recovered from the BMT sufficiently to perform such CNS-mediated reflex activities at similar levels as the normal untreated controls.

Morris water maze test

Acquisition. From days 2 to 6, the mice were tested for their ability to learn the location of the platform placed in the SW quadrant. The normal controls showed a gradual decline in the time needed per trial to find the platform. Although the treated mice (normal and mutant) and untreated mutants also showed a decrease in search time over these days, they were much slower than the normal controls (Fig. 2).

ANOVA for repeated measures showed significant main effects of treatment ($F_{1,43} = 11.62$, $P < 0.002$), genotype ($F_{1,43} = 10.14$, $P < 0.003$), trial ($F_{3,129} = 10.23$, $P < 0.0001$), and day ($F_{4,172} = 10.29$, $P < 0.0001$). This indicates that the performance of the mice was affected by all four parameters: the BMT, the genotype, the sequence of the four trials during the same day, and the sequence of the days from day 2 to day 6. In addition, there were significant interactions between genotype × treatment ($F_{1,43} = 7.83$, $P < 0.01$), trial × treatment ($F_{3,129} = 3.30$, $P < 0.05$), and day × treatment ($F_{4,172} = 2.90$, $P < 0.05$), indicating that the rates of improvement between the two genotypes throughout the four trials of each day and the 5 d during the acquisition phase were different as a result of the BMT. The results were further analyzed with posteriori comparisons (Tukey) as summarized at the top of Fig. 2. It can be seen that the BMT therapy had no effect on improving the performance of the mutant mice, and even retarded the learning performance of normal mice.

Transfer. On day 7, the platform was removed from the SW quadrant and the mice were monitored for the amount of time spent in each quadrant. All four groups were observed to spend more time swimming in the quadrant where the platform was originally located (SW) and less time in the other three quadrants (Fig. 3). When tested with ANOVA for repeated measures, the difference among quadrants was highly significant ($F_{1,129} = 35.54$, $P < 0.0001$). Moreover, a significant interaction was observed between the main effects of quadrant × treatment ($F_{3,129} = 4.63$, $P < 0.02$), indicating that the relative amount of time spent in the four quadrants differed as a result of the BMT. When each quadrant was analyzed separately,

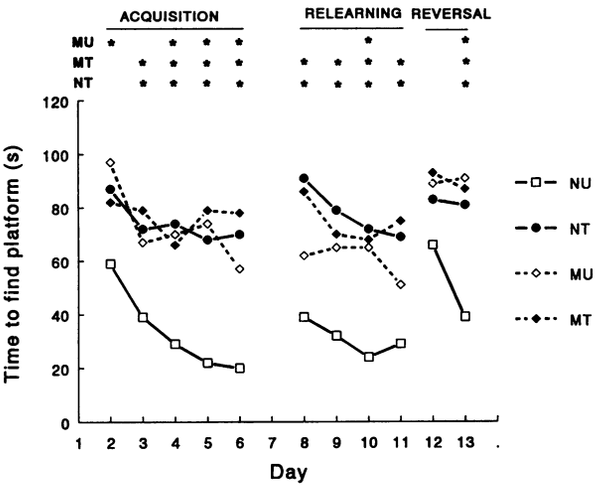

Figure 2. Morris water maze test on normal and gus^{mps}/gus^{mps} mice after bone marrow transplantation. Each animal was released into a water pool from each of the four cardinal points once per day. The time taken to find and land on an invisible platform placed in the pool was recorded and averaged over the four trials as latency per day. Days 2 to 6 comprised the acquisition phase, when the platform was placed in the same location throughout. On day 7, the platform was removed to measure the transfer phase (Fig. 3). Days 8 to 11 comprised the relearning phase, when the platform was replaced to the original position. Days 12 and 13 comprised the reversal phase when the platform was moved to the opposite quadrant. Stars indicate days in which the group differed from the control untreated normal group at the 95% level of confidence. Data are the mean with SEM of 2–8 s, which are not represented in the graphs to prevent cluttering. NU, normal untreated, $n = 17$; NT, normal treated, $n = 12$; MU, mutant untreated, $n = 9$; MT, mutant treated, $n = 9$.

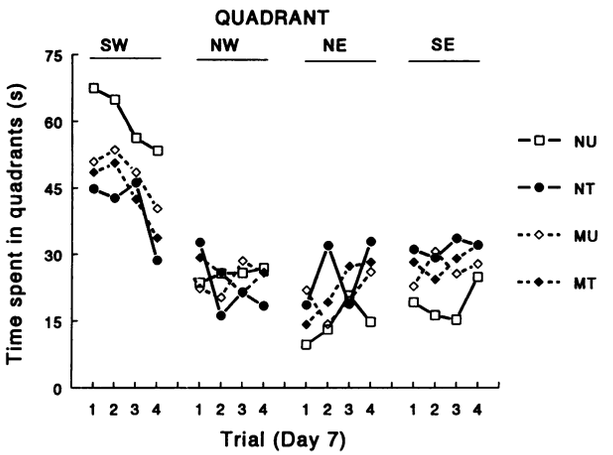

Figure 3. Time spent in each quadrant during the transfer phase of the Morris water maze test (day 7). With the platform removed from the SW quadrant, each animal was released once from each of the four cardinal points (1, 2, 3, and 4), and the amount of time spent in each quadrant was recorded. Data treatment and number of animals are as described in the legend of Fig. 2.

