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Stem cell-based strategies for treating pediatric disorders of myelin

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The pediatric leukodystrophies comprise a category of disease manifested by neonatal or childhood deficiencies in myelin production or maintenance; these may be due to hereditary defects in one or more genes critical to the initiation of myelination, as in Pelizaeus–Merzbacher Disease, or to enzymatic deficiencies with aberrant substrate accumulation-related dysfunction, as in the lysosomal storage disorders. Despite differences in both phenotype and natural history, these disorders are all essentially manifested by a profound deterioration in neurological function with age. A congenital deficit in forebrain myelination is also noted in children with the periventricular leukomalacia of cerebral palsy, another major source of neurological morbidity. In light of the wide range of disorders to which congenital hypomyelination and/or postnatal demyelination may contribute, and the relative homogeneity of central oligodendrocytes and their progenitors, the pediatric leukodystrophies may be especially attractive targets for cell-based therapeutic strategies. As a result, glial progenitor cells (GPCs), which can give rise to new myelinogenic oligodendrocytes, have become of great interest as potential therapeutic vectors for the restoration of myelin to the hypomyelinated or dysmyelinated childhood CNS. In addition, by distributing themselves throughout the deficient host neuraxis after perinatal allograft, and giving rise to astrocytes as well as oligodendrocytes, glial progenitors appear to be of potential great utility in rectifying enzymatic deficiencies. In this review, we focus on current efforts to develop the use of isolated human GPCs as transplantable agents both for mediating enzymatic restoration to the enzyme-deficient brain and for therapeutic myelination in the disorders of congenital hypomyelination.

INTRODUCTION

Oligodendrocytes produce myelin in the postnatal CNS, and their loss or dysfunction is at the heart of a wide variety of diseases of both children and adults, designated the leukodystrophies. Since neurological dysfunction in the leukodystrophies is typically a direct function of myelin absence or loss, a number of cell replacement strategies have been developed with the goal of either replacing myelinogenic cells directly by oligodendrocyte replacement, or supporting their viability through the introduction of non-oligodendrocytes able to restore missing enzymes to an otherwise deficient environment. To accomplish these goals, both neural stem cells and their derived glial progenitor cells (GPCs) have been assessed as potential cell therapeutics for the treatment of a variety of childhood hereditary-metabolic disorders of the brain and spinal cord, including both those manifested by disorders of initial myelination and those reflecting congenital enzymatic deficiency.

THE PEDIATRIC LEUKODYSTROPHIES AS TARGETS FOR GLIAL PROGENITOR TRANSPLANTATION

The early dysmyelinations of the pediatric leukodystrophies comprise especially attractive targets for a progenitor cell-based therapeutic strategy. Children suffer from a variety of hereditary diseases of myelin failure or loss, which include (i) the hypomyelinating diseases, such as Pelizaeus–Merzbacher Disease

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and hereditary spastic paraplegia, X-linked disorders of proteolipid protein production, which represent primary disorders of myelin formation (1); (ii) the metabolic demyelinations and lysosomal storage disorders, such as metachromatic leukodystrophy (MLD), Tay-Sachs, Sandhoff's and Krabbe's diseases, as well as adrenoleukodystrophy and the mucopolysaccharidoses (reviewed in 2) and (iii) gross disorders of tissue loss, such as Canavan's Disease (3) and vanishing white matter disease (4), in which oligodendrocytes are early targets. In addition, a variety of hereditary-metabolic disorders that are manifested by early neuronal loss, such as the organic acidurias and neuronal ceroid lipofuscinoses, are accompanied by early oligodendrocyte loss (reviewed in 2,5).

It is worth noting that besides these genetic disorders of myelin, periventricular leukomalacia, the most common single form of cerebral palsy, may also be due in part to a perinatal loss of oligodendrocytes and their precursors (6–9). As such, cerebral palsy may also be an attractive target for cell-based myelin replacement. Indeed, their mechanistic heterogeneity notwithstanding, all of these conditions include the prominent loss of oligodendrocytes and central myelin, highlighting the potential importance of restoring oligodendrocytes and their progenitor cells throughout this wide spectrum of perinatal disorders. As a group, the leukodystrophies thus comprise attractive targets for therapy based upon the transplantation of GPCs.

