Intraneuronal Aβ as a trigger for neuron loss: can this be translated into human pathology?

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Abstract
In the present review, we summarize the current achievements of modelling early intraneuronal Aβ (amyloid β-peptide) accumulation in transgenic mice with the resulting pathological consequences. Of special importance will be to discuss recent developments and the translation of the results to AD (Alzheimer’s disease). N-terminally truncated AβpE3 (Aβ starting with pyroglutamate at position 3) represents a major fraction of all Aβ peptides in the brain of AD patients. Recently, we generated a novel mAb (monoclonal antibody), 9D5, that selectively recognizes oligomeric assemblies of AβpE3 and demonstrated the potential involvement of oligomeric AβpE3 in vivo using transgenic mouse models as well as human brains from sporadic and familial AD cases. 9D5 showed an unusual staining pattern with almost non-detectable plaques in sporadic AD patients and non-demented controls. Interestingly, in sporadic and familial AD cases prominent intraneuronal staining was observed. Moreover, passive immunization of 5XFAD mice with 9D5 significantly reduced overall Aβ levels and stabilized behavioural deficits. In summary, we have demonstrated that intraneuronal Aβ is a valid risk factor in model systems and AD patients. This feature of AD pathology was successful in identifying novel low-molecular-mass oligomeric Aβ-specific antibodies for diagnosis and therapy.

Introduction
AD (Alzheimer’s disease) represents the most frequent form of dementia and is characterized by two major neuro-pathological hallmarks: (i) extracellular plaques composed of the 40–42 residues Aβ (amyloid β-peptide) peptide [1–3] and (ii) neurofibrillary tangles consisting of abnormal phosphorylated tau protein [4]. The discovery that certain early-onset familial forms of AD may be caused by enhanced levels of Aβ peptides have led to the hypothesis that amyloidogenic Aβ is intimately involved in the AD pathogenic process [5]. In the past, extracellular Aβ has been regarded as the major culprit, whereas more recent evidence now points to toxic effects of Aβ in intracellular compartments [6,7]. In addition, other concepts proposed that soluble oligomers and β-sheets-containing amyloid fibrils are the toxic forms of Aβ [8–10]. Supporting this notion, it has been demonstrated that soluble oligomeric Aβ42, but not plaque-associated Aβ, correlates best with cognitive dysfunction in AD [11,12]. As oligomers are formed preferentially intracellularly within neuronal processes and synapses rather than extracellularly [13,14], the ‘oligomer’ and ‘intraneuronal’ concepts do not exclude each other.

Key words: Alzheimer’s disease (AD), amyloid β-peptide (Aβ), amyloid precursor protein (APP), human pathology, intraneuronal amyloid β-peptide (intraneuronal Aβ), neuron loss.

Abbreviations used: Aβ, amyloid β-peptide; AβpE3, Aβ starting with pyroglutamate at position 3; AD, Alzheimer’s disease; ADDL, Aβ-derived diffusible ligand; APP, amyloid precursor protein; mAb, monoclonal antibody; QC, glutaminyl cyclase.

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Role of soluble oligomers and protofibrils
Soluble oligomers [also described as ADDLs (Aβ-derived diffusible ligands) and/or protofibrils] of Aβ have been discussed to be causally involved in synaptic and cognitive dysfunction in the early stages of AD [15,16]. However, there is no consensus on which aggregation state exerts the highest toxicity in AD. The formation of Aβ oligomers has been repeatedly reported to contribute significantly to the pathophysiological alterations underlying AD and it has been shown that soluble oligomeric Aβ42 and not plaque-associated Aβ correlates best with cognitive dysfunction [11,12]. Oligomers are formed preferentially intracellularly within neuronal processes and synapses rather than within the extracellular space [13,14] and have been demonstrated to cause synaptic alterations [17,18]. These oligomers have been previously demonstrated to inhibit hippocampal long-term potentiation and disrupt synaptic plasticity [19,20] and have been shown to originate from intraneuronal rather than extracellular dimerization [13]. By the use of antibodies raised against synthetic Aβ oligomer assemblies, these oligomers or ADDLs have been detected in up to 70-fold higher concentrations in AD compared with control brains [21]. However, as these oligomers normally occur both inside neurons as well as in a secreted form, it is difficult to attribute the toxic actions to one of the different entities. In addition, it has been shown that Aβ-derived oligomers specifically bind to pyramidal neurons, promoting a rapid decrease in the membrane expression levels of memory-related receptors such as NMDA (N-methyl-d-aspartate) or EphB2 [18]. Concentrations of soluble Aβ clearly distinguished healthy
controls from AD patients and represent a strong inverse correlate of synapse loss [1,22]. In addition to ADDLs, globular Aβ species called globulomers have been found to be toxic [23], leading to suppressed spontaneous synaptic activity by inhibition of P/Q-type calcium currents [24]. Dodecameric Aβ56° oligomers extracted from the brains of APP (amyloid precursor protein) transgenic mice interfere with learning and memory performance in rats [25]. Analysis of neurotoxicity of oligomers derived from synthetic Aβ peptides ranging from monomers to tetramers demonstrated that tetramers have the strongest effect [26]. The conclusion that oligomers are more potent candidates as pathogens is based primarily on experimental evidence demonstrating that natural and synthetic Aβ oligomers impair synaptic plasticity [19,20,27] and memory [25,27,28] and induce loss of synapses [18,29] when applied exogenously into rat cerebral ventricle, cultured brain slices or dissociated neurons. Tomiyama et al. [30] generated APP transgenic mice expressing the E693Δ mutation, which causes neuronal cell death and cognitive impairment by enhanced Aβ oligomerization without fibrillization. The mice displayed age-dependent accumulation of intraneuronal Aβ oligomers from 8 months but no extracellular amyloid deposits even at 24 months. Hippocampal synaptic plasticity and memory were impaired at 8 months of age [30]. Support for these observations comes from a recent report describing a similar APPE693Q mouse model that does not develop plaques at any age studied up to 30 months [31]. Analysis for spatial learning and memory, however, revealed that 12-month-old mice with readily detectable ADDLs displayed a significant delay in acquisition when compared with non-transgenic littermates [31]. Aβ protofibril levels correlate with spatial learning in AD transgenic mice expressing human APP with the Arctic mutation [32], facilitating early intraneuronal Aβ
aggregation [33]. Despite the difficulty in comparing the different studies on oligomeric Aβ species there seems to be converging evidence that they (i) are primarily formed within neurons, (ii) oligomeric Aβ species are more neurotoxic than monomeric or fibrillar Aβ in vitro, (iii) oligomeric Aβ species decrease synaptic activity.

