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REVIEW

The Myelin Mutants as Models to Study Myelin Repair in the Leukodystrophies

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Abstract The leukodystrophies are rare and serious genetic disorders of the central nervous system that primarily affect children who frequently die early in life or have significantly delayed motor and mental milestones that result in long-term disability. Although with some of these disorders, early intervention with bone marrow or cord blood transplantation has been proven useful, it has not yet been determined that such therapies promote myelin repair of the central nervous system. Research on experimental therapies aimed at myelin repair is aided by the ability to test cell replacement strategies in genetic models in which the mutations and neuropathology match the human disorder. Thus, models exist of Pelizaeus-Merzbacher disease and the lysosomal storage disorder, Krabbe disease, which reflect the clinical and pathological course of the human disorders. Collectively, animals with mutations in myelin genes are called the myelin mutants, and they include rodent models such as the *shiverer* mouse that have been extensively used to study myelination by exogenous cell transplantation. These studies have encompassed many permutations of the age of the recipient, type of transplanted cell, site of engraftment, and so forth, and they offer hope that the scaling up of myelin produced by transplanted cells will have clinical significance in treating patients. Here we review these models and discuss their relative importance and use in such translational approaches. We discuss how grafts are identified and functional outcomes are measured. Finally, we briefly discuss the cells that have been successfully transplanted, which may be used in future clinical trials.

Keywords Myelin mutants · Leukodystrophies · Cell transplantation · Myelination · Oligodendrocyte progenitors

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Introduction

The leukodystrophies are a heterogeneous group of inherited disorders of white matter of the nervous system that affect primarily the central nervous system (CNS), but in some cases they also affect the peripheral nervous system (PNS) [1]. They are serious, progressive disorders of myelin that usually present in infancy or childhood, and more rarely in adulthood, and frequently lead to early death. By definition, there is a failure of development of the myelin or its maintenance. This is reflected in disorders in which there is a lack of myelin in the CNS, as seen in Pelizaeus Merzbacher disease (PMD) or where there is myelin breakdown and axons are demyelinated, such as in Krabbe disease. Given the genetic, clinical, and pathological heterogeneity of the leukodystrophies, the approaches to their treatment will vary. The purpose of this review is to present and discuss potential strategies of myelin repair. In the case of the developmental disorders, notably PMD, the goal is to myelinate axons that had never been ensheathed, while in the demyelinating disorders, the goal is to remyelinate axons. It is not known conclusively whether

the cellular interactions, molecular cues, and so forth involved in promoting myelination or remyelination will be different in these two situations [2].

Myelin repair will have 2 important functions: 1) it will restore saltatory conduction [3], and 2) it will act as a form of neuroprotection [3, 4]. Promotion of myelin repair may use 1 of 2 approaches. In the first approach, endogenous neural stem cells or oligodendrocyte progenitor cells (OPCs) are recruited to divide and differentiate into myelinating oligodendrocytes (OLs) [3]. However, in the leukodystrophies, this approach would be unsuccessful unless it was combined with gene therapy to repair the defect in the OL or target cell. Also, this approach remains theoretical at present, although there is promising data from other experimental myelin disorders that pharmacologic targets exist that can influence OPC differentiation [5]. The alternative is to supply an exogenous source of OPCs or immature OLs to initiate repair. Cell transplantation and endogenous cell recruitment may not be mutually exclusive, however, as the cells are transplanted, they may supply the missing or defective enzyme or protein to endogenous OPCs or OLs [6].

The promotion of remyelination or myelination by an exogenous cell source in disorders in which it is delayed or has failed has been extensively tested in a wide variety of animal models. Two approaches have dominated. In the first, focal areas of demyelination are created by the injection of a myelinotoxic compound, usually lysolecithin or ethidium bromide [7, 8]. As endogenous remyelination usually occurs following such injections, prior focal X-irradiation of the spinal cord kills endogenous OPCs and results in inhibition of endogenous repair and the presence of focal areas of persistently demyelinated axons [9]. Thus, these "plaques" of demyelination are targets for implantation of putative myelin-producing cells. More recently, demyelination created by the ingestion of cuprizone, another myelinotoxic chemical that results in myelin loss in restricted areas of the CNS, which has been used as a model to target exogenous cellular repair [10].

The second or alternative approach to exploring myelin repair is to use animals in which the mutations result in a global failure of myelin development or loss as a result of demyelination. Collectively, these animals are known as the myelin mutants [11, 12]. Their greatest usefulness may be where they are used as true models of human neurologic disease. The 2 examples of this are 1) the proteolipid protein (PLP) mutants, which are models of PMD, and 2) the mutants of the galactocerebrosidase (*GALC*) gene, models of Krabbe disease. Although other myelin mutants exist that are used to test the myelinating capacity of exogenous cells, most notably the shiverer (*shi*) mouse, the models of PMD and Krabbe disease provide great opportunities to study repair in a *milieu* that faithfully mimics the human condition, and allows the scaling up required for translational application. This review will discuss the use of the myelin mutants in studying myelin repair and how this has led us to the initiation of current and future clinical trials in patients with inherited myelin disorders.

Diseases to be Targeted by Exogenous Cell Therapy

The leukodystrophies are rare but challenging medical problems, and with few exceptions there are no cures and only supportive therapies (Table 1). The four primary targets are PMD, Krabbe disease, metachromatic leukodystrophy (MLD), and adrenoleukodystrophy (ALD). Exploring therapeutic options for PMD has the advantage of having a number of useful models available, ranging from rodents to a large animal canine model [9, 10]. In addition, a number of transgenic mice and rat exist that can be useful [13]. The majority (>60%) of boys with PMD have a duplication of the *plp1* gene [14, 15], but no naturally occurring duplications exist in animals. PMD can present as a severe disorder with early death, the so-called connatal form, or the classical form of the disease in which the myelin deficit is not as severe and the life expectancy is greater. Hence, the selection of patients and stage of disease at which transplantation should be performed will be important. A clinical trial of transplantation of neural stem cells into PMD patients is already underway at University of California-San Francisco (Clinical trial NCT01391637). We have begun conducting studies of cell transplantation in the rat transgenic model in which overexpression of the PLP gene results in severe dysmyelination and early death [16, 17], similar to the myelin deficient (*md*) rat [18, 19]. This rat, therefore, can model those cases of severe connatal PMD associated with a duplication in the *plp1* gene [20]. It will be important to determine whether the milieu of the CNS in PLP over-expression, hence the majority of PMD patients, is receptive to transplanted cells as it is in the missense mutations (*md* rat and the shaking [*sh*] *pup*) [21–23].

Krabbe disease is an autosomal recessive demyelinating disorder caused by a genetic deficiency in the activity of a lysosomal enzyme (GALC). Affected OLs and Schwann cells are unable to degrade its substrate, galactocerebroside. Unlike other lysosomal storage diseases, however, storage of galactocerebroside in the myelinating cells does not occur, whereas psychosine, a cytotoxic lipid metabolite, accumulates and appears to kill OLs and Schwann cells, leading to rapidly progressive demyelination. Two clinical phenotypes are known in Krabbe disease: 1) the classical infantile form in which initial symptoms develop a few months after birth and severe motor and mental deterioration progress rapidly leading to death at approximately 2 to 3 years, and 2) the late-onset form in which the onset of

Table 1 Leukodystrophies

	Inheritance	Gene defect	Biochemistry	Pathophysiology
Metachromatic leukodystrophy	Autosomal recessive	Arylsulfatase A	Storage of sphingolipid sulphatide	Demyelination in CNS and PNS
Krabbe disease	Autosomal recessive	Galactocerebrosidase	Elevated psychosine levels	Demyelination in CNS and PNS
Adrenoleukodystrophy	X-linked recessive	ABCD1 gene encoding ALD protein	Elevated levels of very long-chain fatty acids	Demyelination in CNS and inflammation
Pelizaeus-Merzbacher disease	X-linked recessive	*plp1*	Absence of PLP or decrease	CNS dysmyelination, PNS involvement rare
Canavan disease	Autosomal recessive	Aspartoacylase	Elevated N-acetylaspartic acid level	Spongy degeneration of CNS
Vanishing white matter disease (childhood ataxia with central hypomyelination)	Autosomal recessive	eIF2B	Increase CSF glycine	Hypomyelination; cyst/cavity formation in cerebral white matter
Alexander disease	Unclear (*de novo* mutations)	Glial fibrillary acidic protein	—	Inclusion body (Rosenthal fibers) in astrocytes
Refsum disease	Autosomal recessive	phytanoyl-CoA hydroxylase (PAHX) or peroxin 7 (PEX7)	Accumulation of phytanic acid in blood, fat, neuron	Hypomyelination
Cerebrotendinous xanthomatosis	Autosomal recessive	Sterol 27-hydroxylase	Accumulation of cholesterol and cholestanol in CNS and tendon	White matter axonopathy

ALD=adrenoleukodystrophy; CoA=coenzyme A; CSF=cerebrospinal fluid; CNS=central nervous system; PLP=proteolipid protein; PNS=peripheral nervous system

clinical signs varies from 3 months to more than 40 years of age, and the neurological deterioration progresses slowly in general. Importantly, the majority of patients with Krabbe disease are infants, and they succumb early in life to severe neurological symptoms [24].

