HYPOTHESIS

Adrenal Medulla Autografts in Parkinson's Disease: A Proposed Mechanism of Action

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ABSTRACT: Concepts emerging from experimental manipulations of neurobiological systems *in vivo* and *in vitro* may provide explanations for some clinical observations following adrenal medulla autografting in patients with Parkinson's disease. These concepts are reviewed, and a proposed mechanism of action of adrenal autografts in Parkinson's disease is advanced.

RÉSUME: Des autogreffes médullosurrénales dans la maladie de Parkinson: un mécanisme proposé. Les concepts provenants des manipulations expérimentales de systèmes neurologiques in vivo et in vitro peuvent expliquer quelques-unes des observations cliniques à la suite des greffes médullosurrénales chez des patients atteints de la maladie de Parkinson. Nous avons fait la critique de ces concepts et avons proposé un possible mécanisme d'action.

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Clinical Observations

The North American experience with adrenal medulla autografts in Parkinson's disease now numbers approximately 100. A consensus is emerging that a proportion of grafted patients show modest to moderate benefits in the form of shorter and less severe off-time, and on-time that is perhaps associated with less dyskinesia.[1] It has been suggested that the on-off phenomenon results, at least in part, from progressive loss of presynaptic nigrostriatal input onto striatal dopamine receptors, and thus, loss of the buffering capacity of the nigrostriatal dopamine uptake system for the large quantities of dopamine that accumulate with exogenous L-dopa replacement. The effect of the adrenal autograft to favourably influence the on-to-off ratio might suggest that the grafts are improving the integrity of the dopaminergic uptake system in striatum, either from provision of dopaminergic cells in the graft, from residual nigral input, or from dopaminergic systems in juxtaposition.

Experimental Studies

Transplantation of neuronal tissue to the nervous system of mammals has been attempted periodically since the end of the 19th century. Over the last fifteen years, conditions for attaining reliable graft survival have been developed, and transplantation of pieces of CNS tissue to various sites within the adult nervous system can now be achieved with high reliability.[2] However, the mechanisms of graft function continue to be delineated. The current understanding implicates a hierarchy of biological phenomena, all of which may act sequentially and/or in concert to achieve the desired response[3](Table 1).

A significant body of experimental data is now accumulating on the grafting of autologous adrenal medulla into mammals with toxin-induced nigrostriatal lesions that produce many features seen in Parkinson's disease. These studies are noteworthy for at least two reasons: first, there is some evidence that the treated/grafted animals show improved motor/behavioural responses that correlate with the presence in striatum of host

Table 1. Proposed Mechanisms of Graft Function*

Negative effects of implant surgery	
Trophic actions on host brain	- from the graft - from the host
Diffuse release of hormones or transmitters	
Reinnervation of host by graft	
Reciprocal connectivity	
Complex integration into host circuitry	

*Adapted from A. Bjorklund, et al., Trends in Neurosciences 1987; 10: 509.

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dopaminergic terminals, and secondly, that the physiological improvement and histological changes are sustained in the absence of viable graft. Thus, the improvement following adrenal cortex grafting appears to be unrelated to the presence of graft cells with dopaminergic potential. In other words, the effect of the graft may be merely to induce a host response which results in an appropriate motor/behavioural improvement possibly by virtue of trophic interactions.

A number of concepts have emerged recently from a variety of manipulations of neurobiological systems *in vivo* and *in vitro* that bear directly on these clinical and experimental observations, and perhaps provide explanations for them. These concepts are discussed under three headings relating to the host milieu; the nature of adult adrenal grafts as compared to fetal neuronal grafts is reviewed; and a hypothesis is advanced.

The Host Milieu

Neurons

The projection from pars compacta of substantia nigra (SNC) is responsible for the dopaminergic innervation of the striatum and is the site of primary insult in Parkinson's disease. Even though there is a marked reduction of dopamine in striatum at the time of diagnosis of Parkinson's disease, evidence from a number of other neuronal systems would suggest that reduction of a neuronal phenotype does not reflect accurately the state of viability of the neurons or the integrity of axons. In other words, in Parkinson's disease the striatal content of dopamine very likely underestimates the true residual nigral innervation of striatum. This point, plus the knowledge that the nigral projection is involved in a progressive degenerative process, is critical to the concept of the priming response that appears to be involved in regeneration of axons.

The idea that a regenerative response by neurons is enhanced if the neuron is injured in some way is not new. Recent experimental[4] observations indicate that the regenerative sprouting response of central axons of mammalian sensory neurons following a dorsal root crush injury is enhanced by at least a factor of 10 if the peripheral sensory axons have been injured previously. In the case of the nigral projection in Parkinson's disease, this neuronal population is injured by virtue of involvement in a degenerative process and, therefore, may be primed to respond in much the same way that sensory neurons are primed, in the experimental paradigm. The molecular basis of the priming effect is not known, but some clues are beginning to emerge and will be discussed.

