REVIEW

New perspectives of tissue remodelling with neural stem and progenitor cell-based therapies

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Abstract Compelling evidence exists that neural stem cell-based therapies protect the central nervous system (CNS) from chronic inflammatory degeneration, such as that occurring in experimental autoimmune encephalomyelitis and stroke. It was first assumed that stem cells directly replace lost cells but it is now becoming clearer that they might be able to protect the nervous system through mechanisms other than cell replacement. In immune-mediated experimental demyelination and stroke, transplanted neural stem/precursor cells (NPCs) are able to mediate efficient bystander myelin repair and axonal rescue. This is dependent on multiple capacities that transplanted NPCs exhibit within specific microenvironments after transplantation. However, a comprehensive understanding of the mechanisms by which NPCs exert their therapeutic impact is lacking. Here we will review some of the most recent evidence — and discuss some of the likely mechanisms — that support the remarkable capacity of NPCs to cross-talk with endogenous cells and to remodel the injured nervous system when applied as novel therapeutic regimes. We foresee that the exploitation of the innate mechanisms regulating these modalities of cell-to-cell communication has realistic chances of revolutionizing most of the actual understanding of stem cell biology and its application to regenerative medicine and CNS repair.

Introduction

A large number of experimental approaches have been carried out in the last decade with neural stem/precursor cells (NPCs) both in acute neurological disease models in which a primary inflammatory mechanism leads to secondary neural degeneration and in chronic disease models in which primary neural degeneration is accompanied by a secondary inflammatory response (Martino and Pluchino 2006). NPCs hold a great potential for the treatment of neurological disorders, not only because of their capacity to integrate into the host tissue, contributing to the replacement of damaged cells but also because of several *bystander* capacities, such as tissue trophic support and immune-regulation (Ben-Hur 2008). These effects are supported by the evidence that transplanted NPCs shape the host environment towards a *rescue mode* for endogenous glial and neuronal cells that have survived primary damage (Martino and Pluchino 2006; Lindvall and Kokaia 2010). Thus, transplanted NPCs promote optimal tissue remodelling, finally leading to clinical recovery. Importantly, this recovery occurs irrespective of the experimental disease characteristics (focal vs multifocal) and is generally associated with only small numbers of transplanted NPCs undergoing terminal differentiation in vivo (Cao et al. 2002; Jeong et al. 2003; Jeong et al. 2003; Chu et al. 2004; Fujiwara et al. 2004). The molecular and cellular mechanisms accounting for this multilayered effect of transplanted NPCs remain far from being fully elucidated.

In this review, we will focus on some of the recent work showing that NPC-based therapeutic strategies are able to

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contribute to remodelling the injured nervous system and discuss potential mechanisms accounting for this effect.

Trophic effects of transplanted neural stem cells

One of the major outcomes of the neuroprotective effects of transplanted NPCs is the significant increase of survival and function of endogenous glial and neuronal progenitors escaping from primary insults (Martino and Pluchino 2006). This phenomenon has broad implications and is usually accompanied by increased availability of a milieu of molecules, such as neurotrophins and growth factors, developmental stem cell regulators and immune modulatory molecules (Martino and Pluchino 2006).

Transplantation studies in experimental neurological diseases

Studies in chronic and relapsing experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis (MS), have first shown that NPCs may enhance endogenous recovery processes by exerting neurotrophic activities (Pluchino et al. 2003; Zhang et al. 2007). Systemically injected NPCs are able to stimulate endogenous oligodendrocyte progenitor cells (OPCs), thus ameliorating the spontaneous remyelination process in EAE (Pluchino et al. 2003). More recently, Einstein et al. have shown that the intracerebroventricular injection of NPCs in a model of extensive demyelination — chronic cuprizone exposure — induces a significant improvement in the remyelination process. Transplanted NPCs have not migrated into the demyelinated area or participated directly in the remyelination process, while remaining mostly undifferentiated. On the other hand, NPCs significantly increased the proliferation of endogenous OPCs through secretion of diffusible factors, such as platelet-derived growth factor-AA (PDGF-AA) and fibroblast growth factor (FGF)-2 (Einstein et al. 2009).

