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Long-lasting impairment in hippocampal neurogenesis associated with amyloid deposition in a knock-in mouse model of familial Alzheimer's disease

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Abstract

Neurogenesis in the adult hippocampus has been implicated in regulating long-term memory and mood, but its integrity in Alzheimer's disease (AD) is uncertain. Studies of neurogenesis in transgenic mouse models of familial AD are complicated by ectopic overexpression restricted to terminally differentiated neurons, while AD cases have been studied only at the pre-senile or end-stage of disease. To investigate further the fidelity of adult neurogenesis, we examined mice carrying targeted mutations in amyloid precursor protein (APP), presenilin-1 (PS-1), or both APP and PS-1, in which FAD-causing mutations have been inserted into their endogenous genes. The latter "double knock-in" mice developed aging- and region-dependent amyloid deposition starting around 6 months, and by 9 months exhibited microglial activation associated with the amyloid. In the 9-month-old dentate gyrus, the double knock-in mutations reduced the numbers of MCM2-positive neural stem and progenitor cells by 3-fold and doublecortin-positive neuroblasts by 2-fold. The reduction in dentate neuroblasts persisted at 18 months of age. The impairment in neurogenesis was confirmed by quantitative Western blot analysis of doublecortin content and was restricted to the hippocampal but not the olfactory bulb neurogenic system. In contrast, neither mutant PS-1 nor APP alone led to amyloid deposition or significant alterations in the two markers. These results demonstrate long-lasting and selective impairment in adult hippocampal neurogenesis in a knock-in mutant mouse model of FAD and suggest a novel mechanism by which amyloid and its attendant microglia-mediated neuroinflammation could contribute to the cognitive and behavioral abnormalities of AD.

Keywords: Amyloid; Presenilin; Neurogenesis; Familial Alzheimer's disease; Neuroinflammation; Neural plasticity

Introduction

Although the majority of cases of Alzheimer's disease (AD) do not have a known direct genetic cause and are considered sporadic, a subset are triggered by inherited mutations in either the amyloid precursor protein (*APP*), presenilin-1 (*PS-1*), or presenilin-2 (*PS-2*) gene (St George-Hyslop, 2000; Tanzi and Bertram, 2005). Excepting its early age of onset, familial Alzheimer's disease (FAD) resembles sporadic forms of the disease in its clinical signs, as well as its slow progression and characteristic neuropathologies, which include regionally restricted amyloid deposition in the brain parenchyma and vasculature, intraneuronal neurofibrillary tangles, amyloid-associated gliosis and neuroinflammation, and the loss of neurons and synapses (Braak et al., 1998; Lleo et al., 2004). The pathogenic mechanisms by which APP, PS-1, and PS-2 mutations cause AD have been the subject of extensive study. When expressed in cellular systems, mice, and humans, the FAD-linked mutants invariably increase production of the Aβ42 variant (Scheuner et al., 1996; Hardy and Selkoe, 2002) that is the major component of parenchymal amyloid plaques (Yang et al., 1994; Savage et al., 1995). In transgenic mice mutant APP overexpression recapitulates the amyloid-associated abnormalities, effects that are accelerated markedly by

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the co-expression of mutant PS-1 and lead to behavioral dysfunction (Games et al., 1995; Hsiao et al., 1996; Holcomb et al., 1998; Ashe, 2001).

The adult mammalian brain contains two major neurogenic systems: the subgranular zone of the dentate gyrus, which provides new neurons for the hippocampus, and the subventricular zone along the lateral ventricle, which supplies the olfactory bulb. In particular, adult hippocampal neurogenesis has been implicated in the regulation of cognition (Rola et al., 2004; Schaffer and Gage, 2004) and mood (Jacobs et al., 2000), behaviors that are a signature abnormality of AD or a frequent co-morbid occurrence, respectively. Thus it is critically important to assess the integrity of this form of neural plasticity in the AD brain and discern the contribution made by altered neurogenesis to the disease process. Presenilins are expressed in neural progenitors and their genetic deletion disrupts developmental neurogenesis (Handler et al., 2000; Wen et al., 2002). Transgenic overexpression of mutant PS-1 in the adult mouse reportedly impairs adult hippocampal neurogenesis (Wen et al., 2004; Chevallier et al., 2005), whereas mutant APP either enhances (Jin et al., 2004a) or impairs it (Haughey et al., 2002). Unfortunately, these studies were all conducted using heterologous promoters that preferentially drive supraphysiological expression in terminally differentiated neurons. As a consequence, the mutant transgenes are not expressed in the neural stem and progenitor cells themselves, and the relevance of these findings for the AD brain is uncertain. Neuroblast numbers are reportedly increased in hippocampus of AD patients (Jin et al., 2004b), but this end-stage analysis leaves unexplored the role of impaired neurogenesis in the onset and progression of the disease and is not substantiated by a study of pre-senile cases of probable AD (Boekhoorn et al., 2006).

