Galactocerebrosidase-deficient oligodendrocytes maintain stable central myelin by exogenous replacement of the missing enzyme in mice

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Globoid cell leukodystrophy (GLD) is a lysosomal storage disease caused by genetic deficiency of galactocerebrosidase (GALC) activity. Failure in catalyzing the degradation of its major substrate, galactocerebroside, in oligodendrocytes (OLs) and Schwann cells leads to death of these myelinating cells, progressive demyelination, and early demise of GLD patients. Transplantation of bone marrow cells and umbilical cord blood have been attempted as a means of enzyme replacement and have shown limited success. It remains unknown whether or how these therapies support survival of GALC-deficient OLs and myelin maintenance. We report that, upon transplantation, GALC-deficient OLs from the twitcher mouse, a model of GLD, achieved widespread myelination in the brain and spinal cord of the myelin-deficient shiverer mouse, which was preserved for the life of the host. GALC immunohistochemistry showed direct evidence for GALC transfer from the shiverer environment to the engrafted mutant OLs *in vivo*. These findings suggest that the mutant OLs can internalize exogenous GALC and maintain stable myelin, demonstrating that exogenous enzyme replacement will be a key strategy in the therapy of GLD.

enzyme replacement | globoid cell leukodystrophy | myelination | transplantation | lysosomal storage disease

Globoid cell leukodystrophy (GLD, also called Krabbe's disease) (1) is a lysosomal storage disease caused by genetic deficiency of activity of a lysosomal enzyme galactocerebrosidase (GALC) that catalyzes the digestion of its major substrate galactocerebroside in myelinating oligodendrocytes (OLs) and Schwann cells (2). GLD is characterized pathologically by rapidly progressive demyelination of the CNS and peripheral nervous system (PNS) and accumulation of macrophages in the demyelinating lesions. The majority of GLD patients (infantile form) succumb to severe neurological symptoms in the first two years of life.

Homozygous twitcher ($GALC^{twi/twi}$; *twi*) mice (3) have a genetic defect in GALC activity (4, 5), show progressive demyelination and accumulation of macrophages in the CNS and PNS, and rarely survive >45 days. These similarities to GLD have enabled the mouse to serve as a bona fide model to explore the pathophysiology and therapy of GLD (6). It has recently been shown that *twi* OLs can internalize exogenous GALC *in vitro*, resulting in their phenotypic correction (7), raising the possibility that *in vivo* enzyme replacement could be used to treat GLD. However, it is essential to evaluate the effect of enzyme replacement on OL function *in vivo*, because *in vitro* correction cannot unequivocally determine the myelinating capacity of the cell or the longevity. Therefore, the aim of this study was to determine whether *twi* OLs form and maintain normal myelin when GALC is provided exogenously in the CNS. In search of a method of enzyme replacement *in vivo*, we transplanted *twi* mutant oligodendrocyte progenitor cells (OPCs) into the brain and spinal cord of homozygous shiverer (*shi*) mice in which OLs do not form compact myelin sheaths because of a deletion in the myelin basic protein (*MBP*) gene (8). The *shi* mouse has previously been used as a host to study cell-transplantation-derived myelination by detecting MBP of donor origin (9, 10).

Here, we report that *twi* OLs can survive and maintain stable myelin in the *shi* environment, which provides the transplanted *twi* cells with the missing enzyme.

Materials and Methods

Mice. Colonies of shiverer (C3Fe.SWV-MBP^{shi}/J) and twitcher (B6.CE-$GALC^{twi}$/J) mice (The Jackson Laboratory) and transgenic mice that express EGFP driven by the 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) promoter (CNP-EGFP) (11) were maintained at the University of Wisconsin. Methods for animal husbandry, surgery, and killing in this study were approved by the University's Animal Care and Use Committee.

Cell Culture. See *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

Transplantation. Oligospheres were dissociated into single OPCs by incubating them in 0.025% trypsin/1 mM EDTA followed by trituration using a fire-polished Pasteur pipette. Neonatal *shi* mice (postnatal day 0–1) of both sexes born to *shi* homozygous pairs were cryoanesthetized and received bilateral intracallosal injection (100,000 cells per 2 μl at each site) through a pulled-glass needle. At 3 weeks, the same animals received laminectomy under isoflurane anesthesia and were injected with 50,000 cells per 1 μl in the dorsal column of the thoracolumbar spinal cord. Cells derived from oligospheres were used for transplantation between 1 and 2 months of culture, and eight different batches of culture used in this study produced the same results.

Histology and Morphometric Analyses. See *Supporting Materials and Methods*.

