Published in final edited form as:

Alzheimers Dement. 2009 July ; 5(4): 340–347. doi:10.1016/j.jalz.2009.03.002.

Amyloid precursor protein transgenic mouse models and Alzheimer's disease: Understanding the paradigms, limitations and contributions

Tyler A. Kokjohn^{1,2} and Alex E. Roher^{1,*}

¹ Sun Health Research Institute, Sun City, AZ

² Midwestern University, Glendale, AZ

Abstract

Transgenic (Tg) mice overexpressing mutant familial Alzheimer's disease (AD) amyloid precursor protein (APP) genes have contributed to the understanding of dementia pathology and support the amyloid cascade hypothesis. Although many sophisticated mice APP models exist, none recapitulates AD cellular and behavioral pathology. The morphological resemblance to AD amyloidosis is impressive, but fundamental biophysical and biochemical properties of the APP/A β produced in Tg mice differ substantially from those of humans. The greater resilience of Tg mice to substantial A β burdens suggests the levels and forms that are deleterious to human neurons are not as noxious in these models. Tg mice have been widely used for testing AD therapeutic agents and demonstrated promising results. Unfortunately, clinical trials resulted in unforeseen adverse events or negative therapeutic outcomes. The disparity between success and failure is in part due to differences in brain environment that separate man and rodent. These observations suggest that the pathogenesis of AD is by far much more intricate than the straightforward accumulation of A β .

Amyloid-beta (A β deposition combined with neurofibrillary tangles (NFT), are important pathological features of Alzheimer's disease (AD). Accumulating toxic forms of A β disrupt neuronal and glial homeostasis and destroy brain and cerebrovascular tissue architecture. A major step toward the exploration of AD pathophysiology and the evaluation of potential treatments was the creation of transgenic (Tg) mice with gross amyloid deposition due to over-expression of human familial Alzheimer's disease (FAD) amyloid precursor protein (APP) mutations. The reproduction of these pathological alterations in APP and presenilin (PS) Tg mice added a powerful confirmation to the "amyloid cascade hypothesis", but although sophisticated mouse models have been created, none faithfully recapitulate the extensive cellular, biochemical and behavioral pathology of AD patients. The preeminence of the amyloid cascade hypothesis as a unique pathogenic factor in sporadic AD (SAD) is being re-examined as a consequence. First, Tg mice reproduction of AD pathology is based on the transfection of mutant APP, PS and tau genes, alone or in combination, that reproduce only some of the pathological changes seen in FAD. Second, no specific mutations in the A β peptide explain the massive pathological amyloid deposition in the most common sporadic form of AD. Third, both A β and tau accumulation are observed in other neurodegenerative disorders.

*Corresponding Author: Alex E. Roher, MD., PhD., 10515 W. Santa Fe Drive, Sun City, AZ 85351, Phone: (623)-876-5465, Fax: (623)-876- 5698, E-mail: alex.roher@bannerhealth.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

More important, these features are widely seen in aged, non-demented (ND) individuals. Fourth, it is well accepted that SAD is multifactorial and pleiotropic in its pathological and clinical manifestations and that these interactions are strongly influenced by risk factors such as apolipoprotein E (ApoE) genotype, cardiovascular pathology and disturbances in lipid and glucose metabolism. Finally, immuno-responsive AD patients vaccinated against A β 42 showed substantial clearance of amyloid plaques from the gray matter, without concomitant dementia abrogation [1;2].

While A β plays a central pathologic role, Tg mice affirm that AD entails more than alterations in APP/A β overproduction, clearance and deposition. Amyloid- β accumulating in the brains of APP Tg mice is neither physically, chemically or functionally equivalent to that characteristic of human AD [3–8]. The APP Tg mice represent a reductionist approach to AD modeling in which massive overexpression of a single gene profoundly alters mouse physiology and behavior. Ultimately, these unfolding, cascading consequences must be explicable in terms of underlying fundamental APP and A β biochemistry. A more complete comparison of A β biochemistry between mice and humans might identify the critical but subtle differences that form part of the full AD pathology repertoire, including dementia. Equally important is that interspecies differences in A β toxicity might explain why recent therapeutic approaches work so consistently and dramatically in the mouse model but not nearly as effectively in AD patients.

The many differences between FAD and SAD, the inherent biochemical complexity of AD and additional complications imposed by the unique species-specific physiological conditions present in Tg mice, have made unraveling the fundamental causes of this dementia a challenging undertaking. Although many APP Tg mice have now been constructed, the scope of this critical review is focused primarily on those models for which detailed biochemical and morphological assessments, and comparisons of A β have been performed in our laboratory. In this synthesis, we combine data from the morphological and chemical analyses of several distinct Tg animals and capitalize on the fact that this body of work, as well as the basic AD A β chemistry that serves as a frame of reference, was performed in a single laboratory to assess the similarities of the models to human AD.

