Targeting small Aβ oligomers: the solution to an Alzheimer's disease conundrum?

William L. Klein, Grant A. Krafft and Caleb E. Finch

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.

William L. Klein*
Northwestern University Institute for Neuroscience and Dept of Neurobiology and Physiology, Northwestern University, 2153 N Campus Drive, Evanston, IL, 60208 USA. Northwestern University's Cognitive Neurology and Alzheimer's Disease Center, Evanston, IL, 60208 USA.
e-mail: wklein@northwestern.edu

Grant A. Krafft
ENH Research Institute and Dept of Molecular Pharmacology, Northwestern University Medial School, Evanston, IL, 60201 USA. Northwestern University's Cognitive Neurology and Alzheimer's Disease Center, Evanston, IL, 60208 USA. Acumen Pharmaceuticals, Inc., Glenview, IL, 60025 USA.

Caleb E. Finch
Ethel Percy Andrus Gerontology Center and Dept of Biological Sciences, University of Southern California, 3715 McClintock Avenue, Los Angeles, CA, 90089 USA.

The amyloid β peptide (Aβ) of Alzheimer's disease (AD) is now showing unexpected structural and biological complexities. Newly recognized forms of the aggregated peptide are soluble, and much smaller than the well-known amyloid fibrils. Moreover, these soluble Aβ assemblies, which exhibit surprising neurological activity, are detected in brain tissue from individuals with AD. Their activity could account for recognized weaknesses of the popular but controversial amyloid cascade hypothesis.

Isolated from AD brains over 15 years ago[1], the Aβ peptide is recognized for its self-aggregation into amyloid. Amyloid comprises large fibrils and β-sheet secondary structure – characterized by Congo red or thioflavin S staining. Aβ peptides consist of 39–43 residues, but the species associated with senile plaques in the brain parenchyma are the less abundant but more hydrophobic 42–43 residue species, which most readily make fibrils. The 40-residue species is found mainly in vascular amyloid. Many studies support the hypothesis that fibrils drive neurodegeneration in AD (the 'amyloid cascade hypothesis' formalized by Hardy and Higgins[2]). *In vivo*, deposits of amyloid constitute a hallmark of AD (for historical background, see http://pubweb.acns.nwu.edu/~wklein/TiNS). Exposure of cultured neurons to synthetic Aβ leads to cell death[3]. Early studies showed that the toxic solutions contained abundant amyloid fibrils in addition to smaller species[4–6]. Other pivotal studies have linked inherited, early-onset AD to various mutations in the amyloid precursor protein (APP) and in presenilins 1 and 2 (PS1 and PS2) that increase production of the highly fibrillogenic $A\beta_{1-42}$ (Refs 7,8). This and other evidence has implicated amyloid fibril-induced nerve cell death as a primary cause of AD.

Nonetheless, in spite of some 5000 publications on Aβ over the past decade, debate over the amyloid cascade hypothesis remains contentious. Most problematic and at the heart of the problem, as argued by Terry and colleagues[9], is the weak correlation between fibrillar amyloid load and measures of neurological dysfunction. In AD, moreover, amyloid deposits often form at a distance from sites of neuron loss. They also develop in cognitively normal individuals who have no evidence of local neuron damage. The best pathological correlate of dementia is loss of synaptic terminals, which correlates poorly with amyloid load[9].

We are left with a fundamental puzzle – if manifestations of disease correlate weakly with amyloid, then what is the role played by Aβ? Findings reviewed below suggest a simple solution: fibrils are not the only neurotoxic form of Aβ, perhaps not even the most significant form for AD. Aβ also assembles into soluble forms (protofibrils and small oligomers; Fig. 1), which could affect neurons, but escape detection by measurements of solid amyloid. Soluble toxins would account for the poor correlation between fibrillar amyloid and disease progression, and could provide a unifying mechanism for AD pathogenesis. Soluble Aβ-derived toxins might also prove to be a crucial target for AD vaccine development.

APP transgenic mice models for AD: CNS deficits without detectable amyloid

A huge clue for understanding Aβ-driven pathogenesis comes from transgenic mice models for AD in which transgenes for human APP (hu-APP) provide elevated brain levels of Aβ. As anticipated, multiple strains show specific AD-like neurological deficits. The surprise is that most of these deficits occur in the absence of amyloid deposits (for a summary, see http://pubweb.acns.nwu.edu/~wklein/TiNS). These animal models thus recapitulate (in an exaggerated manner) the weak correlation between amyloid and disease in humans.

