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Review

Neural stem cells and the quest for restorative neurology

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Summary. A great deal of interest has attracted the attention of researchers on the potential use of (neural) stem cells in cell replacement or restorative therapies for heretofore incurable CNS pathologies such as brain stroke, spinal cord injury, Parkinson's disease or multiple sclerosis. This short perspective illustrates our view of neural stem cell research with a focus on the stem cell concept, on the *in situ* identity of neural stem cells and on selected aspects of embryonic and adult neurogenesis. A brief survey of current stem cell-based experimental literature tries to provide a realistic picture of how far we have gone in the quest to establish a restorative neurology.

Key words: Neuroregeneration, Neurodegeneration, Brain stroke, Multiple sclerosis, Spinal cord injury, Parkinson's disease, Neurogenesis, Stem cells, Stem niche, cellular therapy, subventricular zone, subgranular zone, astrocyte, radial glia, ependymocyte, GFAP

Introduction

Despite the fact that the concept of "stem cell" was introduced decades ago, to date, stem cells can only be defined functionally, not morphologically or phenotypically. A consensus defines stem cells as precursor cells able to propagate themselves indefinitely (long-term self-renewal) and also to generate multiple differentiated cellular progenies (multipotentiality). By extension, in the nervous system, neural stem cells can be defined as a self-renewing subtype of precursor cells with the ability to generate mature neural cell types, neurons and glia. In contrast, neural progenitor cells are defined as proliferative cells with a limited capacity for self-renewal which often generate a single mature neural cell type (unipotentiality). Cells retrospectively fulfilling stem cell criteria (on the basis of their behaviour after isolation) can be obtained from several regions of the embryonic as well as the adult nervous system. Under appropriate conditions, and in the presence of mitogens, neural precursors isolated from neurogenic but also from some non-neurogenic regions can undergo extensive *in vitro* expansion and, therefore, have been proposed as a multipotent renewable source of neural precursors for transplantation in various central nervous system (CNS) diseases. As a matter of fact, there is some evidence for the *in vivo* existence of highly neuropotent embryonic stem cells supporting the generation of the different neural cell types. It is also accepted that stem cells support ongoing neurogenesis in restricted regions of the adult brain. However, because specific markers are not yet available, the precise *in vivo* location and quantity of the endogenous neural stem cells remain basically uncertain (see e.g. Potten and Loeffler, 1990; McKay, 1997; Morrison et al., 1997; Svendsen and Smith, 1999; Fuchs and Segre, 2000; Gage, 2000; Horner and Gage, 2000; Alvarez-Buylla et al., 2001; Blau et al., 2001; Clarke and Frisen, 2001; Morrison, 2001; Temple, 2001a,b; Galli et al., 2003, for selected reviews). In this short perspective we provide our view of neural stem cell research with a focus on the *in situ* identity of these cells and on some aspects of embryonic and adult neurogenesis. Then we summarize current experimental literature regarding stem cell-based therapies with an aim to provide a realistic updated picture of the quest for a cure for heretofore incurable neurological diseases.

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The *in situ* identity of neural stem cells

The discovery of spontaneous neuronal replacement in warm-blooded adult vertebrates has not only broken a long-standing dogma in neurobiology but has also opened new strategies for brain repair (see Nottebohm, 2002, for an excellent historical account and perspective). In the adult mammalian brain, the ongoing genesis of new neurons through the lifespan is now well documented in two regions: the subgranular layer of the hippocampal dentate gyrus and the subventricular zone of the lateral ventricles. From the subgranular layer, after a short-distance migration, newly generated neurons incorporate into the adjacent granule cell layer. From the

subventricular zone, in turn, new neurons reach their destination in the granule and periglomerular layers of the olfactory bulb after a remarkably long-distance migration via the so-called rostral migratory stream. Endogenous stem cells are thought to sustain adult neurogenesis in both of these regions, the most active one being the subventricular zone (see e.g. Alvarez-Buylla et al., 2001, for review).