Rodent A β analogs

Transgenic mice over-expressing human FAD APP mutations develop vascular and parenchymal amyloid deposits morphologically resembling those observed in demented humans. However, the extent of this resemblance is limited. Detailed microscopic examination of Congo red and thioflavin-S-stained Tg mouse plaques reveals that although β -pleated sheet structure is present, other aspects of tertiary and quaternary structure differ between AD mouse models. The compact plaques produced in mice generally exhibit a less dense and a more amorphous morphology than those observed in AD patients [9;10]. In general, while the amyloid plaques of AD patients resist disruption by all but the harshest denaturing agents, the analogs produced in Tg animals, such as the Tg2576 [11], APP23 [12], PDAPP [13], TgCRND8 [14] and to a lesser degree in the Tg-SwDI [15], are completely soluble in detergent-containing buffers. In part this is due to Tg mice A β lacking the extensive post-translational modifications such as Asp isomerizations, Asp and Ser racemizations, Met oxidations, pyroglutamyl cyclizations, intermolecular cross-links and extensive N-terminal degradation that are characteristic and abundant in SAD senile plaque deposits [3;4;7;11–13;16–19]. Amino-terminal truncations due to extensive proteolytic degradation that produce more hydrophobic A β species are prevalent in humans but virtually absent in APP Tg mice [9;11–13]. In addition, approximately 35–40% of total human amyloid plaque mass is an assortment of ancillary glycoproteins and glycolipids that apparently increases their insolubility and resistance to proteolysis [20;21]. These various incongruities may be explained by differences

in lifespan and physiology that allow longer periods of amyloid accumulation while facilitating the deposition of a diverse array of different amyloid-associated proteins in humans. Recent transcriptome analyses revealed that the gene expression pattern of aging in mice extensively differs from that of humans [22].

All APP and PS Tg mouse models engineered thus far express human FAD mutations that result in aggressive, early-onset dementia. These rare mutations have yielded important insights into the amyloid cascade, but when inferring fundamental mechanisms from these studies, it is important to bear in mind that FAD biochemical manifestations differ from those observed in SAD [23]. In addition, Tg mice themselves only partially mimic the amyloidosis of FAD. Transgenic PDAPP mice over-expressing the FAD APP V717F mutation are widely employed as an experimental model for SAD and in A β immunotherapeutic trials. In humans, this mutation results in characteristic early-onset dementia with numerous flocculent amyloid plaques, relatively few compact core plaques and an enormous production of NFT [24]. The biochemical properties of PDAPP Tg mice amyloid deposits were compared to those of a FAD case with the corresponding APP mutation [13;24]. The Tg mice exhibited prominent flocculent amyloid deposits and comparatively few core plaques with substantial thioflavin-S-positive β -pleated sheet structure. However, while the amyloid deposits produced in the human resisted disruption in buffers containing SDS and EDTA, the analogous structures formed in Tg mice disaggregated in such solutions [13]. These studies confirm that although PDAPP Tg mice recreate select aspects of the A β pathology found in humans carrying the corresponding APP mutation, it is an abbreviated recapitulation and does not include other important facets of AD, such as induction of NFT.

The temporal deposition and type of A β also varies among the different species of Tg mice. For instance, in the APP23 at 2 months of age, the levels of the soluble A β in brain tissue are very high and remain as such during the lifespan of the rodent [25], while in the Tg2576, soluble A β levels are very low up to 12–14 months of age and elevate exponentially from this age onward [26]. This is an important feature since the APP23 rapidly accumulates large quantities of vascular amyloid. The Tg2576, on the other hand, deposits a moderate amount of vascular A β at later stages suggesting a direct relationship between the soluble A β fraction and the degree of cerebrovascular amyloidosis [25;26]. Chemical analysis of the APP Tg2576 and the APP23 Tg mice, expressing the Swedish double mutations flanking the N-terminal region of A β , indicates that these models contain abundant quantities of A β monomers (A β :1–37, 1–38, 1–39, 1–40 and 1–42) in their amyloid deposits and no detectable A β dimers and oligomers [11;12;25]. In contrast, using the same analytical tools, other Tg mice such as PDAPP [13], Tg-SwDI [15] and the 3xTg (Roher, unpublished observations) generate large quantities of monomeric, dimeric and oligomeric A β peptides. These observations suggest that internal A β mutations or mutations at the APP C-terminal flanking region of these peptides are necessary for the stable aggregations of A β in Tg models. In SAD, on the other hand, no mutations are needed for the stable association of A β into oligomeric forms, suggesting that post-translational modifications play an important role in A β aggregation and hinder degradation of these peptides [3;5–7;16;21;27–29]. In addition, SAD patients accumulate large quantities of A β 17–42 in diffuse deposits [4], which are not commonly observed in Tg mouse models.

