

Protein aggregates and dementia: is there a common toxicity?

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This review considers some of the recent advances made in the understanding of the pathogenic proteins known to aggregate and be implicated in neurodegenerative dementing disorders. It concentrates on the two most obvious candidates for the role of toxic protein in Alzheimer's disease (AD)— β -amyloid peptide and tau—but also considers other proteins in this disorder and in less common but equally devastating diseases.

J Neurol Neurosurg Psychiatry 2002;**72**:152–161

genesis and suggest shared aetiological mechanisms and therapeutic approaches. In this review we consider some of the recent advances made in our understanding of the pathogenic proteins known to aggregate and be implicated in neurodegenerative dementing disorders, concentrating on the two most obvious candidates for the role of toxic protein in Alzheimer's disease— β -amyloid peptide and tau—but also considering other proteins in this disorder and in less common but equally devastating diseases.

β -AMYLOID PEPTIDE: AN UNDISPUTED TOXIC PROTEIN

Extracellular cerebral amyloid deposition in neuritic plaques is one of the hallmarks of Alzheimer's disease and amyloid deposition itself is one of the earliest pathological abnormalities, preceding paired helical filament tau formation and neurofibrillary tangles (NFTs).¹ Neuritic plaques are relatively insoluble dense cores of 5–10 nm thick amyloid fibrils with a paler staining "halo" surrounded by dystrophic neurites, reactive astrocytes, and activated microglia. The main protein component of amyloid in Alzheimer's disease is the 39–42 amino acid β -amyloid peptide ($A\beta$), which is a normal proteolytic breakdown product of the much larger membrane spanning amyloid precursor protein (APP; fig 1).

The fibrillogenicity of $A\beta$ is dictated by peptide length such that the less common $A\beta_{42}$ variant (~10% of total $A\beta$) is more fibrillogenic than the more commonly produced but shorter $A\beta_{40}$. $A\beta_{42}$ is the main species initially deposited in immature "diffuse" plaques in both Alzheimer's disease and Down's syndrome; it forms a "seed" for future mature neuritic plaque development,^{2,3} a process that takes at least 10–15 years and antedates symptoms by possibly even longer. Although little is known about its normal function, there is a consensus that aggregation of $A\beta$ into amyloid is required for neurotoxicity. The precise nature of $A\beta$ mediated neurotoxicity remains to be elucidated but proposed mechanisms include oxidative stress, free radical generation, altered calcium

A aberrant intracellular or extracellular deposition of self aggregating misfolded proteins is a common finding in primary neurodegenerative disorders such as Alzheimer's disease and other diseases that cause dementia. Although composed of unrelated proteins, these aggregates of initially soluble proteins have many common features. They usually have a dense fibrillar morphology with a high proportion of β -pleated sheet secondary structure, are often ubiquitinated, and are typically resistant to proteolytic degradation. Mutations in the relevant proteins cause familial forms of disease, either by producing abnormally fibrillogenic proteins/peptides and/or by altering the normal intracellular distribution of the protein and thus accelerating the kinetics of aggregation, thereby giving rise to early onset disease.

Formation of protein aggregates may be a critical, albeit lengthy, pathogenic step. If so, their presence should be directly implicated in neurotoxicity; furthermore, aggregate formation should correlate with disease onset and progression, and the regional cerebral location of aggregate deposits should correspond to both the pattern of neurodegeneration and the clinical syndrome. The alternative is that aggregates are not causative but just a consequence of a primary disease process or represent a defensive response to cell death in postmitotic neurons. The presence of mutations in the genes coding for the aggregated proteins in many instances (for example, synuclein, Huntingtin, tau) suggests that protein aggregation is pathogenic—cause rather than consequence. However, the relation between site and extent of aggregation and disease phenotype is far from proved and for some familial conditions the relation between gene affected and protein aggregated is less direct (for example, presenilin-1).

None the less, these abnormal proteinaceous deposits are characteristic disease features, providing remarkable clues about molecular patho-

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Received 26 January 2001
In revised form 30 May 2001
Accepted 27 June 2001

Abbreviations: NFTs, neurofibrillary tangles; $A\beta$, β -amyloid; APP, amyloid precursor protein; PS1, presenilin-1; PS2, presenilin-2; APP_{sw}, APP ectodomain; apoE, apolipoprotein E; apoE^{-/-}, Dab1, disabled-1 intracellular adaptor protein; PTB, phosphotyrosine binding; GSK-3, glycogen synthase kinase-3; PrP^C, normal cellular prion protein

A glossary to terms is located at the end of the review

Table 1 Pathogenic proteins and their aggregates in dementia

Protein/peptide	Disorder	Aggregate	Neuronal location
APP/Aβ	Alzheimer's disease	Amyloid	Extracellular
Tau	Alzheimer's disease, FTDP-17, Pick's disease, CBD, PSP	Paired helical and straight filaments	Cytoplasmic
α-Synuclein	Parkinson's disease, DLB	Lewy body	Cytoplasmic
Huntingtin	Multisystem atrophy	Inclusion body	Glial cells
Prion protein	Huntington's disease	Inclusion body	Cytoplasmic and intranuclear
	Prion disorders	Amyloid	Extracellular

APP, amyloid precursor protein; Aβ, β-amyloid peptide; FTDP-17, frontotemporal dementia with parkinsonism linked to chromosome 17; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; DLB, Dementia with Lewy bodies.

homeostasis, induction of apoptosis, chronic inflammation, and neuronal structural damage.⁴ Although undisputedly toxic to cells in culture when aggregated and almost certainly toxic when injected or overexpressed in animals, some questions remain. In particular, the relative roles of intracellular versus extracellular amyloid and the role of the plaque itself remain to be fully characterised. None the less, most researchers would now accept that the formation and aggregation of amyloid is the most likely starting point of Alzheimer's disease pathogenesis in vivo.

