Replacing cells in multiple sclerosis

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Abstract

Cell transplantation is emerging as a major potential therapeutic approach in the treatment of otherwise untreatable neurodegenerative diseases. In multiple sclerosis (MS), a major direction of current research is to devise strategies that will remyelinate axons and protect them against subsequent ongoing degeneration. Ongoing loss of axons will lead to chronic disability. Oligodendrocytes and their progenitors are lost during multiple relapses in the course of MS and either needs to be replaced from an exogenous source or the remaining progenitors stimulated to differentiate and remyelinate. The successful isolation and purification of human oligodendrocytes from neural or embryonic stem cells offer hope that a source of sufficient cells for translational application might be achievable in the future. Focal repair of strategic lesions followed by more disseminated delivery of exogenous cells will be the short and long-term goals.

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1. Introduction

Novel ideas are emerging on the treatment of multiple sclerosis (MS) that are aimed at promoting remyelination and axonal protection, thus addressing different targets from the current disease modifying drugs which are designed to modify the immune response and lessen inflammation, but which do not address tissue repair or neuroprotection. These new strategies would involve the replacement of lost oligodendrocytes or oligodendrocyte precursor cells (OPCs) from an exogenous source. Replacement of oligodendrocytes could also be promoted, however, by the “rescue” of OPCs that remain in, or near lesions, but appear to be unable to differentiate and successfully remyelinate chronically demyelinated axons [1].

The impetus to explore cell therapy in MS has come from many years of experimental work on the transplantation of myelin-forming cells into the CNS [2–5], and from clinical and experimental experience of neural stem cell therapies in other neurodegenerative diseases [6,7]. There is now a large body of evidence that shows that glial cell transplantation can result in extensive myelin formation by the transplanted cells in a wide range of animal models. To treat MS lesions, human cells will likely be used initially, and the isolation and purification of large numbers of human oligodendrocytes are likely to be the key requirement for the translation of the experimental studies into clinical practice. While advances are being made in isolating human cells, other complementary advances in our knowledge of MS are being reported. Much more is being discovered on the pathology of MS and of imaging lesions that will provide the necessary background information needed prior to transplanting cells.

In this brief review on the topic of cell replacement in MS lesions, I will consider some of these issues and in particular, discuss the replacement of cells in a single, strategic lesion in the CNS. The long-term goal, however, will be to bring about the diffuse dissemination of exogenous cells to repair multiple areas of demyelination throughout the brain and spinal cord.

2. Choice of lesions

There are two major decisions to be made here that remain unresolved. The first choice is whether to target acute or chronic areas of the demyelination and secondly at which site in the CNS could clinically significant lesions be surgically approached. The choice between acute or chronic lesions is
the more complex. In early relapsing–remitting disease, spontaneous remyelination is profuse [8,9]. Indeed, in experiment models of demyelination, especially those caused by myelinotoxic chemicals, complete endogenous remyelination can occur [10]. In MS, remyelination has been described in the presence of inflammatory cell infiltrate, suggesting that OPCs are not inhibited by certain aspects of the immune/inflammatory response, and may actually require this for remyelination to occur. Evidence of the latter comes from a number of experiments. It has been shown that rejection of transplanted cells can result in extensive remyelination by neighboring endogenous OPCs [11], and that in chronic areas of demyelination, extensive remyelination by transplanted cells only occurs when focal areas of inflammation are induced [12]. Likewise, focal inflammation can promote the myelinating capacity of cells transplanted into areas containing normally non-myelinated axons [13]. The dilemma in MS, however, is that acute lesions are likely to spontaneously remyelinate at least early in disease, and implanting cells into such lesion may impede this natural process. Therefore repair of plaques with persistent demyelination associated with fixed and chronic neurological deficits is the most pressing need for cellular therapy. For therapy of such lesions to be useful, however, there must be sufficient axons in the plaque to remyelinate. Single, strategic lesions that might be targeted for focal repair are in the spinal cord, brain stem, cerebellar peduncles or optic nerve. Lesions in the cervical spinal cord that cause either all or the major clinical dysfunction might be the most promising target. Much of the experimental work on focal implantation of cells has been carried out in the spinal cord. However, the caution here is that any transplantation of cells should not cause significant damage to intact myelinated axons in adjacent long-tracts. The optic nerve is an attractive target as focal lesions in a single nerve could be viewed as a low risk target as there is no risk to adjacent CNS tissue. However, the optic nerve has proven to be a difficult anatomic structure to penetrate and transplant cells successfully into because of the thick, tough dura and astrocytic compartmentation that characterize the nerve. The brain stem is clearly a complex site in which to transplant cells because of the critical nuclei throughout the medulla. Both it and the cerebellar peduncles would require stereotactic approaches, but neither should be excluded from consideration.

