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Early-Stage Inflammation and Experimental Therapy in Transgenic Models of the Alzheimer-Like Amyloid Pathology

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Abstract
Background: Intracellular accumulation of β-amyloid (Aβ) is one of the early features in the neuropathology of Alzheimer's disease (AD) and Down's syndrome. This can be reproduced in cell and transgenic animal models of the AD-like amyloid pathology. In a transgenic rat model, our lab has previously shown that the intracellular accumulation of Aβ is sufficient to provoke cognitive impairments and biochemical alterations in the cerebral cortex and hippocampus in the absence of amyloid plaques. ***Objective:*** To investigate an early, pre-plaque inflammatory process in AD-like transgenic models and establish whether the neurotoxic effects of Aβ oligomers and proinflammatory responses can be arrested with minocycline. ***Methods:*** For these studies, we used naïve mice and transgenic animal models of the AD-like amyloid pathology and applied neurochemical, immunohistochemical and behavioral experimental approaches. ***Results:*** In the early stages of the AD-like amyloid pathology, intracellular Aβ oligomers accumulate within neurons of the cerebral cortex and hippocampus. Coincidental with this, behavioral impairments occur prior to the appearance of amyloid plaques, together with an upregulation of MHC-II, i-NOS and COX-2, well-known proinflammatory markers. Treatment with minocycline corrected behavioral impairments, lowered inflammatory markers and levels of Aβ trimers. ***Conclusion:*** A pharmacological approach targeting the early neuroinflammatory effects of Aβ might be a promising strategy to prevent or delay the onset of AD.

In view of the increasing evidence supporting a pathological role for intracellular neuronal accumulation of β-amyloid (Aβ) in Alzheimer's disease (AD), we generated transgenic animal models overexpressing the human amyloid precursor protein – APP751 – bearing the Swedish (K670N, M671L) and Indiana (V717F) mutations under the control of the murine Thy1.2 promoter. These mono-transgenic animals, coded McGill-Thy1-APP, display the AD-like amyloid pathology in the presence of intracellular Aβ immunoreactive material, prior to the deposition of amyloid plaques.

Immunohistochemical analysis and Western blotting revealed that intracellular accumulation of Aβ oligomers

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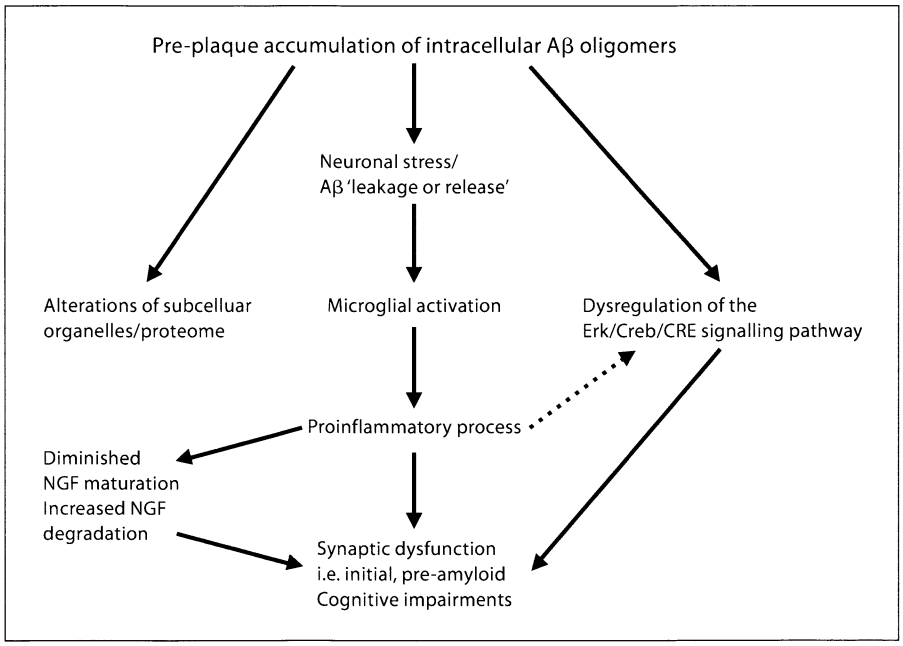

Fig. 1. Schematic representation of the early, pre-plaque components of the AD-like amyloid pathology leading to synaptic dysfunction and cognitive impairments.