Several factors may account for the marked qualitative differences existing between Tg mouse and human AD senile plaques. First, the underlying A β accumulation mechanisms are substantially different. While the factor(s) leading to A β accumulation in SAD have not been elucidated, in most instances, Tg mice employ brute force transcription overexpression of mutant APP genes. Murine APP processing patterns are clearly different from those of AD [13–15]. In addition, the A β peptides produced in Tg animals predictably exhibit proteolytic cleavages that are idiosyncratic for the particular FAD mutation rather than replicating the

activities characteristic of SAD patients. The TgCRND8 and Tg-SwDI mice demonstrated an alteration in the order of APP processing by secretase enzymes that resulted in a novel C-terminal (CT) fragment generation and the accumulation of longer than A β 42 peptides [14; 15]. Although the CT99/83 fragments of APP degradation are less abundant in the TgCRND8 and Tg-SwDI mice than in humans, multiple additional A β -related peptides were also present. This finding suggests that the initial point of the precursor molecule hydrolysis lies upstream of the sites observed in normal and AD individuals, with subsequent sequential secretase cleavages occurring at the γ - and ϵ -positions or elsewhere due to uncharacterized murine endopeptidases [15]. Transgenic PDAPP mice also exhibit an altered CT fragment pattern on Western blots [13] with extended length peptides terminating beyond position 42 as well as N-terminally truncated molecules [13], analogous to those observed in the FAD V717F mutation [24]. In contrast, Tg2576 and APP23 Tg mice accumulate severalfold greater levels of APP than those observed in AD patients, but interestingly, are very efficient in generating A β 40 and A β 42 in contrast to Tg-SwDI [11;12;15;25;26]. These observations reveal substantial strain-specific heterogeneity in proteolytic processing in APP Tg mice. Another explanation for the differing responses of mice and humans could be that, despite the fact that the rodent genome is 14% smaller than that of humans, it has substantially more protease genes than humans [30;31]. The vast overproduction of APP in most Tg animals undoubtedly perturbs and likely overwhelms the entire range of processing enzymes, with implications for cell signaling and homeostasis. Although much of the excess APP is processed to yield A β peptides, the extraordinary pace of production and its scale in most Tg mice may preclude the processing and modification routinely observed in SAD. Along this vein, in the double PS1 and PS2 knock-out mice, enzymatic degradation of membrane-bound substrates is impaired, resulting in the accumulation of membrane-tethered peptide stubs. To compensate, in these mice, there is an increased expression of the calpain-cathepsin enzymes [32].

A growing body of evidence suggests that tertiary and quaternary structures are critical determinants of A β pathogenicity [33], with soluble oligomers of mis-folded A β peptides perhaps representing the most toxic species [34–36]. Transgenic mice accumulate amyloid deposits that stain avidly with thioflavin-S, revealing the presence of extensive β -pleated sheet structure. However, with the exception of the triple APP mutation-expressing Tg-SwDI mice [15], A β oligomers are not produced at levels comparable to those found in AD patients. If A β dimers are a key toxic species, then explaining why most APP Tg mice tolerate substantial A β burdens is easy; they simply lack the most pathologic species. The Tg-SwDI mice expose a logical conundrum for the simple A β dimer abundance model, because they produce and tolerate large amounts of what should be a quite toxic species. One possibility is that the A β 42 dimers produced in Tg-SwDI mice are firmly bound to the vasculature or within the plaque cores that occur in close proximity to the vessels, suggesting that the toxic species are sequestered in such a way that they are rendered less toxic. Another possibility is that the dimers produced in Tg mice are different in structure and pathologic potential from those produced in humans. Binding of the A β -specific “Pittsburgh” compound is less avid in Tg mice, a difference hypothesized to arise as a consequence of the comparative lack of pyroglutamylation in these animals [37]. However, all Tg animals are not equivalent. Examination of synthetic A β 42 peptide carrying the Dutch/Iowa mutation reveals a propensity to produce dimeric structures resistant to IDE degradation. This form was likewise abundant in the Tg-SwDI mice [15]. Finally, it is possible that A β dimers are simply not as neurotoxic in mice as they seem to be in humans for unknown physiological reasons.

Primates and mice diverged about 85 million years ago [38] and consequently exhibit vast differences over a wide range of fundamental attributes, including life span, and an age-dependent repression of broad-spectrum neuronal genes, a feature of humans and Rhesus macaques that is not replicated in mice [22], suggesting that the assumption of evolutionarily conserved biochemical equivalence between human and mouse aging is erroneous. Marked

differences observed in the response to A β injected into aged rodent and primate brains have led to the hypothesis that while the brain becomes more sensitive to A β toxicity with aging, a “species barrier” reduces the deleterious effects in rodents [39].

