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BRAIN RESEARCH

Research Report

Adult mesenchymal stem cells rescue dorsal root ganglia neurons from dying

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ABSTRACT

Mesenchymal stem cells (MSCs) can differentiate into multiple cellular lineages including neuronal cells. However, the positive effect of MSCs on repairing the nervous tissue has not yet been completely understood. In order to investigate the influence of MSCs on a neuronal population, we co-cultured MSCs, obtained by flushing the bone diaphisis from adult Sprague–Dawley rats, with DRG post-mitotic sensory neurons obtained from rat embryos at day E15. Co-cultures were maintained for 2 months. The adult rat MSCs, simply harvested in a pure culture of DRG neurons, allow the long-lasting survival and maturation of neurons otherwise committed to die. Neurons, when co-cultured with rat fibroblasts, do not survive as long as with MSCs and do not mature to the same degree. The rescue effect of MSCs on neurons is achieved only by cellular direct contact. These results provide a valid explanation for the functional improvement reported in some in vivo experiments.

1. Introduction

Mesenchymal stem cells (MSCs) are a bone marrow-derived population which contains multipotent progenitors that can differentiate into multiple cellular lineages such as osteoblasts, chondrocytes, adipocytes (Prockop, 1997; Pittenger et al., 1999; Digirolamo et al., 1999) and also nervous cells (Sanchez-Ramoz et al., 2000; Woodbury et al., 2000; Brazelton et al., 2000; Deng et al., 2001). Adult MSCs have attracted increasing interest for their ability to self-renew, to rapidly expand and to differentiate into various cellular lineages. Moreover, unlike embryonic stem cells (ESCs), adult MSCs can be readily isolated from patients by bone marrow aspiration under anesthesia. The autologous origin of MSCs avoids the risk of immune rejection and, being adult cells, the possibility of tumor development is reduced (Prockop et al., 2000; Brehm et al., 2002). For these reasons, adult MSCs could have a great therapeutic potential even if the mechanisms regulating their differentiation are still unclear.

Previous studies on neuronal differentiation have mainly focused on ESCs, while only recently has the neuronal differentiation potentiality of adult MSCs been affirmed both in vitro (Sanchez-Ramoz et al., 2000; Brazelton et al., 2000; Deng et al., 2001) and in vivo (Chopp et al., 2000; Li et al., 2001; Mahmood et al., 2001; Dezawa et al., 2001; Horwitz et al., 1999; Chen et al., 2001; Akiyama et al., 2002; Cuevas et al., 2004). In vitro, many studies have demonstrated that MSCs, under adequate stimuli, could express typical markers of specific cell lines including glial cells and neurons (Brazelton et al., 2000; Woodbury et al., 2000; Deng et al., 2001). The neuronal differentiation seems to be increased by the release of specific molecules such as Bone Morphogenetic Protein 4 (Wislet-Gendebien et al., 2004), or by experimental conditions that increase the cyclic-AMP level (Deng et al., 2001). However,

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similar studies performed by other researchers have failed to find evidence of neuronal differentiation (Castro et al., 2002; Tondreau et al., 2004; Lu et al., 2004). Some authors have demonstrated that MSCs already express specific nervous markers before any kind of differentiation (Tondreau et al., 2004), others have shown that also chemical toxic stimuli such as Dimethyl Sulfoxide and *b*-mercaptoethanol (Lu et al., 2004) could determine a morphological differentiation of these cells into a nervous-like phenotype. Many authors that have addressed the issue of MSCs differentiation in nervous cells have concluded that the differentiation "from marrow to brain" is not a common phenomenon, that it is often dependent on experimental conditions and that, in some cases, it is an artifact (Lu et al., 2004).

In vivo, some studies have reported that MSCs, when injected into the host, are able to reach the damaged site leading to a functional improvement after spinal cord injuries or ischemic damages (Azizi et al., 1998; Chen et al., 2001; Mahmood et al., 2001; Li et al., 2001; Cuevas et al., 2004; Satake et al., 2004; Wynn et al., 2004), even if only a small quantity of cells were engrafted, and most of them appeared undifferentiated (Akiyama et al., 2002; Zhao et al., 2002). Moreover, some studies have suggested that the different phenotypes acquired by engrafted MSCs could reflect their fusion with host cells (Long and Yang, 2003). These controversial observations in the MSCs field make it very difficult to understand the mechanisms by which MSCs provide therapeutic benefits. In a recent study, Lou and coworkers have demonstrated that bone marrow-derived stromal cells induce the neuronal differentiation of mesencephalic stem cells in Sprague–Dawley rat brain slices (Lou et al., 2003). These observations prompted the present study with the aim of investigating the possible reciprocal interactions of adult MSCs with post-mitotic, embryonic sensory neurons.