Similarly, solid evidence of the neurotrophic effects of transplanted NPCs is also available from experimental models of cerebrovascular diseases (Bacigaluppi et al. 2008). The focal transplantation of the stable, v-myc immortalized human NPC (hNPC) line into the cerebral cortex of mice having undergone collagenase-induced intracerebral haemorrhage (ICH) led to markedly improved behavioural outcomes (Lee et al. 2007a, b). Grafted cells migrated to the haemorrhage core and also to the border of the lesion, while differentiating mostly into astrocytes and to a lower extent into neurons (Lee et al. 2007a, b). Interestingly, NPCs expressed neurotrophic factors including brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), FGF-2, vascular-endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF), thus providing evidence that some of the observed effects were mediated by a multilayered NPC *neurotrophic signature* (Lee et al. 2007a, b).

Several groups have also reported a neuroprotective or neurotrophic effect of NPC transplantation in rodent models of spinal cord injury (SCI). Transplantation of the mouse NPC line C17.2 elicited substantial host axonal re-growth, due to the cellular substrate provided by the graft, which supported extensive growth of both motor and sensory axons even in the absence of stem-cell differentiation. Also, transplanted NPCs were shown to constitutively secrete several neurotrophic factors [nerve growth factor (NGF), BDNF and GDNF]. The authors also transplanted in the same model a genetically modified NPC line expressing neurotrophin-3 (NT-3) to induce neuronal differentiation and axonal sprouting. Interestingly, over-expression of NT-3 resulted in a collateral decrease of the expression of other neurotrophins, thus suggesting the existence of a complex reciprocal interplay between different growth factor signal transduction pathways in NPCs (Lu et al. 2003). More recently, Kusano and colleagues have demonstrated that the transplantation of NPCs expressing NT-3/D15A — a multi-neurotrophin obtained by the modification of human NT-3 with the capacity to bind both TrkB and TrkC — enhances the survival of grafted NPCs, increases myelin formation and leads to modest improvement of hind limb function, even when applied to a complex environment such as the chronically injured spinal cord (Kusano et al. 2010).

Neural stem cell transplants for neurotrophin delivery into the injured CNS

The delivery of diffusible proteins such as neurotrophins to the CNS represents a great challenge due to the protection provided by the blood–brain barrier (BBB). In order to overcome the BBB filter, NPCs might also be genetically modified to foster their innate capacity to secrete neurotrophic factors as well as to use them as 'Trojan horses' delivering the desired diffusible molecules at the site of injury (Kim and de Vellis 2009). NPC-mediated delivery has been achieved with either focal or systemic cell injection, via the blood stream, the lymph, or the cerebrospinal fluid circulation. Transplanted cells are then able to follow the gradient of chemoattractants (e.g., pro-inflammatory cytokines and chemokines) at the site of nervous tissue damage (*pathotropism*) (Muller et al. 2006). We have first demonstrated that mouse NPCs constitutively express functional cell adhesion molecules (e.g., CD44), integrins (e.g., α4, β1) and chemokine receptors (e.g., CCR1, CCR2, CCR5, CXCR3, CXCR4) that regulate tethering, rolling and firm adhesion to inflamed endothelial/ependymal cells, as well as transendothelial migration towards the inflamed CNS after systemic NPC injection in EAE mice (Pluchino et al. 2003; Pluchino et al. 2005). Importantly, these

findings on rodent NPCs have also been confirmed on hNPCs (Mueller et al. 2006; Rampon et al. 2008; Pluchino et al. 2009a, b).

