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The natural and molecular history of Alzheimer'disease

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Abstract. Alzheimer's disease (AD) is a very frequent brain pathology of the elderly, with an etiology by far more complicated than thought in the nineties. In particular, the complexity comes from the coexistence of two degenerating processes, tau aggregation and Aβ deposition, that affect polymodal association brain areas, a feature never observed in non-human primates and difficult to model. Genetic studies have shown that AβPP plays a central role in familial and sporadic AD, but the role of tau has been for a long time understated. To apprehend this role, 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 then hippocampal formation to polymodal association areas to end in primary regions and in many subcortical areas. The cognitive impairment follows exactly the progression of the affected brain regions. In strict parallelism, neocortical Aβ deposits increase in quantity and heterogeneity, suggesting a direct link between both neurodegenerative processes. Altogether, our molecular study suggests that AD is a tauopathy fueled by AβPP dysfunction. Restoring AβPP loss of function seems to be the most efficient therapeutic approach.

1. Introduction

First of all, scientists do not 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 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 lead of research for the scientist working in the Alzheimer field is quite a challenge.

AD is a very complicated disease at the physiopathological level. This 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.

One century after the princeps paper of Alois Alzheimer, the question of the importance 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?

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

Third, AD is one of the rare disease that is totally specific to 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 hippocampal formation. The basic neuropathological criteria of AD, namely plaques and tangles in the association cortex, were never found in other non-human species [1, 2].

2. The "amyloid" period

After the discovery of Glenner and Wong in 1984, showing that plaques result from the aggregation of

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a polypeptide of 39 to 42 amino-acids, successively named A4 then Aβ, a number of great discoveries have shown the importance of physiological events linked to plaques [3]. First of all, the discovery by Hardy's team of mutations on the Aβ protein precursor (AβPP) generated the amyloid cascade hypothesis in 1992 [4]. This theory implies that neuronal dysfunction is generated by amyloid toxicity. Other mutations of FAD located on presenilin 1 and 2 by St Georges Hyslop's team corroborated this cascade hypothesis. Indeed, presenilin cleaves AβPP and patients with PS1 mutations release more Aβ42 species [5]. Then transgenic mice with human APP and PS1 mutated genes developing numerous plaques as well as a possible cognitive impairment, corroborated the hypothesis of John Hardy [6]. Nowadays, all scientists agree that AβPP dysfunction plays a central role in AD etiopathogenesis.

Legitimately, from the amyloid cascade hypothesis, one can conclude that AD is a simple brain disease, with a unique killer, the neurotoxic Aβ peptide, and a unique and simple therapeutic target: the removal or neutralization of Aβ aggregates. However, the accumulation of data on the natural history of sporadic AD, that represents more than 99% of all cases, has progressively changed our perception of AD physiopathology and revealed that neurofibrillary degeneration is the other inescapable feature that explains AD.

3. The Braak stages

Heiko Braak is a German neuropathologist that has observed both lesions, plaques and tangles, at the spatio-temporal level. Using silver staining on large tissue sections of several thousands of brains of patients at different stages of the pathology, he demonstrated that there is a progressive spreading of neurofibrillary degeneration (NFD), along a precise pathway, from the entorhinal and hippocampal formation towards polymodal association then primary brain regions [7]. Alzheimer dementia is observed when a threshold of neurofibrillary degeneration in the association cortical areas is reached, corresponding to stages IV to VI [8].

The role of tangles to explain Alzheimer dementia was so obvious that the Braak stages were incorporated in the consensus criteria for a definite diagnosis of AD in 1997 [9], in addition to the CERAD criteria based only on the number of amyloid plaques.

4. The natural and molecular history of AD

In the same way, our strategy to study AD was the following: first to study the commonest form of AD, sporadic AD; then to develop a strategy similar to Braak, but using molecular probes rather than histological observations. In our Lille Hospital network, with Prof. Pasquier, Dr Lebert, Prof. Maurage, it has been possible to develop a prospective study combining clinical, neuropathological and molecular data. Of course, this approach on sporadic diseases is a difficult one, and took us two decades to be complete. But we found it was the only way to analyze the basic physiopathological events that generate and fuel the disease.

To be as objective as possible, we studied the development of the two degenerating processes that characterize AD, tangles and plaques, using their basic components as markers, namely tau proteins and Aβ peptides. Then we analyzed if these two degenerating processes were interconnected and their relationship to dementia.

