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Prolonged Survival and Remyelination After Hematopoietic Cell Transplantation in the Twitcher Mouse

Abstract. *The twitcher mouse is an animal model of galactosylceramidase deficiency (Krabbe's disease), a human sphingolipidosis. The effects of hematopoietic cell transplantation as potential enzyme replacement therapy were examined in the twitcher mouse. Survival in twitcher mice with transplants was significantly prolonged and was associated with gradual repair of demyelination in peripheral nerves. In contrast, there was no improvement in the neurodegenerative process in the central nervous system after transplantation. These observations indicate that cellular transplantation may effectively provide in vivo enzyme replacement for the peripheral manifestations of genetic storage diseases. Strategies to perturb the blood-brain barrier may be necessary for enzyme replacement to be therapeutic in diseases with central nervous system manifestations.*

The treatment of lysosomal hydrolase deficiency states such as the mucopolysaccharidoses and sphingolipidoses is limited to symptomatic and supportive care, since no definitive therapy is available. Several in vitro studies have indicated that these heritable storage diseases might be treated by specific replacement of the deficient enzyme (*1, 2*). However, isolation and purification of adequate quantities of specific lysosomal hydrolases are impractical, and in vivo administration of limited amounts of enzyme has not been associated with consistent clinical improvement in human recipients (*3*).

Bone marrow transplantation repopulates the reticuloendothelial and lymphohematopoietic systems of the recipient with normal donor cells (*4–6*). Marrow transplants from normal mice into catalase-deficient (*7*) or glucuronidase-deficient (*8*) animals restore normal enzyme activity in the peripheral blood and other tissues of the recipients. Theoretically, bone marrow transplantation may be therapeutic in human storage diseases by providing a proliferating, self-renewing, enzymatically normal cell mass (*9*). The lack of suitable animal models of lysosomal hydrolase deficiency states has precluded the preclinical evaluation of cellular transplant strategies in these disorders.

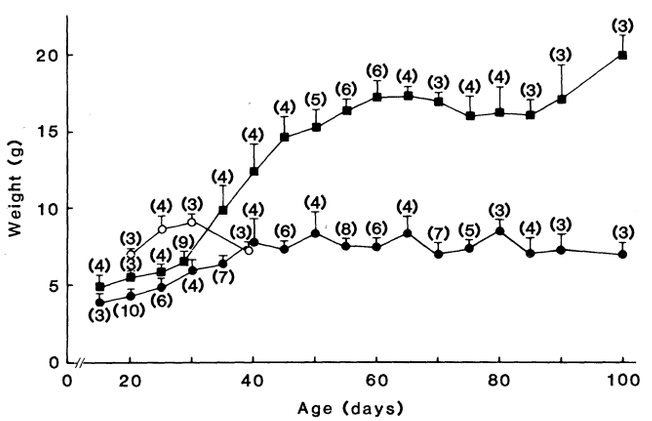

Fig. 1. Weight (mean ± standard error) of untreated twitcher mice, twitcher mice that received hematopoietic cells at 10 days of age, and enzymatically normal control littermates that also received transplants. Numbers in parentheses are the number of animals studied. After the immediate post-transplant period, control littermates gained weight normally. Over the two- to threefold prolongation of life-span the weight of treated twitcher mice did not differ significantly from that of untreated twitchers.

The twitcher mouse, a recently described neurological mutant, appears normal at birth, develops signs of central nervous system (CNS) dysfunction and peripheral demyelination by 3 to 4 weeks of age, and dies from progressive neurodegeneration by 5 to 7 weeks (*10*). Enzymatic studies have shown that affected twitcher mice lack galactosylceramidase, a lysosomal hydrolase that catalyzes the degradation of sphingolipid (*11*). Neuropathologic observations in twitcher mice have demonstrated characteristic periodic acid Schiff (PAS)–positive globoid cells in the central and peripheral nervous systems and extensive demyelination, with mononuclear cell infiltration, of peripheral nerves (*12*). From clinical, enzymologic, and neuropathologic standpoints, the twitcher mouse appears to be an authentic animal model of human galactosylceramidase deficiency (globoid cell leukodystrophy; Krabbe's disease), a progressive neurodegenerative sphingolipidosis (*13*).

