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The natural and molecular history of Alzheimer's disease: Tau is part of the story

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Summary

Alzheimer's disease (AD) is a very common brain pathology of the elderly, with an etiology that is far more complicated than was thought in the 1990s. In particular, the complexity comes from the coexistence of two degenerating processes, tau aggregation and A β deposition, that affect polymodal association brain areas, a feature never observed in non-human primates and one that is difficult to model. Genetic studies have shown that A β PP plays a central role in familial and sporadic AD, but the role of tau has been understated for a long time. The first evidence of this came from the demonstration of the concept of pathological tau proteins in AD and their full biochemical characterization as a major triplet (tau 60, 64, 68) plus tau 72. This concept was extended to most neurodegenerative diseases with dementia, since pathological tau proteins present a disease-specific bar code: a major upper doublet for parkinsonian diseases with dementia (PSP, CBD, Guadeloupe), a lower doublet for Pick's disease, and a single band for myotonic dystrophy. This bar code results from the aggregation of specific tau isoforms and was merged with tau mutations in familial frontotemporal diseases as a more global concept of tauopathy linked to most dementing neurodegenerative disorders .

To apprehend the role of tau in AD, which is 99% sporadic, we have developed a spatio-temporal analysis of tauopathy in many brain areas of hundreds of non-demented and demented patients. This prospective and multidisciplinary study showed us that tauopathy always progresses in the brain along a very precise and invariable pathway, from the entorhinal to the hippocampal formation to polymodal association areas, ending in primary regions and in many subcortical areas. The cognitive impairment follows exactly the progression of the affected brain regions. In strict parallel, neocortical A β deposits increase in quantity and heterogeneity, suggesting a direct link between both neurodegenerative processes. Altogether, our molecular studies suggest that AD is a tauopathy fueled by A β PP dysfunction. Restoring A β PP loss of function seems to be the most efficient and pragmatic therapeutic approach.

Introduction

First of all, scientists never forget that Alzheimer's disease (AD) is a devastating disease, not only for the patient but also for the family, but from a scientific point of view, AD is

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an exciting field of research. At present, we know that this disease is more complicated than expected, with numerous risk factors. Therefore, finding the right area of research for the scientist working in the Alzheimer field is quite a challenge.

AD is a very complicated disease at the physiopathological level, as was observed by Alois Alzheimer himself, who discovered this organic dementing disease with two types of lesions: tangles inside neurons and plaques outside, in the vicinity of degenerating neurons. Alois Alzheimer was probably aware of the importance of intraneuronal lesions, since he also discovered the specific lesions of the fronto-temporal dementia characterized by Arnold Pick, namely Pick bodies of Pick disease. But we should not forget the other early pioneers: Beljahow (1889), Marinesco (Blocq and Marinesco 1982), Redlich (1898), and Leri (1906).

One century after the princeps paper of Alois Alzheimer, the question of the role of plaques versus tangles is still a matter of debate. Which lesion is the cause, which one is a consequence, and more importantly, which one will lead to a treatment?

The complexity of the approach comes from the fact that, on the one hand, the disease is exclusively present in the brain but that, on the other hand, the brain is inaccessible to molecular investigations and well protected behind the blood-brain barrier, the skull and by our cultural, social or religious rules.

Also, AD is one of the rare diseases that is totally specific to the human species. In very old non-human primates, such as the baboon or the rhesus monkey, the presence of tangles is strictly limited to the entorhinal or the hippocampal formation. The basic NIA neuropathological criteria of AD, namely plaques and tangles in the association neocortex, have never been found in other non-human species (Hartig et al. 2000; Schultz et al. 2000).

