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Chronic Expression of Transforming Growth Factor-Beta Enhances Adult Neurogenesis

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Abstract
Neural stem cells reside in two neurogenic regions of the adult brain: the dentate gyrus of the hippocampus (DG) and the subventricular zone (SVZ). Their proliferation, differentiation, migration and survival are modulated by intrinsic and extrinsic signals, forming a neurogenic niche. Brain cytokines have only been recently regarded as possible components of this neurogenic niche. In particular, we have demonstrated that transforming growth factor-β (TGF-β) has a pro-neurogenic effect in the DG in a model of increased neurogenesis by adrenalectomy. We wanted to test whether TGF-β has a similar effect in another neurogenic region, namely the SVZ. To test this possibility, adult rats were injected with adenoviral vectors expressing TGF-β (Ad-TGF) or β-galactosidase (Ad-bgal) in the SVZ and neurogenesis was evaluated 3 weeks later. We have observed that chronic TGF-β expression increased neurogenesis in the ipsilateral hemisphere of Ad-TGF but not in Ad-bgal-treated rats compared to their contralateral side. In addition, an unspecific effect of the adenoviral vector per se could not be totally discarded. We conclude, under our experimental conditions, that TGF-β could enhance adult neurogenesis in the SVZ. This data increase the growing evidence supporting a pro-neurogenic role of anti-inflammatory cytokines in the adult brain.

In the adult mammal brain, there are certain neurogenic regions where neural stem/progenitor cells (NSC) proliferate and have the potential to differentiate into neurons, astrocytes and oligodendrocytes [1]. Adult neurogenesis is regulated by environmental signals collectively defined as the neurogenic niche. Several molecular components of the neurogenic niche have been identified including sonic hedgehog, bone morphogenic proteins, leukemia inhibitory factor and vascular endothelial growth factor [2]. Not only can brain cytokines mediate immune functions but they can also modulate several brain-associated processes independently of the immune system [3]. Only recently, it has been observed that pro-inflammatory cytokines such as IL-6 and IL-1 can diminish and anti-inflammatory cytokines such as transforming growth factor-β (TGF-β) and IL-4 can increment adult neurogenesis, respectively [4]. In particular, we have observed that TGF-β, an anti-inflammatory cytokine, has a positive effect on neurogenesis in the dentate gyrus of the adult hippocampus in a model of enhanced neurogenesis by adrenalectomy in vivo [5]. We wanted to explore the possibility that TGF-β could increase neurogenesis in another neurogenic area, the subventricular zone (SVZ) of the lateral ventricles.

To test this hypothesis, adult male Wistar rats (8–10 weeks old) were unilaterally injected with 1×10^7 particles of adenoviral vectors TGF-β (Ad-TGF) or β-galactosidase (Ad-bgal) as control, under ketamine chlorhydrate (80 mg/kg) and xylazine (8 mg/kg) anesthesia. This treatment allows transgenic expression for at least 14 days

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in the brain ([6] and data not shown). The stereotaxic injections were performed with a 50-μm tipped fine glass capillary directed to the left SVZ at bregma, antero-posterior, +0.5 mm; lateral, +1.5 mm; ventral, –4.0 mm. One week after adenoviral injection, animals were injected intraperitoneally with 50 mg/kg/day of 5-bromo-2′-deoxyuridine (BrdU) during 7 days. Three weeks after adenoviral injection, animals were deeply anesthetized and perfused transcardially with heparinized saline and tissue was fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were removed and postfixed overnight at 4°C in 4% paraformaldehyde in PB, after which they were cryoprotected in 30% sucrose in PB and sliced coronally in 40-μm sections with a sliding cryostat. Animal procedures were performed according to the regulations for the use of laboratory animals of the National Institutes of Health, USA.

The amount of newly born neurons in the SVZ was determined by performing double immunofluorescence against BrdU (a marker of cell proliferation) and doublecortin (DCX, a marker of young neuronal phenotype) with specific antibodies. Briefly, one-in-six series of free-floating sections were incubated for 2 h in 50% formamide at 65°C, washed once in ×2 standard saline citrate (SSC; 0.3 M NaCl, 0.03 M sodium citrate), incubated for 30 min at 37°C in 2 N HCl, rinsed in 0.1 M borate buffer, pH 8.5, and thoroughly washed in Tris-buffered saline (TBS; Tris-HCl 50 mM pH 7.4, NaCl 150 mM). Sections were blocked in 0.1% Triton X-100, 1% donkey serum in TBS (blocking solution) for 40 min at room temperature (RT). Rat anti-BrdU (1:150; Abcam, UK) and rabbit anti-DCX (1:150; Abcam, UK) were diluted in blocking solution and sections were incubated for 72 h at 4°C. Sections were then incubated with Cy2-conjugated donkey anti-rat and Cy3-conjugated donkey anti-rabbit (1:200; Jackson Laboratories, West Grove, Pa., USA) for 2 h at room temperature (RT). Sections were mounted in Mowiol (Calbiochem, La Jolla, Calif., USA). Double-stained cells in the SVZ were quantified using z-scan confocal microscopy (Zeiss LSM 510 confocal-laser scanning microscope equipped with an argon and He/Ne laser that emitted at 488 and 568 nm, respectively) at ×20 magnification. Each cell was analysed along the entire 'z' axis. Between 200 and 600 BrdU-positive cells were counted to calculate double-labeled cell percentages, and the total number of double-labeled cells was estimated.

In the SVZ ipsilateral to the Ad-TGF injection, the number of newly born neurons was increased in comparison to the contralateral side (* $p < 0.05$; fig. 1). No similar effect was observed when the injected and uninjected SVZ of Ad-bgal-treated animals was compared (fig. 1). When the ipsilateral hemispheres of Ad-TGF and Ad-bgal-injected animals were analyzed, a tendency to an increase in neurogenesis was observed in the first group but it did not reach statistical significance (fig. 1), suggesting an effect of the adenoviral injection per se that precluded to draw conclusions from this comparison.

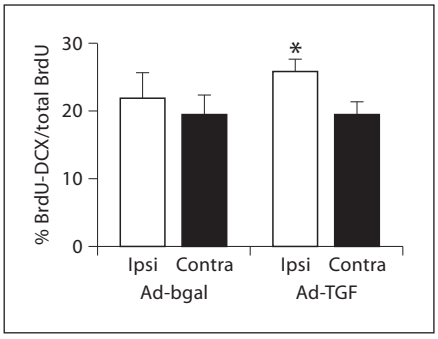

Fig. 1. Effect of TGF-β expression on neuronal differentiation in the SVZ. Quantification of the percentage of double-labeled cells in the SVZ among the BrdU-positive cell population in Ad-TGF-β and Ad-bgal-injected animals. Ad-TGF significantly increases the amount of BrdU/DCX-positive cells. Comparisons were performed using t test analysis (* $p < 0.05$). Values are the mean ± SEM. Ipsi = Ipsilateral; Contra = contralateral to adenoviral injection.

We conclude that chronic TGF-β expression could produce an increment in newborn neurons from the adult SVZ. A contribution of the adenoviral vector per se on this effect could not be discarded. These data support the idea of considering brain cytokines as part of the adult neurogenic niche. It also provides evidence in favor of considering anti-inflammatory cytokines as pro-neurogenic molecules in the adult brain.

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