But where exactly do these outstanding neural progenitors reside? Do they behave comparably? How far do *in vitro* and *in vivo* data go? In the adult mammalian brain, cells expressing the intermediate filament glial fibrillary acidic protein (GFAP, the main marker for astrocytes, tanycytes and ependymocytes) residing in the subventricular zone of the lateral ventricle have been proposed as the neural stem cells giving rise to the ongoing production of olfactory bulb interneurons. However, the *in vivo* identity of this population remains controversial, since one group of researchers proposed an ependymocyte (Johansson et al., 1999) and another one an astrocyte (Doetsch et al., 1999), as the resident stem cell. While the data derived from these studies were robust enough, the interpretation of the results may remain debatable (see e.g. the discussions in Doetsch et al., 2002 and in Brazel et al., 2003) until more specific markers become available. All the same, the proposal that adult neural stem cells share characteristics with astroglia have found more support up to date (Chiasson et al., 1999; Laywell et al., 2000; Capela and Temple, 2002; Imamura et al., 2003). On the other hand, the hypothesis that these particular subventricular astrocytes with neurogenic potential can derive from embryonic radial glia (Alvarez-Buylla et al., 2001) match seminal observations in birds (Alvárez-Buylla et al., 1990; Gray and Sanes, 1992) and recent findings in mammals (Malatesta et al., 2000, 2003; Noctor et al., 2001; Miyata et al., 2001; Tamamaki et al., 2001; Heins et al., 2002) that radial glia behave as stem cells and consequently give rise to neurons. However, even though there is a general acceptance that most adult astrocytes arise from radial glia, because cellular profiles, resembling transitional forms between both cellular types have been repeatly observed, the astrocyte precursor function of radial glia remains largely unknown *in vivo* (see e.g. Zhan, 2001 for review). In fact, the existence of a single cell lineage for all kind of astrocytes is unlikely (Rickmann and Wolff, 1985; Marin-Padilla, 1995; Mi and Barres, 1999; DeAzevedo et al., 2003). A study using metabolic labelling suggests that less than one third of the astrocytes are generated from radial glia (Miyamura et al., 1998). A very interesting recent study (Malatesta et al., 2003) shows that the *in vivo* neuronal progeny of the radial glia differs profoundly between CNS regions. The question remains about the astrocyte lineage and above all, about which mechanism endows a few GFAP-positive cells around the lateral wall of the lateral ventricles and not other in adjacent regions with lifespan neurogenic properties. New developmental studies may hold some keys to uncover the mechanism that regulates the generation of such a diversity and the differentiation of progenitors during CNS ontogeny (e.g. Stenman et al., 2003; Luque et al., 2003).

In the hippocampus, subgranular layer GFAP-positive cells have also been reported to give rise to neurons in the dentate gyrus (Seri et al., 2001), but it is less clear whether they constitute an actual neural stem cell population capable of generating differentiated neurons and glial cells and also of long term self-renewal. Work by some authors suggests that, unlike in the subventricular zone, separate unipotential progenitors generate neurons and astrocytes in the adult subgranular layer (Roy et al., 2000; Seaberg and van der Kooy, 2002). This would imply that the place of the adult endogenous stem cell may be restricted to a single CNS region. Nevertheless, there is very little *in vivo* evidence that in addition to neurons, individual GFAP-positive cells in the adult subventricular zone are capable of generating differentiated astrocytes or other glial cells. If this is the case, it would mean that committed precursor cells, rather than bona fide stem cells support the neurogenic activity of the adult brain. But it is also worth mentioning that up to date there are no examples of cells from any tissue system fulfilling both *in vitro* and *in vivo* stem criteria. In a certainly inspiring recent report (Doetsch et al., 2002) cultured neural stem cells isolated from the adult subventricular zone have been suggested to be derived not from the earlier proposed (Doetsch et al., 1999) endogenous stem cell (type B cell, astrocyte) but from what *in vivo* was considered a transit-amplifying neuronal-commited progenitor (type C cell). Thus, the concept has been put forth for the possibility that manipulations or *in vitro* conditions can re-awaken a latent stem cell program in what can be regarded as "potential stem cells". In this sense, long-term renewal and multipotentiality may be properties shared by multiple cells derived from genuine stem cells, and thus the stem cell concept may be better applied to the potential of cells within the roots of a lineage rather than to a precise "rare" cell type (Doetsch et al., 2002; see also Dupin et al., 2003 for a more recent dramatic example of reversal in precursor hierarchy in differentiated Schwann cells to function as bipotent stem cells). Interestingly, as mentioned above, retrospectively identified neural stem cells can also be isolated from non-neurogenic regions (e.g. Johansson et al., 1999). This may indicate that cells endowed with stem-like potential may exist throughout the CNS, but only those existing within brain areas and which retain a special "niche" or environment remain neurogenically active, while those outside of these "niches" may remain latent (see Galli et al., 2003, and quotes therein).

