Mechanism of extracellular ab oligomer toxicity biology essay

Science, Biology

http://www. ncbi. nlm. nih. gov/pmc/articles/PMC2748841/http://www. ncbi. nlm. nih. gov/pmc/articles/PMC3156817/http://www. pnas. org/content/109/27/11025. fullhttp://www. jneurosci. org/content/30/18/6367. longhttp://www. ncbi. nlm. nih. gov/pmc/articles/PMC2892479/The underlying mechanisms of Amyloid β toxicity in Alzheimer's disease: To be or not to be a PrionAlzheimer's disease (AD) is a neurodegenerative disorder characterized by the progressive loss of cognitive function. The brains of AD patients contain extracellular amyloid plaques composed of amyloid-β (Aβ) peptides as well as intracellular neurofibrillary tangles containing hyperphosphorylated tau protein. The amyloid cascade theory postulates that aggregation of Aβ peptides cleaved from the amyloid precursor protein (APP) and the subsequent spread of Aβ deposits in the brain constitute the earliest key events in the progression of AD. What is Ab: Amyloid β (Aβ) is a small self-aggregating peptide produced at low levels by normal brain metabolism. In Alzheimer's disease (AD), self-aggregation of Aβ becomes rampant, manifested most strikingly as the amyloid fibrils of senile plaques. Because fibrils can kill neurons in culture, it has been argued that fibrils initiate the neurodegenerative cascades of AD. An emerging and different view, however, is that fibrils are not the only toxic form of Aβ, and perhaps not the neurotoxin that is most relevant to AD: small oligomers and protofibrils also have potent neurological activity. Immuno-neutralization of soluble Aβ-derived toxins might be the key to optimizing AD vaccines that are now on the horizon (Klein 2001). These oligomers are not generally stable aggregates; they appear as transient species during the conversion of their monomeric precursors to more massive, stable fibrils, and sometimes

they appear as an ensemble of sizes and shapes. This polymorphic and time-dependent nature of small amyloid oligomers has made it difficult to pin down their assembly pathways, their stoichiometries, their atomic-level structures, their relationship to fibrils, and their pathological actions (New research provides evidence that suggests the ABC cylindrin is a possible model for amyloid oligomers formed by well-studied toxic proteins, such as Ab. share common structural features. For example, studies have suggested oligomers are b-sheet rich (38–40), and several toxic oligomers are recognized by the A11 conformational antibody (41), which also recognizes the cylindrin. laganowsky 2012quantitative correlations between manual microscopiccounts of amyloid plaques in post-mortem brain sectionsand the extent of cognitive symptoms measuredpre-mortem are fraught with methodological challenges. Counting spherical plaques in two-dimensional crosssections provides an imprecise measure of A amountsand misses small and heterogeneous A-assembly forms. Last, the cognitive testing done before the patient’s deathhas often been done with simple, insensitive mentalstatus screens. The advent of specific A enzyme-linked-immunosorbentassays (ELISAs) coupled with western blottingand mass spectrometry has now enabled a more preciseand comprehensive assessment of A qualityand quantity. Such studies indicate that biochemicallymeasuredlevels of soluble A, including soluble oligomers, correlate much better with the presence and degreeof cognitive deficits than do simple plaque counts44–47. Thisevidence, coupled with the fact that large (~20–120-mdiameter) fibrillar plaques present much less A surfacearea to neuronal membranes than do a multitude of smalloligo mers that can

diffuse into synaptic clefts, indicatesthat such soluble assembly forms are better candidatesfor inducing neuronal and/or synaptic dysfunction thanplaques, per se. Importantly, the idea that large aggregates of a disease causingprotein can actually be inert or even protective toneurons has been supported by work on other proteinfoldingdisorders. For example, in cell-culture studies ofHD, less cell death has been observed when large aggregates of polyglutamine-rich huntingtin protein are presentin the cells than when only soluble huntingtin is presentwithout these inclusions48, 49. Analogous findings havebeen reported in a mouse model of spinocerebellar ataxiain which the polyglutamine-rich forms of the ataxin-1protein are expressed50 . However, it must also be pointed out that large plaques of fibrillar A in AD brains typically show surroundingdystrophic neurites, indicating that insolubleaggregates might contribute to neuronal injury. Indeed, fibrillar A deposits have been associated with localsynaptic abnormalities and even with the breakage ofneuronal processes51. The problem is that large, insolubleprotein aggregates are likely to be intimately surroundedby a number of smaller, more diffusible, assemblies (forexample, oligomers). So, it becomes difficult to ascertainwhether the large aggregates are directly inducing localneuronal injury and dysfunction. At the current stageof research, one should not conclude that either large, insoluble deposits or small, soluble oligomers representthe sole neurotoxic entity; indeed, a continuous dynamicexchange between these forms might well be detrimental. Nevertheless, we hypothesize that diffusible oligomershave the principal role, particularly during the earliest, even pre-symptomatic, stages of the AD process. Thus, determining the molecular mechanisms governing

