Early Intraneuronal β-Amyloid Pathology: Do Transgenic Mice Represent Valid Model Systems?

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Abstract: Whereas a plethora of studies focusses on extracellular plaque deposition, only a very limited amount of reports deal with intraneuronal accumulation of Aβ peptides in human AD. However, over the past years, accumulating evidence points to a significant role of intraneuronal Aβ triggering the pathological cascade leading to neurodegeneration in Alzheimer’s disease (AD). Much of the data originate from studies on transgenic mouse models of AD, where initial intraneuronal Aβ accumulation which prede extracellular plaque deposition, has been repeatedly reported. The current review discusses the impact of this finding on the future development of novel mouse models for preclinical research as a basis for therapeutic intervention.

Keywords: Amyloid, Alzheimer’s disease, transgenic mouse model, neuron loss, intraneuronal β-amyloid.

INTRODUCTION

Alzheimer’s disease represents the most frequent form of dementia and is characterized by two major neuropathological hallmarks: (i) extracellular plaques composed of the 40 - 42 residues Aβ peptide [1] and (ii) neurofibrillary tangles, consisting of abnormal phosphorylated Tau protein [2]. There is increasing evidence that, in addition to the well-known extracellular amyloid deposition in the parenchyma, Aβ peptides accumulate inside neurons [3]. It has been hypothesized that this initial accumulation is one of the earliest pathological events, thereby triggering the cascade leading to neurodegeneration [4]. Since their initial generation in the mid 1990s, transgenic mice have been proven to represent valuable model systems reflecting various pathological subfields of AD like plaque deposition, inflammatory changes or behavioral abnormalities (reviewed in [5, 6]). In the present review, we summarize the current achievements of modeling early intraneuronal Aβ accumulation in transgenic mice with their resulting pathological consequences.

Of special importance will be the critical discussion of this observation, because only those models with intraneuronal Aβ accumulation show marked neuron loss.

PROCESSING OF THE AMYLOID Precursor PROTEIN GENERATES Aβ PEPTIDES

The generation of Aβ peptides is due to enzymatic cleavage of the larger precursor APP, which represents a type I membrane protein with a large N-terminal ectodomain and a short intracellular C-terminal domain. Alternative splicing of APP yields eight isoforms with lengths of 677 to 770 amino acid residues, of which APP695 is the primary transcript in neurons [7]. APP can be processed by two different pathways: (i) non-amyloidogenic processing: Cleavage by α-secretase within the Aβ domain releases a secreted form of APP (sAPPα), thereby precluding the generation of Aβ peptides. Different members of the ADAM protein family (a disintegrin and metalloprotease) have been demonstrated to possess α-secretase activity [8, 9]. (ii) amyloidogenic processing: This APP processing pathway results in the cleavage at the β-secretase site, liberating also a secreted form of APP (sAPPβ), leading to the generation of a membrane-associated C-terminal fragment named C99. The β-site APP cleaving enzyme 1 (BACE) belongs to the family of aspartyl proteases and has been identified simultaneously by different research groups [10-13]. Subsequent cleavage of C99 by γ-secretase activity results in the generation of 40 – 42 residue Aβ peptides, as well as a short intracellular APP fragment named AICD. It has been shown that γ-secretase consists of a complex of different proteins including presenilin-1 (PS1) or presenilin-2 (PS2), as well as nicastrin, anterior pharynx defective (APH-1) and presenilin enhancer 2 (PEN-2) (reviewed in [14], Fig. 1).

Fig. (1). Processing of the amyloid precursor protein and generation of its derivatives sAPPα, sAPPβ and Aβ.

Mutations in either APP or in the presenilin genes have been linked to familiar, early onset forms of AD. These cases
represent only a minor portion (~5%), whereas the vast majority of AD cases develop sporadically. Most of the reported APP mutations are located near the secretase cleavage sites and the ameliorate overproduction of Aβ peptides. Some of these mutations (e.g. the Austrian mutation T714I), as well as a couple of PS1 mutations have a drastic effect on the Aβ42/Aβ40 ratio by strongly increasing Aβ42 production with concomitant suppression of Aβ40 secretion [15, 16].