The idea that stem cells are controlled by particular microenvironments or "niches" has been widely invoked, but it has remained a largely theoretical construction since its original formulation by Shonfield in the 70's. They are currently considered to be a subset of cells and extracellular enviroment that can indefinitely house one or more stem cells and control

their self-renewal and progeny production *in vivo* by rationing growth and differentiation factors (for review see Spradling et al., 2001). Mercier and coworkers (2002) have recently revised the neuroanatomy of the subventricular neurogenic "niche". They have presented splendid structural and ultrastructural evidence for highly organized extravascular basal laminae, exclusive to the subventricular zone, which have been termed fractones because of their fractal organization. An individual fractone engulfs numerous processes of astrocytes, ependymocytes, microglial cells and precursor cell types in its folds as well as perivascular macrophages that belong to a fibroblast/macrophage network coursing in the perivascular layer and through the meninges. Different cytokines, growth factors and other molecules may concentrate in and exert their effect from fractones which are most likely involved in adult neurogenesis. Interestingly enough, fractones, similar to those found in the subventricular zone, have also just been described along the walls of the third ventricle (Mercier et al., 2003).

A new report (Imamura et al., 2003) has just confirmed that the predominant neural stem cell isolated from postnatal and adult forebrain, but not early embryonic mouse forebrain, expresses GFAP. But what is the role of intermediate filament proteins such as GFAP in the biology of neural stem cells? Mutant GFAP null mice were shown to develop with apparent normality in earlier reports (Gomi et al., 1995; Pekny et al., 1995) which suggests that this protein may not be essential for neural stem cells or that in its absence its role is compensated. However, GFAP expression has later been shown to be important for normal white matter architecture and blood-brain barrier integrity, and its absence leads to late-onset CNS dysmyelination (Liedtke et al., 1996). GFAP null astrocytes, but not vimentin null astrocytes, have been shown to be a favourable *in vitro* substrate for neuronal survival as well as for neurite growth (Menet et al., 2000, 2001; Hanbury et al., 2003). Also interestingly in the context of this review, a high susceptibility to cerebral ischemic damage (Nawashiro et al., 2000; see, however, Hanbury et al., 2003) and to experimental allergic encephalitis (Liedtke et al., 1998) has been reported in GFAP mutant mice. On the other hand, a weak and diffuse nestin expression pattern has been reported in cultured astrocytes (Menet et al., 2001) and in endothelial and ependymal cells of vimentin and double GFAP-vimentin mutants, with nestin failing to organize itself into a nestin network in reactive astrocytes from double mutants. Defective astrocytic scar formation was observed in an earlier report in the GFAP-vimentin mutants after spinal injury (Pekny et al., 1999). Recently, we have observed an increased sprouting of supraspinal axons including reconstruction of circuits leading to a functional restoration in double mutants after spinal cord hemisection (Menet et al., 2003). In addition, the integration of retinal cell grafts into adult retina has been reported to be vastly improved in the absence of both GFAP and vimentin (Kinouchi et al., 2003). Interestingly enough, while abnormalities in retinal histology were not observed in double mutants by these authors, subtle, non-additive defects, have been shown in Bergmann glia cells in adult cerebellum, an astrocytic population that, likewise retinal Müller glia, normally expresses both proteins (Giménez y Ribotta et al., 2000). In contrast to the relatively subtle developmental defects in the absence of GFAP, its over-expression can be lethal, which led to the discovery that GFAP-coding mutations are responsible for most cases of Alexander's disease, a devasting neurodegenerative disorder (see Messing and Brenner, 2003 for review). It will be positively interesting to further investigate the embryonic and adult germinal zones of such transgenic animals, along with the *in vitro* properties of their isolated precursors, particularly in the context of aging and neurodegeneration.