prion formation and self-propagation in the brain may advance our understanding of the etiology and pathogenesis of neurodegeneration as well as facilitate identification of therapeutic targets, through which effective interventions can be developed. Mechanism of extracellular Ab oligomer toxicity: Recent reports have suggested that there are receptor-mediated mechanisms by which Aβ exerts its toxic effects. For example ADDL has been shown to bind to an N-methyl-d-aspartate (NMDA)-type glutamate receptor (NMDAR) which subsequently causes abnormal calcium homeostasis, in turn leading to increased oxidative stress and synapse loss (De Felice et al 2007). Moreover Magdesian and colleagues showed that by binding to the Frizzled (Fz) receptor, Aβ oligomers can inhibit the Wnt/β-catenin signalling pathway. Inhibition of this signalling pathway also gives rise to tau phosphorylation and neurofibrillary tangles, again leading to cellular dysfunction. Another mechanism by which Aβ oligomers could be toxic was suggested by Vallincius (2008) who noted that as they form the membrane pore they are able to influence the flow of ions in and out of the cell. Essentially it was shown that the oligomers caused an abnormal flow of Ca2+ and disruption of Ca2+ homeostasis could ultimately end in cell death. These observations support the idea that toxicity and cell death might occur via multiple pathways of extracellular Ab aggregate formation. Mechanism of intracellular Ab oligomer toxicity: Wilst it is accepted that Aβ is produced by the cleavage of APP by β-secretase and γ-secretase at the plasma membrane it is also produced within endosomes. Identification of the intracellular protein, endoplasmic reticulum associated binding protein (ERAB), which binds to Aβ, also strongly suggests the existence of

intracellular Aβ. In addition to Aβ being produced intracellularly, previously secreted Aβ that forms the extracellular Aβ pool can be taken up by cells and internalized into intracellular pools through various receptors and transporters, such as the nicotinic acetylcholine receptor, low-density lipoprotein receptor, formyl peptide receptor-like protein 1, NMDAR and the scavenger receptor for advanced glycation end-products [6] (Fig. 2). These receptor-associated Aβ complexes could be internalized into endosomes. Furthermore, in vivo and in vitro proteasome inhibition also leads to higher Aβ levels [62, 63]. As the proteasome is primarily located within the cytosol, these findings strongly suggest that Aβ is also located within the cytosolic compartment. Extracellular Aβ can enter the cytosolic compartment and inhibit the proteasome activity of cultured neuronal cells [62].

Clifford et al. [64] showed that fluorescently labeled Aβ which is injected into the tail of mice with a defective blood–brain barrier (which is common in AD patients) accumulates in the perinuclear cytosol of pyramidal neurons in the cerebral cortex. These observations strongly support the notion that neurons can take up extracellular Aβ in the cytosolic compartment. A recent study by Nimmrich et al. showed that Aβ oligomers can impair presynaptic P/Q-type calcium currents, which are related to neurotransmission and synaptic plasticity in the brain, at both glutamatergic and gamma-amino butyric acid (GABA)-ergic synapses. This impairment is specific for Aβ oligomers, but not for Aβ monomer or fibrils. Even if neurons and other mammalian cells take up fibrillar aggregates by endocytosis, in order for them to nucleate aggregation of endogenous cytoplasmic proteins, which is essential for the ‘ prion-like’ hypothesis, they must escape the intracellular vesicle and gain