The profound disconnection between pathogenesis and amyloid is exemplified by recent results from Mucke and colleagues[12], who engineered multiple transgenic mice strains that overexpressed either mutant or wild-type hu-APP. Amyloid deposits were

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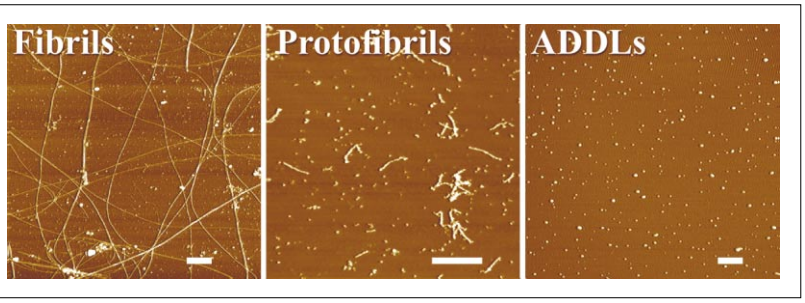

Fig. 1. Different assembled states of amyloid β_{1-42} ($A\beta_{1-42}$). The assembled forms obtained from incubation of synthetic $A\beta_{1-42}$ are highly sensitive to preparation and incubation[10]. Widely differing proportions of insoluble fibrils, soluble protofibrils (PFs) and oligomers are revealed by atomic force microscopy[11]. Typical PF and fibril preparations contain varying levels of small globular molecules, putatively $A\beta_{1-42}$ oligomers; Aβ-derived diffusible ligand (ADDL) preparations initiated from monomeric dimethyl sulphoxide stock solutions are fibril- and PF-free, and (uniquely) comprise oligomers. Scale bar, 200 nm. Fibril, PF and ADDL preparations all show neurotoxicity *in vitro*. Courtesy of Brett Chromy and Blaine Stine.

found in mutant strains, but not in wild type. When scored for synaptophysin immunoreactivity, all mice showed loss of synaptic terminals. APP transgenes thus triggered synapse loss regardless of whether amyloid was deposited. Altered APP expression *per se* was not the culprit because synapse loss and other deficits were unrelated to transgene hu-APP levels[12]. Instead, synapse loss correlated with levels of soluble Aβ (Fig. 2a). This observation is particularly significant because synapse loss is an excellent correlate of cognitive decline in individuals with AD (Ref. 9).

The soluble Aβ hypothesis is in harmony with the idea that small oligomeric species of Aβ drive pathogenesis, as suggested in an earlier study[14]. Transgenic animals with CNS deficits were once considered poor models of AD pathogenesis if they lacked amyloid deposits. Now, in an interesting turnaround, it appears that such animals actually provide good models for pathogenesis by non-fibrillar

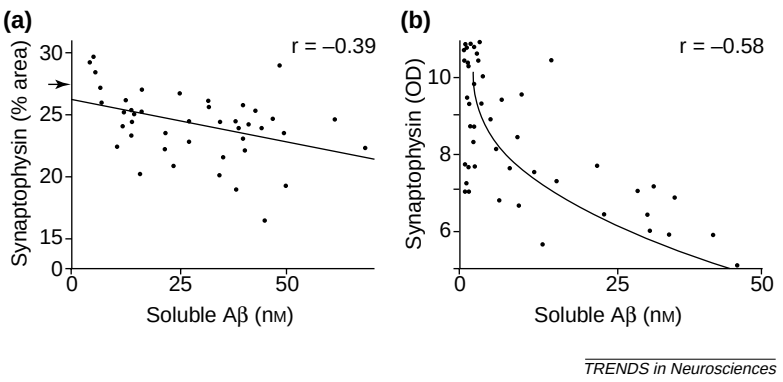

Fig. 2. Synaptic density correlates inversely with soluble amyloid β (sAβ). (a) Transgenic mice carrying human amyloid precursor protein (APP) showed inverse correlations between sAβ in the hippocampus and synaptophysin levels (immunoreactive presynaptic terminals). The immunoassay detected $sA\beta_{1-40}$, $sA\beta_{1-42(3)}$ and C-terminal truncated forms. The graph represents results from seven transgenic lines (three wild-type and four APP mutants associated with AD). (b) Brains from individuals with Alzheimer's disease showed inverse correlations between $sA\beta_{1-40}$ and $sA\beta_{1-42}$ in the entorhinal cortex and superior frontal gyrus, and synaptophysin levels (western blots). nM is equivalent to nM or to pmol Aβ/g brain. (a) Modified, with permission, from Ref. 12; (b) modified, with permission from Ref. 13.

Aβ. Evidence that particular Aβ oligomers occur in transgenic mice has been obtained by Younkin and colleagues, although their mice also produce amyloid[15]. These mice show age-dependent accumulation of oligomeric Aβ, roughly concomitant with onset of behavioral abnormalities.

Plaque-independent CNS pathology can be explained by the neurotoxicity of Aβ oligomers

Aβ monomers are innocuous and must self-associate to become neurotoxic, which until recently was taken to mean that Aβ must assemble into amyloid fibrils[5,6]. However, consistent with the transgenic mice story, recent data show that neurotoxicity can be fibril independent, but still dependent upon self-association.