Aggregated and hyperphosphorylated tau proteins: a powerful marker of neurofibrillary degeneration

Tau proteins are the basic component of neurofibrillary degeneration (NFD), as observed using histological (Brion et al. 1985c) and biochemical means. Using Western blots, we were able to detect and quantify abnormal tau species in AD brains, as they are aggregated, hyperphosphorylated and abnormally phosphorylated (Delacourte and Defossez 1986), in good agreement with the pioneer work of Brion et al. (1985c) and Grundke-Iqbal et al. (1986b). In addition, we were able to develop the concept of pathological tau proteins in AD and, later on, in many other neurodegenerative disorders with dementia. First we detected two abnormal bands in neocortical areas of AD patients (Tau 64 and 68; Flament et al. 1989) (MW are those given in the literature these days) and then a third one using more specific antibodies (Tau 60; Fig. 1; Delacourte et al. 1990). These pathological Tau bands were specifically detected by an anti-PHF absorbed with normal tau proteins. The antibody Alz-50 of Peter Davies, which detects NFD and a group of pathological proteins named A68 so well, was in fact those abnormal tau proteins Tau 64 and 68 (Flament and Delacourte 1990). Our results were corroborated by Lee et al. (1991). At last, using 2D gels and our knowledge that tau proteins contain six isoforms, as shown by Goedert et al. (1992), we demonstrated the presence of a minor and fourth abnormal tau protein at 72 kDa among the bulk of aggregated tau proteins, corresponding to the largest tau isoform (Fig. 1; Sergeant et al. 1997). With a direct

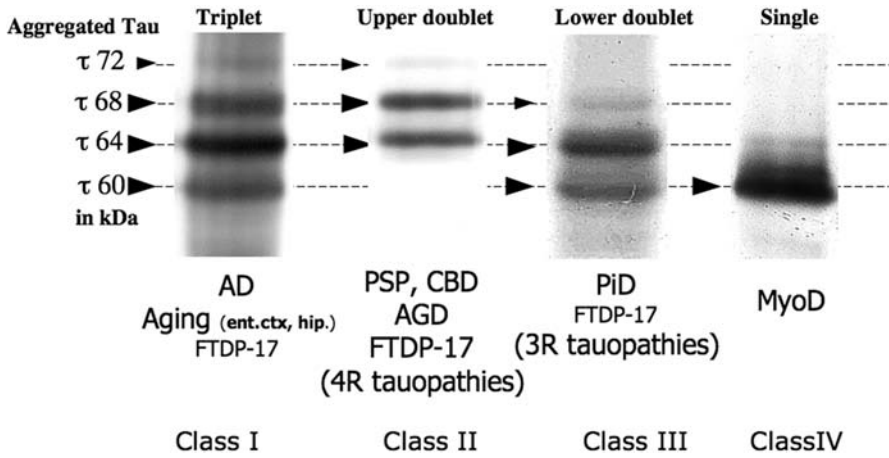


Fig. 1. The bar code of tauopathies. Western blot immunostaining of aggregated tau proteins from brain homogenates of patients affected by Alzheimer's disease (AD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), argyrophilic grain disease (AGD), Pick disease (PiD), dystrophic myopathy (MyoD) and frontotemporal dementia linked to chromosome 17 (FTDP-17). Aggregated tau proteins are specifically stained by AD2, a monoclonal antibody against a phosphorylation site on Ser 396 and 404 of tau. Note that the electrophoretic profile is different for each group of diseases. This is due to the aggregation of specific sets of tau isoforms: all six isoforms for AD, also observed in the entorhinal and hippocampal (ent.ctx, hip.) cortex of aged controls, as well as a few rare diseases including some FTDP-17 with mutations outside exon 10 area; the three isoforms with 4 repeats for PSP, CBD, AGD and most FTDP-17 (4R tauopathies); three repeats for PiD and a few FTDP-17 (3R tauopathies). In MyoD type I and II, the shortest tau isoform is involved in tau aggregates

approach, and to cope with the problem of dephosphorylation during post-mortem delays, we demonstrated that tau are abnormally phosphorylated, since post-mortem tau from AD patients (under the influence of post-mortem dephosphorylation) are more acidic than native tau from post-operative (not dephosphorylated) human brain biopsies (Sergeant et al. 1995).