2. Results

MSCs co-cultured with DRG neurons sustained the neuronal long-lasting survival. As shown in Fig. 1, the number of viable neurons co-cultured with MSCs remained unchanged throughout the observation period, while neurons cultured alone decreased in number and were nearly absent after 2 months (Fig. 1).

Before co-culturing, neurons were small with a few thin processes (Fig. 2A). At day 2, after MSCs were added, the neurons still had the same appearance and did not differ from those of control cultures. The MSCs were stratified on the dish forming a carpet of undifferentiated cells below the neurons (Fig. 2B). After 7–10 days, the neurons cultured without MSCs began to suffer, the neuronal processes were fewer and small and sometimes they had a fragmented appearance, while neurons co-cultured with the MSCs did not show these aspects. After 1 month, cultures in which MSCs were not added showed only a few surviving neurons (Fig. 2D), while in co-cultures neurons had an increased number of thicker and more arborized processes (Fig. 2E). The MSCs formed a carpet of flattened, elongated cells arranged in several layers among which the neuronal processes ran. After 2 months, in control

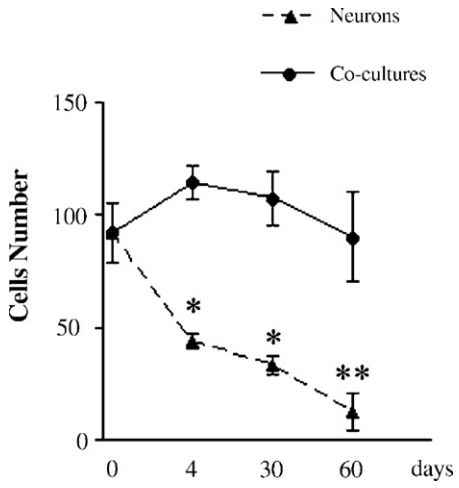

Fig. 1 – Evaluation of the number of viable cells. Primary sensory neurons of co-cultures and neurons cultured alone were plated and counted at the different time points (Day: 0, 4, 30, 60). Cell number was assessed by counting viable cells/optical field and was expressed as a mean ± SD. *$p<0.05$, **$p<0.01$ with respect to Day 0.

cultures, there were a few thin processes and most of neurons were degenerating or already dead (Fig. 2G). On the contrary, in co-cultures, neurons were even larger than before and more differentiated with an extensive dendritic arborization. They were clustered in groups of 3–5 cells (Fig. 2H). The MSCs were immuno-negative for the neuronal marker Neu-N and for the glial marker glial fibrillary acidic protein (GFAP) (data not shown).

In order to verify whether other cellular types different from MSCs were able to support neuronal survival and maturation, we prepared co-cultures with neuronal cells and rat fibroblasts (Fig. 2C). After 1 month, neurons co-cultured with rat fibroblasts were suffering and most of them degenerating (Fig. 2F, compared with Fig. 2E). After 2 months, the fibroblasts were unable to support neuronal survival since almost all neurons were dead (Fig. 2I).

In order to evaluate whether MSCs could have differentiated into neuronal cells, DRG neurons were stained with the vital fluorescent DiI dye. MSCs were then added to the neuronal primary culture, and after 1 month cultures were analyzed with a confocal microscope with an antibody for the detection of the neuronal marker Neu-N and with phalloidin, a fluorescent actin filament-binding toxin which identifies MSCs. Neu-N co-localized exclusively with DiI-stained cells, while there was no evidence of Neu-N expression in MSCs or in DiI negative cells, thus demonstrating that all the neurons derive from the primary neuronal culture and MSCs do not differentiate into neurons (Fig. 3). Moreover, at the different time points, the number of viable neurons, characterized by a birefringent outline absent in dead cells, was not significantly changed (Fig. 1).

In order to evaluate whether the MSCs' supportive role was due to a direct cellular interaction rather than to the release by MSCs of some specific neurotrophic factors, indirect co-cultures and cultures of neurons alone treated with the MSCs' conditioned medium were established. After