THE AMYLOID CASCADE HYPOTHESIS OF ALZHEIMER'S DISEASE

Increased production of the more fibrillogenic Aβ₄₂ has been associated with all of the autosomal dominantly inherited

forms of Alzheimer's disease caused by mutations in the genes encoding APP, presenilin-1 (PS1) and presenilin-2 (PS2).⁵ In addition, Down's syndrome due to trisomy 21 (but only when including the APP gene on proximal chromosome 21q, thus causing a "gene dosage" effect) invariably causes Alzheimer's disease. These findings, coupled with the importance of APP as the precursor of Aβ, have endorsed the "amyloid cascade hypothesis" of Alzheimer's disease, which posits a central aetiological role for Aβ, leading to neurofibrillary pathology and neuronal death (fig 2).

Limitations of the hypothesis include the finding that neuritic plaque counts do not correlate with disease severity as well as do other pathologies such as synaptic loss or numbers of NFTs. However, analysis of total amyloid burden and total Aβ concentrations correlates better with clinical progression

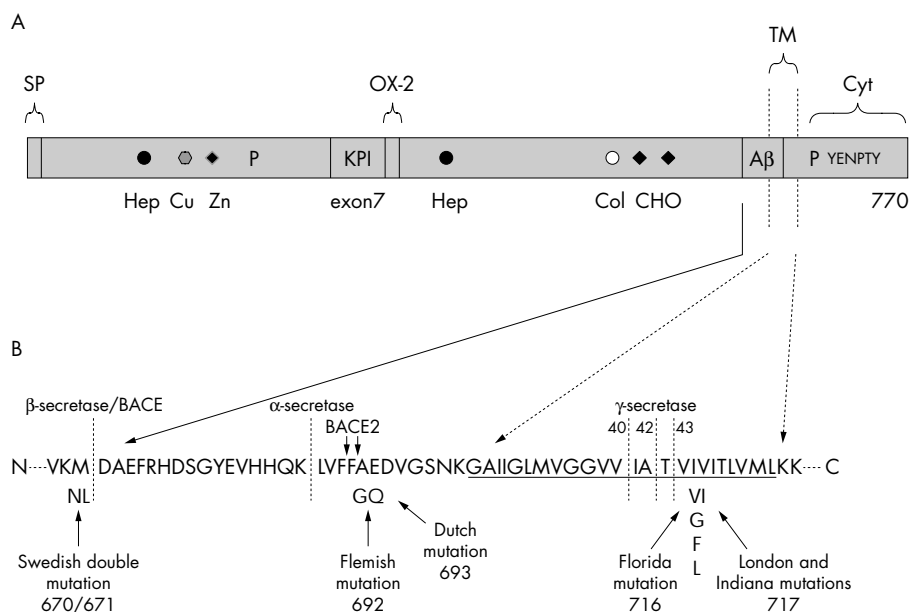


Figure 1 Structure of the amyloid precursor protein. (A) Schematic diagram of the longest APP splice isoform (APP₇₇₀) demonstrating the location of different binding and functional domains. Exons 7 and 8, encoding respectively the Kunitz protease inhibitor domain (KPI) and the MRC-OX2 antigen (OX-2) sequence, are alternatively spliced to generate different APP isoforms. The signal peptide (SP) is cleaved after cotranslational translocation to the membrane of the endoplasmic reticulum. As APP molecules are processed through the endoplasmic reticulum and Golgi apparatus they undergo post-translational modifications at specific sites, including glycosylation (CHO) and phosphorylation (P). APP has several specialised binding domains to interact with components of the extracellular matrix, including heparin (Hep) and collagen (Col), as well as to the metals copper (Cu) and zinc (Zn). The β-amyloid peptide (Aβ) sequence partially resides in the transmembrane (TM) region. The YENPTY re-internalisation motif in the cytoplasmic domain (Cyt) also contains the NPXY motif for binding PTB domains. Re-internalisation of plasma membrane bound APP directs it towards an amyloidogenic pathway. (B) Expansion of the amino acid sequence of Aβ (shown in bold) and the transmembrane region of APP (underlined). Secretase cleavage sites are indicated by dashed lines and the location of pathogenic mutations in APP are shown. Note that the sites of the missense mutations are either flanking or within the Aβ sequence and also near to sites of secretase cleavage. Secreted forms of APP (APP_s) are released into the extracellular space after cleavage of cell membrane bound APP within the Aβ sequence at amino acids 16/17 by α-secretase. The remaining membrane bound C terminal 83 amino acid fragment (C83) is further cleaved by γ-secretase to generate a peptide fragment known as P3. This constitutive secretory pathway precludes formation of intact Aβ. Alternative cleavage of APP by β-secretase generates a slightly truncated secreted APP (APP_s) and a membrane bound 99 residue C terminal fragment (C99). Cleavage of C99 by γ-secretase allows the release of intact Aβ.