Regarding (neural) stem cell plasticity, is there anywhere to stem?

The concept of stem plasticity rests on the ability of a cell to take on different fates in response to environmental signals, both those it might normally acquire *in vivo*, and those that it might not (see e.g. Weismann, 2000; Temple, 2001a, for reviews; see also Frisen, 2002, for further critical discussion of this concept). Several studies, both inside and outside the CNS, have shown that neural stem cells appear to posses a wide developmental potential and functional repertoire whose expression is strongly influenced by extracellular cues (for review see e.g. Galli et al., 2003). Indeed, neural stem cells are claimed to rank among the more plastic of the somatic cells and have been shown to contribute to tissue generated by all three primary germ layers when introduced into a blastocyst (e.g. Clarke et al., 2000; Tropepe et al., 2001). Conversely, stem cells from non-ectodermic origin have been shown to contribute to most, if not all, somatic cell types, the most striking example being a mesenchymal stem cell derived from adult marrow, termed MAPC (multipotent adult progenitor cell) (Jiang et al., 2002a). This late work, along with a remarkable series of recent articles claiming adult stem cell plasticity (see e.g. Galli et al., 2003 for review), has promoted the idea that there is essentially one stem cell type that with the appropiate stimulation will create any kind of cellular progeny. Nevertheless, it seems that most neural-generating stem cells might be increasingly specified (although perhaps not commited) along the course of development. In other words, neural stem cells seem to be regionally and temporally restricted during development to become a particular cell type, so the existence of a single stem cell type through the time, and the existence of a truly pluripotent cell type in the mature nervous system would be unlikely. As a matter of fact, for some authors both views could be compatible. While most neural stem cells might be regionally and temporally specified, there may also be

rare stem cells, perhaps not necessarily of neural origin, still present in the adult nervous system, that have greater plasticity. Shall we be astounded if some of these cellular rara avis do actually exist in the mature brain or if they are hidden for example under the innocent look of cells decorated with GFAP antibodies?. A new report (Jiang et al., 2002b) shows that cells with MAPC characteristics can be isolated not only from bone marrow, but also from brain and muscle. Whether MAPC originally derived from bone marrow are actually existing *in vivo* and, if so, whether they are circulating or all organs contain MAPCs, remains to be seen. The possibility has also been raised that such an amazing plasticity after transplantation could be explained by fusion of donor and host cells, rather than by the actual presence of multipotential or several types of adult stem cells in donor (see e.g. Galli et al., 2003 for a balanced discussion and review). A couple of reports just published simultaneously in Nature have shown that cell fusion is the main source of bone marrow-derived liver cells (Vassilopoulos et al., 2003; Wang et al., 2003).

Stem-cell-based strategies for CNS repair

Stimulated with the armamentarium of accumulated knowledge about stem cell properties a fast-growing community of researchers are engaged in the development of stem cell-based therapies for CNS repair. There are currently two strategies in the quest for developing a restorative therapy: First, tapping the potential of endogenous neurogenesis and/or gliogenesis within the mature CNS and second, transplantation of stem and progenitor cells (isolated from embryonic, foetal or adult tissue) to replace lost populations in brain and spinal cord pathologies.

