

Protein Aggregation in Alzheimer's Disease and Other Neoropathological Disorders

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Abstract: A conspicuous feature shared by Alzheimer's disease as well as a variety of highly prevalent, clinically unrelated neurodegenerative disorders is the occurrence of protein aggregates both intra- and extracellularly. Most of these conditions are characterized at autopsy by the presence of such deposits, typically of fibrillar structure and accompanying extensive neuronal cell loss, displaying a selective brain distribution. The recently discovered similarities of a number of these aggregates with a novel type of experimentally induced protein deposit, formed as a general response to discrepancies in protein turnover and designated the "aggresome", has prompted speculations about the involvement of the ubiquitin-proteasome system in a process fundamental to neurodegeneration. Consistent with this view, protein aggregates have been regarded in a pathogenic connotation, with most aspects of neurologic pathogenesis being largely attributed to their presence in nerve tissues. However, the neurotoxicity of protein aggregates remains ambiguous as direct evidence substantiating it have long remained elusive. A convergence of evidence now support the notion that the actual culprits might comprise the oligomeric, non-fibrillar intermediates that arise early during the aggregation process, termed protofibrils and that the fibrillar end-stage aggregates themselves might actually serve a neuroprotective function. These intermediates ostensibly resolve many puzzling aspects of neurodegeneration and there is evidence that neurotoxicity is one key operational property they may possess. The above attest to the fact that protein aggregation remains a complex issue with a role far more enigmatic than originally thought but nonetheless important for the understanding of the pathological basis of neurodegenerative disorders.

Key Words: AD, SP, NFT, aggregate, Ca⁺² homeostasis, ROS, UPS, aggresome.

INTRODUCTION

In recent years biomedical research has witnessed a dramatic increase in studies on protein aggregation, reflecting a continuously expanding interest in the mechanisms that regulate this process as well as its implications in the molecular pathology and development of disease. Standing in the epicenter of this investigative surge are some of the most prominent debilitating neurodegenerative disorders including Alzheimer's disease (AD), whose meticulously investigated pathologic features are now starting to be promisingly unravelled. Despite the distinctive clinical symptomatology of these conditions their molecular pathologies exhibit a constitutive characteristic found commonly and unexceptionally in most of them: the presence of protein deposits. This feature has attracted attention and allowed inferences for a common link at their mechanistic level.

The case of Alzheimer's disease (AD) is no deviant from this general phenomenon, in fact it could be stated that it has pioneered to its generalisation. Initially described in 1907 by A. Alzheimer as a neuropsychiatric condition affecting the elderly, Alzheimer's disease (AD) is today's most prevalent neurodegenerative disorder, with an estimated 15mil. clinical cases worldwide, 4mil. of which occur in the U.S. alone

[1,2]. It is the primary cause of dementia accounting for 50-60% of reported cases and affects mainly individuals 60-65 years of age (or higher), from both sexes. The main symptoms of AD are changes in personality, behavior and memory loss (dementia), that begins mildly but can become quite severe at the latest stages, imparting speech deprivation and inability to perform even the simplest task (such as recognizing an every-day used object) to its victim. This progressive loss of cognitive function followed by total incapacitation eventually leads to death. Dementia ("senility") is generally defined as an acquired persistent impairment of intellectual functions, severe enough to interfere with daily functioning. It is generally accepted that a certain degree of cognitive decline and the difficulty to absorb complex information with increasing age is normal. Nonetheless, AD is not considered as part of normal aging.

Afflicted brains of AD patients exhibit selective neuronal loss targeted at the cerebral cortex and also in the amygdala and hippocampus of the limbic system [3,4], the presumed anatomical substrates for emotions and memory formation, respectively. Studies using positron emission tomography (PET) have demonstrated a decrease in hippocampal volume that is detectable even a decade before the onset of clinical symptoms [5], suggesting neuronal loss or atrophy as possible causes. The AD-afflicted brain reportedly exhibits degeneration of cholinergic neurons of the basal forebrain [6], a neuronal system that provides the primary cholinergic innervations to both limbic and cortical brain structures. A marked decline in cholinergic markers, such as choline acetyltransferase and acetyl cholinesterase has also been

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found in the cerebral cortex of the AD brain [7] and there is even evidence for apoptosis involving mechanisms employing both intrinsic and extrinsic apoptotic pathways in AD neurodegeneration [8]. On the other hand, as far as atrophy is concerned, postmortem studies have shown that AD patients do exhibit cortical atrophy with a loss of 8-10% of the brain weight every 10 years of disease progression [9], making it possible that both processes might be at work during AD.