One toxic form is the Aβ-derived protofibril (PF), first discovered as an intermediate in $A\beta_{1-40}$-amyloidogenesis[16]. By atomic force microscopy, PFs can be seen to be curvilinear structures of 4–11 nm diameter and <200 nm length[17]. They lack stability at low monomer concentrations but nonetheless can be isolated by molecular sieve chromatography. Isolated PFs, although free of monomers and amyloid fibrils, are bioactive. In cell culture, PFs cause oxidative stress and, eventually, neuronal death[16]. They also elicit rapid electrophysiological changes, inducing membrane depolarization, and increased EPSPs and action potentials[17]. In spite of their thermodynamic instability, PFs apparently occur in CSF, according to a preliminary analysis of individuals with AD (Ref. 18).

Another neurotoxic form of Aβ distinct from PFs comprises small oligomers[19], which are more stable than PFs at low levels of Aβ, particularly for the AD-linked $A\beta_{1-42}$ species. Metabolically derived oligomers accumulate in conditioned cell culture medium without evidence of PFs or amyloid fibrils[20]. Oligomer levels increase after transfection with mutated familial AD presenilins[21], which elevate levels of $A\beta_{1-42}$. Solutions of synthetic $A\beta_{1-42}$ oligomers without fibrils or PFs can be made readily[19] (Fig. 1), although the converse is not true. The stability of fibril-free solutions of toxic oligomers is consistent with the plaque-free pathology of transgenic mice discussed above.

The first evidence that any non-fibrillar Aβ might be toxic to neurons came from experiments in which $A\beta_{1-42}$ was mixed with apolipoprotein J (ApoJ)[22], which is secreted by astrocytes. At substoichiometric ratios, ApoJ inhibited formation of fibrillar Aβ, but, unexpectedly, it did not inhibit Aβ neurotoxicity, as measured by MTT reduction in PC12 cells. Toxicity of these solutions has been verified in live–dead assays in CNS slice cultures[19]. These findings are contrary to the amyloid cascade hypothesis, which predicts fibril blockers should inhibit Aβ toxicity.

By atomic force microscopy, the ApoJ/$A\beta_{1-42}$ toxic preparations are free of PFs and other large structures (Fig. 1). Instead, the toxic molecules, which diffuse through filters, comprise only small Aβ

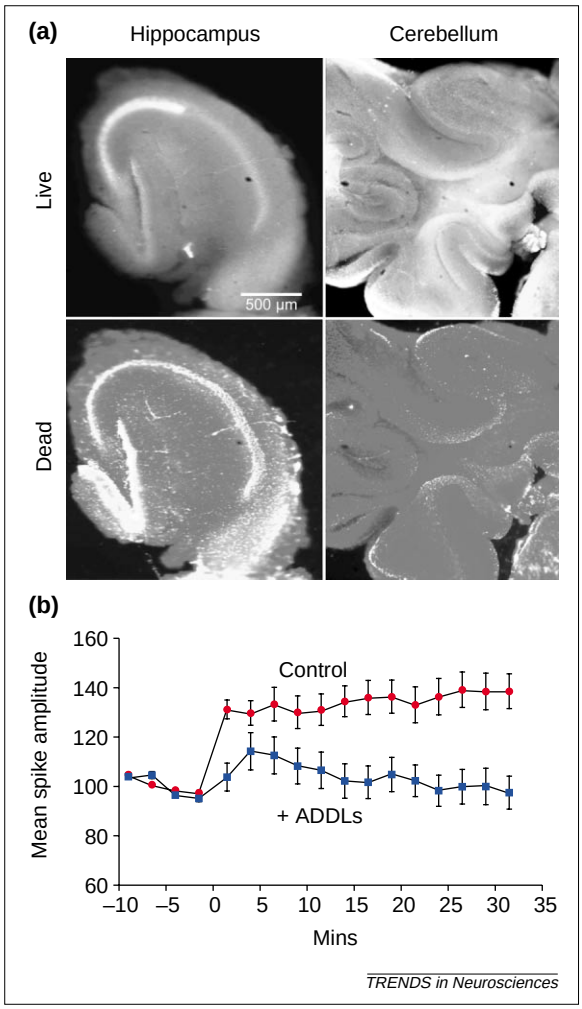

Fig. 3. *In vitro* activities of Aβ-derived diffusible ligands (ADDL): rapid blockade of hippocampal LTP and slower death of neurons. (a) ADDLs are potent neurotoxins that slowly kill hippocampal neurons in mature brain slice preparations[19]. With the live–dead dual fluorescence assay, ADDLs selectively induce death in hippocampal CA1 neurons, whereas a subpopulation of CA3 neurons and cerebellar neurons are resistant (J. Kim and G.A. Krafft, unpublished observations). (b) ADDLs block LTP in hippocampal slice within 1 hr. *In vivo* stereotaxic injections give similar results[24]. As seen here, ADDLs do not block pre-tetanic population spikes, nor do they inhibit EPSPs or LTD (B. Trommer, unpublished observations). The immediate effects of ADDLs thus are selective for particular mechanisms in neuroplasticity. (b) reproduced, with permission, from Ref. 19.