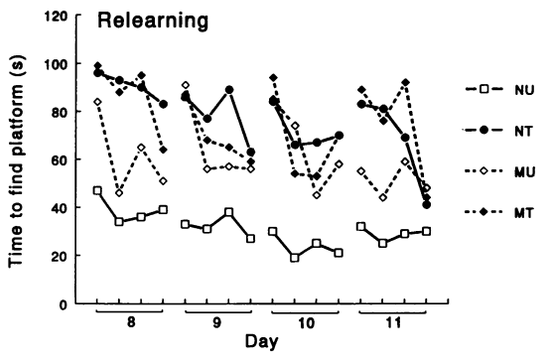

Figure 4. Latency per trial during the relearning phase of the Morris water maze test (days 8–11). From days 8–11, the platform was replaced in the original quadrant (SW). Each animal was released from each of the four cardinal points once per day. The time needed to find the platform was recorded for each trial. Data treatment and number of animals are as described in the legend of Fig. 2.

significant main effects of treatment were found in the SW quadrant ($F_{1,43} = 5.8$, $P < 0.03$), as well as the NE quadrant ($F_{1,43} = 5.04$, $P < 0.03$) and SE quadrant ($F_{1,43} = 5.50$, $P < 0.03$) but not the NW quadrant. Hence, the treated groups (normal and mutant) spent less time in the SW quadrant and more time in the NE and SE quadrants than the untreated groups (normal and mutant) (Fig. 3). Thus, the BMT treatment appeared detrimental for memory retention regardless of the genotype of the animal.

Relearning. From days 8 to 11, the mice were tested as in the "acquisition" phase for their ability to find the platform that now was replaced in the original SW quadrant. On day 8, the average time needed to find the platform of all four experimental groups was longer than those of the last day (day 6) during the acquisition phase (Fig. 2). However, over the subsequent 3 d, only the normal mice (treated or untreated) consistently decreased their search times. The two mutant groups demonstrated somewhat sporadic improvements during this relearning phase. Nevertheless, the treated normals, in spite of demonstrating a similar rate of improvement over the 4 d as the normal controls, clearly were taking much longer than their untreated cohorts to find the platform on each day (Fig. 2).

From ANOVA for repeated measures, a strong linear effect of trial ($F_{1,43} = 38.73$, $P < 0.0001$), as well as an interaction of this effect with treatment ($F_{1,43} = 3.25$, $P = 0.0786$) and with genotype ($F_{1,43} = 4.29$, $P < 0.05$) was observed. This indicates that the latency times differed significantly through the four trials each day, and that this difference was influenced by both the BMT and the genotype of the animals. Further analysis of the individual trials during each day (Fig. 4) indicated that both treated normals and treated mutants showed improvements in search times through the four trials per day in each of the 4 d tested (days 8–11); the untreated mutant mice showed similar improvements for only the first three days of testing, while no improvement was observed on the last day (day 11). The normal controls showed improvements through the four trials for the first day only. During the subsequent three days, they reached a plateau in latency times, showing no more improvements with repeated performance. Therefore, the rapidity with which the animals acquired the maximum amount of learning was in the order: normal control > mutant control > normal treated ≈ mutant treated. Unlike the normal controls, the other three groups took much longer to accomplish the task, and seemed unable to carry over their improved latency from one day to the next. The latency times for the first trial of the day were the same for all 4 d in the treated normals and treated mutants, and for the first 3 d in the untreated mutants—suggesting that each of these days was encountered as a naive experience. The ANOVA for repeated measures showed a strong main effect of treatment ($F_{1,43} = 13.54$, $P < 0.001$), indicating that BMT was a major and detrimental factor in determining the performance of the animals. This was especially evident on the first and last days of testing when both treated groups (normal and mutant) showed a higher latency time than the corresponding untreated cohorts (Fig. 4).