We examined the effects of transplanting hematopoietic cells from normal congenic mice into presymptomatic galactosylceramidase-deficient homozygous twitcher mice. For these studies we used offspring of breeding pairs of C57BL/6J mice heterozygous for the twitcher mutation (+/*twi*). Since affected twitcher mice are asymptomatic for the first 3 to 4 weeks of life, we ascertained their genetic status by determining galactosylceramidase activity in aqueous homogenates of clipped tail tips from 7-day-old offspring (*14*). In our laboratory, galactosylceramidase activity in affected twitcher mice was ≤0.10 nmol/hour per milligram of protein, compared with >0.70 nmol/hour in unaffected control littermates. Twenty-four hours before hematopoietic cell transplantation, affected mice and enzymatically normal (+/+) control littermates received 900-rads of total body irradiation from a ^{137}Cs source at a dose rate of 120 rads per minute. At 10 days of age each mouse received an intraperitoneal injection of bone marrow cells (1.0×10^7 to 1.5×10^7) and spleen cells (3.5×10^7 to 5.0×10^7) freshly obtained from 6- to 8-week-old female congenic C57BL/6J mice that had normal galactosylceramidase activity. In addition, these donor mice were homozygous for the A isozyme of erythrocyte glucosephosphate isomerase 1 (GPI-1A). Since C57BL/6J mice, including those with the twitcher mutation, are homozygous for the B isozyme of GPI-1, we were able to assess hematopoietic engraftment after transplantation by electrophoretic analysis of GPI-1 isozyme patterns in 5-μl samples of blood from recipient mice

(*15*). All mice were housed in sterilized plastic cages with their respective dams until weaning. Both untreated twitcher mice and recipients with transplants received unlimited standard laboratory feed and acidified water (*p*H 2.0 to 2.5) containing neomycin (500 μg/ml), polymyxin B (13 μg/ml), and trimethoprim-sulfamethoxazole (100 μg of trimethoprim per milliliter).

At selected times after transplantation we killed recipient twitcher mice and control littermates. We removed their brains and sciatic nerves, which were then fixed in buffered Formalin, sectioned, and stained with PAS and Luxol fast blue (LFB) (*16*) to assess globoid cells and myelination, respectively.

Untreated twitcher mice appeared normal for the first 21 to 28 days of life, then developed truncal tremor, gait incoordination, hind limb weakness, and head drop. Progressive neurodegeneration and failure to thrive led to death. The mean life-span of 51 untreated twitcher mice was 40 days (range, 28 to 47 days). In contrast, mean survival in 12 twitcher mice that had received hematopoietic cells was 80 days (range, 56 to 108 days). This difference in survival is statistically significant ($P < 0.001$, Wilcoxon rank-sum test). Electrophoretic analysis of recipient blood samples showed that hematopoietic engraftment, as assessed by the presence of donor erythrocyte GPI-1A, had occurred by 2 to 3 weeks after cell transplantation. Complete conversion to donor hematopoiesis occurred 6 to 7 weeks after transplantation. The magnitude and progression of truncal tremor was similar in both untreated and treated twitcher mice. However, gait, foraging, and grooming behaviors were qualitatively less impaired in mice with transplants. Despite prolongation of survival, the weight of twitchers with transplants did not differ significantly from that of untreated mice (Fig. 1).

Sciatic nerves from untreated 40-day-old twitcher mice showed perineural edema, increased interstitial cellular infiltrate, and total loss of myelin sheaths (Fig. 2A). In treated twitcher mice there was histological evidence of progressive repair of demyelination in peripheral nerves. By 30 to 50 days after transplantation a few patchy areas of remyelination were apparent, although edema and interstitial infiltrate were still present. By 80 days there was extensive but incomplete regeneration of myelin sheaths and decreased perineural edema in sciatic nerves, although mononuclear cells were still abundant (Fig. 2B).

