2 Clinical Prospects for Neural Grafting Therapy for Cortical Lesions

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2.1 INTRODUCTION

The goal of this chapter is to assess how close the hypothesis of neural grafting to enhance nervous system function may be to clinical reality, and the problems yet to be resolved before it is applicable clinically.[1–5] This chapter focuses on neural

grafting for lesions of the cerebral cortex arising from epilepsy, stroke, and head injury. Additional chapters will include further information on neural grafting for spinal cord injury (Chapter 3) and Parkinson's disease (Chapter 8). Although clinical neural grafting has been performed primarily in the context of Parkinson's disease,[6] many issues relevant to this disease do not necessarily transfer to lesions of the cerebral cortex and hippocampus.

For example, Parkinson's disease involves grafting of a dopaminergic phenotypic cell that possesses a large, diffuse axonal elaboration with a modulatory function, rather than specific synaptic relay systems as noted in glutaminergic synapses within the cortex and hippocampus. Thus, the goal of grafting in cortical lesions is usually to functionally replace part of a highly organized relay system, whereas in Parkinson's disease the goal is to replace dopaminergic innervation nonspecifically to the striatum.

Clinical treatments for acute lesions of the cerebral cortex and hippocampus such as head injury and stroke have focused almost exclusively on cytoprotection and prevention of secondary damage within the early period after the lesion.[4] While a moderate reduction in the number of neurons damaged may facilitate recovery, in many instances this format of treatment was clearly insufficient clinically because recovery was less than optimal. Moreover, the spontaneous neuronal replacement that transpires via proliferation of endogenous stem/progenitor cells after injury appears to be very restricted, ephemeral, and nonfunctional.[7]

Few available treatments are aimed at enhancing recovery of function of surviving neuronal elements.[2] Additionally, recovery can be accompanied by aberrant axonal plasticity of surviving neurons, characterized by inappropriate innervation of denervated synaptic regions.[8,9] One consequence of such inappropriate recovery is the late occurrence of epilepsy due in many instances to isolation of hyperexcitable regions, but which may still exert an untoward effect on the intact brain.[8,10] Restoration of normal afferent brain control over autonomous, hyperexcitable regions may be critical to both restore function and alleviate epilepsy. Exogenous transplants of multipotent progenitor or stem cells may play a role not only in epilepsy, but also in head injury, stroke, and degenerative disease.[11–17]

At both early and late time points after hippocampal or cortical lesions, one method to enhance recovery and restore function may be grafting of committed embryonic neural cells even though the goals may differ.[1,4] Neural grafting acutely after a lesion may provide additional unformed neuronal elements that may then insinuate and become integrated into the host circuitry, potentially enhancing overall recovery.[1,18,19] Early grafting may also change the acute milieu, decreasing death among host cells. Late after a lesion, when the damage is stable, neural grafts may be competent to enhance actual appropriate circuitry reconstruction.[3] This is likely accomplished by:

1. Providing correct target neurons for host axons
2. Furnishing proper afferent axons to host neurons
3. Inducing withdrawal of aberrantly formed synaptic contacts

These events together may suppress hyperexcitability and restore afferent control in autonomous regions. Embryonic neural grafts have the dual advantage of surviving the transplantation trauma and anoxia and possessing competence for considerable axonal growth into the adult host CNS.[1,3,20] In Parkinson's disease, for example, neural grafts have been used to treat a stable, long-term disorder by adding ectopic but critical dopaminergic re-innervation.[6]

The goal of circuitry reconstruction with neural grafts requires appropriate neuronal elements for the host region that are capable of becoming functionally integrated within the host. Many other possible goals and mechanisms can be achieved by neural transplantation including release of neurotrophic factors or neurotransmitters and replacement of glial cells.[16,21–23] However, the specific requirement for circuitry reconstruction leads to a hypothesis as to what an ideal graft may be.[3] An ideal graft would have certain characteristics:

1. Adequate survival of the transplanted neurons within the host environment (at least 20% of grafted neurons)
2. Appropriate dispersion or migration of the transplanted cells to restore host neuronal cell layers (leaving few cells at the transplant site)
3. Normal cellular development including acquisition of region-specific dendritic complexity, synapses and intrinsic characteristics
4. Appropriate elaboration of both local circuit and long-distance axons for synaptic connectivity into the host
5. Attraction of a significant number of specific afferent axons from the host

While these requirements are rigorous, the quantitative measurement of these characteristics may lend credence to exertion by the graft of a specific, defined role in the host, as opposed to a nonspecific or non-neuronal effect.[1,3]