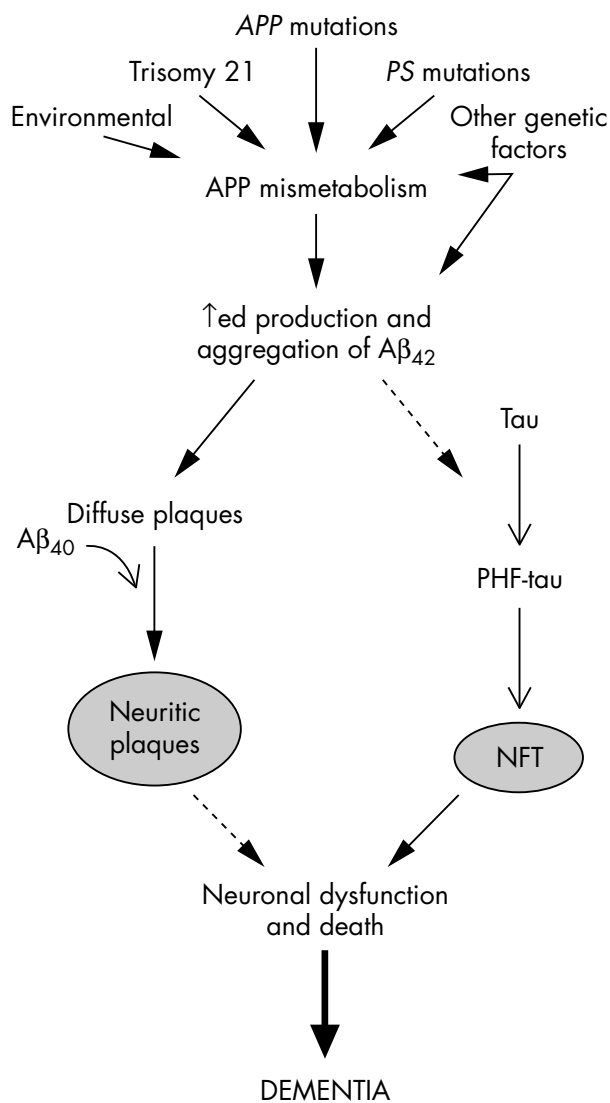


Figure 2 The amyloid cascade hypothesis. A model of the sequence of pathogenic events in Alzheimer's disease. The broken lines indicate possible, but not definitively proved, pathways. The pathway on the left (closed arrowheads) charts the evolution of the amyloid pathology and the pathway on the right (open arrowheads) is for the tau/cytoskeletal pathology. PHF, paired helical filament; NFT, neurofibrillary tangle.

than do analyses of plaques alone.⁶ Another difficulty is that, 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.⁷ Finally, no direct link has yet been established between APP and/or Aβ and the NFT pathology. Interestingly, mutations in the tau gene indisputably cause NFTs and neurodegeneration (see below) but are not associated with amyloid pathology, whereas mutations in APP and the presenilins cause both amyloid and tau pathology. Thus tau is genetically downstream of APP and the presenilins, but this does not rule out an important aetiological role for NFTs in the clinical picture. One possibility is that abnormalities in APP metabolism result in dysregulation of intracellular signalling pathways leading to aberrant phosphorylation of tau, NFT formation and neurodegeneration. However, to date, most transgenic mice overexpressing mutant or wild type APP or presenilins have not developed convincing

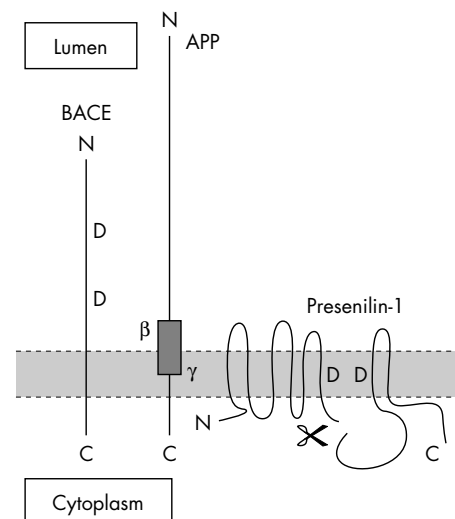


Figure 3 β-Secretase- and γ-secretase cleavage of APP. Schematic diagram depicting endoplasmic reticulum/Golgi membrane bound APP, BACE, and PS1. D, conserved aspartic acid residues that are candidate active sites for proteolytic activity (see text). Note that these sites are located in the luminal ectodomain of BACE, whereas in PS1 they reside within transmembrane regions 6 and 7, respectively, of the N and C terminal fragments of PS1. The endoproteolytic cleavage site of PS1 is indicated.

NFT pathology, even in the presence of substantial amyloid deposits reminiscent of Alzheimer's disease.⁸

APP, Aβ, AND THE FORMATION OF PLAQUES

The proteolytic processing and intracellular trafficking of APP is complex and occurs through several routes (fig 1). The normal function of APP is not yet clear but aberrant processing of APP causing either increased production, decreased removal, or the production of abnormally amyloidogenic Aβ is possibly the primary pathogenic event in Alzheimer's disease.⁹ Ubiquitously expressed and highly evolutionarily conserved, APP is a membrane spanning protein comprising a large extracellular/intraluminal amino(N)-terminal ectodomain, a single membrane spanning domain, and a short intracellular/cytoplasmic carboxyl(C) terminal domain (fig 1).

There are, however, other pathways for APP processing that provide routes for the generation of intact Aβ. Two proteolytic enzyme activities (β-secretase and γ-secretase) have been defined that allow Aβ to be excised from within APP (fig 1). β-Secretase cleaves APP at the N terminus of Aβ, generating a C99 fragment, and γ-secretase cleaves APP after the C terminus of Aβ—that is, actually within the hydrophobic transmembrane region. In the past 2 years extraordinary progress has been made in the identification and characterisation of these two secretases that hopefully should eventually lead to novel rational therapeutic targets for Alzheimer's disease.

The same candidate for β-secretase has been identified by several independent groups using different experimental approaches and has been named "BACE" (β-site APP cleaving enzyme; chromosomal location is 11q23.3) or "Asp2" or Memapsin2 as it is a membrane bound aspartyl protease.^{10–14} BACE is brain enriched and expression levels are much higher in neurons than in glia. It is a transmembrane protein with a short cytoplasmic tail and has been localised predominantly to the lumen of the Golgi apparatus and also to endosomes. There are two conserved Asp(Thr/Ser)Gly(Thr/Ser) motifs in its luminal ectodomain. These two motifs form the active site and, because of their orientation, have access to the β-cleavage site in the ectodomain of APP (fig 3). In support of BACE

being β -secretase are that overexpression results in increased cleavage of APP at known β -secretase sites,^{10,11} and inhibition of BACE activity results in decreased β -cleaved products.^{10,12}

A homologue, BACE2/Asp1, has also been identified, indicating that there is a family of these novel membrane spanning aspartyl proteases. The gene for BACE2 is in the critical Down's syndrome region of chromosome 21.¹⁵ Neuronal expression of BACE2 is very low,¹⁶ however, and BACE2 predominantly cleaves within the A β sequence rather than at the N terminus.¹⁷ These findings tend to mitigate against a role for BACE2 in Alzheimer's disease pathogenesis in Down's syndrome but might implicate BACE2 in the Alzheimer's disease pathology associated with the "Flemish" APP mutation, the site of which is beside the newly identified BACE2 A β cleavage site (fig 1).