At the histological level, AD pathology is intimately related to the presence of proteinaceous deposits and a high oxidative stress component, both of which are not mutually exclusive and there is evidence that these may even be interrelated in a positive-feedback mechanism. The protein deposits observed in AD patients are the extracellularly occurring senile plaques (SPs), made up primarily of the 4kDa A_β (beta-amyloid) peptide and the intracellular neurofibrillary tangles (NFTs), whose major constituent is a hyperphosphorylated form of the microtubule-associated protein (tau). These deposits constitute the histopathological hallmarks and most widely accepted markers of AD diagnosis currently available.

The pathogenesis of AD is uncertain. The two contending theories about it maintain that accumulation of A_β peptides in extracellular aggregates (amyloid cascade hypothesis) [10] as opposed to accumulation of hyperphosphorylated tau in intraneuronal neurofibrillary tangles (NFTs) are, respectively, the initiating events of the disease. An emerging notion, however, is that protein aggregation itself as a result of impairment or overwhelming of the protein degradative machinery (the ubiquitin-proteasome system and auxiliary pathways) is the fundamental event to pathogenesis and the underlying cause of dementia, with its end-stage aggregates representing merely pathologically inert products. But exactly which aspect or stage of it is crucial remains highly debated [11,12].

PROTEIN AGGREGATES IN ALZHEIMER'S DISEASE

Senile Plaques (SPs) & A_β

Senile plaques (SPs) are amyloidogenic deposits of fibrillar structure occupying the interneuronal spaces in brain tissue. They comprise fibrils made up of A_β 40 and A_β 42, two peptides derived from the proteolytic processing of the amyloid precursor protein (APP), of 40 and 42 amino acids in length, respectively. APP is a ubiquitously expressed, highly evolutionarily conserved membrane glycoprotein, containing a single transmembrane domain, part of which comprises the A_β sequence. Its function is unknown, but it does not appear to be required for viability since deletion of the APP encoding gene in mice does not significantly influence life expectancy, although it does correlate with alterations in locomotive behavior and gliosis in adult subjects [13]. By proteolytic processing of APP from the α- and γ-secretase enzymes, A_β is released into the extracellular space where it progressively becomes deposited in the form of straight, unbranched fibrils of 7-10nm in diameter, highly enriched in β-sheet structure [14]. A_β is by far the major constituent of SPs accounting for ~90% of their mass, of which A_β 40 is the most abundant species (>60-70%), while

the rest is occupied by A_β 42 (~15%) and minor amounts of other peptide species such as A_β 1-28, A_β 3-34, A_β 1-39 [1,15]. Of the two major A_β variants generated, A_β 42 appears to have a much higher fibrillogenic capability [16]. Increased generation of this variant is associated with most of the APP-linked mutations that lead to the familial form of Alzheimer's disease (FAD) [17]. FAD is characterised by early onset of pathology (<50 years) and accounts for nearly 5% of all AD cases [18]. Almost all of the reported FAD mutations have as a consequence the upregulation of the A_β 42 variant [10], including mutations in the two presenilin genes (PS1 and PS2), that are believed to conduct the γ-secretase activity of APP processing. The increased fibrillogenic capability of A_β 42 might be the operational property underlying its pathogenicity since another variant of A_β 40, termed A_β 40_{ARCTIC} has been identified in a novel form of APP-linked FAD in a Swedish family that causes AD [19]. This variant generated by a E693G substitution in the APP gene, that falls within the A_β sequence at position 22, (E22G) [19] appears to share a high fibrillogenic propensity, comparable with that of A_β 42, a feature apparently sufficient to make it pathogenic albeit not by virtue of SP formation but rather protofibril formation enhancement [19,20], the presumed precursor aggregates of the end-stage SP deposits. These findings argue for a neurotoxic property of A_β 42, that depends on generation of these oligomeric non-fibrillar species. Perhaps, the most compelling evidence in favor of this view, though, comes from the case of Down syndrome (DS) patients; individuals with trisomy for chromosome 21. Abnormally high accumulation of oligomeric, non-fibrillar A_β 42 deposits is consistently detected in these patients, a phenomenon attributed to a possible gene dosage effect of the APP gene (chromosomal location: 21q). While adults suffering from DS almost invariably develop AD-like dementia when they survive beyond their thirties and forties, DS children exhibit A_β 42 deposition early in their life, before any plaque and tangle formation and AD-like dementia are detected [21].