MLD is an autosomal recessive disorder resulting from a mutation in the *arylsulfatase* A gene, which leads to profound demyelination of the CNS and PNS. MLD can present as an infantile, juvenile, or adult onset disorder and the late infantile onset cases (<2 years) are likely the most relevant target for cell therapy. The severe involvement of the PNS in MLD and in Krabbe disease provides greater challenges in developing remyelinating strategies compared to PMD. A knockout of the *arylsulfatase* gene has been generated [25], but there is only scattered demyelination in the nervous system of the mouse, which nonetheless appears to be corrected in symptomatic mice by hematopoietic cell transplants that were transduced to overexpress the gene [26]. However, the limited demyelination makes the knockout mouse less useful to test exogenous cell-induced remyelination. Transplantation of adult mouse neural stem cells into the MLD knockout mouse brain resulted in improvement in the biochemical defect, but no differentiation of cells into OLs [27], probably because the lack of demyelination and the need for OLs.

ALD, which is the most common leukodystrophy in children, results from a mutation in the peroxisomal membrane protein gene ABCD1 [28, 29]. The disease is striking in its phenotypic variation. The early onset, childhood cerebral form has profound subcortical demyelination associated with marked inflammation [30]. In contrast, the adult onset phenotype (adrenomyeloneuropathy) has notable long-tract degeneration in the spinal cord and no prominent demyelination [30, 31], hence it is an unlikely target for myelin repair. Attempts to generate an animal model of childhood ALD using a mouse knockout strategy have failed to yield a model with similar pathology [31]. An alternative model in which OLs are targeted that could be used to examine cell-based remyelination relative to ALD is the peroxin-5 conditional knockout mouse [32]. Peroxin-5 is involved in transport of proteins into peroxisomes, and its deletion results in a mouse with a progressive, late onset neurologic disease associated with demyelination, axon loss, and inflammation [32]. In addition, the mouse had an accumulation of very long chain fatty acids in the brain, a hallmark of ALD. It could, therefore, serve as a surrogate model of ALD to test exogenous cell remyelination, but axon loss may be an impediment.

Two other leukodystrophies with extensive white matter changes may be considered for cell-based therapy. The infantile form of Alexander disease has profound frontal lobe myelin pathology [1]. The disease has been found to result from mutations of the glial fibrillary acidic protein gene [33], but attempts to create an animal model using

knockout or knockin strategies have failed to produce any myelin changes [33]. The final disease to be considered is Canavan disease, which results from mutations in the aspartoacylase gene, leading to profound vacuolation of white matter as is found in a knockout of the gene [34, 35] and in a point mutation in the rat model [36]. However, there is little or no evidence that the white matter change leads to significant demyelination, hence promoting remyelination would not seem to be a useful therapeutic strategy. A lack of demyelination in this disease in the presence of extensive white matter vacuolation is not unique. Mitochondrial DNA mutations in a canine disorder [37] and in Kearns-Sayre syndrome in humans [38] lead to profound myelin vacuolation, but little demyelination. Transplanting OPCs into the aspartoacylase knockout mouse has shown that they differentiate into mature OLs [39] and transplanted cells may therefore be a source of the missing enzyme to cross-connect endogenous cells. Other, newer and less well-characterized developmental disorders of myelin exist that may be future targets for remyelination therapy. Vanishing white matter disease is a more recently recognized white matter disorder that might be considered, yet axonal loss can be severe, perhaps limiting the chances of successful myelin repair [40].

A key feature of the leukodystrophies in regard to remyelination is the degree of axon loss in each. Although they are all primary myelin diseases, there is almost always some axon loss, as pure demyelinating disorders with no loss of axons is rare. Thus, in PMD some axon loss has been reported [41] as it has in Krabbe disease [24] and ALD [30, 31]. However the degree of axon degeneration may not be significant in terms of producing long-term disability, as is thought to happen in multiple sclerosis (MS) [42].

Myelin Mutants used as Models of Myelin Repair

The myelin mutants are a heterogeneous collection of animals, some of whose mutations make them models of individual human leukodystrophies (Table 2). Others have no known human analog or their mutation is unknown, yet they can provide opportunities as models with which to study exogenous cell-based remyelination. The largest and most homogenous group is those with disorders in the *plp1* gene, which are models of PMD. However, the most commonly used myelin mutant in transplant experiments is the *shi* mouse, which has a mutation in the myelin basic protein (*MBP*) gene, but it is not a model of human disease. All of these mutants have predominantly developmental defects of myelination. In contrast, myelin mutants also exist in which there is a loss of myelin (i.e., demyelination). Collectively, therefore, these mutants offer a broad range of opportunities to study myelin repair.

The PLP Mutants

These mutants form the largest group of myelin mutants and are models of the human disorder PMD. The *plp1* gene is located on the X chromosome, hence the mutations are inherited as X-linked recessive disorders. Mutations in the *plp1* gene have been found in mice (the jimpy [*jp*] mouse and its allele [jpmsd]), the rat (myelin deficient [*md*] rat), the dog (the shaking pup [*sh*] *pup*), and in the pig (mutation not defined) [12]. In regard to cell transplantation, only the *md* rat and the *sh pup* have been used in any detail. The *md* rat has a point mutation in the third exon of the *plp1* gene [43] and develops tremors at approximately 10 days of age, seizures at 18 to 20 days, and it dies at 24 to 25 days [18, 19]. There is practically no myelin throughout the CNS, with a reduction in mature OLs through their early apoptosis [44]. The scarce myelin sheaths present in the md rat lack the normal double leaflet intra-period line [45]. Transplants into the *md* rat have been performed from postnatal day (P) 0 to P7. This allows transplanted cells from 2 to 4 weeks to divide and migrate, integrate and myelinate axons before the demise of the rat. We have also transplanted cells into fetal *md* rat pups following laparotomy and injection through the uterine wall, but this is a challenging and time-consuming endeavor [46]. A longer lived strain of the *md* rat has been reported that could be of use in studying longer post-transplant time points, and data from such an animal is described as follows [47]. However, this rat is no longer available. The usefulness of the *md* rat is that it is an excellent model in which to test the myelinating capacity of cells transplanted as either allografts or xenografts, but its early death limits the time that grafted cell function can be monitored. The presence of extensive myelin formation and determination that the myelin is PLP positive are clear indicators (as in *shi* with MBP) that the myelin has resulted from the transplanted cells.

The *sh pup* is a canine mutant, first described by Griffiths et al. [48, 49] in Glasgow in 1981. It is inherited as an X-linked recessive with a point mutation in the second exon of the *plp1* gene [50]. Affected pups develop a notable tremor at 6 to 10 days of age. They are never able to ambulate, but they can survive far beyond 2 years if hand raised. They develop seizures from 2 months that can be controlled with anti-convulsants. The myelin deficiency is not as severe as in the *md* rat, but their usefulness lies in their longevity and the greater size of the CNS compared to rodents that allows a "scaling-up" of cell therapy toward trials in PMD. A magnetic resonance image (MRI) of the canine brain has shown clear differences in myelination between wild-type and mutant dogs [51]. Myelin in the *sh pup* is weakly PLP-positive, so the proof of myelinating capacity of transplanted cells comes from the increased density of myelin compared to nontransplanted animals [52].

