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Dendritic Degeneration, Neurovascular Defects, and Inflammation Precede Neuronal Loss in a Mouse Model for Tau-Mediated Neurodegeneration

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Adeno-associated virus (AAV)–mediated expression of wild-type or mutant P301L protein tau produces massive degeneration of pyramidal neurons without protein tau aggregation. We probed this novel model for genetic and structural factors and early parameters of pyramidal neurodegeneration. In yellow fluorescent protein–expressing transgenic mice, intracerebral injection of AAV-tauP301L revealed early damage to apical dendrites of CA1 pyramidal neurons, whereas their somata remained normal. Ultrastructurally, more and enlarged autophagic vacuoles were contained in degenerating dendrites and manifested as dark, discontinuous, vacuolated processes surrounded by activated astrocytes. Dendritic spines were lost in AAV-tauP301L–injected yellow fluorescent protein–expressing transgenic mice, and ultrastructurally, spines appeared dark and degenerating. In CX3CR1$^{EGFP/EGFP}$-deficient mice, microglia were recruited early to neurons expressing human tau. The inflammatory response was accompanied by extravasation of plasma immunoglobulins. α2-Macroglobulin, but neither albumin nor transferrin, became lodged in the brain parenchyma. Large proteins, but not Evans blue, entered the brain of mice injected with AAV-tauP301L. Ultrastructurally, brain capillaries were constricted and surrounded by swollen astrocytes with extensions that contacted degenerating dendrites and axons. Together, these data corroborate the hypothesis that neuroinflammation participates essentially in tau-mediated neurodegeneration, and the model recapitulates early dendritic defects reminiscent of "dendritic amputation" in Alzheimer's disease. ***(Am J Pathol 2011, 179:2001–2015; DOI: 10.1016/j.ajpath.2011.06.025)***

Tauopathies include a wide variety of primary disorders including Pick's disease, progressive supranuclear palsy, corticobasal degeneration, and frontotemporal dementia, as well as the most frequent secondary tauopathy, Alzheimer's disease (AD). In AD, the intracellular inclusions in somata and processes consist of highly phosphorylated protein tau and develop concomitant with or subsequent to intracellular accumulations of amyloid peptides, presumably in multivesicular bodies. Subsequently, extracellular amyloid plaques develop together with neurofibrillary tangles and inflammation, which combined define the postmortem pathologic findings in AD. The relative timing and molecular relation between amyloid and tauopathies are still debated, whereas the link to kinases such as GSK3β is becoming accepted.[1–5] Although aggregation of phosphorylated protein tau into filamentous inclusions in soma and neuropil is characteristic and diagnostic of all tauopathies, the neurotoxic phosphorylated tau species that damages synapses and neurons remains elusive. By analogy to amyloids, it is not the final tau deposits but the intermediate tau oligomers that were first suspected to cause disease; however, their cellular sites of action and the mechanisms whereby neurons succumb in tauopathy remain to be defined.

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Progressive staging of AD is clinically based on symptoms, cognitive examination, and brain imaging. Postmortem pathologic staging of AD is based on tauopathy visualized using immunohistochemistry (IHC) with monoclonal antibody AT8, which is specific for phosphorylated protein tau.[6] The follow-up study by Braak and Braak[2] revealed that transient tauopathy in the dendritic segments located in the stratum lacunosum moleculare causes "dendritic amputation." Of note, tau-related dendritic defects are an early, albeit transient, phenomenon in stages II and III, preceding the tauopathy in soma of pyramidal neurons in later stages of AD. The stratum lacunosum moleculare is the "connection hub" of the dendritic tree of CA1 pyramidal neurons with incoming myelinated axons of the temporoammonic path, which originates in the entorhinal cortex (medial and lateral layers II and III).[7] Thereby, the stratum lacunosum moleculare confers the direct connection between the two brain regions that are the first to be affected by pathologic features of AD, and primarily by tauopathy.[2,6,8]

