β-amyloid oligomers and prion protein

Fatal attraction?

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The relationship between Alzheimer disease (AD) and prion-related encephalopathies (TSE) has been proposed by different points of view. Recently, the scientific attention has been attracted by the results proposing the possibility that PrPᶜ, the protein whose pathologic form is responsible of TSE, can mediated the toxic effect of β amyloid (Aβ) oligomers. The oligomers are considered the culprit of the neurodegenerative process associated to AD, although the pathogenic mechanism activated by these small aggregates remain to be elucidated. In the initial study based on the binding screening, PrPᶜ was identified as ligand/receptor of Aβ oligomers, while long-term potentiation (LTP) analysis in vitro and behavioral studies in vivo demonstrated that the absence of PrPᶜ abolished the damage induced by Aβ oligomers. The high affinity binding Aβ oligomers-PrPᶜ has been confirmed, whereas a functional role of this association has been excluded by three different studies. We approached this issue by the direct application of Aβ oligomers in the brain followed by the behavioral examination of memory deficits. Our data using PrP knockout mice suggest that Aβ 1–42 oligomers are responsible for cognitive impairment in AD but PrPᶜ is not required for their effect. Similarly, in two other studies the LTP alterations induced by Aβ 1–42 oligomers was not influenced by the absence of PrP. Possible explanations of these contradictory results are discussed.

AD is the main cause of dementia in elderly, with prevalence that doubles every five years of age from the 0.5% at 65 years old. AD is characterized by the cerebral accumulation of Aβ in extracellular plaques and the formation of intracellular neurofibrillary tangles constituted by hyperphosphorylated tau protein; the two pathological features are sequentially associated.[1] TSE are rare transmissible neurodegenerative disorders with heterogeneous phenotypes,[2] the genetic cases are associated with mutations of prion protein genes.[3] The conformational change from the cellular (PrPᶜ) to the pathological (PrPˢᶜ) form of prion protein characterized the TSE and PrPˢᶜ is considered the infectious agent that propagates by autocatalytic mechanism in the host.[4] The presence of PrP mutations favors the shift from PrPᶜ to PrPˢᶜ. Both AD and TSE belong to the group of neurodegener-ative disorders associated to protein misfolding. A common molecular mechanism has been proposed to explain the pathogenesis of these disorders characterized by the extracellular accumulation of misfolded proteins. Despite the enormous differences in terms of incidence (ten case AD/10^3/year, one TSE/10^6/year, 10,000-fold less) genetic and neuropathological studies have proposed possible associations in specific populations or in single subjects between AD and TSE. Several evidences indicate that polymorphism at residue 129, a key element in TSE, might also influence the susceptibility to AD.[5-7] More recently, a direct interaction between Aβ and prion protein has been proposed by several points of view. The studies by Hooper's group reported that PrPᶜ can influence the metabolism of amyloid precursor protein (APP) and the

production of Aβ, (reviewed in ref. 8). A series of proteolytic passages mediated the formation of Aβ from APP, the amyloidogenic pathway is initiated by the cleavage by β secretase (BACE) producing soluble βAPP and the C-terminal C99 membrane bound fragment, whereas the following cleavage mediated by γ secretase complex forms Aβ and the amyloid intracellular domain (AICD). Alternatively, the non-amyloidogenic pathway α secretase cleaves APP at in the middle of Aβ sequence forming soluble αAPP and C-terminal fragment. Multiple peptidases, including neprysil, endothelium-converting enzyme and insulin degrading enzyme are involved in Aβ degradation. The accumulation of Aβ is consequence of an imbalance between the formation and the degradation of the peptide. Apparently PrP^c can influence this mechanism through a reduction of β secretase cleavage. The initial results were produced in a human neuroblastoma cell line where the overexpression of PrP^c was associated with a reduction of Aβ production, whereas when PrP^c was silenced by siRNA the Aβ levels increased. An increase of Aβ was also observed in PrP knockout mice due to a reduction in APP cleavage by BACE. Further investigations by the same authors indicate that PrP^c can interact directly with BACE when both are localized in lipid raft, where β secretase cleavage preferentially occurs and glycosaminoglycans played an essential role in mediating this interaction. A regulation of PrP expression by AICD fragment is suggested by the data of Vincent et al.[9] where the traslocation of this fragment acting as transcription factor interacting with the promoter of PrP. Based on this evidence, Kellett and Hooper[8] proposed a potential feedback loop between AICD and PrP^c. The production of AICD and Aβ is inhibited by PrP^c by the modulation of β secretase, in turn an excess of AICD activated the PrP^c synthesis. Thus, the physiological production of Aβ appears controlled by PrP^c. This mechanism is interesting but its effective role in vivo is controversial. Recently Calella et al.[10] showed that double APP/PS1 transgenic mice Aβ and APP levels were not altered by the different expression of PrP^c. However, in this case the presence of PS1 mutation might alter the metabolic pathway associated to Aβ production and the PrP^c is no more effective.

