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EARLY-ONSET SUBICULAR MICROVASCULAR AMYLOID AND NEUROINFLAMMATION CORRELATE WITH BEHAVIORAL DEFICITS IN VASCULOTROPIC MUTANT AMYLOID β-PROTEIN PRECURSOR TRANSGENIC MICE

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Abstract—Cerebral microvascular amyloid β protein (Aβ) deposition and associated neuroinflammation are increasingly recognized as an important component leading to cognitive impairment in Alzheimer's disease and related cerebral amyloid angiopathy (CAA) disorders. Transgenic mice expressing the vasculotropic Dutch/Iowa (E693Q/D694N) mutant human Aβ precursor protein in brain (Tg-SwDI) accumulate abundant cerebral microvascular fibrillar amyloid deposits exhibiting robust neuroinflammation. In the present study, we sought to determine if the unique amyloid pathology of Tg-SwDI mice was associated with deficits in behavioral performance. Behavioral performance tests that assessed a variety of psychological functions, including overall activity, motor ability, balance and strength, anxiety, impulsivity, and learning were conducted on homozygous Tg-SwDI mice and similarly aged wild-type C57Bl/6 mice. Our results indicate that Tg-SwDI mice were impaired in the performance of the Barnes maze learning and memory task at 3, 9, and 12 months of age. While more widespread cerebral microvascular Aβ pathology was evident in older animals, the evaluation of the Aβ pathology in the 3 months old transgenic animals revealed specific accumulation of microvascular amyloid and markedly elevated numbers of reactive astrocytes and activated microglia restricted to the subiculum. These findings indicate that early-onset accumulation of subicular microvascular amyloid and accompanying neuroinflammation correlates with impaired performance in the learning and memory task in Tg-SwDI mice. © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: cerebral microvascular amyloid, cognitive impairment, neuroinflammation, subiculum, transgenic mice.

One of the primary pathological features of Alzheimer's disease (AD), as well as other related disorders, is the accumulation of amyloid β protein (Aβ) in the parenchyma and cerebral vasculature of the brain (Selkoe, 2001). Aβ is formed by the sequential cleavage of amyloid β-protein precursor (APP) by β- and γ-secretases. Parenchymal Aβ deposits come in two forms: diffuse plaques and fibrillar plaques. Although both types of plaque deposits have been associated with dementia, fibrillar deposits show dystrophic neurites and evidence of inflammation including activated microglia (Selkoe, 2001; Walsh and Selkoe, 2004). Cerebral vascular Aβ deposits are largely of fibrillar form and are associated with a localized neuroinflammatory response and cognitive impairment (Vinters, 2001; Jellinger, 2002; Rensink et al., 2003; Bailey et al., 2004; Atterns and Jellinger, 2004; Greenberg et al., 2004). Furthermore, recent studies suggested that dementia shows a better correlation with microvascular Aβ accumulation than parenchymal Aβ accumulation (Thal et al., 2003; Atterns and Jellinger, 2004). However, direct demonstration of microvascular amyloid-induced cognitive impairment is lacking.

Two mutations within the Aβ region of the APP gene that result in severe, early onset cerebral amyloid angiopathy (CAA) are the Dutch E22Q and Iowa D23N mutations (Levy et al., 1990; Van Broeckhoven et al., 1990; Grabowski et al., 2001). Recently, we created a line of transgenic mice (Tg-SwDI) that express human Swedish/Dutch/Iowa mutant amyloid β precursor protein in the brain and consequently develop early-onset, robust accumulation of fibrillar Aβ in the cerebral microvasculature (Davis et al., 2004). This accumulation occurs despite the mice expressing low levels of human APP and producing low levels of human mutant Aβ (Davis et al., 2004; Deane et al., 2004). The insufficient removal of the Dutch/Iowa mutant Aβ across the blood–brain barrier into circulation appears to contribute to this extensive accumulation (Deane et al., 2004; Davis et al., 2006). These mice were further shown to exhibit vascular degeneration and neuroinflammation specifically around the sites of microvascular amyloid deposits (Miao et al., 2005a,b). Nevertheless, the relationship between cerebral microvascular amyloid-related pathological changes and behavioral performance in this unique model was unknown.

In the present study, we characterized the behavioral performance of Tg-SwDI mice as compared with non transgenic C57Bl/6 wild-type mice. Since the impact of cerebral microvascular amyloid pathology on behavior has not been assessed, we tested the mice on several inter-

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Abbreviations: ANOVA, analysis of variance; APP, amyloid β-protein precursor; Aβ, amyloid β protein; CAA, cerebral amyloid angiopathy; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; PBST, PBS containing 0.05% Tween 20; Tg-SwDI, transgenic mice expressing neuronal vasculotropic Dutch/Iowa (E693Q/D694N) mutant human amyloid β precursor protein.

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supporting tests that examined a range of psychological functions, including overall activity, motor ability, balance and strength, anxiety, impulsivity, and learning. Our results show that Tg-SwDI mice exhibit a specific deficit in the performance of a learning and memory test. This behavioral deficit was detected in Tg-SwDI mice as early as 3 months of age. Analysis of the Aβ pathology at this early age revealed accumulation of microvascular amyloid and highly elevated numbers of reactive astrocytes and activated microglia restricted to the subiculum. These findings indicate that early-onset accumulation of subicular microvascular amyloid and neuroinflammation is associated with impaired performance in a spatial learning and memory task in Tg-SwDI mice.