Table 2 Animal Models of Leukodystrophy

	Human disease	Affected gene	Neuropathology	Therapies tested	Longevity
Jimpy mouse [12]	PMD	*plp1*	Dysmyelination	—	21 days
Myelin deficient rat [18, 19]	PMD	*plp1*	Dysmyelination	Cell* transplantation [62, 64, 116]	23-24 days
Shaking pup (canine)	PMD	*plp1*	Dysmyelination/ hypomyelination	Cell* transplantation [66]	>2 years
PLP deficient-transgenic mice [117, 118]	PMD	*plp1*	Hypomyelination demyelination axon degeneration	—	
PLP overexpressing mice [13]	PMD	*plp1*	Hypomyelination/ dysmyelination-demyelination/ axonal degeneration	—	23 days-1 year
PLP overexpressing transgenic rat [16, 17]	PMD	*plp1*	Dysmyelination	—	23-24 days
Shiverer mouse [53]	—	MBP	Dysmyelination	Cell* transplantation [6, 18, 67, 119] (many others)	120 days
PEX5 transgenic mouse [32]	ALD/MS	PEX5	Demyelination/ axon loss/ inflammation	—	12 months
Long Evans shaker rat [56, 57]	—	MBP	Dysmyelination/ demyelination	Cell* transplantation [116]	6-9 months
Taiep rat [59–64]	—	Unknown	Dysmyelination/ demyelination	Cell transplantation	>18 months
Twitcher mouse [65]	Krabbe disease	Galactocerebrosidase	Demyelination (CNS, PNS); globoid cell accumulation	Cell* transplantation [78], ERT [94, 120], BMT [121], BMT-based gene therapy [98]	35-45 days
Arylsulfatase A (ASA) knockout mouse [25]	Metachromatic leukodystrophy	ASA	ASA accumulation without notable demyelination	ERT [122] , gene therapy [123], BMT-based gene therapy [26, 124]	2 years
Aspartoacylase knockout mouse [34]	Canavan disease	Aspartoacetylase	Myelin vacuolation, no demyelination	Cell transplantation	2-9 months
Tremor rat [36]	Canavan disease	Aspartoacetylase	Myelin vacuolation	—	1 year
X-ALD knockout mouse [125]	Adrenoleukodystrophy	X-ALD gene	No demyelination	_____	6 months
GFAP knock-in mutant mouse	Alexander disease	GFAP	Rosenthal fibers, no myelin abnormality	—	11 months

ALD/MS=adrenoleukodystrophy/multiple sclerosis; BMT=bone marrow transplantation; ERT=enzyme replacement therapy; PLP=proteolipid protein; PMD=Pelizaeus-Merzbacher disease

*In this citation, “cell” implies to myelination-competent cells, such as neural stem cells and oligodendrocyte progenitor cells

The MBP Mutants

Shiverer (*shi*) is an autosomal recessive mutant in which there is a major deletion in the *mbp* gene resulting in a severe reduction of myelin in the CNS [53]. Ultrastructural analysis demonstrates that the scattered, thin myelin sheaths that are present lack a major dense line [53]. Homozygous mice have a notable tremor from 12 days of age forward, and develop seizures at approximately 30 days of age, with death at approximately 120 days. The generalized absence of myelin makes the analysis of transplant-induced myelination straightforward. First there is significantly more myelin than in controls, and the myelin is MBP positive [6, 54, 55]. Further confirmation that this myelin is not endogenous can be seen on electron microscope (EM), in which the myelin has a major dense line and a thicker sheath than compared to the thin myelin sheaths of the host [55].

The Long Evans shaker (*les*) rat is also an MBP mutant, but it has been used much less than the *shi* mouse in myelin repair research, and nonetheless it offers additional opportunities to the *shi*. The mutation is also autosomal recessive and results from the insertion of a retrotransposon into intron 4 of the *mbp* gene, leading to a failure in gene transcription and hence failure of production of the MBP [56]. As with the *shi*, the *les* rat has practically no myelin throughout the CNS, but a striking difference is that the spinal cord initially has myelin, especially in the ventral column, but this is all lost by 4 to 6 weeks of age [57]. Thus, the *les* rat has features of both dysmyelination and demyelination. Like the *shi*, it develops seizures from 4 to 6 weeks of age, but lives much longer if carefully reared, and it can survive for as long as 9 months of age or even longer in rare cases. The loss of myelin is associated with a marked microgliosis [58]. The longevity of *les* and the larger nervous system provides some advantages to *shi*.

The *taiep* Rat

The *taiep* is an autosomal recessive mutant found on a Sprague Dawley background [59]. It is a unique myelin mutant that initially demonstrates hypomyelination, and then severe demyelination of white matter in the brain, optic nerves, and the fasciculus gracilis and corticospinal tract of the dorsal column of the spinal cord [59]. The early failure of normal myelination and subsequent demyelination result from the progressive accumulation of microtubules in OLs [59, 60]. This results in a disturbance in the polarity of microtubules and a disruption in the transport of molecules and mRNAs that utilize the microtubular network within the cytoplasm of OLs [61–63]. We have mapped the *taiep* gene to rat chromosome 9 [64] and are continuing efforts to identify the gene and its mutation. The usefulness of the *taiep* is that it lives for as long as 2 years of age with progressive neurologic dysfunction, and it can be used to test exogenous cell repair of *demyelinated* axons unlike that in *shi* or the *plp1* mutants, and also in older recipients than is possible with other rodent mutants.

The twitcher Mouse

The twitcher (*twi*) mouse is a model of Krabbe disease, also known as globoid cell leukodystrophy [65]. The mouse lacks the GALC enzyme due to a nonsense mutation in the *GALC* gene [66]. Affected *twi* mice (on B6 background) develop a tremor at P10, fail to thrive, and develop rapidly progressive neurological dysfunction with paresis leading to hind limb paralysis after P30. They do not survive beyond 45 days. However, an extended lifespan has been reported when its genetic background is altered [67, 68]. The neuropathology is characterized by the infiltration of periodic acid schiff (PAS)-positive macrophages (so-called globoid cells) concomitant or prior to demyelination both in the white matter of CNS and PNS. Demyelination progresses in an orderly manner as myelin develops in the white matter [69].

In addition to the *twi* mouse, other animal models of Krabbe disease that have spontaneous mutations in the *GALC* gene have been identified. These include dogs [70, 71], cats [72], and the rhesus monkey [73]. Although none of these have been used to study myelin repair, the canine and primate models as large animal models, provide the opportunity to scale-up remyelination therapies.

Outcome of Cell Grafting in the Myelin Mutants

Historically, the outcome of glial cell transplantation into the myelin mutants was assessed primarily on the myelin produced. Thereafter, evaluation of cell survival, migration, division, and so forth were explored. Finally, the potential for cell grafting leading to functional improvement has been studied.

Myelination

The first transplants in the *shi* mice or *md* rats were performed to test whether cells could myelinate host axons on transplantation and not as a test for future therapies. Thus, the initial studies provided proof-of-principle only that this was feasible. The *shi* transplants were carried out in the laboratory of the late Madeleine Gumpel, Salpetriere, Paris, and on the *md* rat by 1 of us (IDD) at the University of Wisconsin-Madison in the 1980s. These experiments showed that there was focal myelination in the brain of *shi* mouse with some migration of cells from the site of implantation of fragments from the olfactory bulb [74, 75]. The myelin was MBP positive and had a normal major

dense line [76]. In the *md* rat, transplantation of cell suspensions from either CNS or PNS (Schwann cells) into the spinal cord resulted mainly in focal myelination in the dorsal column, which was either PLP-positive in the case of CNS suspensions, or P0-positive in the case of Schwann cells [21]. The myelin produced by CNS grafts had a normal intraperiod line. Migration of cells away from the site of transplantation, with myelin present in the lateral column was seen in 1 recipient (Fig. 1a). Since these early reports, numerous other studies have confirmed the ability to generate large, yet predominately focal areas of myelin in *shi* (MBP-positive) and the *md* rat (PLP-positive) (Fig. 1). In the case of spinal cord transplantation, visual inspection of the spinal cord on removal from the recipient may show an obvious white "streak" in the dorsal or ventral column, which corresponds to an area of myelin made by the transplanted cells (Fig. 2). In the *md* rat, in some cases almost the entire dorsal column can be myelinated at the site of engraftment [22, 23, 77]. In both the *shi* and *md* rat, myelin made by transplanted cells is characteristically thin, similar to that seen in endogenous remyelination. However in some instances myelin made by the transplanted cells is normal thickness, possibly relating to time post-grafting (Fig. 1d) [52, 55]. Repair of a myelin deficiency is also required by some axons in the grey matter. In *md* transplants and in the *sh pup*, this has been documented and it appears that the degree of myelination is equivalent to that seen in normal grey matter (Fig. 1f, g).