Among candidate neurotrophic factors to be delivered, GDNF has shown a potent neuroprotective effect on a variety of neuronal injury models, such as stroke and Parkinson's disease (Zhang et al. 2002; Kobayashi et al. 2006). However, its effects are generally transient and need consecutive administrations to obtain long-standing results. NPCs over-expressing GDNF can provide better neuroprotective effects, as shown with hNPCs delivering GDNF in animal models of Parkinson's disease (Behrstock et al. 2006). After striatal transplantation, these cells were able to release physiologically relevant levels of GDNF (up to 8 weeks and 3 months after transplantation, in rat and monkey respectively), increasing host dopaminergic neuron survival and fibre outgrowth (Lin et al. 1993). NPC transplantation into mice with collagenase-induced ICH (Lee et al. 2009), or into rats with middle cerebral artery occlusion (MCAo) (Chen et al. 2009), also led to improved functional recovery and increasing overall cell survival. Interestingly, treated MCAo rats also displayed a significant increase of the synaptic proteins synaptophysin and post-synaptic density protein (PSD)-95, suggesting an enhanced neuronal function and a possible reconstruction of endogenous neural circuitries after the grafting (Chen et al. 2009). Other neurotrophic factors, such as BDNF and VEGF, have been over-expressed in NPCs obtaining comparable results in SCI (Kim et al. 2009a, b) or ICH (Lee et al. 2007a, b; Lee et al. 2010) models.

Regulation of the immune response

A variety of stem cells — including hematopoietic stem cells, mesenchymal stem cells (MSCs) and NPCs — display the potential to promote in situ immune modulation, neuroprotection and tissue regeneration, thus contributing to the idea that these properties are likely due to a shared stemness-related functional signature (Uccelli et al. 2008) and (Martino et al. 2011). The observation that mouse and human NPCs share the expression of a variety of functional immune-like receptors (e.g., cell adhesion molecules and pro-inflammatory chemokine receptors) (Martino and Pluchino 2006) has led to a number of studies that aimed at highlighting the extent of the crosstalk between NPCs and immune cells. The immune regulatory actions of transplanted NPCs have been described in acute and chronic EAE, SCI, stroke and neurometabolic diseases, among others.

Effects in the injured CNS

Considerable evidence of the immune modulatory capacity of NPCs has derived from transplantation studies through different routes on the EAE model. Intracerebroventricularly injected NPCs were able to migrate into white matter tracts and attenuate brain inflammation, reducing demyelination and axonal pathology and ultimately the clinical severity of both acute and chronic EAE mice. This effect correlated with a reduction of perivascular infiltrates and of CD3$^+$ T cells and an increase in CD25$^+$ and CD25$^+$/CD62L$^+$ regulatory T cells, as well as a significant reduction in the brain expression of two markers of inflammation, the inter-cellular adhesion molecule (ICAM)-1 and its ligand, lymphocyte function-associated antigen (LFA)-1 (Einstein et al. 2003). Also, systemically injected NPCs induced apoptosis of blood-borne CNS-infiltrating encephalitogenic T cells, thus protecting against chronic neural tissue loss as well as disease-related disability (Pluchino et al. 2005).

In vitro, NPCs increase the apoptosis of proteolipid protein (PLP)139–151-specific Th1 pro-inflammatory (but not Th2 anti-inflammatory) cells selectively through the engagement of death receptors, including FasL, TRAIL and APO3L, on the surface of NPCs (Pluchino et al. 2005). Mouse NPCs also inhibit T cell activation and proliferation in response to T cell receptor (TCR)-mediated stimuli (e.g., concanavalin A and anti-CD3/anti-CD28) but not in response to TCR-independent stimuli (e.g., phorbol myristate acetate/ionomycin) (Fainstein et al. 2008). Fainstein et al. also demonstrated in vitro that NPCs suppress T-cell activation but without active induction of cell death. Further studies of NPC-T lymphocyte interactions suggest that part of the anti-proliferative effect of NPCs might depend on the inhibition of IL-2 and IL-6 signalling on T and B lymphocytes respectively (Fainstein et al. 2008). NPCs have also shown to have a direct inhibitory effect on the proliferation of rat EAE-derived lymphocytes in response to myelin oligodendrocyte glycoprotein (MOG) and to concavalin A (ConA) in vitro (Einstein et al. 2003). A recent study by Knight et al. shows that mouse NPCs have a selective pro-apoptotic effect on Th17 pro-inflammatory (but not Th2 anti-inflammatory) cells in vitro via a FasL-dependent mechanism, identifying the axis Fas-Birc3 as a novel survival pathway for NPCs (Knight et al. 2010).