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

Tau proteins are the basic component of NFD, as observed using histological and biochemical means. Aggregation of tau is easy to observe at the biochemical level, rendering very convenient the quantification of NFD. Indeed, aggregated tau proteins are not dephosphorylated by phosphatases during post-mortem delay, while normal tau proteins are dephosphorylated. Therefore, phosphorylated tau proteins of human brain homogenates detected with phospho-dependant antibodies are those that are aggregated. Using western blots, we have been able to detect and quantify abnormal tau species in AD brains, in that they are aggregated, hyperphosphorylated and abnormally phosphorylated [10], in good agreement with the results of Brion [11] and Iqbal [12]. In addition, we were able to demonstrate that tau proteins in AD are reliable markers of the degenerating process. First we were able do detect two abnormal bands in neocortical areas (Tau 64 and 68) [13], then a third one using more specific antibodies (Tau 60) [14]. These pathological Tau bands were specifically detected by an anti-PHF absorbed with normal tau proteins. The antibody Alz-50 of Peter Davies, that detects so well neurofibrillary degeneration and a group of pathological proteins named A68,

was in fact immunostaining those abnormal tau proteins Tau 64 and 68 [15]. This was confirmed later on by Trojanowski's team [16]. At last, using 2D gels and our knowledge that tau proteins contain 6 isoforms [17], we demonstrated the presence of a minor and fourth abnormal tau protein at 72 kDa [18] (MW are those given in the literature these days).

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. In PSP and CBD, we observed a specific characteristic upper doublet (Tau 64 and 69), due to the aggregation of tau isoforms with 4 repeats (4R tauopathy) [19,20], while in Pick's disease, there is a lower doublet (Tau 60 and 64), resulting from the aggregation of 3R isoforms [21,22]. Other diseases have other tau profiles such as the singulet in myotonic dystrophy (DM1) [23,24], and soluble tauopathy in dementia lacking distinctive histology (DLDH) [25]. For DLDH, an heterogeneous group, it has been clearly shown by Zhukareva et al. that a subgroup has a defect in the synthesis of tau proteins [26].

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 this unique and late answer to different types of neuronal insults. Indeed, many demented disorders result from a defect of tau proteins [27]. Therefore, the question was to determine the natural history of tau pathology in the aging human brain that develops or not Alzheimer's disease.

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

6.1. 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 neurofibrillary degeneration at the biochemical level, using the triplet of abnormal tau proteins as a marker. In Alzheimer brains, we observed that tau pathology always extends along ten stages, corresponding to ten 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. The progression was categorized into ten 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) [28].

6.2. Lessons given by tau staging

6.2.1. Relationship with Braak staging

Together, there is a perfect agreement on the pathway of progression of the degenerating process described by Braak, ranked from stage I to VI at the histochemical level, and our staging at the biochemical level. Surprisingly, our biochemical approach was more precise than the neuropathological one, in that we observed precisely that the temporal pole was affected just after the hippocampus and prior to the inferior temporal cortex. This step is included in our staging. Also we were able to distinguish a transentorhinal stage prior to the entorhinal stage and then to the hippocampal stage, showing that the scalpel can also makes the approach very precise.

6.2.2. Relationship with aging

At the present time, we have probably studied more than 500 patients comprising non-demented patients and demented patients with different neurodegenerative brain diseases, at the exception of prion diseases. First we observed that all patients aged over 75 years, controls or affected by a brain pathology, had at least a tau pathology in the entorhinal formation, and very frequently in the hippocampal formation. Since 100% of patients have a tau pathology at the age of 75 years, this means that tau pathology is an inevitable degenerating process that occurs in the human brain (Fig. 1). This vulnerability to tau pathology in the entorhinal and hippocampal formations is also present in a few non-human species such as the baboon or the rhesus monkey [1,2]. Immunohistochemistry is more sensitive than biochemistry to analyze the formation of tau pathology in some specific subsets of neuronal populations. Braak demonstrated that neurofibrillary degeneration can begin very early in the human brain. Using the same histological approach and antibodies against specific tau phosphorylated sites such as ser 199P, we

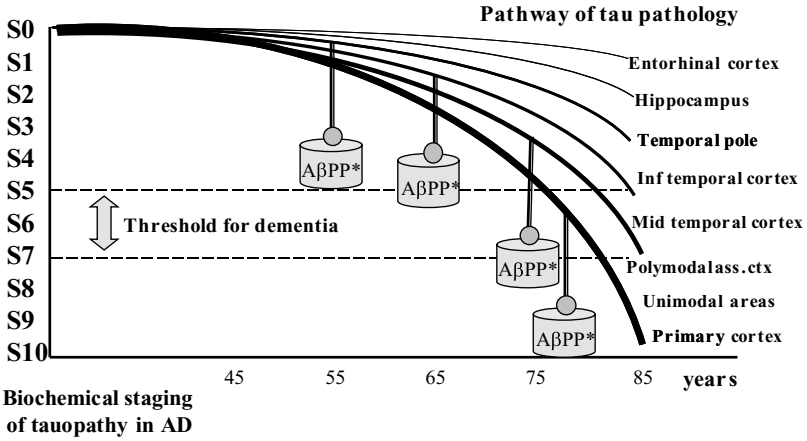

Fig. 1.

observed similar findings. Together, these studies show that tauopathy is observed in 2 patients out of 10 at the second to third decade of age. At the age of 50 years, probably 1 patient out of 2 has a small but significant entorhinal tau pathology. The frequency increases with age to be constant at the age of 75 years using either histochemical of biochemical means (Fig. 1).