NEURAL STEM AND GPCS FOR CELLULAR THERAPY

Neural stem cells, defined as the self-renewing and multilineage-competent derivatives of the early neuroepithelium (reviewed in 10), are most prevalent in the developing central nervous system, yet remain within the ventricular subependyma of all adult vertebrates that have been studied (reviewed in 11). As such, neural stem cells can be isolated to purity from both the fetal (12,13) and adult (14–17) human forebrain. Although neural stem cells can give rise to neuronal and glial populations alike, a large body of studies have focused on their ability to generate GPCs of the brain and spinal cord (reviewed in 18). GPCs may be generated from both tissue and embryonic stem cell-derived neural stem cells, but they may also be isolated directly from tissue, including from both fetal and adult human brain (19–21). In the normal adult brain, GPCs disperse and persist widely throughout the parenchyma, within which they reside as relatively primitive neural precursors; when removed from the local tissue environment and raised *in vitro*, they are able to generate neurons as well as both astrocytes and oligodendrocytes (19,22). Yet *in vivo*, they appear restricted to glial fate, and appear to generate either or both astrocytes and oligodendrocytes depending upon their local signal environment. As such, GPCs may serve as transit amplifying intermediates between the ventricular zone neural stem cells and their terminally differentiated glial daughters. *In vitro*, both fetal and adult-derived GPCs are able to give rise to both astrocytes and oligodendrocytes, but adult glial progenitors differ markedly from their fetal counterparts in their slower turnover and greater ease of oligodendrocytic maturation and myelination (21,23).

Since GPCs can give rise to both oligodendrocytes—the sole myelinating cell type of the adult CNS—and astrocytes—the most prevalent cell type of the adult human CNS, and a key regulator of brain metabolic homeostasis—they have been assessed as potential therapeutic vectors in a variety of diseases with prominent glial involvement, especially in the congenitally hypomyelinating and lysosomal storage disorders. Glial progenitors are competent to differentiate as myelinogenic oligodendrocytes after transplantation (21,24–26), as a result of which they have been tested extensively in models of acquired adult demyelination, including both experimental allergic encephalomyelitis and spinal cord injury. However, their more immediate value may be in mediating the myelination of congenitally dysmyelinated hosts (21), since central oligodendrocytes are the primary, and often sole, victims of the underlying disease process. Indeed, given the relative availability and homogeneity of human oligodendrocyte progenitor cells, the disorders of myelin formation and maintenance may be especially compelling targets for cell-based neurological therapy. In addition, by distributing themselves throughout the deficient host neuraxis after perinatal allograft (27,28), both neural stem cells and glial progenitors appear to be of potential great utility in rectifying enzymatic deficiencies.

MYELIN REPLACEMENT IN EXPERIMENTAL MODELS OF CONGENITAL HYPOMYELINATION

A number of groups have assessed the potential of cell-based treatment for congenital dysmyelination, in genetic models of hypomyelination. The most common target of these attempts has been the shiverer mouse, a dysmyelinated mouse deficient in myelin basic protein (MBP), which was the first model of congenital hypomyelination in which some degree of remyelination could be accomplished through a cell transplant-based strategy (29,30). Whereas these first attempts used fetal brain tissues and dissociates thereof, later efforts were directed at using defined donor cell populations for this purpose. Snyder and colleagues (31) first reported context-dependent differentiation and myelination of myc-transduced murine neural stem cells in shiverer mice, and Schwartz and colleagues (32) subsequently reported the widespread dispersal and myelin production by EGF-expanded neural stem cells. In light of the isolation of adult human GPCs by Roy *et al*. (20), Windrem *et al*. (21) then transplanted enriched populations of human GPCs of both fetal and adult origin into newborn shiverer mice. In these experiments, fetal GPCs were extracted from the late second-trimester forebrain, and adult GPCs from surgically resected subcortical white matter, by fluorescence-activated or immunomagnetic sorting based upon the antigenic phenotype $A2B5^{+}$/PSA-$NCAM^{-}$, which identifies human GPCs with reasonable specificity and sensitivity (21). (Although these cells are often referred to as oligodendrocyte progenitors because of their myelinogenic potential, we use here the terminology glial progenitor cell, which recognizes the multilineage competence of the cells, while passing no judgment as to their oligodendrocyte or