GALC Activities. See *Supporting Materials and Methods*.

Results

Mutant OPCs. OPCs were derived from primary oligosphere cultures of neonatal *twi* mice carrying the CNP-EGFP transgene as an oligodendroglial marker (see Fig. 6, which is published as supporting information on the PNAS web site). The transplanted cells consisted of 95.1 ± 0.4% $NG2^+$, 4.4 ± 1.3% $GFAP^+$, and 26.4 ± 4.4% $O4^+$ (mean ± SD of three separate experiments).

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Abbreviations: CNP, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; GALC, galactocerebrosidase; GLD, globoid cell leukodystrophy; MBP, myelin basic protein; OL, oligodendrocyte; OPC, oligodendrocyte progenitor cell; PNS, peripheral nervous system; *shi*, homozygous shiverer; *twi*, homozygous twitcher.

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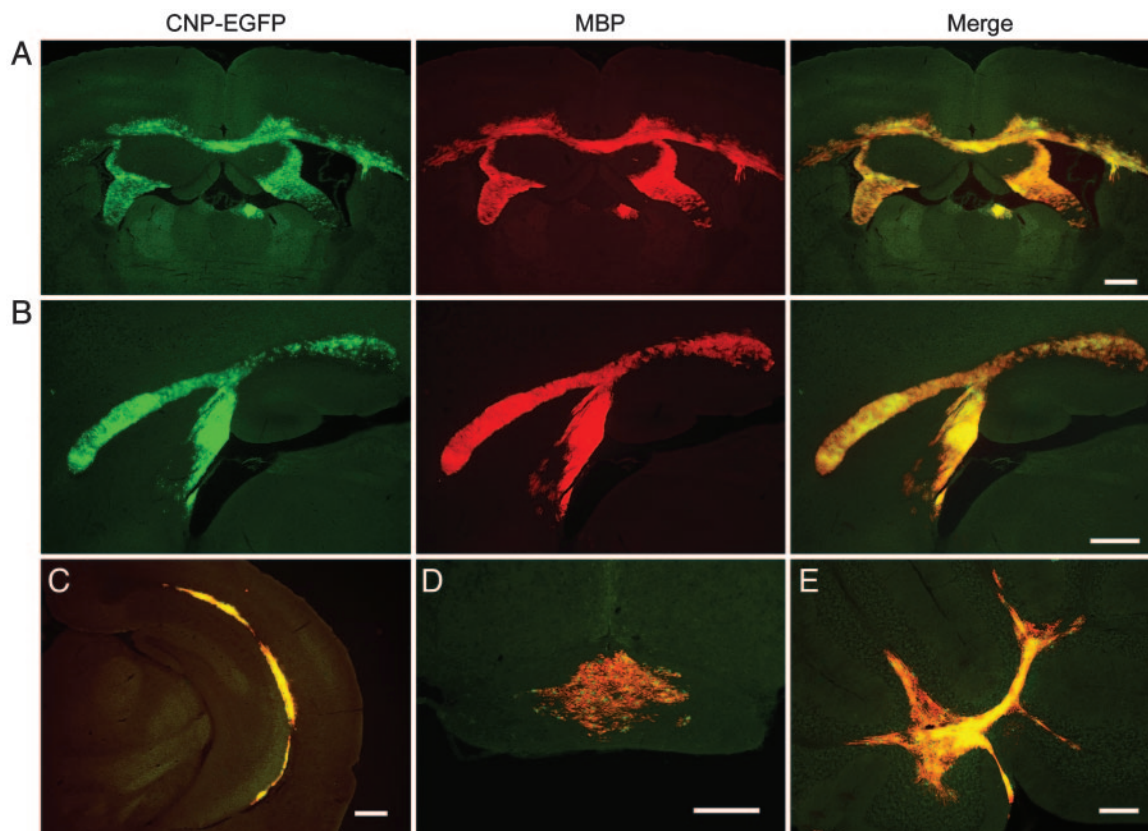

Fig. 1. Widespread *twi*/CNP-EGFP grafts in the *shi* mouse brain 4 months after transplantation. Extensive distribution of *twi* mutant OLs/myelin in coronal (*A*) and sagittal (*B*) sections of *shi* brains revealed by EGFP signal (green), MBP immunolabeling (red), and their merged images. (*C–E*) Merged images of CNP-EGFP and MBP immunolabeling. (*C*) The *twi*/CNP-EGFP OL-derived myelination extending toward the ventral part of the external capsule at the midbrain level, showing long-distance migration of donor cells. The grafts were also found in areas distant from the injection sites, such as optic chiasm (*D*) and cerebellar white matter (*E*). *C* and *D* are coronal sections, and *E* is a sagittal section. [Scale bars: 500 μm (*A–C*) and 200 μm (*D* and *E*).]