NPCs also suppress T-cell proliferation, at least in part, by reactive production of the soluble mediators nitric oxide (NO) and prostaglandin E2 (PGE2). High levels of NO and PGE2 are in fact induced in T cells when co-cultured with NPCs. In addition, inducible NOS (iNOS) and microsomal type 1 PGES (mPGES-1) are detected in NPCs in co-culture with T-cells, suggesting that NO and PGE2 production in NPCs is induced by exposure to activated T cells (Wang et al. 2009). Interestingly, genetically engineered NPCs expressing IL-10 — an effective anti-inflammatory cytokine that efficiently suppresses EAE (Croxford et al. 2001) — showed enhanced ability to induce remyelination, neuronal repair and immune suppression

(both in periphery as well as in the CNS), as compared to non-engineered NPCs, when systemically injected in EAE mice (Yang et al. 2009).

Human NPCs (hNPCs) suppress the proliferation and alter the cytokine secretion profiles of xenogeneic (e.g., marmoset) antigen-specific and allogeneic mitogen-activated T cells both through direct cell-to-cell contacts and via the release of soluble mediators into the culture supernatant (Kim et al. 2009a, b; Pluchino et al. 2009a, b). In contrast to the mouse NPC counterparts, hNPCs have a limited cytotoxicity towards T cells in vitro, given that CD95L is only barely detectable on their surface. Meanwhile, cytokine-exposed hNPCs express high levels of tumor necrosis factor (TNF)-α, resulting in a higher cytotoxic potential against monocytic-macrophagic cells (Ricci-Vitiani et al. 2007).

Work on rodent models of stroke has shown a critical role of the two CCL2/CCR2 and CXCL12/CXCR4 axes, in trans-endothelial recruitment and intraparenchymal migration respectively, of intravascularly delivered NPCs (Imitola et al. 2004; Darsalia et al. 2007; Andres et al. 2011). NPCs injected systemically in MCAo mice are mainly found in the perilesional area, where they maintain an undifferentiated phenotype (Bacigaluppi et al. 2009; Sun et al. 2010). In the ischemic brain, the interplay between transplanted NPCs and the inflammatory environment is a multi-sided process that involves several mechanisms and cell types (Bacigaluppi et al. 2008). First, transplanted NPCs are able to vary the bioavailability of immune mediators. An increase in the gene expression levels of VEGF, CXCL12/SDF1-α and TGF-β was first observed in mice subjected to systemic injection of NPCs 4 hours after MCAo (Capone et al. 2007). On the other hand, the sub-acute systemic injection of NPCs in MCAo mice produced significant down-regulation in the ischemic brain hemisphere of multiple RNAs involved in inflammation, including *lfng*, *Tnfa*, *ll1b* and *Lepr* (Bacigaluppi et al. 2009). Furthermore, the hyperacute NPC transplantation in experimental ICH has been shown to significantly reduce the number of infiltrating neutrophils in the perihematomal areas 3 days after ICH (Lee et al. 2008). Lee et al. also found a reduction in both the levels of inflammatory mediators and the numbers of activated macrophages in lymphoid organs. Consistently, splenectomy performed before ICH eliminated the positive effect of NPC transplantation on brain oedema and inflammatory infiltrations (Lee et al. 2008).

Several studies have also pointed out at the crucial role of the interaction between transplanted NPCs and microglia/macrophages, though with controversial results. On one side, transplantation of both human and mouse NPCs has been shown to markedly increase the number of infiltrating CD11b^{+} myeloid cells and to modulate their activation in the brain of MCAo mice receiving cell transplantation, with an associated positive effect on motor function and axonal sprouting (Capone et al. 2007; Daadi et al. 2010). In particular,

CD11b^{+} cells were found to have an activated morphology when surrounded by intravenously injected NPCs after transplantation (Capone et al. 2007). These studies contributed to building up the hypothesis that microglia activation might be required for transplanted NPCs to exert their neuroprotective action through secretion of growth factors, including IGF-1, VEGF, TGF-β and BDNF (Capone et al. 2007). These results are in line with the reported exacerbation of the ischemia-dependent brain injury in MCAo mice that were selectively ablated of CD11b-positive microglia (Lalancette-Hebert et al. 2007). On the other hand, two other studies have shown after NPC transplantation a significant reduction of microglia/macrophages in the brain of mice with either ischemic or hemorrhagic stroke, with improved neuronal survival and locomotor functions (Lee et al. 2008; Bacigaluppi et al. 2009).