astrocytic bias.) When introduced as highly enriched isolates, both fetal and adult-derived donor GPCs spread widely throughout the white matter, ensheathed resident mouse axons and formed antigenically and ultrastructurally compact myelin (Figs 1 and 2). Specifically, the donor GPCs dispersed widely throughout the shiverer forebrain white matter, such that single neonatal injections of GPCs into the lateral ventricles and adjacent callosum yielded abundant donor cell infiltration of the entire corpus callosum, fimbria and internal and external capsules, as well as the deep subcapsular white matter to the level of the cerebral peduncles (21) (Fig. 1A). Importantly, addition of a single intracerebellar injection at birth proved sufficient to substantially infiltrate the cerebellar white matter and peduncles, allowing cell dispersal throughout the brainstem (Fig. 1A).

The human donor GPCs developed as astrocytes and myelinating oligodendrocytes in a context-dependent fashion, such that those donor cells that engrafted presumptive white matter developed as oligodendrocytes, whereas those invading cortical and subcortical gray developed largely as astrocytes (Fig. 1B). The majority of donor cells engrafted the white matter, so that within 3 months after a single intracerebral injection of donor GPCs into neonatal shiverer mice, the hosts typically expressed MBP throughout the entire extents of their corpus callosa and internal capsules, to and beyond the cerebral peduncles (21).

Donor-derived myelin effectively ensheathed host axons, as noted by both confocal imaging and the ultrastructural observation of donor-derived myelin with major dense lines, indicating effective myelin compaction (Fig. 1F–I). In addition, confocal analysis revealed the presence of nodes of Ranvier between donor-derived myelinated segments, and the paranodal expression of Caspr protein suggesting functionally appropriate nodal architecture (Figs 1J and 2E). Importantly, those animals given additional cell injections into their cerebellar and brainstem white matter ultimately manifested widespread myelination, pari passu with their more extensive donor cell dispersal. These multiply transplanted animals exhibited prolonged survival relative to untreated shiverers and manifested progressively denser and more complete axonal ensheathment and myelination with time (Fig. 2). In light of the widespread dispersal of donor GPCs, their high-density engraftment and myelination, and their architecturally appropriate and quantitatively significant ensheathment of host axons, these results thus indicated the feasibility of neonatal progenitor cell implantation as a means of treating—and perhaps rescuing—the congenital disorders of myelin.

IDENTIFYING THE BEST CELLULAR AGENTS FOR TREATING THE LEUKODYSTROPHIES

Cell transplantation-based strategies for treating the demyelinating diseases require the acquisition of human neural and GPCs in both high purity and high yield. To address this need, several antibody-based methods for isolating GPCs from mixed cell populations have been developed (reviewed in 33,34). Most notably, the selective isolation and purification of both fetal and adult human GPCs, by both surface antigen-based fluorescence-activated cell sorting and magnetic cell sorting, have allowed the assessment of these cells as potential restorative agents in a variety of animal models of congenital dysmyelination (21,26). In shiverer mice, we noted that fetal and adult-derived GPCs behaved quite differently after neonatal xenograft. Isolates of human GPCs derived from adult white matter myelinated recipient brain much more rapidly than did fetal GPCs; adult-derived progenitors achieved widespread myelination by just 4 weeks after graft, whereas cells derived from late second-trimester fetuses took over 3 months to do so (21). The adult GPCs also generated oligodendrocytes more efficiently than fetal glial progenitors and ensheathed more axons per donor cell. In contrast, fetal glial progenitors emigrated more widely and engrafted more efficiently, differentiating as astrocytes in gray matter regions and oligodendrocytes in white matter (Fig. 1B).