Transplantation into the Brain and Spinal Cord. At 4 months of age, 8 of 12 transplanted *shi* brains contained CNP-EGFP$^+$ grafts extending at least 2.5 mm in the corpus callosum rostrocaudally. These mice also showed CNP-EGFP$^+$ grafts in the spinal cord extending at least 2.5 mm along the dorsal column rostrocaudally. The remainder ($n = 4$) were not used for further analyses, because only sporadic and scattered CNP-EGFP$^+$ cells were found in cryostat sections, and fewer CNP-EGFP$^+$ cells were found near the injection site in the spinal cord.

Four months after intracallosal transplantation of *twi*/CNP-EGFP OPCs, the donor-derived OLs and their resulting myelin were found throughout the corpus callosum of *shi* mice and also in other white-matter tracts of the brain (Fig. 1). The hippocampal fimbria and the corpus callosum were predilection sites of migration and engraftment (Fig. 1 *A–C*). The grafts found in areas distant from the injection sites (Fig. 1 *G* and *H*) suggest that the transplanted *twi*/CNP-EGFP OPCs are capable of migration throughout the brain.

In the spinal cord, *twi* donor cells migrated widely in the white matter around the injection site (Fig. 2*A*). In four engrafted mice, *twi* OLs myelinated the white matter almost completely, as viewed in cross sections (Fig. 2*B*). Scattered donor OLs were also found in the gray matter, providing myelin sheaths around axonal projections. Compared with the extensive patchy demyelination in *twi* mice, *twi*/CNP-EGFP myelin was more evenly and densely distributed in the white matter, although it was less dense than the wild-type myelin (Fig. 2 *B* and *C*). There was no MBP immunoreactivity in the untransplanted *shi* spinal cord (Fig. 2*C*). Toluidine blue staining showed extensive myelination in all columns of the spinal cord (Fig. 2 *D–G*). Electron microscopy showed multiple myelin wrapping by *twi* OLs (Fig. 2*H*). *twi*/CNP-EGFP OLs myelinated 95.5% of *shi* axons (15.1 $\pm$ 1.9 myelinated axons and 0.7 $\pm$ 0.1 nonmyelinated axons per 100 μm^2 in the ventral column at 4 months of age, mean $\pm$ SD). The donor myelin was significantly thinner than wild-type myelin (G ratios of 0.66 $\pm$ 0.10, $n = 170$ for the donor myelin versus 0.56 $\pm$ 0.07, $n = 171$ for wild-type myelin, mean $\pm$ SD; n denotes the number of axons scored from three animals; $P < 0.0001$, Mann–Whitney U test). However, this result does not appear to be due to *twi* mutation, because transplanted wild-type CNP-EGFP OLs myelinated *shi* axons in the same manner (see Fig. 7, which is published as supporting information on the PNAS web site).

Stability of the Graft. The persistence of scattered nonmyelinated axons in the transplanted *shi* spinal cord raised the possibility that *twi* OL death/demyelination could have taken place in parallel with myelination. Thus, we determined the frequency of donor-cell death by using TUNEL assay, because OLs die by apoptosis in both GLD patients (12) and *twi* mice (13). As expected, TUNEL$^+$ cells were scattered throughout the spinal white matter of moribund *twi* mice at 45 days of age (Fig. 3 *A* and *B*). There were sporadic TUNEL$^+$ apoptotic cells in the engrafted *shi* mice at 4 months of age (Fig. 3 *C* and *D*). However, the numbers of TUNEL$^+$ cells in the graft and in the wild-type and untransplanted *shi* mice were negligible compared with the *twi* spinal cord (Fig. 3*A*). Unfortunately, natural death of *shi* mice at ≈4 months of age did not allow us to observe extended life span of the engrafted *twi* OLs beyond