Many, if not most, of the work previously cited is from the transplantation of cells into the neonate. This would seem to match the likely scenario well in treating children with leukodystrophies. However, transplantation of OPCs has also been carried out in adult *shi* mice [78–80] and in the *sh pup* [52] with successful myelination. This is not always true in all of the myelin mutants, as we and others have had little success in transplanting OPCs into the CNS of the *taiep* rat. If, however, the white matter is "primed" to become mildly inflammatory, transplanted cells myelinate *taiep* axons [81]. The question of stability of myelin made by transplanted cells is important, as this is a prerequisite in translational use. The *shi* mouse is usually limited by the shortened lifespan of the mouse, but grafts and their myelin have been noted to survive for as long as 120 days. Division of human glial restricted progenitors in *shi* has

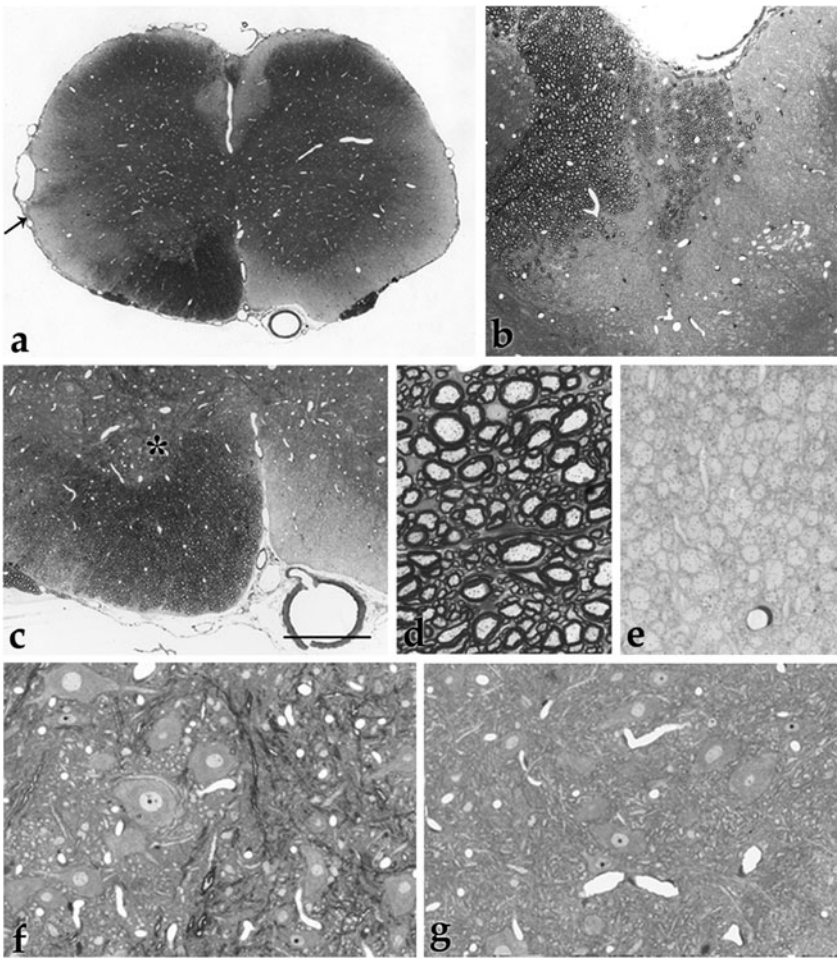

Fig. 1 Transplantation of myelin-producing cells into the myelin deficient (*md*) rat. Eight weeks after transplanting a mixed glial cell preparation from wild-type spinal cord, there was extensive myelination in the dorsal and ventral columns, with a small patch of myelin in the ipsilateral lateral column (arrow). (**a**) Details of the myelination of the dorsal (**b**) and ventral (**c**) column in adjacent sections are shown on higher power. Myelination of the complete ventral column on the left side of the cord enlarges it to almost twice the size of the nonmyelinated right column (**c**); the complete myelination and nonmyelination in these areas, respectively, are shown in (**d**) and (**e**). In addition, the left ventral grey matter (***c**) is appropriately myelinated compared to the right side (**f** and **g**, respectively)

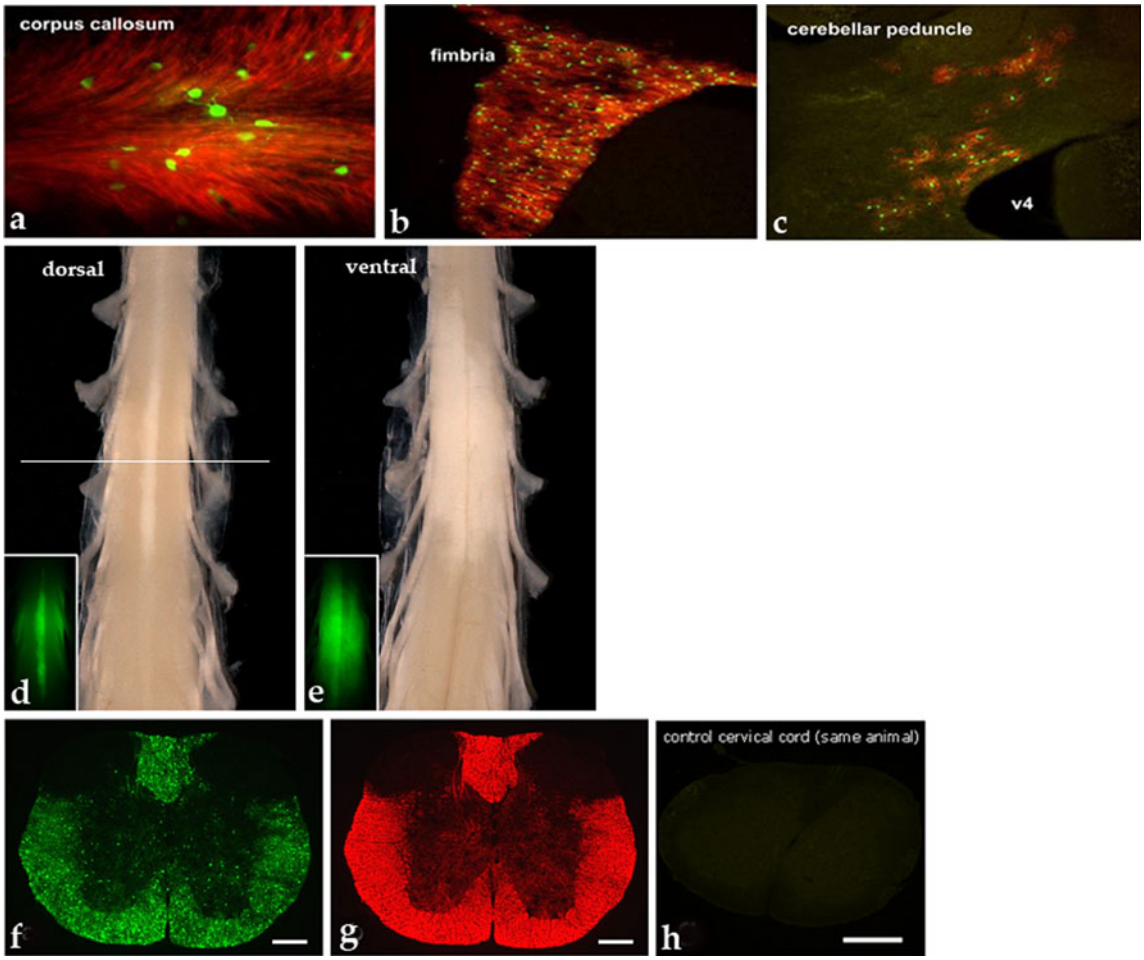

Fig. 2 Myelination of the brain and spinal cord in the shiverer (*shi*) mouse. A *shi* mouse at 120 days of age that was transplanted at P0 in the brain with 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNP)-green fluorescent protein (GFP) transduced oligodendrocyte progenitor cells derived from oligospheres, and P21 in the spinal cord. Three areas from the brain (**a-c**) show GFP-positive cells (yellow/green) scattered throughout the brain associated with myelin basic protein (MBP)-positive myelin (red). The spinal cord when viewed both from the dorsal (**d**) and ventral (**e**) views shows a white strip (**d**) and broad white band (**e**) that marks myelin made by the transplanted cells. The insets show the fluorescent signal that corresponds with myelin deposition. The white line through the cord (**d**) marks the areas of the cord shown in transverse section in (**f**) and (**h**). In (**f**), GFP-positive cells are seen at high density in the white matter and also in appropriate numbers in grey matter. These cells have completely myelinated the cord (**g**) compared to a segment from the cervical cord of the same mouse, which is MBP negative (**h**)

been demonstrated by Ki67 labeling [55]. However, the ongoing myelination in these transplants was not ascribed to an increase in the production of myelinating OLs by the transplanted cells [55]. In more recent work by Windrem et al. [55] in which the global repair extended the lifespan of recipient *shi* mice there is survival of myelin as long as 1 year of age and longer. We have also found evidence of cell and myelin survival, 6 and one-half months after transplantation in the *sh pup* [52]. This longevity has been extended further in a transplant study in the *taiep* rat. Transplantation of OPCs derived from *lac*Z-transduced oligospheres resulted in survival of cells in the dorsal column, 18 months after injection, with widespread myelination by the transplanted cells (Fig. 3) (Zhang and Duncan, unpublished data).