A crosstalk between misfolded proteins was proposed by Morales et al.[11] In this study it has been shown that the inoculation of experimental scrapie in transgenic mice overexpressing human APP (Tg2576) showed an acceleration of onset of clinical symptoms and a shortening of survival time, modest but significant, compared to wild-type littermate. However, neither the histopathological profile of TSE lesions nor the strain feature of the inoculum (RML) was found altered by the passage in Tg 2576 mice. On the other hand, an increase of brain inflammation and Aβ deposition was observed in Tg2576 animals inoculated with experimental scrapie. Furthermore, the determination of PrP^{Sc} at the same post inoculation days in transgenic or wild-type mice showed a clear increase accordingly with the anticipation of symptoms. Based also on the cross seeding demonstrated in vitro using synthetic Aβ and recombinant PrP, the authors explained the synergistic effect in the two diseases as consequence of a direct interaction between the two proteins favoring the protein misfolding. Heterogeneous seeding between different amyloidogenic proteins has been shown by in vitro and in vivo findings;[12,13] this can occur also in the human diseases under appropriate conditions.

The more attractive finding on the interaction between Aβ and PrP^c came from the evidence shown by Lauren et al.[14] indicating PrP^c as a, or "the," receptor capable to mediate the neurotoxic effect of Aβ oligomers. Oligomers, soluble small aggregates of Aβ, are considered responsible for synaptic and cognitive dysfunction as well as neurodegenerative effect in AD. When the neurotoxicity of Aβ peptides was initially described, it was strictly associated to their fibrillogenic capacity;[15,16] however, we found that the toxicity of Aβ peptide could be independent of its aggregation state.[17] Successively, the studies of Walsh and Selkoe demonstrated that protofibrils and oligomers rather than fibers can be responsible of neurodegeneration in AD.[18] In agreement with this concept is the strong correlation between the synaptic loss with cortical levels of soluble Aβ

species rather than with plaques distribution in AD patients.[18-20] In vitro and in vivo studies have now indicated that soluble Aβ oligomers impair synaptic plasticity, inhibiting hippocampal LTP, the electrophysiological correlate of synaptic plasticity.[21-23] Memory impairment and LTP inhibition have also been detected in AD mouse models before plaque deposition in the brain parenchyma.[24-26] The mechanism through which Aβ oligomers act remains uncertain, but interactions have been reported with several receptors such as nicotinic, insulinic and glutamatergic receptors, leading to detrimental effects on synaptic plasticity and spine formation.[27-29] Thus, using an oligomeric preparation according to Klein's group (ADDL),[30,31] Lauren et al.[14] identified the PrP^c as Aβ oligomers ligand based on the screening of cDNA expression library of more than 200,000 proteins. The authors further confirmed these results by the evaluation of the affinity of PrP^c for Aβ oligomers in various conditions. The functional consequence of this association was investigated in vitro by the electrophysological determination LTP suppression induced by Aβ oligomers, in PrP knockout cells, this inhibition was completely abolished. While the binding of Aβ oligomers to PrP^c is a result foreseen because PrP^c has hydrophobic domains that can attract the oligomers, specific interaction with functional consequence was not obvious. Using surface plasmon resonance (SPR) technique and in vivo experimental model we investigated the ability of PrP^c to bind Aβ oligomers and its involvement in their action.[32] Several types of Aβ aggregates isolated from biological sources have been used in rats to test the involvement of Aβ oligomers in memory impairment.[33] In our study we determined which Aβ assemblies are responsible for memory deficit in C57BL/6 receiving into lateral ventricle infusions of initial state, oligomers or fibrils of synthetic Aβ$_{1-42}$ and assessed their cognitive performance in the novel object recognition task.[34,35] We demonstrated that synthetic Aβ oligomers can induce an immediate memory impairment in mice; the effect was detectable at a nanomolar concentration of Aβ oligomers (10–50 nM). In the same conditions, freshly solubilized Aβ