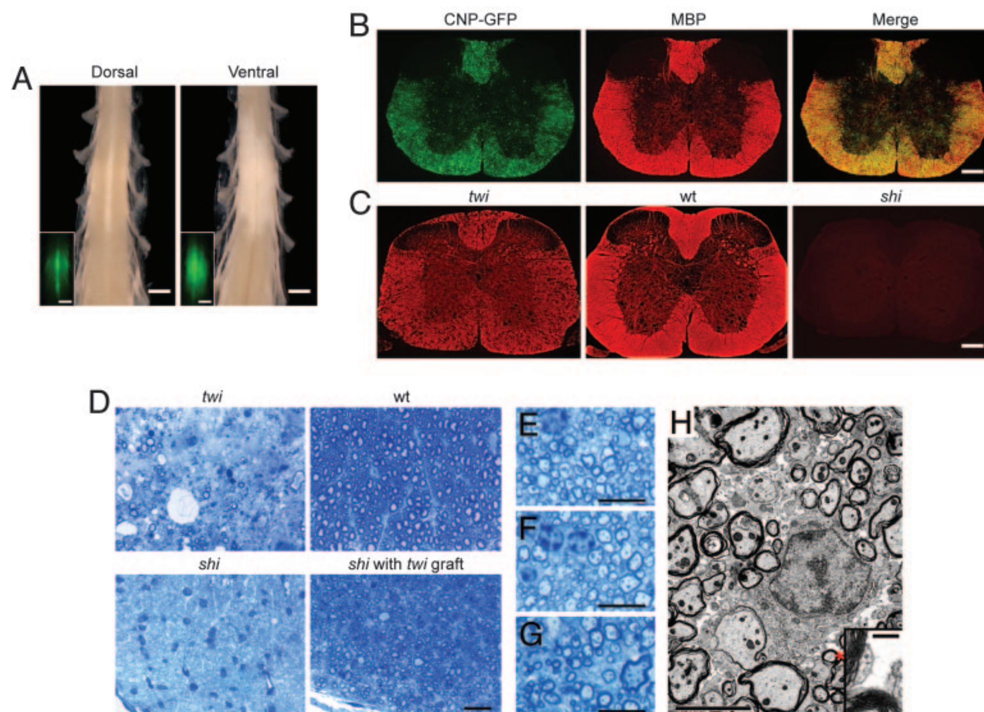

Fig. 2. Widespread *twi*/CNP-EGFP grafts in the *shi* spinal cord at 4 months of age. (*A*) Distribution of *twi*/CNP-EGFP OL/myelin was visible to the naked eye as white patches in the spinal cord of *shi* mice. Epifluorescence at 488 nm excitation (*Insets*) corresponds to the white patches, confirming that the myelinating cells were of donor origin. (*B*) In the cross section of *A*, the donor OLs/myelin spread over almost the whole white matter, shown by EGFP fluorescence (green), MBP immunolabeling (red), and their merged image. (*C*) MBP immunolabeling of the thoracolumbar spinal cord of 45-day-old moribund *twi*, 4-month-old wild-type, and 4-month-old untransplanted *shi* mice. (*D*) Toluidine blue myelin staining of the ventral column of thoracolumbar spinal cord. Despite severe demyelination in the 42-day-old moribund *twi* mouse, the transplanted *twi*/CNP-EGFP OLs myelinated numerous axons in the *shi* host. Myelin sheaths were absent in the untransplanted *shi* mouse. *twi*/CNP-EGFP OLs myelinated *shi* axons extensively in the dorsal (*E*), lateral (*F*), and ventral (*G*) columns. (*H*) Representative electron micrographs from the engrafted ventral column of *shi* spinal cord. (*Inset*) A higher magnification of the area indicated with *, showing well compacted myelin. [Scale bars: 1 mm (*A*), 200 μm (*B* and *C*), 20 μm (*D*), 10 μm (*E–G*), 5 μm (*H*), and 200 nm (*H Inset*).]

this point. However, the fact that few TUNEL$^+$ *twi* OLs were present in the graft of 4-month-old *shi* mice (Fig. 3 *C* and *D*) suggests that the transplanted *twi* OLs could have lived and maintained myelin for a longer period. That these cells lived approximately three times longer than the life span of *twi* mice (40–45 days) suggests that the *shi* CNS environment corrected the phenotype of the mutant OLs.

Evidence for GALC Enzyme Transfer from the *shi* Environment to *twi* OLs. Our next objective was to determine whether the activity and protein expression of GALC were altered in the engrafted *shi* white matter and, more importantly, whether there was evidence for GALC transferred into the mutant OLs. Interestingly, the level of GALC activity in *shi* mice was significantly higher than normal ($P < 0.0001$, Fig. 4*A*). Bu *et al.* (14) have recently reported that the number of OLs in *shi* mice continuously increased up to 60 days of age as a result of NG2$^+$ cell proliferation. We confirmed an increased number of *shi* OLs, even at 4 months of age (see Fig. 8, which is published as supporting information on the PNAS web site). Hence, the elevation of GALC activity level in *shi* mice is, likely, a reflection of the proliferation of OLs. In *shi* mice transplanted with *twi*/CNP-EGFP OPCs, the level of enzyme activity in the transplant was less than in the *shi* host ($P < 0.0001$) but was slightly higher than normal (Fig. 4*A*). It appears that the GALC-deficient, myelination-competent *twi* OLs and the GALC-producing, myelin-deficient *shi* OLs balanced the levels of GALC activity to support enzyme correction in *twi* OLs.