As rightly pointed out by Nottebohm (2002) these two strategies are likely to vie for primacy, and it may occur that there are special conditions or part of the CNS that will benefit from stem cell transplantations or from the activation on endogenous progenitors. Perhaps, if adult neurogenesis is locally restricted to the forebrain, then this region would be better suited for autogenous repair than regions elsewhere which in turn would be elegible for transplantation strategies. Manipulation of endogenous neural precursors may became an alternative therapy or a complementary therapy to stem cell transplantation for neurodegenerative diseases and CNS injury. Certainly, we might be in for additional surprises. Actually, neurodegeneration could be conceived as a collapse of endogenous neuroregeneration. A primary deficit in neural stem cell proliferation, migration, or differentiation might contribute to net cell loss and consequently neuronal circuit disruption in Parkinson's, Huntington's or Alzheimer's diseases (e.g. Armstrong and Barker, 2001). Validation of this certainly provocative hypothesis could have profound implications for future treatment of these and other incurable neural disorders. In the meantime, it is already increasingly difficult to keep ahead of all the developments in the field. A plethora of stem cell-based research studies hold the promise to cure the hithertho uncurable; brain stroke, spinal cord injury, Parkinson´s disease and multiple sclerosis being among the cases in point.

Brain stroke

Whereas acute stroke treatments focus on thrombolytic and neuroprotective molecules, stroke recovery represents a new and relatively untested target for therapeutics dealing with the structural and functional plasticity of the damaged brain. Cell transplantation holds promise as a treatment to enhance neurological recovery after a stroke. Porcine foetal cells, stem cells, immortalized cell lines, and marrow stromal cells are under intense investigation in experimental and clinical stroke recovery trials (see Savitz et al., 2002; Cairns and Finklestein, 2003, for reviews). Seminal observations of accelerated proliferation of brain neuronal precursors after forebrain ischemia in mice (e.g. Takagi et al., 1999) have opened new research avenues to investigate the mechanisms of fuctional recovery after stroke.

Recently, Zhang and coworkers have shown that grafted adult cultured subventricular zone cells selectively migrated toward ischemic boundary regions in the adult rats with stroke. Moreover, a significantly enhanced functional recovery was obtained when cells were intracisternally transplanted 48 hours after the stroke (Zhang et al., 2003). The site of implantation of stem cell grafts probably plays a key role in stroke recovery. Differential effects of intraparenchymal and intraventricular grafts indicate that different mechanisms might be involved in recovery from cognitive and sensorimotor deficits induced by stroke. In addition, both the intact and lesioned hemispheres appear to exert a tropism for grafted stem cells, suggesting local repair processes and plastic changes in contralateral pathways (Modo et al., 2002). High migration patterns and comparable results have been observed for transplanted embryonic stem cells using magnetic resonance imaging in rats with stroke (Hoehn et al., 2002). Multipotent progenitors from bone marrow mesenchyma have been able to generate cells exhibiting neuronal and glial phenotypes, and ameliorate neurological deficits after grafting into ischemic rats (Zhao et al., 2002). However, the morphological features of the grafted cells were poorly differentiated, spherical in nature and with few processes. Thus, these authors considered that the functional recovery of grafted animals was mediated by proteins and trophic factors secreted by the transplanted stem cells rather than by the full integration of transplanted cells in the host brain circuitry. Interestingly enough, bone marrow-derived circulating endothelial progenitor cells have been shown to contribute to the vascular substructure of the choroid plexus and to participate in cerebral neovascularization after focal ischemia (Zhang et al., 2002). More recently, a hopeful

report of a related field shows the safety, feasibility and benefits of autologous progenitor cell transplants (bone marrow or circulating blood-derived progenitor cells) in a randomised clinical trial in patients with ischemic heart disease due to myocardial infarction (Assmus et al., 2002).