access to the cytoplasm. The aggregates investigated in the cell culture studies described above are too polar to diffuse passively across lipid bilayers and too large to pass through transmembrane pores or transporters. Nonetheless, extracellular aggregates containing polyglutamine42, tau39 and α-synuclein34 have now been shown to enter cells and cause seeding of endogenous proteins. Deep-etch electron microscope images of polyglutamine aggregates shortly after internalization into cultured cells revealed ‘ naked’ aggregates on the cytoplasmic face of the plasma membrane. There was no evidence of a surrounding membranous structure42, suggesting that aggregates can penetrate the plasma membrane in the absence of vesicular uptake. Studies in artificial systems on α-synuclein oligomers show that they can render lipid bilayer membranes permeable to fluorescent dyes, suggesting that α-synuclein aggregates can intercalate directly into lipid membranes50. This provides a potential means by which aggregates could exit from endosomes or perhaps cross the plasma membrane directly. Finally, it is possible that tunnelling nanotubes — 50–200 nm diameter actin-rich hollow filaments seen between interconnected cells in culture51 — can act as transport conduits for prion-like protein aggregates, as has been suggested for PrP51 (FIG. 2c). The destabilization of intracellular membranes may also contribute to the presence of cytosolic Aβ. A high proportion of autophagy-related vesicular structures, which would suggest impaired maturation of autophagosomes to lysosomes, has been found in the AD brain, but not in the normal brain [65]. Although most Aβ formed in endosomes is normally degraded within lysosomes, Aβ can accumulate in lysosomes in the AD brain. Aβ within the lysosomal

compartment destabilizes its membrane [66], which would also lead to the presence of Aβ in the cytosolic compartment. Recent observations by Yuyamaet al., [67] showing GM1 accumulation in early endosomes, support the idea that intracellular GM1 could also induce Aβ oligomer formation. Recently, we found formation of toxic high-MW (50–250 kDa) soluble Aβ oligomers by the cytosolic molecular chaperone protein, prefoldin, in vitro [68The toxicity mechanism of intracellular Aβ oligomers also remains unclear. Microinjection of Aβ or a cDNA-expressing cytosolic Aβ induces the cell death of primary neurons and the simultaneous formation of low-MW Aβ oligomers [80]. Furthermore, intracellular Aβ accumulation is closely correlated with apoptotic cell death via the P53-BAX pathway [81]. Recently, Mousnier et al. [82] reported a possible prefoldin-mediated proteasomal protein-degradation pathway. It is therefore plausible that Aβ oligomer–prefoldin complexes could bind to proteasome, causing proteasome dysfunction and subsequent cell death. This idea is supported by interaction studies between Aβ oligomers and proteasome, which showed that the proteasomal function was inhibited while interacting with Aβ [63]. Impairment of proteasomal function by the Aβ oligomer also leads to age-related pathological accumulation of Aβ and tau protein [63]. Recent research has shown that the dysfunction of autophagy, a lysosomal pathway for degrading organelles and proteins, is related to neurodegenerative diseases, including AD and PD [65, 76]. These observations support the idea that the toxicity mechanism of intracellular oligomers may be different from that of extracellular oligomers (Fig. 2). However, more studies, particularly those focused especially on the proteolysis system in AD brains, are

necessary to understand AD pathology in relation to intracellular soluble Aβ oligomers. The requirement of Tau for Ab toxicity: Although both tau tangles and Ab fibrils are hallmarks of AD, it has long been assumed that tau plays a secondary role to amyloid-β toxicity as it has been suggested that tau pathology could be induced by Ab (Terwel et al 2008). One example s that by using intracerebral injections of Ab the hyperphosphorylation of tau can be exacerbated and accelerate the neurofibrillary tangle formation in P301L mutant tau transgenic mice (Go¨tz et al., 2001). However this view has recently been challenged by the observations that tau deficient neurons (-/-) are protected from amyloid-β toxicity (Rapoport et al 2002). In one particular study tau deficiency was also shown to rescue memory deficits in APP transgenic mice (Roberson et al., 2007). What is currently known about the mechanisms behind this protective function is that it is the disruption of the interaction between NMDA receptor subunits (NRs) and the postsynaptic density protein 95 (PSD95) which prevents excitotoxic damage (Chin et al 2005). As tau interacts with the kinase Fyn which in turn phosphorylates the NMDA receptor 2 subunit (NR2), facilitating the interaction of NRs with PSD-95 (Nakazawa et al., 2001), it follows that tau facilitates this interaction and thus a necessary component for the prevention of Ab toxicity. However the precise mechanisms by which tau influences Ab toxicity and how it can prevent excitotoxic damage remained unclear, that was until a study done nearly a decade later by the Gotz lab shed some light on this question (Ittner et al 2010). The researchers began by producing transgenic mice (Dtau74) that express only the amino terminal projection domain (PD) of tau, causing a deficient tau/Fyn interaction, and crossed them with APP (Ab forming) and