Transgenic Mice and Tau Pathology

Despite exhibiting a massive and often rapid accumulation of A β peptides, none of the APP Tg mice examined replicated the full spectrum of AD pathology. Notably, these animals failed to produce dementia-correlated NFT structures. This suggests that subtle aspects of the amyloid cascade disease process are not duplicated in these mice and that the presence of A β is necessary, but not sufficient to induce the *tau*-involved pathological changes typical of SAD and some FAD patients [24]. It may be important to recognize that these limitations mean that with Tg mice we are studying the direct effects of A β overproduction and deposition, not AD. Transgenic mouse models may suffer proportionately less deleterious effects than AD patients and therefore respond to amyloid disruption/removal therapies far better in terms of cognitive function recovery than even minimally demented humans who are contending with a broader scope of pathology. Eliminating amyloid deposits by A β immunization may represent a cure in Tg mice, but notwithstanding the apparent total disruption of senile plaques in immunized patients with SAD, successful clearance of these structures could neither reverse nor halt the progression of dementia [1].

Triple Tg (3xTg) mice, carrying the APP Swedish mutations K670N/M671L, the presenilin-1 M146V mutation and the tau P301L mutation in the C57BL/6 mice background, were developed as an AD model by Oddo et al. [40]. This widely employed Tg model is of paramount importance because it develops intracellular A β and extracellular plaque deposits as well as tau pathology similar to that observed in AD. In addition, these mice demonstrate altered long-term potentiation (LTP), synaptic dysfunction and age-related learning deficits and have potential for testing therapeutic interventions against AD [41;42].

However, as is the case for APP Tg mice, it may not be correct to view the 3xTg mice as duplicators of the amyloid cascade as it unfolds in humans [43]. The key APP/A β and tau genes are overexpressed simultaneously by transcriptional forcing methods, while tau pathology emerges subsequent to A β production in AD. Nevertheless, this model is useful to study the dynamic interactions between human A β and tau proteins in mice and further biochemical characterization is needed.

Vascular amyloidosis in Tg mice and AD

Another important difference between AD and Tg mice relates to the distribution and morphology of the A β 40 and A β 42 peptides associated with the vasculature. In humans, the deposition of cortical A β associated to the capillary network affects the form of globular cores, resembling pussy-willows that are mainly composed of A β 42. Amyloid associated to the arteriolar and small cortical arteries is in the form of sheets and contains a mixture of A β 40 and A β 42. Larger leptomeningeal vessels, on the other hand, mostly contain A β 40 organized in large concentric sheets replacing the smooth muscle cells [27]. This clear pattern of amyloid deposition is unique to humans as well as its composition and chemical structure [44]. With the exception of the APP23 [12] and the Tg-SwDI [15] Tg mice, which show some cores of amyloid encroached upon cortical and thalamic microvasculature, respectively, other Tg mice do not entirely replicate the vascular amyloid composition and pattern of AD. The unique distribution of vascular A β in AD is related to the drainage of the cerebral interstitial fluid, which occurs along the periaxonal spaces that extend from capillaries to the major leptomeningeal cerebral arteries and drain into the lymphatic vessels of the head, eventually reaching the systemic venous circulation [45;46]. It is along the interstitial fluid drainage pathways that A β accumulates. The size of this system in humans is by far larger and much

more complex than that of rodents. Obstruction of the periarterial spaces by amyloid deposition in the cortical regions results in the accumulation of interstitial fluid and dilation of the periarterial spaces in the white matter and edema [47], with dire pathological consequences for both gray and white matter. Again, APP Tg mice do not recapitulate these important changes in brain hemodynamic physiology and chemistry [48] that are important components of AD dementing process.

The Tg-SwDI mice express very low levels APP and exhibit a unique pattern of amyloid deposition and composition. In addition to diffuse cortical amyloid deposits, thioflavin-S-positive, senile, core-like structures were observed in close association with the thalamic vasculature, but absent in the cerebral cortex [15]. Unlike the situation with the parenchymal tissue plaque analogs of other APP Tg mice, these vascular-associated core plaques were resistant to physical disruption and were more reminiscent of the recalcitrant vascular amyloid deposits observed in other Tg mouse strains. The Tg-SwDI mice also produced large quantities of dimeric, insulin degrading enzyme (IDE)-resistant A β , a feature unique to this animal [15]. This finding is significant because the thalamic vascular-associated amyloid pathology produced in the Tg-SwDI strain is far more extensive in total scale and scope than in other APP Tg mice [15]. Similar to APP23 and Tg2576 Tg mice, which produce high levels of A β , Tg-SwDI animals tolerate the presence of enormously elevated levels of A β .