Interestingly, overexpression of human APP carrying FAD-linked mutations in transgenic mice, also produces amyloid plaques and elevated amounts of both A_β 40 and A_β 42 [22], which are, similarly to DS, not accompanied by extensive neurofibrillary pathology or pronounced neuronal loss [23]. Despite the absence of neurofibrillary tangles (NFTs), these animals display some learning and memory deficits [24] and neuronal loss is observed only in some of them confounded in the immediate vicinity of a subset of the A_β deposits, suggesting a focal pattern of neurotoxicity for these aggregates, if any [25]. Obviously, though, this does not necessarily implicate SP formation in the neurodegenerative process. Indeed, transgenic studies of FAD-associated APP mutations have been able to produce pathologies that only remotely resemble that of human AD and in most cases wherever some cognitive impairment has been observed, the correlation was stronger between levels of A_β 42 and soluble A_β fractions rather than actual SP count [26]. In these transgenic models, A_β deposition within plaques proceeds in elevated levels in the cortex with increasing age albeit with limited neuronal degeneration. In contrast, soluble A_β levels increase dramatically [27] but amyloid deposition maintains a selective distribution pattern

suggesting that elevated A β levels are not sufficient to cause plaque formation and allowing inferences for a threshold effect-driven mechanism and the existence of region-specific factors that either promote or inhibit A β deposition [28].

When formed, the anatomical distribution of SPs overlaps with areas where neurodegeneration is most extreme. Their correlation, however, with disease development is not very clear since plaques can also be found in elderly, non-demented individuals and plaque count numbers, even when present, do not seem to follow disease severity as closely as other pathologies, like synaptic loss or NFT numbers, do [29,30]. Unlike the stereotypical development and hierarchical distribution of NFTs, amyloid plaque distribution is variable in Alzheimer's disease brain and plaques are not necessarily detectable in high quantities in cerebral regions where cell death and loss occurs [31]. Rather total amyloid burden and total A β_{42} concentrations correlate better with clinical progression than do analyses of plaques alone [32, 36]. This has spurred the concept that the ratio of A β_{40} /A β_{42} might be a determining factor for cognitive decline and brain nosology in AD. The "seeding" hypothesis epitomizes the view of A β_{42} as the critical pathologic agent, wherein A β_{42} is considered as the "seed" factor that nucleates the plaque formation process and A β_{40} is subsequently incorporated forming progressively larger deposits that culminate in the SPs observed at autopsy [33]. The higher the rate at which this process takes place, the higher the deviation from the symptomatic phenotype and accompanying dementia. It is noted, that in this process the pathogenic mediator is considered to be the soluble A β fraction and not the SP itself. In line with this view, soluble A β levels measured in postmortem brains can distinguish between people who have plaque pathology but no dementia and those who exhibit clear clinical symptoms of dementia in addition to plaque formation [34]. In short, soluble A β levels evidently correlate better with disease severity than do senile plaque levels alone [35,36]. Not surprisingly, this observation has added credence to the proposition that aggregation of amyloid into SPs might be less damaging than originally thought and there are numerous studies effectively supporting this view by demonstrating the deleterious effects of soluble A β oligomers, specifically against long term potentiation of neurons [37,38], a phenomenon that is considered as the forerunner of neurodegeneration and dementia.

The view that plaque free A β can be the neurotoxic intermediate is supported by various other lines of evidence and it provides answers to many puzzling aspects of AD pathology that cannot be accounted for by an end-stage SP-mediated pathogenic mechanism. First and foremost, the observation that fibrous protein aggregates occur after the earliest observation of a behavioral and/or neuropathological phenotype, has cast doubts on a possible pathogenic role for SPs [39,40]. Additionally, studies in APP transgenic mice showing that synaptic loss [41] and spatial learning deficits correlate with soluble A β in the absence of SPs [42] have reinforced the concept of soluble A β -based pathogenicity. It has even been demonstrated that passive immunization with an antibody against A β peptides is capable of reducing levels of A β peptides and reversing memory deficits in APP

transgenic mice even though the number of A β plaques is not reduced [43].