tau -/- mice. Then coimmunoprecipitations (coIPs) were undertaken in order to test the interaction between NRs and PSD-95. A reduced interaction was shown by markedly less coimmunoprecipitation between the receptors and PSD-95 in both Dtau74 and tau -/- mice compared to wild types. Then by using purified synaptosomes the researchers ruled out the possibility that it was the level of receptor subunits that had decreased but instead a reduction of the phosphorylation of the NR2 subunit. This would then suggest that expression of Dtau or a lack of tau leads to a defective interaction between NRs and PSD-95. Then in order to explore how disruption of this interaction might influences memory deficits APP/Dtau74 and APP/tau-/- mice were compared using the water T maze. It was found that not only did both mice expressing Dtau74 and tau-/- show improved memory function to the level of wild typed but also improved mortality rates. These results would then suggest expression of Dtau or a lack of tau leads to a reduction in Fyn mediated NR2 phosphorylation, ultimately meaning a rescuing of the memory deficits and high mortality rates usually seen in APP mice. Then following the hypothesis that disruption of this interaction plays a key role in protecting against memory deficits, the authors sought to substantiate their claim by directly perturbing the NR/PSD- 95 interaction . This was done by treating primary cortical cultures with the Tat-NR2B9c peptide which has been shown to protect from NMDA induced excitotoxicity (Aarts et al., 2002). It was subsequently found that targeting this interaction protects against Ab-mediated toxicity in vitro. Moreover by repeating the experiment in vivo it was found that perturbing NR/PSD-95 interaction is sufficient to prevent memory deficits in APP mice. Also mice treated with Tat-NR2B9c had a much

higher survival rate, suggesting that treatment with such a peptide could be therapeutic even if only given once. Essentially what this study has contributed to the field should not be overlooked as it highlights the importance of tau to Ab toxicity and AD pathology in general. It raises the possibility of new drug targets as it could be argued that reducing overall tau levels or targeting of the tau dependent interactions of NRs and PSD-95, could be beneficial for future Alzheimer's treatments. It should be noted that this is not the only study to have made this conclusion as the necessity of tau to Aβ toxicity was also supported by Vossel et al (2010) who found that reducing tau levels prevented the impairment of axonal transport seen with Aβ toxicity. The requirement of cellular prion protein (PrP) in Ab toxicity: It has been demonstrated that soluble Ab oligomers suppress long term potentiation of the Schaffer collateral pathway when hippocampal slices are pre incubated with Ab42 oligomer preperations (Walsh et al 2002). However it was recently shown that this inhibition can be stunted if the preparation is used on slices from prion protein null mice (PrP-/-) (Lauren et al 2009). The authors suggested that this could be because PrPc acts as a receptor for the Ab oligomers and supported this claim by showing that when treated with an anti-PrP antibody (6D11) which prevents the binding of Ab oligomers to PrPc, LTP inhibition was rescued. Thus it was concluded that PrPc functions as a receptor of Ab and mediates the Ab inhibition of synaptic plasticity making it necessary for the synaptic deficits seen in AD patients. However this study was done in vitro using synthetically derived Ab species which cannot accurately reflect the interaction of Ab and PrPc in vivo with brain derived Ab. It was then a study carried out in the same lab which sought to explore