Reversal. On days 12 and 13 of the test, the platform was changed to the NE quadrant of the pool (Fig. 2). On the first day of this phase, all experimental groups showed latency times similar to that of the first day of the acquisition phase. On the second day, however, the normal controls quickly reduced their latency times, while the other three groups showed little improvement. ANOVA for repeated measures indicated significant main effects of genotype ($F_{1,43} = 7.91$, $P < 0.01$) and trial ($F_{1,43} = 4.45$, $P < 0.01$), while the main effects of treatment ($F_{1,43} = 3.21$, $P = 0.0800$) and day ($F_{1,43} = 3.87$, $P = 0.0556$) were significant only at the 90% level of confidence. This indicates that the latency times were affected by the genotype of the animals, by the sequence of the four trials each day, and, to a lesser degree, by the BMT as well as the day of the tests. Follow up tests (Tukey) confirmed that the performance of the normal controls on day 13 was significantly different from the other three groups ($P < 0.05$). Therefore, BMT was clearly detrimental to the cognitive performance of normal animals, as shown by the poor performance of the treated normal group. It also did not improve the performance of the transplanted mutants.

Lysosomal enzyme analysis

The effect of BMT on normalizing the deficient β-glucuronidase activity and the elevated β-hexosaminidase and α-galactosidase

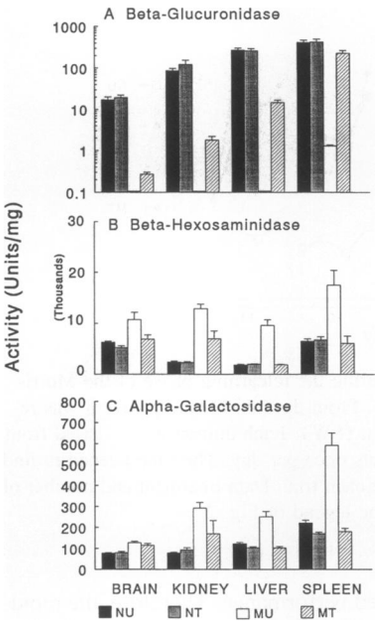

Figure 5. Lysosomal enzyme activities in various organs of the normal and gus^{mps}/gus^{mps} mice after bone marrow transplantation. At about 20 wk of age, animals were sacrificed and their organs removed for protein and lysosomal enzyme assays. Each organ was assayed in duplicate or triplicate. The means for the different animals were averaged (±SEM). NU, normal untreated, $n = 8$; NT, normal treated, $n = 6$; MU, mutant untreated, $n = 4$ (3 for the brain); MT, mutant treated, $n = 5$.

activities in the gus^{mps}/gus^{mps} mice was variable. Consistent with previous observations (10), the degree of enzymatic normalization depended on the organs studied. β-Glucuronidase activity in the brain, kidney, liver, and spleen of treated mutants was restored to 1.6, 2.1, 5.5, and 55.3%, respectively, of those of the normal controls (Fig. 5 *A*). The untreated mutants, as expected, had < 0.5% (range 0–0.35%) of normal activity. The transplanted normals showed no difference from the normal control in these biochemical profiles. ANOVA, followed by post hoc tests (Tukey) revealed that treated mutants differed from untreated mutants in all the organs tested, thus indicating the effectiveness of bone marrow transplant in restoring at least some β-glucuronidase in the mutants. When the secondary elevation of other lysosomal enzyme activities was examined, the levels of β-hexosaminidase (Fig. 5 *B*) and α-galactosidase (Fig. 5 *C*) activities also had decreased to near normal levels in all organs except for β-hexosaminidase activity in the kidney and α-galactosidase activity in the brain and kidney (Fig. 5, *B* and *C*). In these organs, even though the levels were still significantly higher than normal, they were lower than those of the untreated mutants—again confirming the biochemical efficacy of the BMT. When the peripheral blood smear of the transplanted mutants was assessed for the percent of donor cell types as an indicator of the level of engraftment in the hemopoietic system, the average±SEM was 29.7±3.9% (range 11–52%).

Discussion

Although BMT performed in neonates improved many of the biochemical (Fig. 5), clinical, and pathological abnormalities (11) of the gus^{mps}/gus^{mps} mice, it did not ameliorate the behavioral abnormalities of the mutants. Neither the grooming test (Fig. 1) nor the Morris water maze test (Figs. 2–4) indicated any significant difference in performance between the treated and untreated mutants. The β-glucuronidase enzyme was delivered to the brain in treated mutants, but its activity was only 1.6% of normals (Fig. 5).

The sequence and frequency of grooming activities in rodents have a strong genetic basis and are developmentally regulated (17). Peripheral and central control pathways (28, 29) including central neural structures such as the corpus striatum (30, 31) and the caudal brain stem (32) have been implicated in the complex and stereotypical set of grooming movements innate to rodents (see 33 for review). It is likely then, that the CNS pathology of the untreated mutants, such as cytoplasmic vacuolations in the neurons, glia, and mesenchymal cells in the brain (7), contributed to the depression in baseline body and stimulated face grooming seen here (Fig. 1) and in earlier experiments (8). That the treated mutants were not significantly different in baseline body grooming from the normal controls may be attributed to the presence of an outlier which increased the time spent in body grooming for treated mutants by 50%. However, one cannot exclude the outlier (animal M-2, level of engraftment 12%, as assessed from blood smear, unpublished observation) as there may have been variability in the degree of bone marrow engraftment and/or in the time when engraftment occurred.