Grafting into cerebral cortex or hippocampus to facilitate circuitry reconstruction may be radically different from the grafting treatment of Parkinson's disease. For example, grafts into the striatum of dopamine-enriched tissue are intentionally ectopic, and do not appear to develop long-distance axonal growth despite the fact that embryonic dopaminergic axons are inherently capable of such growth.[6] The other major difference is the type of neuron that is grafted and its neurotransmitter type because dopamine neurons possess much more diffuse and larger axonal terminal synaptic fields than the more typical glutamatergic neurons and GABAergic neurons considered in hippocampal or cortical grafting. Thus, only some parallels may be noted between the two different regions, but issues of graft tissue survival and integration remain paramount for both.[3,24,25]

The hippocampus represents a critical model region for cerebral cortex in general for the analysis and testing of grafting treatments because all the elements present throughout the neocortex are noted in some form in the hippocampus, including the various types of principal cells and interneurons and the intervening neuropil. The purpose of this chapter is to first describe the preclinical data for neural grafting. Second, the clinical situations to which hippocampal or cortical neural grafting may be applicable will be analyzed, in addition to potential graft sources and their

limitations. Finally, the bridge between preclinical research and clinical usefulness and applicability will be discussed.

2.2 GRAFT CELL INTEGRATION: PRECLINICAL STUDIES

We defined graft integration into the host on a quantitative, cellular basis specifically to assess circuitry reconstruction.[26–28] Neural grafting has many other goals, for example, provision of an enzyme or neurotransmitter, furnishing cells to form myelin sheaths for host axons, and production of growth factors or metabolic products. Our hypothesis of cellular integration applies primarily to the goal of making a graft an integral part of synaptic circuitry within the brain. The specific measurable aspects of integration include:

1. Cell survival, directly comparing the number of cells transplanted and those recovered *in vivo* at different postgrafting time points
2. Cell dispersion and migration away from the graft site
3. Graft cell differentiation into region-specific neuronal phenotypes
4. Graft cell local and long-distance efferent synaptic interactions with the host neurons
5. Graft cell afferent connectivity with appropriate host axons

Graft integration may be differentially analyzed for various cell types, including embryonic neurons and immature stem cells.[28,29] Figure 2.1 is a schematic of the results of these preclinical studies.

2.2.1 Graft Cell Labeling

Assessment of graft integration requires a unique label for the grafted neurons so that their survival, migration, and differentiation fate after transplantation may be followed.[30] Genetically engineered cells may be labeled with a permanent, gene-based label (such as green fluorescent protein or beta galactosidase).[31] Prior to harvesting embryonic postmitotic cells, embryonic neurons may be efficiently labeled with a DNA label such as the thymidine analog 5-bromodeoxyuridine (BrdU)[26] by injecting the maternal host during times of neurogenesis for those cells. Because the cells are postmitotic and committed after embryonic harvesting, the neurons retain the BrdU label permanently.

After harvesting embryonic cells, fluorescent labels such as rhodamine dextran (RDA) may be used.[30] Serial sections through the host can define the location and developmental fate of the grafted cells and the percent of survival and degree of migration and/or dispersion can be calculated.[26] The label also allows confirmation of the graft cells when double-labeled with a second marker specific for the graft cell phenotype, long-distance connectivity, or physiology. For analysis purposes, the placement of micrografts (10,000 to 30,000 cells) is much more definitive than the use of larger but more therapeutic macrografts of $>1 \times 10^6$ cells. The smaller number of cells within micrografts can be explicitly counted and tracked using

