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Cell proliferation is reduced in the dentate gyrus of aged but not young Ts65Dn mice, a model of Down syndrome

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Abstract

Reduced number of neurons is a common feature in Down's syndrome (DS) brains. Since reduced neuronal number also occurs in the dentate gyrus of Ts65Dn mice (TS), a model for DS, hippocampal cell proliferation and survival were analyzed in young and old TS mice. For evaluating proliferation and survival, half of the mice were sacrificed 1 day, and the other half 30 days after the last bromodeoxyuridine injection, respectively. No difference was found in the number of proliferating or surviving cells of young TS and control mice. An age-associated decline in total cell number and density has been found in both genotypes, this decline being more pronounced in TS animals. Thus, aged TS mice showed reduced cell proliferation and density of surviving cells compared to CO mice. Due to the putative involvement of newborn cells in the dentate gyrus in learning processes, the reduced proliferative capacity found in TS mice could be involved in the cognitive problems found in this model of Down syndrome.

Keywords: Down's syndrome; Ts65Dn; Neurogenesis; Aging

A widely recognized feature of Down's syndrome (DS) is the reduction in the number of neurons in several brain regions, including hippocampus [9]. Ts65Dn mice (TS) are trisomic for the region of mouse chromosome 16 orthologous to human chromosome 21, spanning the region just proximal to Gabpa/App cluster to Znf295 (~23 Mb). As a genetic model for DS, the TS mouse shares many neural phenotypic characteristics of DS, including alterations in neural morphology [12,13,16], neurochemistry [5], electrophysiology [20], and cognition [7,12]. It also provides the opportunity to study underlying neurodevelopmental pathology.

Reduced hippocampal neurons have been found in the dentate gyrus of TS mice [13,16]. In this regard, it is noteworthy that the hippocampus is one of the regions where new neurons are born in adult mammalian brain [14,15,25,26]. In the dentate gyrus, stem-cell proliferation takes place in the subgranular zone (SGZ) at the border between the granule cell layer (GCL) and hilus. The multipotent progenitor cells continuously divide and give rise to neurons, astrocytes and oligodendrocytes. Newborn neurons migrate into the GCL and establish functional connexions in the dentate molecular region [26]. Therefore, it could be hypothesized that the reduction of neuronal density in the dentate gyrus of TS mice could be related to a restricted degree of neurogenesis. This would be of special relevance, as newborn neurons are likely to be involved in the establishment of long-term potentiation (LTP) and to contribute to spatial learning and memory, since hippocampal neurogenesis is important for spatial learning and memory, not only in early stages but also in adulthood and aging [19,22,24]. In fact, manipulations that enhance neurogenesis in the dentate gyrus, such as voluntary running and environmental enrichment, also enhance learning and LTP [14,15,25,26]. Interestingly, both, LTP and learning are deteriorated in TS mice [7,23].

The aim of the present study was to analyze whether neurogenesis is also present in TS mice. Since early aging is a recognized feature of DS, the study was extended to compare neurogenesis in young and adult TS mice. For this purpose, cell proliferation and survival was analyzed in the dentate

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gyrus of young and old TS mice through labeling with bromodeoxyuridine (BrdU).

Male TS mice were bred in the Faculty of Medicine colony, from Ts65Dn females and B6EiC3HF1 male breeders provided by the Robertsonian Chromosome Resources (The Jackson Laboratory, Bar Harbor, Maine, USA). Control mice (CO) were the non-trisomic littermates. In order to determine the presence of trisomy, animals were cariotyped at the age of 6–8 weeks following the method of Davisson and Akeson [5]. Cell proliferation and survival were studied in 34 young (3–5 months) and 44 old (13–15 months) mice. Half the animals were used to evaluate cell proliferation and the other half to evaluate cell survival.

BrdU was administered at the age of 3–5 months for young mice, and at 13–15 months for old mice. All mice received one daily i.p. injection of 50 μg/g of BrdU in sterile 0.9% NaCl solution for 12 days. To evaluate cell proliferation and survival, animals were sacrificed and perfused with 4% paraformaldehyde on days 1 and 30 after the last injection, respectively.

After perfusion, all brains were post-fixed overnight in paraformaldehyde at 4 °C and transferred into 30% sucrose. Coronal sections of 40 μm thickness were cut in a cryostat. Free-floating sections were used in the determination of BrdU-labeling. Every sixth 40 μm section was used.