RELEVANCE OF INTRANEURONAL Aβ ACCUMULATION IN AD AND DOWN SYNDROME PATIENTS

The occurrence and relevance of intraneuronal Aβ accumulations in AD have been a matter of controversial scientific debate. First reports showing that Aβ is initially deposited in neurons before occurring in the extracellular space date back roughly 20 years [17, 18]. More recently it has been shown that neurons in AD-vulnerable region accumulate Aβ42 and it has been further suggested that this accumulation precedes neurofibrillary tangles and extracellular Aβ deposition [3]. Consecutively a variety of reports has been published demonstrating Aβ in neurons of AD [19-22] and Down syndrome (DS) patients [23-25]. However, a recent study found intracellular Aβ immunoreactivity during the entire life span in control subjects and DS patients, leading to the suggestion that this represents rather a feature of normal neuronal metabolism than a pathological alteration. As the authors found the strongest intraneuronal Aβ in brain structures that are not highly vulnerable to AD-associated changes, they believe that intraneuronal Aβ immunoreactivity is not a predictor of brain amyloidosis or neurofibrillary degeneration [26]. In general, reports on intraneuronal Aβ accumulation in AD remain scarce. Recent reports have shown that modifications of the staining method have crucial influence on the detection of Aβ peptides in neurons. Whereas pretreatment of AD tissues using formic acid enhances the immunological detection of extracellular plaques, it might have an opposing effect on intracellular Aβ peptides. Heat-induced antigen retrieval has been proposed to have the most significant effect, whereas enzymatic treatment alone is not sufficient [27, 28]. Fixation might also be an important point, as intraneuronal Aβ detection in mice is well-documented [29-36], however, these tissues are usually fixed by transcardial perfusion in a narrow time frame, whereas human material as a general rule is subjected to much longer post-mortem intervals.

ALTERNATE SOURCES OF INTRANEURONAL Aβ AND Aβ SYNAPTOTOXICITY

A second mechanism that contributes to intracellular Aβ accumulation is uptake from the extracellular space, in addition to intraneuronal Aβ production. It has been previously shown that cells that were treated with synthetic Aβ peptides, selectively accumulate Aβ42 and that this internalization could be prevented under conditions that block endocytosis [37]. On the subcellular level, these internalized Aβ42 peptides seem to accumulate especially in the endosomal/lysosomal system which lead to lysosomal permeability and membrane damage [38, 39]. One possible internalization mechanism might be cell-surface receptor-mediated uptake via the α7 nicotinic acetylcholine receptor [40], but also passive diffusion of extracellular Aβ through the plasma membrane has been suggested [41]. A selective Aβ42 uptake was revealed in the CA1 subfield of rat organotypic slice cultures, whereas other hippocampal regions like CA3 and dentate gyrus remained almost unaffected [42]. Primary neurons from APP transgenic Tg2576 mice accumulate Aβ peptides and have been shown to undergo selective reductions in synaptic levels of PSD-95 and the glutamate receptor subunit GluR1 compared to the wild-type situation [43]. As Aβ has been implicated in the depression of AMPA receptor currents, thereby regulating synaptic activity [44], reduced levels of GluR1 provide a molecular basis for Aβ-induced AMPA current alterations [43]. In addition, it has been shown that Aβ-derived oligomers (ADDLs) specifically bind to pyramidal neurons, promoting a rapid decrease in the membrane expression levels of memory-related receptors like NMDA or EphB2 [45]. In hippocampal neuron cultures, ADDLs stimulated the excessive formation of reactive oxygen species (ROS) through a mechanism requiring NMDA receptor activation. Interestingly, the memory-preserving drug memantine, which represents a NMDA receptor antagonist, completely protected against these toxic effects, indicating that ADDLs bind to NMDA receptors and trigger neuronal damage through receptor-dependent calcium influx [46]. These oligomers have been previously demonstrated to inhibit hippocampal long-term potentiation and disrupt synaptic plasticity [47, 48] and have been shown to originate from intraneuronal rather than extracellular dimerization [49]. 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.

TRANSGENIC ALZHEIMER MOUSE MODELS WITH INTRANEURONAL Aβ ACCUMULATION

Early intraneuronal Aβ accumulations preceding amyloid-plaque deposition has been consistently reported in a variety of transgenic AD mouse models in the last years. These models differ either in the expressed APP isoform, the respective APP mutation, the expression level of APP, the promoter construct used to drive APP expression, as well as in the fact whether they are single or multi-transgenic (co-expression of Presenilin-1 or Tau) (see Table 1). First evidence for intracellular Aβ pathology came from a model expressing APP695 with the Swedish (KM 670/671NL), Dutch (E693Q) and London (V717I) mutations in combination with transgenic mutant PS1 with the M146L mutation [35]. Aβ peptides were detected in hippocampal and cortical neurons before any plaque pathologies were detectable. A stronger phenotype with early intraneuronal Aβ accumulation has been detected in another model expressing APP751 with the Swedish and London mutations, also in combination with the above-mentioned PS1 transgenic mouse. Strong Aβ staining was identified in somatodendritic compartments of neurons in subiculum, CA1 region of the hippocampus, as well as in cortical areas in young mice [29, 36]. It has been shown that with incremental extracellular pathology an attenuated intraneuronal Aβ immunoreactivity was noted [36, 50]. This finding corroborates an observation in Down syndrome (DS) patients, where consistently strong cellular Aβ staining was reported in young patients, with a
progressive decline paralleling deposition and maturation of extracellular amyloid plaques [24].