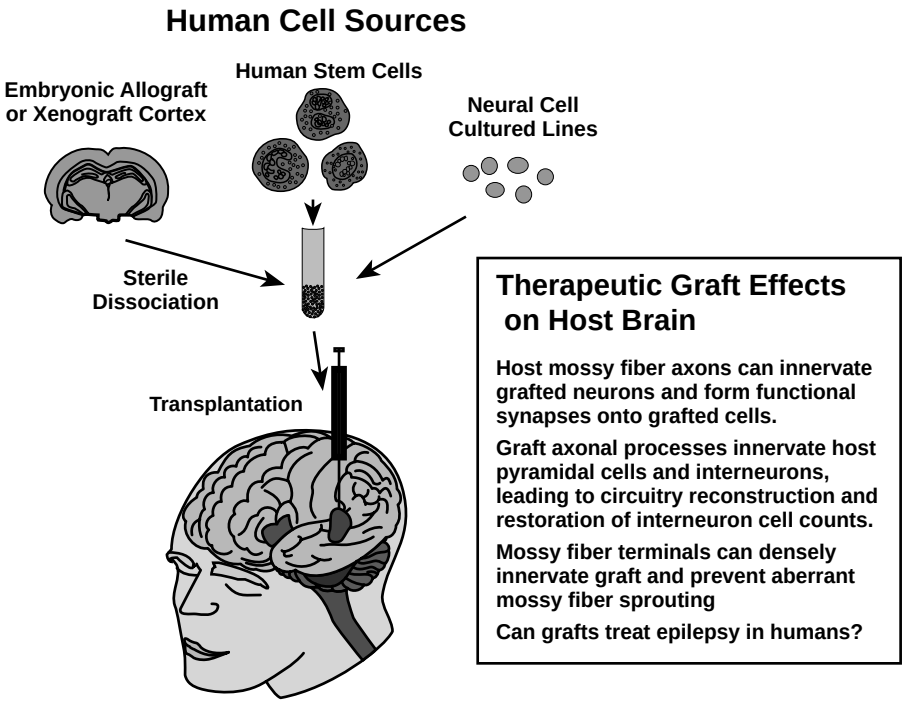

FIGURE 2.1 (See color insert following page 146.) Cortical grafting studies. First, cell sources include human or porcine embryonic cortex or hippocampus, various types of progenitor or stem cells, or cultured cell lines, most derived from neuronal tumors. After dissociation and transplantation, the fate of the transplanted cells can be assessed for synaptic integration within the host. In rodent models, therapeutic graft effects on the host include the formation of mossy fiber synapses onto grafted neurons, amelioration of postlesion interneuron loss, and prevention of aberrant mossy fiber sprouting. Whether grafts can ameliorate epilepsy remains to be analyzed in rodents and humans although the framework has been established.

unbiased cell counting methods. Unique labels form the critical basis for evaluation of graft integration within the host to unambiguously identify the grafted cells within the host.[26]

2.2.2 Graft Cell Survival

Cell survival can be assessed in terms of the number of cells grafted compared to those recovered later *in vivo*. In normal or intact hosts and grafts performed late after lesions, only 18 to 30% of grafted hippocampal cells survived.[26] In contrast, dopaminergic grafts in models of Parkinson's disease showed far poorer survival ranging from 3 to 20% in various studies. However, at early time points following lesions, the degree of survival was much greater (60 to 80%), particularly in young adults.[26] This enhanced survival is due primarily to the enhanced neurotrophic factor environment present up to 10–14 days following a lesion and the potential effects of denervation.[32] Grafts soon after lesions were well tolerated and considerably

enhanced, compared to the intact cortex and the situation after resolution of the lesion.[26] At time points equivalent to a fully healed human lesion or with aging, the hippocampus and cortex become much less receptive to grafts. Graft augmentation techniques are required to enhance graft survival and integration.[33,34]

2.2.3 Graft Cell Migration and Dispersion

Breaking down the separate aspects of integration led to some surprising results for embryonic hippocampal grafts. First, during development time when embryonic neurons are removed (at embryonic day 19), these cells have completed programmed migration along the radial glia into their respective layers. Second, the total distance of migration for embryonic hippocampus is short — less than 0.1 mm. Therefore, after grafting, these cells show minimal specific migration to appropriate cell body layers, and most remain clumped within 0.5 mm of the grafting site.[26] In an attempt to enhance dispersion, grafts were transplanted as a suspension rather than as tissue, but migration still remained minimal. This lack of capability for migration within the host requires more accurate placement of multiple grafts directly within the degenerated cell layer because only appropriately placed grafts of certain cell types show capacity for specific connectivity.[27,35,36] One of the promises of stem cells may be that enhanced migration capability could lead to recapitulation of hippocampal and cortical architecture.[15]

Grafts that are nonspecific to the lesioned region, such as striatal tissue placed within the lesioned hippocampus, demonstrate poor survival and little capacity for integration.[9] Thus, it is critical that appropriate cellular elements are placed into locations most suitable for their development. They differ from dopaminergic grafts for Parkinson's disease that appear to function better when placed ectopically within the striatum, and grafted as strands rather than as a dissociated cell suspension.[6] In the hippocampus, the various types of functions that improve with appropriate placement include afferent circuitry (mossy fiber terminals on grafted CA3 neurons) and efferent circuitry to both the deafferented CA1 region and commissural projection areas.[9,27,35]

2.2.4 Synaptic Graft–Host Interactions

Connectivity between graft and host requires that host fiber tracts have access to the graft and that graft axons are able to recognize and follow host axon guidance pathways.[3,27,36] A graft placed as a chunk of tissue, rather than as dissociated cells, encourages local connections to form within the graft, discouraging connections between the surrounding brain and the graft.[37] Axon guidance pathways differ, depending on the inherent wiring and pattern of connections.[20,27] For example, hippocampal CA3 cell grafts can demonstrate contralateral commissural efferent projections if located near the degenerated CA3 cell layer, which apparently provides the appropriate molecular signals for such long-distance connectivity. We have termed this capability the *axon guidance pathway*, which for commissural connections appears to be specific.