3. Cells within the plaque

If a single, chronic MS plaque is to be targeted for cell replacement, it is important to consider the milieu into which cells might be transplanted. The chronic MS plaque is not acellular (Fig. 1). Within areas of chronic demyelination as seen in Fig. 1, there are many astrocytes, likely some cells of the oligodendrocyte lineage, most likely OPCs, microglia and perhaps some residual lymphocytes and macrophages. It appears that OPCs within such a chronic plaque differentiate poorly or if they do, are unable to normally ensheathe and remyelinate axons [14,15]. Attempts to remyelinate such areas by transplantation of exogenous cells will not need to remove these cells. Experimental studies have shown that myelination or re-myelination occurs in the presence of endogenous cells of the oligodendrocyte lineage, although it has been suggested host OPCs may inhibit exogenous cells from remyelinating lesions. An animal model in which chronic areas of demyelination resemble those of on MS plaque would be extremely helpful here to test this question. Such a model could also be used to study the means to promote endogenous repair. Ideally, such lesions would consist of chronically demyelinated (over 4–6 months) axons in a gliotic milieu in which inactive OPCs are preserved. In our studies on the transplantation of glia into the shaking pup mutant, we were able to show that OPCs transplanted into the mature mutant CNS in which there is a marked gliosis, were able to myelinate a large number of axons at the site [4]. Thus astrocytes and the gliosis seen in MS plaques may not prevent transplanted cells from myelinating axons, although in severely gliotic lesions this may restrict migration of OPCs and ensheathment of axons. Further testing of this question in appropriate models is desirable.

4. Cells to be used for replacement

This remains the greatest impediment to the translational application of cellular therapy in the repair of focal lesions. The choices of cell type ranges from cells of the oligodendrocyte lineage, to Schwann cells and olfactory ensheathing cells. We have focused our efforts on oligodendrocytes and OPCs. Unlike rodent or other animal tissue oligodendrocytes derived, it has proven difficult to isolate large numbers of OPCs from human tissue. The in vitro conditions required to maintain human OPCs and promote their division are different from other species [16]. We have shown, as have others, that OPCs can be isolated from neurospheres, grown from the striata of the human fetal brain [16]. We have also shown that OPCs can be differentiated from human embryonic stem cells (hESCs) [17]. However, in both cases, the number of cells was insufficient for translational purposes. However, other investigators have shown more promising data. In the case of fetal or even the adult brain, it is possible to isolate relatively large numbers of OPCs by the use of progenitor cell-selective reporter gene

Fig. 1. A) A chronic MS plaque, stained for myelin. Note that there are many cells throughout the plaque but no evidence of perivascular accumulation of T cells. The identity of cells within the plaque is possible through appropriate immunolabeling (modified from Prineas and Wright, 1978, with permission). B) To replace cells of the oligodendrocyte lineage, lost over time in the plaque, persistent OPCs, either in the plaque or closely adjacent, might be recruited. It is likely that such cells will need to proliferate before they ensheathe and remyelinate axons. To recruit dormant, endogenous OPCs a number of approaches might be used, which are noted. As more becomes known about the molecular differences between myelination and remyelination, so the ability to control the appropriate temporal and spatial expression of transcription factors in OPCs might be critical for successful endogenous repair. C) Exogenous repair by the injection of OPCs may result in complete repair of large lesions if sufficient numbers of cells are delivered, or divide appropriately on implantation and differentiate into myelinating cells.
expression \cite{18}. Cells are transduced with a fluorescent reporter gene such as green fluorescent protein under the control of the myelin gene CNP. Fluorescent positive cells can then be isolated and purified by fluorescent activated cell sorting (FACS) \cite{18}. FACS sorting using antibodies against surface antigens of human fetal or adult progenitor cells can also be used to isolate populations of cells for transplantation \cite{19}. Likewise, with human embryonic stem cells, Keirstead and colleagues have successfully differentiated ESCs to OPCs with a resultant 90% purity \cite{20}. It will be important to test the remyelinating capacities of OPCs derived as cited above, in models that mimic both acute and chronic MS, most likely in experimental allergic encephalomyelitis.

5. Analyses of the outcome of cell replacement

An important component of this new therapy will be to devise outcome measures that can critically evaluate the success of the cell replacement strategy. The goal of focal repair, however, is to improve or restore function; hence detailed neurological evaluation must be the primary method of judging success. However, imaging of the lesion or lesions will also be important for two reasons. Firstly, it may be possible to follow cells after implantation if they are labeled with superparamagnetic iron particles (SPIO) prior to transplantation \cite{21–23}. Secondly, the MRI appearance of the plaque and whether it has the features of remyelination (although more needs to be known about the MRI characteristics of remyelination) will be important to evaluate. It would seem likely that progress in imaging, using contemporary imaging techniques such as diffusion tensor, will provide the prerequisite surrogate markers of axon survival and CNS remyelination within the next few years.

6. Conclusion

Remyelination of demyelinated axons in MS is a rational and important strategy that will help to restore function to axons and protect them against ongoing loss. While current and future medical therapies may prove successful in limiting the early loss of axons, the greatest challenge appears to be remyelinating chronically demyelinated axons in non-repairing lesions. It may not be possible, however, to devise experimental models that represent such a disease stage, allowing the testing of transplant-induced repair. Thus transplanting cells into chronic, focal MS lesions may be the final “experiment.” However, such a clinical trial in MS patients may be the only way to determine whether exogenous cells are capable of surviving, differentiating and myelinating axons, in plaques where endogenous OPCs are present, but incapable of repair.

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References