oligomers[19]. Toxic oligomers also form without ApoJ, indicating that ApoJ might act as a chaperone, decreasing fibril growth from small 'seeds' while enabling monomers to form semi-stable oligomers. We have given the toxic oligomers the abbreviation ADDL (Aβ-derived diffusible ligands). Depending on conditions, ADDL preparations can contain predominantly trimers–hexamers, with larger oligomers of up to 24 mers[23]. ADDL-sized species are evident in some PF preparations[16], but the possible conversion between ADDLs and larger species remains unexplored.

ADDLs show important regionally selective neurotoxicity, sparing neurons in the cerebellum while selectively killing neurons in hippocampal CA1 region and entorhinal cortex (Fig. 3a). This specificity models the regional pattern of neurodegeneration in AD. By contrast, conventional $A\beta_{1-42}$ aggregates containing mostly fibrils do not spare cerebellar neurons, either in slice or cell cultures[17].

Oligomer-induced memory dysfunction before neuron death

Research focusing on the electrophysiological impact of Aβ oligomers suggests a new concept for early-stage AD. Declarative memory impairment occurs early in AD and typically is attributed to nerve cell death[25]. An additional mechanism for memory loss is suggested by the rapid inhibition by ADDLs of LTP – a classical paradigm for synaptic plasticity and memory mechanisms[26]. Complete inhibition takes place in less than 1 hr *in vivo*[24] and in culture (Fig. 3b). Unpotentiated population spikes, EPSPs and LTD are unaffected[19] (B. Trommer, unpublished observations). Chemical variants of Aβ that lack neurotoxicity or the capacity to form fibrils also block LTP (Ref. 27). Furthermore, LTP can be impaired in some transgenic mice with normal synaptic terminals, fast synaptic transmission and short-term plasticity[28]. Overall, these results indicate that novel mechanisms independent of neuron death could contribute to mild cognitive impairments in early AD.

Signal transduction

The kinetics and specificity of LTP inhibition suggest that ADDLs could target signal transduction. This possibility is untested for LTP, but knockout experiments have implicated an LTP-related protein tyrosine kinase, Fyn (Ref. 29), in ADDL-induced neuron death[19]. The role of Fyn in LTP is presumably downstream from glutamate receptors, to which it must be tethered by the scaffold protein PSD95 (Ref. 30). Fyn signaling pathways also lead to upregulation of reactive oxygen species[31], known to be associated with ADDL- and fibril-induced neurotoxicity through a mechanism recently attributed to iron release from aconitase[32]. Fyn has been linked specifically to the memory dysfunction associated with murine AIDS (Ref. 33) and was recently found to play a role in prion signal transduction[34]. Of particular significance for AD are the observations that Fyn is coupled through GSK3β to tau phosphorylation[35,36] and that its activity is increased 400% in tangle-positive neurons in AD-affected brains[37]. It has been suggested that Fyn signaling might be affected by ADDLs via specific cell-surface toxin receptors[19], but intracellular ADDL–Fyn association is also possible[24].

We propose a dual-toxin hypothesis for AD that comprises three stages of neural dysfunction and degeneration (Fig. 4). The hypothesis derives in part from the rapid impact of ADDLs on LTP, along with the weak correlation of amyloid with pathogenesis. Because Aβ is also a normal metabolite, produced through highly regulated proteolysis[38], we speculate that transient physiological elevations in $A\beta_{1-42}$ (which should exponentially favor oligomerization) could exert physiological control. With increased exposure and dose, the oligomers would convert from physiological to pathogenic molecules in AD.