From these results one could speculate that entorhinal tau pathology is an age-associated process, but in fact it is more a vulnerability that is revealed during aging. Indeed, we have been able to study the brain of non-demented centenarians. A few of these patients had a very mild entorhinal tauopathy, demonstrated the absence of a direct link with aging.

6.2.3. Brain lesion burden in mild cognitive impairment (*MCI*)

There is no clinical method to determine if a patient with MCI has incipient AD. Some will progress to AD with dementia, or have a benign form of MCI without progression. Our prospective study led us to collect all data on the cognitive status as well as the extent of tau and Aβ pathology of patients with MCI [28,29]. We observed that all 13 MCI patients from our brain bank had a tau pathology, but not necessarily Aβ pathology. Furthermore, all patients of our prospective study with a mild tau pathology did not have MCI, probably because tau burden for these patients was compensated by neuronal plasticity. These results are in perfect agreement with those of the Mesulam group [30] showing that tau pathology is more closely related to cognitive impairment than is Aβ. However, from our knowledge of Aβ aggregation in AD (following chapters), we know that the presence of Aβ deposits in the brain of MCI patients, as well as a decrease of Aβ x-42 in the CSF [31], is the marker of incipient AD.

6.2.4. The threshold for dementia

Another interesting point of our staging of tau pathology is the clinical status of patients at stage 7, with a mild to moderate tau pathology in polymodal association areas. All of them are cognitively impaired, but at very different levels. Altogether, we observed that the patients that are fully demented at stage 7 have generally in addition a significant vascular pathology. The logical explanation is that vascular pathology has an additional deleterious effect on neuronal plasticity, that decreases the compensation effect of the not-yet affected neuronal populations, and therefore increases the cognitive deficit. These observations strengthen the idea that clinical impairment results from an imbalance between a progressing degenerating process and decreasing compensatory effects from not-yet affected neurons (Fig. 1). The best illustration comes from Parkinson disease, with extra-pyramidal signs expressed only if more than 50 to 90% of dopaminergic neurons are affected.

6.2.5. The mechanism of progression of tau pathology

The mechanism of tauopathy spreading 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 progressive supranuclear palsy (PSP). Neurodegeneration in PSP is observed first in the brain stem, then in the striatum, to conquer after the primary motor frontal neocortical area (Broadmann area 4), then the unimodal

frontal areas and at last a spreading in all neocortical and limbic areas [20]. In other words, the basic mechanism of tau spreading in sporadic tauopathies is likely starting in a specific vulnerable neuronal population (layer II of the entorhinal formation in AD; occulomotor nuclei for PSP). Then, this local tauopathy will destabilize the connected neuronal populations that had a cross-talk with the vulnerable area, and this degenerating process will extend, with a domino effect, to other neuronal populations along a neuron-to-neuron propagation phenomenon [32]. Knowing better this mechanism of propagation will certainly open therapeutic strategies for AD as well as for other sporadic tauopathies and synucleopathies.

6.3. The relationship between tauopathy and amyloidosis in aging and sporadic AD

It is not surprising that tau pathology is well correlated with 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. AβPP dysfunction is the best candidate, as revealed by genetic studies. Therefore, we quantified all AβPP metabolic products to see a possible relationship with the different stages of tau pathology. AβPP holoproteins, AβPP-CTFs and Aβ species were analyzed in the different brain areas of all our non-demented and demented patients. First, Aβ species were studied. Insoluble Aβ-42 and -40 species were fully solubilized and quantified after their extraction in formic acid. In order to simplify the interpretation of the results, we propose the following biochemical staging for the quantification of either Aβ 40 or Aβ x-42 aggregates [29]:

Aβ quantification (μg/g of tissue)	Stage
From trace to 2.5	1
2.5 to 5	2
5 to 10	3
10 to 25	4
25 to 50	5
50 to 100	6
100 to 200	7
200 to 400	8
400 to 800	9
Over 800	10

