Neurobiology of Aging 34 (2013) 2564–2573

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Accelerated tau pathology with synaptic and neuronal loss in a novel triple transgenic mouse model of Alzheimer's disease

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ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 11 April 2013
Accepted 4 May 2013
Available online 5 June 2013

Keywords:
Alzheimer
Tau
Neuron loss
Transgenic mice
APP
Hyperphosphorylation
Aβ
Neurodegeneration
Synaptic pathology

ABSTRACT

There is pivotal evidence that tau pathology can be triggered by amyloid-β (Aβ) pathology in experimental systems. On the other side, studies on human brain specimen have elucidated that tau pathology may occur before amyloid pathology is present indicating that in principle tau pathology could also trigger Aβ aggregation. To address this question, we have crossed 5XFAD mice coexpressing human mutant APP695 with the Swedish, Florida, and London mutations and human mutant presenilin-1 (PS1) with the M146L and L286V mutations with the PS19 model overexpressing human mutant tau with the P301S mutation. The resulting triple transgenic model 5XFAD/PS19 has been characterized at 3 and 9 months of age. A dramatic aggravation of hyperphosphorylated tau pathology together with a dramatically increased inflammatory response and a loss of synapses and hippocampal CA1 neurons in aged 5XFAD/PS19 mice were observed. Extracellular amyloid deposits were unaltered. These data support the assumption of tau pathology being downstream of amyloid pathology, suggesting that both pathologies together trigger the severe neuron loss in the hippocampus in the 5XFAD/PS19 mouse model.

1. Introduction

Alzheimer's disease (AD) is a fatal neurodegenerative disorder representing the most common form of dementia in the elderly population. The main neuropathologic alterations comprise the deposition of extracellular plaques, the intracellular accumulation of neurofibrillary tangles (NFTs), and progressive loss of neurons and synapses. Whereas senile plaques contain amyloid-β (Aβ) peptides of various lengths, NFTs are comprised of microtubule-associated protein tau that is hyperphosphorylated at different positions. NFT pathology correlates much better with the cognitive status and disease severity than extracellular amyloid plaque pathology (Giannakopoulos et al., 2003); however, the upstream role of Aβ is now widely accepted (Blurton-Jones and Laferla, 2006). According to the prevailing amyloid cascade hypothesis, the formation of NFTs and the concomitant loss of synapses and neurons result from disequilibrium of Aβ production and clearance and are, therefore, regarded as downstream events (Hardy and Allsop, 1991). This hypothesis is supported by genetic evidence, as mutations not only in the gene encoding the amyloid precursor protein (APP) but also in presenilin-1 (PS1) and PS2, which are important components of the γ-secretase complex, lead inevitably to familial AD with an early disease onset (Bertram et al., 2010). Very recently, a novel APP mutation has been identified that protects against AD and cognitive decline in the elderly without AD (Jonsson et al., 2012; Kero et al., 2013). On the other hand, it is a matter of controversial discussion whether tau in turn influences amyloid pathology. Thorough neuropathologic analyses using large numbers of AD patients with different stages have demonstrated that NFT pathology is one of the earliest pathologic alterations (Braak and Braak, 1997; Duyckaerts and Hauw, 1997), and there is evidence from human postmortem studies that tangles might antecede amyloid plaque formation (Braak and Del Tredici, 2011; Duyckaerts et al., 2009; Schonheit et al., 2004).

The mechanism underlying the influence of Aβ on tau is not entirely clear. However, it has been shown that tau is essential for Aβ toxicity, as primary cultured neurons from tau knockout mice are resistant to Aβ exposure (Rapoport et al., 2002). Subsequent in vivo studies have demonstrated that premature mortality and memory deficits could be rescued in APP transgenic mice bred on a tau knockout background (Roberson et al., 2007). In bigenic mouse models expressing both mutant human APP and mutant tau transgenes, increased tau phosphorylation in comparison with tau single transgenic mice has been described (Hurtado et al., 2010; Lewis et al., 2001; Perez et al., 2005; Ribe et al., 2005) and

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http://dx.doi.org/10.1016/j.neurobiolaging.2013.05.003

phospho-tau–positive hippocampal mossy fibers and dystrophic neurites have also been detected in APP transgenic models harboring endogenous wild-type tau (Blanchard et al., 2003). In addition, mice overexpressing APP with the E693Δ mutation, leading to high levels of intracellular oligomeric Aβ, show phospho-tau–positive hippocampal mossy fibers that are absent in control mice overexpressing human wild-type APP (Tomiyama et al., 2010).

In the present report, we crossed the previously described 5XFAD mouse model (Jawhar et al., 2012; Oakley et al., 2006) coexpressing human mutant APP695 with the Swedish, Florida, and London mutations and human mutant PS1 with the M146L and L286V mutations with the PS19 model overexpressing human mutant tau with the P301S mutation (Yoshiyama et al., 2007). The resulting triple transgenic model 5XFAD/PS19 coexpressing APP and PS1 transgenes under the control of the murine Thy1 promoter and mutant tau under the control of the murine prion promoter has been characterized in detail by biochemical and neuropathologic analyses at 3 and 9 months of age. Our data indicate a dramatic aggravation of hyperphosphorylated tau pathology together with a dramatically increased inflammatory response and a loss of synapses and hippocampal CA1 neurons in aged 5XFAD/PS19 mice, whereas the amount of extracellular amyloid deposits is not significantly altered.