(manly monomeric form) or fibrils were not active. In addition, a 10-day later retest without further oligomer injection found that the memory performance was fully restored showing that the deficit was transient. When we distinguished the effects of Aβ oligomers on memory encoding/consolidation or retrieval we found that they inhibited the encoding/consolidation of information, without affecting its retrieval if properly stored. In fact, the i.c.v. injection of Aβ oligomers before acquisition of the information during the familiarization phase prevented the information being either encoded or consolidated. In contrast, when the Aβ oligomers were applied only 24 h after the information had been processed, no deficit was detected, suggesting that Aβ oligomers do not abolish the retrieval of stabilized information, but do prevent its encoding or consolidation. Memory processing requires NMDA receptor activation and intracellular signaling leading to AMPA receptor trafficking, synthesis of new proteins and formation of dendritic spines.[36-38] In another study using APP23 transgenic mice we found direct evidence that electrophysiological function mediated by NMDA receptors was altered before the formation of Aβ plaques.[39] Thus, our findings indicate the interaction with NMDA receptors as responsible of the detrimental activity of Aβ oligomers.

When Aβ oligomers were injected i.c.v. in PrP knockout mice, we found that the effect of Aβ oligomers on memory was preserved and comparable to that of wild-type mice. We also tested the involvement of PrP^C in mediating Aβ oligomer toxicity in vitro, by investigating their effect on survival of primary hippocampal neurons from wild type or $Prnp^{0/0}$ cells. Aβ oligomers were toxic to both $Prnp^{+/+}$ and $Prnp^{0/0}$ hippocampal cells, consistent with the conclusion that their adverse effects are independent of PrP^C. In the past we already published results comparing PrP 106–126 neurotoxicity with the toxic effect of Aβ 25–35 in $Prnp^{0/0}$ hippocampal cells, the effect of PrP 106–126 disappeared while the toxicity of Aβ fragment was similar to that found in wild-type neurons.[40] On the other hand, surface plasmon resonance (SPR) confirmed a high-affinity interaction between PrP^c

and Aβ oligomers. PrP^c from mouse brain homogenates was captured on the sensor surface of SPR chips by either 3F4 or 94B4, two anti-PrP^c antibodies. The captured protein was actually PrP^c since no signal was detected when flowing brain homogenate coming from $Prnp^{0/0}$ mice. PrP^c captured by both 94B4 and 3F4 maintains its ability to bind 6D11, an anti-PrP antibody directed against the epitope 93–109, i.e., the region suggested to be involved in the interaction with Aβ oligomers. In these conditions Aβ oligomers bound PrP^c specifically, while Aβ initial state and fibrils did not. These data were confirmed by Chen et al.[41] who specifically analyzed the binding of Aβ oligomers to PrP^c using both SPR and side-directed spin labeling. These authors found that in addition to the previously postulated 95–110 region, the segment 23–27 of PrP^c is critically important for effective binding to Aβ oligomers. The affinity of synthetic Aβ for recombinant PrP was confirmed by Calella et al.[10] using less sophisticated technique. The functional role of Aβ oligomers/PrP^c interaction was further investigated by Strittmatter's group crossing PrP$^{0/0}$ mice with APP/PS1 transgenic mice.[42] The memory deficit exhibited by APP/PS1 aged mice was completely rescued when the animals were grown on PrP$^{0/0}$ background. The APP/PS1/PrP$^{0/0}$ mice not only performed better cognitively but also had a significant reduction of mortality compared to APP/PS1/PrP$^{+/+}$. According to these results the absence of PrP^c was sufficient to avoid the cognitive deficits, presumably due to Aβ oligomers, and to improve the general conditions of an AD animal model. In a collaborative study, the same group recently reported that a treatment with an anti-prion antibody for two weeks can also complete nullified the cognitive impairment observed in the APP/PS1 transgenic mice.[43] However two other papers did not confirm the influence of PrP^c on Aβ oligomers activity. Calella et al.[10] showed that either the ablation or the overexpression of PrP^c had no effect on the impairment of hippocampal LTP in double APP/PS1 transgenic mice. The suppression of LTP found in APP/PS1 mice was not altered when PrP was overexpressed or nullified, importantly these results were obtained

