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Aβ increases neural stem cell activity in senescence-accelerated SAMP8 mice

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ABSTRACT

Neurogenesis persists in the adult brain as a form of plasticity due to the existence of neural stem cells (NSCs). Alterations in neurogenesis have been found in transgenic Alzheimer's disease (AD) mouse models, but NSC activity and neurogenesis in sporadic AD models remains to be examined. We herein describe a remarkable increase in NSC proliferation in the forebrain of SAMP8, a non-transgenic mouse strain that recapitulates the transition from healthy aging to AD. The increase in proliferation is transient, precedes AD-like symptoms such as amyloid beta 1-42 [Aβ(1-42)] increase or gliosis, and is followed by a steep decline at later stages. Interestingly, in vitro studies indicate that secreted Aβ(1-42) and PI3K signaling may account for the early boost in NSC proliferation. Our results highlight the role of soluble Aβ(1-42) peptide and PI3K in the autocrine regulation of NSCs, and further suggest that over-proliferation of NSCs before the appearance of AD pathology may underlie neurogenic failure during the age-related progression of the disease. These findings have implications for therapeutic approaches based on neurogenesis in AD.

1. Introduction

Neurogenesis persists as a form of plasticity in few adult brain areas, namely the subventricular zone-olfactory bulb system (SVZ-OB) and the hippocampal formation, due to the existence of neural stem cells (NSCs) (reviewed in (Ihrie and Alvarez-Buylla, 2011; Ming and Song, 2005; Zhao et al., 2008)). Although the implications of adult neurogenesis for brain function are still under intense investigation, in vivo studies in animal models, in combination with computational approaches, have so far uncovered an important role in OB- and hippocampus-dependent learning and memory tasks (Deng et al., 2010; Lazarini and Lledo, 2011). Alterations in neurogenesis have been reported during ageing and in age-related neurodegenerative diseases, including Alzheimer's disease (AD), in which deficits in olfaction and in hippocampal function are commonly found (Lazarov and Marr, 2010; Winner et al., 2011). However, the mechanisms underlying defective neurogenesis during healthy and pathological ageing remain to be fully elucidated.

NSCs in the SVZ (also referred to as type B cells) are located beneath the ependymal layer that lines the lateral ventricles. Type B cells are predominantly out of the cell cycle in a quiescent state, although they eventually proliferate and engage in a neurogenic cascade. Most of the active type B NSCs divide asymmetrically to generate intermediate self-amplifying progenitors (type C cells), which in turn give rise to type A precursors that become specified to the neuronal lineage (Doetsch et al., 1997). Type A neuroblasts migrate through the rostral migratory stream (RMS) to the OB, where they mature and integrate into pre-existing neuronal circuits as granule and periglomerular interneurons (Carleton et al., 2003; Lois and Alvarez-Buylla, 1994). Thus, given this cell type progression, a reduction in adult NSC activity ultimately results in defective neurogenesis and reduced granule cell numbers in the olfactory bulb (Imayoshi et al., 2008).

A dysfunction in neurogenesis has been observed in multiple transgenic mouse models of AD and in human AD brain tissue. Contradictory findings have been reported, with some studies

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pointing to an increase in proliferation of progenitor cells in the neurogenic areas, and others showing decreases in proliferation and survival of newly generated neurons (Chuang, 2010; Haughey et al., 2002; Lazarov and Marr, 2010; Marlatt and Lucassen, 2010; Rodriguez and Verkhratsky, 2011; Winner et al., 2011). For the most part, it remains to be clarified whether AD-related alterations in neurogenesis reflect changes that specifically affect NSC activity. Regardless of the mechanism, it has been proposed that neurogenic defects may contribute to the progression of the neuropathology (Lazarov and Marr, 2010). Hence, understanding neurogenesis and NSC behavior at early pre-symptomatic disease stages may provide new insights into the pathogenesis of AD.

The majority of AD cases are sporadic late-onset forms of the disease, whereas most animal models correspond to autosomal early-onset familial forms of AD that account for <5% of total AD cases. Ageing is the major risk factor for sporadic AD, so there is a need in the field to use models that mimic the late-onset age-dependent development of AD pathology. The senescence accelerated mouse prone 8 (SAMP8) strain has been recently proposed as a suitable model to study both ageing changes and AD, given that SAMP8 mice develop AD lesions in an age-dependent manner (Morley et al., 2012). SAMP8 mice also develop peripheral changes with age that are not features of AD. The strain was spontaneously generated from AKR/J mice after selective breeding pairs by Dr. Takeda (Takeda et al., 1981). The mice display some of the neuropathological features occurring in AD, such as increased oxidative stress, amyloid beta (Aβ) deposits, gliosis and tau phosphorylation. A high increase in reactive oxygen species (ROS) is detected in SAMP8 mice at early ages (Yasui et al., 2003), and lipid peroxidation and carbonyl damage have also been described in older SAMP8 mice compared to the senescence-accelerated mouse resistant SAMR1 strain used as a control (Alvarez-Garcia et al., 2006; Petursdottir et al., 2007; Rodriguez et al., 2008). The increase in ROS production may be explained by the reduction of several antioxidant enzymes (Kurokawa et al., 2001; Okatani et al., 2002; Sato et al., 1996). Regarding Aβ pathology, SAMP8 shows an age-related increase in β-amyloid precursor protein (APP) production and its mRNA (Kumar et al., 2000; Morley et al., 2000; Nomura et al., 1996). Moreover, cognitive impairments in old SAMP8 mice could be reversed using antisense oligonucleotides directed against APP mRNA or following the administration of antibodies to Aβ (Kumar et al., 2000, 2001; Morley et al., 2002; Poon et al., 2004). In addition to these alterations, SAMP8 mice develop neurochemical and cognitive abnormalities found in AD patients, including progressive memory decline, and it has been suggested that SAMP8 provide a more encompassing picture of human late-onset AD (Pallas et al., 2008). Thus, SAMP8 studies may be a good complement to those performed in AD transgenic models.

