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Neural precursor cells inhibit multiple inflammatory signals

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ABSTRACT

Intravenous neural precursor cell (NPCs) injection attenuates experimental autoimmune encephalomyelitis by reducing autoreactive T cell encephalitogenicity in lymph nodes *in vivo*. Here we examined NPC–lymphocyte interactions *in vitro*. NPCs inhibited the induction of T cell activation marker IL-2-Receptor α, ICOS, PD-1 and CTLA-4 and inhibited T cell proliferation. NPCs inhibited T cell activation and proliferation in response to Concavalin-A and to anti-CD3/anti-CD28, which are T cell receptor (TCR)-mediated stimuli, but not in response to phorbol myristate acetate/ionomycin, a TCR-independent stimulus. The suppressive effect was not mediated via downregulation of CD3ε or induction of apoptosis. We next examined NPCs effects on inflammatory-cytokine signaling. NPCs impaired IL-2-mediated phosphorylation of JAK3 in lymphocytes, and inhibited IL-6 mediated proliferation of B9 murine hybridoma cells. In conclusion, NPCs ameliorate TCR-mediated T cell activation and inhibit inflammatory cytokines' signaling in immune cells. These findings may underlie the broad anti-inflammatory effects of NPCs *in vivo*.

Introduction

Neural stem cells were initially introduced as a potential source of regenerating cells for neurological diseases. Specifically, in demyelinating diseases, transplantation of neural precursor cells (NPCs) and oligodendrocyte progenitors was suggested for remyelination (reviewed by Ben-Hur et al., 2005; Blakemore and Franklin, 2000; Duncan, 1996).

Recent data indicate, however, that neural precursors possess also immuno-modulatory properties (Einstein et al., 2003, 2006, 2007; Pluchino et al., 2005). This effect was first shown in the acute model of experimental autoimmune encephalomyelitis (EAE) in Lewis rats (Einstein et al., 2003), which is a model for disseminated brain inflammation with little or no demyelination. Intracerebroventricular (ICV) transplantation of neural spheres led to a significant reduction in inflammatory markers in the brain in conjunction with amelioration of disease severity (Einstein et al., 2003). The clinical relevance of the anti-inflammatory properties of neural precursors was further demonstrated in the myelin-oligodendrocyte-glycoprotein model of EAE in C57Bl/6 mice, where attenuation of brain inflammation by ICV transplanted NPCs reduced axonal injury and demyelination, resulting in improved clinical outcome (Einstein et al., 2006; Pluchino et al., 2005).

T cell reactivity against brain derived antigens takes place in lymph nodes (LNs) (de Vos et al., 2002), and T cells undergo profound functional changes in the peripheral lymphoid organs before infiltrating the CNS in EAE (Flugel et al., 2001). Thus, peripheral lymphoid organs are crucial for the induction of immune responses to brain derived antigens (Widner et al., 1988). Following intravenous (i.v.) injection, neural precursors attenuated EAE by a purely peripheral immuno-suppressive effect, involving the homing of NPCs to LNs (Einstein et al., 2007). NPCs inhibited the proliferation of LN derived T cells and significantly reduced their encephalitogenicity (Einstein et al., 2007). While this peripheral effect is crucial for EAE inhibition by i.v. delivery of NPCs, it is still unclear how direct intracerebroventricular (ICV) NPC transplantation inhibits the secondary inflammatory processes induced by infiltrating immune cells in the EAE brain.

Here we sought to further characterize the inhibitory effect of NPCs on lymphocytes. We utilized a co-culture system to show that NPCs inhibit T cell receptor (TCR)-mediated T cell activation. NPCs inhibited also interleukin 2 (IL-2) and interleukin 6 (IL-6)-mediated signal transduction in other cell systems. We conclude that neural precursors interfere with the signaling of multiple inflammatory-cytokine receptors. These diverse actions may underlie a broad anti-inflammatory effect of NPCs.

Results

NPCs inhibit T cell activation and proliferation following polyclonal activation

Multipotential neural precursors were isolated from newborn C57BL/6 cerebral hemispheres, and expanded in spheres consisting

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mainly of polyasialic acid–neural cell adhesion molecule (+), nestin+, and NG2(−) cells. We first examined the response of the T cell subpopulation in naïve lymph node cells (LNCs) stimulated with ConA in the absence versus the presence of NPC spheres. To this end we examined by FACS analysis the expression on Thy1.2+ T cells of several cell surface markers that are up-regulated at different time points following stimulation. ConA stimulation increased the expression of CD25 (IL-2Rα) at 24 h, CTLA-4 (CD-152), ICOS (inducible co-stimulator) and PD-1 (programmed death-1) at 48 h. Co-culturing with NPC spheres at a NPC–LNC ratio of 1:2.5 inhibited the rise of the above molecules. As compared to control-stimulated LNCs, the prevalence of CD25+ T cells in co-cultures was reduced by 62±13% (Fig. 1A; $p=0.002$), confirming our previous results (Einstein et al., 2007). In addition, the mean fluorescence intensity (MFI) of CD25 was reduced in co-cultures by 52±6% (data not shown). Similarly, the prevalence of ICOS+ T cells was reduced by 75±6.5% (Fig. 1B; $p=0.01$) and ICOS MFI by 63.5±9% (data not shown). CTLA-4+ T cells were reduced by 67±14% (Fig. 1C; $p=0.04$), and CTLA-4 MFI by 76%±5.6% (data not shown), and PD-1+ T cells by 57±9% (Fig. 1D; $p=0.03$) and PD-1 MFI by 50±7.5% (data not shown). The proliferation of LNCs was examined by ^{3}H-thymidine and BrdU incorporation assays. ConA stimulation caused a 50–100 fold increase in ^{3}H-thymidine incorporation as compared to naïve LNCs. Co-culturing with NPCs inhibited ConA induced proliferation in a dose dependent manner, reaching 95±3%