Modulation of microglia/macrophages infiltrates by transplanted NPCs seems to be crucial also in other disease models, including Alzheimer's disease and SCI. Decreased microgliosis has been observed in a rat model of Alzheimer's disease after transplantation of rat embryonic NPCs (Ryu et al. 2009). Ziv et al. also reported a synergistic effect between NPC transplantation and T-cell-based vaccination in mice with SCI. Myelin-specific T cells induced transplanted NPCs to migrate to the site of injury, while also instructing the local macrophages/microglial cells at the injury site towards a neuroprotective phenotype, which led to tissue preservation, increased neurogenesis and improved functional recovery (Ziv et al. 2006).

Finally, some more recent evidence from our laboratory shows that, when sub-acutely transplanted in a contusion model of SCI, undifferentiated NPCs accumulate and survive around the lesion site. There, transplanted NPCs establish contact through cellular junctions with phagocytic cells and astrocytes and profoundly regulate the expression levels of inflammatory mediators. Interestingly, transplanted NPCs skewed the inflammatory infiltrate of the injured spinal cord by reducing the proportion of 'classically-activated' (M1) macrophages, in turn promoting the healing of the injured cord (Cusimano et al. 2012).

Effects in secondary lymphoid organs

In parallel to the pathological evidence that NPC transplantation increases the frequency of T-cell apoptosis at the CNS (Pluchino et al. 2005), other studies have shown that systemically injected NPCs also (if not predominantly) inhibit EAE by a peripheral immune modulation in lymph nodes (Einstein et al. 2007; Pluchino et al. 2009a, b). Einstein et al. first showed that EAE-derived lymph node cells were strongly inhibited by NPCs in the production of pro-inflammatory cytokines in response to MOG(35-55) peptide. Furthermore, primed T cells from mice treated with

NPCs were also deficient in their ability to adoptively transfer EAE, thus suggesting a long-lasting inhibition of the encephalitogenicity of the T cells (that are transferred to a naïve host) rather than an effect specific for the in vivo environment (Einstein et al. 2007).

A specific and almost exclusive targeting of the peripheral immune system was reported in SJL mice with PLP-induced EAE in which NPCs had been injected subcutaneously (Pluchino et al. 2009a, b). NPC-injected EAE mice showed significant clinical improvement, despite transplanted cells never being consistently found in the CNS. Instead, undifferentiated NPCs were consistently found to accumulate and persist at perivascular areas in draining lymph nodes, where they interacted with lymph node cells. Ex vivo analyses of the lymph nodes from NPC-transplanted EAE mice showed that NPCs hindered the activation of myeloid dendritic cells (DCs), in turn limiting the expansion of antigen-specific encephalitogenic T cells at sites of antigen presentation. Also, transplanted NPCs accumulating in lymph nodes modulated the in situ increase of major stem cell fate determinants, including the bone morphogenetic proteins (BMPs)-4 and -7, sonic hedghehog (Shh) and the BMP antagonist Noggin, which were released by both transplanted NPCs and immune cells and promoted the survival of NPCs outside the CNS. NPCs specifically affected DC maturation in response to TNF-α or toll-like receptor (TLR) agonists in vitro and impaired DC function through BMP-4 production both in vivo and in vitro (Pluchino et al. 2009a, b).