A trend: soluble oligomers correlate better than fibrils with neurodegeneration

A crucial question that needs to be answered is whether nonfibrillar Aβ toxins exist in individuals with AD. Although we do not have the final answer, a

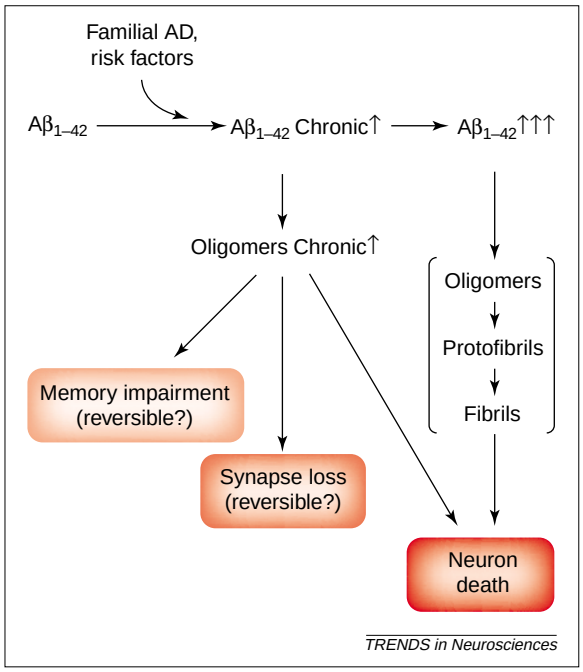

Fig. 4. Pathogenic amyloid β_{1-42} ($A\beta_{1-42}$) cascades. Alzheimer's disease (AD) is linked to chronic elevations in brain levels of $A\beta_{1-42}$, which are associated with familial risk factors for AD (mutations in presenilins 1 and 2, and amyloid precursor protein) and possibly other risk factors (head trauma, apolipoprotein E4 alleles). Thermodynamic stability of the elevated $A\beta_{1-42}$ can result from its self-association into oligomers, which are relatively stable at low concentrations, both for synthetic[19] and metabolically derived $A\beta_{1-42}$ (Ref. 20). The Aβ cascade would at first impair memory mechanisms (as observed experimentally in loss of LTP). As oligomers increasingly accumulate and duration of exposure expands, synaptic terminals would be damaged or destroyed; eventually, synaptic regression and other ADDL-induced effects will lead to neuron death. The structurally higher order protofibrils and macroscopic fibrils, which require higher concentrations of $A\beta_{1-42}$ for assembly, will eventually accumulate to toxic levels as well, participating with Aβ-derived diffusible ligands in an overall pathogenic Aβ cascade.

shift in thinking is illustrated by a recent comment from Beyreuther and colleagues, who first characterized the *APP* gene: 'Fibrillar Aβ has historically been viewed as the primary candidate for the neurotoxic element in Alzheimer's disease…[but recently we have] observed a clear correlation between the severity of the disease and what has been described as the soluble amyloid component'[39,40]. Strong evidence to support this conclusion comes from Lue *et al.*, who showed that synapse loss in AD correlates with soluble Aβ, but not with amyloid[13]. There is a remarkable correspondence in the apparent neurotoxic dose–response to soluble Aβ in AD and amyloid-free transgenic mice (Fig. 2b). It is noteworthy that brains from cognitively normal 80-year olds have significant levels of soluble $A\beta_{1-42}$ (Ref. 41). Multiple analyzes of human cortex agree well in their measurements of soluble Aβ levels in normal as well as in diseased brain, with AD brain consistently showing a major increase: more than 30-fold (see http://pubweb.acns.nwu.edu/~wklein/TiNS).

Identifying the specific species of soluble Aβ will be difficult, as levels are low and the peptides are sensitive to solution conditions. Nonetheless, five research groups found that AD brains contain oligomeric Aβ. Small SDS-stable oligomers were reported by Wisniewski *et al.* as early as 1994 (Ref. 42) but were considered as only intermediates on the way to becoming toxic amyloid. Roher and colleagues found that complex mixtures of water-soluble oligomers, detectable in normal brain, were elevated 12-fold in individuals with AD (Ref. 43). Moreover, dimers isolated from AD brains can kill neurons indirectly by acting through microglia[44]. According to Guerette *et al.*, dimers appear to be a minor species[45] and they have no rapid electrophysiological impact. Sodium dodecyl sulfate-stable oligomers can be obtained from insoluble amyloid fractions from aging human cortex[46], whereas CSF contains Aβ aggregates that could be protofibrils[47]. Final answers regarding the nature of Aβ toxins in AD might depend on antibodies that target unique epitopes of particular Aβ-derived species. Motivation to develop such antibodies has gained a tremendous boost from exciting new results showing that Aβ antibodies could be therapeutic.

What would be the target of an optimum Alzheimer's vaccine?

Antibodies designed to combat the CNS effects of Aβ are now in early-stage clinical trials[48]. Immunization against Aβ neurotoxicity represents a major new approach to AD therapeutics and is based on the revolutionary findings of Schenk and colleagues[49]. If immunization is established as a safe procedure, its optimum use will require knowledge of the therapeutic mechanism and the specific antigen(s) being targeted.