Fibers from many regions of the hippocampus terminate in the septum. Therefore grafts placed in most regions of the hippocampus can robustly send efferent

fibers into the septum. These embryonic graft neuron axons demonstrate competence to follow innate host axon guidance pathways and are not susceptible to inhibitory molecules such as myelin-associated glycoproteins along the host pathways, unlike adult host axons. The locations of these axon guidance pathways in the host may be highly specific, requiring accurate placement of the grafts to achieve access. Afferents into the graft develop readily, particularly mossy fiber ingrowths, if the grafted cells are their natural target cells (i.e., CA3 cells), demonstrated both physiologically by direct slice neuronal recordings and anatomically by Timm's histochemical staining.[9,20,30] Likewise, short-distance outgrowth from embryonic grafts was demonstrated to be both dense and appropriate (from CA3 grafts to denervated regions of the CA1 subfield and the dentate gyrus), indicating that embryonic grafts can develop both appropriate afferent and efferent connections in the hippocampus.[28]

2.2.5 Preclinical Graft Cell Sources

Aspects of integration have been well defined for embryonic hippocampal cells, as discussed above.[26] However, these cells are not optimal in the sense that they are not "ideal" grafts, particularly because of limited supply, ethical issues, and lack of ability to migrate within the host after transplantation. Therefore, hippocampal stem cells with pyramidal neuronal phenotypes have also been analyzed as alternatives to embryonic cells.[12,28,29] These cells likely arise from the posterior subventricular zone and form neurospheres *in vitro* in the presence of mitogenic factors such as epidermal growth factor (EGF) or fibroblast growth factor (FGF).[29,38–40]

Neurospheres are large collections of undifferentiated cells that develop in specific culture conditions from clonal stem cells removed from *in vivo* subventricular zones and contain both stem cells and their progeny. However, these cells show limited differentiation into neurons *in vitro* and *in vivo*, and may require conditioning with appropriate neurotrophic factors to enhance neuronal differentiation both prior to and after transplantation.[29] For example, physiological development and fiber outgrowth may be limited, even for cells resembling pyramidal neurons, due to their limited differentiation and axon growth. Further, the milieu of the injured brain could adversely affect differentiation of stem cells into neurons as a result of inadequate positional cues. Thus, hippocampal stem cells (and neural stem cells in general) are very promising, but will clearly require priming into partially differentiated region-specific neurons prior to grafting to fully achieve their differentiation and connectivity specific to the site of grafting. Whether this differentiated phenotype will be maintained after grafting, particularly for prolonged periods, will require further research.[11,13,15,38–44]

Immortalized cell lines have also been developed to obviate logistical problems from the use of fetal and embryonic stem cells. However, cell lines are limited by their potential to form tumors and degree of differentiation into true neurons capable of integration into the host. In addition, another goal of therapy using cell lines (NT2N cells) is to produce exogenous proteins needed in the CNS for particular disorders instead of completely integrating into existing circuitry. The human embryonal carcinoma cell line NT2N exhibits many properties of neuroepithelial precursor cells.[45,46]

2.3 CLINICAL CONTEXTS

This chapter focuses on clinical entities — primarily lesions of the cerebral cortex including the hippocampus. Grafting proposed for spinal cord treatment will be discussed in Chapter 3; subcortical grafting for Parkinson's disease is discussed in Chapter 8.

2.3.1 Cortical Lesions: Early Postlesion Grafting

Common lesions of the cerebral cortex (including the hippocampus) include head injuries, particularly cerebral contusions, and cerebral infarcts. Both head injury and stroke may be accompanied by extensive tissue damage and early neuronal replacement via grafts may facilitate structural and functional recovery by adding unformed neural elements to assist in circuitry reconstitution.[18,19,47] Such facilitation could consist of enhancing regional recovery and also preventing aberrant regeneration that may accompany cortical recovery in the form of compensatory sprouting of neighboring axons and inappropriate innervation of denervated synaptic sites.[9] The relatively unformed neuronal characteristics of neural grafts and their enhanced short- and long-distance axonal collateral growth in the adult, host CNS, may facilitate host recovery far beyond what would be obtained with either innate axonal regrowth alone or endogenous stem/progenitor cell proliferation and differentiation.[7]

The environment, within a few days after the lesion, appears particularly conducive to graft survival and integration.[26] This favorable host environment may be due to an enhancement in the level of neurotrophic factors in the vicinity of the lesion[32] as a result of astrocytic hypertrophy, microglial activation, and enhanced neurotrophic gene expression in surviving neurons.

For clinical grafting purposes, hippocampal grafts could be placed directly within the appropriate cell layers by stereotactic injection. However, neocortical suspension grafts may require multiple small injections into the neocortex on the border of the damaged region because direct injection into a severely damaged (or ischemic) area may provide minimal tissue nutrition and support for initial growth of axons. Preclinical studies of grafts into ischemic regions suggest excellent integration of embryonic cells into the appropriate tissue.[18,47] Histological demonstration of surviving cells and behavioral changes are considered to define "graft" effect in many studies. Depending on the goal of the graft, if circuitry reconstitution is desired, clear evidence of actual restoration of the damaged circuitry at the cellular level of analysis should be present. In other words, graft cell presence in the host does not necessarily imply circuitry reconstruction or appropriate synaptic interactions with host neurons. Physiological study of the grafted neurons and their synaptic interactions with the host is critical to fully define mechanisms of graft action on circuitry.[30]

