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Perspectives in Neural Transplantation

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During the last decade fetal neural tissue transplant to replace damaged neural tissue in adult brain has emerged as a possible therapeutic modality for management of several neurological disorders. However, a number of unsolved problems still remain for its routine clinical use. This review summarises the current status and indicates future direction of research.

Key Words: Review, Neural transplantation, Rat, Primate, Human, Degeneration, Rejection, Problems

Introduction

One of the most exciting development in Neurosciences in the past two decades has been the demonstration that the fetal neural tissue can be successfully transplanted into adult brain. This graft used either as a small solid "plug" or as cell suspension grows, differentiates, develops organotypic characteristics and gets integrated into host brain with the development of two way fibre connections. Grafted neurons synthesize the appropriate neurotransmitter depending upon its ontogenic nature. This has opened new possibilities for the study of basic problems of developmental biology and plasticity and has tantalising potentials for therapeutic use. It is no surprise then that it aroused keen interest amongst neurosurgeons who face daily the unsurmountable problems due to lack of regeneration potentials of the diseased and damaged mature neurons of the central nervous system. Rapidly accumulating data from large number of centres, based on morphological, biochemical, physiological and behavioural studies involving different regions and systems of the brain, primarily in rats, unequivocally demonstrated that such grafts could reverse the consequences of brain damage and result in varying degree of recovery of lost functions. Prompted by these studies we at the All India Institute of Medical Sciences, New Delhi initiated some work in early 1980s, which was further strengthened by the creation of a National Facility supported by Department of Science and Technology, Government of India. This presentation is based on the lessons learnt from our studies and a review of the current status of the subject globally. Since the results of our studies have already been published in a

series of papers, it is not proposed to go in details here but only highlight some of our salient observations which have direct relevance for future human therapeutic trials (Gopinath et al. 1987, Verma et al. 1989, Shetty et al. 1991a, b, c, Tandon et al. 1990, Gopinath et al. 1991, Tandon 1992, Tandon et al. 1992).

Studies on Rats

Fetal ventral mesencephalic tissue was transplanted in the anterior chamber of the eye, the lateral ventricle, and the striatum of adult rats. Successful grafts could be achieved consistently. Detailed morphological studies including light and electron microscopy, immunohistochemistry, Horse radish peroxidase transport and labelling, Golgi staining and quantitative morphometry were carried out at varying intervals after transplantation. Unlike most other such studies which were terminated at 3 to 4 months interval, we extended our observations up to 2 years. The unique finding of these experiments was the observation of premature ageing of the transplanted neurons already seen in stray neurons after 6 months. The ageing changes were characterized by appearance of clear spaces and membrane-bound vacoules, paucity of organelles specially rough endoplasmic reticulum and increasing accumulation of lipofuscin granules in the cytoplasm. With the passage of time increasing numbers of neurons were involved with greater severity of such changes. This led to progressive degeneration of the grafted neurons, so that by 18 months there were very few healthy surviving neurons. This process continues unabated as seen in animals followed up to 2 years. We are not sure of the precise pathogenesis of these changes. However, the possibility of slow immune rejection cannot be ruled out, even though we have so far not observed any definite evidence of active reaction in and around the transplant. Investigations are currently going on to detect immunocompetent lymphocytes and macrophags, and MHC presenting cells. Although so far ours were the only observations in respect to premature ageing, recently Helms et al. (1993) have observed the same in fetal neostriatal allografts in monkeys. In any case these findings raise an important question, hitherto not addressed in other studies, regarding the long term fate of the therapeutic transplants. It is interesting to note that Sladek (1988), and Lindvall (1991) even in their studies with much shorter follow up than ours observed survival of 10-20% of the grafted neurons. They, however, failed to ascribe any reason for it.