In parallel to these observations on rodent NPCs, hNPCs have been shown to interfere with a number of key DC functions, such as the differentiation of myeloid precursor cells (MPCs) into immature DC (iDC) and the maturation of iDC to functional (antigen-presenting) mature DCs. A significant impairment of the differentiation of CD14$^+$ MPCs into CD1a$^+$ iDCs has been reported when MPCs were cultured with GM-CSF and IL-4 in presence of hNPCs (Pluchino et al. 2009a). In the same study, hNPCs influenced the upregulation of the co-stimulatory molecules CD80, CD86 and MHC-II on LPS-treated DCs, thus impairing their capacity to induce a proliferative allogeneic response in mixed leukocyte reaction in vitro (Pluchino et al. 2009a).

Whether most of the immune regulatory effects of systemically injected NPCs act directly in the CNS or in the periphery is still an unsolved question. Peripheral lymphoid organs have been demonstrated to play an important role in the regulation of the immune responses to myelin antigens in EAE and within these organs a very sophisticated modulation of T-cell self-reactivity is known to take place (Flugel et al. 2001; de Vos et al. 2002; Mohindru et al. 2004). A very recent study has showed that the preventive intravenous administration of NPCs ameliorates EAE by selectively inhibiting the differentiation of encephalitogenic T helper 17 (Th17) through secreted factors. Again, injected NPCs were rarely detectable in CNS tissue sections. Remarkably, the authors achieved the very same clinical recovery with irradiated NPCs or even NPC supernatants. By focusing on the mechanisms of the immunoregulatory capacities of NPCs, Cao et al. have identified leukemia inhibitory factor (LIF) as the key factor responsible for the observed inhibition of Th17 cell differentiation, as well as elucidated the signalling pathway behind this novel mechanism of action, where LIF antagonizes interleukin (IL)-6-induced Th17 cell differentiation through ERK-dependent inhibition of STAT3 phosphorilation (Cao et al. 2011).

Cell-to-cell interactions and the formation of atypical ectopic stem cell niches

A highly specialized microenvironment supports endogenous NPCs (Doetsch 2003) and is essential for aspects of stem-cell biology and regenerative medicine. This niche is constituted by different components in which cell–to-cell interactions and an array of diffusible signals are the key elements. In this context, an intimate association with endothelial cells and the physical proximity to the vasculature have been described as playing a major role (Shen et al. 2008; Tavazoie et al. 2008; Kokovay et al. 2010).

Interestingly, well-defined areas whose molecular nature appears to be reminiscent of prototypical germinal stem cell niches have been observed to form around perivascular areas of the inflamed CNS following NPC transplantation (Pluchino et al. 2005), as well as in brain tumors (*atypical ectopic niches*) (Calabrese et al. 2007). These areas are established around infiltrated perivascular areas, show endothelial cells at the centre and contain transplanted NPCs, inflammatory cells from the blood stream, activated macrophages/microglia and reactive astrocytes (Fig. 1). We have described the sustained cross-talk that occurs between the different cell components of the niche and that probably regulates the long-term survival and behaviour of transplanted NPCs (Pluchino et al. 2005; Bacigaluppi et al. 2009; Pluchino et al. 2009a, b). Also, we have provided correlative evidence that, depending on local inflammatory milieu, transplanted NPCs may either remain in the niche (i.e., in an undifferentiated state), or move out from the niche, thereby acquiring a terminally differentiated phenotype (Pluchino et al. 2005, 2009a, b). Indeed, the description of these atypival ectopic niches still suffers from the lack of a final mechanistic proof of the cellular and molecular events leading to their formation, as well as regulating the therapeutic plasticity of transplanted NPCs.

Here we postulate that these entities may behave as anatomically atypical, although highly specialized, ectopic microenvironments that regulate long-term survival and behaviour of transplanted NPCs (Pluchino et al. 2005, 2009a, b). We believe that our case is made even stronger by the evidence