THE PRESENILINS AND γ -SECRETASE

The presenilin proteins (PS1 and a homologue PS2) are 463 and 448 amino acid proteins with predicted eight transmembrane domain serpentine structures (fig 3) and are localised mainly to the membranes of the endoplasmic reticulum and cis Golgi apparatus.¹⁸ Constitutive endoproteolysis within a large cytoplasmic loop domain gives rise to N and C terminal fragments that form stable heterodimeric complexes that seem to be the principal functional form. The PS proteins have been reported to have a wide range of functions, including control of Notch mediated developmental morphogenesis, subcellular transport, and apoptosis, but it is becoming increasingly apparent that the PS proteins regulate γ -secretase activity.

PS1 knockout mice are embryonic lethal or die shortly after birth and display severe disruption of somitogenesis and axial skeleton development plus forebrain neurodevelopmental abnormalities,^{19,20} a phenotype similar to Notch knockout mice.²¹ Remarkably, cultured neurons from PS1 knockout embryos demonstrate normal α -secretase and β -secretase activities but substantially diminished γ -secretase activity, resulting in a five-fold decrease in A β production (and also the P3 peptide) along with an accumulation of α -secretase and β -secretase generated C terminal APP stubs—that is, C99 and C83.^{22,23} Knocking out both PS1 and PS2 eradicates γ -secretase cleavage of APP.²⁴ In transmembrane regions 6 and 7 of PS1 there are two aspartic acid residues (Asp257 and Asp385, respectively) that are conserved between all members of the PS family and that are candidate sites for intramembranous protease activity. Mutating either of these aspartic acid residues to alanine reduces γ -secretase cleavage of APP.²⁵ Asp385, in particular, is required for cleavage of APP by γ -secretase, whereas both sites seem to participate in Notch endoproteolysis.²⁶ That some γ -secretase activity resides in PS1 has been supported by studies using known transition state specific inhibitors of γ -secretase activity to bind, label, and identify PS1 and also by detecting γ -secretase activity in PS1 associated complexes.^{27–29}

Taken together, these findings have led to the hypothesis that PS1 might directly or indirectly regulate γ -secretase, perhaps as a diasparyl cofactor or by post-translational modification of γ -secretase and/or APP, or indeed might actually be γ -secretase itself. However, the therapeutic viability of inhibiting γ -secretase could be limited as this may deleteriously affect Notch signalling pathways that are involved in haematopoiesis and myogenesis as well as neurodevelopment.

APOLIPOPROTEIN E, APP, AND LATE ONSET ALZHEIMER'S DISEASE

The $\epsilon 4$ allele of the gene encoding apolipoprotein E (ApoE) is the most robust susceptibility gene so far to be identified for late onset Alzheimer's disease. Possession of one $\epsilon 4$ allele lowers the age of onset by 5 years and two $\epsilon 4$ alleles by 10 years. A

large population survival study has shown that the risk attributable to the apoE $\epsilon 4$ genotype is disease modifying—by lowering the age of onset—rather than causative.³⁰

How ApoE modifies Alzheimer's disease pathogenesis is not yet known. As with the genetic defects in APP, PS1 and PS2, the apoE $\epsilon 4$ effect seems to be mediated through A β either by enhanced aggregation or reduced clearance. For example, crossing apoE knockout mice (apoE^{-/-}) with mutant APPV717F transgenic mice results in mice at 6 months with only sparse diffuse A β deposits compared with more widespread amyloid deposition in APPV717F:apoE^{+/+} mice.³¹ Moreover, there is an increased A β load in patients with Alzheimer's disease with an apoE $\epsilon 4$ genotype.³² ApoE4 related pathogenic mechanisms could be related to isoform specific activity—for example, ApoE4 has been shown in vitro to have a greater avidity for binding A β compared with the other isoforms³³ and a lower efficacy for interacting with tau and protecting it from hyperphosphorylation and possible NFT formation.^{34,35}

Cerebral ApoE receptors are abundantly expressed on neurons but ApoE is actually produced by astrocytes. ApoE mediates phospholipid and cholesterol mobilisation and distribution and is active in membrane remodelling and maintenance of synaptic plasticity.³⁶ It could be that apoE4 performs these reparative functions less effectively than the E3 or E2 isoforms, thereby bringing forward the onset of dementia in persons developing Alzheimer's disease.

APOE, REELIN, AND ADAPTOR PROTEINS IN ALZHEIMER'S DISEASE

ApoE also functions in the reelin signalling pathway, which is essential for the proper migration of developing neurons. At the neuronal surface, reelin binds to both the very low density lipoprotein (VLDL) receptor and the ApoE receptor-2 (ApoER2), two members of the family of low density lipoprotein (LDL) receptors to which ApoE also binds. This induces phosphorylation of the disabled-1 (Dab1) intracellular adaptor protein (a protein that provides a molecular "scaffolding" for the formation of multiprotein complexes), which is required for propagation of the intracellular reelin signal; defects in this pathway can apparently cause hyperphosphorylation of tau similar to that found in Alzheimer's disease.³⁷ Dab1 also binds to a sequence within the cytoplasmic domain of APP containing the NPXY re-internalisation motif (fig 1), which regulates endocytosis of cell surface APP.³⁸ The NPXY motif also acts as a binding site for proteins that contain a PTB (phosphotyrosine binding) domain, a well characterised type of modular protein-protein interaction domain. In addition to binding to the Dab1 PTB domain, the cytoplasmic domain of APP also binds to members of two other PTB domain containing adaptor protein families, the Fe65s and the X11s.³⁹

Both Fe65 and Fe65L1 have been reported to enhance trafficking of APP through the secretory pathway and promote secretion of APP_{sol}, and, in the case of Fe65, to also increase secretion of A β in cultured cells.^{40,41} The X11s seem to have the opposite effect on APP processing in that the neuron specific X11 proteins X11 α and X11 β stabilise intracellular APP and decrease secretion of A β from cultured cells.^{42–45} Of note, XIIX and XII β have been reported to bind to the C-terminus of PS1 and to mediate PS1 binding to the cytoplasmic domain of APP.⁴⁶

It has been proposed that Fe65 can simultaneously bind, via its two PTB domains, to NPXY motifs within the cytoplasmic domains of both APP (fig 1) and the low density lipoprotein (LDL) receptor related protein (LRP), another member of the LDL receptor family, while Dab1 binds to a second NPXY motif in the cytoplasmic domain of LRP.³⁸ Via its extracellular domain, LRP has been reported to bind KPI domain containing APP isoforms, promote reinternalisation of APP and thereby increase secretion of A β .⁴⁷ Thus, in summary, a theoretical framework is beginning to emerge, centering

around a complex series of interactions between the cytoplasmic domain of APP, various cytoplasmic adaptor proteins and members of the LDL receptor family, that functionally couples ApoE to intracellular signalling, phosphorylation of tau, and processing of APP.