whether interaction with PrPc is necessary for memory impairment in AD (Gimbel, Nygaard, Coffey, Gunther, Lauren, Gimbel & Strittmatter 2010). To do this the experimenters looked at how PrPc interaction affects spatial learning and memory and other markers of neurodegeneration in transgenic mouse models of AD (APPswe/PSen1 E9 mice) which either have or lack PrPc. To measure spatial learning and memory the Morris water maze was used which as a hidden platform submerged beneath the water and mice are trained to a particular platform location which is then reversed and mice must learn the new location. The AD model mice with PrPc have severe impairments to their ability o learn the new location whereas mice lacking PrPc have similar latencies to find the platform as wild type mice. Moreover the AD model mice exhibit an age dependant decline reminiscent of human AD patients that was not seen with those lacking PrPc. This study also looked at some other hallmarks of AD and the dependence of PrPc in mediating them. For example the effect of PrPc on axon degeneration was tested by examining deficits in 5-HT immunoreactive axon fibres of the cerebral cortex in AD mice with and without PrPc. It was subsequently found that when these mice had no PrPc there was no deficit and the fibres resembled those of wild type mice instead. It was then this result which led the researchers to suggest PrPc is necessary for axon degeneration in AD. Another phenotype of AD is synapse loss and by using both presynaptic and postsynaptic markers, anti-synaptophysin and anti-PSD-95 respectively, in different areas to detect synapse loss. In the transgenic mice with intact PrPc there is a reduction in the immunopositive area but when there is a lack of PrPc this immunopositive response is unchanged, indicating no synapse loss. An

additional phenotype is reduced lifespan as the transgenic AD mice had a 60% survival rate between 0-12 months compared to those lacking PrPc who only had a 96% survival rate, suggesting that even reduced survival is mediated by PrPc. Ultimately what this study shows is that PrPc interaction with Ab oligomers is necessary for many of the phenotypic deficits seen in in vivo Ab oligomer toxicity. This research is an important step in the right direction when it comes to uncovering the underlying mechanisms of Ab toxicity but unfortunately, as is the case with many complex diseases, the literature is not as unitary and coherent as we would like. In particular a recent study by Balducci et al (2010) agreed that Ab oligomers bind to PrPc but in contrast to the study previously mentioned they found PrPc to not be necessary for memory impairment. However, whilst this seems to completely contradict the work by the Strittmatter lab, it could be argued that the methodological differences between experiments are so vast that they cannot be measuring the same thing. This refers to the fact that the study by Gimbel et al (2010) used AD transgenesis as a model but Balducci et al (2010) on the other hand injected mice with a synthetic Ab oligomer preperation. In addition they also measure a different type of memory, object recognition memory which measures the ability of a mouse to learn and remember which the familiar object is compared to a novel object. It was found that mice lacking PrPc behave normally by preferring the novel object and once injected with Ab they avoid the novel object, suggesting PrPc is not essential for this memory deficit. However it should be noted that preference reversal is by no means an absence of memory, something that the Strittmatter group also highlighted. Bearing this in mind it could be argued

that memory impairment is indeed rescued by knocking out PrPc as there is no clear deficit in memory per se in this experiment. Alternatively, Ab oligomers could affects novelty preference in a way that is not dependent on PrPc, propably via its effect on monoaminergic pathways which are known to influence anxiety behaviour, leading to abnormal novelty preference. Essentially this is not a complete picture of the role PrPc has in Ab oligomer toxicity but it does shed some light on the importance of this protein to AD pathogenesis. It does have some therapeutic implications as blocking the interaction of Ab and PrPc could alleviate several of the debilitating deficits brought on by AD, most importantly the loss of memory. It should be borne in mind however that all the aforementioned experiments used a genetic knockdown of PrPc so mice lacked this protein since birth and so the results cannot be generalized to assume that if binding or PrPc expression was blocked at the onset of AD symptoms it would have the same desired effect. On a more optimistic note however it was suggested by Gimbel et al (2010) that binding of Ab to PrPc functions downstream of Ab plaque formation which would implies that it is not required for Ab plaque formation. This then suggests that the impairments caused by AD are not correlated with the plaques and instead supports the hypothesis that the plaques themselves are not toxic but that perhaps the soluble oligomer form of Ab is the main cause of toxicity. Mechanism of Ab oligomer spreading:

The propagation of prion-like protein inclusions in neurodegenerative diseases - goedert

