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Glial Progenitor–Based Repair of Demyelinating Neurological Diseases

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Oligodendrocytes are the sole source of myelin in the adult central nervous system (CNS), and their loss or dysfunction is at the heart of a wide variety of diseases of children and adults [1]. In children, the hereditary and metabolic leukodystrophies accompany the vascular dysmyelination (defective formation or breakdown of an immature myelin sheath, usually involving biochemical or genetic abnormalities) of periventricular leukomalacia as major sources of neurologic morbidity [2]. In adults, oligodendrocytic loss is causal in hereditary and inflammatory diseases as diverse as the metabolic leukoencephalopathies and multiple sclerosis (MS) [3]. Demyelination, defined as destruction, removal, or loss of the mature myelin sheath of a nerve or nerves, is also a prominent feature of vascular and traumatic injury, such as typifies spinal cord injury, traumatic brain injury, and stroke [4]. In addition, demyelination is noted in conditions of functional deterioration as varied as Alzheimer's disease [5], normal aging [6], and schizophrenia [7], although in the latter disorders, demyelination may attend rather than predict pathologic change. In light of the extraordinary range of disorders to which oligodendrocytic loss or demyelination may contribute and the apparent relative homogeneity of central oligodendrocytes and their progenitors, the demyelinating diseases are especially attractive targets for cell-based therapeutic strategies. As a result, oligodendrocyte progenitor cells (OPCs) have become a target of study for those interested in restoring myelin to demyelinated regions of the diseased or injured CNS. In this article, the authors focus on current efforts to develop the use of isolated human OPCs as transplantable agents for mediating therapeutic remyelination.

Stem cells for cellular therapy

Neural stem cells (NSCs) are undifferentiated neuroepithelial cells capable of proliferation, self-renewal, and derivation of lineage-restricted differentiated glial and neuronal cells [8,9]. NSCs are found in discrete ventricular zone germinal compartments in the adult brain, and their derived neuronal progenitors similarly reside within discrete regions of the ventricular wall, olfactory subependyma, and dentate gyrus of the hippocampus (Fig. 1) [10–12]. In contrast, the glial progenitor derivatives of NSCs seem to disperse and persist widely throughout the adult brain parenchyma. Additionally, glial progenitors reside in the ventricular zone and in tissue parenchyma [13]. These cells are relatively primitive neural precursors and are able to generate neurons as well as astrocytes

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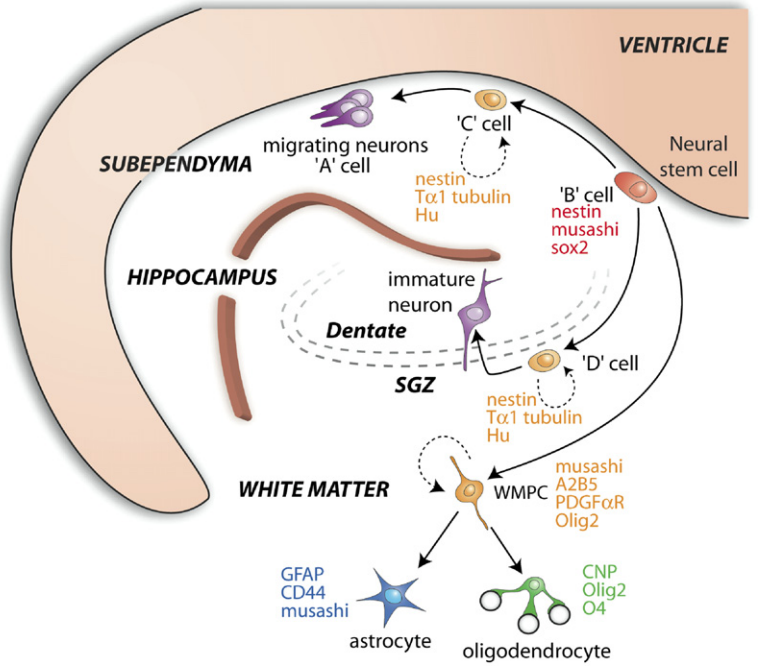