OTHER GENETIC FACTORS IN ALZHEIMER'S DISEASE

ApoE $\epsilon 4$ clearly does not account for the entire familial component in late onset Alzheimer's disease. A genome scan has identified several other candidate linkage sites including sites on chromosomes 12 and 10.⁴⁸ An intronic polymorphism in the gene coding for $\alpha 2$ -macroglobulin ($\alpha 2$ M; located on chromosome 12), which binds to LRP and also to A β , has been reported by one group to be associated with increased risk for late onset Alzheimer's disease.⁴⁹ Although $\alpha 2$ M is an attractive candidate, this finding has not been substantiated by most other independent groups and the chromosome 12 locus remains to be identified. Most recently, linkage has been confirmed to a region of chromosome 10q.^{50–51} One possible candidate here is the insulin degrading enzyme (IDE),⁵² which has been identified as having a role in the degradation and clearance of A β secreted by neurons and microglia.⁵³ Undoubtedly, the search for new genetic risk factors for Alzheimer's disease will continue and it would be most unusual if these did not turn out to have a role in modulating APP processing and generation of A β .

TAU: THE PROTEIN OF TANGLES

Tau was first implicated as a protein involved in the pathogenesis of Alzheimer's disease when it was discovered to be a major component of the neurofibrillary tangle described by Alzheimer himself.^{54–55} In tangles highly phosphorylated tau aggregates, in the form of paired helical filaments, gradually replace the normal neuronal cytoskeleton. However, some questions remain—is tau aggregation a primary pathogenic event or a secondary epiphenomenon? Does tau phosphorylation occur before or after aggregation? And is it the aggregation that is pathogenic or the loss of the normal function of tau?

Tau is a normal component of the neuronal cytoskeleton, a microtubule associated protein, expressed predominantly in axons where it functions to stabilise microtubules which are essential for fast axonal transport. This function of tau is regulated at the genomic and the proteomic level. Genetic regulation of the function of tau is by differential splicing—there are six isoforms of tau in CNS, differing by possession of N-terminal inserts of unknown function and by possession of three or four imperfect repeats that bind to microtubules. Tau with four microtubule binding domains binds microtubules better and this is reflected in the predominance of three repeat tau in the relatively unstable and dynamic developing brain. Proteomic regulation of tau function is by phosphorylation—the more phosphorylated tau fails to bind and stabilise microtubules as well as unphosphorylated tau. In parallel with the genetic regulation of tau function, developmental brain tau is more highly phosphorylated than mature adult brain tau. (For more detailed reviews of the biology of tau in health and disease see Spillantini and Goedert,⁵⁶ Lee,⁵⁷ and Lovestone and Reynolds.⁵⁸)

Although it was known for more than 10 years that tau was aggregated in Alzheimer's disease, it took a genetic disease to finally demonstrate that tau pathology could be primary in dementia.⁵⁹ Mutations in the tau gene were found first in frontotemporal degeneration with parkinsonism linked to chromosome 17, a disorder with predominant tau positive aggregations in glia as well as in neurons.⁶⁰ Other tau inclusion disorders (now subsumed under the term the tauopathies) include frontal lobe dementia, corticobasal degeneration, and progressive supranuclear palsy.⁶¹ Mutations in tau have now

been found in families with diverse frontal lobe dementing conditions including some which very closely resemble Pick's disease^{62–63} and polymorphic variation in tau has been associated with progressive supranuclear palsy.⁶⁴

The mutations in the autosomal dominant tauopathies are of two types—intronic mutations that disrupt the splicing of tau⁶⁵ and missense mutations that alter the function of tau.^{66–68} Both the splicing of tau, resulting in the relative proportion of three and four repeat isoforms, and the function of tau (through phosphorylation) are normally tightly regulated. It is likely that it is loss of this normal regulation that somehow results in tau aggregation, although it should be noted that in vitro the mutations also increase tau aggregation itself.^{69–70} Transgenic mice carrying tau mutations exhibit behavioural and neuropathological correlates of the disease process and are the first in vivo models of tau aggregation.⁷¹ Thus the first question was answered—tau aggregates are indeed a sign of primary pathology and not a secondary epiphenomenon. Tau aggregation without amyloid pathology is sufficient to cause a dementia in mice and in humans and hence the first question is answered—tau can clearly be a pathogenic protein.

Ironically this genetic demonstration of the importance of tau pathology also demonstrated the primacy of amyloid in Alzheimer's disease. As mutations in tau give rise to tangles but mutations in APP give rise to plaques and tangles it follows that amyloid must, biochemically, precede tau pathology in Alzheimer's disease. This confirms the observational studies on Down's syndrome brain where it was shown that amyloid deposition preceded tangle aggregation.⁷² However, somewhat against these findings are those of Braak and Braak who demonstrated in normal brain a gradual accumulation of highly phosphorylated tau, normally taken as a sign of pathology, that precedes amyloid deposition and dementia, by decades.⁷³ In fact, tau phosphorylation is in itself not pathological but the extent of tau phosphorylation can be. In foetal brain tau is very highly phosphorylated, in normal brain tau is moderately phosphorylated, in postmortem normal brain tau becomes rapidly dephosphorylated but in Alzheimer's disease brain tau phosphorylation is maximal and stable. All the evidence points to one enzyme, glycogen synthase kinase-3 (GSK-3), being the predominant tau kinase in brain, although other kinases also phosphorylate tau (reviewed in Lovestone and Reynolds⁵⁸).