In contrast, there was no evidence of improvement in the CNS manifestations of murine galactosylceramidase deficiency after hematopoietic cell transplantation. Truncal tremor, an indicator of CNS dysfunction, was qualitatively similar in treated and untreated twitcher mice. The number of PAS-positive globoid cells in the brain increased progressively at 30, 50, and 80 days after transplantation. Despite prolongation of survival and repair of peripheral neuropathy in treated twitcher mice, there was no apparent amelioration of CNS degeneration, and death in these recipients was attributable to progressive CNS dysfunction.

To our knowledge, this is the first demonstration that cellular transplantation from congenic normal donors is associated with significant prolongation of survival in an enzyme-deficient animal. Although bone marrow transplantation successfully replaces the missing enzyme in acatalasemic (*7*) or glucuronidase-deficient (*8*) mice, untreated animals that lack these enzymes are asymptomatic and have normal life-spans. Mice with sphingomyelinase deficiency (Niemann-Pick disease) undergo neurological degeneration and death by 10 to 12 weeks of age (*17*). Bone marrow transplantation from congenic normal donors at 4 weeks apparently reduces accumulation of sphingomyelin in the liver, spleen, and bone marrow of Niemann-Pick mice, but the survival of affected recipients does not differ from that of untreated enzyme-deficient mice (*18*). The investigators did not perform marrow transplants in younger sphingomyelinase-deficient mice. We found no difference in survival between symptomatic twitcher mice given hematopoietic cell transplants at 21 to 28 days of age and untreated affected twitchers. These observations strongly suggest that hematopoietic cell transplants must be undertaken at relatively early ages to affect survival in murine sphingolipidoses.

It also appears that transplantation of hematopoietic cells is associated with subsequent remyelination in peripheral nerves. After orthotopic transplantation of demyelinated sciatic nerves from twitcher mice into enzymatically normal congenic recipients, reestablishment of myelin sheath formation does not occur until 4 to 9 months later (*19*). Galactosylceramidase activity remains low in twitcher nerves 1 to 2 months after they are grafted into normal mice, but is consistently normal by 4 to 9 months (*20*). After hematopoietic cell transplantation,