Although these results have been promising and illustrative of the potential of cell therapy in the leukodystrophies, the need for more global myelin repair is essential. In this regard, 3 studies in the *shi* mouse and 1 in the *sh pup* standout. In the first of these, Mitome et al. [82] transplanted green fluorescent protein (GFP)-transduced neural stem cells into the lateral ventricle(s) of neonatal *shi* mice and the cisterna magna, and examined the mice 12 weeks later. They showed extensive spread of the cells throughout the putative white matter of the brain that was MBP-positive, with the spread of MBP-positive myelin, into the cervical spinal cord. This was the first real evidence of extensive repair in *shi*. In the second study, we transplanted OPCs into the brain at P0 to P1 and into the dorsal column of the thoracolumbar spinal cord of the same *shi* mice

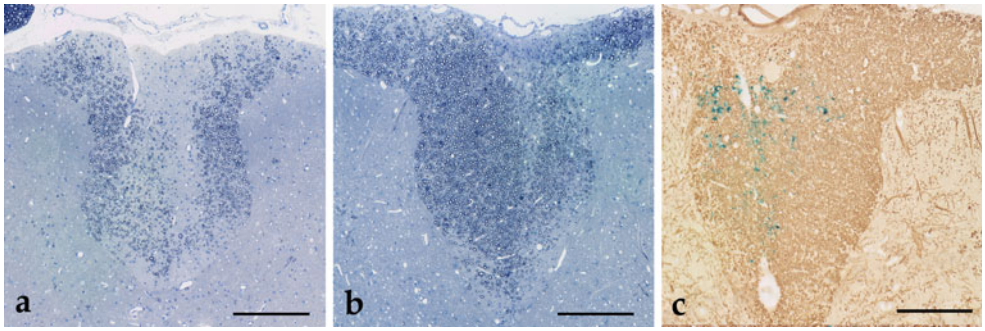

Fig. 3 Long-term survival of grafted cells. Sections from the thoracic cord, dorsal column of a 1 and one-half-year-old *taiep* rat, transplanted at 8 days of age with lacZ-positive oligodendrocyte progenitor cells derived from oligospheres. In (**a**) a few millimeters from the site of injection, it can be seen that the fasciculus gracilis (fg) and corticospinal tracts are poorly myelinated. In contrast, at the site of injection (**b**), almost the entire dorsal column is myelinated. In a myelin basic protein (MBP)-stained section close to the site of the transplant, the dorsal column appears well-myelinated, and notably, a patch of lacZ-positive cells is present. (**a**, **b**) toluidine blue; (**c**) MBP

3 weeks later [6]. At 120 days, the mice were examined and GFP-positive OPCs were found to have extensively migrated throughout the brain, associated with MBP-positive myelin patches, and across the entire spinal cord at the site of injection (Fig. 2). The most compelling evidence of global repair in *shi*, however, comes from the study of Windrem et al. [55]. In this study, they generated a *shi*-rag2 knockout mouse into which they transplanted human fetal glial precursors. These mice were transplanted at P0 with a total of 300,000 cells divided between 5 injection sites, with 4 that were bilateral injections in the anterior and posterior corpus callosum, and the fifth into the cerebellar peduncle. The first striking evidence from these mice was the prolonged survival of 21% of the mice for as long as 1 year of age, with improvement in their neurologic dysfunction, in particular their ambulation and lessened seizure frequency. Examination of the brains of these mice showed remarkable dispersion of GFP-positive cells that increased with time. The human origin of these cells was confirmed with an antibody to human nuclear antigen associated with MBP reactivity throughout the brain. Extensive myelination was also seen in the spinal cord and approximately 80% of the axons in the upper cervical and corticospinal tract were myelinated. This study unequivocally demonstrated the power of exogenous cell therapy, albeit in the mouse. It is clear that multiple sites of transplantation are required, yet it is not clear why only 20% of the mice displayed this widespread integration of human cells and survived.

Glial cell transplantation in the *sh pup* has also been illustrative of the degree of myelination that can be achieved from focal transplantation. Indeed the study of Archer et al. [52] was the first to demonstrate that myelin made by transplanted cells myelinate areas of the size comparable to some MS plaques in a large animal model, hence this could be of clinical significance. Transplanting fetal canine glia into the spinal cord resulted in almost complete myelination of the dorsal column, unilaterally in the lateral column and complete myelination of the ventral columns (Fig. 4). This transplant-induced myelin was found along 20 mms of the spinal cord and was 10 times the volume achieved in the *md* rat shown in Fig. 1. An outstanding goal will be to use the *sh pup* to follow myelin repair *in vivo* using MRI.

Tracking Cells and Their Fate After Transplantation

Although a number of methods have been used to label cells prior to transplantation, the 2 methods most frequently used are to transduce the cells to express a reporter gene, usually β-galactosidase (*lac*Z) or GFP [6, 55, 83]. In the case of *lac*Z, the spinal cord or brain at the site of transplantation is incubated overnight in X-gal solution [23]. In 24 hours, a blue reaction product is seen at the site, which will frequently overlap with a white patch seen grossly prior to X-gal staining [23]. More recently, GFP has become the standard method of tracking cells and has some advantages over *lac*Z, as cell tracking is performed by shining a fluorescent light on the transplanted tissue and positive areas can be selected for further studies (Fig. 2). As with *lac*Z, the areas of positive fluorescence usually overlap with a patch of myelin (Fig. 2). However, in the brain, given the localization of white matter compared to the spinal cord, it is difficult to follow cells without first trimming the brain into slices (Fig. 2). In the case of xenografts, in most instances human cells grafted into animal models, human-specific antibodies, such as human nuclear antigen are extremely useful in following cell survival and migration (Fig. 5). Most recently, *GFP* gene transduction of human neural precursors has been shown to track long distance migration of cells when transplanted into focal demyelinated lesions of the nude mouse spinal

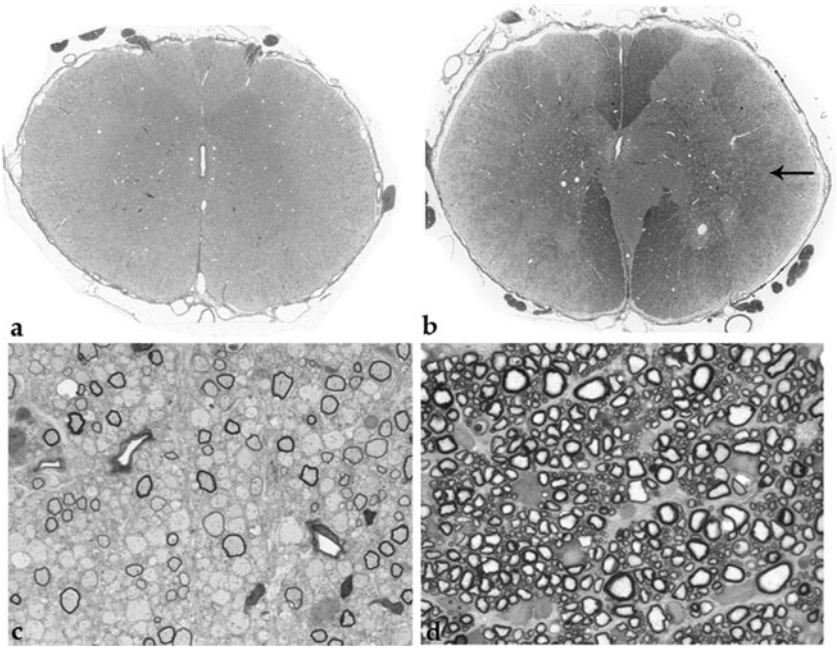

Fig. 4 Scaling up of transplant-induced myelination. Thoracic spinal cord from a 9-week-old shaking (*sh*) *pup* transplanted at 1 week of age with a glial cell suspension from the brain of an E-45 day fetal pup. More than 5 cm from the transplant site, the white matter appears poorly myelinated (**a**) and on higher power contains only scattered myelinated fibers (**c**). At the site of injection (**b**), a large part of the dorsal column is myelinated, as are the ventral columns and the deep part of the right lateral column (arrow). On higher power of the ventral column, all axons are myelinated (**d**)

cord [83]. The migration, well beyond the lesion site, suggests that transplanted cells do have the ability to migrate through normal white matter [83].