Under stimulated conditions, face grooming was significantly depressed not only in the untreated mutants but also in the treated mice, compared with the normal controls. The decrease seen in the treated mutants indicates that the therapy was not effective in restoring such neurologically mediated reflex actions. However, the depression observed in treated normals suggests that the therapy itself may have been detrimental.

The performance of the animals during the Morris water maze test provides further insight to the behavior of the animals. The untreated mutants were slower than the normal controls in learning the location of the platform during the acquisition phase (Fig. 2). It has been suggested (8) that the untreated mutants are slower due to physical constraints such as synovial fluid retention in limb joints, skeletal deformities resulting in abnormal gait, and reduction in adipose tissue as reservoirs for metabolic energy (2, 7). However, the treated normals, which did not manifest these physical abnormalities, were just as slow to find the platform, suggesting that the untreated mutants indeed do have neurological deficits and that the BMT procedure is detrimental to neurological functions in general.

The latter hypothesis is supported by the results of the "transfer" tests on day 7 of the Morris water maze. Both treated groups (normal and mutant) spent less time in the quadrant where the platform was originally located than the untreated (normal and mutant) mice (Fig. 3). Thus the treated mice were unable to retain and transfer the memory from their previous learning experience during the acquisition phase. Hence, BMT was detrimental to memory retention regardless of the genotype of the treated animals.

The untreated mutants, and treated normals and mutants, were also unable to carry over their learned experience from one day to the next. This was particularly evident during the "relearning" phase, when the platform was replaced in the original quadrant (Fig. 4). In fact, the two treated groups showed no improvement in behavior over the 4 days of testing whereas the untreated mutants were able to learn at least on the

last day of testing (Fig. 4, days 8–11). When the platform was placed in a new position, only the untreated normal controls were able to adopt new strategies to locate the platform (Fig. 2). The results of the transfer, relearning, and reversal phases of the Morris water maze test not only confirm the memory deficits of the gus^{mps}/gus^{mps} mice (8), but also demonstrate that BMT was unable to restore these deficits. Even the treated normal mice were unable to perform spatial tasks requiring more complex cognitive functions as successfully as the normal controls.

Previous studies show that the brains of the transplanted mutant mice have focal areas of decreased lysosomal storage in the meninges and ependyma cells, but not in the neurons, perivascular cells, or glia (11). Consequently, the first assumption to interpret the lack of neurological functional improvement is that the level of β-glucuronidase did not reach either the critical level or the appropriate cell types in the CNS. However, this alone cannot account for the behavior of the treated mutants. Even the treated normal mice were observed to perform far below the level of the untreated normal controls and similarly to the mutants (treated or untreated), in most instances.

Hence, in general, the lack of improvement in the transplanted mutants and the regression in behavior in the transplanted normals (Figs. 1–4) can be largely attributed to the transplant procedure itself, and most likely to radiation-induced toxicity in the CNS. Mice exposed to 2 Gy showed focal thinning of the cerebellar granule cell layer of the brain (11). Furthermore, loss of Purkinje's cells, disorganization of the cortical layer, and a reduction in cerebellar mass were evident at higher radiation doses (11). In addition, the immature state of the neonate mice is particularly susceptible to radiation-induced damages (unpublished observation), even though the incomplete closure of the blood–brain barrier at this stage of development in the newborn mice (34) is a distinct advantage for the delivery of lysosomal enzymes into the brain.

In conclusion, it has been demonstrated that, even though biochemical improvement was obtained, BMT in the newborn period did not improve the neurological function of the mutant gus^{mps}/gus^{mps} mice. Furthermore, the therapy proved deleterious to the cognitive functions of even normal animals. Hence, an alternate approach to the prevention of disease progression may require temporary enzyme replacement with less invasive techniques during the newborn period (35). This can be followed by BMT after the animals have matured enough to withstand the transplant procedure (11). However, other approaches such as somatic gene therapy with autologous (14, 36) or non-autologous (37) cell implant are more benign and may well be the preferred treatment. This will certainly obviate the harmful effects of marrow ablation and still be capable of delivering β-glucuronidase immediately after birth. It is also clear from this study that the gus^{mps}/gus^{mps} mice not only provide a good model to study the restoration of biochemical and pathological deficits, but the restoration of neurological function as well. The latter, which can be determined by monitoring grooming activities and performance during the Morris water maze test, is an important adjunct when studying the efficacies of preexisting and new therapies on animal models of human neurodegenerative diseases.

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