In this study, we sought to analyze proliferation and neurogenesis in the adult SVZ-OB system of SAMP8 mice throughout ageing. We investigated whether alterations in proliferation precede AD-like symptoms and whether these changes specifically affect adult NSCs, the cellular type at the top of the adult neurogenic lineage. Our results indicate that NSCs over-proliferate before the appearance of AD pathology, and highlight the role of soluble Aβ(1-42) peptide and PI3K signaling in the regulation of NSC proliferation during the early stages of AD.

2. Method

2.1. Animals

SAMP8 males were compared to senescence accelerated resistant 1 (SAMR1) males, which are genetically related to SAMP8 but resistant to accelerated senescence (Takeda et al., 1981). SAMP8 and SAMR1 strains were kindly provided by Dr Mercè Pallàs from the Universitat de Barcelona. Crl:CD1(ICR) male mice were purchased from Charles River (Les Oncins, France). Mice were maintained under specific pathogen free (SPF) conditions. Breeding characteristics were the same as those described by Tanaka et al. (Tanaka et al., 2005), and all manipulations were approved by the Animal Care Committee of the Instituto de Salud Carlos III.

2.2. BrdU injection

5-bromo-2′-deoxyuridine (BrdU) solution was prepared in saline solution (0.9% NaCl) as previously described (Moreno-Estelles et al., 2012) and was injected intraperitoneally (at a dose of 50 mg/kg of body weight) 3 times every 2 hours. Mice were sacrificed 1 hour after the last injection.

2.3. Fixation and tissue processing

Collection and 4% paraformaldehyde (PFA) fixation of brain tissue were carried out as described elsewhere (Moreno-Estelles et al., 2012), with the exception that animals were anesthetized with isoflurane (Isoba vet, 100% w/w). The brains were coronally sectioned in a cryostat (Leica Microsystems, Heidelberg, Germany) using Tissue-Tek (Sakura Finetek Europe, Alphen aan den Rijn, The Netherlands). The resulting 30-μm sections were kept at −20 °C. For the anatomopathological examination of other organs, tissue was fixed in formalin and was embedded in paraffin. Sections 6 μm were collected and were stained with hematoxylin and eosin.

2.4. Immunostaining of tissue sections and quantification

For immunohistochemistry, 30-μm-thick coronal sections were collected in series of 10 slides. Each slide contained an anterior–posterior (AP) reconstruction of 6 brain sections separated 300 μm between them. Thus, the distance between the sections analyzed was 300 μm. The area of analysis was defined rostrally by the anterior tip of the crossing of the corpus callosum and caudally by the appearance of the ventral hippocampal commisure, which coincides with the joining of the 2 lateral ventricles with the third ventricle (Ferron et al., 2007). Approximately, the AP coordinates from bregma for the SVZ serial sections included in the analysis were the following: 1.1 mm, 0.8 mm, 0.5 mm, 0.2 mm, and −0.1 mm (Paxinos and Franklin, 2004). BrdU-containing cells were visualized using the peroxidase method and the DAB substrate as described elsewhere (Brey et al., 2003). However, some modifications were included: the primary monoclonal anti-BrdU antibody (Dako, 1:300) was incubated 16 hours at room temperature, and the secondary horse biotinylated anti-mouse antibody (Vector, 1:400) was incubated 1 hour at room temperature. The number of $BrdU^+$ cells was counted under a Nikon Eclipse 50i H550S microscope with a ×40 objective. For the Nestin and BrdU double staining, anti-Nestin (Abcam, 1:100) was used. For GFAP (Dako, 1:300), SOX2 (R&D, 1:50) and BrdU (Dako, 1:300) triple immunofluorescence, antigen retrieval was performed as described in (Jiao et al., 1999) with 3 times repetition of 10-minute incubation in 10 mmol/L sodium citrate buffer, pH 6. Tissue sections corresponding approximately to 0.2 mm AP from bregma were captured in a Leica Spectral SP5 confocal microscope and analyzed with LAS AF Lite Leica confocal imaging software.

For GFAP (Dako, 1:300) and S100B (Sigma-Aldrich, 1:500) immunofluorescence, hippocampal sections corresponding approximately to −1.6 mm AP from bregma were captured in a Zeiss Axio Imager A1, using a Zeiss AxioCam MRm camera and were analyzed with ImageJ software (available online from the National Institutes of Health).