Fig. 1. Stem and progenitor cells of the adult human brain. This diagram illustrates the basic categories of progenitor cells in the adult brain and their lineal relations as well as markers and combinations thereof that permit their enrichment. The human periventricular NSCs (*pink*) generate at least three populations of potentially neurogenic transit-amplifying progenitors of neuronal and glial lineage (*blue*). These include the neuronal progenitor cells of the ventricular subependyma, those of the subgranular zone of the dentate gyrus, and the glial progenitor cells of the subcortical white matter. Each transit-amplifying pool may then give rise to differentiated progeny appropriate to their locations, including neurons (*purple*), oligodendrocytes (*green*), and parenchymal astrocytes (*orange*). A- through C-cell stage terminology is derived from Alvarez-Buylla and Garcia-Verdugo [61]. Markers defining each stage have been reviewed previously [4]. CNP, cylcic nucleotide phosphodiesterase; GFAP, glial fibrillary acidic protein; PDGFαR, platelet-derived growth factor α receptor; SGZ, subgerminal zone; WMPC, white matter progenitor cell. (*Adapted from* Goldman S. Stem and progenitor cell-based therapy of the human central nervous system. Nat Biotechnol 2005;23(7):863; with permission.)

and oligodendrocytes when removed from the local tissue environment and raised in vitro [14–16]. Yet, in vivo, they seem restricted to glial fate and can generate astrocytes and oligodendrocytes, depending on their local signal environment. As such, these cells seem to serve as transit-amplifying intermediates between the ventricular zone NSC and its terminally differentiated glial daughters. Importantly, these cells may be isolated to purity and are able to generate myelinogenic oligodendrocytes on transplantation [16].

Deriving optimal cellular vectors for the leukodystrophies

Cell transplantation–based strategies for treating the demyelinating diseases require the acquisition of human neural and glial progenitor cells in high purity and high yield. To this end, the authors have established methods for the selective isolation and purification of fetal and adult OPCs and have assessed the competence and efficiency of each as a potential vector for cell therapy of the congenitally dysmyelinated CNS (Fig. 2) [3,17–19]. Interestingly, the authors noted that fetal and adult-derived OPCs behave quite differently after neonatal xenograft [3]. Isolates of human OPCs derived from adult white matter myelinated recipient brain myelinated much more rapidly than did fetal OPCs; adult-derived progenitors achieved widespread myelination by just 4 weeks after graft, whereas cells derived from late second-trimester fetuses took longer than 3 months to do so. The adult OPCs also generated oligodendrocytes more efficiently than fetal glial progenitors and ensheathed more axons per donor

