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The therapeutic use of stem cells for myelin repair in autoimmune demyelinating disorders

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Abstract

Spontaneous remyelination occurs in multiple sclerosis (MS) patients. However, this process is not robust enough to promote a functional and stable recovery of the myelin architecture in demyelinated areas of the central nervous system (CNS). As a consequence of this incomplete reparative process, the disease invariably progresses and patchy areas of demyelination–in which axonal damage and/or loss is a constant accompanying factor–increase over time and lead to the accumulation of irreversible neurological deficits. Thus, the development of cell-based therapies aimed to promote multifocal remyelination in MS represents one of the most challenging areas of investigation. Several cell-replacement strategies have been developed in the last few years. However, most of these therapeutic approaches–although consistently able to form new myelin sheaths around the transplantation site–are unrealistic owing to the mutifocality of the demyelinating process and the inability to in vitro growth and differentiate large number of myelin-forming cells. Recently, promising cell-replacement therapies based on the use of stem cells have been proposed. However, before envisaging any potential human applications of such therapies we need to confront with some preliminary and still unsolved questions: (i) the ideal stem cell source for transplantation, (ii) the route of cell administration, (iii) the differentiation and persistence of stem cells into the targeted tissue and, last but not least, (iv) the functional and long-lasting integration of transplanted cells into the host tissue.

Keywords: Stem cell; Neural stem cell; Bone marrow; Demyelination; Remyelination; Neuroprotection; Transplantation; Multiple sclerosis; Cell-therapy

Since the first transplant of Schwann cells (SC) within focal areas of primary demyelination produced in the spinal cords of rats by means of local injections of 6-amino-nicotinamide [1], we have witnessed a spur of experimental cell-based transplantation approaches aimed at repairing myelin damage within the central nervous system (CNS). New promising sources of myelinogenic cells for transplantation purposes (i.e. SC, oligodendrocytes, oligodendrocyte precursors, olfactory bulb ensheathing cells, adult and embryonic stem cells) have been characterized, and new cell-replacement strategies have been proposed. A better understanding of the dynamics of spontaneous endogenous remyelination has been achieved, and insights concerning the process of remyelination driven by site-specific transplantation of myelin-forming cells have been uncovered. This has led to the first clinical trial in multiple sclerosis (MS) patients based on transplantation of autologous SCs into demyelinating areas of the brain. The study–although safe in its transplantation procedure–was discontinued in 2003 as no evidence of SC survival was found in the first three implanted patients (see http://www.myelin.org/06232003.htm). The negative results of this pioneering approach have cooled down most of the expectations raised by the last 25 years of successful experimental cell therapies based on the use of myelin-forming cells. New cell-based therapeutic strategies aimed to promote remyelination are therefore required.

Somatic stem cells might represent an alternative source of cells that can be used to promote myelin repair. Encouraging preliminary results have been obtained by transplanting adult neural stem cells (aNSCs) (Fig. 1) into rodents affected by CNS demyelination [2–7]. However, although somatic stem cells may integrate within the CNS and possibly repair the myelin damage, further studies are required to assess the in vivo plasticity of these cells and the safety and efficacy of this therapeutic approach. Further-

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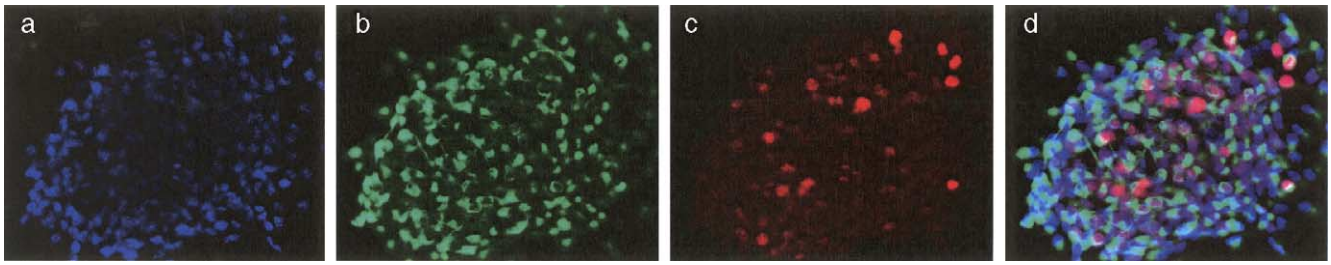

Fig. 1. Adult neural stem cells (aNSCs) for transplantation in CSN demyelination. Double immunofluorescence of mouse subventricular zone (SVZ)-derived NSCs grown as neurospheres in vitro and labelled with antibodies against nestin (b, green), and the proliferation marker Ki67 (c, red). Nuclei have been counterstained with dapi (a, blue). Merged image of a–c is shown in d. Magnification 40×.

more, there are some additional questions we need to confront with before prospecting any potential human application of such therapies: (i) the ideal stem cell source for transplantation; (ii) the route of cell administration; (iii) and, the differentiation and persistence of the transplanted cells into the targeted tissue. Last but not least, (iv) functional and long-lasting integration of transplanted cells into the host tissue has to be achieved.