The mechanism of soluble A β pathogenesis is believed to be intimately related to their oligomerization reaction into ordered fibrils (fibrillization). This ordered oligomerization of soluble A β peptides into pre-fibrillar kinetic intermediates, designated "protofibrils" has been reported to cause neurological dysfunction in association with or independently of neuronal degeneration, in more than one instances [44-46]. The selectivity of neurodegeneration might also exhibit age-dependence as demonstrated by experiments involving the injection of fibrillar A β , proving non-toxic to the young adult rhesus brain while causing profound neuronal loss, tau phosphorylation and microglial proliferation in the aged one [47]. Such findings suggest that age-dependent changes in the composition of the local cerebral matrix may contribute to AD [1]. All of these studies including those that dissociate neuronal loss from pathology, can be reconciled by postulating that synaptic loss [41], which may be a precursor to neuronal loss, is sufficient to produce AD symptomatic phenotypes. The formation of protofibrils may induce synaptic loss followed by neuronal loss on one hand and fibril formation on the other, accounting for both pathology and SP presence.

Finally, an interesting issue pertaining to A β and its involvement with neuropathology is the presence of this peptide in the intraneuronal space. A β has been found to accumulate in lysosomal or late endosomal compartments of AD-afflicted neurons presumably via proteolytic processing of immature APP or internalization of intact, cell membrane bound APP that subsequently receives α - and β -secretase cleavages by presenelins, which also localize in the ER [48]. Interestingly enough, the intracellular accumulation of A β has been shown to antedate SP formation in transgenic models of AD, much like the onset of clinical symptoms [49]. In the operational context of AD pathogenesis, this initial accumulation of A β is believed to induce a nucleation-dependent aggregation mainly driven by the highly fibrillogenic A β_{42} species, which is then maintained and exacerbated by the subsequent addition of aberrantly folded APP and amyloidogenic fragments of APP, that would otherwise be degraded, into the amyloid core in a fashion reminiscent of the prion replication process [50]. Many of the key pathological events of AD are believed to be directly related to the intracellular accumulation of A β , such as the production of reactive oxygen species (ROS) and lipid peroxidation products and ultimately the leakage of the lysosomal membrane with concomitant release of heparan sulfate and lysosomal hydrolases into the cytosol [51]. But perhaps the most important implication of intracellularly accumulating A β is its involvement in the induction of apoptotic pathways. This induction is believed to be mediated by proteolytic products of APP, mainly A β_{42} [52,53], which promote caspase activation through ER- or mitochondrial-stress involving pathways [54]. The effected stresses, activate these apoptosis specific proteases in a yet incompletely understood mechanism, which, in turn, culminate in the observed neuronal death and probably some other pathologically related upstream events as well, such as the cleavage of tau, which is regarded as a prerequisite for tau's incorporation into NFTs [54]. Although the above

outlined pathogenic mechanism can explain some of the more enigmatic features of Alzheimer's pathogenesis, like the focal nature of senile plaques and the precedent of SP and NFT appearance symptomatology, it does not account for cognitive impairment in the presence of limited neuronal loss as documented in postmortem and transgenic model studies [25,55] and despite being able to provide some clues it is far from being regarded as the full picture of AD etiology.

Neurofibrillary Tangles (NFTs) & Tau

The existence of fibrillar precursors is somewhat more established in the case of tau and its cognate aggregates, the neurofibrillary tangles (NFTs); the second pathological hallmark of AD and most closely correlated with the progression and extent of severity of the disorder [56]. The precursors of NFTs, designated paired helical filaments (PHFs), are pairs of thin filamentous structures of 10nm in diameter, twisted around one another in a helix with a periodicity of 80 nm and a width varying from 8 to 20 nm [57]. NFTs are formed by the association of arrays of PHFs, which populate the intraneuronal space and gradually replace the normal cytoskeleton, becoming deposited within neurones of the hippocampus, the entorhinal cortex and amygdalae of demented patients [58]. They are predominantly composed of abnormally hyperphosphorylated and ubiquitylated tau protein [59,60], an abundant, low-molecular-weight, microtubule associated protein (MAP), encoded by a single copy gene on chromosome 17. The assembly of tau into NFTs also requires a proteolytic cleavage event, that is most likely attributed to caspase activity [61], with caspase 6 featuring as a possible candidate [62], a finding that has revived attention on the possible roles of apoptosis in AD pathogenesis.

Tau is predominantly localized in the axons of central (CNS) and peripheral nervous system (PNS) neurons but it is also expressed at very low levels in CNS astrocytes and oligodendrocytes [63]. It is a normal component of the neuronal cytoskeleton with multiple and important functions in the assembly of microtubules (MTs), their stabilization against dynamic instability and in bridging them with other cytoskeletal filaments [64,65]. Its function is regulated by the level of its phosphorylation, which can be performed on at least 79 of the potential phosphate-acceptor Ser/Thr residues identified on the longest tau isoform [66]. In normal brain, the equilibrium between phosphorylation and dephosphorylation of tau modulates the stability of the cytoskeleton and consequently axonal morphology. In the highly phosphorylated, developmentally associated state, tau (and other MAPs) is presumed to contribute to a less stable MT cytoskeleton required for rapid axonal growth and plasticity, whereas in its low phosphorylated state, found routinely in the adult brain, tau contributes to the MT stabilization required for axonal maturation and maintenance [67].

