

Review Article

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Autologous transplantation in the central nervous system

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Cell transplantation has been proposed to replace lost neurons in the diseased brain, and after injuries to the central nervous system (CNS). Strategies for cellular therapy in the CNS consist primarily in heterologous transplantations. Despite the CNS being an immunologically privileged site, immune rejection of intracerebral transplants remains a concern. In addition, the use of immunosuppressive drugs, like cyclosporine, is a major constraint associated with heterologous transplantations. Autologous transplantation is therefore viewed as the model of choice for cellular therapy. With the recent progress in somatic cell nuclear transfer (SCNT) research, and the confirmation that neurogenesis occurs in the adult brain and neural stem cells (NSCs) reside in the adult CNS, new opportunities for autologous transplantations are being considered for the CNS, and are promising.

Key words Adult neurogenesis - cellular therapy - embryonic stem cells - olfactory ensheathing cells - neural stem cells - plasticity - somatic cell nuclear transfer

The central nervous system (CNS) is an immunologically privileged site, where grafts survive longer than in other organs and tissues¹. Therefore, allogeneic and xenogeneic transplantations are candidate strategies to replace lost neurons in neurodegenerative diseases, and after injuries to the CNS. Cell types from various sources have been considered and evaluated for therapy in the CNS, and have shown promising results in preclinical studies, and clinical trials². Despite the CNS being an immunologically privileged site, immune rejection of intracerebral transplants remains a concern. On the one hand, genetically matching the donor-recipient and

the use of immunosuppressive drugs to avoid tissue rejection are major constraints to allogeneic transplantations. Finding a matching donor represents a challenge, and immunosuppressive treatments, like cyclosporine, are not very well tolerated by patients, and may also compromise the effectiveness of the transplanted cells³. On the other hand, encapsulation of xenogeneic cell lines has been suggested as a way to avoid immune responses. However, there are examples of immune response against encapsulated cells resulting in graft destruction. Further, the possibility of transmissible zoonoses, the host long-term response to xenograft and the ethical issues

over grafting of non human tissues into the human CNS, are major limitations to xenotransplantation⁴. In fact, it is now clear that the CNS does not display absolute immunological privilege, and that the immune response of the CNS to grafted tissue depends on a number of variables, which include the phylogenetic relationship of donor to the host, its composition, mode and site of implantation³. In addition, in the context of neural grafting, there will be inevitable damage to the blood-brain barrier (BBB), further weakening the immune protection of the CNS. Altogether, these data support autologous transplantation as a model of choice for cellular therapy in the CNS. Autologous transplantation obviates the need of donor-recipient matching and the use of immune-suppressive drugs, conditions that would favour successful graft integration, survival and recovery. In this review, we will discuss about the various cell types considered, and evaluated for autologous transplantation in the CNS.

Lineage fated cells

Chromaffin cells and sympathetic neurons: Cells from the sympathoadrenal (SA) lineage, chromaffin cells - the neuroendocrine cells of the adrenal medulla - and sympathetic neurons, mostly release noradrenaline, though some of them produce and release dopamine⁵. Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease primarily associated with the loss of dopaminergic neurons in the substantia nigra. SA cells also express dopaminotrophic factors, like glial-derived neurotrophic factor (GDNF) and transforming growth factor- β , which protect dopaminergic neurons from degeneration. Hence, SA cells have been proposed for the treatment of PD. Chromaffin cells and sympathetic neurons have been transplanted, either freshly isolated or after expansion in culture, into the denervated striatum and reported to exert beneficial effects in animal models of PD, and in clinical trials⁵. Since the proportion of dopaminergic cells in SA tissue transplanted is very low *i.e.*, only 1 per cent of the entire adrenal chromaffin cell

population releases dopamine, the beneficial activity of transplanted adrenal cells on PD symptoms may result from its neurotrophic effect, rather than from the release of dopamine. One of the advantages of SA transplantation is the ability to isolate cells from the patients, allowing autologous transplantations. However, the survival of adrenal medulla grafts, either chromaffin cells or sympathetic neurons, is low in animal models of PD and extremely low after grafting in PD patients. Hence, despite the ability to isolate and purified adrenal cells in extremely high quantities and to perform autologous transplantation, this approach is no longer pursued clinically.