Analysis of the subcellular localization of Aβ peptides by means of double-fluorescence experiments in these mice revealed that intracellular Aβ colocalized with lysosomal markers and less frequently with markers of the trans-Golgi network (TGN). Using electron microscopy, Aβ has been detected in the lumen of multivesicular bodies (MVBs) [50], corroborating an earlier report in Tg2576 mice showing that Aβ42 is localized predominantly to MVBs within pre- and postsynaptic compartments [51]. In Tg2576 mice an altered synaptic morphology has been recognized which preceded extracellular amyloid deposition [51] and it has been shown in a subsequent report that Aβ42 aggregates into oligomers within endosomal vesicles, as well as in neuronal processes [52]. This is particularly interesting as it has been hypothesized that Aβ oligomers have a significant impact on the pathological alterations underlying memory deficits in AD patients, as they have been demonstrated to disrupt synaptic plasticity and to inhibit hippocampal long-term potentiation [47, 48].

Another interesting model expresses APP with the Swedish and London mutations on a homozygous mutant PS1 knock-in background (APP/PS1KI). These animals show early extracellular amyloid deposition at the age of 2 months, which is preceded by strong intraneuronal Aβ accumulation in hippocampus, cortex and motor neurons of the spinal cord. Staining with the dye Thioflavin-S indicated that the intraneuronal Aβ is aggregated and is present in the form of β-pleated sheets. Further pathological features are severe axonal degeneration, as well as motor and cognitive deficits starting at the age of 6 months [30, 53-55].

Mice expressing human APP with the Arctic mutation (E693G) have been recently developed [31, 32]. This mutation is particularly interesting as it has been demonstrated that protofibrils are produced in a much higher rate and in larger quantities than wildtype Aβ [56]. The available mouse models express APP with both the Swedish and the Arctic mutation. A transgenic line named tg-APPArcSwe mice has been created under the control of the murine Thy1 promoter. Strong intraneuronal Aβ immunostaining was observed at 2 months of age, which preceded plaque formation occurring at 5-6 months of age [32]. Another mouse line named ArcAβ uses the murine prion protein promoter to express APP695 with the Swedish and Arctic mutations. These mice show intracellular Aβ staining already at 3 months of age, with increasing amounts during aging. Interestingly, these mice develop age-dependent cognitive impairments, as shown by deficits in behaviour tasks like the Y-maze or a two-way active avoidance paradigm [31].

Recently a triple transgenic mouse model has been developed expressing both APP and Tau on a mutant PS1 knock-in background (3xTg-AD mice) [34]. Intracellular Aβ is apparent between 3 and 4 months in these mice and precedes the deposition of extracellular Aβ peptides starting at the age of 6 months.

Table 1. Overview of Currently Available Transgenic Mouse Models of Alzheimer’s Disease in Which Intraneuronal Aβ Accumulation has been Reported. In Addition, Data on the Onset of Extracellular Plaque Deposition, the Occurrence of Neuron Loss and Reported Behavioural Deficits are Given (n.a. – not analyzed)

<table>
<thead>
<tr>
<th>Transgenic mouse model</th>
<th>Mutation APP</th>
<th>Mutation PS1</th>
<th>Promoter</th>
<th>Plaque onset</th>
<th>Neuron loss</th>
<th>Behavior deficits</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP695SDLx PS1M146L</td>
<td>Swedish, Durch, London</td>
<td>M146L</td>
<td>PDGF (APP), HMG-CoA (PS1)</td>
<td>8m</td>
<td>no</td>
<td>n.a.</td>
<td>[35]</td>
</tr>
<tr>
<td>APP751SLx PS1M146L</td>
<td>Swedish, London</td>
<td>M146L</td>
<td>Thy1 (APP), HMG-CoA (PS1)</td>
<td>3m</td>
<td>Yes (17m)</td>
<td>n.a.</td>
<td>[29, 36, 57]</td>
</tr>
<tr>
<td>Tg2576</td>
<td>Swedish</td>
<td>-</td>
<td>Hamster Prion Protein</td>
<td>12m</td>
<td>no</td>
<td>yes</td>
<td>[43, 51, 52, 58]</td>
</tr>
<tr>
<td>3xTg-AD</td>
<td>Swedish</td>
<td>M146V</td>
<td>Thy1 (APP, Tau), PS1 knock-in</td>
<td>6m</td>
<td>n.a.</td>
<td>yes</td>
<td>[34, 59]</td>
</tr>
<tr>
<td>APP751SL/PS1KI</td>
<td>Swedish, London</td>
<td>M233T, L235P</td>
<td>Thy1 (APP), PS1 knock-in</td>
<td>2m</td>
<td>yes (6m)</td>
<td>yes</td>
<td>[30, 53-55]</td>
</tr>
<tr>
<td>Tg-APP(ArcSwe)</td>
<td>Swedish, Arctic</td>
<td>-</td>
<td>Thy1</td>
<td>5-6m</td>
<td>n.a.</td>
<td>n.a.</td>
<td>[32]</td>
</tr>
<tr>
<td>arcAβ</td>
<td>Swedish, Arctic</td>
<td>-</td>
<td>Mouse Prion Protein</td>
<td>7m</td>
<td>n.a.</td>
<td>yes</td>
<td>[31]</td>
</tr>
<tr>
<td>5xFAD</td>
<td>Swedish, Florida, London</td>
<td>M146L, L286V</td>
<td>Thy1 (APP, PS1)</td>
<td>2m</td>
<td>yes</td>
<td>yes</td>
<td>[33]</td>
</tr>
<tr>
<td>APP-Au</td>
<td>Austrian</td>
<td>-</td>
<td>PDGF</td>
<td>-</td>
<td>n.a.</td>
<td>subtle</td>
<td>[60]</td>
</tr>
</tbody>
</table>
At this time point synaptic plasticity was already strongly compromised in these mice, as shown by impaired long-term potentiation (LTP) [34]. Intracellular Aβ accumulation is functionally linked to cognitive impairment in these mice, as they develop deficits in long-term retention at the age of 4 months, a time point prior to plaque deposition where only intracellular Aβ is present [59].