Interestingly enough, using the same approach, we demonstrated that these tau aggregates were different in other neurodegenerative dementing disorders and that there is a code-bar of tauopathies (Fig. 1). In progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), we observed a specific characteristic upper doublet (Tau 64 and 69), due to the aggregation of tau isoforms with 4 repeats (4R tauopathy; Flament et al. 1991; Buee Scherrer et al. 1996; Sergeant et al. 1999), whereas in Pick's disease, there is a lower doublet (Tau 60 and 64), resulting from the aggregation of 3R isoforms Buee Scherrer et al. 1996; Delacourte et al. 1998; recently confirmed by Dickson and co-workers de Silva et al. 2006). Other diseases have other tau profiles, such as a single band in myotonic dystrophy (DM1; Vermersch et al. 1996; Sergeant et al. 2001) and soluble tauopathy in dementia lacking distinctive histology (DLDH; Vermersch et al. 1995). For DLDH, a heterogeneous group, Zhukareva et al. (2000) clearly showed that a subgroup has a dramatic decrease of normal tau proteins levels.

All these specific biochemical signatures and different sets of tau isoforms aggregated in specific subsets of neuronal populations began to demonstrate that tangles are not such a unique and late answer to different types of neuronal insults. Indeed, many dementia disorders result from a defect of tau proteins, and tau mutations are causal in familial frontotemporal dementia (FTD; Goedert and Spillantini 2000). Our concept of pathological tau proteins was a basis for a more global concept of tauopathy, adapted first for familial diseases but also true for sporadic diseases. Therefore, the question was to determine the contribution of tau pathology to AD etiology.

The spatio-temporal biochemical pathway of tau pathology in aging and sporadic AD

Tau pathology spreading in cortical areas is invariable and hierarchical

A prospective and multidisciplinary study of more than 200 cases, including 70 non-demented patients, was undertaken. We gathered clinical and neuropathological data and, in parallel, studied the presence of NFD at the biochemical level, using the triplet of abnormal tau proteins as a marker. In Alzheimer brains, we observed that tau pathology always extended along 10 stages, corresponding to 10 brain areas that are successively affected. Paired helical filaments (PHF)-tau pathology was systematically found to be present in variable amounts in the entorhinal and hippocampal regions of non-demented patients aged over 75 years. When tau pathology was found in other brain areas, it was always along a stereotyped, sequential, hierarchical pathway (Fig. 2). The progression was categorized into 10 stages according to the brain regions affected: transentorhinal cortex (S1), entorhinal cortex (S2), hippocampus (S3), anterior temporal cortex (S4), inferior temporal cortex (S5), mid temporal cortex (S6), polymodal association areas (prefrontal, parietal inferior, temporal superior) (S7), unimodal areas (S8), primary motor (S9a) or sensory (S9b, S9c) areas, and all neocortical areas (S10; Delacourte et al. 1999).

The mechanism of progression of tau pathology

Determining the mechanism of the spread of tauopathy is likely to open relevant therapeutic avenues in the neuroprotection domain. From the study of AD, we observe that this spreading is not diffuse but, on the contrary, along precise neuron-to-neuron connections, from the limbic structures toward the neocortical association areas. Interestingly enough, we observe a similar mechanism of spreading in other sporadic tauopathies, such as PSP. Neurodegeneration in PSP is observed first in the brain stem, then in the striatum, the primary motor frontal neocortical area (Brodmann area 4), the unimodal frontal areas and at last spreading into all neocortical and limbic areas (Sergeant et al. 1999). In other words, the basic mechanism of tau spreading in sporadic tauopathies likely starts in a specific vulnerable neuronal population (layer II of the entorhinal formation in AD; oculomotor nuclei for PSP). Then, this local tauopathy destabilizes the connected neuronal populations that had a cross-talk of neurotrophic factors with the primary set of vulnerable neurons, and this degenerating process will