In Schenk's experiments, amyloid-producing transgenic mice were injected with solutions of full-length $A\beta_{1-42}$, aged overnight in buffer. Such aged Aβ solutions typically contain amyloid fibrils together with a mixture of smaller species which, in our experience, typically include ADDLs. Vaccination produced a startling reduction in amyloid that also appeared to benefit neuritic structure. Similar vaccination of other transgenic mice AD models preserved performance in memory tasks measured by the Morris water maze[48,50]. Most recently, amyloid burden also has been reduced by passive immunization with Aβ antibodies[51]. Thus, perhaps for AD, as well as stroke[52], therapeutic antibodies will potentially penetrate the blood–brain barrier.

It has been suggested that antibodies to Aβ might indirectly activate microglia[49], which leads to clearance of Aβ either before or after plaque formation. Alternatively, various Aβ-derived toxins could be targeted directly. A potentially therapeutic single chain antibody obtained by phage display dissolves fibrils *in vitro* and prevents toxicity in PC12 cells[53,54]. This antibody targets the N-terminal amino acids that play a role in Aβ self-association. Aside from therapeutic value, these and other antibodies should be powerful tools for identifying toxic epitopes, which

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should provide molecular insights for the development of traditional small organic molecule drugs.

An important unanswered question is whether immunization provides benefit to amyloid-free transgenic mice models. Given the neurological deficits in plaque-free transgenic mice, and the correlation between soluble Aβ and synapse loss, we hypothesize that major benefit from vaccines comes from immuno-neutralization of ADDLs or PFs, independently of plaque disappearance. ADDLs are immunogenic at low doses, more so than Aβ monomers, and oligomer-selective antisera show neuroprotection in nerve cell culture experiments[55]. If the model in Fig. 4 is correct, therapeutic antibodies designed to target oligomers could ultimately intervene early in AD.

References

1 Glenner, G.G. and Wong, C.W. (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* 120, 885–890

2 Hardy, J.A. and Higgins, G.A. (1992) Alzheimer's disease: the amyloid cascade hypothesis. *Science* 256, 184–185

3 Loo, D.T. *et al.* (1993) Apoptosis is induced by beta-amyloid in cultured central nervous system neurons. *Proc. Natl. Acad. Sci. U. S. A.* 90, 7951–7955

4 Howlett, D.R. *et al.* (1995) Aggregation state and neurotoxic properties of Alzheimer beta-amyloid peptide. *Neurodegeneration* 4, 23–32

5 Lorenzo, A. and Yankner, B.A. (1994) Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. *Proc. Natl. Acad. Sci. U. S. A.* 91, 12243–12247

6 Pike, C.J. *et al.* (1993) Neurodegeneration induced by beta-amyloid peptides *in vitro*: the role of peptide assembly state. *J. Neurosci.* 13, 1676–1687

7 Selkoe, D.J. (1997) Alzheimer's disease: genotypes, phenotypes, and treatments. *Science* 275, 630–631

8 Scheuner, D. *et al.* (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med.* 2, 864–870

9 Terry, R.D. (1999) The neuropathology of Alzheimer disease and the structural basis of its cognitive alterations. In *Alzheimer Disease* (Terry, R.D. *et al.*, eds), pp. 187–206, Lippincott Williams and Wilkins

10 Busciglio, J. *et al.* (1992) Methodological variables in the assessment of beta amyloid neurotoxicity. *Neurobiol. Aging* 13, 609–612

11 Stine, W.B.J. *et al.* (1996) The nanometer-scale structure of amyloid-beta visualized by atomic force microscopy. *J. Prot. Chem.* 15, 193–203

12 Mucke, L. *et al.* (2000) High-level neuronal expression of Aβ 1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J. Neurosci.* 20, 4050–4058

13 Lue, L.F. *et al.* (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am. J. Pathol.* 155, 853–862

14 Hsia, A.Y. *et al.* (1999) Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. U. S. A.* 96, 3228–3233

15 Kawarabayashi, K. *et al.* Age dependent changes in brain, cerebrospinal fluid, and plasma amyloid β protein in the Tg2576 transgenic mouse model of Alzheimer's disease. *J. Neurosci.* (in press)

16 Walsh, D.M. *et al.* (1999) Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates. *J. Biol. Chem.* 274, 25945–25952

17 Hartley, D.M. *et al.* (1999) Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons. *J. Neurosci.* 19, 8876–8884

18 Pitschke, M. *et al.* (1998) Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. *Nat. Med.* 4, 832–834

19 Lambert, M.P. *et al.* (1998) Diffusible, nonfibrillar ligands derived from Aβ1–42 are potent central nervous system neurotoxins. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6448–6453

20 Podlisny, M.B. *et al.* (1995) Aggregation of secreted amyloid β-protein into sodium dodecyl sulfate-stable oligomers in cell culture. *J. Biol. Chem.* 270, 9564–9570

21 Xia, W. *et al.* (1997) Enhanced production and oligomerization of the 42-residue amyloid β-protein by Chinese hamster ovary cells stably expressing mutant presenilins. *J. Biol. Chem.* 272, 7977–7982