These transplant experiments have also provided information on cell survival and division after engraftment. Although there has been little documentation of the death of transplanted glia in these mutants, the number of pyknotic nuclei at 4 and 24 hours after transplantation of OPCs into the dorsal column of *md* rats was found to be approximately 56% and 25% at each time point [84]. This significant cell death is typical of what occurs following any neural cell transplant and success of the transplant likely means that surviving cells must divide. Autoradiography using tritiated thymidine has shown that cell division of transplanted OPCs does occur [85], hence this must be the reason for the extensive areas of myelination seen at transplant sites. Mitotic signals within the neuropil presumably are responsible, possibly originating from unen-

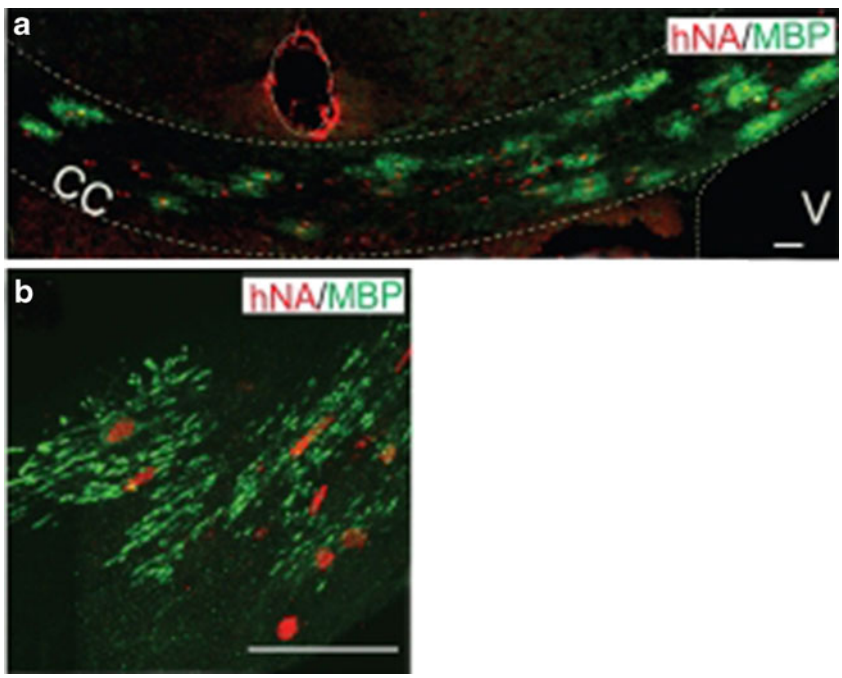

Fig. 5 Myelination by oligodendrocyte progenitor cells derived from human embryonic stem cells. Three months after transplantation into the shiverer (*shi*) mouse brain at P0, grafted cells are found scattered throughout the corpus callosum that are human nuclear antigen-positive and associated with myelin basic protein (MBP)-positive myelin (**a**). The human cells can be seen to be associated with multiple MBP-positive internodes (**b**). (Reproduced and modified from Hu et al., 2009:136:1443–1452 [11])

sheathed axons or endogenous glia. It has been shown when OPCs are co-grafted with the neuroblastoma cell line, B-104, which is known to maintain OPCs in a dividing, nondifferentiating state, there is an increase in division of the grafted cells [85]. In addition, when B-104 cells are transplanted distant to the site of grafting of OPCs, the latter migrate toward the B-104 site, suggesting a chemotactic gradient [85]. Hence, it can be concluded that it is possible to both stimulate division of transplanted cells and direct their migration.

To follow cells *in vivo*, cells can be tracked following their labeling with iron nanoparticles that are detectable by MRI. This has been demonstrated both *ex vivo* and *in vivo* in the md rat and migrations of cells followed longitudinally in the les rat spinal cord [86, 87]. Neural stem cells induced to over-express Olig-2, which were derived from a luciferase-GFP-actin transgenic mouse and transplanted into a mouse fed cuprizone, were detected by bioluminescence imaging in the brain of live mice [88].

Functional Outcome of Transplantation of Cells into the Myelin Mutants

From a translational standpoint, the key question is whether transplanting stem cells or OPCs into the CNS will have therapeutic significance and improve or restore function and not just result in focal myelin repair. Based on 25 years of research on glial cell transplantation, this can be judged at 3 levels: 1) the myelination resulting from cell therapy results in the formation of normal nodes of Ranvier, 2) there is neurophysiologic evidence of secure conduction in areas of myelination, and 3) extensive myelination resulting from transplants results in behavioral improvement.

Nodes of Ranvier

Restoration of normal conduction is dependent on the presence of normal nodes. Although it might be expected a *priori* that myelin made by transplanted cells, which extended along several consecutive internodes would be associated with normal nodes of Ranvier, this required formal proof. Study of the paranodal areas of myelin formed by mouse glial cells transplanted into the *md* rat implied that nodes are formed normally after transplantation [22]. Confirmation of this has been documented in 3 recent studies, all of which were following transplantation of cells into the *shi* mouse. In the first study, adult mouse neural stem cells were injected into *shi* spinal cord and the area examined 6 weeks later [11]. In areas of MBP-positive compact myelin, the ultrastructure of nodes and paranodes, and the molecular architecture of these areas were studied. EM studies showed the formation of normal paranodal loops. Immunolabeling for Na and K channels and Caspr localization at nodes and paranodes was normal [11]. In a similar study in *shi* mice, but transplanting human glial precursors, 3 areas of the CNS (i.e., the corpus callosum, cervical spinal cord, and optic nerves) were studied in a similar manner to the previous report, and showed normal nodal and paranodal Na, K, and Caspr localization [55]. This has recently been confirmed in further *shi* transplants with human cells [83].

Restoration of Conduction

The first report of restoration of conduction pre-dated the reports showing the production of normal nodes of Ranvier. In this study, *md* rats were transplanted with a mixed glial preparation and sacrificed at 15 to 17 days postsurgery. The spinal cord at the site of cell transplant was removed and placed in a brain chamber. The site of engraftment and myelin made by the cells was visible by a naked-eye inspection [89], (Fig. 1), hence it was possible to place stimulating and recording electrodes over both myelinated and nonmyelinated areas of the spinal cord. Areas with myelin repair showed a threefold increase in conduction velocity. Other parameters of normal conduction, such as frequency-response parameters were normal [89]. In the first study of node of Ranvier formation in *shi* previously noted [79], the authors recorded spinal cord-evoked potentials by stimulating and recording from the dorsal column of the thoracic spinal cord. Nerve conduction velocity was improved through the transplant site, although not restored to normal [79]. Conduction velocity in transplanted *shi*-rag2 mice was restored close to normal and was significantly faster than nontransplanted mutants [83].

Improvement in the Phenotype Following Transplantation

The great majority of reports of cell therapy in the myelin mutants have not reported any improvement in behavioral outcome. Two studies, however, have documented phenotypic improvement, both in *shi* mice. Mice that were transplanted with the mouse neural stem cell line, known as C17.2 at P0 were followed for 2 to 8 weeks [90]. The cells, which expressed lacZ, were found to have migrated quite extensively throughout the brain. Myelination was determined by Western blots for MBP, but there was no immunohistochemical demonstration of MBP. EM demonstrated more myelinated axons than normal. It was suggested that the transplanted cells lessened the tremor in the mutant compared to controls using recordings of tail movements [90]. The most definitive proof of restoration of the phenotype, however, comes from the Windrem et al. [55] study of transplantation of human cells into the *shi*-rag2 mouse brain (see previously). The mice that survived

for more than 1 year had fewer seizures, less tremors, improvement in mobility, and so forth. To all intents, these mice were "cured." This result is enormously important for 2 reasons: 1) the phenotype of a mutant with global lack of myelin can be reversed, and 2) human progenitor cells are capable of accomplishing this when transplanted as xenografts. Presumably, scaling up of such grafts using similar human cells could lead to even greater myelination of the neonatal human brain.