5xFAD mice express human APP with the Swedish, Florida (I716V) and London mutations, together with mutant PS1 (M146L, L286V) under the control of the murine Thy1 promoter [33]. The earliest intracellular Aβ accumulation could be detected at 1.5 months of age, immediately preceding extracellular plaque deposition occurring at the age of 2 months. The intraneuronal Aβ is in an aggregated state, as shown by Thioflavin-S staining and synaptic deficits were detected by reduced levels of the synaptic markers syntaxin and PSD-95 at the age of 9 months. Memory impairment, as shown by a reduced Y-maze performance and deficits in trace fear conditioning, became evident already at the age of 4 - 5 and 5 - 6 months respectively, compared to wildtype control animals [33, 61].

AD MOUSE MODELS WITH NEURON LOSS

Despite of a plethora of transgenic AD mouse models expressing various APP isoforms and mutations, efforts modelling significant neuronal loss were, until recently, not successful [62, 63]. First evidence for significant hippocampal neuron loss (~14%), was reported in the APP23 mouse model, where the number of CA1 neurons was inversely correlated with CA1 plaque load [64].

Using unbiased stereologic methods, a loss of CA1-3 neurons in a magnitude of ~30% was detected in 17-month-old APP/PS1 transgenic mice, compared to age-matched PS1 control animals. Interestingly, the plaque load was approximately 10% smaller than the level of hippocampal pyramidal cell loss in these mice, indicating a loss of neurons at sites of Aβ aggregation but additionally also distant from extracellular Aβ deposits [57].

Another model showing massive hippocampal neuron loss is the abovementioned APP/PS1KI mouse model [30]. At the age of 10 months an extensive neuron loss (~50%) in the hippocampus was reported, that correlated with the accumulation of intraneuronal Aβ and Thioflavin S-positive intracellular material and was already detectable at the age of 6 months [30].

The recently described 5xFAD mice underscore the potential influence of intraneuronal Aβ accumulation on the loss of neurons. Cresyl violet staining revealed a reduced number of cortical layer 5 neurons, a region with robust intracellular Aβ immunoreactivity. The same holds true for the subiculum where neurons where pale or entirely missing [33]. However, unbiased stereological neuron quantifications are needed to disclose the onset and severity of neuron loss in this model.

CONCLUSION

In summary, mouse models have been proven to be valuable model systems to study the influence of intraneuronal Aβ accumulation on the course of AD pathology. However, as data on human patients is limited so far, we do not know whether the alterations reported in mouse models really reflect the human pathological situation. Transgenic mouse models still represent an “artificial system” reflecting only parts of the human pathology. The variation in the reported transgenic constructs (comprising different APP isoforms with either single or multiple mutations) and the combination of transgenes by the use of double-transgenic mice complicates the comparison of different studies. As these entire models use miscellaneous promoter constructs to drive the expression of the transgene (resulting in varying expression levels in a variety of different CNS regions and cell types) the potential influence of intraneuronal Aβ on behaviour and neuron loss has to be evaluated with caution. These models are useful in deciphering the effects of intraneuronal Aβ accumulation and its pathological effects, on neuronal integrity and brain function. Validation of this concept is important and can only be achieved after successful drug development.

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REFERENCES

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