**Therapeutic role of oligomeric pyroglutamate Aβ**

Besides the concept that oligomeric forms of Aβ are toxic, it is of interest that in addition to full-length Aβ peptides starting with an aspartate at position 1, a variety of different N-truncated Aβ peptides have been identified in AD brains. Ragged peptides including phenylalanine at position 4 of Aβ have been reported as early as 1985 by Masters et al. [34]. In contrast, no N-terminal sequence could be obtained from cores purified in an SDS-containing buffer, leading to the assumption that the N-terminus could be blocked [35,36]. The presence of AβpE3 (Aβ starting with pyroglutamate at position 3) in AD brain was subsequently shown using MS of purified Aβ peptides, explaining at least partially the initial difficulties in sequencing Aβ peptides purified from human brain tissue [37]. The authors reported that only 10–15% of the total Aβ isolated by this method begins at position 3 with AβpE3. Saido et al. [38] subsequently showed that AβpE3 represents a dominant fraction of Aβ peptides in senile plaques of AD brains. Recently, we generated a new mouse model selectively expressing AβpE3–42 in neurons, and demonstrated for the first time that this peptide is neurotoxic *in vivo* leading to neuron loss and an associated neurological phenotype [39]. The N-terminal pyroglutamate formation can be catalysed by QC (glutaminyl cyclase), which can be pharmacologically inhibited by QC inhibitors, both *in vitro* [40] and *in vivo* [41]. QC expression was found to be up-regulated in the cortex of patients with AD and correlated with the appearance of pyroglutamate-modified Aβ. Oral application of a QC inhibitor resulted in reduced AβpE3–42 burden in two different transgenic mouse models of AD as well as in a transgenic *Drosophila* model. Interestingly, treatment of these mice was accompanied by reductions in Aβ1–40/42, diminished plaque formation and gliosis, as well as improved performance in context memory and spatial learning tests [41].

Recently, we have identified low-molecular-mass AβpE3 oligomers, which can be detected by 9D5, a novel mouse mAb (monoclonal antibody) [42]. Interestingly, 9D5 stained abundant intraneuronal Aβ accumulation in sporadic and familial AD brain (Figure 1). 9D5 did not cross-react with Aβ1–42 monomers, dimers or other higher-molecular-structured aggregates, indicating that these oligomers present a unique and novel epitope. The therapeutic potential of 9D5 was demonstrated in passively immunized 5XFAD mice as plaque load and Aβ levels were reduced and behavioural deficits were normalized. In an ELISA using 9D5 as capture antibody, we could show that the signal was significantly lower in plasma of AD patients as compared with non-demented controls. We believe that
our observation represents a novel therapeutic mechanism rescuing AD pathology and related behavioural deficits. Several studies demonstrated that N-terminal-specific Aβ antibodies showed significant beneficial effect in AD mouse models. Bard et al. [43] and Buttini et al. [44] studied the optimal antibody response for reducing neuropathology in PDAPP transgenic mice. Immune sera with reactivity against different Aβ epitopes and mAbs with different isotypes were examined for efficacy and showed that antibodies against the N-terminal regions of Aβ were able to invoke beneficial effects. Saido et al. [38] suggested that AβPE3–42 is generated step-by-step from its precursor Aβ1–42 by N-truncation and subsequent glutamate to pyroglutamate conversion. We therefore assume that reducing Aβ1–42 by passive immunization (reviewed in [45]) will also reduce AβPE3–42 levels and the resulting oligomeric forms. Saido et al. [38] have already suggested that Aβ1–42 is N-terminally truncated by one or more unknown protease(s), resulting in depletion of the first two amino acids aspartate and alanine. The consequence is a free N-terminal glutamate residue, which is turned into pyroglutamate in AβPE3–42 (Figure 2).

### Summary

We strongly believe that intraneuronal AβPE3 oligomers represent an important pathological step appearing at a time point when behavioural deficits occur. Interrupting this toxic pathway by specifically reducing these oligomers also has an impact on other Aβ peptides as shown, for example, by the reduction in general plaque load. In conclusion, we have therefore demonstrated for the first time that oligomeric AβPE3 represents a novel Aβ entity, which can be detected by specific antibodies serving as promising tools for diagnosis and therapeutic intervention of AD.

### References


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