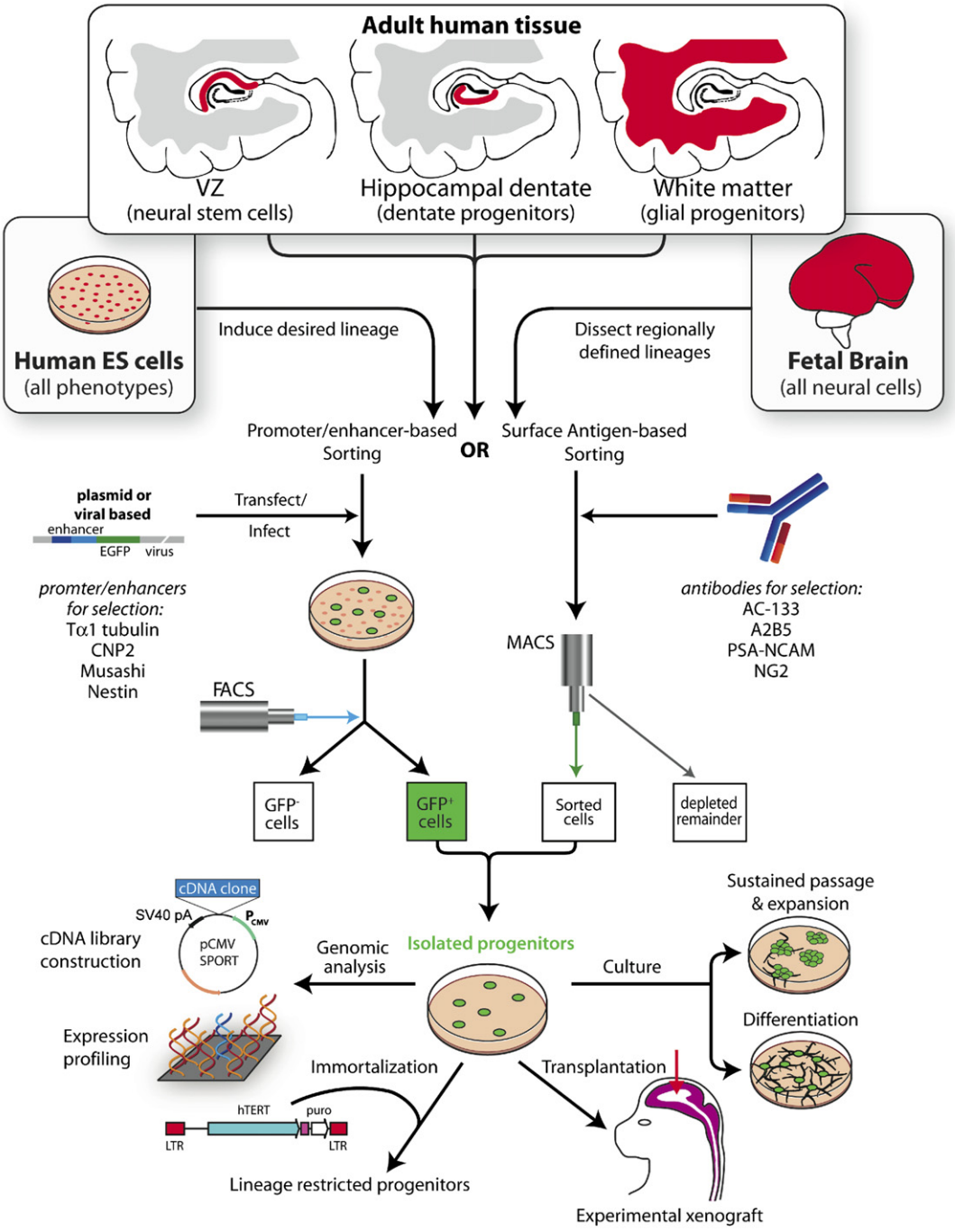

Fig. 2. Sources, isolation, and use of defined progenitor phenotypes. This figure schematizes the methods of isolating prospectively defined glial progenitor phenotypes from a variety of human cell and tissue sources and highlights potential strategies to achieve clinical trial. CNP2, 2,3-cylcic nucleotide phosphodiesterase; EGFP, enhanced green fluorescent protein; ES, embryonal stem; FACS, fluorescence-activated cell sorting; GFP, glial fibrillary protein; hTERT, human telomerase reverse transcriptase; LTR, long terminal repeat; MACS, magnetic cell sorting; pCMV, plasmid cytomegalo virus vector; puro, puromycin; PSA-NCAM, polysialylated-neural cell adhesion molecule; VZ, ventricular zone. (*Adapted from* Goldman S. Stem and progenitor cell-based therapy of the human central nervous system. Nat Biotechnol 2005;23(7):864; with permission.)