The mechanisms of transmission usually uniquely associated with prion diseases are increasingly becoming associated with many

neurodegenerative diseases and are now being described by some as " prion-like" in their behaviour (Aguzzi & Rajendran 2009). This is because the proteins implicated in most neurodegenerative diseases share some key characteristics with the infectious cycle of seeding and spreading that is seen with prion pathogenesis in which a misfolded conformer of the prion protein (PrPsc), induces further misfolding of endogenous prion protein (PrP). This mechanism of seeding is the way PrPsc self propogates and ultimately leads to aggregation and toxicity. In fact there has been a growing number of reports showing that self propagating protein aggregates could be at the crux disease progression in Alzheimers. One recent study has shown that intracerebral injection of brain extracts of human Alzheimer patients or from Alzheimer model mice into transgenic mice expressing human amyloid precursor protein (APP), accelerated Aβ aggregation (Kane et al., 2000 and Meyer-Luehmann et al., 2006). However this idea is not a novel one as it was first suggested in the 90’s when Aβ plaque formation was seen in the brains of primates after injection of brain extracts of human Alzheimer patients (Baker et al., 1994). However in order to define a disease as prion-like, they must directly mimic the mechanisms by which the prion protein exerts its toxic effect. For example cells ‘ infected’ by aggregates must continuously synthesize the non-aggregated form, the transmissible aggregate must be released from cells and aggregates must be able to bind and enter the recipient cell. This mechanism of action has been shown both in vivo and in vitro over recent years, supporting the hypothesis and fuelling the debate over whether Alzeimer’s behaves as a prion disease. One study used a similar format to those original reports suggesting a prion link, and

saw that injection of brain extract containing tau aggregates causes widespread aggregation of normal human tau in transgenic mice, mirroring the seeding mechanism (Clavaguera et al., 2009). These kinds of observations have led to many researchers arguing that Alzheimer's may spread via a non-cell-autonomous mechanism within the nervous system. However in order to provide some more definitive evidence that Ab behaves like a prion it would need to self-propagate in the absence of any auxiliary cofactors, as is the case with prion proteins (Legname et al 2004). Previous studies looking at Aβ aggregation in vivo using synthetic Aβ peptides have not been successful thus far leading to the questioning of whether cofactors may be essential for the prion-like pathogenesis. A recent investigation has however shed some light on this debate by providing the first evidence that Aβ deposition can be induced in Tg mice when inoculated with brain-derived purified Aβ fibrils or synthetic Aβ peptides alone (Stohr et al 2012). This was done firstly by crossing mice who express luciferase reporter under the control of the glial fibrillary acidic protein (Gfap) promoter, with Tg mice expressing human APP to produce Tg(APP23: Gfap-luc) mice. Then Bioluminescence imaging (BLI) was used to monitor Aβ spreading in these mice as when this protein accumulates it causes astrocytic gliosis, resulting in up-regulation of the Gfap promoter. These mice showed an increase in the BLI signal at 9d old which correlates with the deposition of Aβ in the brain. Then to assess whether Ab spreads in a prion-like fashion the Tg(APP23: Gfap-luc) mice were intracerebrally (ic) inoculated in the right cerebral hemisphere with brain homogenates from two types of Alzheimers mutant mice, either the Tg(APP23) mice or the Tg(CRND8) mice which express both

the Swedish and Indiana mutations. This caused an increase in the BLI signal but stayed low in those mice receiving the control non Tg inoculation and was not significantly different from the uninoculated mice. Moreover, not only was Ab deposition higher in mice inoculated with Ab aggregates but unilateral inoculation of both synthetic and brain derived Ab induced bilateral deposition. The spreading seen with the synthetic Ab shows that it is the Ab itself and not any auxiliary factors that may have been copurified along with the homogenous Ab. Therefore the prion like behaviour of Ab is inducible by Ab alone and this form of spreading has also been seen in AD patients (Braak & Braak 1991), indicating that this is unlikely to be an artifact of the inoculation but a part of the pathogenesis of AD. Essentially the investigators were able to demonstrate that Aβ peptides alone are sufficient for the widespread formation of Aβ aggregate assemblies similar to that of synthetic prions composed of recPrP alone (Legname et al 2004) and therefore it is not dependent on any possible cofactors. Although both brain derived and synthetic Ab were capable of inducing prion like spreading it should be borne in mind that there are still fundamental differences between these aggregates and their possible mechanisms of action. Firstly this study highlighted that the synthetic Aβ inocula contained 100fold more Aβ peptide than the brain derived Ab. It should be noted that the authors made a valid point when stating that the increased peptide levels did not influence the subsequent level of deposition as the level of deposition postinoculation was below the level of detection by BLI. This was the case for both synthetic and brain derived inoculations and so the results cannot be due to residual peptides from the inocula. However this is an observation which has also