Phosphorylation by GSK-3 renders tau less capable of binding microtubules and microtubules less stable and more liable to depolymerise.⁷⁴ This raises the second unanswered question in relation to tau aggregation. It is possible that tau phosphorylation, after all a normal process probably being regulated on a moment to moment basis as the cytoskeleton responds to extracellular signals, does not precede tau aggregation but simply occurs as tau is accumulated into stable, insoluble aggregates, perhaps preceded by proteolytic events.^{75–76} In truth there is little to argue for phosphorylation being chicken or egg at present. Our own laboratory is interested in the finding that both tau mutations and tau phosphorylation have the effect of disassembling tau from microtubules and result in tau accumulation in the cytoplasm and, by the evidence that under certain conditions tau will readily self aggregate in vitro.⁷⁷ It would seem plausible at least that any event, be it mutation, polymorphism, or phosphorylation, that resulted in tau accumulation in the cytoplasm would result in increased self aggregation and hence tangles. However, plausibility can be deceptive. It is transgenic animals that will demonstrate whether tau phosphorylation precedes or follows aggregation.

The third question is whether the aggregation of tau results in disease as a direct result of aggregation or whether there is loss of function of tau that might contribute to the disease phenotype. Tau as noted above stabilises the microtubule cytoskeleton and microtubules are essential for axonal transport. As axonal transport is necessary to replenish synaptic

proteins then a failure of transport would result in loss of neuronal function. In disease aggregation of tau is mirrored by loss of microtubules. Is this a sign of the loss of the normal function of tau resulting in the loss of normal microtubules? The same thing is seen in cell models of Alzheimer-like tau phosphorylation⁷⁴ but it might equally be that the gross accumulation of tau aggregates is in itself toxic to the cell. The finding that it is loss of microtubules (and not for instance loss of the actin cytoskeleton) that occurs in Alzheimer's disease may be a coincidence—a toxic gain of function—and not a sign of loss of function.

Tau aggregation is unequivocally associated with Alzheimer's disease and other neurodegenerative diseases and to this extent tau is a toxic protein in dementia. Tau, like the other proteins associated with dementia has a tendency to self aggregate. However, whether it is the tau aggregation that results in disease (toxic gain of function) or whether clinical phenotypes also result from a loss of the normal function of tau remains to be seen.

SYNUCLEIN AND THE LEWY BODY

In addition to amyloid fibrils the plaque of Alzheimer's disease contains many other proteins—the so called non-amyloid component (NAC). One of these proteins was found to be synuclein,⁷⁸ a presynaptic membrane associated protein known to be important in learning in songbirds.⁷⁹ However, synuclein came to prominence as a toxic protein in dementia after the demonstration that the fibrillar inclusion bodies in dementia with Lewy bodies and of Parkinson's disease were composed largely of this protein.^{80–82} Genetics again demonstrated that this was core to the pathology and not an epiphenomenon when mutations were found in a family with autosomal dominant Parkinson's disease.⁸³ Synuclein aggregation also occurs in multisystem atrophy within glial inclusion bodies.⁸⁴ A new classification of the neurodegenerative diseases has emerged from these findings—the tauopathies to include frontotemporal degeneration with parkinsonism and progressive supranuclear palsy by contrast with the synucleinopathies to include multiple system atrophy, Parkinson's disease, and dementia with Lewy bodies. Alzheimer's disease is simultaneously an amyloidopathy (similar to familial British or Danish dementia) and a tauopathy. Mouse and fly models of synuclein overexpression generate a phenotype resembling Parkinson's disease with dopaminergic loss and Lewy body-like inclusions.^{85–86}

Like other of the proteins we have been considering (tau for example) synuclein is a normal protein that is developmentally regulated—becoming associated with the synapse on maturity.^{87–88} Mice lacking synuclein display a subtle phenotype indicative of dopaminergic neuronal abnormality.⁸⁹ Again similar to tau, mutations in the synuclein gene result in a protein that self aggregates more readily than wild-type synuclein^{90–92} and, having aggregated, mutant synuclein can act as a nidus for aggregation of both wild type and mutant synuclein.⁹³ However, the mutation also seems to reduce turnover of synuclein by the proteosome.⁹⁴ Thus there is another symmetry with tau as mutations in both genes result in proteins that, at least in vitro and in cultured cells, show increased self aggregation and increased cytoplasmic accumulation.

PRION PROTEIN AND TRANSMISSABLE SPONGIFORM ENCEPHALOPATHIES

The prion disorders are rare transmissible diseases of the CNS with long incubation periods that affect humans and other animals. Classically, there is variable vacuolation of the neuropil giving rise to a “sponge-like” appearance in the brain, neuronal loss, gliosis, and extracellular amyloid plaques. The main constituent of the amyloid is PrP^{Sc}, which is named after the sheep prion disorder scrapie and is a modified

partially protease resistant and more aggregable form of the normal cellular prion protein (PrP^C).⁹⁵ Prion disorders can be inherited, infectious, or sporadic in origin. In addition, inherited forms can also be infectious—that is, they can be transmitted from one animal to another after either deliberate or accidental/iatrogenic inoculation with affected tissue. The appearance of variant Creutzfeld-Jakob disease, thought to be due to ingestion of material from cattle with bovine spongiform encephalopathy, has demonstrated that disease associated PrP^{Sc} can in certain instances cross a species barrier.⁹⁶

Human PrP^C is a 253 amino acid membrane associated glycoprotein expressed by neurons and to a lesser extent by astrocytes. An N-terminal 22 amino acid signal peptide is cleaved off after translation and the C-terminal 23 residues are also subsequently removed, following which a glycosyl phosphatidyl inositol (GPI) molecule is attached to serine230. This GPI molecule links mature PrP^C to the outer cell surface where PrP^C can be endocytosed and recycled within the cell. The C-terminal two thirds of the protein has a three helix bundle structure, whereas the N-terminal third is less obviously structured but does include four copper binding octapeptide (PHGGGWGQ) repeat motifs. Binding of transitional copper ions possibly protects against copper toxicity and also has a role in PrP^C antioxidant function.⁹⁷ PrP^C may also have a role in synaptic function as it is axonally transported to synaptic boutons and some mice in which the prion gene (Prnp) has been knocked out (Prnp^{0/0}) have abnormalities in long term potentiation.⁹⁸