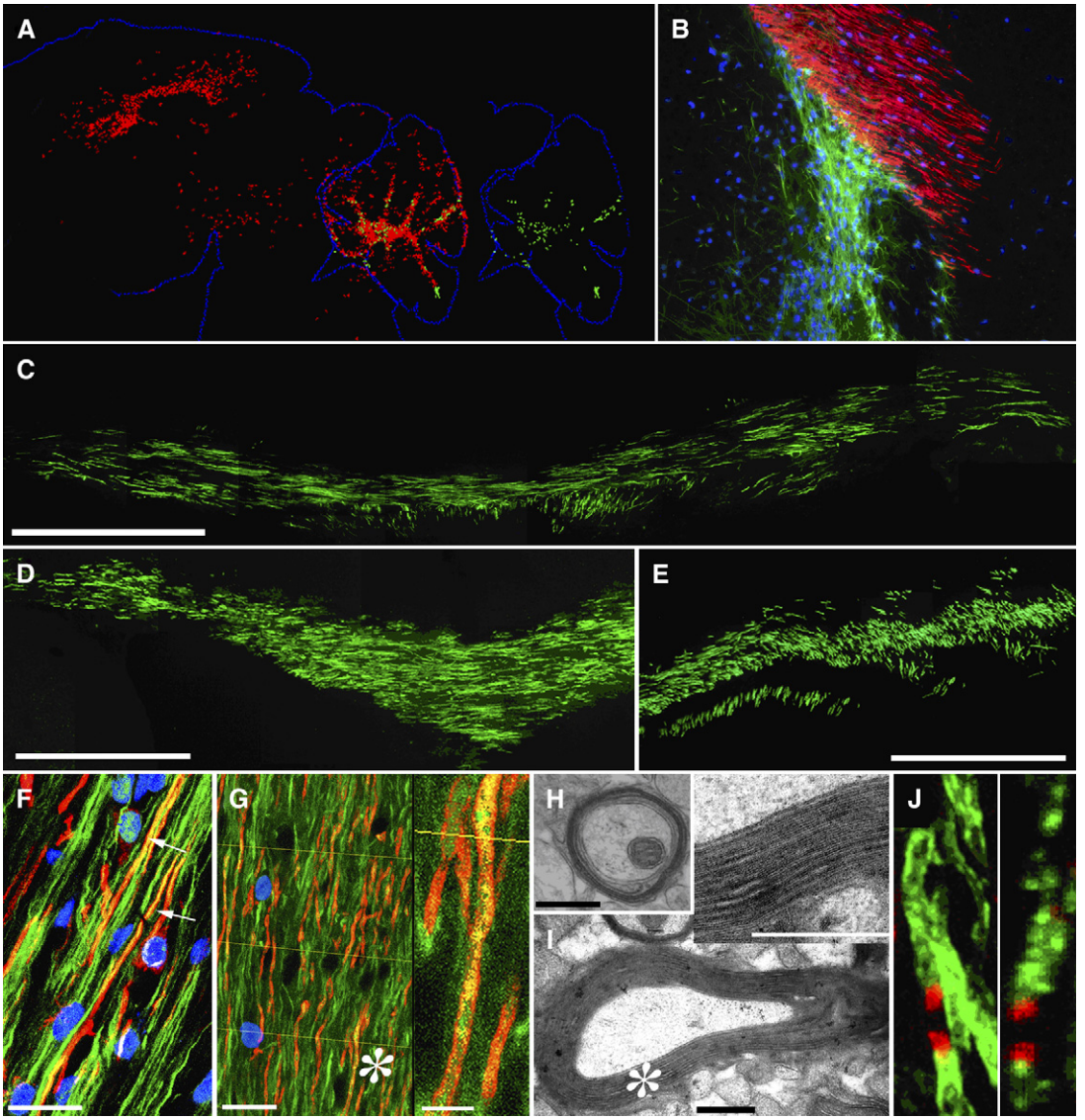

Fig. 3. Myelination by engrafted human OPCs. (*A*) Implanted human fetal OPCs myelinated extensive regions of shiverer mouse forebrain. This animal was injected on postnatal day 0 into the corpus callosum, cerebellar peduncles, and cisterna magnum with 1×10^5 cells at each site and then killed at day 60 and stained for human nuclear antigen (*red*) to identify donor cells. (*B*) Striatocallosal border of a shiverer brain 3 months after engraftment with human fetal OPCs (human nuclear antigen, *blue*). Donor-derived MBP (*red*) is evident in the callosum, whereas donor-derived glial fibrillary acidic protein–positive (*green*) astrocytes predominate in the striatum and along the ventricular wall. OPCs were thus recruited as oligodendrocytes or astrocytes in a context-dependent manner. (*C–E*) Extensive MBP expression by sorted human OPCs implanted into homozygote shiverer mice as neonates indicates that large regions of the corpus callosum (C, D; different mice) have myelinated by 12 weeks (MBP, *green*). (*E*) OPCs myelinated fibers throughout the dorsoventral extent of the internal capsules. (*F*) Confocal micrograph shows triple immunostaining for MBP (*red*), human antinuclear antibody (*blue*), and neurofilament protein (*green*). In this image, all MBP immunostaining is derived from the sorted human OPCs, whereas the neurofilament protein–positive axons are those of the mouse host. Arrows identify murine axons ensheathed by human MBP. (*G*) Two-micrometer deep composite of optical sections taken through the corpus callosum of a shiverer recipient killed 12 weeks after fetal OPC implantation. Shiverer axons were scored as ensheathed when the yellow index lines intersected a neurofilament protein–positive axon abutted on each side by MBP. The asterisk indicates the field enlarged in the inset. (*H–I*) Representative electron micrographs of 16-week-old

cell. In contrast, fetal glial progenitors emigrated more widely and engrafted more efficiently, differentiating as astrocytes in gray matter regions and as oligodendrocytes in white matter (Fig. 3) [3].

The divergent behaviors of fetal and adult-derived glial progenitors raise the possibility of distinct uses for different disease targets. Fetal progenitors may prove more effective for treating disorders of dysmyelination attributable to enzymatic deficiency, such as those that occur in lysosomal storage disorders, because the extensive migration of fetal progenitors better ensures 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 postinflammatory demyelinated lesions. The authors consider each of these clinical situations in turn.