The divergent behavior of fetal and adult-derived glial progenitors suggests their respective use for different disease targets. Fetal glial progenitors may prove more effective for treating disorders of dysmyelination due to enzymatic deficiency, such as occur in lysosomal storage disorders, since the extensive migration of fetal progenitors better assures their uniform and widespread dispersal, whereas their astrocytic differentiation and invasion of gray matter may offer the correction of enzymatic deficits in deficient cortex. In contrast, adult OPCs, by virtue of their oligodendrocytic bias and rapid myelination, may be most appropriate for diseases of acute oligodendrocytic loss, such as subcortical infarcts and post-inflammatory demyelinated lesions. The potential use of cell therapeutics in treating these primarily adult disorders of acute demyelination has recently been reviewed elsewhere (35), and will not be otherwise discussed here.

CELL-BASED STRATEGIES FOR TREATING LYSOSOMAL STORAGE DISORDERS

In the metabolic disorders of myelin, such as Krabbe's and Canavan's Diseases, oligodendrocytes are essentially bystanders, killed by toxic metabolites emanating from cells deficient in one or more critical enzymes (2,5,36). Since the engraftment of GPCs is associated with astrocytic as well as oligodendrocytic production, and since both the subcortical gray and cortical gray are infiltrated with donor-derived astrocytes after early implantation, fetal glial progenitors would seem an especially promising vehicle for the distribution of enzyme-producing cells throughout otherwise deficient brain parenchyma. On that basis, several groups have begun to assess the ability of enzymatically competent, effectively wild-type GPCs to delay or ameliorate the signs and symptoms of the lysosomal storage disorders and other metabolic leukodystrophies. Indeed, perinatal grafts of fetal progenitor cells might prove a means of simultaneously myelinating and correcting enzymatic deficiencies in the pediatric leukodystrophies. The lysosomal storage disorders present especially attractive targets in this regard, since wild-type lysosomal enzymes may be released by integrated donor cells and taken up by deficient host cells through the mannose-6-phosphate receptor pathway (37). As a result, a relatively small number of donor glia may provide sufficient enzymatic activity to correct the