Grafting may have other goals beyond circuitry reconstitution and these goals should be fully specified for each type of transplant. Embryonic grafts placed within a few days after an acute lesion have been shown to provide several clearly beneficial effects for the host. First, there is a clear anatomical and physiological demonstration of afferent connectivity from the host onto grafted cells when cells

specific to the lesioned site are grafted.[26,27,30,35] Second, early appropriate grafts can permanently prevent the development of aberrant supragranular mossy fiber sprouting following development of mossy fiber sprouts into the graft.[9] Third, the apparent down-regulation of glutamate decarboxylase and calcium binding proteins in GABAergic interneurons following a CA3 lesion can be reversed by placement of appropriate (as opposed to inappropriate or control grafts) embryonic hippocampal grafts at 45 days postlesion.[35] These graft influences on the host strongly indicate that cellular graft integration can exert a positive influence on host lesion recovery.

2.3.2 Cortical Lesions: Late Postlesion Grafting

Most preclinical studies of graft integration focused on early transplantation after a lesion, particularly 10 to 14 days postlesion due to the propitious effects of the host environment on graft survival.[1,26,32] However, in many clinical situations such as chronic epilepsy and Parkinson's disease, the host environment many years after the lesion has occurred is resistant to graft integration (similar to normal cortex).[26] This host resistance may worsen with age.[3,33,34] In contrast to immediate results after a lesion, when the extracellular environment, postsynaptic cells, and presynaptic axons are all ready to attempt circuitry restoration, late after a lesion all three critical elements have returned to a more quiescent and less facilitating state.

Thus, grafts placed late after a lesion may require significant enhancement of the number of cells transplanted, their readiness to re-innervate the host, and critical placement.[34] It may be possible to also prepare the host prior to the graft with a small lesion sufficient to induce a glial reaction (for example, placement of a probe 7 to 10 days ahead of time and subsequent placement of the cells) or with pregraft infusion of neurotrophic factors. Because of the difficulty with graft integration at such late postlesion times, fewer preclinical investigations focused on overcoming this resistance have been performed although these barriers to graft cell survival are important to analyze clinically.

2.3.3 Neural Grafts for Treatment of Epilepsy

The lifespan incidence of seizures shows a dramatic increase at the extremes of young and old ages, particularly seizures due to lesions of the brain including those arising from head injuries, strokes, tumors, and Alzheimer's disease. In younger patients one of the most common seizure types is partial complex, resulting from mesial temporal sclerosis (MTS).[8] Most types of lesions that lead to later epilepsy involve neuronal and tissue loss and this is exemplified by MTS.

One concept of lesions resulting in hyperexcitability and eventually epilepsy is that an autonomous region becomes disconnected from the normal afferent host control. This autonomous region persists in demonstrating intrinsic hyperactivity, possibly manifest as an interictal focus, and can under some conditions lead to seizure propagation within the remainder of the brain.[8] One hypothesized role of grafts is to reconnect the autonomous area directly to the host. Another hypothesis involves modulation of other systems that can suppress seizures, for example, noradrenergic, serotonergic, midbrain, or cholinergic inputs.[11,21,48–50]

Animal models of convulsions and epilepsy reflect large numbers of types and categories of the human disease. For example, numerous mutants show early onset of generalized seizures,[48,51,52] kindling models of seizures,[16,21,49,53] and many models of irritants that can lead to convulsions. Several hippocampal models may reflect some features of partial complex seizures and anatomically may resemble aspects of MTS.[3,8,9,32,53] Late manifestations in these animal lesions often resemble the human situation and include aberrant mossy fiber sprouting, permanent down-regulation of calcium binding proteins in the CA1 subfield and dentate gyrus, and loss of glutamate decarboxylase within major fractions of interneurons.[35]

Embryonic grafts into animals following kainic acid lesions demonstrate a number of positive effects on the host that are indicative of a high degree of graft integration. Hippocampal grafts receive afferents from the host dentate granule cells (mossy fibers).[20] If the grafted CA3 cells are sufficiently numerous, the result can be amelioration of aberrant mossy fiber sprouting, indicating that these axons prefer an appropriate rather than inappropriate target.[9] Based on *in vivo* studies, the CA3 grafts develop long-distance connections, including to the contralateral CA3 region and to the septum. Long-term *in vitro* tissue studies using organotypic hippocampal cultures indicated a dense local connectivity established to the CA1 region. These graft efferents can also lead to a powerful re-innervation of the CA1 region, restoring glutamate decarboxylase in the GABAergic interneuron population as compared to lesion-only hosts.[35] All these beneficial effects confirm that graft integration may be sufficient to reconstruct the hippocampal circuitry after a kainic acid lesion. However, *in vivo* EEG and behavioral studies are needed to confirm beneficial effects on lesion recovery and host electrographic or clinical seizures. A stable rodent model of seizures following a lesion is clearly needed to assess how well these grafts may ameliorate seizures.[7,12]