Childhood disorders of myelin as targets for glial progenitor transplantation

Tens of thousands of children in United States have a variety of diseases of myelin failure or loss, including the hereditary leukodystrophies associated with lysosomal and peroxisomal enzymatic deficiency and the less common congenital hypomyelinations, such as Pelizaeus-Merzbacher disease (Table 1) [4,20]. Even more common pediatric afflictions, such as cerebral palsy, may be attributable largely to a perinatal loss of oligodendrocytes and their precursors [20]. More specifically, children have a variety of hereditary diseases of myelin failure or loss, including the following [20–22]:

1. The hypomyelinating diseases, such as Pelizaeus-Merzbacher disease and hereditary spastic paraplegia, which represent primary disorders of myelin formation
2. The metabolic demyelinations and lysosomal storage disorders, such as metachromatic leukodystrophy (MLD) and Krabbe's disease as well as adrenoleukodystrophy
3. The myelinoclastic disorders of frank white matter loss, such as vanishing white matter disease and Canavan's disease, in which oligodendrocytes are early targets
4. A variety of hereditary metabolic and lysosomal storage disorders that are manifested by early neuronal loss but later predictable demyelination, such as the gangliosidoses, organic acidurias, and neuronal ceroid lipofuscinoses

In addition, periventricular leukomalacia, the most common single form of cerebral palsy, may be partially attributable to a perinatal loss of oligodendrocytes and their precursors [21,23,24]. Their mechanistic heterogeneity notwithstanding, all 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 on the transplantation of glial progenitor cells (see Table 1).

Neonatal delivery of oligodendrocyte progenitor cells as a treatment for congenital leukodystrophies

To assess the potential of cell-based treatment for congenital dysmyelination, Windrem and colleagues [3,17,19] transplanted sorted human OPCs

shiverer homozygotes implanted with human OPCs 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 electron microscopic confirmation of myelination. (*I*) High-power confocal images of MBP-positive donor-derived myelin sheaths (*green*) at internodal junctions, characterized by expression of Caspr protein (*red*) at the paranodal borders. (*Left*) Z-stack composite. (*Right*) Single 0.4-μm optical section. Caspr staining confirmed nodes of Ranvier between adjacent donor-derived myelinated segments [62]; these results suggest physiologically appropriate conduction support by donor-derived myelin. Scale: 20 μm (*F*), 40 μm (*G*), and 1 μm (*H–I*). (*From* Goldman SA, Lang J, Roy NS, et al. Progenitor cell-based myelination as a model for cell-based therapy of the CNS. Ernst Schering Foundation Symposium. In: Morser J, Nishikawa S-I, Schoeler H, editors. Stem cells in reproduction and the brain, vol. 60. Berlin: Springer-Verlag; 2006. p. 204; with permission. *Individual photos adapted from* Goldman S. Stem and progenitor cell-based therapy of the human central nervous system. Nat Biotechnol 2005;23(7):865; and Windrem MS, Nunes MC, Rashbaum WK, et al. Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat Med 2004;10(1):94. Reprinted by permission from Macmillan Publishers Ltd.)

Table 1
Central demyelinating pathologic findings

Perinatal disease targets	Adult disease targets
Hereditary leukoencephalopathies	**Vascular leukoencephalopathies**
Congenital dysmyelination	*Subcortical stroke*
Pelizaeus-Merzbacher disease	*Diabetic leukoencephalopathy*
Vanishing white matter disease, 18 q syndrome	*Hypertensive leukoencephalopathy*
Hereditary leukodystrophies with demyelination (eg, ALD, Cockayne's disease, Canavan's disease)	
Lysosomal storage diseases with primary or secondary demyelination (eg, MLD, Krabbe's disease, Niemann-Pick disease, Tay-Sachs/Sandhoff's disease)	
Cerebral palsy	**Inflammatory leukoencephalopathies**
Periventricular leukomalacia	*Radiation-induced demyelination*
Spastic diplegias of prematurity	*Multiple sclerosis*
	Transverse myelitis
	Traumatic injury
	Spinal cord demyelinative injury
	Metabolic disease
	Central pontine myelinolysis
	Posthypoxic demyelination

These pediatric and adult demyelinating and dysmyelinating diseases represent possible targets of cellular transplantation.

Abbreviations: ALD, adrenoleukodystrophy; MLD, metachromatic leukodystrophy.

of fetal and adult origin into newborn shiverer mice, a dysmyelinated mouse deficient in myelin basic protein (MBP). In this set of experiments, fetal OPCs were extracted from the late second-trimester forebrain and adult OPCs were extracted from surgically resected subcortical white matter by fluorescence-activated or immunomagnetic sorting based on the antigenic phenotype A2B5-positive/polysialylated-neural cell adhesion molecule (PSA-NCAM)–negative, which identifies human OPCs with reasonable specificity and sensitivity (see Fig. 2). When introduced as highly enriched isolates, fetal and adult-derived donor OPCs spread widely throughout the white matter, ensheathed resident mouse axons, and formed antigenically and ultrastructurally compact myelin [3].