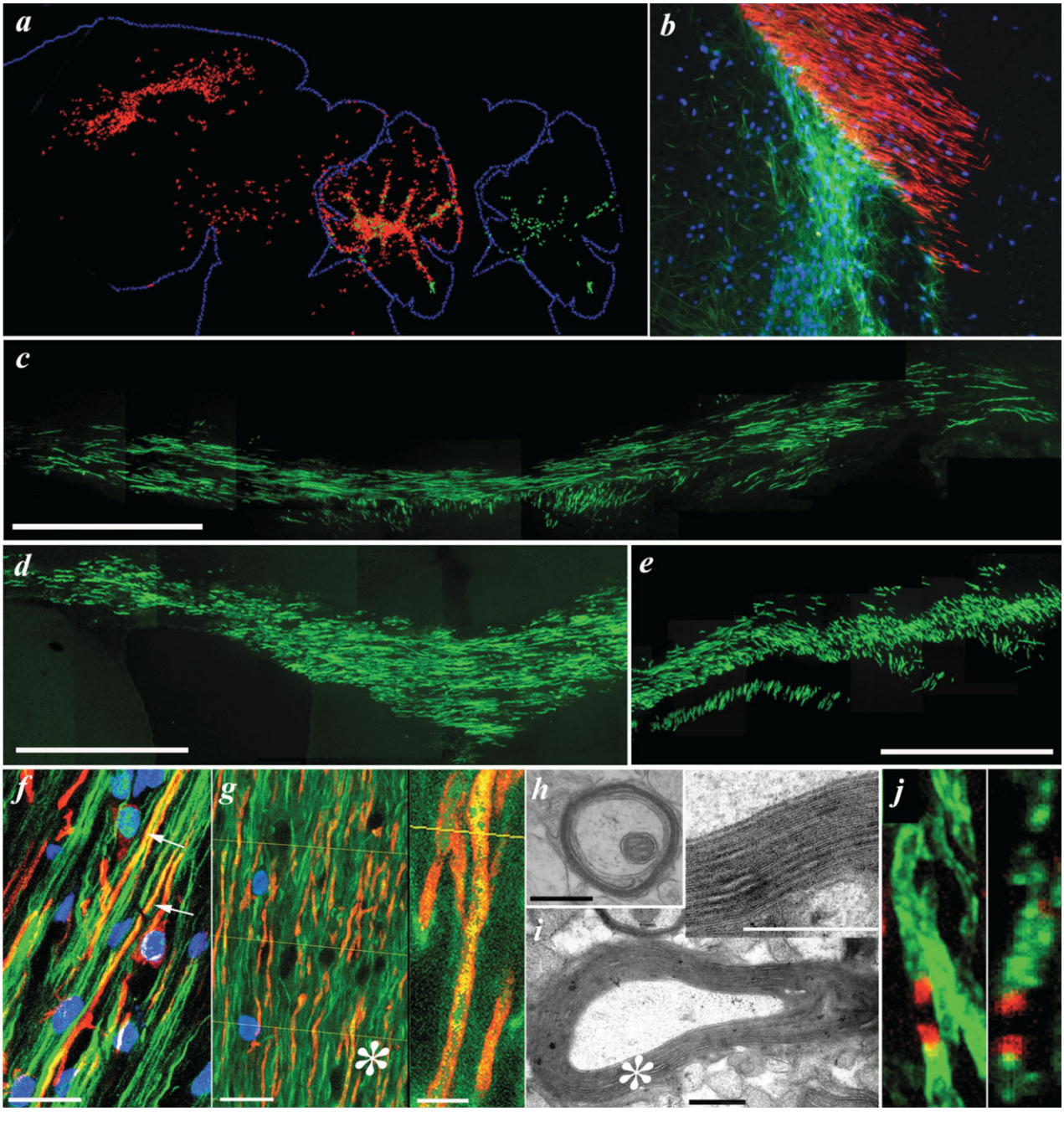

Figure 1. Myelination by engrafted human OPCs. (**A**) Implanted human fetal glial progenitors myelinated extensive regions of shiverer mouse forebrain. This animal was injected on P0 into the corpus callosum, cerebellar peduncles and cisterna magnum with 1×10^5 cells at each site, then sacrificed at day 60 and stained for human nuclear antigen (red) to identify donor cells. (**B**) The striatocallosal border of a shiverer brain, 3 months after engraftment with human fetal GPCs (hNA, blue). Donor-derived MBP (red) is evident in the callosum, whereas donor-derived $GFAP^+$ (green) astrocytes predominate in the striatum and along the ventricular wall. GPCs were thus recruited as oligodendrocytes or astrocytes in a context-dependent manner. (**C–E**) Extensive MBP expression by sorted human GPCs, implanted into homozygote shiverer mice as neonates, indicates that the corpus callosum (C–D, different mice) and internal capsules (E) have myelinated by 12 weeks (MBP, green). (**F**) A confocal micrograph showing a triple immunostain for MBP (red), human ANA (blue) and neurofilament protein (NF, green). In this image, all MBP immunostaining is derived from the sorted human GPCs, whereas the NF^+ axons are those of the mouse host. Arrows identify murine axons ensheathed by human MBP. (**G**) A 2 μm deep composite of optical sections taken through the corpus callosum of a shiverer recipient sacrificed 12 weeks after fetal OPC implantation. Shiverer axons were scored as ensheathed when the yellow index lines intersected an NF^+ axon flanked on each side by MBP. The asterisk indicates the field enlarged in the inset. (**H** and **I**) Representative electron micrographs of a 16-week-old shiverer homozygote implanted with human GPCs shortly after birth. The images show shiverer axons ensheathed by densely compacted myelin. The asterisk indicates the field enlarged in the inset. Inset: major dense lines are noted between lamellae, providing EM confirmation of myelination. (**J**) High-power confocal images of MBP^+ donor-derived myelin sheaths (green) spanning myelin internodes, characterized by expression of Caspr protein (red) at the paranodal segments. Left: a z-stack composite; right, a single 0.4 μm optical section. Caspr staining thus confirmed nodes of Ranvier between adjacent donor-derived myelinated segments; these results suggest physiologically appropriate conduction support by donor-derived myelin. Scale: (F), 20 μm; (G), 40 μm; (H–I), 1 μm. Reprinted from Keyoung and Goldman (35); adapted from Windrem *et al*. (21).