with a particular attention to minimize the influence of the genetic background of the mice used. Finally, Kessels et al.[44] using two different methods, showed that Aβ$_{1-42}$ oligomers mediated LTP suppression was independent from the presence of PrP^c. Organotypic hippocampal slice neurons infected with a virus that increased the production and secretion of Aβ showed a depression of synaptic activity in both wild-type or PrP$^{0/0}$ slices. Similar effect in both conditions was found when a reduction of neuronal spines was induced by the APP overexpression or direct application of Aβ$_{1-42}$ oligomers in organotypic hippocampal slices. The authors replicated the LTP inhibition induced by Aβ$_{1-42}$ oligomers but this effect in their hands was not influenced by the absence of PrP^c.

Taken together these results are in agreement on two relevant points, first, the capacity of oligomers deriving from synthetic Aβ$_{1-42}$ peptide, not only bioderived preparation as suggested by others,[23] to induce an impairment of synaptic plasticity and the memory deficits both in vitro and in vivo. Second point, the Aβ$_{1-42}$ oligomers do exhibit a high affinity for PrP^c, as originally shown by Lauren et al.[14] and the 95–110 domain is involved in this binding capacity. The discrepancies emerge when we consider functional aspects consequent to the physical interaction between Aβ$_{1-42}$ oligomers and PrP^C, relevant and consistent for one group, poorly or inexistent for the others. Differences in methodological approaches could explain these conflicting results although the consistency of the other two aspects such as Aβ$_{1-42}$ oligomer detrimental action and their binding to PrP^C, would argue against this possible explanation. As suggested by Lauren et al.[45] in their reply, the different electrophysiological results in vitro, could be for instance consequence of the different Aβ$_{1-42}$ oligomers concentration used. To rule out this possibility, in our experimental conditions we tested either our original preparation of Aβ$_{1-42}$ oligomers, obtained with an incubation of Aβ$_{1-42}$ peptide overnight at 4°C and the Aβ$_{1-42}$ oligomers preparation obtained following the procedure described by Lauren et al.[14] Accordingly to the atomic force microscopy (AFM) analysis the peptide solution at 22°C

included not only oligomers but also few other species, fibers and protofibers, while at 4°C only spherical oligomers were detectable. However, the two preparations exerted similar effects either in the SPR binding studies or in the behavioral tests, thus suggesting that in our hands oligomer concentration and the presence of other chemicophysical species did not influence the biological effect. We must, however, take into account that if small changes in the methodological conditions can nullified the role by PrP^c as mediator of the Aβ_{1-42} oligomer detrimental action on synaptic function, the relevance of the evidence becomes negligible. On the other hand, however the results obtained in APP/PS1/PrP$^{0/0}$ mice, where the absence of PrP^c can completely rescue the behavioral deficits, are in favor of a central role of PrP^c in Aβ_{1-42} oligomers toxicity. As mentioned above the PrP$^{0/0}$ background was shown to affect also the survival of APP/PS1 transgenic mice indicating a favorable influence apparently behind the simple interaction with Aβ_{1-42} oligomers activity. Furthermore, as raised by Peretti,[46] both APP and PS1 transgene in the mice used by Strittmatter's group are under the control of prion promoter. This make the Aβ production and the presence of PrP^c strictly associated over the physiological conditions. Several aspects thus remain to be clarify before to drive any firm conclusion. The interaction between Aβ_{1-42} oligomers and PrP^C has been tested in a single experimental model, and the positive effects of PrP^C ablation in transgenic mice were observable only at twelve months of age when amyloid plaques were already detectable. For this reason, the use of different strains is essential to confirm the relevance of PrP^c in vivo. For instance, in APP23 transgenic mice we have shown that memory deficits occurred before the deposition of Aβ in plaques.[39] A possible explanation of these results is that soluble oligomers inducing memory deficits are formed and they actively affect the cognitive behavior in an early pathological phase that precedes the plaques formation. An association between the appearance of oligomers and memory deficits was found by Lesne et al.[29] while profibrils formation and behavioral deficits before plaque formation have been shown in transgenic