Using both donor cell sources, the OPCs dispersed widely throughout the neonatal shiverer forebrain white matter, such that single neonatal injections of OPCs 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. Although the dorsal brain stem was not infiltrated by cells introduced to the forebrain, addition of a single intracerebellar injection at birth proved sufficient to infiltrate the cerebellar white matter, peduncles, and dorsal brain stem substantially, allowing donor engraftment contiguous with that of the forebrain and ventral brain stem. These cells migrated as widely as did native and immortalized murine NSCs, which have been reported to be similarly capable of context-dependent differentiation and myelination [25,26].

The human donor OPCs developed as astrocytes and myelinating oligodendrocytes. Remarkably, they did so in a highly context-dependent fashion, such that those donor cells engrafting presumptive white matter developed as oligodendrocytes, whereas those invading cortical and subcortical gray developed largely as astrocytes [3]. Most donor cells engrafted the white matter, such that after neonatal intraventricular and intracallosal injection, the corpus callosum typically expressed MBP throughout its mediolateral extent, along its entire length in the sagittal plane, and throughout the dorsoventral extent of the internal capsules to the cerebral peduncles [3]. Donor-derived myelin effectively ensheathed host shiverer axons, as validated by confocal imaging and ultrastructural observation of donor-derived myelin with major dense lines, indicating effective myelin compaction, of which native shiverer oligodendrocytes are incapable. 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. Given the widespread dispersal of donor cells, their high-density engraftment and myelination, and their architecturally appropriate and quantitatively significant ensheathment of host axons, these results strongly suggested the feasibility of neonatal progenitor cell implantation as a potential therapeutic strategy in the congenital disorders of myelin formation.

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 [20,22,27]. Because the engraftment of glial progenitor cells is associated with astrocytic as well as oligodendrocytic production and because the subcortical and cortical gray matter is infiltrated with donor-derived astrocytes after early implantation, 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 and effectively wild-type glial progenitor cells 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 [28]. The lysosomal storage disorders present especially attractive targets in this regard, because 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 [29]. As a result, a relatively small number of donor glia may provide sufficient enzymatic activity to correct the underlying catalytic deficit and storage disorder of a much larger number of host cells [30].

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 on engraftment of transduced NSCs into recipient mice [31], although functional benefits accruing to engraftment-associated enzyme expression have not yet been reported. Similarly, Pellegatta and coworkers [32] recently engrafted twitcher mice, a murine model of Krabbe's globoid cell leukodystrophy, with cultured NSCs transduced to overexpress 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 by Pluchino and colleagues [33,34].

As an alternative to the use of neural or glial progenitor cells for enzymatic replacement in the CNS, Kurtzberg and colleagues [35] have reported clinical benefit in infants with Krabbe's disease transplanted with allogenic umbilical cord blood stem cells. Asymptomatic patients with Krabbe's disease receiving these cell grafts exhibited slower disease progression than unimplanted controls and those transplanted after symptom onset [35]. Indeed, the marked differences in outcome between patients implanted before and after symptom development strongly suggest the wisdom of initiating treatment as early as possible after genetic diagnosis in these children; this may prove the case with OPCs as well as with umbilical and hematopoietic sources of engraftable cells, assuming that the therapeutic intent is for enzyme replacement.

It is worth noting that despite the promise of using nonneural cell grafts in some enzyme-deficiency–associated demyelinating diseases, many of these require replacement of enzymes only expressed by neural and glial cells, and thus require neural cell grafts. By way of example, MLD is characterized by deficient expression of arylsulfatide A (ARSA), which results in sulfatide misaccumulation and oligodendrocyte loss. Hematopoietic stem cell grafts have proven unable to correct the CNS manifestations of this disorder [36,37], which have instead responded to glial progenitor cell grafts in experimental models of MLD [38].

Challenges for the use of oligodendrocyte progenitor cell 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. Few 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, however, and these values likely need to be obtained for each disease target. Similarly, effective cell doses, delivery sites, and time frames 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, because 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, particularly 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.