Table 1. Effects of early, pre-plaque treatment of McGill-Thy1-APP mice with minocycline on neurochemical and behavioral parameters

Untreated Tg mice	Minocycline-treated Tg mice
i-NOS ↑↑ MHC-II ↑↑ COX-2 ↑↑ Behavioral impairments Aβ trimers ↑↑↑	Normalized i-NOS, MHC-II and COX-2 Correction of behavioral impairments Aβ trimers ↑

occurs as early as at 1 week in mice, while amyloid plaque deposition was detected after 3 months of age. The oligomeric nature of the Aβ peptides was confirmed by dot blot and immunohistochemistry of unfixed frozen sections to rule out the possibility that immunoreactivity of fixed tissue was due to artificial aggregates.

Our model also displayed early behavioral impairments in the performance of the Morris Water Maze task. Interestingly, learning and memory deficits occurred when the amyloid pathology was limited to intracellular Aβ oligomerization within pyramidal neurons of the cerebral cortex and hippocampus, prior to the deposition of plaques. Learning and memory were progressively impaired with age and with increasing Aβ load.

In addition, upregulation of specific proinflammatory markers followed the early intracellular accumulation of Aβ oligomers. In particular, Western blotting of hippocampal homogenates revealed an increase in the levels of i-NOS, MHC-II and neuronal COX-2 in Tg animals compared with age-matched non-Tg littermates. COX-2 immunoreactivity was detected in areas associated with Aβ-bearing neurons.

We further stained brain sections from Tg animals with Iba-1, a structural marker for microglia, and morphologically distinguished an early activation of these cells prior to plaque deposition. In contrast, stained sections from control animals revealed resting microglial cells, with a small flattened soma and long, thin, highly ramified processes as opposed to activated cells with larger soma and retraction and thickening of the processes. Immunohistochemical analysis further revealed a recruitment of activated microglial cells around neurons with intracellular accumulation of Aβ oligomers.

The appearance of an early inflammatory response coincidental with the intracellular accumulation of Aβ suggested that inflammation is at least one of the earliest neurotoxic effects of the peptide. Therefore, targeting this early phenomenon with minocycline, which is well

known for its anti-inflammatory actions in the central nervous system, which crosses the blood brain barrier and displays limited toxicity of the gastrointestinal tract [for review, see 1], might delay or prevent the AD-like amyloid pathology. As proof of principle, we treated Tg animals with 50 mg/kg of minocycline for a month prior to the appearance of extracellular amyloid plaques, sacrificed them at 3 months of age and observed a decrease in the levels of inflammatory markers. Behavioral deficits were also corrected. Unexpectedly, early treatment also lowered the levels of Aβ trimers (table 1).

In these studies, we have shown that intracellular Aβ oligomerization in transgenic models of the AD-like amyloid pathology is an early phenomenon and prominent in pyramidal neurons of the cerebral cortex and hippocampus. The accumulation of intracellular Aβ oligomers is coincidental with behavioral impairments and proinflammatory responses observed in AD-like transgenic models prior the appearance of amyloid plaques. Furthermore, it is likely that Aβ oligomers unleash the proinflammatory responses as injections of synthetic Aβ oligomer preparations into the hippocampus of naïve rats can elicit a similar inflammatory process, as well as a dysregulation of the nerve growth factor (NGF) metabolism [2]. The NGF dysmetabolism appears very early in the AD-like amyloid pathology and an indication of this can be observed in the brains of MCI sufferers [3].

These Aβ-induced inflammatory changes and behavioral deficits can be arrested with minocycline treatment in both naïve rats (injected with Aβ oligomers in the hippocampus) and in the McGill-Thy1-APP transgenic models. Our lab has previously shown that the pathological intracellular accumulation of Aβ also disrupts the CREB-CRE gene regulation pathway [4–6], which is essential to synaptic plasticity. It is therefore likely that all the above factors affect synaptic function and collectively explain the early cognitive impairments detected prior to the appearance of amyloid plaques, as schematically represented in figure 1.

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