Fibroblasts: Fibroblasts can be easily isolated from individuals and grown *in vitro*, providing a source of tissue for autologous transplantation. To take advantage of these properties for the treatment of CNS diseases and injuries, fibroblasts have been genetically engineered, to express trophic factors or to produce neurotransmitter-synthesizing enzymes⁶. Pre-clinical studies reveal that fibroblasts genetically engineered to express brain-derived neurotrophic factor or acetylcholine promote neuronal survival, regeneration, and functional recovery in animal models of CNS diseases and injuries^{7,8}. Recently, fibroblasts genetically engineered to express nerve growth factor, transplanted in patients with Alzheimer's disease, have been shown to improve the patients' abilities to recover⁹, validating such strategy for cellular therapy. However, the main concerns over the use of genetically engineered cells, and particularly fibroblasts, for cellular therapy reside over the long-term expression of the transgene, and the need to develop vectors allowing sustained expression of the transgene¹⁰.

Olfactory ensheathing cells: Olfactory ensheathing cells (OECs) are a specialized population of glial cells that ensheath olfactory axons in the olfactory bulb (OB), the region of termination of the olfactory sensory axons in the CNS, and the olfactory mucosa, the olfactory epithelium of the nasal cavity¹¹. OECs have unique properties. They reside both inside and

outside the CNS. They are non-myelinating glial cells that play a role in guiding the axons as they grow from the olfactory mucosa through the lamina propria to the OB. OECs have therefore properties of both astrocytes and Schwann cells, in being able to live within the CNS and in assisting axonal growth, respectively. Because of their properties to support axonal growth, OECs have been proposed for cellular therapy in the nervous system, particularly for spinal cord injuries¹²⁻¹⁵. Preclinical studies reveal that when transplanted into the spinal cord after injury, like after corticospinal tract lesions, OECs are associated with myelinating regrowing axons and functional improvements in locomotor abilities. These results have been reported for OECs from the OB, but also from the nose¹⁶. As OECs of the olfactory mucosa can be obtained by simple biopsy from the nose in all individuals without affecting the sense of smell and grown *in vitro*, OECs represent a potential candidate for autologous transplantation in the nervous system, particularly for spinal cord repair¹⁷. Human OECs have been reported to remyelinate spinal cord injuries in rodent after transplantation, and are being evaluated in phase I clinical trial, in which autologous OECs are transplanted in the injured spinal cord of paraplegic humans¹⁸. Genetically engineered OECs to produce growth factors, like GDNF, promote survival and regenerative mechanisms within the host tissue, further supporting the potential of OECs for cellular therapy¹⁵. Altogether, these results show that OECs represent a promising strategy for cellular therapy by autologous transplantation in the nervous system, particularly for spinal cord injury.

Stem cells

Embryonic stem cells: Embryonic stem cells (ESCs) are the self-renewing pluripotent cells derived from the inner cell mass of blastocysts. ESCs have the potential to generate all the lineages of the individual. As such, ESCs hold the promise to cure a broad range of diseases and injuries, particularly of the CNS¹⁹. With the advances in somatic cell nuclear transfer

(SCNT), there is the potential to generate isogenic ESCs, carrying a set of chromosomes identical to that of an individual. SCNT consists in isolating nucleus of a somatic cell type, like fibroblast, from an individual and transfer it into an enucleated oocyte from a female donor. By mechanisms still unknown, the cytoplasm of the oocyte reprogrammes the chromosomes of the somatic cell's nucleus, and the cloned cells develop into a blastocyst from which ESCs can be derived²⁰. Isogenic ESCs, carrying the genetic make-up of the individual, are therefore unlikely to be rejected after transplantation back into the individual. SCNT has been successfully applied to clone various animals, and to derive ESCs from various species, supporting SCNT has a valid strategy for generating isogenic ECS lines for transplantation²¹. However, ethical issues over the destruction of human blastocysts remain a major limitation for the therapeutic application of SCNT. Further, the generation of isogenic ESCs by SCNT from human remains to be established²².