A Japanese and a Swedish group have claimed most hopeful results of neural replacement therapy after experimental ischemic injury by recruitment of endogenous neural progenitors (Arvidsson et al., 2002; Nakatomi et al., 2002). Nakatomi and coworkers have shown that the activation of murine endogenous progenitors after ischemic injury leads to massive regeneration of hippocampal pyramidal neurons. Subsequent to the ischemic injury the progenitors were shown to migrate (most probably from the subventricular hippocampal arch, rather than from the subgranular dentate zone) towards the hippocampal pyramidal field where they appeared to integrate as new neurons. The infusion of growth factors appears to strongly improve this response as well as the fuctional outcome (Nakatomi et al., 2002). Arvidsson and coworkers have shown that ischemia generated by cerebral middle artery occlusion leads to a marked increase in cell proliferation in the subventricular zone. New stroke-generated neurons along with neuroblasts probably formed before the lesion, were shown to migrate into the damaged striatum, where they expressed markers of developing and mature striatal medium-sized spiny neurons (Arvidsson et al., 2002). Both studies certainly expand the possibility of novel neuronal cell regeneration therapies for brain stroke recovery and perhaps other neurological diseases.

Parkinson's disease

Parkinson's disease affects four million people worldwide. The symptoms of the disease are tremor, bradykinesia, rigidity and postural instability. The disease involves the death of dopaminergic neurons of the nigrostriatal pathway. The ultimate cause of Parkinson's disease remains unknown (Olanow and Tatton, 1999). Experimental models of the disease are based on surgical techniques or the use of specific neurotoxins affecting the dopaminergic nigrostriatal system. Transplants of dopamine-producing neurons into the striatum have been used to partially reverse Parkinsonism symptoms in animal models (Arenas, 2002; Lindvall, 2003). In humans, however, although early uncontrolled clinical reports were enthusiastic, the outcome of the first randomised, double-blind, controlled study using intrastriatal transplants of foetal mesencephalic tissue (Freed et al., 2001) was quite controversial and raised doubts and ethical concerns about the cell replacement strategy in Parkinson's disease patients. Stem cells are in the mind of everyone as an obvious research avenue because first of all they could be useful to generate large numbers of dopaminergic neurons in standardized and quality-controlled protocols. Neurons with some dopaminergic characteristics have been generated from stem cells, but in many cases the survival after grafting in experimental models of Parkinson's disease was limited, and it was also unclear whether they functioned as normal intrinsic dopaminergic neurons (Arenas, 2002; Lindvall, 2003, for reviews). A series of recently published articles (Björklund et al., 2002; Kim et al., 2002; Yang et al., 2002; Nishimura et al., 2003), has greatly encouraged the use of embryonic stem cells in cell replacement therapy for Parkinson's disease. Björklund and coworkers have shown that transplanted undifferentiated embryonic stem cells can develop spontaneously into dopamine neurons which can restore cerebral function and behaviour in an animal model of Parkinson's disease. Tumour formation in a proportion of the transplanted animals was, however, a concern. Similar results have been reported by Yang and coworkers using a clonal cell line of undifferentiated neural stem cells. Upon transplantation into the intact or 6-hydroxydopamine-lesioned striatum, cells withdraw from the cell cycle, migrate extensively in the host striatum and most of them express dopamine, sometimes improving motor behaviour. The authors consider it conceivable that the adult brain contains sufficient intrinsic cues to direct the specific expression of dopaminergic traits in immature multipotential neural stem cells (Yang et al., 2002). Kim and coworkers and Nishimura and coworkers have both presented encouraging data by using differentiated stem cells. It has been shown that mouse embryonic stem cells can generate a highly enriched population of dopaminergic cells exhibiting electrophysiological and behavioural properties like those of neurons from the midbrain (Kim et al., 2002). After transplantation into the brains of rat with Parkinson's symptoms the cells survived for 2-3 months while reverting the symptoms. Under the conditions described, the authors did not observe dividing cells in the analysed grafts. In the study by Nishimura and coworkers, however, one out of ten transplanted mice developed a tumour. Additional long-term data are clearly needed to investigate the *in vivo* proliferative potential of embryonic stem-derived cells.