It has repeatedly been claimed that the recovery of function is due to integration of the graft with the host, development of neuritic processes from and into the graft and restoration of neuronal circuitry (Lund & Hauschka 1976, Bjorklund & Stenevi 1979, Bakay et al. 1987, Wictorin et al. 1988, 1989). While we observed neuritic processes across the interface between transplant and host brain and healthy looking synapses in electron micrographs, it was obvious to us that under the best of circumstances a transplant cannot reproduce the intricate circuitry of the intact animal. This is supported by a number of other studies (Perlow et al. 1979, Lindvall 1991, Dunnett 1991). This then raises the important question regarding the precise mechanism of recovery of function which has been unequivocally established in numerous studies. In case impaired function is due to deficiency of a specific neurotransmitter as in Parkinson's disease or a specific hormone as in the case of hypogonadal mutants it is easy to surmise that recovery can occur in the absence of fully developed circuitry due to the release of the appropriate transmitter or the hormone by the graft. While this may be true for some of the conditions, it can not account for recovery observed in others.

There is growing evidence to suggest that the beneficial effect of the transplant is dependent upon graft derived trophic or growth factors. These factors help in regeneration of

the host nervous tissue. Bankiewicz et al. (1990) and Kordower et al. (1991) produced experimental evidence to substantiate this hypothesis in their MPTP treated monkey model. They both found that factors other than dopamine replacement were responsible for the dopaminergic sprouting of neuritic processes of host origin. The beneficial effect on the graft of exogenously provided nerve growth factor (NGF) or the co-transplantation of peripheral nerve as a source for it further support this contention. While it is felt that there may be other trophic factors mediating this effect, so far except for NGF there is no information available regarding the nature of such factors. The changes indicating premature senescence of the grafted neurons observed by us may well be related to deficiency of some trophic factor in the long term. Further critically planned experiments are necessary to resolve this issue. At the moment it would be correct to state that the precise mechanism of graft-derived functional recovery in lesioned animals is ill-understood.

In addition to the above major unresolved problems, there are a number of others of more technical nature which may be significant for successful therapeutic application of this procedure. Thus, the questions like the optimal developmental window for harvesting the donor tissue, its optimal size, relative merit of solid versus cell suspension, fresh versus cryopreserved or cultured, precise location of transplant, and the number of sites need to be investigated further. However, no further comments will be made here on these issues.

Studies on Rhesus Monkeys

We believed that the results of experiments on sub-human primates will be more relevant for ultimate application to human therapy than the vast amount of information already available on rats and other smaller mammals. Soon after establishing a reliable and reproducible technique for transplantation in rats, work was initiated on rhesus monkeys (Gopinath et al. 1989). Details of these studies have recently been summarised by us (Tandon et al. 1994) but the most striking observations relate to the unsuspected difficulties in achieving a successful transplant in this species.

Notwithstanding reports of successful transplantation in African green monkeys, squirrel monkeys and marmosets (Gash et al. 1985, Redmond et al. 1986, Annett et al. 1990), and even a few in rhesus (Bakay et al. 1987, Bankiewicz et al. 1988 & 1991, Fiandaca et al. 1988), our observations indicate that unlike lower mammals and lower subhuman primates, neural transplantation in the more highly evolved rhesus is beset with the problem of immune rejection. Inspite of very carefully conducted fetal neural transplantation both subcortical and deep intraparenchymal in 19 monkeys, we had practically no long term surviving grafts. Unlike in rats we observed necrosis, perivascular cuffing, infiltration of mononuclear leucocytes, macrophages, and Gitter cells in and around grafts. Immunocytological investigations demonstrated MHC presenting cells and both T and B cells in the region of the transplant. No doubt we observed scattered healthy looking surviving neurons with their processes and occasionally even a cluster of such neurons with a well developed neuropil in some of our grafts even as late as six months after transplantation. Nevertheless, the overall impression is that in the absence of exogenously administered immunosuppressants, as advocated by Bankiewicz et al. (1990) and Lindvall (1991) immune rejection is a rule rather than exception in these highly evolved primates.

