

Two New Leads for Therapeutic Neuroprotection Against Alzheimer's Disease

a report by

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Dementia is a devastating clinical state frequently occurring in the aged population. Two figures reflect well the gravity of this problem: dementia occurs in 1% of the total population and almost one of two persons at the age of 90 years is demented. More than 70% of dementias are provoked by Alzheimer's disease, either alone or in conjunction with brain vascular pathology.

Hypotheses for the Cause of Alzheimer's Disease

Despite the overwhelming number of scientific publications in the field of Alzheimer's disease, the characterisation of mutations in familial Alzheimer's disease – and the important involvement in time and money of numerous private and academic research centres – the diagnosis of Alzheimer's disease is still a problem and there is no aetiological treatment available. Scientists concur that Alzheimer's disease is characterised by two different degenerating processes: amyloidosis, with the extra-cellular deposition of a 40 amyloid beta (A β) to 42 A β peptide into spherical plaques, named amyloid plaques; and neurofibrillary degeneration, with the intra-neuronal aggregation of tau proteins into abnormal filaments.

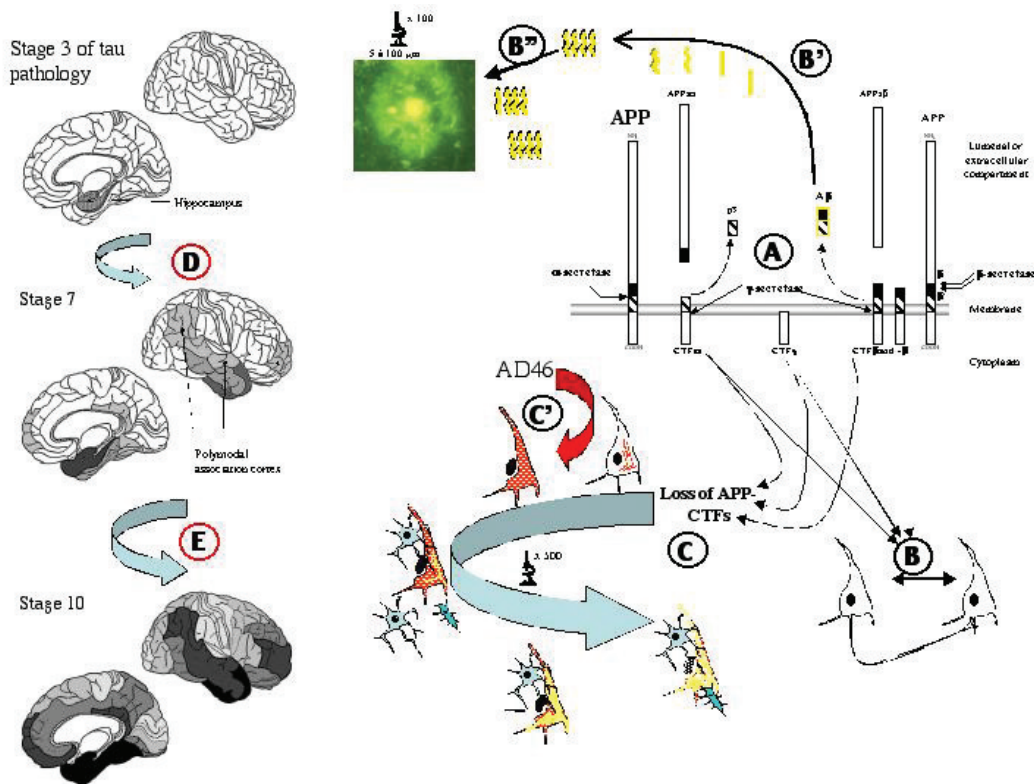
There is also no doubt that the dysmetabolism of amyloid precursor protein (APP), the precursor of A β peptide, is responsible for Alzheimer's disease. Indeed, mutations of APP, or of presenilin 1 (PS1) or

presenilin 2 (PS2), which is part of the gamma secretase complex to release A β , provoke familial autosomal dominant Alzheimer's disease, with full penetration. Aside from these facts, all other information related to the spatiotemporal physiopathology of Alzheimer's disease is a matter of heated discussion. The precise cause of sporadic Alzheimer's disease, representative of 99.7% of all cases, is still unknown. The amyloid hypothesis would support that the aggregated or oligomerised A β peptide is the neurotoxic agent killing neurons.¹ This theory, which is not shared by all scientists,² but has the merit of being simple, has been tested with a vaccination approach.³ The therapeutic trial on 300 vaccinated patients has been terminated, due to serious adverse events, i.e. inflammatory meningoencephalitis.^{4,5} Other new trials should be developed in the near future. Secretase modulators could also be another means of eliminating the neurotoxic A β agent.⁶

Other solid but totally different hypotheses could explain neurodegeneration in Alzheimer's disease. They are more relevant to the complexity of the human brain physiology. APP has, on its primary sequence, numerous potential functional regions that could be responsible for neurotrophic activity or signalling functions.⁷ The carboxy-terminal fragments (CTFs) of APP, which are released after alpha, beta and gamma secretase cleavages, very likely have an important role in neuronal physiology (see *Figure 1*). For example, it has been shown that the