Alzheimer's disease and vascular pathology often exhibit an intricate, perhaps co-dependent relationship [47;49]. Whatever role vascular disease and inflammation play in AD promotion and progression, one thing seems certain: APP Tg mice develop a variable degree of amyloid deposits in the brain vasculature. Unlike the case for the senile plaque analogs in Tg mice, the vascular deposits are more resistant to immuno-disruption and thus mimic the situation present in AD [2;50]. Although parenchymal deposits decreased subsequent to anti-A β immunization, vascular deposits increased [2;51]. Once the vasculature of Tg mice becomes amyloid burdened, the dynamic flow of A β out of the brain tissue may be impaired [25] and this situation may not be completely reversible by vaccination [52;53]. Moreover, there have been reports of microhemorrhages in vaccinated Tg mice [54;55] and signs of vasogenic edema in A β -immunized humans (Alzforum, August 11, 2008; <http://www.alzforum.org/new/detail.asp?id=1894>).

Recent follow-up studies of AN-1792-vaccinated patients suggest that clearance of vascular amyloid deposits may occur in a subset of patients or over long-term treatment [1]. However, it is impossible to judge the degree of vascular amyloid deposition or damage, if any, that existed in any of these patients prior to immunization. Studies of Tg mice as well as humans [2;51;54], have suggested an increased vascular pathology associated with vaccination-induced disruption of parenchymal amyloid plaques. Some experiments have demonstrated that in Tg mice immunotherapy reduced plaque burden despite the presence of substantial vascular pathology [56]. However, ELISA quantifications of total A β were not performed although the vascular accumulation of A β strongly suggests that disrupted plaque components exit the brain slowly or not at all. The results of the first human immunization trial revealed that even patients with mild AD may have already sustained sufficient vascular damage to prevent disrupted A β from egressing the brain [2]. That may mean anti-A β immunotherapy will be most effectively employed as a preventive rather than a therapeutic method. This increases the sense of urgency to vaccinate or treat patients before cognitive problems are evident. Concerns over potential deleterious effects on the brain vasculature as a consequence of anti-A β immunotherapy have prompted investigators to assess novel antibodies with differing binding specificities and chemistry in Tg mice [54;57]. These studies suggest that specific antibody forms coupled with dose modulation may produce beneficial effects with minimal collateral vascular damage.

Although mutant APP genes are overexpressed within the brain, it is important to remember that in humans, A β peptides are both produced and found outside the CNS. Several tissues in the periphery express APP isoforms and harbor substantial levels of A β peptides. While any beneficial function(s) for A β is uncertain, by the same token these molecules could contribute to as yet undefined processes in the vasculature and other peripheral sites [58]. The implications for AD immunotherapy are obvious, but at the same time remain frustratingly obscure.

A β therapies in Tg mice and humans

Immunization of Tg mice against A β 42 resulted in the apparent reduction of plaque formation and the regression of existing plaque deposits [52], although total A β peptide levels remained elevated along with a redistribution of A β in the brain [59–61]. Despite the sharp dichotomy between the physicochemical properties of SAD patient and Tg mouse senile plaque amyloid, A β vaccination of humans with mild to moderate SAD also resulted in an apparent reduction of plaque deposits [1;2;51;62–64]. This equivalency in the outcome of plaque disruption suggests that APP Tg mice are faithful models of the initial phases of the amyloid cascade in humans. However, studies undertaken *in vivo* on a real time basis have revealed that amyloid plaques rapidly emerge and disappear in Tg mice [65]. The fact that murine amyloid deposits are substantially more labile than their human counterparts suggests that plaques could be far more dynamic over the entire lifespan of Tg mice as well. These observations bolster the idea that the most effective amyloid disruption therapies must commence with the first clinical evidence of dementia or earlier.

The APP Tg mice models have been widely used for the testing of several AD potential therapeutic agents [52;66–70]. Overall, in the Tg mice, the various curative approaches demonstrated promising results such as improved learning, significant reduction in amyloid plaques, a decrease in soluble and insoluble A β burden in brain and a reduction of A β in plasma. Unfortunately, clinical trials to date have resulted in unforeseen adverse events or failed to produce clinically beneficial outcomes. The disparity between therapeutic success and failure must in part be due to differences in the complexities of the brain biological environment, metabolism and lifespan that separate humans from rodents. Furthermore, these observations suggest that the pathogenesis of SAD is far more complex and intricate than the straightforward accumulation of A β peptides.