EXPERIMENTAL PROCEDURES

Tg-SwDI mice

All work was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996) and was approved by the Stony Brook University Institutional Animal Care and Use Committee. The minimal number of mice was used to obtain statistical significance, and all procedures used were minimally invasive to reduce any suffering of the animals. The generation of Tg-SwDI mice on a pure C57Bl/6 background was as described (Davis et al., 2004). For the behavioral studies and subsequent pathological analyses, all Tg-SwDI were homozygous for the human Swedish/Dutch/Iowa APP transgene. Homozygous Tg-SwDI mice developed more extensive pathology earlier but remained highly fertile and exhibited no early lethality. There were three age cohorts of behavioral testing at ages 3, 9, and 12 months. Each cohort consisted of 12–14 Tg-SwDI and wild-type C57Bl/6 mice. Each group consisted of an even mix of male and female animals. Mice were weighed at the beginning of each week of behavioral testing.

Tissue preparation

Mice were killed with an overdose of 2.5% avertin, the brains were immediately removed and bisected in the mid-sagittal plane. One hemisphere was snap-frozen and the other hemisphere was placed in 70% ethanol, followed by xylene treatment and embedding in paraffin for immunohistochemical and histological analyses.

Immunochemical analysis of cerebral Aβ peptides

Soluble pools of Aβ40 and Aβ42 were determined by using an specific enzyme-linked immunosorbent assay (ELISA) of carbonate extracted mouse forebrain tissue and subsequently the insoluble Aβ40 and Aβ42 levels were determined by ELISA of guanidine lysates of the insoluble pellets resulting from the carbonate extracted brain tissue (Johnson-Wood et al., 1997; DeMattos et al., 2002). Total Aβ40 and Aβ42 levels were determined by combining the soluble and insoluble levels of each form.

Soluble Aβ oligomers were analyzed in Tris-buffered saline (TBS)–soluble forebrain fractions using dot blot analysis. Briefly, brain fractions were loaded to nitrocellulose membranes (Schleicher and Schuell, Hertogen-Bosch, The Netherlands). Blots were washed for 15 min in PBS containing 0.05% Tween 20 (PBST), preincubated with blocking solution (1% milk powder in PBST), washed three times with PBST, and subsequently incubated with primary polyclonal anti-oligomer antibody OC11 and peroxidase-labeled secondary antibodies (Amersham, Arlington Heights, IL, USA). Detection was performed by chemiluminescence according to the description of the manufacturer (Biorad Laboratories, Hercules, CA, USA) and quantitated using BioRad VersaDoc system and the Quantity One software.

Immunohistochemical analysis

Immunohistochemistry and histology were performed as previously described (Davis et al., 2004; Miao et al., 2005a). Briefly, sections were cut in the sagittal plane at 10 μm thickness using a microtome, deparaffinized and rehydrated. Antigen retrieval was performed by treatment with proteinase K (0.2 mg/ml) for 10 min at 22 °C for Aβ and collagen staining, and by 10 mM sodium citrate solution (pH 9.0) for 30 min at 90 °C in a water-bath for activated microglia staining. Primary antibodies were detected with horseradish peroxidase–conjugated or alkaline phosphatase–conjugated secondary antibodies and visualized either with a stable diaminobenzidine solution (Invitrogen, Carlsbad, CA, USA) or with the Fast Red substrate system (Spring Bioscience, Fremont, CA, USA), respectively, as substrate. Sections were counterstained with hematoxylin. Cerebral microvascular amyloid burden in the regions of the subiculum, thalamus and fronto-temporal cortex was respectively quantified on the same set of systematically sampled Aβ-immunostained sections using stereological principles as described (Long et al., 1998). The following antibodies were used for immunohistochemical analysis: mouse monoclonal antibody 66.1, which recognizes residues 1–5 of human Aβ (Deane et al., 2003) (1:200), rabbit polyclonal antibody to collagen type IV for identification of microvessel (1:100, Research Diagnostics Inc., Flanders, NJ, USA), mouse monoclonal antibody to glial fibrillary acidic protein (GFAP) for the detection of astrocytes (1:300, Chemicon, Temecula, CA, USA), mouse monoclonal anti-keratan sulfate antibody for the detection of activated microglia (clone: 5D4, 1:200, Seikagaku Corporation, Tokyo, Japan).

Quantitative analysis of reactive astrocyte and activated microglia cell densities

Total numbers of astrocytes and activated microglia in the subiculum, dentate gyrus, thalamus, and fronto-temporal cortex regions were estimated using a computerized stereology system (Stereologer, Systems Planning and Analysis, Alexandria, VA, USA). Every tenth section was selected and generated 10–15 sections per reference space in a systematic-random manner. Immunopositive cells were counted using the optical fractionator method with the dissector principle and unbiased counting rules (Long et al., 1998).

Behavioral testing

The following battery of behavioral tasks was utilized in the analysis of Tg-SwDI and wild-type mice. The measurements on all tests were either mechanically scored or objectively scored by the experimenters and each task was conducted by multiple two person teams.

Digiscan. On test day 1, an assessment of overall activity was performed using a commercial Digiscan activity monitor (Columbus Instruments, Columbus, OH, USA). Each mouse was placed in a plastic tub cage measuring 43.2 cm long by 21.6 cm wide by 20.3 cm deep for 5 min. Horizontal movements were recorded automatically. An experimenter blind to treatment condition counted unsupported rearings and wall-supported rearings from videotape records of the sessions.

Open field. Day 2 of testing assessed open field activity. The open field apparatus was 54×54 cm and is enclosed by a 19 cm high wall marked with lines forming 36 9 cm^2 squares along the floor of the apparatus. The mice were placed in the open field for 2 min each and the number of lines the mouse crossed and number of times the mouse traversed the center four squares were recorded from videotape. Day 3 of testing assessed 2 min of open field activity again except that a novel object, e.g. a clear 6 cm^3 plastic box filled with pennies, was present in the center of

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