22 Oda, T. *et al.* (1995) Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1-42) and forms slowly sedimenting A beta complexes that cause oxidative stress. *Exp. Neurol.* 136, 22–31

23 Chromy, B.A. *et al.* (1999) Stability of small oligomers of $A\beta_{1-42}$ (ADDLs). *Soc. Neurosci. Abstr.* 25, 2129

24 Klein, W.L. (2000) Aβ toxicity in Alzheimer's disease. In *Contemporary Clinical Neuroscience: Molecular Mechanisms of Neurodegenerative Diseases* (Chesselet, M.F., ed.), p. 47, Humana Press

25 Gomez-Isla, T. *et al.* (1996) Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J. Neurosci.* 16, 4491–4500

26 Soderling, T.R. and Derkach, V.A. (2000) Postsynaptic protein phosphorylation and LTP. *Trends Neurosci.* 23, 75–80

27 Chen, Q.S. *et al.* (2000) Impairment of hippocampal long-term potentiation by Alzheimer amyloid beta-peptides. *J. Neurosci. Res.* 60, 65–72

28 Chapman, P.F. *et al.* (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat. Neurosci.* 2, 271–276

29 Grant, S.G. and Silva, A.J. (1994) Targeting learning. *Trends Neurosci.* 17, 71–75

30 Tezuka, T. *et al.* (1999) PSD-95 promotes Fyn-mediated tyrosine phosphorylation of the *N*-methyl-D-aspartate receptor subunit NR2A. *Proc. Natl. Acad. Sci. U. S. A.* 96, 435–440

31 Altun-Gultekin, Z.F. and Wagner, J.A. (1996) Src, ras, and rac mediate the migratory response elicited by NGF and PMA in PC12 cells. *J. Neurosci. Res.* 44, 308–327

32 Longo, V.D. *et al.* (2000) Reversible inactivation of superoxide-sensitive aconitase in Aβ1–42–treated neuronal cell lines. *J. Neurochem.* 75, 1977–1985

33 Sei, Y. *et al.* (1996) Altered brain fyn kinase in a murine acquired immunodeficiency syndrome. *FASEB J.* 10, 339–344

34 Mouillet-Richard, S. *et al.* (2000) Signal transduction through prion protein. *Science* 289, 1925–1928

35 Lesort, M. *et al.* (1999) Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase. *J. Neurochem.* 72, 576–584

36 Lee, G. *et al.* (1998) Tau interacts with src-family non-receptor tyrosine kinases. *J. Cell Sci.* 111, 3167–3177

37 Shirazi, S.K. and Wood, J.G. (1993) The protein tyrosine kinase, fyn, in Alzheimer's disease pathology. *NeuroReport* 4, 435–437

38 Gabuzda, D. *et al.* (1993) Inhibition of beta-amyloid production by activation of protein kinase C. *J. Neurochem.* 61, 2326–2329

39 Guerrette, P.A. *et al.* (1999) Oligomeric Aβ in PBS–soluble extracts of human Alzheimer's brain. *Soc. Neurosci. Abstr.* 25, 2129

40 McLean, C.A. *et al.* (1999) Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* 46, 860–866

41 Funato, H. *et al.* (1998) Quantitation of amyloid beta-protein (A beta) in the cortex during aging and in Alzheimer's disease. *Am. J. Pathol.* 152, 1633–1640

42 Frackowiak, J. *et al.* (1994) Non-fibrillar beta-amyloid protein is associated with smooth muscle cells of vessel walls in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 53, 637–645

43 Kuo, Y.M. *et al.* (1996) Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. *J. Biol. Chem.* 271, 4077–4081

44 Roher, A.E. *et al.* (1996) Morphology and toxicity of $A\beta_{1-42}$ dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. *J. Biol. Chem.* 271, 20631–20635

45 Guerette, P.A. *et al.* (1999) Oligomeric Aβ in PBS-soluble extracts of human Alzheimer brain. *Soc. Neurosci. Abstr.* 25, 2129

46 Enya, M. *et al.* (1999) Appearance of sodium dodecyl sulfate-stable amyloid beta-protein Aβ dimer in the cortex during aging. *Am. J. Pathol.* 154, 271–279