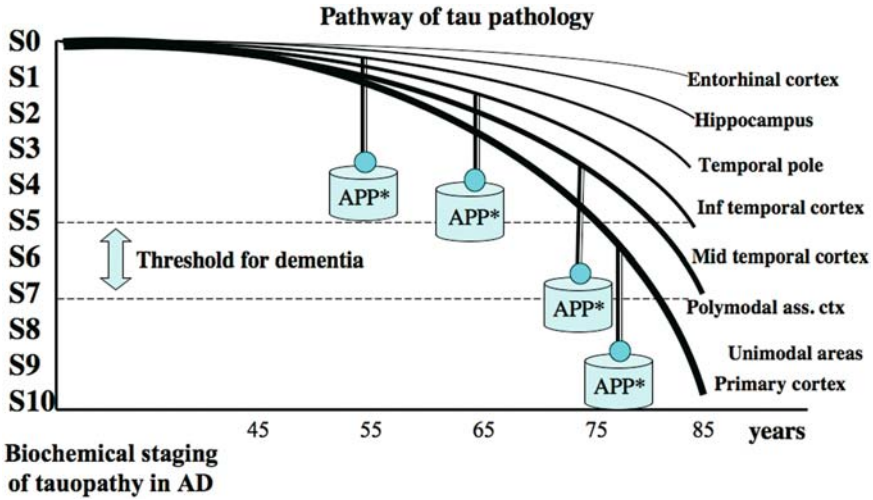


Fig. 2. Pathway of tau pathology in aging and in AD. First, neurofibrillary tangles (i.e., tau pathology) are age-related but not age-dependent brain lesions. They appear in the entorhinal cortex of 20% of people with an average age of 25 years. The ratio increases at 50% at the age of 50 years to affect all people at the age of 70 years or older, as shown by Braak and Braak 1997 and in our study. This vulnerability varies dramatically among individuals. A few nonagenarians in our study were very mildly affected. Therefore, the entorhinal formation is a vulnerable area that is always affected by tau pathology at old age (stages 1 and 2 of tau pathology). Second, tauopathy in aging tends to spread from the affected vulnerable area to other connected neuronal populations, along a neuron-to-neuron propagation that resembles a chain reaction or a domino effect. This spreading can be observed up to the temporal pole (stage 4 of tau pathology) without A β deposition. Third, the extension of tauopathy toward polymodal association areas is systematically observed in the presence of A β x-42 deposits (amyloid stage of 1 to 4), as if these aggregates, directly (neurotoxicity) or indirectly (markers of A β PP dysfunction) were fueling tau spreading. This step represents the beginning of incipient AD. Fourth, after neocortical extension of tauopathy, when neuroplasticity will no longer be able to compensate for the progressing neurodegenerative process, clinical impairment and dementia will appear. The cognitive impairment observed in AD is well explained by the brain areas that are successively affected by tau pathology, from mild cognitive impairment (stages 3 to 6) to the different AD stages, from stage 6 to stage 10 of tau pathology. The amyloid burden will also increase (stages 5 to 10), paralleling tau staging. Fifth, tau pathology will continue its conquest of the brain, through primary regions and subcortical areas, to kill the patient, directly or indirectly

extend, with a domino effect, to other neuronal populations through a neuron-to-neuron propagation phenomenon (Delacourte 2000). Understanding this mechanism of propagation better will certainly open up therapeutic strategies for AD as well as for other sporadic tauopathies and synucleopathies (Deramecourt et al. 2006).

The relationship between tauopathy and amyloidosis in aging and sporadic AD

It is not surprising that tau pathology is well correlated to cognitive impairment, since it shows the neurodegeneration process and its extent. However, we do not know the

factors that generate tauopathy and its extension in brain areas. A β PP dysfunction is the best candidate, as revealed by genetic studies. Therefore, we quantified all A β PP metabolic products to locate a possible relationship with the different stages of tau pathology. A β PP holoproteins, A β PP-CTFs and A β species were analyzed in the different brain areas of all our non-demented and demented patients.

First, A β species were studied. Insoluble A β -42 and -40 species were fully solubilized and quantified, and we were able to propose a biochemical staging of amyloidosis on a scale from 0 to 10 for the quantification of either A β 40 or A β x-42 aggregates (Deramecourt et al. 2006; Delacourte et al. 2002a).