The primary sequences of PrP^C and PrP^{Sc} are identical. So how can PrP^{Sc} be pathogenic? It is now generally believed that the differences between PrP^{Sc} and PrP^C reside at the level of their tertiary and quaternary structures, in particular an increased β -sheet content in PrP^{Sc}, and that these differences confer pathogenicity on PrP^{Sc}.⁹⁹ Indeed, these conformational differences endow PrP^{Sc} with the ability to convert normal host encoded PrP^C to PrP^{Sc} and thereby facilitate and extend the disease process in an infectious manner. PrP^{Sc} thus truly seems, even in the absence of any nucleic acid, to be a “proteinaceous infectious particle”. What mediates this conversion process is not known but it seems to occur as a late post-translational event, either at the cell surface or after endocytosis of PrP^C. What is increasingly clear though is that expression of normal host PrP^C is required for disease. Transgenic mice not expressing any PrP^C cannot be infected with diseased tissue and neurons not expressing PrP^C do not have PrP^{Sc} induced toxicity.⁹⁹

Mutant forms of PrP^C that cause inherited prion disorders also have an altered conformation that makes them pathogenic and also sometimes infectious. About 10%–20% of human prion disease is inherited in an autosomal dominant fashion. A wide range of point mutations and insertions in Prnp on chromosome 20 has been identified that cause inheritable prion disorders. For example, the P102L mutation causes Gerstmann-Straussler-Scheinker syndrome, D178N causes either fatal familial insomnia or Creutzfeld-Jakob disease, and E200K causes a Creutzfeld-Jakob disease-like disease in Libyan Jews. In the N-terminal half of PrP^C there is a five repeat region (amino acids 51–91) composed of one nona-repeat and the four copper binding octa-repeats. Mutant PrP^C variants carrying up to nine extra octa-repeats have been identified in patients presenting with various familial prion disorder phenotypes. Persons homozygous for a common methionine-valine polymorphism at codon 129 of PrP^C are at increased risk for both sporadic (methionine) and iatrogenic (valine) Creutzfeld-Jakob disease. Interestingly, this polymorphism can also influence the phenotype of the D178N mutation; those with valine 129 on the mutant allele develop familial Creutzfeld-Jakob disease whereas methionine 129 is associated with fatal familial insomnia.

As with most of the proteins discussed in this review, it is not yet fully clear how, or even if, PrP^{Sc} and mutant PrP^C actually cause neurodegeneration. A peptide corresponding to PrP106–126 can mimic PrP^{Sc} neurotoxicity, an effect that requires the presence of host PrP^C and also microglia, suggesting that oxidative stress may be involved.¹⁰⁰ It could also be possible that PrP^{Sc} inhibits the normal cellular antioxidant function of PrP^C and this further contributes to neurodegeneration.¹⁰¹ Understanding the normal biology of PrP^C, the mechanisms that convert it to the PrP^{Sc} isoform and how this and mutant PrP^C isoforms are associated with cell death will no doubt illuminate the mysteries surrounding this most unusual protein and lead to the identification of novel therapies.

HUNTINGTIN AND THE POLYGLUTAMINE TOXIC PROTEINS

Huntington's disease (HD) was not expected to be a disorder associated with aggregation of proteins, toxic or otherwise. By contrast with the other neurodegenerative disorders we have been considering, inclusion bodies are not part of the characteristic pathology of the condition. It came as something of a surprise, therefore, to discover the hitherto hidden Huntington's disease inclusions and it took a transgenic mouse model to find them. In an effort to generate a model of Huntington's disease, mice were initially generated overexpressing the 5'-end of the human Huntington's disease gene with CAG repeat expansions.¹⁰² Perhaps surprisingly, these mice developed a neurodegeneration strikingly similar to that of Huntington's disease and careful pathological studies revealed the accumulation of ubiquitinated huntingtin containing lesions in neuronal nuclei prior to neurodegeneration.¹⁰³ Similar lesions, aggregates of both nuclear and neuropil ubiquitinated huntingtin protein fragments were then revealed in the brains of those with Huntington's disease.^{104–106} The normal huntingtin protein shows little tendency to self aggregate but in vitro, as well as in these animal models and in disease, huntingtin with amino terminal polyglutamine expansions readily self aggregates.¹⁰⁷

The increased tendency of mutated huntingtin to aggregate is a direct result of the change in structural properties resulting from increased polyglutamine length in the protein.¹⁰⁸ In cells in culture expression of the polyglutamine expansion also results in ubiquitinated intraneuronal and perineuronal lesions and increases the vulnerability of the cells to apoptosis, with a correlation between expansion size and toxicity.^{105 109–111} Whether it is the nuclear or the cytoplasmic accumulation of huntingtin that results in neuronal toxicity is not entirely clear. Both occur in models and in disease¹¹² and it may be that nuclear entry after proteolysis is a secondary step just as ubiquitination is likely to be. In line with this is a mouse transgenic expressing the human huntingtin gene using a yeast artificial chromosome which has demonstrable pathology before the appearance of nuclear aggregates.¹¹³

The finding that expression of only a minimal segment of the gene containing a poly-CAG repeat is sufficient to cause disease in animal models demonstrates, more clearly than for other neurodegenerative disorders, that it is a toxic gain of function rather than loss of the normal function of the resulting protein. The same is true for other polyglutamine triplet repeat disorders such as dentatorubro-pallidolusian atrophy. Questions remain in particular regarding the mechanism whereby the aggregations cause disease, possibly by interfering with neurotransmission¹¹⁴ and whether, for example, proteolysis is a necessary step.¹¹⁵