47 Pitschke, M. *et al.* (1998) Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. *Nat. Med.* 4, 832–834

48 Helmuth, L. (2000) Alzheimer's congress. Further progress on a beta-amyloid vaccine. *Science* 289, 375

49 Schenk, D. *et al.* (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like

pathology in the PDAPP mouse. *Nature* 400, 173–177
50 Arendash, G. *et al.* (2000) Behavioral consequences of vaccination with human β-amyloid in mutant APP + PS1 transgenic mice. *Soc. Neurosci. Abstr.* 26, 1059
51 Bard, F. *et al.* (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat. Med.* 6, 916–919
52 During, M.J. *et al.* (2000) An oral vaccine against NMDAR1 with efficacy in experimental stroke and epilepsy. *Science* 287, 1453–1460
53 Frenkel, D. *et al.* (2000) Immunization against Alzheimer's beta-amyloid plaques via EFRH phage administration. *Proc. Natl. Acad. Sci. U. S. A.* 97, 11455–11459
54 Frenkel, D. *et al.* (2000) Modulation of Alzheimer's beta-amyloid neurotoxicity by site-directed single-chain antibody. *J. Neuroimmunol.* 106, 23–31
55 Klein, W.L. *et al.* (2000) Oligomer/conformation-dependent Aβ antibodies. *Soc. Neurosci. Abstr.* 26, 1285

The link between excitotoxic oligodendroglial death and demyelinating diseases

Carlos Matute, Elena Alberdi, María Domercq, Fernando Pérez-Cerdá, Alberto Pérez-Samartín and María Victoria Sánchez-Gómez

Oligodendrocytes, the myelinating cells of CNS axons, are highly vulnerable to excitotoxic signals mediated by glutamate receptors of the AMPA and kainate classes. Receptors in these cells are commonly activated by glutamate that is released from axons and glial cells. In addition, oligodendrocytes contribute to the control of extracellular glutamate levels by means of their own transporters. However, acute and chronic alterations in glutamate homeostasis can result in overactivation of AMPA and kainate receptors and subsequent excitotoxic oligodendroglial death. Furthermore, demyelinating lesions caused by excitotoxins can be similar to those observed in multiple sclerosis. This, together with the effect of AMPA and kainate receptor antagonists in ameliorating the neurological score of animals with experimental autoimmune encephalomyelitis (an animal model of multiple sclerosis), indicates that oligodendrocyte excitotoxicity could be involved in the pathogenesis of demyelinating disorders.

Carlos Matute*
Elena Alberdi
María Domercq
Fernando Pérez-Cerdá
Alberto Pérez-Samartín
María Victoria Sánchez-Gómez
Departamento de Neurociencias, Universidad del País Vasco, 48940-Leioa, Spain.
*e-mail: onpmaalc@lg.ehu.es

Enhanced glutamate signaling can lead to excitotoxicity, a phenomenon whereby overactivation of glutamate receptors (GluRs) triggers cell death. Excitotoxicity was first described in the late 1950s in retinal neurons[1]. Later, Olney and co-workers[2] found that this vulnerability is shared by all central neurons that contain GluRs. Thereafter, glutamate excitotoxicity has been implicated in acute injury to the CNS and in chronic neurodegenerative disorders[3–5].

The concept of excitotoxic cell death has recently been expanded to CNS glia. Glial cells include astrocytes, oligodendrocytes and microglia, which are distributed throughout the CNS. Classical studies have assigned to glia various roles that contribute to the support of neuronal function[6]. In addition, in the past few years it has been shown that astrocytes and oligodendroglia can actively participate in neurotransmission[7–9]. Strikingly, oligodendrocytes, which myelinate axons and constitute the vast majority of cells in the white matter, are highly vulnerable to overactivation of GluRs (Refs 10–12). This feature led to the proposal that oligodendroglial excitotoxicity might also be involved in the pathogenesis of demyelinating diseases[13–15], which are characterized by the destruction of myelin, oligodendrocyte cell death and inflammation[16,17]. This review discusses the current knowledge of the determinants of glutamate signaling in oligodendrocytes, the vulnerability of these cells to glutamate excitotoxicity and the evidence pointing to the relevance of this process in demyelinating disorders of the CNS.

All major types of CNS glial cells participate in glutamate signaling

Glutamate activates ionotropic and metabotropic receptors present in neurons and glial cells. Ionotropic GluRs can directly mediate excitotoxicity[18]. According to pharmacological, electrophysiological and molecular properties, ionotropic GluRs are classified as AMPA (subunits GluR1–4), kainate (subunits GluR5–7 and KA1–2) and NMDA (subunits NR1 and NR2A–D) receptors[18–20]. However, it should be noted that in spite of this nomenclature, kainate activates both AMPA and kainate receptors[21].

AMPA and kainate receptors are commonly present in astrocytes and oligodendrocytes[22–25] as well as in microglia[26,27]. By contrast, NMDA receptors are rare or absent in these cells[23]. Importantly, AMPA receptors in differentiated oligodendrocytes (Table 1), both *in vitro* and *in situ*, lack the GluR2 subunit, a feature that renders them permeable to Ca^{2+} (Ref. 31). In addition, the kainate receptor subunit GluR6, which is expressed in oligodendrocytes is edited to a

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