If the senile plaques of human AD are intrinsically more neurotoxic than their Tg mouse analogs, it is possible that disrupting such structures by immune or other methods could produce an array of serious consequential changes in patients. In addition, such adverse events, if they occur in Tg mice, could be too subtle to recognize easily. Senile plaques, despite their apparent toxicity to surrounding cells might represent the optimal means to sequester toxic monomeric/oligomeric A β [6;71]. Unless all A β released from plaques is promptly degraded or removed, deposit disruption may be a treatment that ultimately turns out to be worse than the original disease. Postmortem examination of AN-1792-vaccinated patients, both with and without meningoencephalitis, revealed an unexpected and potentially ominous finding [2]. Although senile plaques were disrupted, there was no proportionate decrease in total A β levels within the brain tissue. These findings reveal that disrupted plaque A β was unable to exit the brain and suggest that some of this material ended up in the white matter. The toxic and inflammatory properties of A β suggest that a permanent elevation of these peptides will ultimately be deleterious to white matter function with profound implications for cognitive function.

Conclusions

Deposition of A β may be the penultimate link in a much longer chain of pathological events. Processing of APP is both dynamic and variable, and subtle changes may underlie synaptic

functional declines and neuronal death [72;73]. The precise temporal sequence of concerted protease processing determines whether or not the toxic A β species are produced at all [72]. In addition to A β peptides, APP proteolysis yields a variety of larger products that affect LTP [74] and transcription-mediated signaling processes, including glycogen synthase kinase-3 β expression which promotes tau hyperphosphorylation, all of which potentially impact cognitive functions [75;76]. Studies of FAD mutations have revealed that A β is of central importance and that even subtle alterations in the relative mix of APP proteolytic peptides yield profound effects on neuropathology, cognition and dementia development that can be manifested even in the absence of a cardinal defining AD lesion, the senile plaque [24]. Recent investigations of several AN-1792-vaccinated patients, who survived for extended periods, revealed that all ultimately died with dementia despite an apparent therapy-induced absence of amyloid deposits [1]. The factors that both influence or are influenced by APP processing remain undefined, but may include fundamental energy deficiency [71;72;77–79] arising as a consequence of increasing vascular deficiencies [47;49;80], mitochondrial failure [81–84] or other stressing mechanisms and co-morbidities associated with aging decay that, in principle, could exert a broad range of synergistic impacts beyond amyloid deposition.

Despite of the anatomical and physiological differences between humans and rodents, Tg mouse models have and will continue to represent promising venues to investigate the amyloid cascade and the efficacy of anti-A β immunization and other therapeutic interventions. It is important to recall the underlying complexity and long-lasting effects of AD as both Tg mice and AD therapies are evaluated. Depending on the precise chronicle of the disease process, remediation of late-stage pathology may reverse only a fraction of the damage to the brain. Extrapolating Tg mouse results to humans may be even more complicated because the instigating events of complex aging processes, and hence, the exact pathway to disease, is not necessarily identical in each individual. This implies that just as we should not demand the perfect and comprehensive AD model, we may wish to re-think how we assess therapeutic interventions such as immunization in rodents. It may be better to evaluate the relative success or failure of amyloid disruptive treatments by focusing on that specific target and outcome rather than anticipating and demanding a global AD cure. The pathology underlying AD is complex and its management or ultimate treatment may be equally complicated.

Acknowledgments

We are in debt to Dr. Mark Emmerling, Dr. Adrian Friday and Chera Maarouf for valuable advice. Supported by NIA grant RO1-AG19795

Reference List

1. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, et al. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 2008;372:216–223. [PubMed: 18640458]
2. Patton RL, Kalback WM, Esh CL, Kokjohn TA, Van Vickle GD, Luehrs DC, et al. Amyloid-beta peptide remnants in AN-1792-immunized Alzheimer's disease patients: a biochemical analysis. *Am J Pathol* 2006;169:1048–1063. [PubMed: 16936277]
3. Roher AE, Lowenson JD, Clarke S, Wolkow C, Wang R, Cotter RJ, et al. Structural alterations in the peptide backbone of beta-amyloid core protein may account for its deposition and stability in Alzheimer's disease. *J Biol Chem* 1993;268:3072–3083. [PubMed: 8428986]
4. Gowing E, Roher AE, Woods AS, Cotter RJ, Chaney M, Little SP, et al. Chemical characterization of A beta 17–42 peptide, a component of diffuse amyloid deposits of Alzheimer disease. *J Biol Chem* 1994;269:10987–10990. [PubMed: 8157623]
5. Kuo YM, Emmerling MR, Vigo-Pelfrey C, Kasunic TC, Kirkpatrick JB, Murdoch GH, et al. Water-soluble Abeta (N-40, N-42) oligomers in normal and Alzheimer disease brains. *J Biol Chem* 1996;271:4077–4081. [PubMed: 8626743]