2. Materials and methods

2.1. Transgenic mice

The generation of 5XFAD mice (Tg6799) has been described previously (Oakley et al., 2006). In brief, 5XFAD mice express the 695-amino acid isoform of the human APP (APP695) carrying the Swedish/London/Florida mutations under the control of the murine Thy1 promoter. In addition, human PS1 carrying the M146L/L286V mutations is expressed also under the control of the murine Thy1 promoter. 5XFAD mice used in the present study were backcrossed for more than 8 generations to C57Bl/6J wild-type mice to obtain an incipient congenic line on a C57Bl/6J genetic background (Jawhar et al., 2012). PS19 mice overexpressing human tau with the P310S mutation under the control of the murine prion protein promoter (Yoshiyama et al., 2007) were purchased from Jackson laboratories (B6;C3-Tg(Prnp-MAPT*P301S)PS19Vle/J). Mice used in the present study were backcrossed for more than 5 generations to C57Bl/6J wild-type mice. All animals were handled according to the German guidelines for animal care. Only female mice were used in the present study.

2.2. Immunohistochemistry on paraffin sections

Immunohistochemistry was performed on 4-μm sagittal paraffin sections as described previously (Wirths et al., 2002). In brief, sections were deparaffinized in xylene and rehydrated in a series of ethanol. After treatment with 0.3% H_2O_2 in 0.01 M phosphate-buffered saline (PBS) to block endogenous peroxidases, antigen retrieval was achieved by boiling sections in 0.01 M citrate buffer with pH 6.0, followed by 3-minute incubation in 88% formic acid. Nonspecific binding sites were blocked by treatment with 4% skim milk and 10% fetal calf serum in PBS for 1 hour at room temperature, before the addition of the primary antibodies. The following antibodies were used: 24311 (pan-Aβ, 1:500; rabbit, Supplementary Fig. 1), Glial fibrillary acidic protein (GFAP) (1:2000, rabbit, Synaptic Systems, Goettingen, Germany), MC1 (1:500, mouse, generous gift of P. Davies), AT8 (1:500, mouse, Thermo Scientific, Bonn, Germany), and β3-tubulin (1:1000, rabbit, Millipore, Schwalbach, Germany) and the synaptic markers synapsin-1 (1:1000, mouse), synaptobrevin (1:500, rabbit), and synaptoporin (1:500, rabbit, all from Synaptic Systems, Goettingen, Germany). Primary antibodies were prepared in PBS supplemented with 10% fetal calf serum and incubated overnight in a humid chamber at room temperature followed by incubation with biotinylated secondary antibodies (1:200, DAKO, Glostrup, Denmark) for 1 hour at 37 °C. Staining was visualized via the ABC method using the Vectastain kit (Vector Laboratories, Burlingame, CA, USA) and diaminobenzidine (DAB) as chromogen providing a reddish-brown color. Counterstaining was carried out with hematoxylin.

2.3. Quantification of Aβ plaque load, GFAP staining, tau pathology, and synaptic markers

Extracellular Aβ load (using antiserum 24311 without hematoxylin counterstaining) was evaluated in the hippocampus (Bregma 1.08–1.32) using an Olympus BX-51 microscope equipped with an Olympus DP-50 camera and the ImageJ software (version 1.47b, National Institutes of Health [NIH], USA). Serial images of ×40 magnification (hippocampus) were captured on 4 sections per animal that were 30 μm afar from each other. Using ImageJ, the pictures were binarized to 8-bit black and white images, and a fixed intensity threshold was applied defining the DAB staining. Measurements were performed for a percentaged area covered by DAB staining (Breyhan et al., 2009). Accordingly, for GFAP staining quantification, images of ×40 (hippocampus) and ×200 (cortex and thalamus) were captured, and the astrocyte-covered areas were analyzed as described previously. For this analysis, a protocol modified from Vilaplana and Lavialle (1999) using ImageJ has been used applying fixed intensity threshold levels. AT8 and MC1 were used to quantify phospho-tau pathology in the CA1 and CA3 regions of the hippocampus (×400), the spinal cord (×40), and the cortex (×100). Antibodies against synapsin-1, synaptobrevin, and synaptoporin were used to quantify mossy fiber density in the CA3 region of the hippocampus (×400). The following animal models were used in these analyses: 5XFAD, PS19, and 5XFAD/PS19 ($n = 3$ each per time point).

2.4. Quantification of CA1 neuron numbers

To assess neuronal loss in the CA1 region of the hippocampus, sagittal brain sections (Bregma 1.08–1.32) of 3- and 9-month-old 5XFAD, PS19, and 5XFAD/PS19 mice ($n = 3$ per time point) were stained with hematoxylin. Neuronal nuclei were identified according to their size and characteristic appearance clearly differing from glial cells. Images of the CA1 layer were taken at ×400 using an Olympus BX-51 microscope equipped with a DP-50 camera. The number of CA1 neurons in a defined area per section ($n = 3$ per animal) was counted using the manual cell counting tool implemented in ImageJ (version 1.47b, NIH).

2.5. Western blot analysis

Brain hemispheres were homogenized in lysis buffer (750 mM NaCl, 50 mM Tris/HCl, 2 mM EDTA, supplemented with protease and phosphatase inhibitors, pH 7.4) in a wt/vol ratio of 1:10. After centrifugation at 17,000 × g at 4 °C for 20 minutes, supernatants were stored as soluble fractions at −80 °C until use. Pellets were resuspended via sonication in 2% sodium dodecyl sulfate (including protease and phosphatase inhibitors) and centrifuged again. Supernatants were saved as insoluble fractions at −80 °C until use. For western blotting, 30 μg total protein was loaded per lane of a 4%–12% VarioGel (Anamed, Groß-Bieberau, Germany). Following electrophoresis, proteins were transferred to nitrocellulose membranes (GE Healthcare, Freiburg, Germany) using a semi-dry transfer protocol. After transfer, membranes were incubated in Tris-buffered