Surprisingly, we observed a parallel and synergistic effect of A β PP dysfunction (as visualized by A β deposition) on the neuron-to-neuron propagation of tau pathology. Indeed, tau pathology can be found in the hippocampal area without A β deposits, as mentioned by Braak and Braak (1997). In contrast, the extension of tau pathology in polymodal association areas was systematically found in the presence of A β deposits (A β stages 4 to 10), as if these A β species, directly or indirectly, were necessary to stimulate the progression of tau pathology (Fig. 2). Altogether, our results clearly demonstrated that amyloid deposits do not precede tau pathology in sporadic AD, as claimed in the amyloid cascade hypothesis based upon familial cases (Hardy and Higgins 1992b; Hardy and Selkoe 2002). Also, a systematic analysis of tauopathy, amyloidosis and synucleopathy in sporadic Lewy body disease (LBD) revealed a similar pattern. Indeed, the extension of synucleopathy in neocortical areas is observed in the presence of amyloid deposits (Deramecourt et al. 2006). Interestingly enough, our proteomic analysis of the first A β 42 deposits that appear in the aging human brain and in incipient AD are not the full length A β 1-42, but N-truncated species. In other words, the first A β species that initiate amyloidosis are not physiological species, but pathological species. This finding was observed at the biochemical and immunohistochemical levels, in the brain of patients affected by AD but also LBD (Deramecourt et al. 2006). This discovery could improve dramatically the vaccination approach (Sergeant et al. 2003).

Relationship between Tau pathology and A β PP dysmetabolism

The parallelism and synergy between tau and A β aggregation led us to search an A β PP molecular event linking the two degenerating processes. A β PP is a ubiquitous protein found in all cell types of all species, suggesting a basic and important role that remains to be identified. A neurotrophic activity for A β PP and secreted s-A β PP is often mentioned (Turner et al. 2003). Therefore, a loss of function of A β PP rather than a gain of toxic function of A β could also be a reasonable hypothesis to explain the stimulation of tau pathology and neurodegeneration (Fig. 2). Complementary to this study of A β species, we found no obvious modification of A β PP holoprotein, but all A β PP-CTFs were found to be significantly diminished during the course of AD and well correlated with the progression of tau pathology (Sergeant et al. 2002). An important role of gamma stub, also named AICD (A β PP intracellular domain), as a possible transcription factor could explain its involvement in the disease if these fragments are lacking (Cao and Sudhof 2001; von Rotz et al. 2004; Pardossi-Piquard et al. 2005).

In fact these observations directly lead to other therapeutic strategies concentrated around the concept of a loss of function of A β PP stimulating tau pathology, in good agreement with other teams who contend that A β may be a player, but A β PP is central (Neve and Robakis 1998; Neve 2001; Lee et al. 2004c). From our study on tau and A β in the human brain, the stimulation of the non-amyloidogenic pathway seems to be the more powerful and less risky way to decrease A β production and simultaneously to stimulate the production of sAPP α , a neurotrophic factor, and AICD, a possible transcription factor, which should delay tau pathology (Delacourte 2006).

Conclusion

Altogether, many converging studies show that AD is not a pure pathology of A β ; neither is it a pure tauopathy. We propose the following definition: AD is a tauopathy fueled by A β PP dysfunction (Fig. 2). The natural and molecular history of sporadic AD shows that both A β PP and tau are equally involved in the etiopathogenesis (Fig. 2). Both are also therapeutic targets and the good news is that β APPists and tauoists must work together. From observations of the human brain, relevant animal models are most likely those that will demonstrate a synergy between A β PP and tau lesions. Some interesting models have already been described (Gotz et al. 2001; Lewis et al. 2001). Another one with a severe neuronal loss is also interesting to understand the loss of function of A β PP as well as the role of intracellular A β deposition (Casas et al. 2004).

At last, one can see that most dementing neurodegenerative disorders are tauopathies, that most demented patients have a tau pathology in neocortical areas, and that many different types of tau dysfunction lead to dementia: mutations on tau gene in FTDP-17 (frontotemporal dementia with Parkinsonism linked to chromosome 17; Spillantini et al. 1998c); the haplotype H1H1, which is a risk factor for PSP and CBD (Baker et al. 1999); the abnormal tau splicing in DM1 (Sergeant et al. 2001); tau-less DLDH (Zhukareva et al. 2001); and the vulnerability of specific brain areas to tauopathy, as observed in the entorhinal cortex and hippocampus for AD (Delacourte et al. 2002b) or in the brain stem nuclei for PSP and CBD (Sergeant et al. 1999; Dickson 1999; Caparros-Lefebvre et al. 2002). In conclusion, tau is a key player in most dementing neurodegenerative disorders.