SCIENTIFIC COMMENTARY

Neural stem cell-mediated immunomodulation: repairing the haemorrhagic brain

Neural stem/precursor cells (NPCs) are broadly proposed as an alternative cell source to repair brain damage upon transplantation. NPC-driven brain repair has variably been shown in several pre-clinical models of neurological disorders. However, a comprehensive knowledge of the different mechanism(s) by which such cells exert their therapeutic potential is still lacking. While the replacement of lost or damaged cells was until a few years ago assumed to be the prime therapeutic mechanism of stem cells, it is now clear that transplanted somatic stem cells may simultaneously instruct several therapeutic mechanisms not confined to cell replacement on its own. Combining the overall therapeutic potential of NPCs in neurological disease, the concept of *therapeutic plasticity* has recently been proposed (Martino and Pluchino, 2006).

The brain repair potential of transplanted stem cells in stroke-like conditions has solid pre-clinical evidence. It has been shown, with variable results, that NPCs—transplanted either systemically or intraparenchymally—display peculiar *pathotropism* toward specific sites of ischaemic damage, survive within the host for long periods of time, and establish a functional cross-talk with the different cell types of the (micro)environment. This NPC-dependent operational behaviour was first demonstrated to be capable of promoting CNS tissue recovery in mice with experimental bacterial collagenase-induced intracerebral haemorrage (ICH) (Jeong *et al.*, 2003) or acute middle cerebral artery occlusion-induced ischaemic stroke (Chu *et al.*, 2004). Interestingly, transplanted NPCs promoted significant functional recovery despite predominantly accumulating in an undifferentiated state at the borders of damaged areas. Therefore, therapeutic mechanism(s) different from the expected contribution of cell replacement have been postulated. Among these alternative mechanisms, that encompassing the release by transplanted NPCs of molecules ultimately acting as neuroprotectants (*bystander* or *chaperone* effect) has been supported by much converging evidence (Martino and Pluchino, 2006).

In this issue of *Brain*, a study by Soon-Tae Lee and colleagues (Lee *et al.*, 2007) identifies an additional *bystander* mechanism by which human NPCs injected through the blood-stream ameliorate clinico-pathological signs of experimental bacterial collagenase VII-induced ICH. Hyperacute i.v.—but not hyperacute intracisternal (i.c.) or delayed

i.v.—NPC injection promoted a profound anti-apoptotic and anti-inflammatory effect leading to a significant reduction of the brain oedema ipsilateral to the haemorrhage. In parallel, the authors observed down-regulation of the pro-inflammatory cytokines tumour necrosis factor (TNF)α and interleukin (IL)-6 both in the brain and in secondary lymphoid organs. Furthermore, in hyperacute i.v. transplanted ICH rats the great majority of injected cells accumulated and persisted (up to 35 days post-transplantation) within the marginal zone area of the spleen, with very few (if any) NPCs being detected within the brain, lung, liver or lymph nodes. An NPC-mediated peripheral immunomodulatory effect taking place in the spleen was then postulated. While the mechanism sustaining the peripheral NPC-mediated therapeutic effect was not fully detailed at the molecular level, *in vitro* co-cultures suggested that NPCs might reduce the capacity of activated (LPS-triggered) macrophages to secrete the pro-inflammatory cytokine TNFα (but not IL-6) upon cell-to-cell contact.

The data by Lee *et al.* resonate with previous reports showing that terminal differentiation of NPCs may not represent the essential pre-requisite for the overall therapeutic efficacy of such cells upon systemic transplantation in CNS diseases (Martino and Pluchino, 2006). However, the demonstration that NPCs may also exert their therapeutic properties in ischaemic brain disorders when operating outside the CNS is certainly novel.

The reported data further confirm the concept that peripheral inflammation has to be viewed as an integral part of the brain-confined process occurring in ischaemic brain disorders and that interfering with such pathway(s) might have relevant therapeutic implications. Although in their article, Lee and colleagues only inferentially demonstrated this concept (e.g. splenectomy prior to ICH was able to reduce the brain oedema and the number of perihaematomal inflammatory cells), increasing evidence from animal and clinical studies suggests that both innate and adaptive immunity contribute to sustain the inflammatory mechanisms that occur as a primary consequence of ICH. It is certain that cellular (including erythrocytes, leukocytes, macrophages) and soluble (e.g. plasma proteins such as thrombin, plasmin, etc.) inflammatory components enter the brain from the blood-stream within a few hours after ICH and induce a surge of inflammatory and cytotoxic

cytokines and chemokines which, in turn, contribute to the progression of ICH-induced cellular injury (Hartl et al., 1996; Matsuo et al., 1994; Power et al., 2003).

While further emphasizing the concept that systemic somatic stem cell transplantation is indeed a way to promote CNS recovery in inflammatory conditions, the work by Lee et al. demonstrates that the *therapeutic plasticity* of transplanted NPCs is very much dependent on the timing of transplantation. Since the first demonstration that systemically (i.v. and intrathecally) injected NPCs exert neuroprotective and anti-inflammatory effects in mice with CNS-confined inflammatory disorders (Ben-Hur et al., 2003; Pluchino et al., 2003, 2005), a spur of research indicating that this timing-dependent therapeutic effect might change in response to environmental needs has been provided. When injected systemically in the effector phase of a chronic CNS inflammatory condition (e.g. after disease onset), NPCs preferentially accumulate in the CNS where they create a milieu of cell regulators (e.g. morphogens, neurotrophins, cytokines, chemokines) capable of promoting neuroprotection and blockade of inflammation blockade at the site of tissue damage (*bystander neuroprotection*) (Pluchino et al., 2003, 2005). When injected systemically during the induction phase of a chronic CNS inflammatory condition (e.g. before disease onset), NPCs preferentially home to secondary lymphoid organs where they interfere with adaptive immune responses (e.g. generation of encephalitogenic effector T cells) and, as a secondary phenomenon, prevent the occurrence of CNS inflammation (Einstein et al., 2007).

The work of Lee and colleagues further supports the concept that the *therapeutic plasticity* is a true functional signature of somatic stem cells. Strictly depending on when injected into a live host suffering from a tissue specific disease (inflammatory versus degenerative), transplanted somatic stem cells are likely to possess some unique therapeutic *adaptive* functions. This is due to the fact that they display an extraordinary capacity to find routes towards favourable *atypical niches*, where they survive and act as therapeutic weapons through interaction with different cell types in the (micro)environment (Pluchino et al., 2005; Jin et al., 2006; Calabrese et al., 2007; Einstein et al., 2007; Lee et al., 2007; Thored et al., 2007). Nonetheless, the recent demonstration that other sources of somatic stem cells (e.g. mesenchymal, haematopoietic)—with very low capabilities of neural (trans) differentiation—may show equally significant bystander capacities and promote CNS repair (Escolar et al., 2005; Zappia et al., 2005), further proves the relevance of somatic stem cell-dependent alternative therapeutic mechanisms. The next challenge for these therapies—which must be considered of pivotal importance—will be to regulate tightly the therapeutic mechanisms that somatic stem cells may initiate *in vivo*.

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