Transplantation of Cells into the *twi* Mouse

As a model of Krabbe disease, a number of studies have been carried out to repair the demyelinating lesions seen in this leukodystrophy using the *twi* mouse. Because of the complexity of the pathophysiology of the disease, it appears likely that a combined therapeutic approach, perhaps including exogenous cell-based remyelination will be necessary. In most lysosomal disorders, the affected cells can function when the missing enzyme is replaced, even exogenously [6, 91–93]. Therefore, replacing GALC in oligodendrocytes and Schwann cells, either endogenously or exogenously, is critical as a therapeutic approach in Krabbe disease. For example, enzyme replacement by intravenous administration of synthetic GALC has moderately improved the clinicopathology of twi mice [94]. However, the effect is limited because the GALC protein does not cross the blood brain barrier to reach the CNS. The question, therefore, is whether cell-based enzyme replacement therapies in the CNS of *twi* mice will be successful. When normal OPCs were transplanted into the *twi* brain, the extent of myelin formed by these cells was not as extensive as seen in *shi* mice (Kondo and Duncan, unpublished observations). Interestingly, however, these areas showed less active microglial activation and absence of globoid cell-like macrophages, suggesting that the transplanted cells had modulated the host response at the transplant site (Fig. 6). In 1 study, a neural stem cell line genetically modified to overproduce GALC was transplanted into the *twi* brain [78]. The donor cells appeared to have migrated, proliferated, and finally distributed widely in the brain, although the proportion of donor cells that differentiated into myelinating OLs is unclear. Even though the *twi* mice is a model of severe demyelination, a considerable amount of endogenous myelin remains in the nervous system (Fig. 7a-d). Therefore, the use of an appropriate marker is necessary to identify donor-cell derived myelin. We found it beneficial to use OPCs derived from CNP-GFP transgenic mice, in which the CNP promoter drives OL specific GFP expression [95], and thus further immunohistochemical demonstration of OLs is not required [6] (Fig. 7e,f).

Atlhough the local transplantation of myelination competent cells is a powerful experimental approach, its clinical limitation in Krabbe disease will be the need to promote cell migration throughout the entire nervous system, including the CNS and PNS. To date, such global migration of exogenous cells, although not myelin-producing cells, has been achieved by bone marrow transplantation (BMT) and umbilical cord blood transplantation (UCBT). We have shown such migration in *twi* mice following BMT (Fig. 7g-n) (Kondo and Duncan, unpublished). Indeed BMT [96] and UCBT [97] have been performed in patients and proven beneficial, although these therapies have minimal effects on symptomatic patients. Macrophages derived from the BMT

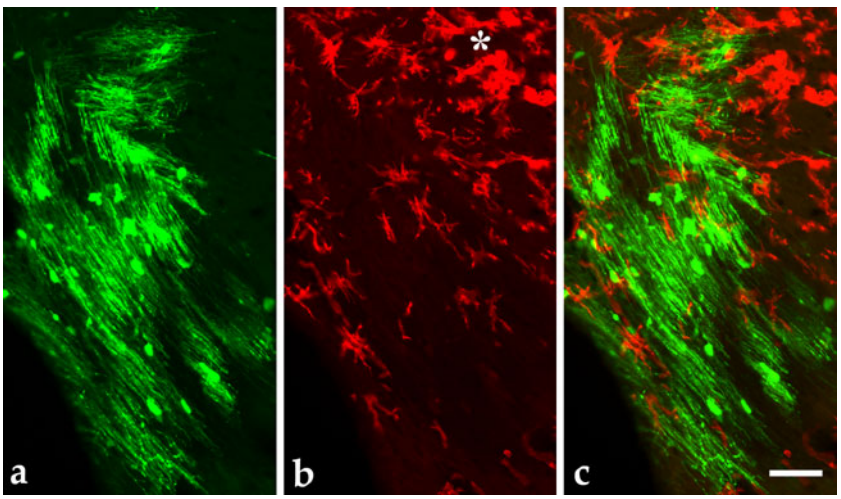

Fig. 6 Normal donor oligodendrocytes subsides host inflammation. The hippocampal fimbria of a 45-day-old twitcher (*twi*) mouse transplanted with CNP-green fluorescent protein oligodendrocyte progenitor cells at P10 (**a**). Without engraftment, the white matter of *twi* mice accumulates a number of macrophages intensely immunoreactive for CD45 (***b**). However, microglia/macrophages are scarce or less active in the engrafted area as evidenced by the donor-derived oligodendrocytes and myelin (**c**). Scale bar, 50 μm

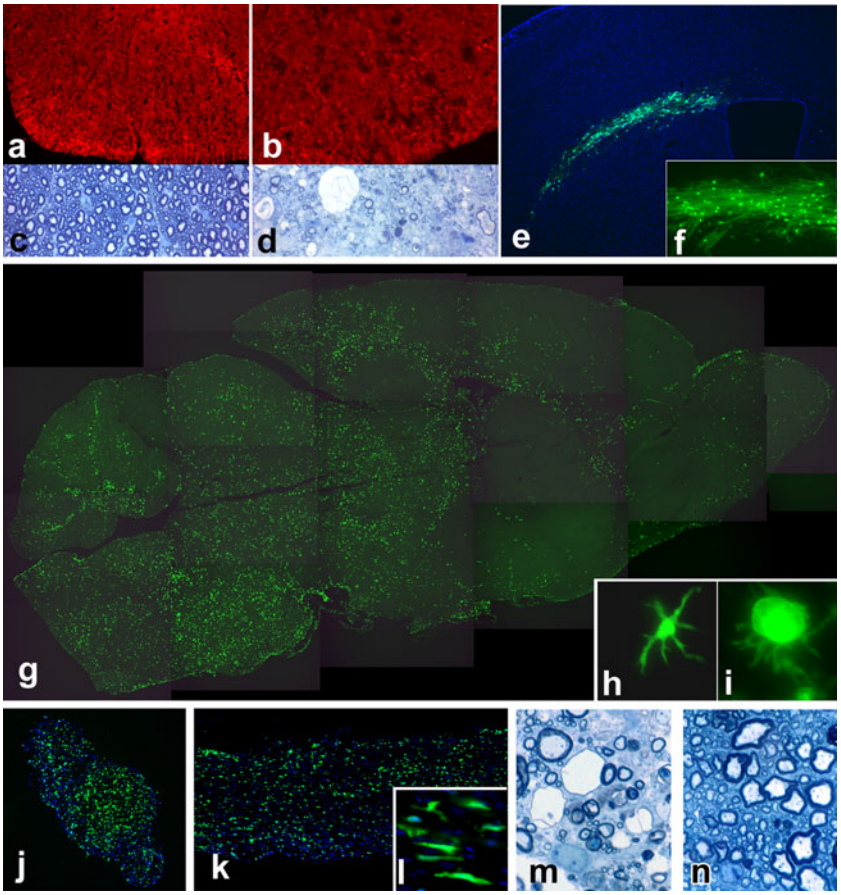

Fig. 7 Transplantation studies in twitcher (*twi*) mice. (**a**-**d**) Immunohistochemical staining for myelin basic protein (**a**, **b**) and toluidine blue myelin staining (**c**, **d**) in the ventral white matter of the cervical spinal cord from wild-type control (**a**, **c**) and *twi* (**b**, **d**) mice at P45. Note that some myelin basic protein (MBP) immunoreactivity, as well as a number of myelinated axons remains even at a moribund stage of *twi* mice, requiring a proper marker to identify transplanted cells. Punched out MBP-negative areas in (**b**) correspond to macrophages. (**e**-**f**) galactocerebrosidase competent primary oligodendrocyte progenitor cells were cultured from the neonatal brain of CNP-green fluorescent protein (GFP) transgenic mice and transplanted into the corpus callosum of twitcher (*twi*) pups at P1 to P3. CNP promoter-driven GFP expression identifies the donor-derived myelin and oligodendrocytes in the brain of *twi* mice at P45 (**e**). Note that the cells have only migrated moderately in the white matter. Donor-derived myelin (diffuse green) and oligodendrocytes (intense green) are demonstrated in the inset (**f**) at higher magnification. (**g**-**n**) Donor-derived GFP + cells (green) in the nervous system of *twi* mice after bone marrow transplantation (BMT). The sagittal view of the brain shows that the white matter is the predilection site of migration (**g**). These GFP + infiltrating cells are of the monocyte lineage and show a branched morphology (**h**, cerebellar cortex) and a "globoid"-like morphology (I, cerebellar peduncle) at higher magnification. A transverse (**j**) and a longitudinal section (**k**) of the sciatic nerve demonstrate GFP + macrophages infiltrated in the peripheral nervous system. An enlarged image of macrophages from (**k**) is shown in the inset (**l**). Blue in (**j**-**i**) is 4'-6-Diamidino-2-phenylindole counter-staining. Toluidine blue staining of the ventral white matter of the cervical spinal cord in untreated-*twi* (**m**) and *twi* mice 5 weeks after BMT (**n**) demonstrate an improvement in myelination by BMT

and UCBT infiltrate the donor nervous system and provide neighboring myelinating cells with intact GALC [6, 91, 92]. We demonstrate that transplanting *twi* cells into the *shi* mouse, lead to their rescue *in vivo* through the uptake of endogenous GALC, thus providing proof that this approach is translationally significant. Modifying these cells to allow better enzyme replacement could be a promising approach in treating symptomatic patients. Recently, for instance, enhancing GALC expression by lentiviral gene transduction in hematopoietic stem cells prior to their transplantation into *twi* mice has been reported, which moderately enhanced the effect of BMT [98].