The phosphorylation status of tau in AD brains is modulated by the action of different protein kinase and phosphatase systems that appear to lead to structural and conformational changes in this protein, thus affecting its binding with tubulin and the capacity to promote

microtubule assembly [68]. The phosphorylative regulation of tau has been predominantly attributed to at least two distinct kinases: glycogen synthase kinase-3 (GSK3), the principal tau kinase in the brain, and Cdk5, the kinase that is involved in laminin activation and normal neurogenesis [69,70]. Especially GSK3 exhibits selective and elevated tau phosphorylation activity even after heat shock, which is sufficient to suppress the rest of the putative tau kinases (ERK1/2, JNK and cdk5), suggesting a central role for this kinase in NFT formation [71]. Phosphatase activity targeted to tau has been verified for the PP2A, PP2B (calcineurin), PP2C phosphatases and to a lesser extent for PP1 (protein phosphatase 1) [72].

The initial observations that FAD-associated mutations in APP and PS genes, and that A β neurotoxic aggregates are invariably accompanied by tau hyperphosphorylation and NFT formation, had led to the belief that A β deposition is a primary event in AD development and one that is required for manifestation of NFT pathology [73]. However, the observation that aggregation without amyloid pathology is sufficient to cause neurodegeneration both in mice and in humans and the identification of a novel group of neurodegenerative diseases, characterised by distinct tau pathology but no apparent amyloid deposition has now come to contest this view [32]. These disorders, collectively called tauopathies, include conditions such as frontotemporal dementia (FTD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease (PiD) and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), all sharing the common feature of NFT presence in specific brain regions as a common pathological hallmark, in which tau is almost invariably found in an aggregated state [74]. Additionally, they all seem to be associated with mutations in the tau gene (missense or intronic), which appear to markedly enhance aggregation [75]. The role of phosphorylation of tau in the aggregation procedure, however, remains somewhat less well understood. It is generally accepted that extensive phosphorylation of tau decreases its ability to bind to MTs [76] but it is not certain whether this modification is a direct etiologic event of AD pathogenesis. The beneficial effects on AD progression by specific inhibition of GSK3 imposed by lithium salts [77] and, inversely, the contribution of PP1 and PP2A phosphatase inhibition to MT destruction and axonal degeneration argue in favor of a critical intervention of phosphorylation towards NFT formation [78]. However, whether this has direct consequences or is an epiphenomenon of AD pathology remains to be determined.

PROTEIN AGGREGATION AND NEURO-PATHOLOGY

Postulated Effects of Protein Aggregates on Neuron Physiology

The effects of the protein aggregates observed in the AD-afflicted brain with respect to their pathogenicity have been the subject of intensive and detailed studies since the time these pathological lesions were originally identified. A lot of mechanisms have been proposed and experimentally verified for the observed neuronal loss in the affected brain regions, most of which involve either the generation of reactive

oxygen species (ROS) and induction of oxidative stress or the perturbation of Ca^{+2} homeostasis.

Numerous studies have implicated A β in the induction of oxidative stress as an underlying cause of pathogenesis in AD. The contributory role of both soluble and aggregated A β in the generation of oxidative radicals is well documented and has been demonstrated both *in vivo* and *in vitro* by experiments that leave little support against it [79,80]. The mechanisms of A β -dependent ROS formation can be mainly categorised in direct and indirect. In the former, which include the participation of transition metal ions (Fe^{+2} , Cu^{+2}), ROS are generated from peroxides through Fenton chemistry reactions by these ions bound to A β moieties [81]. Indirect pathways involve activation of the receptor for advanced glycation end-products (RAGE) [82], prolonged excitatory state of neurons by perturbation of the glutamate release/clearance cycle [83] and induction of inflammatory mediators (e.g. IL-1, TNF- α) from astrocytic and reactive microglial populations [84]. Aggregated A β has been found to bind and activate RAGE or type 2-scavenger receptors, leading to the downstream activation of NADH oxidases [85] that catalyse formation of superoxide ($\text{O}_2^{\bullet-}$) and subsequent generation of hydrogen peroxide (H_2O_2) and related species [86]. Similarly, increased excitability of neurons by glutamate induces oxidative processes either by binding to the NMDA receptors [87] or by competing for the cysteine-antiporter system and thereby depleting intracellular glutathione (GSSH) pools from neurons and glia [88]. Additionally, elevation of intracellular Ca^{+2} levels via ionotropic glutamate receptor stimulation leads to calmodulin-dependent activation of nitric oxide synthase (NOS) and the generation of reactive nitrogen species (RNS), such as the highly neurotoxic peroxynitrite (ONOO-) [89]. The activation of NOS is also known to be mediated by the inflammatory factors released from activated astrocytes and microglia, which, besides causing upregulation of NOS expression, can increase oxidative load by induction of cyclooxygenase 2 (COX-2) [90], an enzyme with a proven contributory role to oxidative cell damage in neurodegeneration [91].