Other types of grafts have been proposed for amelioration of kindling-induced seizures, particularly locus coeruleus grafts that contain norepinephrine-producing cells[21] and cholinergic grafts.[49,54] Further, the antiepileptogenic outcome of specific neural grafting in the latter studies was linked to the degree of graft-derived noradrenergic or cholinergic innervation of the stimulated brain region. Nevertheless, these findings are not clinically relevant for application of neural grafting to epilepsy, particularly MTS, because lesions of noradrenergic or cholinergic neurons are not present in the human condition. GABAergic grafts (those containing inhibitory interneurons predominantly) have also been suggested[16,25,55] due to the considerable inhibitory effect of GABA (the main inhibitory neurotransmitter) on seizures.[50]

Some hippocampal grafts have been shown to function as a heterotopia, particularly when chunks (rather than suspensions) of hippocampal tissue are placed.[37,54] These heterotopic grafts are inherently epileptogenic and can actually induce seizures in the host — a highly undesirable situation. When hippocampal tissue is placed as a chunk graft, the internal recurrent circuitry tends to form within the graft instead of an external connection between the host and graft through the graft–host interface. These internal connections within a graft may reinforce the innate hippocampal tendency toward hyperexcitability and seizures. Thus, integration of the graft into the host circuitry and appropriate afferent control over the graft are critical for both

lesion recovery enhancement and possible treatment of the epilepsy. If grafts are placed early after a lesion, then prevention of development of an epileptic focus, a true anti-epileptogenic therapy, could possibly result.[9,35]

2.3.4 Neural Grafts for Treatment of Stroke

Stroke is one of the leading causes of death and causes severe disability. Treatments are limited to prevention and the acute setting. After a cerebrovascular accident has occurred, only supportive therapies are available. Grafting would seem ideal to replace lost cells.[56] Currently, stroke is one of the most active areas for cell transplantation, with small Phase I and Phase II trials completed for select basal ganglia (deep) strokes in humans.[5,19] The basis of these initial human studies lies in the success of animal studies although synaptic integration has not been fully analyzed in such a deep model of hemorrhage. Many stroke models exist, but the rodent model of the middle cerebral artery (MCA) occlusion has many clinical similarities to conditions seen in humans during cerebral ischemia. Much success has been achieved in this area with experimental functional recovery.[18]

Transplantation in stroke models has shown improvement in behavioral dysfunction as early as 1 month after grafting using an immortalized cell line as the cell source although animals required immunosuppressants to maintain the robust effects of the grafts. However, even non-immunosuppressed animals showed improvement in comparison to control animals. These studies have justified the use of human-derived NT2N neurons in stroke by showing functional improvements in the animals. Finally, the grafts produced no obvious deleterious effects.

2.3.5 Neural Grafts for Treatment of Severe Head Injury

Traumatic brain injuries and head injuries have very limited treatment. Most therapies are aimed at controlling intracranial pressure in the acute phase, but such supportive measures aim to decrease secondary cell loss rather than enhance recovery. Cell transplantation has a two-fold strategy: (1) to decrease the initial inflammatory reaction that leads to cell death and (2) to replace lost cells from the primary and secondary injuries. Initial studies showed poor survival of grafts in injured areas, but animal models of lesions showed considerably enhanced survival within a few days of the initial injury.[3,26] In addition, multiple sources of cells have also been used in this paradigm. Strategies have evolved to improve survival of grafts and integration, such as cografting of neural stem cells with supportive cells or substrates. Partial functional recovery has been shown with cografts including marrow-derived stromal cells and fibronectin.[57,58] In addition, developed cell lines have been successfully grafted.[17]

The primary model of neural grafting for treatment of head injury has focused on early addition of unformed elements to cortical areas (and potentially to areas of white matter shear injury), then allowing these elements to participate in the overall recovery and rehabilitation of the patient. However, measurement of improved outcome with the wide range of severity of head injury may be very difficult compared to measuring stroke outcomes.

2.4 CLINICALLY APPROPRIATE GRAFT CELL SOURCES

Potential sources of cells include various forms of embryonic neural cells, non-neural cells, tissue cultures cell lines, and pluripotent stem cells. All these groups can be further subdivided depending on the times they are acquired and the sources from which they are initially derived including autografts (from individual patients), allografts (from another individual of the same species), and xenografts (from a different species, for example, another mammal). All the initial human Parkinson's grafting studies, for example, were performed with autografts (adrenal medulla harvested at the same surgical session and reimplanted into the brain) or allografts from mixed embryonic cadaver donors.[6]

Embryonic porcine xenograft cells have also undergone clinical trials because mammalian embryonic cells appear to substitute well across species.[1,59,60] All these cells (with perhaps the exception of directly derived autografts) now require extensive FDA approval for reimplantation strategies. The approval requires that sterility and safety be ensured during processing of any *in vitro* maintenance or tissue culture and during direct reimplantation, particularly since these cells require direct brain implantation.