Glia

One of the responses to acute or subacute injury of the nervous system involves the astrocytic reaction known as reactive astrocytosis which involves both hypertrophy and proliferation of this cell population. There is little evidence that a quantifiable astrocytic response is seen in striatum in Parkinson's disease, but experimental data indicate that reactive astrocytosis accompanies grafting of adrenal to the primate caudate.[5] However, a mitogenic response by glia is not the only phenotypic alteration in the region of injury. Data are accumulating to suggest that injury results in increased specific activity of neurotrophic factors in the region, and that the same molecular species involved in regulation of neurotrophic activity also produce astrocytic proliferation *in vitro*.

Although there is little direct evidence, it is generally believed that glial elements of the nervous system are important sources of neurotrophic factors for neurons. In some parts of the nervous system neurons may provide trophic support for innervating presynaptic populations. There is also emerging evidence that target regions of innervating neuronal populations are regionally specialized and perhaps supply growth factors specific to the populations of innervating neurons. This certainly appears to be the case of NGF, whose mRNA is found at high levels in olfactory bulb, hippocampus, and cerebral cortex — regions of forebrain that receive cholinergic innervation from basal forebrain nuclei.[6] In hippocampus, there is evidence to suggest that much of the $mRNA^{NGF}$ is provided by pyramidal and granular neurons.[7] The cholinergic neurons of basal forebrain supplying these regions of cortex bear high-affinity receptors for NGF.[8,9] Recent data have emerged to indicate that axonal injury and/or the accompanying inflammatory response has profound effects on the biochemical phenotype of glial elements in the region of the injury. Following peripheral nerve axotomy, the denervated perineural elements distal to the cut show an increase in $mRNA^{NGF}$ and product. Messenger RNA^{NGF} remains elevated only if there is a source of macrophages that accompanies the subsequent inflammatory response. The molecular basis of this response is related to release by macrophages of a soluble mediator known as interleukin-1 (IL-1).[10]

Recently, two trophic factors for dopaminergic neurons have been detected in striatum[11,12] and one of these factors is known to be increased following injury to striatum. The role of macrophages and/or IL-1 in these observations is not known but IL-1 has been shown to be mitogenic for glial elements in the CNS.[13]

While diffusible growth factors for neurons, whose list continues to grow, possibly play an important role in maintenance and protection of neurons, there is emerging evidence that some immobilized or extracellular matrix-associated ECM neurotrophic factors are expressed following CNS trauma in the region of the glial response to insult. It is noteworthy that many of these proteins are expressed during early growth and development of the nervous system.

Inflammatory Cells

Recent data indicate that cells with a macrophage/microglia phenotype are found in regions of neuronal degeneration in the SNc, as well as in the striatum in Parkinson's disease.[14] These cells may function to remove debris of degenerated neurons and axons by phagocytosis, but because of other specialized functions, these cells may be responsible for the priming effect on the injured neurons.

Following an implant of autologous adrenal, the graft likely remains devitalized. In response to the presence of the devitalized graft and the disruption of the blood-brain barrier, there is appearance of inflammatory cells. Macrophage/monocyte cells are prominent in the region of the graft and likely play a role in phagocytosis of cellular debris.[5]

Macrophages are known to be rich sources of soluble mediators which activate other cells of the immune system. Within the

nervous system a number of functions are attributed to resident macrophage/microglia cells (Table 2).[15] A family of molecules known as interleukins released by macrophages, and having autocrine influences on macrophages, have been implicated in some of these interactions, and as alluded to above, one of the interleukins, IL-1, is known to be both mitogenic and stimulatory for glial cells of the central and peripheral nervous systems. IL-1 is also produced by astrocytes that have been activated by macrophages, setting the stage for an enhanced astrocytic response by autocrine and reciprocal activation mechanisms. If the resident macrophages in the parkinsonian striatum prior to grafting behave as do other activated macrophages, a stage is set, such that the local environment of the striatum could provide specific priming signals for axons and glia, while the inflammatory reaction to the graft could be the quantitative boost required for proliferation of astrocytes, reciprocal activation of macrophages, and increases in mRNA and product of neurotrophic factors from the astrocytic population.

Graft Milieu

The rationale behind the use of adrenal medulla for grafting in PD relates to the potential for the medullary cells to synthesize and release dopamine. Other potential benefits of the adrenal medulla might include the ability of the support cells to provide trophic materials for nigral dopaminergic neurons of the host, since one might postulate that the phenotype of the support cell milieu of the adrenal is similar to that of the striatal support cell milieu, given the similar monoaminergic phenotype of the neuronal cells.