Oxidative stress is also presumed to mediate subsequent perturbation of Ca^{+2} homeostasis via additional pathways besides ionotropic glutamate receptor stimulation, through the deregulation of some equally critical ion transport systems, including ion channels (Ca^{+2} , K^+ channels), ion pumps (Ca^{+2} pump, Na^+/K^+ ATPase), ion exchangers and cotransporters ($\text{Na}^+/\text{Ca}^{+2}$ exchanger, K^+/Cl^- cotransporter), which are vital for neurotransmission and synaptic function [92]. Altered Ca^{+2} homeostasis is believed to be of fundamental importance in the development of AD pathology since it represents a vital element of synaptic transmission. The exact sequence of events is supposedly instigated by raised levels of oxygen radicals, which in turn cause alteration in Ca^{+2} homeostasis and tau phosphorylation, a step also believed to be A β induced [93]. Studies performed on cultured cortical neurons and SH-SY-5Y neuroblastoma cell lines, however, provide evidence for a different sequence of events for A β -mediated neurotoxicity, in which Ca^{+2} influx represents the initial step that precedes presentation of an oxidatively stressed environment and tau phosphorylation [94]. Whatever the

initiating event, both oxidative stress and alteration of Ca^{+2} homeostasis are known to culminate in neurological lesions in the AD brain that either manifest as neuronal loss due to apoptosis or necrosis, or synaptic loss [81]. Apoptosis through oxidative stress has been extensively studied and it is known to involve one of many distinct pathways that include mitochondrial dysfunction and cytochrome c release, caspase and calpain non-specific activation, lipid peroxidation, DNA damage and protein oxidation, some of which are also replicated in necrosis and in processes leading to loss of Ca^{+2} homeostasis [81,92].

It is not certain whether A β is required in an aggregated (SP) or soluble state to produce the aforementioned effects. Some reports sustain the view that aggregation of A β into fibrils is a prerequisite for unveiling of its pro-oxidant activity and that this property is concentration-dependent, exactly like aggregation of A β is [95]. Irrespective of what the verdict on this matter might be though, it is almost certain that an insidious aggregate species inherent to the fibrillization process is bound to possess at least some potent neurotoxic properties. An interesting aggregate species that has recently received much attention with respect to this aspect are the oligomeric intermediates designated "protofibrils", which are presumed to have capabilities deleterious for neuronal function and integrity. Protofibrils, were initially recognized as short, flexible assemblies of A β , approximately 5 nm in diameter and 200 nm in length [17,96] but have been detected thenceforth adopting a variety of sizes and fibrillar conformations [97]. Most strikingly, these species can cause selective neuronal cell death [44-46,98] and synaptic dysfunction, an event that is considered as primary in the pathogenesis but may actually be sufficient to induce symptoms of neurologic disorder (cognitive decline is believed to be related, at least initially, with the interference of processes that constitute major steps of cholinergic neurotransmission) [99,100]. Protofibrils can form structures, capable of perturbing membranes in a pore-like mechanism and consequently mediate loss of Ca^{+2} homeostasis and additional oxidative burden [101]. The ability to form these unusual, non-specific channels that indeed can allow flux through their structure has been verified by experiments that demonstrate an increased dye leakage from liposomes composed of phosphatidylcholine in the presence of A β fragments [102]. The concept of protofibrils as the neuropathologic mediator is substantiated by various lines of evidence but perhaps most convincingly by the observations that antibodies directed against them eliminate A β -mediated toxicity to cultured neurons [103] and that this neurotoxicity is not affected by the administration of reducing agents [104]. Additionally, protofibrils have been demonstrated to inhibit hippocampal long-term potentiation *in vivo*, a property not shared by their fibrillar, end-stage aggregate counterparts [37,105]. Finally, a link between the proposed A β dependence of NFT formation might be provided by these fibrillar intermediates as they have been shown to conduct both GSK3 activation [106] and NFT induction [107].