Adult dysmyelinating diseases as therapeutic targets

In adults, oligodendrocytic loss is causal in disease as diverse as the vascular leukoencephalopathies; traumatic spinal cord and brain injury; and MS and its variants, transverse myelitis and optic neuritis. To date, most experimental models of transplant-based remyelination have focused on MS, an incurable physically and psychologically debilitating disease characterized by an inflammatory loss of myelin and a degenerative axonal loss. The attraction of MS as a therapeutic target is based on its high incidence, and extraordinary prevalence, given its typical onset in youth and protracted disease course. More than 200 young adults are diagnosed each week, with more than 300,000 affected in the United States alone. As a result, the National Institute of Neurological Disease and Stroke (NINDS) estimates that MS alone costs the nation more than $2.5 billion annually. The clinical course in MS is heterogeneous; the presentation in 80% of patients is initially relapsing and remitting, with the remainder having progressive deterioration from the onset (primary progressive) [39]. Within 10 years, approximately one half of patients with relapsing and remitting disease develop progressive features (secondary progressive). Historical longitudinal data suggest that half of the patients with MS are likely to lose the ability to walk unaided within 15 years of diagnosis and that overall life expectancy is reduced by roughly 10 years [40,41].

Conventional immunosuppressive and immunomodulatory therapies, such as β-interferon, Copaxone, pulsed intravenous steroids, and cyclophosphamide, are limited in their control of disease progression [42]. These treatment options are most effective during the relapsing-remitting phase of MS, although mitoxantrone has been approved for use in slowing the progression of chronic progressive disease [43]. Natalizumab has shown promise in providing longer term relief of disease, but its use has been clouded by the appearance of progressive multifocal encephalopathy in several treated patients [44]. In response to the difficulties in developing pharmacologic immune suppression as a treatment strategy in MS, bone marrow transplantation after immune ablation has been attempted, although its morbidity, especially after allogeneic graft, has proven rate limiting [45–47].

As a result of these limitations of current therapy, OPC engraftment has been assessed in a variety of models of adult acquired demyelination. The systemic administration of NSCs in mice subjected to experimental allergic encephalomyelitis resulted in some degree of local engraftment and improved remyelination [34]. Indeed, these NSCs seemed to persist as undifferentiated cells and exerted neuroprotective effects by inducing programmed cell death of blood-borne, CNS-infiltrating, proinflammatory T helper (T_H) 1 (but not anti-inflammatory T_H2) cells [48,49]. In this way, the injected NSCs acted as local immune suppressants, thereby slowing disease progression within these animals with experimental allergic encephalomyelitis. Indeed, these mice exhibited decreases in relapse rates and axonal loss as well as in demyelination. In addition, most engrafted stem cells remained undifferentiated, which is of potential benefit in providing reserve in a chronic and relapsing inflammatory disease like MS [33,50,51].

In contrast to the immune suppressive strategy incorporated by systemic NSC administration, OPCs may be administered directly to demyelinated brain as a means of direct remyelination. Using this approach, when Windrem and colleagues [17] transplanted adult human OPCs directly into lysolecithin-induced demyelinating lesions within the adult rat brain, they observed that the cells quickly matured as oligodendrocytes and myelinated residual denuded host axons but with relatively low efficiency compared with the robust myelination noted using similar donor cells in congenitally

hypomyelinated brain [3,17]. Thus, although donor glial progenitor cells would seem to be effective cellular vectors for remyelination, the complexity of the adult disease environment may make such adult targets less approachable than their pediatric counterparts [52]. At the least, any cell-based therapeutic strategies for adult demyelination, especially those intended to remyelinate acute and chronic lesions of MS, require aggressive disease modification and immunosuppression as adjuncts to cell delivery.

Cell-based strategies for treating the injured or diseased spinal cord

The spinal cord has been an attractive target for cell-based therapeutic attempts, partially because of the dearth of available treatment options for spinal cord injury and trauma and partially because of the relative simplicity of spinal cord neural circuits compared with those of the brain. Most importantly, many traumatic cord injuries are primarily demyelinative; the sensory tracts of the posterior columns and the descending motor tracts of the corticospinal pathways are frequent victims of flexion-extension and contusion injuries of the cord. Indeed, such traumatic disruption as well as spinal cord stroke and iatrogenic perioperative occlusion, can result directly in the segmental loss of the superficial white matter tracts. These surface pathways are also especially predisposed to ischemic damage after cord edema, with the draining veins on the posterolateral surfaces of the spinal cord being especially predisposed to congestion and hypoperfusion when edematous. As a result, the transplantation of myelinogenic glial progenitors has been an especially appealing strategy for treating spinal cord injury. Indeed, this approach has been considered as a potential treatment for the more restricted segmental demyelinations, such as those that occur in transverse myelitis. Rao and colleagues [53] demonstrated that glial-restricted progenitors implanted into the contused rat spinal cord dispersed widely, with astrocytic and oligodendrocytic maturation. Although neither myelination nor the net efficiency of oligodendrogliogenesis was reported in this study, it clearly suggested the utility of glial progenitor grafts in carefully selected spinal injuries, especially those with limited involvement of the posterior columns and lateral funiculi (see the article elsewhere in this issue by Belegu and his colleagues).