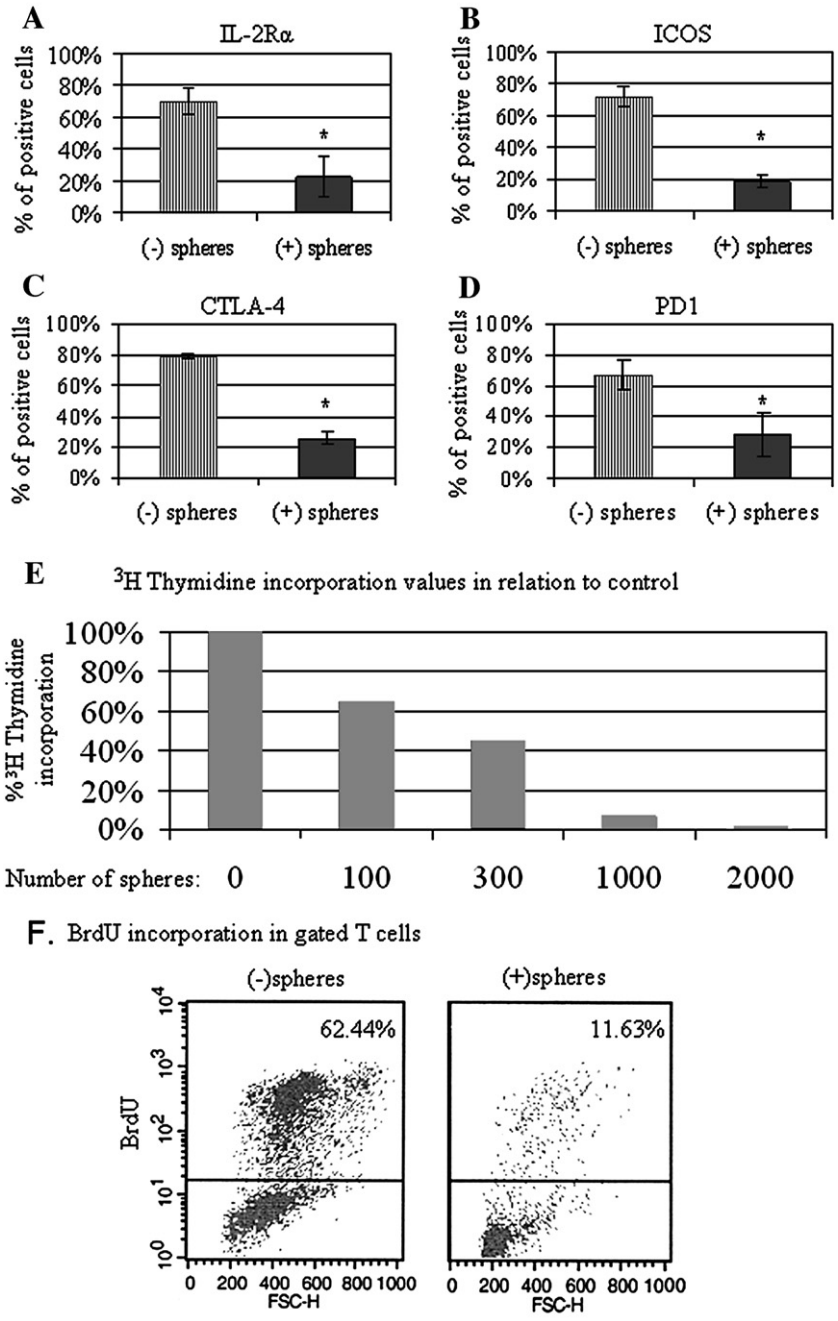

Fig. 1. NPC spheres inhibit T cell activation and proliferation. ConA-stimulated lymph node cells were incubated with or without NPC spheres, and examined at several time points for the expression of cell surface markers on the T cell subpopulation. FACS analysis showed that co-culturing with NPC spheres caused significant reduction in IL-2Rα positive T cells at 24 h (A), and in ICOS (B), CTLA-4 (C) and PD1 (D) positive T cells at 48 h. Co-culturing with NPC spheres diminished LNC and T cell proliferation as shown by ^{3}H Thymidine (E) and BrdU (F) incorporation, respectively. *- $p \leq 0.05$ by standard t test.