The formation of NFTs and their contribution to AD pathogenesis has been less extensively studied and the insights into how they might bring about deleterious effects on neurons stand poor in comparison with what is known

about A β . In fact, even the role of NFTs as neurotoxic mediators has lately come under challenge, since not only is there inadequate evidence in the affirmative but also stereological studies have suggested that NFT formation might be the event that drives the survival of those NFT-bearing neurons, which are observed in the post-mortem examinations of AD patients [108]. If NFTs are indeed deleterious, one fundamental but still unresolved issue pertaining to their neuronal effects is whether their pathogenicity arises from a loss-of-function or a gain-of-function event; in other words, if it is the dissociation of microtubules and the collapse of their cytoskeletal network *per se* or the acquirement of a novel, ostensibly toxic function of hyperphosphorylated tau that is the underlying cause of pathology. It is conceivable that an occurrence of the magnitude of cytoskeletal disintegration, especially for a neuronal cell, would undoubtedly be deleterious, leading to cell death within a short period of time and without the need for intervention of any additional cytotoxic factors. The MT network is vital for neuronal function as it is employed for conducting the bidirectional transport of various membrane organelles and macromolecules between neuronal cell bodies and synaptic terminals. Axonal transport is necessary to replenish synaptic proteins, mitochondria and synaptic vesicles from the neuronal cell bodies to the synaptic endings, therefore, its elimination would result rapidly to synapse degeneration. Retrograde transport of neurotrophic signals from synaptic terminals to neuronal cell bodies is also vital for neuron maintenance and such failure would readily commit neurons to apoptotic pathways [109].

On the other end of this matter, the toxic gain-of-function possibility could be substantiated on the grounds of tau-mediated aggregate formation and a toxic role of NFTs. The presence of intracellular aggregates, which apparently exhibit strenuous resistance to the ubiquitin-proteasome system (UPS) could pose serious threats by interfering with the delicately balanced protein turnover cycle. A novel type of intracellular aggregate, termed the "aggresome", that is increasingly being regarded as a general response to UPS distortions, is found to share some common features with NFTs, such as the localization of ubiquitin and heat shock proteins (Hsps) but more importantly the association with neurodegenerative disorders. One of the postulated effects of aggresomes is the inhibition of the UPS, essentially reflected by their imperviousness to attacks by Hsps and the 26S proteasome. This property could easily be projected to the NFTs found in dementia, as there is currently no evidence indicating that 26S proteasomes can degrade proteins in filamentous structures [110]. Even by abiding to this scenario, though, the critical link to the development of neuropathology is still missing and, surprisingly enough, might not be sufficient to explain pathogenicity at all, as recent evidence indicates that UPS inhibition might in fact facilitate increases in synaptic contact numbers and synaptic strengthening instead of weakening [111]. Even further, the formation of NFTs itself might not represent a primary event at all as studies in transgenic animal models of AD have been shown to develop tauopathy and concomitant neurodegeneration in the absence of NFTs [112] making the role of NFT formation even less understandable in the context of AD nosology.

Albeit sound and quite rational, the above propositions for a possible NFT-mediated neurotoxic mechanism still remain speculative and, hence, inadequate to categorically incriminate NFTs as the pathogenic mediator. That is mainly because NFTs have not been found to be obligatory for the development of AD, as studies in the past have shown that the disease can manifest in the absence of neurofibrillary pathology [113] and NFT presence can, indeed, be an unreliable criterion for clinical diagnosis of dementing syndromes like AD [114]. Reversely, NFTs have also been found in the brains of non-demented individuals [115], but perhaps the most guilt discharging evidence for NFTs derives from studies, which have, rather unexpectedly, suggested that neurons can actually survive for many years bearing NFTs intracellularly, a prospect that has spurred thoughts, which place NFTs in a neuroprotective and survival-promoting context, rather than their original neurotoxic one [116,117]. In view of this novel concept, it has been suggested that NFT-bearing neurons, which are routinely observed in AD autopsies might simply represent those that have survived the neurotoxic insults of the disease, instead of actual diseased neurons on their way to demise.

