"Use of neural stem cells to induce neuroprotection in inflammatory CNS disorders"

Background and Description of Invention. Transplantation of neural stem precursor cells in patients affected by central nervous system (CNS) disorders characterized by chronic inflammation (e.g. multiple sclerosis, brain tumors, ischemic stroke) has limited therapeutic impact due to recurrent or persisting inflammation that targets and kills both CNS-resident and transplanted cells. Scientists at San Raffaele Scientific Institute have been able to describe a novel immunomodulatory mechanism that boosts such limited therapeutic effect by transplanting undifferentiated adult neural stem/progenitor cells (aNPC), which promote direct neural cells replacement by acquiring *in vivo* terminally differentiated phenotype *(Pluchino et al., Nature* 2005*; Martino G, Pluchino S. Nat Rev Neurosci.* 2006*. Review;* Pluchino et al., *Ann Neurol.* 2009*)*. Furthermore, upon systemic injection, aNPC are able to exert a neuroprotective effect by inducing *in situ* programmed cell death of blood-borne CNS-infiltrating pro-inflammatory Th1, without affecting anti-inflammatory Th2 cells in the inflamed CNS perivascular area.

The CNS inflammatory microenvironment dictates aNPCs cell fate, and therefore their therapeutic efficacy as follows:

- when neurodegeneration prevails, transplanted aNPCs acquire a mature phenotype and thus replace damaged neural cells, while
- when neuroinflammation predominates, transplanted aNPCs survive to recurrent inflammatory episodes by retaining both an undifferentiated phenotype and notable proliferating capacities.

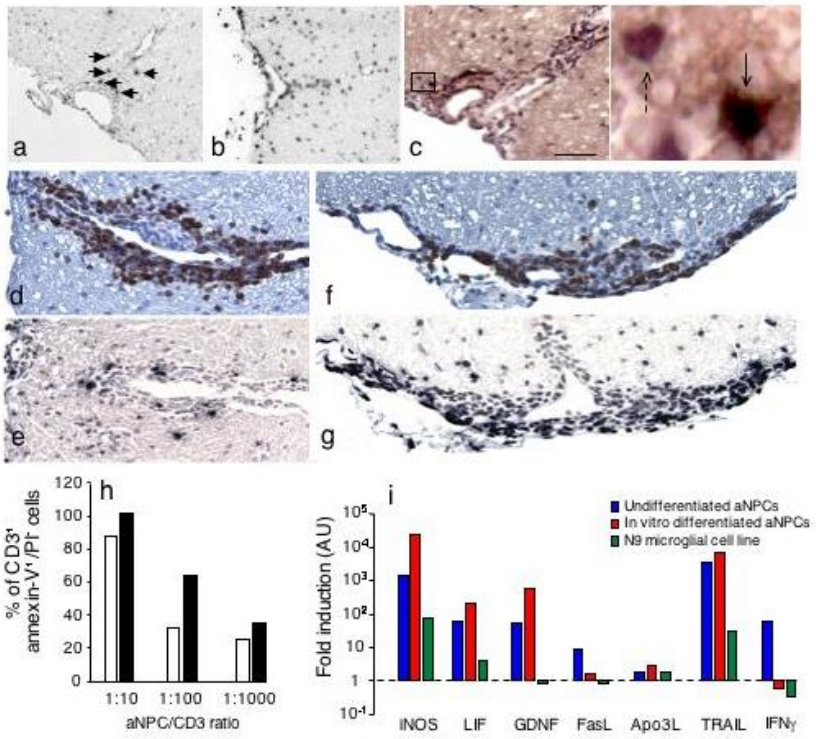

Figure 1. In vitro and in vivo analysis of CD3+ cells undergoing apoptosis. **a** and **b**, Spinal cord perivascular areas stained for TUNEL from either sham- (**a**) or aNPC-treated R-EAE mice (**b**, 20X magnification). Few apoptotic cells (arrows) are visible in **a**, while the great majority of the cells surrounding the blood vessel in **b** are TUNEL+ (black dots). **c** Spinal cord perivascular area double stained for TUNEL (dark grey) and CD3 (dark brown) (dashed arrow, TUNEL+CD3- cell; solid arrow, TUNEL+CD3+; Scale bar, 30 μm). **d**-**g**, Representative consecutive (5 μm-tick) spinal cord sections – stained for CD3 (brown dots in **d** and **f**) or TUNEL (black dots in **d** and **f**) – showing perivascular areas from sham-treated (**d** and **e**) or aNPC-injected (**f** and **g**) R-EAE mice (40X magnification). Nuclei in **d** and **f** have been counterstained with haematoxilin. The great majority of apoptotic cells expressing CD3 – which are significantly increased in aNPC-treated mice ($p< 0.005$ vs. sham-treated) – are confined within perivascular inflamed CNS areas,as early as 2 weeks p.t. (30 dpi). **h**, CD3/CD28 activated spleen-derived lymphocytes undergo apoptosis (AnnexinV+/PI- cells) when co-cultured with aNPCs (single well, black bars; trans-well, white bars). **i**, Pro-inflammatory cytokine-conditioned aNPCs express mRNA of pro-apoptotic molecules. Arbitrary units (AU) represent fold induction of mRNA levels between conditioned and non-conditioned cells.

Patent information. The international patent application was published as WO2007015173. Patent pending in Europe.

Stage of Development. Scientists transplanted subventricular zone (SVZ)-derived syngenic adult NPCs (aNPC) in a mouse model of chronic-recurrent autoimmune CNS inflammation, namely relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE). While assessing their therapeutic potential, they have been able to demonstrate that during R-EAE inflamed CNS perivascular areas function as ideal, although atypical, niche-like microenvironments where transplanted cells can survive for long-term (up to 3 months post-transplantation) as *bona fide* aNPCs. It has also been demonstrated that systematically injected syngenic aNPCs use constitutively activated integrins and functional chemokine receptors to selectively enter the inflamed CNS.

Potential Applications and Competitive Advantages. Undifferentiated aNPCs have relevant therapeutic potential in chronic inflammatory CNS disorders because they display immune-like functions that promote long-lasting neuroprotection in inflamed CNS perivascular area on the one hand, and brain repair on the other. Among their competitive advantages we have:

- aNPC-mediated apoptosis of blood-borne CNS-infiltrating encephalitogenic T cells, promoting long-lasting neuroprotection in chronic inflammatory CNS disorders;
- Selective accumulation of intravenously-injected aNPCs within CNS inflamed areas using constitutively functional homing molecules (e.g., a4 integrins and GPCRs) canonically used by pathogenic CNS-infiltrating blood-borne lympho- and mono-cytes;
- Preferential maintenance of an undifferentiated phenotype upon aNPCs transplantation, thus potentially escaping the chronic CNS-reactive autoimmunity;
- *In vivo m*aintenance of their proliferation capacity for up to 100 days after aNPCs transplantation, thus potentially modulating their fate *in vivo* (proliferation vs quiescence vs migration and differentiation) in response to specific environmental signals (e.g., cytokines, chemokines, stem cell regulators).

We seek a commercial partner with a strong pipeline in chronic CNS disorders, to further explore the clinical use of adult neural stem cells for therapeutic applications.

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