6. Roher AE, Chaney MO, Kuo YM, Webster SD, Stine WB, Haverkamp LJ, et al. Morphology and toxicity of Abeta-(1–42) dimer derived from neuritic and vascular amyloid deposits of Alzheimer's disease. *J Biol Chem* 1996;271:20631–20635. [PubMed: 8702810]
7. Kuo YM, Emmerling MR, Woods AS, Cotter RJ, Roher AE. Isolation, chemical characterization, and quantitation of A beta 3-pyroglutamyl peptide from neuritic plaques and vascular amyloid deposits. *Biochem Biophys Res Commun* 1997;237:188–191. [PubMed: 9266855]
8. Giulian D, Haverkamp LJ, Yu J, Karshin W, Tom D, Li J, et al. The HHQK domain of beta-amyloid provides a structural basis for the immunopathology of Alzheimer's disease. *J Biol Chem* 1998;273:29719–29726. [PubMed: 9792685]
9. Roher AE, Kokjohn TA. Appraisal of AbetaPP Transgenic Mice as Models for Alzheimer's Disease Amyloid Cascade. *Curr Med Chem Immun Endo & Metab Agents* 2003;3:85–90.
10. Roher AE, Kokjohn TA. Of mice and men: The relevance of transgenic mice Abeta immunizations to Alzheimer's disease. *J Alzheimers Dis* 2002;4:431–434. [PubMed: 12446974]
11. Kalback W, Watson MD, Kokjohn TA, Kuo YM, Weiss N, Luehrs DC, et al. APP transgenic mice Tg2576 accumulate Abeta peptides that are distinct from the chemically modified and insoluble peptides deposited in Alzheimer's disease senile plaques. *Biochemistry* 2002;41:922–928. [PubMed: 11790115]
12. Kuo YM, Kokjohn TA, Beach TG, Sue LI, Brune D, Lopez JC, et al. Comparative analysis of amyloid-beta chemical structure and amyloid plaque morphology of transgenic mouse and Alzheimer's disease brains. *J Biol Chem* 2001;276:12991–12998. [PubMed: 11152675]
13. Esh C, Patton L, Kalback W, Kokjohn TA, Lopez J, Brune D, et al. Altered APP processing in PDAPP (Val717 --> Phe) transgenic mice yields extended-length Abeta peptides. *Biochemistry* 2005;44:13807–13819. [PubMed: 16229470]
14. Van Vickle GD, Esh CL, Kalback WM, Patton RL, Luehrs DC, Kokjohn TA, et al. TgCRND8 amyloid precursor protein transgenic mice exhibit an altered gamma-secretase processing and an aggressive, additive amyloid pathology subject to immunotherapeutic modulation. *Biochemistry* 2007;46:10317–10327. [PubMed: 17705508]
15. Van Vickle GD, Esh CL, Dausgs ID, Kokjohn TA, Kalback WM, Patton RL, et al. Tg-SwDI transgenic mice exhibit novel alterations in AbetaPP processing, Abeta degradation, and resilient amyloid angiopathy. *Am J Pathol* 2008;173:483–493. [PubMed: 18599612]
16. Kuo YM, Webster S, Emmerling MR, De LN, Roher AE. Irreversible dimerization/tetramerization and post-translational modifications inhibit proteolytic degradation of A beta peptides of Alzheimer's disease. *Biochim Biophys Acta* 1998;1406:291–298. [PubMed: 9630681]
17. Atwood CS, Perry G, Zeng H, Kato Y, Jones WD, Ling KQ, et al. Copper mediates dityrosine cross-linking of Alzheimer's amyloid-beta. *Biochemistry* 2004;43:560–568. [PubMed: 14717612]
18. Miravalle L, Calero M, Takao M, Roher AE, Ghetti B, Vidal R. Amino-terminally truncated Abeta peptide species are the main component of cotton wool plaques. *Biochemistry* 2005;44:10810–10821. [PubMed: 16086583]
19. Hung LW, Cicciotosto GD, Giannakis E, Tew DJ, Perez K, Masters CL, et al. Amyloid-beta peptide (Abeta) neurotoxicity is modulated by the rate of peptide aggregation: Abeta dimers and trimers correlate with neurotoxicity. *J Neurosci* 2008;28:11950–11958. [PubMed: 19005060]
20. Roher AE, Palmer KC, Capodilupo J, Wakade AR, Ball MJ. New biochemical insights to unravel the pathogenesis of Alzheimer's lesions. *Can J Neurol Sci* 1991;18:408–410. [PubMed: 1933690]
21. Roher AE, Palmer KC, Yurewicz EC, Ball MJ, Greenberg BD. Morphological and biochemical analyses of amyloid plaque core proteins purified from Alzheimer disease brain tissue. *J Neurochem* 1993;61:1916–1926. [PubMed: 8229002]
22. Loerch PM, Lu T, Dakin KA, Vann JM, Isaacs A, Geula C, et al. Evolution of the aging brain transcriptome and synaptic regulation. *PLoS ONE* 2008;3:e3329. [PubMed: 18830410]
23. Maarouf CL, Dausgs ID, Spina S, Vidal R, Kokjohn TA, Patton RL, et al. Histopathological and molecular heterogeneity among individuals with dementia associated with Presenilin mutations. *Mol Neurodegener* 2008;3:20. [PubMed: 19021905]
24. Roher AE, Kokjohn TA, Esh C, Weiss N, Childress J, Kalback W, et al. The human amyloid-beta precursor protein770 mutation V717F generates peptides longer than amyloid-beta-(40–42) and flocculent amyloid aggregates. *J Biol Chem* 2004;279:5829–5836. [PubMed: 14645225]