Protein Aggregates in other Neurodegenerative Disorders

The observation of protein aggregates has lately started to emerge as a common theme in neuropathological disorders, hence fuelling speculations about a common mechanism inherent to the process of neurodegeneration. It is characteristic that most of these debilitating conditions are typified by the presence of their cognate aggregates, such as Lewy bodies (Parkinson's disease), prion plaques (Creutzfeldt-Jakob's disease), Bunina bodies of superoxide dismutase (Amyotrophic lateral sclerosis), poly-glutamine protein aggregates (Huntington's disease), Pick bodies (Pick's disease), all of which are related, in one way or another, with the perturbation of the quality control system that monitors the deployment of functional, natively folded proteins into the intracellular space and the re-direction of misfolded, non-functional and aggregation prone proteins to the UPS for degradation. Distortion of the UPS is suspected in most if not all of these conditions and it has been experimentally verified for some of them [118,119]. The formation of aggresomes upon UPS inhibition both in experimental systems and pathological samples indicates that this phenomenon might be more than a circumstantial occurrence. The striking similarities of aggresomes with many of these types of aggregates both at the biochemical and the operational level attests to the view that the protein turnover machinery is primarily targeted in these neurologic disorders [120,121]. However, there are some notable exceptions in this theory, especially as far as AD is concerned: the UPS is not affected or by any means interacts with extracellular amyloid deposition that gives rise to the commonly observed SPs, making it difficult to envisage a way by which a targeted cellular system remains uninfluenced by the presence of one of the two major hallmarks associated with disease development.

More importantly though, the role of most of these disease associated aggregates as causative or consequential factors of pathogenesis remains controversial as an

increasing number of studies points away from a neurotoxic impact and, what is more, might support an entirely opposite role attributing neuroprotective functions to them [122,123]. These observations reinforce the possibility that the characteristic protein deposits observed in the vast array of neurodegenerative disorders are simply an end point of disease generated by some initial insult, unrelated to these end-stage aggregates. Crossing out these aggregates as the pathogenic agents, however, raises the question of which factors qualify as possible candidates for the role. This apparently beleaguering matter could be explained by the introduction of the precursor species of these end-stage aggregates into the clinical picture as major mediators of pathology. Such a theoretical scheme may hold true as it can provide answers to many bewildering and originally unanticipated aspects of these disorders, such as the manifestation of pathological symptoms well before the formation of the end-stage aggregates or even in the complete absence of them. The "ion channel hypothesis" for protofibrils is a promising model, which can contribute significantly to the delineation of the underlying pathogenic mechanisms and studies demonstrating the generalised manifestation of these aggregation intermediates over a wide spectrum of these disorders is an encouraging finding in this context [124]. Additionally, the explanation of pathology in the absence of neuronal death is one more important issue that has been demonstrated with respect to protofibril formation and this is a critical aspect, which needs to be addressed as it can not only give insights to the initiating processes of neuropathology but also provide important targets for disease prevention and therapeutic intervention. Finally, a point that deserves noting with regard to the enigmatic properties of the end-stage aggregates is that a pragmatic approach towards the disclosure of their true role in neuropathogenesis will need to take into account the inherent heterogeneity of these species, as not all might necessarily have the same impacts on cellular physiology and a distinction between neurotoxic and neuroprotective should be investigated in close relationship to their biochemical composition and cellular localization as well. It is conceivable that some types of aggregates can pose a threat to cells depending solely on the identity of their constituent proteins (the sequestration of vital, irreplaceable factors into aggregates will harm the cell to a much higher degree than the sequestration of functionally disposable factors). It is important therefore that these issues become realized in the study of protein aggregates and their associated neurodegenerative disorders, so that valuable leads can be drawn from the experimental evidence obtained both from model systems and pathological investigations.

To conclude, the issue of protein aggregation has clearly proved that it compels further and much more detailed study as its mechanism and pathogenic implications go beyond what was initially anticipated. The original understanding that the observed end-stage aggregates are the pathologic mediators, which dates back as far as their original respective discoveries, currently seems inadequate to explain the full disease mechanism of these conditions. One issue that gradually is becoming more clear though, is that at least as far as the end-stage aggregates are concerned, their role is most likely irrelevant to the central disease mechanism and it

can not be excluded that they might serve a neuroprotective function, as well. The evidence in agreement with this view are becoming progressively stronger and difficult to ignore, leaving the challenging quest for the actual culprits still on. Continuing this undoubtedly daunting task, however, should not be a source of deterrent as the current knowledge status has indicated the right directions and with an optimistic approach it can be expected, that definite answers will soon be provided to this biological phenomenon and the devastating conditions, with which it is associated.

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