Fig. 1 Schematic view of an atypical ectopic perivascular niche, which is established in the inflamed CNS after NPC transplantation. NPCs selectively accumulating at the level of a perivascular area interact with blood-borne T lymphocytes, macrophages and microglial cells and reactive astrocytes. The mechanisms of cell-to-cell interactions in the atypical ectopic perivascular niche include the secretion of paracrine factors as well as the establishment of cellular contacts
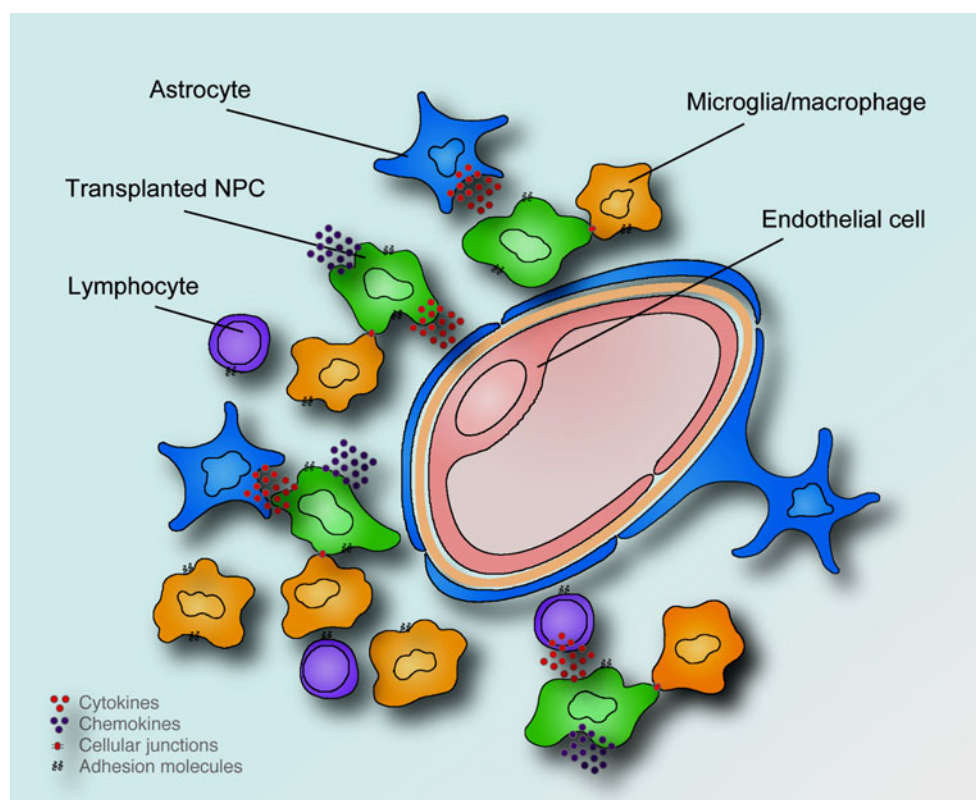
that in multifocal inflammatory CNS disorders, non-neural somatic stem cells (e.g., bone-marrow-derived, umbilical cord blood, or MSCs) also specifically travel through biological fluids (e.g., the blood, the lymph, the cerebrospinal fluid) towards inflamed CNS areas, where they persist — usually at the level of perivascular areas — and promote functional and tissue recovery (Chu et al. 2004; Ziv et al. 2006; Gerdoni et al. 2007; Bacigaluppi et al. 2009). Thus, the formation of CNS atypical ectopic niches can be also hypothesized as the functional requisite for the therapeutic activity of somatic non-hematopoietic stem/precursor transplanted cells.

The observation of the atypical ectopic niches has also shown a remarkable flexibility of transplanted NPCs upon exposure to precise microenvironments in vivo. To gather this remarkable adaptation capacity, we have first proposed the provocative concept of *therapeutic plasticity* (Pluchino and Martino 2008). This embraces the propensity of transplanted stem cells to accumulate and survive undifferentiated at the level of the atypical ectopic perivascular niches (Pluchino et al. 2005, 2009a, b) — while engaging highly sophisticated programs of cell-to-cell communication and/or instruction with endogenous neighbouring cells. Direct intercellular communication between transplanted NPCs and the host pathological tissue at the level of these atypical niches may therefore constitute a crucial mechanism behind the therapeutic protective effects of NPCs in vivo, overall leading to tissue repair and clinico-pathological benefits (Pluchino et al. 2010).