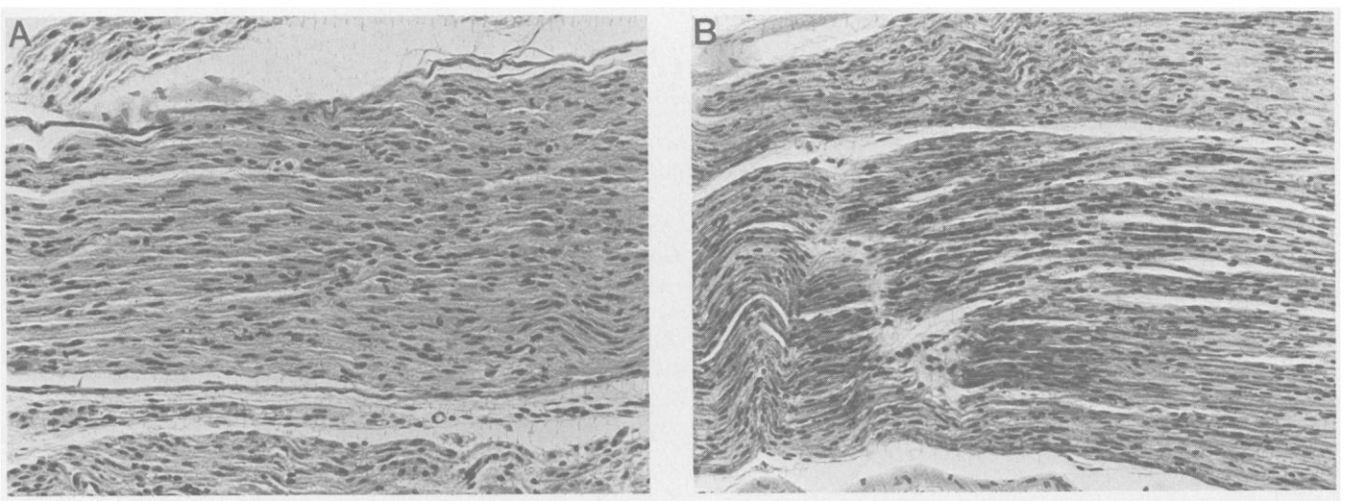

Fig. 2. (A) Sciatic nerve from a 40-day-old affected untreated twitcher mouse. The nerve sheaths are completely demyelinated, and perineural edema with mononuclear cell infiltrates is present. (B) Sciatic nerve from a 90-day-old twitcher mouse that received a hematopoietic cell transplant at 10 days of age. Substantial remyelination is indicated by dark, LFB-positive areas. Perineural edema is diminished, but mononuclear cells are abundant. (Stain, LFB/PAS; magnification, ×140)

the rate of remyelination is more rapid than that observed in orthotopically grafted sciatic nerves: repair is evident by 1 to 1½ months and is extensive by 2½ to 3 months. The delay in onset of remyelination in nerve graft experiments may be due to the surgical grafting procedure and tissue healing processes, variables absent from our studies.

Hematologic chimerism was complete in twitcher mice 6 to 7 weeks after transplantation. In contrast, remyelination was minimal after 6 to 7 weeks and was not extensive until 11 weeks, at which time remyelination was still incomplete. These findings may reflect the kinetics of reconstitution of the reticuloendothelial system after hematopoietic cell transplantation. The cell populations of the reticuloendothelial system, including hepatic Kupffer cells (*4*), pulmonary alveolar macrophages (*5*), and other tissue macrophages, become donor in origin after bone marrow or hematopoietic cell transplantation (*6*). However, attrition of recipient phagocytic cells and proliferation of donor macrophage populations occur over a long period (*6*) compared with the relatively rapid establishment of donor-derived lymphohematopoiesis.

The specific mechanisms responsible for the repair of peripheral nerves in galactosylceramidase-deficient mice are not well elucidated. In normal mice given orthotopic transplants of twitcher sciatic nerves, autoradiographic studies fail to demonstrate migration of host Schwann cells into the demyelinated twitcher graft (*19*). Furthermore, no demyelination occurs when normal sciatic nerves are transplanted into twitcher mice, and no macrophage infiltration or accumulation of characteristic twitcher Schwann cells is detected in these grafts (*19*). The observed rate of regeneration of myelin sheaths in affected twitcher mice after hematopoietic cell transplantation may indicate gradual replacement of autologous macrophages that have infiltrated the demyelinated peripheral nerves with enzyme-containing donor phagocytes. The exogenous galactosylceramidase elaborated by these donor cells might be taken up by twitcher Schwann cells and thus lead to normalization of myelin formation by these host cells.

The lack of objective improvement of neurodegeneration in the CNS of twitcher mice after hematopoietic cell transplantation contrasts sharply with the repair of peripheral demyelination in these recipients. That exogenous lysosomal hydrolase might repair biochemical and functional defects in cells of the CNS is supported by in vitro studies that demonstrate cellular uptake of purified enzyme and subsequent degradation of substrate in hexosaminidase-deficient human glial cells (*2*). However, the blood-brain barrier (*21*) is normally impermeable to the entry of systemically administered exogenous enzymes and other proteins (*22*). Our observations suggest that, in vivo, the blood-brain effectively prevents entry of galactosylceramidase, elaborated by normal donor cells, into the brains of twitcher mice.

Whether it involves purified exogenous enzyme, cellular transplantation, or specific gene replacement, any potential therapeutic strategy in lysosomal storage diseases with CNS manifestations must address the obstacle of the blood-brain barrier (*23*). Procedures that induce reversible permeability of the blood-brain barrier include hypercarbia and intracarotid injection of hyperosmolar agents (*25*). Cellular transplantation or specific gene replacement (to provide an ongoing source of lysosomal hydrolase) and concomitant or subsequent perturbation of the blood-brain barrier (to allow entry of enzyme into the brain) may provide optimal therapy in storage diseases with CNS manifestations. The twitcher mouse is an appropriate preclinical model with which to examine these combined therapeutic approaches.

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