1. J Hardy and D J Selkoe, "The Amyloid Hypothesis of Alzheimer's Disease: Progress and Problems on the Road to Therapeutics", *Science*, 297 (2002) 5,580, pp. 353–356.
2. A Motluk, "Could the Proposed Treatments for Alzheimer's make this Debilitating Disease even Worse?", *New Scientist*, 177 (2003), p. 34.
3. D Schenk, P Seubert, et al., "Immunotherapy with Beta-amyloid for Alzheimer's Disease: A New Frontier", *DNA Cell Biol.*, 20 (2001) 11, pp. 679–681.
4. P L McGeer and E McGeer, "Is There a Future for Vaccination as a Treatment for Alzheimer's Disease?", *Neurobiol. Aging*, 24 (2003) 3, pp. 391–395.
5. J C Dodart, K R Bales, et al., "Immunotherapy for Alzheimer's Disease: Will Vaccination Work?", *Trends Mol. Med.*, 9 (2003) 3, pp. 85–87.
6. D J Selkoe and D Schenk, "Alzheimer's Disease: Molecular Understanding Predicts Amyloid-based Therapeutics", *Ann. Rev. Pharmacol. Toxicol.*, 43 (2003), pp. 545–584.
7. M P S Mattson, S W Barger, et al., "Cellular Signaling Roles of TGF-beta, TNF-alpha and Beta APP in Brain Injury Responses and Alzheimer's Disease", *Brain Res. Brain Res. Rev.*, 23 (1997) 1–2, pp. 47–61.

Figure 1: The Synergy of APP-Tau Pathologies in Alzheimer's Disease



APP is a trans-membrane protein cleaved by different secretases, at the alpha, beta, beta prime and gamma sites (A). The cleavages release several APP-CTFs, which likely have a neurotrophic activity (B) and release also, in parallel, a beta peptide (B'). A decrease of APP-CTFs is observed in AD, which enhances tau pathology and neurodegeneration (C). AD46 (C') is also an early co-factor of tau pathology. The stimulated tau degenerating process will progress in brain areas, along cortico-cortical connections, from stages 1 to 3 (entorhinal and hippocampal formation) to stage 10 (all neocortical areas, via polymodal association areas (stage 7)) (left part, D, E).

APP intracellular domain (AICD) fragment translocates to the nucleus with Fe65, likely as a transcription factor complex.^{8,9} AICD could be, either directly or indirectly, the 'survival factor' for neurons and, therefore, a therapeutic target.

A third hypothesis is also gaining significance. In the amyloid cascade hypothesis, tau pathology is considered as a late consequence.^{1,10} Today, it is known that most dementing disorders are 'tauopathies', in that many different tau defects can generate neurodegeneration, from mutations, abnormal splicing to post-translational decrease of tau levels.^{11,12} Also, the sequence of brain areas affected by tau pathology in Alzheimer's disease is well correlated with the sequence of altered cognitive functions, from the hippocampal area and short-term

memory impairment to affected polymodal association areas associated with aphasia, apraxia and agnosia (see Figure 1).

The observations of normal and affected human brains show that the explanation for the physiopathology of Alzheimer's disease is likely to be global (spatiotemporal) and complex. It is striking to observe that tau pathology and amyloidosis are always developing in parallel in the diseased brain, from the earliest stages to the end of the disease.¹³ A synergy between both degenerating processes is the likely explanation for this. Therefore, the aetiology and the leads for therapy could be linked to APP and tau processes and on their possible interaction.

The synergy of APP/Aβ and tau pathologies on the

8. W T Kimberly, J B Zheng, et al., "The Intracellular Domain of the Beta-amyloid Precursor Protein is Stabilized by Fe65 and Translocates to the Nucleus in a Notch-like Manner", *J. Biol. Chem.*, 276 (2001) 43, pp. 40,288–40,292.
9. X Cao and T C Sudhof, "A Transcriptionally Active Complex of APP with Fe65 and Histone Acetyltransferase Tip60", *Science*, 293 (2001) 5,527, pp. 115–120.
10. J A Hardy and G A Higgins, "Alzheimer's Disease: The Amyloid Cascade Hypothesis", *ibid.*, 256 (1992) 5,054, pp. 184–185.
11. L Buée, T Bussiere, et al., "Tau Protein Isoforms, Phosphorylation and Role in Neurodegenerative Disorders", *Brain Res. Brain Res. Rev.*, 33 (2000) 1, pp. 95–130.
12. V M Lee, M Goedert, et al., "Neurodegenerative Tauopathies", *Ann. Rev. Neurosci.*, 24 (2001), pp. 1,121–1,159.
13. A Delacourte, N Sergeant, et al., "Non-overlapping but Synergetic Tau and APP Pathologies in Sporadic Alzheimer's Disease", *Neurology*, 59 (2002) 3, pp. 398–407.

one hand and, on the other, the fact that tau pathology progresses along cortico-cortical connections, suggest that Alzheimer's disease neurodegeneration is a chain reaction phenomenon and that neuroprotection could play a decisive role in slowing down the fatal destabilisation of neuronal populations. Other therapeutic alternatives other than the removal of amyloid deposits exist. Two of these, linked to APP loss of function and to a co-factor of tau pathology, are presented briefly in this article.