Here we investigated the integrity of adult brain neurogenesis using the APP/PS-1 double knock-in mutant mouse, in which FAD-causing mutations were targeted into their endogenous genes (Reaume et al., 1996; Siman et al., 2000; Siman and Salidas, 2004). The resulting mice express mutant APP and PS-1 without the complications of ectopic expression and overexpression inherent to transgenic approaches, and their brains exhibit AD-type amyloid deposition and microgliosis. Using markers for neural stem and progenitor cells, along with differentiating neuroblasts, we evaluated the impact of mutant APP, PS-1, and the double knock-in mutations on neurogenesis in the adult brain.

Materials and methods

Gene-targeted mouse lines

The APP knock-in, PS-1 knock-in, and APP/PS-1 double knock-in mouse lines were created using gene targeting in embryonic stem cells and have been described and characterized previously (Reaume et al., 1996; Siman et al., 2000; Flood et al., 2002; Siman and Salidas, 2004). For the APP line, the Swedish double point mutation was introduced into the APP gene of the CD-1 outbred mouse strain by changing the sequence in exon 17 encoding the Lys-Met at codons 670–671 to one encoding Asn-Leu, and the 3 variant codons within the Aβ domain changed from the mouse to the human sequence. The neomycin selection cassette reduced transcription of the APP gene, and so the cassette was removed from the intron upstream of the targeted exon 17 using the Cre recombinase, and the resulting APP knock-in mutant mice were bred to homozygosity (APP KI/KI line). A similar strategy was used to introduce the FAD-linked P264L mutation into the mouse PS-1 gene and remove the neomycin selection cassette, also in the CD-1 background. The mRNA and protein expression of APP and PS-1 were confirmed by Northern and Western blot analyses as being equivalent between the targeted and control lines. The APP homozygous mutant mice were crossed with PS-1 heterozygous mutant mice to generate offspring of 3 different genotypes: APP KI/KI+PS-1 WT/WT, APP KI/KI+PS-1 KI/WT, and APP KI/KI+PS-1 KI/KI. The latter line is referred to as the APP/PS-1 double knock-in mouse. APP WT/WT+PS-1 KI/WT mice were crossed with one another to generate offspring of two additional genotypes for experimentation: APP WT/WT+PS-1 WT/WT, and APP WT/WT+PS-1 KI/KI. The mice were given free access to food and water and maintained under veterinary supervision in strict compliance with all standards for animal care and investigation established in the "Guide For the Care and Use of Laboratory Animals" (National Academy Press ISBN# 0-309-05377-3).

The genotypes of the APP and PS-1 alleles were determined by a PCR strategy. For PS-1, the following primers were used: forward—GCT GGA GCA ATG CTG TGT TA; reverse—GAG ATG GCT TAC GGG TTG AG. The amplified product is 190 bp for the wild-type PS-1 allele and 280 bp for the mutant knock-in allele. For APP, the following primers were used: forward—CAC ACC AAG AAG TAC AAT AGA; reverse—CCT GGG TTG TAG GGA CTG TAC TTG. In this case, the amplified product is 214 bp for the wild-type APP allele and 298 bp for the mutant knock-in allele.

Immunocytochemistry and cell counting

Male and female mice of either 8–9 months or 18–24 months of age were anesthetized deeply with an overdose of pentobarbital, then perfused transcardially with ice-cold 0.1 M sodium phosphate (pH 7.4; PB) followed by freshly prepared 4% paraformaldehyde in PB. Brains were carefully dissected, left in fixative for 4 h at 4°C, cryoprotected in 20% sucrose in PB overnight, blocked, and then frozen in dry ice/2-methylbutane at −40°C and stored at −80°C. Sagittal sections 40 μm thick were prepared with a sliding microtome and collected sequentially into ten series, with each series starting from the medial border of hippocampus and extending laterally to the start of the ventral hippocampus. The total number of sections between these anatomical boundaries did not vary as a function of APP or PS-1 genotype. A minimum of two series of sections each were immunostained for MCM2, a marker for neural stem and progenitor cells (Maslov et al., 2004), or doublecortin, a marker for immature neuroblasts (Nacher et al., 2001; Rao and Shetty, 2004). Thus, each marker was evaluated on a minimum of 10 sections per mouse. Goat antibodies to MCM2 and