In addition to the positive effect of *twi*/CNP-EGFP graft (i.e., building myelin in the myelin-deficient host), the transplantation surgery itself might have contributed to the reduction in the abnormally elevated GALC activity of *shi* mice, because the sham-operated group showed a moderate decrease (Fig. 4*A*, $P < 0.0001$, compared with *shi* mice). As the control experiment, we transplanted dead OPCs. Injecting vehicle without cells may not be sufficient as a control, because a considerable number of donor OPCs are expected to die immediately after transplantation.[¶] A possible explanation for this sham-surgery-induced reduction in GALC activity is that the dead cell transplantation could cause tissue damage in the host through inflammation (15). Nevertheless, the *twi*/CNP-EGFP transplants normalized the GALC activity in *shi* mice, and the extent of this activity was significantly larger than the possibly deleterious reduction by the sham transplantation ($P = 0.0012$ between *shi* mice with *twi* OPC graft and *shi* mice with sham surgery).

To demonstrate localization of GALC in *twi* cells, we used an immunofluorescent, confocal-microscopy approach. GALC immunolabeling in the spinal white matter (Fig. 4*B*) paralleled the results of GALC activity (compare Fig. 4 *A* and *C*). In confocal microscopy, GALC immunolabeling in the graft was found mainly in the *shi* parenchyma, presumably in OLs; granular GALC immunoreactivity was localized in all *twi*/CNP-EGFP OLs examined ($n = 40$), indicating that the donor cells internalized the enzyme (Fig. 4*D*). In previous studies, *twi* sciatic nerve grafted into the sciatic nerve of normal or trembler mutant mice showed improved myelination in the graft (16, 17). The

¶Zhang, S. C., Wagner, D. & Duncan, I. D., 29th Annual Meeting of the Society for Neuroscience, Oct. 23–28, 1999, Miami Beach, FL (abstr.).

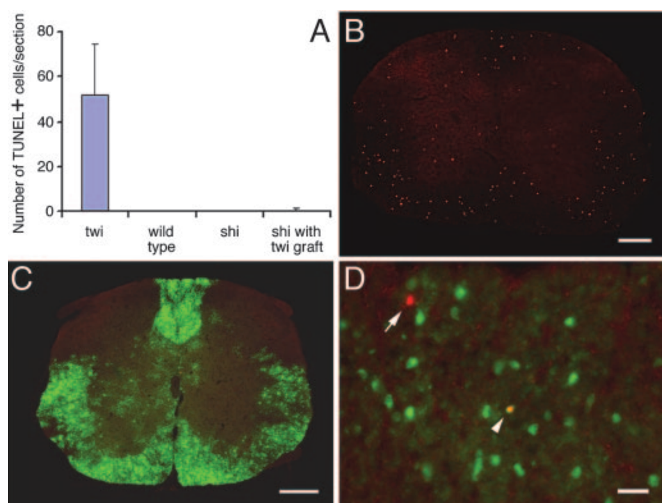

Fig. 3. Stable integration of *twi*/CNP-EGFP OLs in the *shi* spinal cord. (*A*) The number of TUNEL$^+$ cells was frequent in *twi* and rare in wild-type, *shi*, and the transplanted *shi* mice. (*B–D*) Data are the mean + SD number of TUNEL$^+$ cells per 20-μm section. TUNEL$^+$ cells (red) were found mostly in the white matter of *twi* mice (*B*) and were negligible in the *shi* mice with *twi*/CNP-EGFP graft (*C*) (TUNEL in red and CNP-EGFP in green) where, if found, 40% of TUNEL$^+$ cells were the *twi* OLs (arrowhead in *D*), and the rest were not (arrow). [Scale bars: 200 μm (*B* and *C*) and 20 μm (*D*).]

finding was indicative of GALC enzyme replacement in *twi* Schwann cells. However, no evidence was provided for localization of GALC in the mutant cells. Moreover, it is essential to know whether phenotypic correction by GALC replacement occurs in OLs that myelinate multiple CNS axons in a more complex manner with elaborate processes.

Cell fusion of the donor OPCs to *shi* host cells could account for the GALC localization in *twi*/CNP-EGFP OLs; such a phenomenon has been reported in more immature donor cells, such as hematopoietic stem cells (18), but has not been recognized in OPCs. It appears unlikely that this phenomenon has occurred in our study, because no cells with double nuclei were observed in DAPI-counterstained EGFP$^+$ OLs in the graft (confirmed with 2,000 EGFP$^+$ cells from the brain and spinal cord of three transplanted mice).