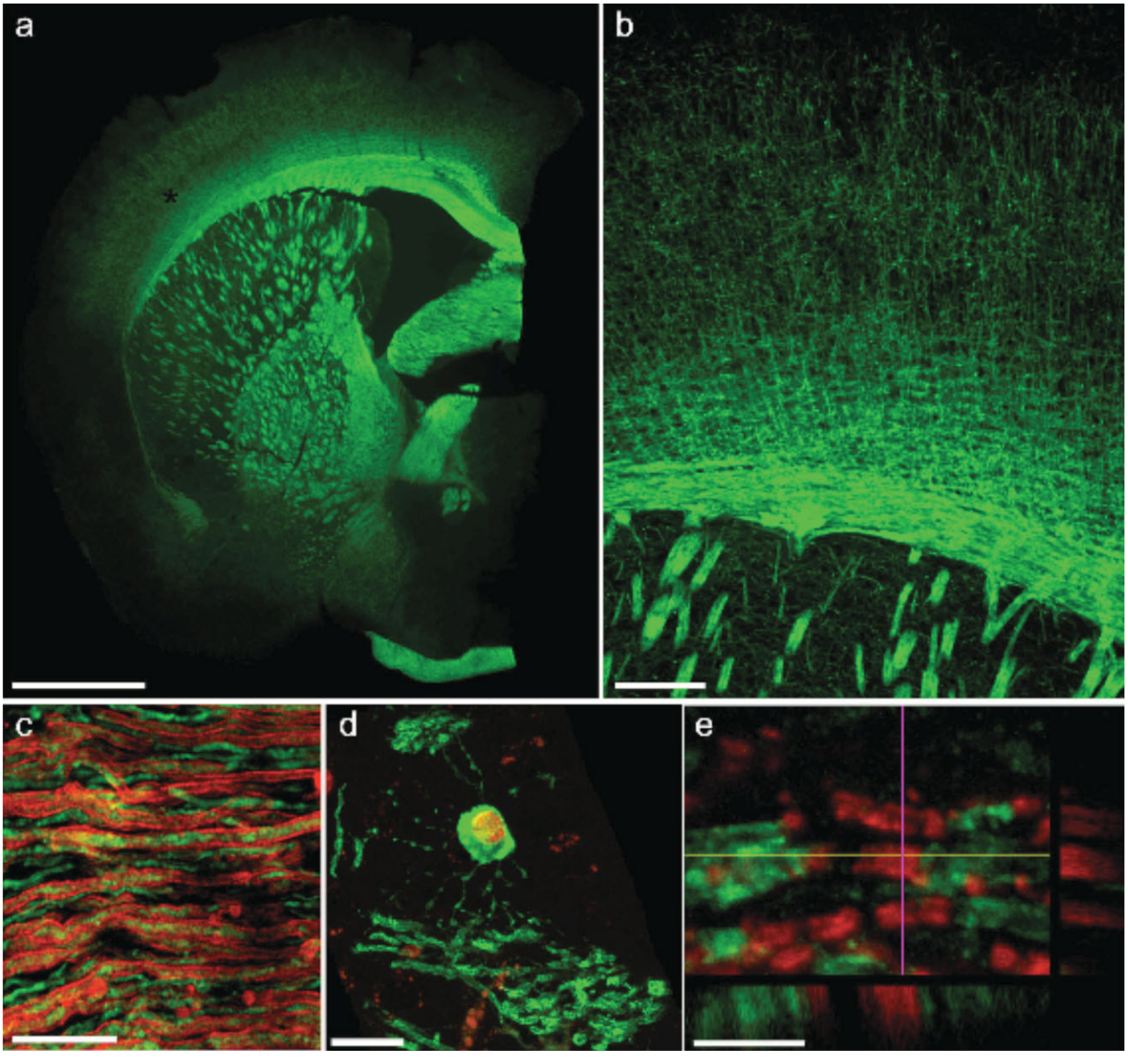

Figure 2. Donor human GPCs can stably and efficiently reconstitute myelin-deficient host white matter. These images are taken from either a 1-year-old (**A**–**C**) or a 35-week-old (**D** and **E**) double homozygous shiverer × rag2 null immunodeficient and myelin-deficient mouse, implanted at birth with $A2B5^{+}$/$PSA\text{-}NCAM^{-}$ sorted human GPCs. (A) A low magnification coronal image of the transplanted shiverer × rag2 null forebrain, immunostained for MBP (green); as in Figure 1, since the shiverer is MBP-deficient, all of the MBP immunoreactivity here is perforce of human origin. (B) A higher magnification view illustrates the high efficiency myelination of intrastriatal and lower cortical as well as callosal fibers. (C) A confocal image showing the donor myelin (MBP, red)-ensheathed host axons (neurofilament, green), imaged in the cervical spinal cord of a 1-year-old transplanted shiverer × rag2 null mouse. (D) An individual human oligodendrocyte (human nuclear antigen, red; MBP, green), imaged as a 1 μm stack of optical sections in the striatum of a 35-week-old neonatally transplanted shiverer. This single-donor cell can be seen to extend projections to, and concurrently ensheath, well over a dozen fibers within the plane of view. (**E**) An optical section through the cerebellar white matter of the same 35 week transplanted shiverer, manifesting the normal nodal organization of its donor-myelinated axons. Caspr (a paranodal protein), red; Caspr2 (a juxtaparanodal marker), green. Scale: (A), 1 mm; (B), 200 μm; (C), 10 μm; (D), 20 μm; (E), 5 μm.

underlying catalytic deficit and storage disorder of a much larger number of host cells (38).

The cell-based rescue of enzymatically deficient host cells by wild-type donor NSC implantation was first noted in a mouse model of Sly's Disease (MPS-VII), in which myc-transduced NSCs were implanted neonatally and observed to migrate widely and restore lost enzymatic function broadly in the recipient forebrain (28). The