BrdU inmunohistochemistry was performed as described by Malberg et al. [19]. DNA denaturation was conduced by incubating sections for 2 h in 50% formamide/2× SSC at 65 °C, followed by several PBS rinses. Sections were then incubated for 30 min in 2N HCl and then 10 min in boric acid. After washing with PBS, sections were incubated for 30 min in 1% H_2O_2. After blocking with PBS-TS (PBS, 5% goat serum, 0.1% Triton X-100), cells were incubated with monoclonal mouse anti-BrdU (Roche, 1:600) overnight. Sections were then incubated for 90 min with biotinylated donkey anti-mouse IgG (Vector Laboratories, 4 μg/ml) followed by amplification with an avidin–biotin complex ABC Elite reagent (Vector Laboratories) and diaminobenzidine was used as chromogen (Vector Laboratories).

Sampling of cell proliferation was done through the whole extent of the SGZ, whereas cell survival was assayed throughout the GCL and SGZ in its rostrocaudal extension. The resulting number of BrdU-positive cells was related to the granule cell layer volume multiplying the value by 6, because every sixth section has been used.

To evaluate cell density, the GCL area was determined on sections of the tissue adjacent to those used to evaluate cell survival, stained with cresyl-violet. BrdU-positive cells were detected using an optical microscope (Axiolab, ZEISS) and analyzed with Axiovision AC 4.1 (ZEISS) software. The volume was calculated considering section thickness and number.

All animal procedures met the guidelines of the European Communities Directive 86/609/EEC regulating animal research.

ANOVAs were performed to analyze total cell number and density followed by Bonferroni post hoc tests to analyze individual differences between groups. All the analysis was done using SPSS for Windows, version 11.0.

In the young mice, the number of proliferating cells in TS mice was not significantly different from that of CO mice as shown in Figs. 1A, 2A and B. Similarly, there was no significant difference in the number of BrdU-positive cells that survived one month after treatment (survival) in young TS and CO mice [ANOVA 'genotype': $F(1,33) = 0.0$, n.s.].

In old animals, TS mice showed significantly lower cell proliferation compared to CO [$F(1,43) = 6.2$, $p < 0.05$]. However, no significant difference was found in cell survival between aged TS and CO mice (Figs. 1B, 2C and D).

During their lifespan, a significant fall in proliferation [ANOVA 'age': $F(F(1,38) = 65.52)$, $p < 0.001$] and survival [$F(1,40) = 51.65$, $p < 0.001$], was observed in all groups (Table 1). A larger number of BrdU-labeled cells were observed in young CO (Fig. 2A) and TS (Fig. 2B) animals when compared to aged mice (Fig. 2C and D). Table 1 shows that TS mice presented a larger reduction of the proliferative capacity than CO mice (87.9% in TS and 73.4% in CO mice), whereas decrease in cell survival was similar in both groups (75.7% in TS and 77.0% in CO mice).

Changes in GCL volume were found between the different groups of young and old TS and CO mice [$F(3,31) = 10.44$,

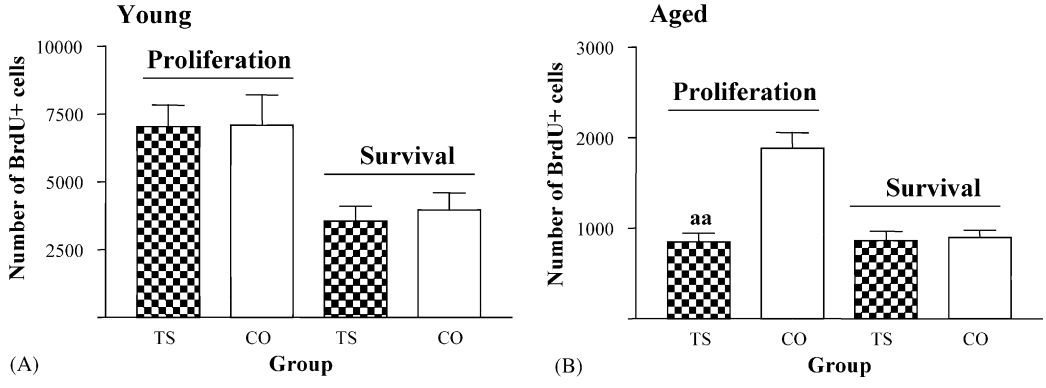

Fig. 1. Mean ± S.E.M. of the number of newborn and surviving BrdU-positive cells in the hippocampal dentate gyrus of young (A) and aged (B) TS and control mice. $^{aa}p < 0.01$ TS vs. CO, Bonferroni tests after significant ANOVA.