Activation of Microglia/Macrophages. Infiltration of numerous macrophages, often multinucleated and "globoid," is a unique pathology in the demyelinating lesion of GLD and its animal models including *twi* mice. It has been postulated that undegraded galactocerebroside induces globoid-cell formation (19). Thus, it was important to determine whether macrophages accumulate in the *twi*/CNP-EGFP graft. Moribund *twi* mice (postnatal day 45) showed highly activated microglia and macrophages in the spinal white matter (Fig. 5*A*). Compared with resting microglia in wild type mice (Fig. 5*B*), microglia in *shi* mice were partially activated, with increased number and CD45 immunoreactivity (Fig. 5*C*). In the *twi*/CNP-EGFP graft of *shi* spinal cord 14 weeks after transplantation, microglia were sporadically further activated (Fig. 5*D*). However, no macrophage accumulation was observed. When wild-type CNP-EGFP OPCs were transplanted, the extent of microglial activation was similar to that of *twi*/CNP-EGFP OPCs, showing sporadic microglia with thick processes, increased cell volume, and CD45 immunoreactivity (see Fig. 9, which is published as supporting information on the PNAS web site). Thus, this slight activation of microglia may relate to factors in transplantation instead of *twi* mutation *per se* (e.g., a mismatch of the major histocompatibility complex haplotype, EGFP antigenicity, and toxicity of EGFP).

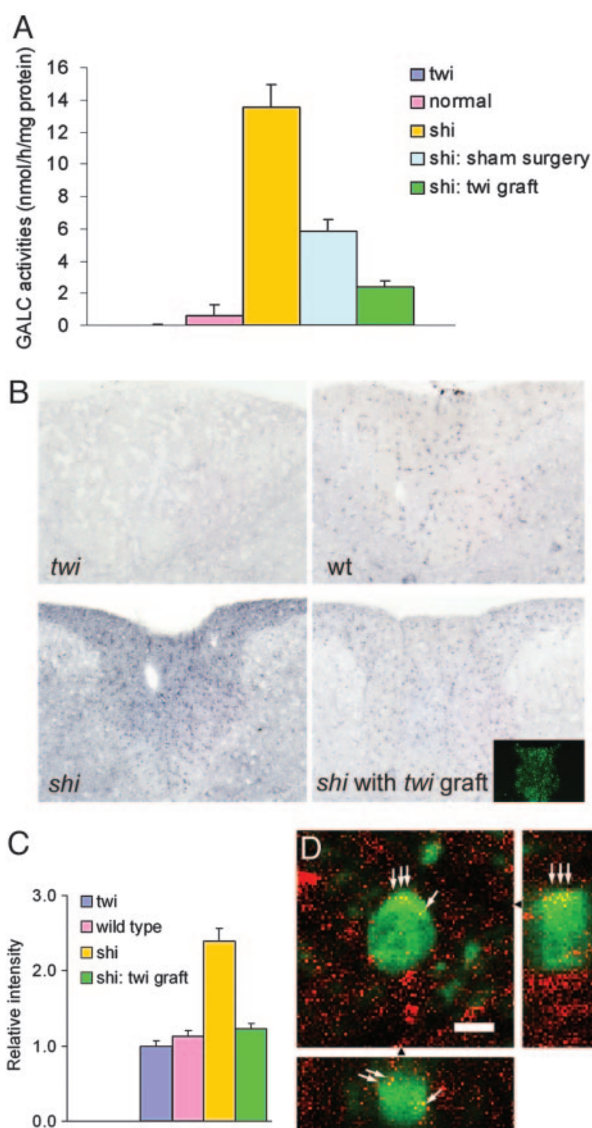

Fig. 4. Increased activity and expression of GALC in the *shi* spinal cord and their reduction after *twi*/CNP-EGFP OPC transplantation. (*A*) GALC activities of the excised dorsal column of the thoracolumbar spinal cord from *twi*, normal (*MBP*$^{shi/+}$), *shi*, *shi* with dead *twi*/CNP-EGFP OPC transplantation (sham surgery), and *shi* with *twi*/CNP-EGFP OPC transplantation. Data are mean + SD (n = 3–6 in each group). (*B*) GALC immunolabeling of the dorsal spinal cord of *twi*, wild-type, *shi*, and the transplanted *shi* mice. (*C*) Optical density of GALC immunoreactivity in the dorsal column was expressed as relative intensity to the optical density for *twi* mice. Data are the mean + SD. (n = 3 in each group). (*D*) Confocal images of GALC immunolabeling (red) showed the localization of GALC in a *twi*/CNP-EGFP OL (arrows) in the graft. Reconstructed orthogonal images are presented as viewed in the *x–z* (*Lower*) and *y–z* (*Right*) planes. [Scale bars: 100 μm (*B*) and 10 μm (*D*).]