same group subsequently reported expression of β-hexosaminidase upon engraftment of transduced neural stem cells into recipient mice (39). Importantly, when human neural stem cells were transplanted to the neonatal β-hexosaminidase-deficient Sandhoff's mouse, this approach yielded not only significant engraftment-associated enzyme expression, but also a corresponding functional and survival benefit to the engrafted hosts (27). Similarly, Pellegatta *et al.* (40) recently engrafted twitcher mice, a murine model of

Krabbe's globoid cell leukodystrophy, with cultured neural stem cells transduced to over-express galactocerebrosidase, the enzyme deficient in Krabbe's disease. Although the engrafted cells did not survive well in the highly inflammatory twitcher brain, they migrated appropriately to active sites of demyelination, in a manner akin to that noted in adults with experimental allergic encephalomyelitis by Martino *et al.* (41,42). One might hope that in recipients immunosuppressed to reduce both local inflammation and donor cell rejection, future trials may be better able to assess the capacity of engrafted neural stem or progenitor cells to restore lost structure and function in the lysosomal storage disorders.

It is worth noting that as an alternative to the use of neural or GPCs for enzymatic replacement in the CNS, Kurtzberg and colleagues (43) have reported clinical benefit in infants with Krabbe's disease transplanted with allogeneic umbilical cord blood stem cells. Asymptomatic Krabbe's patients receiving these cell grafts exhibited slower disease progression than both unimplanted controls and those transplanted after symptom onset. Indeed, the marked differences in outcome between patients implanted before and after symptom onset strongly suggest the wisdom of initiating treatment as early as possible after genetic diagnosis in these children; this may prove the case with GPCs as well as with umbilical and hematopoietic cell sources, at least when the therapeutic intent is enzyme replacement.