been made of synthetic PrP and is thought to derive from a structural disparity between the amyloid fibrils composed of recombinant PrP (recPrP) and PrPSc from the brain (Cobb et al 2007, Wille et al 2009). It could be argued that if they are structurally different then perhaps the synthetic Aβ aggregates are a unique strain with distinct characteristics that mean more of the peptide is necessary to induce self propagation. If this is the case and synthetic Ab is somehow functionally different as well as structurally, there must be caution when using them as models of Ab pathogenesis. Just because the end product, which we see as the formation of Ab fibrils, can be caused by synthetic Ab, it does not mean to say the underlying mechanisms governing this formation is identical to brain homogenous Ab. Thus it would be premature to take such findings and make assumptions about how Ab aggregated form fibrils and possibly lead to toxicity. Another cautionary thought to take away from such findings is that if Ab spreading can be induced by injection of synthetic Ab aggregates then there is a danger that immunization with synthetic Aβ peptide could be at risk of accelerated deposition. So whilst such immunization techniques have been shown to reduce Aβ deposition and improve cognition (Janus et al 2000), the long-term effects could be damaging. This is emphasized by the finding of a clinical trial of Aβ immunization that had to be was stopped because some patients developed meningoencephalitis (Orgogozo et al 2003). Studies have shown that experimental aggregate propagation of tau35 or Aβ plaques18 in mouse models is often not accompanied by neuronal loss. Three possible scenarios might explain this observation. First, aggregate deposition could simply precede neuronal loss and other pathological changes. Second, aggregate

propagation may be a critical factor—but not in itself sufficient—for neurodegeneration; in this case transmitted aggregates could cooperate with unknown factors that are in limited supply in the mouse models. Third, the progression of protein aggregation and neurodegeneration could be entirely unrelated and independent events. ConclusionDespite the similarities between prion and neurodegenerative diseases such as Alzheimers we cannot overlook the simple fact that it is prion diseases alone which are truely infectious and capable of transmission between organisms. It is then for this reason that Aguzzi coined the term prionoid to distinguish prion-like mechanisms functioning within a single organism from genuine infectious prion diseases (Aguzzi, 2009 and Aguzzi and Rajendran, 2009). If prion-like transmission has a role, it seems more likely to contribute to the gradual spreading of neuropathological changes in the brains of afflicted individuals. Importantly, the possible existence of extracellular intermediates in the progression of what previously have been considered strictly cell-autonomous intra-cellular disorders, provides a hitherto unappreciated extracellular stage in pathogenesis. This extracellular step in the pathogenesis may represent a more readily accessible target for novel therapeutic intervention. In conclusion, it seems that the 'seeding Aβ extracts' and the 'toxic Aβ oligomer extracts' are both biochemically ill-defined mixtures of Aβ peptides, and it remains unclear whether specific species are responsible for these two aspects of the disease process. It is likely that the seeding Aβ species isolated from brain are in complex equilibrium with the postulated toxic Aβ oligomer conformations. The structural explanation for both properties will remain, however, elusive but

nevertheless important. Despite a plethora of studies stretching back over two decades, identifying the toxic Aβ species has proved difficult. Debate has centred on the Aβ fibril and oligomer. Despite support from numerous experimental models, important questions linger regarding the role of the Aβ oligomer in particular. It is likely a huge array of oligomers, rather than a single species, which cause toxicity. Reappraisal of the role of the Aβ fibril points towards a dynamic relationship with the Aβ oligomer within an integrated system, as supported by evidence from microglia. However, some continue to doubt the pathological role of amyloid β, instead proposing a protective role. If the field is to progress, all Aβ oligomers should be characterised, the nomenclature revised and a consistent experimental protocol defined. For this to occur, collaboration will be required between major research groups and innovative analytical tools developed. Such action must surely be taken if amyloid-based therapeutic endeavour is to progress.