However, the really important step forward that results from the clear demonstration of toxic protein aggregates in Huntington's disease is towards therapy. Steps are being made to reduce aggregation of Huntington's disease protein, including the demonstration that formic acid dissolves

aggregates,¹¹⁶ that antibodies directed against the molecule also reduce aggregation formation,¹¹⁷ that the heat shock protein chaperones might also reduce toxicity,^{118 119} that inhibiting caspase cleavage reduces aggregation,^{108 120 121} and that proteosome inhibition increases aggregation.¹²²

CONCLUSIONS

Are there toxic proteins causing dementia? Not really. All the disorders we have considered, and others that we have not, are associated with inclusion bodies formed from proteins normally present and functioning in the brain. Whether it is the aggregation of these proteins that is toxic or whether it is loss of the normal function of these proteins that is toxic is not clear and some evidence suggests that the latter leads to the former in any case. There are some clear differences between the disorders—the amyloid aggregates of Alzheimer's disease are extracellular, the other aggregates are intracellular, some neuronal, some glial. For Alzheimer's disease other proteins—the presenilins, APOE—have been identified through genetics that seem to alter the formation of the protein; no such proteins are known in the other disorders although the search is on.

Are there common aetiological mechanisms? It is possible, for example, that a decrease in protein degradation underlies these conditions. The proteosome might be a common final pathway to different dementias. Most recently it has been shown that protein aggregation inhibits the ubiquitin-proteosome system leading to a positive feedback loop whereby this inhibition results in less protein degradation, more aggregation, more inhibition of the system and, presumably, in vivo, cell death.¹²³

Despite some questions remaining to be answered, the identification of fibrillar aggregates in many neurodegenerative disorders raises the potential for therapy directed at preventing or clearing these aggregates. Different approaches are under consideration as illustrated by the search for disease modifying agents in Alzheimer's disease. One approach is to prevent or slow down the formation of the protein—for example, α -secretase promotion, or BACE and γ -secretase inhibition, to prevent A β formation. To prevent hyperphosphorylation of tau GSK-3 inhibitors might have some value—and one such, lithium—is in common use in another context. Such an approach makes complete sense but caution should be expressed as even A β is likely to have a normal, if unknown, role in the adult brain. If it proves difficult to block prevention of the peptide then prevention of its aggregation by inhibition of conversion from native to β -sheet structure or blocking "nucleus" formation might be possible. Compounds to prevent aggregation are already approaching clinical trial phase in Alzheimer's disease. However, by far the most exciting development has been the demonstration that even when formed, there is considerable merit in clearing amyloid plaques. In a transgenic mutant APP overexpressing mouse, passive and active vaccination against A β both cleared existing plaques and reversed cognitive decline.^{124 125} The same approach is unlikely to be of use against intracellular inclusions but might be used against other extracellular aggregates—PrP^{Sc} for example. Finally efforts to reduce the effects of the toxicity of aggregates might find some common therapeutic goals across these disorders. From the finding that aggregation of proteins occurs in many different dementias, it is tempting to draw the conclusion that there are common pathological processes. There may be, but equally protein aggregation might be a common final pathway in neurodegeneration. However, the prospect that there might be common therapeutic strategies, if not common actual therapies, is an exciting one and one that should spur further research into toxic protein aggregation in dementia.

GLOSSARY OF TERMS

Amyloid is generic term for abnormally aggregated proteins that usually have adopted a β -pleated sheet secondary structure.

Apoptosis is a form of programmed death for individual cells. It is characterised morphologically by plasma membrane blebbing and nuclear chromatin condensation and fragmentation. This all occurs in the absence of cell swelling or disintegration of other organelles and eventually serves to target the cell for phagocytosis by surrounding macrophages or microglia. The events leading to apoptosis are tightly regulated and involve a complicated cascade of enzymatic activities, including various members of the *caspase* family of enzymes.

Constitutive endoproteolysis is enzymatic cleavage within a protein that is continuously occurring, to a greater or lesser degree, independently of other cellular events.

Endocytosis is the process whereby the cell surface membrane progressively invaginates to eventually form a discrete membrane bound vesicle within the cytoplasm. Any membrane bound protein and the contents of this vesicle are thereby internalised from extracellular space into the cell body. Cell surface membrane bound APP can be reinternalised in this manner such that its *ectodomain* (or large extracellular domain) is no longer protruding into extracellular space but is now actually within a cellular vesicle.

A **heterodimer** is a complex comprised of two different proteins; a **heterotrimeric complex** contains three different proteins; a **homodimeric complex** contains two of the same protein, etc.

Notch is a single membrane spanning cell surface protein that acts in cell signalling and has a central role in neurodevelopment. Similar to APP, it undergoes endoproteolysis within its transmembrane domain. This seems to be due to the same γ -secretase that cleaves APP to generate A β and is also dependent upon the presenilin proteins.

Phosphorylation is the biochemical process in which a phosphate group is attached to another molecule. For proteins this is achieved by kinase enzymes. Phosphorylation of proteins is a key method of regulating their activity.

Protein structure can be described in four different levels of complexity. **Primary** structure is simply the linear sequence of amino acids. **Secondary** structure is essentially the α -helical and β -strand arrangements formed by short sequences of continuous amino acids. **Tertiary** structure is how these α -helices and β -strands, plus intervening less well structurally defined sequences, are positioned relative to each other in 3 dimensional space. **Quaternary** structure is how larger well defined protein subunits relate to each other in three dimensional space. To some extent the secondary structure of a protein can be deduced from the primary structure. However, tertiary and quaternary structures are not readily predictable and solving structure at this level requires complicated analysis.

Ubiquitin is a small conserved intracellular protein that binds other proteins, targeting them for degradation. Once a protein is ubiquitinated it is directed towards the proteasome, which is a multiprotein complex containing a range of protease enzymes. The proteases break down peptide bonds between amino acids in the ubiquitinated protein. After degradation of the protein, ubiquitin molecules are released and recycled

ACKNOWLEDGEMENTS

Work in our laboratories is supported by the Wellcome Trust, the MRC, The EU, the Alzheimer's Society and The Alzheimer's Research Trust.

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