In addition to hierarchical (mother-to-daughter) communication, cells may also exchange information horizontally. Mechanisms of communication include secretion of growth factors, hormones, cytokines, chemokines and small molecular mediators (Gnecchi et al. 2008); cell-to-cell (adhesion) contacts (Vultur et al. 2004); cell-to-cell interactions via tunnelling nanotubules (Gerdes et al. 2007); and even secretion of circular membrane vesicles (Thery et al. 2009). Each of these modalities might in principle contribute to the overall therapeutic effect of transplanted NPCs.

A number of recent pieces of evidence also suggest that NPCs are able to communicate with the host cells via cellular contacts. For instance, functional gap junction formation has been shown to allow exogenous NPCs to rescue host neurons and their projections in animal models of Purkinje neurodegeneration. Gap junctions permitted the trans-cellular delivery of homeostasis-modulating molecules, as well as directly influenced host network coordinated activity via $Ca2^+$ waves. Moreover, hypoxic preconditioning of NPCs before the in vitro engraftment increased Connexin 43 expression and improved subsequent communication with host cells (Jaderstad et al. 2010). Specifically in mice with PLP-induced EAE, we showed that subcutaneously injected NPCs accumulated at secondary lymphoid organs and established consistent anatomical contacts with lymph node cells, through polarized microvilli, cytoplasmic expansions, or intercellular junctions. Transplanted NPCs were also occasionally found in deeper

contact and enclosing resident lymph cells, up to membrane fusion (Pluchino et al. 2009a, b). More recently, we have observed formation of atypical ectopic niches after focal NPC transplantation into mice suffering from a severe contusion SCI. Within this experimental setting, 4–5.5% of transplanted NPCs distributed all over the analysed segment of the cord — including the lesion epicentre — at 1 week after transplantation. This went down to 0.5–1% of surviving NPCs 6 weeks later. Most transplanted NPCs appeared organized at the boundaries of the injured tissue, in very close proximity to Iba1$^+$ cells and blood vessels. The analysis of cell-to-cell interactions at the level of the perivascular niches revealed the presence of tight contacts, up to junctional coupling, between NPCs and macrophages, which were established via Connexin 43. None of the NPCs at the boundaries of the injury expressed major markers of differentiation, whereas a small percentage of them stained for the astroglial lineage marker GFAP and the helix–loop–helix transcription factor Olig2. Immunogold electron microscopy allowed us to describe the ultrastructure of *atypical ectopic niches* following NPC transplantation (Cusimano et al. 2012).

We therefore sense that there is enough correlative evidence for envisaging that the injury (inflammatory) microenvironment is likely to play as a critical component to foster/influence the establishment of atypical ectopic niches (Fig. 1) and in turn to maintain cell-to-cell communication between transplanted NPCs and endogenous cells (Martino et al. 2011).

Conclusions

Consistent evidence challenges the old view that non-haematopoietic stem cell therapies, including those using NPCs, protect the injured CNS exclusively throughout cell replacement (Rossi and Cattaneo 2002). It is in fact now quite well-established that the transplantation of NPCs via biological routes remarkably promotes the repair/healing of the CNS via several *bystander* capacities that transplanted NPCs exhibit within specific in vivo microenvironments after transplantation. The establishment of atypical ectopic niches after NPC transplantation is potentially the in vivo microenvironment where most of these NPC capacities are observed. After having established a significant role of diffusible secreted neuroprotective and immune regulatory molecules in this, recent evidence highlights the potential of cellular junctional coupling, as well as the transfer of different levels of information between transplanted NPCs and endogenous cells.

In parallel to the development of clinical trials looking at the safety of these novel NPC-based therapeutics, some of which have just started (Aboody et al. 2011), we should keep focusing on the mechanisms regulating the molecular actions of therapeutically plastic NPCs more thoroughly.

Among a number of potential actions that NPCs have adapted to different in vivo microenvironments, we foresee that the exploitation of the mechanisms regulating their modalities of intercellular communication has realistic chances of revolutionizing the actual understanding of stem cell biology and its application to the clinics for CNS repair.

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