2.4.1 Embryonic Neural Cells

No ideal graft donor cells currently exist.[3] All tissue sources show significant limitations from both scientific and ethical viewpoints.[61] While embryonic neural cells currently demonstrate the best integration, specific migration of postmitotic neurons is highly limited, thus impeding appropriate cell distribution in the host. Additionally, embryonic allografts and xenografts impose ethical burdens because abortion is the tissue source, because they alter the innate human characteristics of brain and mind, and because of the risk of rejection.[6,59,60,61] However, xenograft embryonic cells have the advantage of availability in large numbers, particularly from porcine sources. They appear capable of substituting for human cells of similar origin based on equivalent neuronal sizing and lengths of projections.[60]

Other advantages of embryonic neural tissue are the ready, appropriate growth of embryonic axons into the host CNS, the known, postmitotic fates of the cells, and their excellent survival in a relatively anoxic host environment directly after grafting. However, because human trials of allografts for Parkinson's disease have shown marginal improvement and unexpected side effects, enthusiasm for any form of embryonic cell transplant is now considerably diminished.[6]

2.4.2 Cultured Cell Lines

Various types of precursor cell populations have been immortalized using oncogenes or telomerase. These cells offer the potential benefit of generating clonally identical cells, with innate genetic rules (such as temperature elevation) for inhibition of further mitoses.[1,11,13,16,22,23,45] In addition, spontaneously generated tumor (hNT) cells have been subcloned and characterized, and these tumor lines have indefinite capability for mitotic activity. Some show differentiation with retinoic acid, but these are usually not inherent CNS cells and quantitative assessment of their actual (rather

than projected) integration into the CNS remains limited. Also, the tumor genotype remains, and long-term questions about tumor reversion *in vivo* remain in spite of short-term differentiation *in vitro*.

Thus, these tumor cell lines have the advantage of ready availability and unlimited numbers but the worries about appropriateness for various grafting purposes and the residual risk of tumor escape remain, although such escape does not appear to have occurred during short-term preclinical testing. One initial clinical trial using such cells involved deep intracerebral hemorrhage.[19] For most of these cell lines, their abilities to differentiate into neurons capable of CNS integration have not been fully tested in ways similar to the ways embryonic cells have been tested. Thus, histological evidence of cell survival has often led to the premature conclusion that the cells are integrated within the CNS circuitry; for most cell lines, this analysis remains to be done. Although they are convenient and available in large numbers, such cell lines may not readily behave as CNS neurons due to their origins as immature cells.

2.4.3 Non-Neural Cells

The idea that differentiated cells have limited choices of progeny has recently been challenged. The concepts of transdifferentiated and dedifferentiated CNS cells have potentially supplied new populations of cells for transplantation.[62–65] Transdifferentiation involves taking cells such as bone marrow stromal cells and forming neural progenitor cells. Dedifferentiation is exemplified by glial progenitor cells that can form neurons. Many questions still surround this cell source.

Other cell types include transfected fibroblasts, glial cells, and multiple types of non-neural systemic cells such as lymphocytes that may transfer specific functions to the nervous system even though they are not able to function as neurons. Interest in most available non-neural cells has waned as newer forms of cells have become available, particularly various forms of stem cells.

2.4.4 Pluripotent Progenitor and Stem Cells

True embryonic stem (ES) cells can differentiate into all embryonic derivatives.[40] However, these cells require the highest number of cues for subsequent differentiation. They are isolated very early from the inner cell masses of embryonic blastocysts. Relatively few experiments have been done using this very primitive cell population because of the great difficulty in forcing differentiation along various lines. These cells offer several advantages, particularly their general CNS fate capability and rapid cell division to create large numbers of cells, although access to blastocysts is highly limited in the U.S. today. These cells give rise to slightly more differentiated and regional stem cells derived from ventricular and subventricular zones, and then further along the path of commitment are progenitor cells.[40] All these cell types are actively being pursued for transplantation paradigms for recovery of function.[12,14,15,28,29,41–44,66]

Various types of multipotent, self-renewing neural progenitor or stem cells show considerable promise but differentiation into a specific lineage remains

difficult to control before and after grafting particularly when grafted into a lesioned CNS.[13,28,31,42,44] It was initially thought possible that a lesioned brain might direct specific differentiation of otherwise unformed cell transplants, but it was realized subsequently that differentiation is difficult to maintain after grafting. Furthermore, the exact differentiation potential of neural stem cells obtained from distinct brain regions after grafting into different areas of the lesioned adult brain is mostly unknown — particularly whether neural stem cells from different brain areas produce neurons specific to their region of origin or specific to the site of their grafting. Addressing these concerns directly will help determine whether we must use different kinds of neural stem cells to treat different types of neurodegenerative disorders based on the area of the brain afflicted. For example, hippocampal stem cells may be specific for repair of a lesioned hippocampus in epilepsy, mesencephalic stem cells for Parkinson's disease, and striatal stem cells for Huntington's disease, etc.