APP-CTF Loss in Alzheimer's Disease

The development of APP and tau-degenerating processes in the brain of numerous patients that were followed prospectively and with a multidisciplinary approach were investigated. Several hundred brains were analysed, from spared brains to those with full-blown Alzheimer's pathology. Tau and A β aggregates were analysed at the quantitative and qualitative levels, as well as full-length APP and APP-CTFs. This study led to the observation of three main points:

- a progressive spreading of tau pathology from the entorhinal regions to all neocortical areas, via 10 stages of progression (see *Figure 1*);¹⁴
- a simultaneous, diffuse and progressive aggregation of A β 42 species in neocortical areas;¹⁵ and
- a significant decrease of APP-CTFs associated with 'Alzheimerization'.¹⁶

This last observation is important since it could show the real cause of Alzheimer's disease: a loss of neuroprotection factors. If APP-CTFs are survival factors, their disappearance could lead to neuronal depression and facilitate their neurodegeneration. This simple observation could open a therapeutic avenue, directing the amyloid aggregation upwards, based on the restoration of normal APP-CTF levels and functions in neurons. Cellular and transgenic mice are likely to demonstrate which signalling pathway needs to be stimulated for this recovery of APP-CTF levels. Pharmacological agents working on secretase activities are still good candidates, but the focus will be on APP-CTF levels and not on A β production, which is not modified in sporadic Alzheimer's disease. Therefore, restoration of APP-CTFs is a new strategy to slow neurodegeneration.

Tau Pathology – A Target for a Neuroprotection Approach

The spatiotemporal analysis of tau pathology in the ageing human brain, as well as at the very first stages of the Alzheimer's process, provides clues to therapy. The systematic development of tau pathology has been demonstrated in the entorhinal, then the hippocampal, formation in ageing. Tau pathology is always found in these regions at the age of 75 years. This degenerating process is age-related, but not age-dependent; it is likely to be one of numerous possible tauopathies observed in the human brain.^{13,15} This tau pathology has several characteristics:

- a slow dynamic of development, explaining why it is age-related;
- an aptitude to spread along neuronal connections;
- a progression that can progressively reach all neocortical neurons, if boosted by specific co-factors; and
- the phenomenon of being totally 'human-specific'.

The development of the initial age-related entorhinal/hippocampal tauopathy towards other brain areas is always associated with the presence of amyloid deposits, suggesting that Alzheimer's disease is a tauopathy fuelled by APP dysfunctions (see *Figure 1*).

The proteomic approach of brain lesions at the earliest stage of Alzheimer's disease, using the criteria to establish a biochemical diagnosis of Alzheimer's disease (CEBDAD) biochemical criteria for the diagnostic,¹⁵ led to the discovery of an early marker associated with tau pathology.¹⁷ This marker, named AD46, is a monoclonal antibody that detects the abnormal association of tau proteins with the alpha chain of adenosine triphosphate (ATP) synthase. This protein, which is encoded by the nuclei genome, is a mitochondrial regulating subunit of the complex V of oxidative phosphorylation. Aggregation of ATP synthase alpha chain is an early and constant event of neurofibrillary degeneration, demonstrating that this protein is potentially a diagnostic tool. This protein is involved in apoptosis and cell signalling. A loss of ATP synthase alpha chain function could simultaneously increase tau aggregation and enhance neurodegeneration. Therefore, ATP synthase alpha chain is likely a therapeutic target to slow down

14. A Delacourte, J P David, et al., "The Biochemical Pathway of Neurofibrillary Degeneration in Aging and Alzheimer's Disease", *ibid.*, 52 (1999), pp. 1,158–1,165.

15. A Delacourte, N Sergeant, et al., "Tau Aggregation in the Hippocampal Formation: An Ageing or a Pathological Process?", *Exp. Gerontol.*, 37 (2002) 10–11, pp. 1,291–1,296.

16. N Sergeant, J P David, et al., "Progressive Decrease of Amyloid Precursor Protein Carboxy Terminal Fragments (APP-CTFs), Associated with Tau Pathology Stages, in Alzheimer's Disease", *J. Neurochem.*, 81 (2002) 4, pp. 663–672.

17. N Sergeant, A Watzet, et al., "Association of ATP Synthase Alpha-chain with Neurofibrillary Degeneration in Alzheimer's Disease", *Neuroscience*, 117 (2003) 2, pp. 293–303.

neurofibrillary degeneration. This approach is completely new but nevertheless promising.

There are different possible therapeutic strategies to fight Alzheimer's disease. From the natural and

molecular history of this disease, general consensus is that the main pragmatic target is to slow down the dynamic of progression of the two main degenerating processes. Experimental models for drug screening should soon be available. ■