Yet despite the promise of using non-neural cell grafts in some enzyme deficiency-associated demyelinating diseases, many of these require replacement of enzymes expressed only by neural and glial cells and will thus necessarily require neural cell grafts. For example, MLD is characterized by deficient expression of arylsulfatase A, which results in sulfatide misaccumulation and oligodendrocyte loss. Mesenchymal and hematopoietic stem cell grafts have proven unable to correct the CNS manifestations of this disorder (44), yet experimental models of MLD have responded well to GPC grafts (45). Similarly, the neuronal ceroid lipofuscinoses will likely require neural cell grafts for their cell-based treatment, as the enzymes deficient in this class of disorders are largely neural in their normal expression. In this regard, a recently initiated trial to assess the use of human neural stem cell allografts in treating Batten's disease (NCL2) speaks to the efforts that may be anticipated in developing the use of engrafted neural stem and GPCs as vehicles for intracerebral enzyme replacement, in both the lysosomal storage disorders as well as other genetic disorders of brain metabolism characterized by substrate misaccumulation or aberrant catabolism.

CHALLENGES FOR THE USE OF GPC GRAFTS IN THE PEDIATRIC LEUKODYSTROPHIES

One might hope that in recipients immunosuppressed to reduce donor cell rejection, engrafted progenitors may indeed prove competent to prevent progressive demyelination in the lysosomal storage disorders and metabolic leukodystrophies. However, little data currently exist with regard to the number or proportion of wild-type cells required to achieve local correction of enzymatic activity and substrate clearance in any storage disorder, and these values will likely need to be obtained for each disease target. Similarly, effective cell doses, delivery sites and time frames will need to be established in models of congenital hypomyelination before clinical trials of progenitor-based therapy can be contemplated. Moreover, the efficiency of myelination required for significant benefit remains undecided, as functional improvement may require remyelination over much if not the entire linear extent of each recipient axon. These caveats notwithstanding, there is reason for optimism that cell-based therapy of the pediatric myelin disorders, in particular for the primary dysmyelinations such as Pelizaeus–Merzbacher disease, vanishing white matter disease and the spastic diplegic forms of cerebral palsy, may not be far off.

ACHIEVING ABUNDANCE AND ESCAPING IMMUNE REJECTION: EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENTIAL CELLS AS SOURCES OF TRANSPLANTABLE PROGENITORS

The practical limitations on both fetal and adult cell acquisition for human allograft have driven research on deriving tissue-specific progenitor cells from human embryonic stem (hES) cells. Oligodendrocytes derived from hES cells were recently shown to myelinate demyelinated foci in spinal cord contusions (46). This latter observation paralleled earlier studies that reported myelination in the injured spinal cord by implanted murine ES cells (47). However, neither of these studies isolated glial progenitors or oligodendrocytes prior to transplantation, and neither followed animals for the long periods of time required to ensure the long-term survival and phenotypic stability of the engrafted cells. In particular, these ES-based approaches may prove limited by the potential for tumorigenesis, in particular, by the potential for any persistent undifferentiated ES cells in the donor pool to yield either teratomas or undifferentiated neuroepithelial tumors after implantation (48). As a result of these considerations, stringent selection for, and purification of, committed GPCs will have to be applied so as to completely deplete donor cell populations of any undifferentiated ES cells before hES cell-based therapy may be safely contemplated. Until that time, the implantation of tissue-derived GPCs will necessarily be the more clinically feasible option.

OVERVIEW

In most developmental disorders of myelination, resident OPCs are themselves either lost—as in prenatal stroke and cerebral palsy—or diseased—as in the hereditary and metabolic leukodystrophies. In such cases, it is likely that for the foreseeable future at least, remyelination may only be accomplished by a transplantation-oriented approach. Nonetheless, the apparent efficiency with which donor cells can disperse and myelinate the otherwise dysmyelinated CNS in experimental models provides a sound basis for optimism that cell-based remyelination may provide an effective means for treating both infants and children with congenital disorders of myelin formation. We can reasonably predict that disorders of myelin formation, such as Pelizaeus–Merzbacher Disease or periventricular leukomalacia, of myelin maintenance, such

as vanishing white matter disease, and of postnatal demyelination, such as occurs in the lysosomal storage disorders, might all become targets of GPC-based therapeutic trials in the coming years.

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Conflict of Interest statement. None declared.

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