Adeno-associated virus (AAV)–mediated gene transfer of mutant amyloid precursor protein and of wild-type (WT) and mutant P301L protein tau in the hippocampus of WT mice replicates pathologic features of AD including intracellular and extracellular amyloid accumulation and phosphorylation of protein tau. Pyramidal neurodegeneration was evident only in mice injected with AAV-tau, without formation of large aggregates of protein tau or tangles.[1] This model robustly recapitulates neurodegeneration *in vivo*, which is largely lacking in transgenic mouse models of amyloidopathy and tauopathy.[4,5,9,10] We observed that protein tau, present in an apparent oligomeric state, effected cellular demise via non-apoptotic mechanisms, and a large panel of markers implicated inflammation as a major actor.[1] The third major defect in AD-affected brain, neuroinflammation, was thereby implicated in the novel AAV-based model. In degenerating brain, inflammation is chronic and thought to contribute essentially to the disease process, although inflammatory reactions are proposed to provide protection.[11–14] In this respect, amyloid is analyzed most extensively because of experimental and clinical studies of anti-inflammatory compounds and vaccination against amyloid in AD. Far less is known of the mechanisms by which protein tau contributes to neurodegeneration and neuroinflammation.

We analyzed the reciprocal relations of tauP301L-induced dendritic degeneration, gliosis, and vascular defects as contributing early to the subsequent neurodegeneration. Expression of mutant protein tau was initially evident in dendrites, causing degeneration of distal neuronal compartments, reminiscent of dendritic amputation in AD-affected brain.[2] Degenerating neuronal axons and dendrites contained more and larger autophagic vacuoles and were surrounded by swollen astrocytic soma and processes. Moderate inflammatory reactions preceding the death of CA1 pyramidal neurons are proposed to increase the permeability of the blood-brain barrier (BBB), which was remarkably selective. The combined data are consistent with the hypothesis that tauP301L-mediated neurodegeneration is initiated by distal dendritic and axonal injury, closely associated temporally and spatially with early inflammatory responses and vascular defects. The combined effects eventually lead to annihilation of CA1 pyramidal neurons, which subsequently abrogate the neuroinflammatory reaction and plasma protein extravasation, corroborating their contribution to neurodegeneration.

Materials and Methods

Animals and Stereotaxic Injection

Adult WT FVB/N mice aged 3 to 4 months and of both sexes were used for most studies. To visualize neurodegeneration and microgliosis, we also studied yellow fluorescent protein (YFP)–expressing transgenic mice [B6.Cg-TgN(Thy1-YFP-H)2Jrs] and CX3CR1-deficient mice (B6.129P-Cx3cr1^{tm1Litt}/J) (Jackson Laboratory, Bar Harbor, ME).[15,16] Recombinant AAV vectors of hybrid serotype 1/2 to express either enhanced green fluorescent protein (EGFP) or mutant tauP301L under control of the human synapsin 1 gene promoter were used as described.[1,17]

Surgical procedures and unilateral intracerebral injection of viral particles into the left hemisphere were performed as described.[1] In brief, stereotactic injection of 2 μL viral suspension containing 10E8 t.u. was at coordinates 1.94 mm posterior, 1.4 mm lateral, and 2.2 mm ventral relative to bregma.[1,18]

All experiments were performed by certified researchers conforming to regional, national, and European regulations concerning animal welfare and animal experimentation, and were authorized and supervised by the university animal welfare commission (Ethische Commissie Dierenwelzijn, Katholieke Universiteit Leuven).

Immunohistochemistry

At indicated times after infection, mice were anesthetized using pentobarbital (Nembutal) and perfused transcardially using 7 mL ice-cold saline solution for 2 minutes. Brains were removed rapidly and fixed overnight in 4% paraformaldehyde for subsequent IHC analysis on 40-μm free-floating coronal vibratome sections. Primary antibodies were either affinity-purified polyclonal antibodies or mouse monoclonal antibodies that were biotinylated or horseradish peroxidase–labeled (Table 1) to avert cross-reaction with mouse immunoglobulins (see *Results*). Immune reactions were developed using streptavidin–horseradish peroxidase complex for biotinylated monoclonal antibodies or via a three-step method for polyclonal antibodies or directly for horseradish peroxidase–labeled primary antibodies using diaminobenzidine as chromogen.[1] Sections were counterstained using hematoxylin, dehydrated by passage through a graded series of alcohol and xylol, and mounted using DePeX mounting medium (Sigma-Aldrich Corp., St. Louis, MO) for microscopic analysis. For confocal analysis, after incubation with primary antibody, sections were processed using secondary antibodies coupled to Alexa Fluor 488 or 594 (Molecular Probes, Inc., Eugene, OR) counterstained with DAPI and mounted using Mowiol mounting