Notwithstanding unequivocal statements by Bjorklund et al. (1982), Brundin et al. (1985), Perlow (1987) and Fiandaca et al. (1988) that they observed little or no evidence of rejection Geyer et al. (1985), Mason et al. (1984) and Bakay and Barrow (1988) cautio-

ned against such a complacent view. Rao et al. (1988) summarised the status thus, "while a clear picture of a single graft rejection mechanism has not emerged yet, current experiments suggest a pattern of events. Essentially, the grafts exist in a metastable immune balance, that can be disrupted by a variety of factors. Each one on its own may not be sufficient to provoke a rejection response, but once a critical threshold is reached a cascade of events may occur which precipitate a classical immune response". As mentioned earlier most of these studies were carried out in rats. It may be mentioned that Morihisa et al. (1984), Bakay et al. (1987) and Sladek et al. (1988) in their experiments on rhesus monkeys found only few or no surviving neurons 3-8 months after transplantation. It would be interesting to investigate, as we are currently doing, the factors predisposing immune rejection. The possibility that the evolutionary status of a species may be one of these cannot be dismissed without further studies. In any case it is now generally accepted that the so-called " immuno-priviledged" status of brain is only partially true. The implications of these observations for human therapeutic transplants are only too obvious. It is, therefore, not surprising that contrary to the assertions of Hitchcock and his group (1990), currently most other neurosurgeons, put their transplanted patients on immunosuppressive therapy (Lindvall et al. 1990, 1992, Madrazo et al. 1990).

Status of Neural Transplantation in Parkinson's Disease

The first human transplantation of autologous adrenal medullary cells was performed by Backlund in March, 1982 and the second in May, 1983 (Backlund et al. 1985). However, while the Swedish group returned to the laboratory after these two operations Madrazo in Mexico enthusiastically followed this lead, with some modifications and claimed dramatic results (Madrazo et al. 1988). Failing to achieve identical results, most other surgeons, who tried these operations, gave it up. Already in 1987 Lindvall had observed that the results "have been negative so far—they have shown that we cannot help the patient with this approach as is done today". Transplantation of adrenal medulla has now been given up owing to its unpredictable and at best transitory benefits, unacceptable surgical risks, and undesirable behavioural complications. Laboratory studies are, however, still continuing to improve these results. Cunningham et al. (1991) used genetically altered astrocytes to provide NGF, while Kordower et al. (1990) and Date et al. (1993) have experimented with cografts of pretransected peripheral nerve with adrenal medulla for the same purpose. Olson et al. (1991) have attempted to supplement these grafts with intraputaminal infusion of nerve growth factor for treatment of patients with Parkinson's disease (PD).

It is, however, generally accepted that as of today the most suitable tissue for treatment of patients with PD is fetal substantia nigra—over 100 patients have so far received implants of human fetal mesencephalic tissue into the striatum. Some general improvement of motor symptoms along with reduced requirement of drugs has been reported. In addition evidence of graft survival and significant increase of fluorodopa uptake has been demonstrated in a few patients (Freed et al. 1992, Spencer et al. 1992, Swalf et al. 1992). We have recently summarised some of the unresolved problems in clinical use of neural transplant (Tandon & Gopinath 1994). These unresolved problems, requiring further experimental study, have prompted Widner (1993) to conclude, "there exists at present no treatment of PD based on intracerebral transplantation".

Alternatives to Fetal Tissue as Transplants

Practical and ethical considerations add to the problems of using fetal tissue for trans-

plantation. In view of these concerns efforts are underway to find alternate sources as grafts. Gash et al. (1986) demonstrated the feasibility of transplanting cultured cells of neuroblastoma made amitotic. These could be transfected with the gene for the appropriate enzyme like tyrosine hydroxylase (TH) to produce dopamine or for a growth factor which will help regeneration of the host tissue. Genetically engineered astrocytes or fibroblasts capable of producing L-dopa are being investigated (Gage et al. 1987, Fisher et al. 1991). The results are promising, but still too preliminary so the search for "better cells for brain repair" continues (Bjorklund 1993).

Conclusion

There is little doubt that fetal neural tissue from specific regions of the brain can be transplanted to replace damaged neurons of identical origin and function. There are still several unanswered questions which prevent routine application of this technique for human therapy. The practical difficulty of obtaining human fetal tissue and the associated ethical consideration have prompted search for alternative source of cells for repair. It is obvious that during the last decade or so we have travelled a long way to demonstrate that repair of the central nervous system and restoration of lost neurological function, hitherto considered as impossible, is an achievable goal within a foreseeable future. This will, however, depend upon a better understanding of the complex mechanisms underlying cell damage, regeneration, growth and long term survival.

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