mice combined Arctic and Swedish mutation.[47] If this interpretation is correct, it is assumable that the ablation of PrP^C and, consequently its binding to Aβ oligomers, would abolish the cognitive decline also in these conditions. The appropriate controls are also necessary for the treatment with anti-PrP antibody in APP/PS1 mice, such as the treatment with an antibody that recognizes a domain of PrP not involved in the binding with Aβ_{1-42} oligomers.

However the main problem raised by the functional role of PrP^c as mediator of oligomer toxic effects is the unconvincing concept that PrP^c is responsible for more that 50% of Aβ_{1-42} oligomers binding to neuronal membrane. A recent study has shown that initial exposure of Aβ_{1-42} oligomers, within one hour, affect the membrane mobility of metabotropic glutamate receptors (mGluR5). The Aβ_{1-42} oligomers induced a clustering of mGluR5 in hippocampal cells that elevate the intracellular calcium, potentially responsible of synaptic deterioration.[48] Similar effect was obtained with artificial crosslinking of mGluR5 that increased the intracellular calcium and promote synaptotoxicity. According to this study the interaction of Aβ_{1-42} oligomers with mGluR5 is specific, and the application of an antibody against the extracellular domain of these receptors reduced the binding of oligomers of about 40%. Interestingly, the reduction of Aβ_{1-42} oligomer binding after incubation with the anti-prion antibody (6D11) was also confirmed in this study, but when the antibodies anti-prion and anti-mGluR5 were applied together no increase of the effect with respect to the single antibody was observed. Even with the addition of a third antibody directed against NR1, NMDA receptor subunit, that also reduced the binding of Aβ_{1-42} oligomers by itself, but the total effect did not go over the 30% of binding reduction. Although the incubation time of this experiment was very brief (15 min), no further reduction of the binding was found. The proximity of the different antigens with the Aβ_{1-42} oligomer binding site might explain the result.

On the other hand, the hippocampal cells cultured from mGluR5$^{0/0}$ mice showed a reduction of the Aβ_{1-42} oligomers binding sites in the neuronal spines of

about 80%, in contrast with the findings of Lauren et al.[14], which showed in PrP$^{0/0}$ a 60% reduction of the oligomers binding sites. Several other proteins have been proposed as receptors for Aβ_{1-42} oligomer, such as the voltage-gated calcium channel,[49] the angiotensin II receptor[50] or the nicotinic receptors.[51,52] Furthermore, a new concept of a multi-component assembly required for stabilizing the toxic accumulation of Aβ oligomers at the synaptic membrane involving NMDA receptors has been recently proposed.[53] Taken together, these results indicate that the quantification of Aβ_{1-42} oligomer binding sites is approximate and the relevance of one or the other potential receptors is strongly influenced by the research conditions used.