At this point, the weight of evidence from experimental studies suggests that adrenal grafts (not previously exposed to NGF) do not survive the implant procedure for periods that would explain the longevity of the motor/behaviour response. Since there are no markers for support cell populations, and since the marker for the neuronal elements of the graft may have disappeared before the death of the cells, it remains possible that some surviving neuronal and support cells continue to have an influence. It is unlikely, however, that this influence is related to the release of DA from grafted adrenal medulla.

The survival of grafts in the mammalian CNS had been a topic of considerable interest in the past. It is now established that there is a very narrow time window limited to fetal life, beyond which grafts of neural tissue to the CNS do not survive. In other words, the regenerative capacity of the neuronal cells of the graft is dependent on the age of the grafted tissue.

The properties of fetal and early neonatal neurons that permit survival and an appropriate regenerative response are only now beginning to be elucidated. In the case of fetal neuronal grafts, the CNS milieu into which they are placed seems appropriate to support survival, expression of a differentiated phenotype, synaptic connectivity, and correction of motor/behaviour deficits following specific lesions (see Table 1). This fetal neuron behaviour in the mature CNS differs significantly from that of lesioned mature neurons. These observations might suggest that the fetal neuronal cells possess properties that are qualitatively and/or quantitatively different from those of more mature neurons of the same type.

An examination of the properties of neurons that permit an appropriate regenerative response indicates that a time period during and shortly after neurogenesis maximizes the success of axonal regeneration and functional recovery following an insult *in vivo*. The observations suggest that the ability to regenerate is a consequence of growth periods where the neurons display a molecular phenotype that permits appropriate interactions with the extracellular milieu, and thus, appropriate regenerative growth.[16] While there is no direct proof at this time, it is possible that the success of fetal neuron transplants relates, at least in part, to the same molecular properties that permit a successful regenerative response *in vivo*. Bearing in mind the potential functions of resident microglia of the nervous system (Table 2), the role of macrophage/microglia elements that are present in large numbers during phases of CNS development remains to be determined in regard to the success of fetal grafting.[17]

Table 2. *Possible Functions of Macrophages in Nervous System**

Development:	phagocytosis ECM catabolism (proteinase) growth factor and inhibitor production
Homeostasis:	neurotransmitter hormone & peptide processing
Lipid Turnover:	ganglioside, phospholipid
Inflammation and Repair:	release of mediators, proteinases, cytotoxins, growth factors
Immune Responses:	antigen processing

*Adapted from V.H. Perry and S. Gordon, Trends in Neurosciences 1988; 11: 273.

What properties of fetal neurons might promote survival, neurite outgrowth and differentiation? It is now established that embryonic neurons can synthesize and release molecular species that support neurite outgrowth. These molecular species belong to three general classes, which appear to be functionally interactive-enzymes, cell adhesion molecules (CAM's), and proteoglycans. Embryonic neurons have on their cell surfaces at least three types of enzyme species that could facilitate interactions with the milieu of growth; these include proteolytic enzymes such as a metalloprotease and a urokinase,[18] and a galactosyltransferase activity which uses glycosylated molecular species of the ECM such as laminin and HSPG's as adhesive substrates.[19] The second class of molecules produced by neurons are the CAM's[20] which function to promote cell adhesion between neurons, between neurons and glia, and between neurons and the ECM. A third class of molecules are the proteoglycans which are associated with the neuronal cell surface and are released into the perineuronal milieu where they interact with defined species of the ECM.[21] Proteoglycans can also interact with CAM's and can act as acceptor substrates for galactosyltransferases. It is not yet clear if any of these molecular species play a role in the mature nervous system, but it is of interest and pertinent to the present discussion to note that CAM's change their molecular and binding properties between embryonic life and adult life,[20] and that CNS proteoglycans are at high levels during early stages of development and then decrease to very low levels as the nervous system matures.[22]

These considerations may bear upon the failure of adult neuronal and adrenal medulla grafts to survive, express their pheno-

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type, and make appropriate synaptic connections when grafted to the nervous system.

Hypothesis

The data available at this time suggest that autologous adrenal medulla grafts have modest beneficial effects in some patients with Parkinson's disease. As depicted in schematic form in Figure 1, the nature of the beneficial effects produced by the grafts suggests that residual nigrostriatal dopaminergic processes, primed by previous insult and the presence of microglia, may sprout and/or express quantitatively more of their differentiated phenotype such that the nigral buffering capacity for surges of dopamine is partially restored. The data further suggest that the type of autologous graft has little to do with the observed response since there is little evidence that graft is surviving at a time when clinical response is observed. Evidence has been presented to suggest that a prolonged inflammatory response resulting from the presence of devitalized graft could mediate the observed effects. The proposed molecular basis of the response has been reviewed and may involve, at least in part, the release of soluble mediators from macrophages which invade the graft site, have mitogenic effects on astrocytes, and stimulatory effects on these cells resulting in reciprocal stimulation of the macrophages and sustained release of neuronal growth factors which influence axons in the vicinity.