The overall differentiation into neurons improves with progenitor cells that are more rather than less differentiated (i.e., subventricular zone cells versus embryonic stem cells from blastocysts), but still remains a critical issue. Thus, characterization of molecular mechanisms that control the fate of neural stem/progenitor cells after grafting into different regions of the lesioned adult CNS in experimental models is necessary prior to their routine clinical use as treatments for neurodegenerative disorders.[11,12,15,38,43] While the promise remains that stem cells may eventually be directed to function as neurons *in vivo*, this promise has yet to be clearly realized in preclinical studies.[15]

2.5 SIDE EFFECTS OF NEURAL GRAFTS

Unlike a medical therapy that can be suspended, graft treatments currently are irreversible because no clinically applicable method to destroy or eliminate a neural graft *in vivo* has been developed.[6] Potential deleterious side effects of grafts include increased seizures,[54] transmission of a virus or tumor to the host, induction of rejection, and difficult-to-treat problems related to the disease, for example, dyskinesias noted with neural grafts in Parkinson's disease.[6]

In all such instances, it may be helpful to have a method to noninvasively remove the graft or alter it selectively without damage to the host brain. This problem is peculiar to neural grafts because they normally require diffuse placement as cell suspensions or chunks of cells and thus cannot be removed surgically without causing extensive damage. This is particularly true if the grafts are capable of migration to specific regions, in which case their diffusion and insinuation into the brain preclude direct forms of removal.

In animals, graft cells can be labeled before transplantation with a triggerable stealth toxin that releases singlet oxygen only when specifically triggered (chlorin E6).[67] Without the appropriate trigger, the cells develop normally and are indistinguishable from control grafts. However, upon illumination with even a low level of infrared light (at 720 nm), the chlorin E6 releases massive singlet oxygen that can destroy the grafted cells selectively *in situ* and show minimal host

damage. Other methods of selectively destroying grafts include allografts and immunotoxins that may attack xenografts selectively. Some forms of triggerable genes may also be transfected into graft cells to allow initiation of selective cell death *in situ* without host damage. These methods may be helpful to extinguish any side effects from grafted cells by virtually destroying the cells selectively within the milieu of the brain.

2.6 CLINICAL APPLICABILITY AND CHALLENGES IN TRANSLATION

How far an experimental surgical treatment must be developed prior to initial human application remains a very difficult, almost unregulated, and contentious question. One set of guidelines generally outlines preclinical studies needed along with human experimentation requirements.[68] As an example, grafting of cultured tumor cells into deep basal ganglia lesions after intracerebral hemorrhages was performed in patients[19] following extensive preclinical testing.[18] Another example is the application of porcine embryonic cells to humans for Parkinson's disease and potentially for hippocampal or cortical use.[60] Clearly, a cell source should be FDA-approved for initial human trials of cell lines in terms of safety and freedom from transmissible diseases and neoplasias. Preclinical evidence should support a specific intended use. These requirements have clearly been met for treatment of Parkinson's disease, as confirmed by the large number of proposed and performed clinical trials for embryonic cell grafts.[1,4,6]

Methods are likewise needed to enhance graft functioning at late, stable post-lesion phases likely used to treat neurological disorders. Such methods could include enhancing the extent of survival of grafted cells using pretreatment of donor or host cells with distinct neurotrophic factors and other factors such as caspase inhibitors[24,34,69] that suppress the apoptotic deaths of grafted cells during the early postgrafting period. At this juncture, the most appropriate donor cells for hippocampal grafting may be porcine embryonic cells from the age of gestation directly after hippocampal neurogenesis (10 to 12 weeks of gestation in the human, slightly earlier in the porcine model).[60] Since the FDA has now imposed extensive requirements for processing implanted cells, a method should be established to determine appropriate sterility, cell numbers, and presence of contaminants. These requirements may facilitate the standardization of grafting studies.

While much of this chapter discussed the mechanisms underlying graft integration into a host, by the time when human grafting experiments are pursued for neurological disorders, these principles will not be known in human subjects although they presumably will have been developed in animal models. Most medications helpful in treating seizures, head injuries, and strokes now have known bases from laboratory studies but this was not true at their market introduction. Thus, there is no need to have actual mechanistic understanding for a treatment to go forward and become FDA approved. On a scientific basis, however, such mechanistic underpinnings are critical to understand and improve treatments. In summary, the neurobiology of graft integration, survival, and differentiation is not yet fully mapped or

understood. Assuming an appropriate graft cell source becomes available for further human testing, a critical approach to host integration of the graft and mechanistic treatments of neurological disorders will be needed if this form of restorative neurosurgery is to become a long-term, viable treatment option.[1,4,41]

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