Spinal cord injury

Spinal cord injury (SCI) is a major cause of disability which generally strikes down young and healthy people. The functional loss that occurs after traumatic SCI results from the initial injury which is immediate and irreversible, and from the reactive cascade, which leads to a subsequent secondary lesion. This secondary neuronal damage is associated with pathological changes in endogenous neurotransmitter systems, including autodestructive factors such as free radicals and excitatory amino acids. Axonal regeneration at the injury site is extremely rare due to (i) the formation of glial scar involving both activated microglia and astroglia, (ii) the presence in the adult

CNS tissue of inhibitory or non-permissive molecules, and (iii) the poor presence of neurotrophic factors or a stimulating substrate for promoting axonal growth (Giménez y Ribotta and Privat, 1998). No practical treatment is yet available. In contrast, there are several reports showing a limited degree of axonal regeneration and functional recovery using a diversity of experimental strategies in animal models of spinal cord injury (Gimenez y Ribotta et al., 2002, David and Lacroix, 2003). Transplants using differentiated embryonic neural stem cells have shown that these populations have the capability of generating neuronal and glial cells and achieving some remyelination and functional recovery after spinal cord lesions (McDonald et al., 1999, Ogawa et al., 2002). Transplants of differentiated clonal neural precursors from adult human brain obtained from surgical resections have also been shown to establish functional peripheral myelin in the rat spinal cord (Akiyama et al., 2001). On the other hand, grafts of undifferentiated embryonic stem cells into the intact or lesioned adult rat spinal cord appear to be restricted to a glial lineage (Cao et al., 2001). *In vitro* priming of these embryonic precursors to a neuronal phenotype demonstrates that they can indeed differentiate into several types of neurons in the normal adult rat spinal cord, but such differentiation appears to be inhibited in the injured spinal cord (Cao et al., 2002). Therefore, manipulation of the microenvironment in the injured spinal cord or manipulation of stems cells prior to transplantation appear as necessary to induce neuronal differentiation in a replacement strategy. Interestingly, the neurogenic capacity of adult spinal cord precursors has been shown after transplantation into the adult dentate gyrus (Shihabuddin et al., 2000). On the other hand, its has been reported that in response to spinal cord injury, ependymal cell proliferation increases dramatically to generate migratory cells that differentiate to astrocytes and participate in scar formation (Johansson et al., 1999). A more recent study supported the view that ependymal cells of the central spinal canal are indeed neural stem cells, can proliferate according to the severity of the injury, and generate reactive astrocytes within the ependyma (Takahashi et al., 2003). In addition, there is also evidence that in the adult rat spinal cord, a significant number of endogenous neural progenitors are present in parenchymal regions beyond the periventricular area, and the immunohistochemical analysis suggested that proliferative progenitors emerge through the gray and white matter in the lesioned spinal cord (Horner et al., 2000; Yamamoto et al., 2001). Thus, the widespread occurrence of resident neural progenitors in the adult spinal cord also guarantees future research efforts to activate their restorative potential after trauma. In addition to the spinal cord, a recent publication (Riess et al., 2002) has shown that a neural stem cell clone survives, differentiates and improves motor function after traumatic brain injury. Interestingly, cells were found to differentiate to just neuronal (contralateral to the lesion), or neuronal and astroglial (ipsilateral), but not to oligodendroglial phenotype.

Multiple sclerosis

Multiple sclerosis is a chronic inflammatory and demyelinating autoimmune disease of the CNS affecting around one million people worldwide. Paralysis, blindness, loss of sensation and lack of coordination are among the possible outcomes of the disease. Most existing treatments are focused on blocking the immunological attacks on a wide front (Steinman, 2001). A seminal study illustrated that undifferentiated embryonic stem cells transplanted into a demyelinating disease animal model differentiated into oligodendrocytes which myelinated host axons (Liu et al., 2000). A recent very hopeful report (Pluchino et al., 2003) showed that intravenous or intraventricular injection of *in vitro* expanded adult stem cells isolated from the subventricular zone induces recovery in experimental autoimmune encephalomyelitis, a chronic animal model of multiple sclerosis. Interestingly enough, like the surface of attacking immune cells, the neural precursor cells also appeared to express the adhesion molecule intergrin $\alpha 4$. Thus, perhaps they use it to move from the ventricular system or from the blood into the brain. The neural precursors are claimed to be there involved in processes that decrease the levels of inflammatory molecules, to reduce the astrocytic scarring associated with CNS inflammation, to give rise to new pools of oligodendrocytes and neurons and to produce growth factors such as CNTF that may provide a restorative milieu. The potential of this strategy to treat this and other disorders such as Alzheimer's disease, also characterized by very extensive or global neuropathie, is obvious.