Cells that have been Transplanted in the Myelin Mutants

Practically all stages of the developmental lineage of the OL have been transplanted into the myelin mutants (as recently reviewed) [99–102]. It is likely that the best cells for myelin repair are those at the more immature stage of the lineage. Thus, neural stem cells, OPCs, and perhaps very immature OLs will be the choice. Although OPCs and OLs can be derived from dissociated cell preparations from the freshly isolated CNS, the more utilized technique is to culture the cells as free-floating preparations, either as

neural stem cell neurospheres [103] or OPC oligospheres [104–106]. The former can give rise to neurons, astrocytes, and OLs on withdrawal of the growth factors basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) [103]. However, few OLs are derived from these cells *in vitro*, but when transplanted into a dysmyelinated background they can generate large numbers of myelinating OLs [80, 107, 108]. Hence, it appears that cues in the milieu promote this oligodendrogenic switch and/or factors in the culture inhibit OL differentiation. If the growth factors, bFGF and EGF are switched slowly for B104-conditioned media, neurospheres can be converted *in vitro* into pure collections of OPC oligospheres that will differentiate appropriately in culture to mature, membrane-bearing, myelin gene expressing OLs (Fig. 8) [105, 106]. Oligospheres can be derived directly from dissociated striatum and grown in the presence of B-104 conditioned medium [104].

A similar switch from neurospheres to oligospheres has not been successful with human cells, confirming the difference in molecular signals between human and animal cells (Zhang, unpublished data). This has been successfully demonstrated with mouse embryonic stem cells (ESCs) that can be differentiated into neural stem cells that will give rise to myelinating cells on transplantation into the *md* rat [109]. These cells are now commonly used in transplantation experiments, being easy to transduce with a variety of genes. The current hope is that human OPCs can be similarly derived from ESCs. The derivation and use of human ESC lines, however, is associated with lingering ethical difficulties. In addition, the generation of a large population of OLs from human ESCs has proven a technical challenge for most laboratories, with the exception of Nistor et al. [110] who used a novel selection method of embryoid bodies and retinoic acid to promote neural differentiation and reported a purity of as much as 95% OLs in their cultures. Hu et al. [111] reported considerably restricted OL differentiation and highlighted the importance of sonic hedgehog (SHH) in inducing human precursor cells along the OPC differentiation process. They further discovered that expansion of Olig2+/Nkx2.2+ pre-OPCs with FGFs inhibits OPC specification through downregulation of SHH signaling [111]. This finding highlights the similarity and difference between human and rodents in OPC specification. It also explains why FGF-expanded human neurospheres rarely convert to OPCs. When these cells were transplanted into *shi*, they showed patches of MBP-positive areas and human nuclear antigen-positive cells in the corpus callosum (Fig. 6). However, the future may lie with induced pluripotent stem cells (iPSCs), as these cells can be generated on a patient basis by culturing fibroblasts from a skin biopsy and used as autologous grafts following their reprogramming back to an embryonic-like state and then differentiation to a neural lineage [112]. Indeed, OPCs and immature OLs have been derived from human iPSCs in such a fashion, albeit technical issues need to be improved for more efficient OPC generation [113]. However, there are apparent differences between ESCs and iPSCs [113] that suggest the effort should proceed with both cell types to define the best and safest source of myelinating OLs. In addition, a recent study of transplantation of mouse iPSCs has raised a caution that even autologous cells may generate an immune response in the recipient [114].

Greater success to date in demonstrating the myelinating capability of cultured human OPCs has come from the fetal and adult brain using immunomagnetic sorting with antibodies to the Poly-Sialated Neural Cell Adhesion Molecule (PSA-NCAM) and A2B5, surface antigens that are present

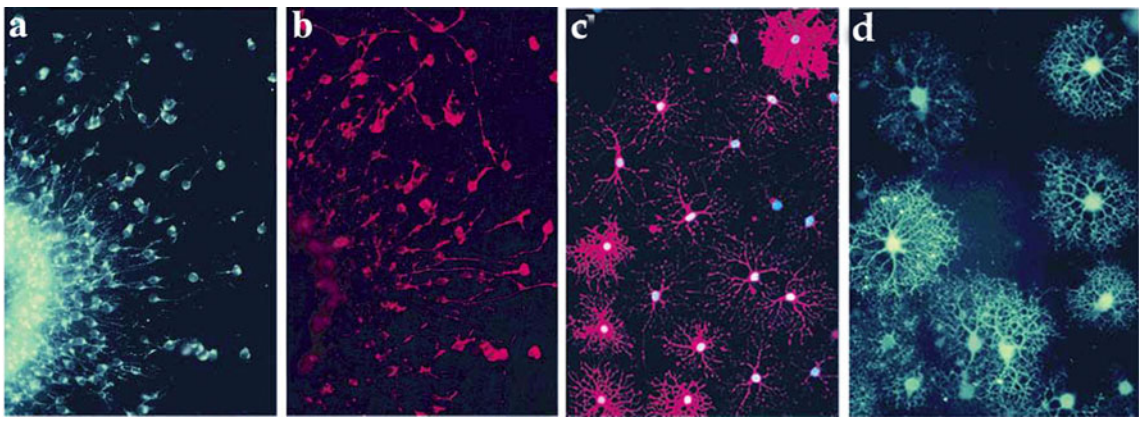

Fig. 8 Oligodendrocyte development *in vitro* from oligospheres. Representative outgrowth and differentiation of oligodendrocyte progenitor cells from an oligosphere derived from canine striatum. Cells in the oligosphere and those during early migration are Platelet Derived Growth Factor Receptor (PDGFR)-α positive (**a**), whereas only those that have left the sphere are positive for A2B5 (**b**). In the presence of 0.5% fetal bovine serum, cells start to differentiate into O1 positive early oligodendrocytes (OLs) (**c**) at 4 days *in vitro* then at 6 days *in vitro*, express MBP (**d**), and have the highly branched, membrane-producing appearance of mature OLs. (Modified from Zhang et al, 1998)

at different stages of neural and glial differentiation [115]. Purified preparations were transplanted into the *shi* mouse and cells from both fetal and adult sources myelinated *shi* axons, although paradoxically, adult cells myelinated more efficiently and faster than fetal cells [115]. In a recent study, the gliogenic and myelinating capacity of human neural progenitors from different areas of the brain of the first trimester fetus were investigated, and the oligodendrogenic potential of cells from the ventral telencephalon at 7.5 weeks of gestation demonstrated on transplantation into the *shi* mouse [83]

Conclusions

There is optimism that cell replacement therapies will become a therapeutic approach in the leukodystrophies in the near future. Each disease may take a different approach or combined approaches to treatment. A cautionary note will be that exogenous cell therapy will only be successful if there is sufficient numbers of axons surviving to be remyelinated. This might be best evaluated by contemporary MRI, and treatment instigated at a sufficiently early stage of the disease prior to axon loss. In PMD, the ability to deliver large numbers of OPCs at multiple sites and more than 1 occasion, may make it the most straightforward of the leukodystrophies to treat. In Krabbe disease and MLD, cell therapy may be part of a complex treatment strategy that will repair the brain and/or supply the missing enzyme to endogenous OLs and Schwann cells in a global fashion. Defining these therapies in appropriate animal models with some outcome measure (or measures) of success will be a prelude to instigating clinical trials.

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