Furthermore, robust evidence indicates that the interaction between oligomers and cellular membrane can occur without a specific mediation of any proteic entity.[54-56] The Aβ_{1-42} oligomers are inserted in the membrane with change in the membrane fluidity and alteration of citoarchitecture or even internalized within the cells. Several findings have demonstrated that Aβ oligomers are directly incorporated into neuronal membranes and form calcium-permeable ion channels (amyloid channels).[57,58] This concept, originally proposed by Arispe et al.[59], is based on the measurement of ionic current through artificial bilayer membrane exposed to Aβ_{1-42} peptide. Similar results were obtained also with neurotoxic prion peptides 106–126 and 82–146.[60,61] In a recent paper a structural model of oligomers constituted by tetrameric and hexameric β-sheet subunits has been proposed on the basis of chemicophysical and biochemical evidence.[62] Along this line a potential therapeutic approach has been proposed based on the possibility to block with small molecules the amyloid channel and consequently to antagonize Aβ oligomers neurotoxicity.[58] Using an original cellular model, other authors have recently shown that the disruption of lipid rafts affect the toxicity of Aβ oligomers.[63] Using proteomic and immunocytochemistry approaches we found that Aβ toxic species crossed the plasma membrane and accumulated in cells through binding to a variety of internal proteins.[64] In neuroblastoma cell line, Aβ oligomers
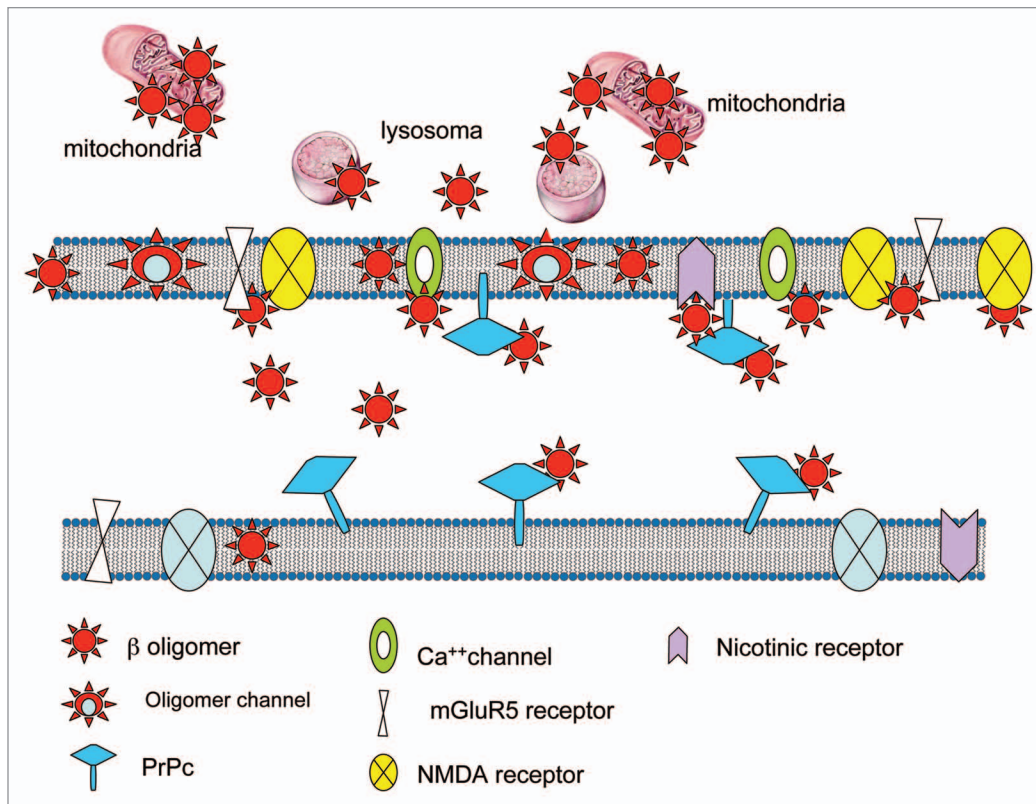
Figure 1. Multiple interactions of Aβ oligomers with different cellular structures.

interact with a large number of proteins located on the cytoskeleton and in the endoplasmatic reticulum. On the other hand, an accumulation of Aβ oligomers was found in the mitochondrial compartment of APP transgenic mice supporting the growing evidence of a major role of mitochondria in AD pathogenesis[65] and underlining the capacity of Aβ oligomers to activate an intraneuronal neurotoxic pathway. In conclusion, the molecular mechanisms responsible for Aβ oligomers synaptotoxicity is extremely complex and the interaction with the neuronal membrane can occur at various levels with multiple processes (**Fig. 1**). In specific conditions it is possible that PrP^c might exert a relevant role, probably as consequence of a sequestration of Aβ oligomers rather than a functional activity directly associated to the protein. Taken together, all this evidence makes us conclude that we are still very far away from proving the absolute relevance of this phenomenon in AD pathogenesis.

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