Concluding remarks

Neural stem cell research portraits itself as an ultra-fast moving field. The very concept of stem cell is fluxing because every now and then it is challenged with new experimental data that in turn open avenues of new queries and new uncertainties. Particularly, because of the difficulty to harmonize the *in vivo* and *in vitro* studies highlighting differences between stem and more restricted progenitor cells, there is a current trend towards the abandonment of rigorous definitions of stem and progenitor cells in favour of more wide concepts (Seaberg and van der Kooy, 2003). The finest of the attempts to develop an amended conceptual definition of adult tissue stem cell (Loefler and Roeder, 2003) has not been matched by a comparable achievement that provides a definition of embryonic stem cell. One wonders if this has something to do with the fact that *in situ* most, if not all, of the so-called embryonic stem cells are not self-renewing or self-mantaining as the embryo continues to develop. In addition, even if the need for molecular markers in the field is clear, it appears conceptually misleading to consider stemness

(or neural stemness) as a specific property that can be determined at one point in time (e.g. by gene or protein profiling with microarray technology) without putting the cells to functional test (Loefler and Roeder, 2003). To our perception the fascinanting changing nature of the current concept of stem cells mirrors the extreme dynamism of this area of research, perhaps in a much similar manner to the modifications suffered by earlier key biological ideas in highly dynamic research fields (consider e.g. Genetics and the accumulated changes in the concept of the gene). Thus, even irrespectively of the availability of prospective markers, it is easy to envisage an increasing difficulty to obtain a definition of stem cells which gives an account for all stem cells processes. The more we learn about the molecular biology of stem cells, the more dispensable becomes a given concept about it (presumably, no one will include all the details that are discovered at the molecular level).

It is increasingly evident that a given isolated (neural) progenitor may acquire a broader developmental potential as a consequence of proliferation in culture. Genes responsible for *in vivo* regional and temporal specification of neural fates are likely to switch on/off in the culture dish. Perhaps the expression patterning of some of these genes may eventually be responsible for changes in growth factor sensitivity regulating (neural) lineage determination (in a way that could be better described as de-differentiating). On the other hand, regardeless of the possibility of the actual existence of multipotent progenitors or progenitors capable of cell fusion, what is clear is that there would be a change in the pattern of gene activation. We can only hope to either find a way to understand such a change or, at least, to make it possible.

It is obvious that several scientific issues should still be addressed before we can begin with stem cell-based clinical trials for human CNS repair. To understand how to drive differentiation along specific pathways, to improve specific cell sorting and isolation, to control cell proliferation and cell-cell interactions or immune responses that might mediate graft rejection or to assess the long-term stability of transplanted cells are among the cases in point. The development of extra-safety measures, such as the incorporation of genetically engineered "suicide cassettes" in cells to be used for human transplantations (Schuldiner et al., 2003), should be more than welcome. But beyond all reasonable hesitation, these days of exciting developments are shifting faster than ever an ancient view of fatality about the outcomes of CNS damage, since the oldest written record of the word brain is in The Edwin Smith Surgical Papyrus. Even though there is no guarantee that all the research activity displayed for the development of stem cell-based therapies will give way to clinical solutions for CNS repair, the level of hopfulness is today higher than ever for heretofore uncurable spinal cord injury, brain stroke, multiple sclerosis or Parkinson's disease. However, a routine restorative neurology department does yet not exist in hospitals. …An ailment to be treated. This is the quest.

Note added in proof: After acceptance of this manuscritp two new studies (alvarez-Dolado et al., 2003, Nature 425: 968-973; Weimann et al., 2003, Nature Cell Biol. 5: 959-966) identified cell fuison as the mechanism underlying contribution from bone-marrow-derived stem cells to Purkinje neurons in cerebellum.

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