Although the pathogenesis of GLD primarily lies in the lack of GALC activity, it is not known whether the macrophage accumulation plays an important role in OL death and progressive demyelination. In this study, the survival of *twi* OLs in the *shi* host could be due to the absence of macrophage accumulation and not to the enzyme replacement. Scaravilli and Suzuki (16) noted the absence of macrophage accumulation in the *twi* sciatic nerve grafted in normal mice but did not exclude the possibility that *twi* Schwann cells survived in the host because of the lack of macrophage accumulation. Recently we have created macrophage-deficient *twi* mice by crossing the *twi* mouse with a mutant mouse deficient in

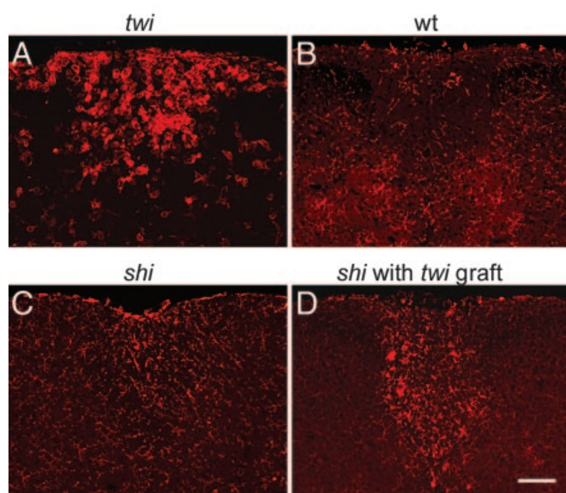

Fig. 5. Absence of macrophage accumulation in the *twi*/CNP-EGFP graft. CD45 immunolabeling (red) of the dorsal column of thoracolumbar spinal cord in 45-day-old moribund *twi* mice (*A*), 60-day-old wild-type mice (*B*), and 4-month-old untransplanted *shi* mice (*C*). Microglia in the engrafted dorsal column of *shi* mice were sporadically further activated; however, there was no macrophage accumulation (*D*). (Scale bar: 100 μm.)

macrophage-colony-stimulating factor (20). We confirmed that this double mutant had no macrophage accumulation in the CNS white matter yet showed demyelination comparable with or even more severe than that in control *twi* mice.‖ Thus, the absence of macrophage accumulation (Fig. 5*D*) is not a likely explanation for why engrafted *twi* OLs maintained stable myelin in *shi* mice.

Discussion

In both GLD and its animal counterpart, the *twi* mouse, myelination of both the CNS and PNS begins normally, yet, shortly after its formation, myelin is lost through progressive demyelination. Thus, both OLs and Schwann cells have the initial capacity to synthesize normal myelin in these disorders, albeit in reduced amounts (21, 22), raising the question whether they could be protected from ensuing death and myelin loss. Here, we show that replacement of GALC enabled *twi* OLs to maintain stable and widespread myelin throughout the CNS, when these cells were transplanted into another mutant background. The *shi* mouse serendipitously provides advantages to *twi* cells as the increase in OLs and their progenitors in *shi* mice (14), even in the face of their persistent failure to myelinate axons, provides an extraneous source of GALC that the transplanted *twi* cells can use.

The mechanism of GALC transfer into the mutant OLs is yet to be determined. The mannose-6-phosphate receptors (MPRs) cycle among the Golgi, endosomes, and the plasma membrane, internalizing exogenous lysosomal enzymes and mediating transport of acid hydrolases to lysosome (reviewed in ref. 23). In the CNS, immunohistochemical localization of MPRs is found mostly in neurons (24, 25). Because OLs are also reported to express MPRs, perhaps to a lesser extent (26), the cells are likely to internalize GALC through MPRs. However, MPR-mediated internalization of GALC into cultured *twi* OLs is only partially blocked by the addition of mannose-6-phosphate (7), suggesting other mechanisms of GALC internalization, such as direct pinocytosis. As to the source of exogenous GALC in this study, *shi* OLs may secrete the enzyme by certain means. The diffuse GALC immunoreactivity, in addition to the strong intracellular labeling in the white matter of *shi* spinal cord (Fig. 4*B*), may indicate the presence of secreted GALC in the neuropil. In addition, direct cell-to-cell transfer of lysosomal enzyme independent of MPRs could be possible, because it occurs between lymphocytes and fibroblasts (27), although the phenomenon is enzyme-specific (28) and has not been tested for GALC or for OL-to-OL.