Whatever the organ or tissue necessities, the "gold standard" cell for replacement therapies has to be inherently plastic. Stem cells can meet this criterion since they are intrinsically able either to adapt their terminal cell fate to different environmental needs (differentiation plasticity) or to trans-differentiate (developmental plasticity). Moreover, stem cells represent a potentially unlimited source of myelin-forming cells while either more mature or even post-mitotic myelin-forming cells are difficult to manipulate and can be expanded in vitro only scarcely. Both embryonic stem cells (ES) and aNSCs might represent the ideal cell source for cell replacement-based therapies in myelin CNS disorders. aNSCs showed the potential to repair demyelinating lesions by acquiring a preferential glial cell-fate once in vivo transplanted into rodents suffering from either acute or chronic autoimmune inflammatory demyelination [3–6]. ES cells have been able to differentiate into glial cells and re-ensheat in vivo demyelinated axons when transplanted in animal models of either genetically determined or chemically induced demyelination [8–12]. However, transplantation of ES cells have been complicated by the formation of heterologous tissues and teratomas within the organ of transplantation [10,13–15].

Other somatic stem cells of non neuronal origin have been recently used to repair the myelin sheath in vivo. Rats with an acute demyelinated lesion of the spinal cord showed varying degrees of remyelination–which was proportional to the number of injected cells–after systemic or intralesional injection of acutely isolated mononuclear bone marrow-derived stem cells (BMSCs) [16,17]. Moreover, bone marrow-derived stromal cells induced remyelination and improvement of axonal conduction velocity once transplanted by direct microinjection into the demyelinated spinal cord of immunosuppressed rat [18]. These results, although encouraging, are still too preliminary to draw any meaningful conclusion about the therapeutic use of BMSCs in demyelinating disorders.

The route of cell administration represents another key issue for stem cell transplantation in multifocal CNS diseases. While direct intralesional cell transplantation can be instrumental in focal CNS disorders (e.g. Parkinson's disease or spinal cord injury), alternative approaches have to be established in multifocal CNS disorders (e.g. MS), where multiple CNS injections would be impractical. Interestingly enough, some recent experiments have shown that stem cells (e.g. bone marrow cells, mesenchymal cells, aNSCs) may reach multiple areas of the CNS once injected into the blood stream (i.v.) or into the cerebrospinal fluid circulation (i.c.) of rodents with multifocal demyelinating disorders of inflammatory origin [3,4,6]. This specific homing has been explained, at least in part, by the constitutive expression by transplanted stem cells of a wide array of inflammatory molecules such as adhesion molecules (i.e. integrins, selectins, immunoglobulins, etc.), cytokines, chemokines, and chemokine receptors [4,19–24].

Ideally, once in the target organ, transplanted stem cells should differentiate into the appropriate daughter cells and persist as longest as needed at the site of engraftment. Very little is known about the mechanisms instructing the terminal differentiation of stem cells in vivo, however, there is strong evidence that the local micro-environment might dictate the fate choice of transplanted uncommitted stem cells. In this respect, undifferentiated multipotent aNSCs or even totipotent ES cells, transplanted in different experimental neurological conditions, have shown considerable capacity to restrict their terminal fate to tissue-specific cues and replace non-functioning neural cells of different lineages, including myelin-forming cells [4,10–12]. It has been shown that also BMSCs may give rise to myelin-forming cells once transplanted in vivo into demyelinated areas [16,17]. However, developmental transdifferentiation of BMSCs into neural stem cells–although clearly described–has been recently disputed by studies showing that this is a rare event in vivo and that most of "transdifferentiated" BMSCs are transplanted cells whose nuclei are fused with those of endogenous resident neural cells [25–28].

Finally, the functional integration of stem cells at the site of homing/transplantation is the most critical issue. Although indications that stem cells—whatever their tissue of origin—can reach the target organ and differentiate into the appropriate lineage exist, there is still scarce evidence that these cells can reconstruct the 3D brain architecture and give raise to properly functioning cells integrating into the brain circuitries. So far, most studies on aNSCs or BMSCs have relied strictly on morphological or immunohistochemical evidence [29]. Further studies fulfilling several strict criteria are therefore necessary to determine whether a stem cell has generated a functional neuronal or glial cell.

In conclusion, the intrinsic complex nature of MS—in particular its chronicity and multifocality—the presence of both inflammatory (acute myelin and axon destruction) and degenerative (chronic axonal loss) features—poses great limitations for cell-based remyelinating therapies. Although promising results have been obtained using stem cell-based therapies in pre-clinical settings, the great challenge for the next future is to understand how to use, in a reliable, safe and reproducible fashion, these cells in order to hopefully achieve a complete functional and anatomical rescuing of myelin architecture.

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