Future trends: embryonic stem cells as a source of transplantable progenitors and endogenous mobilization

The practical limitations on 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 [54]. This latter observation paralleled earlier studies that reported myelination in the injured spinal cord by implanted murine embryonic stem (ES) cells [55]. Neither of these studies isolated glial progenitors or oligodendrocytes before transplantation, however, and neither followed animals for the long periods required to ensure the long-term survival and phenotypic stability of the engrafted cells. In particular, these ES cell–based approaches may prove limited by the potential for tumorigenesis, particularly by the potential for any persistent undifferentiated ES cells in the donor pool to yield teratomas or germinomas after implantation. As a result of these considerations, stringent selection for, and purification of, committed glial progenitor cells must be applied so as to deplete donor cell populations of any undifferentiated ES cells completely before hES cell–based therapy may be safely contemplated. Until that time, the implantation of tissue-derived glial progenitor cells is necessarily the more clinically feasible option (see the article elsewhere in this issue by Rao and colleagues).

In broader terms, however, cell therapy of the demyelinating disorders is unlikely to effect clinically significant restoration of function without concurrent modulation of the disease environment in such a way as to favor cell integration and axonal regeneration while suppressing ongoing disease pathologic change. As a result, cell therapy of the demyelinating diseases is likely to evolve as multimodal strategies, necessarily accompanied by treatments designed to support neuronal survival [56] and synaptogenesis [57] as well as engraftment and differentiation. It is important to note that in most diseases of the brain and spinal cord, resident OPCs are themselves lost, as in stroke and major injury, or diseased, as in the hereditary and metabolic leukodystrophies. In such cases, it is likely that remyelination may only be accomplished by a transplantation-oriented approach. Nonetheless, by virtue of their widespread and abundant distribution in the developing and adult brain, OPCs may also prove intriguing

targets for pharmacologic inductive strategies intended to mobilize and use endogenous progenitor cell pools [58]. In diseases of multifocal transient demyelination, such as MS, the ability to mobilize endogenous progenitor pools pharmacologically and direct their daughters to oligodendrocyte lineage might have great utility, and strategies intended to accomplish this are now under active investigation [59,60,63].

Summary

Clinically responsible stem cell–based therapy of the diseased CNS mandates a rigorous determination of those diseases most amenable to this approach. By virtue of the relative homogeneity of the affected phenotype, the availability of appropriate animal models, and the accessibility of highly enriched preparations of donor OPCs, diseases affecting the glial compartment stand out as especially promising initial targets for transplant-based therapy of CNS disease. Using a common strategy of glial progenitor cell implantation, pediatric diseases as diverse as the leukodystrophies, lysosomal storage diseases, and cerebral palsy as well as such adult-acquired demyelinations as transverse myelitis, MS, and subcortical stroke may all be approachable as therapeutic targets. Moreover, the same technologies used to enrich progenitor cells for transplantation yield isolates amenable to immortalization and gene expression analyses as well. As a result, isolated glial progenitor cells may prove useful not only as cellular vectors for transplantation but as tools for understanding the signaling pathways and growth control of native progenitors in vivo. Using such information, endogenous glial progenitor cells can be targeted for directed mobilization and phenotypic induction, whether by cognate cytokines or by their small molecule mimetics. Indeed, by mobilizing endogenous progenitors in vivo, it may be possible to mitigate the need for transplantation in disorders like the ischemic and inflammatory demyelinations, in which large accessible stores of endogenous progenitors may persist locally. In contrast, in diseases involving the widespread loss of cells and disorders in which endogenous progenitor cells themselves are lost or deficient, such as the congenital leukodystrophies and lysosomal storage disorders, therapeutic strategies based on cell transplantation are necessary. Together, these distinct approaches highlight the potential utility of glial progenitor cell–based therapy in diseases of the pediatric and adult CNS.

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