Adult neural stem cells: Neural stem cells (NSCs) are the self-renewing multipotent cells that generate the main cell types of the nervous system, neurons, astrocytes and oligodendrocytes. NSCs hold the promise to treat a broad range of CNS diseases and injuries. The recent confirmation that neurogenesis occurs in the adult brain and NSCs reside in the adult CNS in mammals, including human, provides new alternatives for cellular therapy²³. Self-renewing multipotent NSCs have been isolated and characterized from various areas of the adult CNS including the spinal cord, and from various species, including from human (biopsies and post-mortem tissues). Adult-derived neural progenitor and stem cells have been transplanted in animal models, and shown functional engraftment, supporting their potential use for therapy²³. The ability to isolate and culture neural progenitor and stem cells from adult tissue open the opportunity to perform autologous transplantation, in which neural progenitor and stem cells would be isolated from an undamaged area of the CNS, expanded *in vitro*, and grafted back to the

patient to repair the CNS. However, autologous transplantation of adult-derived neural progenitor and stem cells is not without limitations, like the potential serious consequences of an invasive surgical procedure and the possible permanent damage done to harvest the patient cells. Neurogenesis also occurs in the olfactory mucosa, the sense organ of smell that produces new olfactory sensory neurons throughout adulthood, including in human²⁴⁻²⁸. Neural progenitor and stem cells have been isolated and characterized from the adult olfactory mucosa of human, providing a source of tissue for therapy²⁹. Since olfactory mucosa-derived neural progenitor and stem cells can be isolated by simple biopsy from the nose, olfactory mucosa-derived neural progenitor and stem cells provide a promising candidate for autologous transplantation in the nervous system¹⁷. Adult NSCs also present several advantages over other cell types for cellular therapy, like non-neuronal cell types, as they permit the rewiring of the CNS, over ESCs that have the risk to form tumours upon grafting, and without the ethical and political hurdles associated with SCNT and the use foetal-derived cells for therapy. Altogether, these studies show that adult NSCs provide an alternative source of tissue for cellular therapy in the CNS, candidate for autologous transplantation.

Adult stem cell plasticity: Adult stem cells are multipotent: they generate cell types of tissues from which they are derived. Several studies have reported that adult-derived stem cells might not be restricted to generate tissue specific cell types, but may have a broader differentiation potential than previously thought^{30,31}. The ability to give rise to neuronal lineages has been reported for stem cells derived the hematopoietic system³², bone marrow³³, skin³⁴, skeletal muscle³⁵, adipose tissue³⁶, as well as umbilical cord³⁷. The mechanisms underlying such phenomenon remain to be fully determined. It is hypothesized that the broader potential of adult stem cells is controlled by the microenvironment in which they reside. Stem cell niches are specialized microenvironments that regulate stem cells activity.

Removing stem or progenitor cells from their original environment that regulate their fate, may lead to the expression of molecules that would confer the stem cells a new fate, under a new environment³⁸. The evidences that adult-derived stem cells have a broader potential suggest that alternative sources of adult stem cells may be of use for cellular therapy, particularly for the CNS, as NSCs could be derived from other tissues, like the skin, permitting autologous therapies in which a patient's own cells are removed from the skin, grown in a dish and transplanted back into the patient. However, the broader potential of adult stem cells remains the source of debates and controversies³⁰. Some reports have raised concerns that the broader potential of adult-derived stem cells could derive from phenomenon like cell transformation, transdifferentiation or fusion. All of which can affect the use of adult stem cells for therapy. For example, cell transformation may be associated with aberrant cell growth, and the risk of tumour formation upon grafting. So, a therapeutic effect could be associated with potential risk for the patients. In all, the "true" potential adult stem cells remains to be fully characterized, before such strategy can be used for therapy.

Conclusion

Although autologous transplantation represents a model of choice for cellular therapy in the CNS, yet only a few sources of tissues are candidates for isogenic transplantation, each with their respective merits and limitations. Among them, adult NSCs represent a promising model. There are several questions that remain to be answered related to the use of neural progenitor and stem cells for cellular therapy, like the uncertain potential to establish the right connections, to differentiate to specific neuronal phenotypes, the optimal time for transplantation, the optimal number of cells delivered, the site and route of administration. Future studies will aim at demonstrating the potential of adult NSCs for therapy, and for autologous transplantation.

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