The quantities of both Aβ species were compared to the extent of tau pathology, as well as to cognitive impairment. Aβ x-42 aggregates were observed at the early stages of tau pathology in non-demented patients and all along AD pathology (Aβ stages 1 to 4), while Aβ x-40 aggregates are markers of the last stages of AD. During the progression of the disease, Aβ x-42 aggregates increase in quantity and heterogeneity (Aβ stages 4 to 10), in close parallelism to the extension of tau pathology. But unexpectedly, there was no spatial overlap between Aβ aggregation that is widespread and heterogeneously distributed in cortical areas and tau pathology that is progressing sequentially, stereotypically, and hierarchically. Hence, there is a synergetic effect of AβPP dysfunction on the neuron-to-neuron propagation of tau pathology. Indeed, tau pathology can be found in the hippocampal area without Aβ deposits, as mentioned by Braak [33]. In contrast, the extension of tau pathology in polymodal association areas was systematically found in the presence of Aβ deposits (Aβ stages 4 to 10), as if these Aβ species, directly or indirectly, were necessary to stimulate the progression of tau pathology (Fig. 1). Altogether, our study clearly demonstrated that amyloid deposits do not precede tau pathology in sporadic AD, as mentioned in the amyloid cascade hypothesis. Interestingly enough, our proteomic analysis of the first Aβ 42 deposits that appear in the aging human brain and in incipient AD are not full length Aβ 1–42, but N-truncated species. In other words, the first Aβ species that initiate amyloidosis are not physiological species, but pathological species. This was observed at the biochemical and immunohistochemical levels. This discovery could improve dramatically the vaccination approach [34].

7. Relationship between tau pathology and amyloid β protein precursor dysmetabolism

The parallelism and synergy between tau and Aβ aggregation led us to search an AβPP molecular event linking the two degenerating processes. AβPP is an 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 AβPP and secreted sAβPP is often mentioned [35]. Therefore a loss of function of AβPP rather than a gain of toxic function of Aβ could be also a reasonable hypothesis to explain the stimulation of tau pathology and neurodegeneration (Fig. 1).

Complementary to this study of Aβ species, we found no obvious modification of AβPP holoprotein and its secreted sAβPP in correlation with the pathol-

ogy. However, AβPP -CTFs were found to be significantly diminished during the course of AD and well correlated with the progression of tau pathology [36]. Beta, alpha and gamma stubs were also significantly decreased in the brain tissue of individuals having an inherited form of AD linked to mutations of presenilin 1, showing a general defect common to familial and sporadic forms of AD. An important role of gamma stub, also named AICD (AβPP intracellular domain), as a gene regulator could explain its involvement in the disease if these fragments are lacking [37].

In fact these observations directly lead to other therapeutic strategies concentrated around the concept of a loss of function of AβPP stimulating tau pathology, in good agreement with other teams mentioning that Aβ may be a planet, but AβPP is central [38–40]. From our study on tau and Aβ in the human brain, we propose the following criteria for a good anti-Alzheimer drug: the drug should 1) reduce the production of Aβ x-42 species and in parallel 2) should stimulate the production of the "good" AβPP-CTFs, namely the alpha and gamma stubs. Theoretically, this drug should be able to reduce or to stop the deleterious effect of AβPP dysfunction, and therefore to stop the burden that fuels tau pathology and provoke dementia in AD.

8. Conclusion

Altogether, many converging studies show that AD is not a pure pathology of Aβ, nor it is a pure tauopathy. We propose the following definition: AD is a tauopathy fueled by AβPP dysfunction. The natural and molecular history of sporadic AD shows that both AβPP and tau are equally involved in the etiopathogenesis (Fig. 1). Both are also therapeutic targets and the good news is that βAPists and tauoists must work together. From observations of the human brain, relevant animal models are most likely those that demonstrate a synergy between AβPP and tau lesions. Some interesting models have already been described [41,42]. Another one with a severe neuronal loss is also interesting to understand the loss of function of AβPP as well as the role of intracellular Aβ deposition [43].

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 (fronto temporal dementia with Parkinsonism linked to chromosome 17) [44], the haplotype H1H1 which is a risk factor for PSP and CBD [45], the abnormal tau splicing in DM1 [24], tau-less DLDH [26], and the vulnerability of specific brain areas to tauopathy as observed in the entorhinal cortex and hippocampus for AD [46], or in the brain stem nuclei for PSP and CBD.

9. Pathway of tau pathology in aging and in Alzheimer's disease

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 et al and in our study. This vulnerability varies dramatically among individuals. A few nonagenarians of our study were very mildly affected. Therefore, the entorhinal formation is a vulnerable area 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 population, 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 Aβ deposition.

Third, the extension of tauopathy toward polymodal association areas is systematically observed in the presence of Aβ x-42 deposits (amyloid stage of 1 to 4), as if these aggregates, directly (neurotoxicity) or indirectly (markers of AβPP dysfunction) were fueling tau spreading. This step represents the beginning of incipient AD.

Fourth, when neuroplasticity will be no more able to compensate the progressing neurodegenerative process, clinical impairment and dementia will appear. The cognitive impairment observed in Alzheimer's disease 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, in parallel to tau staging, with an amyloid staging between 5 and 10.

Fifth, tau pathology will continue its conquest of the brain toward primary regions and subcortical areas.

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