twi OLs survived for up to 4 months after transplantation in the *shi* recipient, suggesting that longevity of these cells is achievable. In this study, the transplanted mutant OPCs were capable of developing into mature OLs in the presence of GALC in the environment, and they maintained stable myelin for the long term. It has been reported that a substantial number of OLs appear to be newly formed in parallel with their death in the spinal white matter of *twi* mice (29). Thus, the mechanism of GALC-replacement therapy in GLD would be to cease this turnover of OLs and enable myelin maintenance and repair by supporting the survival of OLs derived from endogenous OPCs.

GALC replacement in GLD might seem an obvious therapy, yet it faces at least two important challenges. First, enzyme replacement to protect the myelinating cells must take place very early in life, because children with the classical infantile form of the disease develop disability within the first few months of life and rarely survive beyond two years. To date, only early bone marrow (30) and umbilical cord blood transplantation (31) have been shown to have any beneficial effect in GLD. The mechanism by which these therapies improve function is not clear, although it has been postulated that GALC is supplied to mutant OLs and Schwann cells by donor-derived microglia/macrophages (32). Direct evidence for this mechanism, however, is lacking. Regrettably, these therapies have minimal effects on the symptomatic patients with infantile GLD, who represent the majority of the cases, probably because the levels of GALC activity in the CNS provided by the donor cells are not sufficient to reverse rapid progression of the disease. The second major challenge to enzyme replacement in GLD is that GALC must be supplied to the entire nervous system as both the CNS and PNS are severely affected. Preservation or remyelination of the CNS might prolong survival and quality of life, but demyelination of the PNS is responsible for severe disability. Recently, intracerebroventricular or intracranial injections of an adenoassociated virus encoding the *GALC* gene in neonatal *twi* mice resulted in high levels of GALC activity in the brain, leading to moderate improvement in neurological symptoms and life span (33). Interestingly, neuronal cells were most efficiently transduced with the *GALC* gene. It is not clear whether the virus also infected OLs to a lesser extent or the transduced neurons secondarily delivered GALC to OLs. Our study supports the idea that the mutant OLs can survive and maintain myelin solely by exogenous GALC *in vivo*. Eventual death of the animals, however, emphasizes that therapies targeting focal areas of the brain, spinal cord, or peripheral nerve through gene therapy or cell transplantation have critical limitations for complete cure of the disease.

To overcome the difficulties described above, rapid and systemic delivery of GALC is crucial. Our results of enzyme transfer *in vivo* strongly support efforts to deliver the exogenous enzyme by the systemic administration of recombinant GALC (34); i.p. GALC injection appeared to improve the demyelinating pathology of PNS in *twi* mice but had little effect on the CNS. Perhaps a sufficient level of GALC activity was not achieved in the CNS, leaving a question whether the strategy of exogenous enzyme transfer is effective in OLs in the CNS. Our present study clearly shows that the mutant OLs can survive and maintain central myelin for the long term when a sufficient amount of GALC is provided. Thus, whether the enzyme can cross the blood–brain barrier (BBB) is a key factor for success. In fact, enzyme-replacement therapy has been performed successfully in

‖Kondo, Y. & Duncan, I. D., 34th Annual Meeting of the Society for Neuroscience, Oct. 23–27, 2004, San Diego (abstr.).

some lysosomal storage diseases, such as type I Gaucher disease, Fabry disease, and mucopolysaccharidosis I, in which somatic organs are mainly affected (35). However, poor transfer of enzymes to CNS target cells across the BBB has made it difficult to apply this approach to neurodegenerative lysosomal storage diseases (36). Indeed, Urayama *et al.* (37) have recently demonstrated that MPR-mediated transport of β-glucuronidase from blood to brain parenchyma occurs only in early postnatal life and is down-regulated by 7 weeks of age in mice. This limitation, however, could be overcome by high doses of enzyme and a long-term treatment (38). Another approach to deliver GALC would be transplantation of GALC-producing (ideally -secreting) cells that have been transduced with the GALC gene. Blood-borne macrophages and microglia may be among the best candidates, because they have the potential to cross the BBB and populate widely in the CNS and PNS (39). Development of such methods for enzyme delivery will benefit GLD and, potentially, other lysosomal storage diseases with severe CNS pathologies, such as Tay–Sachs and Sandhoff disease.

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