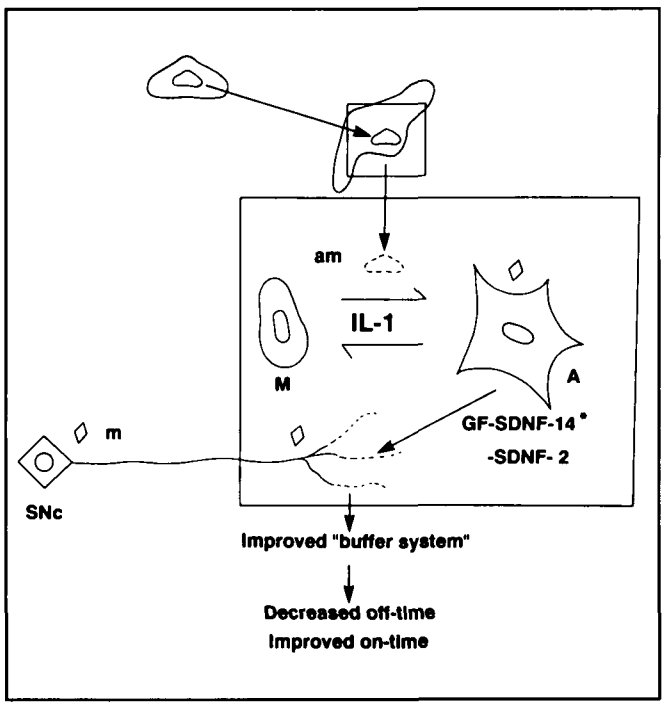

Figure 1 Schematic representation of a proposed mechanism of action of autologous adrenal medulla grafts; legend: am - adrenal medulla graft; m - microglia; M - activated macrophages; A - reactive astrocytes; SNc - dopaminergic neurons from pars compacta of substantia nigra innervating striatum; IL-1 - interleukin-1; GF - growth factor; SDNF - striatum-derived neurotrophic factor; 14 - 14000 daltons; 2 - 2000 daltons; asterisk refers to the striatum-derived growth factor that is present at increased concentrations following injury to striatum.

The recent findings of two classes of neurotrophic factors for mesencephalic dopaminergic neurons, one of which (asterisk, Figure 1) is present at higher specific activity following striatal lesions, might support this scenario. A trophic effect on residual nigrostriatal axons in the striatum resulting in differentiation and/or sprouting could possibly enhance the buffering capacity of the nigral projection and result in the effects on off-time and on-time observed in some grafted patients.

Two other clinical observations deserve comment — the reported bilateral nature of the improvement, and the periventricular location of the graft. Bilateral improvement could be consistent with release into CSF of growth factors and/or macrophage- and astrocyte-derived interleukins, which would then influence directly or indirectly a dopaminergic system that was primed to respond by virtue of involvement in a chronic degenerative process.

While a number of hypotheses have been raised to explain the clinical improvement seen with periventricular grafts to caudate, it is worth mentioning that, as in lower vertebrates, neuroepithelial cell populations of periventricular or subependymal regions of nervous system may possess properties that are favorable for regenerative responses.[16] Therefore, the subependymal region may provide a milieu that is more favourable for initiating the observed host response.

These suggested scenarios do not indicate a specific role for autologous adrenal medulla tissue as grafts. It is suggested that these grafts play only a non-specific role and are effective because they remain devitalized and promote a prolonged host inflammatory response resulting in restorative trophic actions on susceptible host neurons (Table 1).

In this regard, it is perhaps worthwhile to note that R.H. Meyers reported in 1942[23] transient and variable improvement in some symptoms of patients with Parkinson's disease who had undergone extirpation and sectioning of caudate and other brain regions. In light of the present discussion, it remains a possibility that the effects observed by Meyers were related to production of an abbreviated host inflammatory response in those regions of the brain where neurons and axons were primed to respond.

The clinical experience with autologous adrenal medulla grafting in Parkinson's disease had provided some useful insights into restorative responses of human brain regions involved in a chronic neurodegenerative process. While early data suggest some symptomatic improvement in grafted patients, it remains to be determined whether the improvement will be sustained, and whether it represents the earliest beneficial influence on the natural history of Parkinson's disease. Patience and detailed follow-up of currently grafted parkinsonian patients are now required to determine if this clinical experience will complement and extend the scientific rationale for neural grafting in degenerative neurological disease.

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