25. Kuo YM, Beach TG, Sue LI, Scott S, Layne KJ, Kokjohn TA, et al. The evolution of A beta peptide burden in the APP23 transgenic mice: implications for A beta deposition in Alzheimer disease. *Mol Med* 2001;7:609–618. [PubMed: 11778650]
26. Kuo YM, Crawford F, Mullan M, Kokjohn TA, Emmerling MR, Weller RO, et al. Elevated A beta and apolipoprotein E in A betaPP transgenic mice and its relationship to amyloid accumulation in Alzheimer's disease. *Mol Med* 2000;6:430–439. [PubMed: 10952022]
27. Roher AE, Lowenson JD, Clarke S, Woods AS, Cotter RJ, Gowing E, et al. beta-Amyloid-(1–42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. *Proc Natl Acad Sci U S A* 1993;90:10836–10840. [PubMed: 8248178]
28. Roher AE, Kuo YM. Isolation of amyloid deposits from brain. *Methods Enzymol* 1999;309:58–67. [PubMed: 10507016]
29. Lowenson JD, Clarke S, Roher AE. Chemical modifications of deposited amyloid-beta peptides. *Methods Enzymol* 1999;309:89–105. [PubMed: 10507019]
30. Puente XS, Lopez-Otin C. A genomic analysis of rat proteases and protease inhibitors. *Genome Res* 2004;14:609–622. [PubMed: 15060002]
31. Puente XS, Sanchez LM, Overall CM, Lopez-Otin C. Human and mouse proteases: a comparative genomic approach. *Nat Rev Genet* 2003;4:544–558. [PubMed: 12838346]
32. Mirnics K, Norstrom EM, Garbett K, Choi SH, Zhang X, Ebert P, et al. Molecular signatures of neurodegeneration in the cortex of PS1/PS2 double knockout mice. *Mol Neurodegener* 2008;3:14. [PubMed: 18834536]
33. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* 2007;8:101–112. [PubMed: 17245412]
34. Watson D, Castano E, Kokjohn TA, Kuo YM, Lyubchenko Y, Pinsky D, et al. Physicochemical characteristics of soluble oligomeric Abeta and their pathologic role in Alzheimer's disease. *Neurol Res* 2005;27:869–881. [PubMed: 16354549]
35. Walsh DM, Selkoe DJ. Oligomers on the brain: the emerging role of soluble protein aggregates in neurodegeneration. *Protein Pept Lett* 2004;11:213–228. [PubMed: 15182223]
36. Walsh DM, Selkoe DJ. A beta oligomers - a decade of discovery. *J Neurochem* 2007;101:1172–1184. [PubMed: 17286590]
37. Morrisette DA, Parachikova A, Green KN, LaFerla FM. Relevance of transgenic mouse models to human Alzheimer disease. *J Biol Chem*. 2008
38. Li, CK.; Ting, SY. Evolutionary relationships among rodents. In: Luckett, WP.; Hartenberger, JL., editors. *A multidisciplinary analysis*. Plenum, NY: Springer; 1985. p. 35-58.
39. Yankner BA, Lu T, Loerch P. The aging brain. *Annu Rev Pathol* 2008;3:41–66. [PubMed: 18039130]
40. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron* 2003;39:409–421. [PubMed: 12895417]
41. Pietropaolo S, Feldon J, Yee BK. Age-dependent phenotypic characteristics of a triple transgenic mouse model of Alzheimer disease. *Behav Neurosci* 2008;122:733–747. [PubMed: 18729626]
42. Pietropaolo S, Sun Y, Li R, Brana C, Feldon J, Yee BK. The impact of voluntary exercise on mental health in rodents: a neuroplasticity perspective. *Behav Brain Res* 2008;192:42–60. [PubMed: 18468702]
43. Mastrangelo MA, Bowers WJ. Detailed immunohistochemical characterization of temporal and spatial progression of Alzheimer's disease-related pathologies in male triple-transgenic mice. *BMC Neurosci* 2008;9:81. [PubMed: 18700006]
44. Roher, AE.; Kuo, YM.; Roher, AA.; Emmerling, MR.; Goux, WJ. Chemical analysis of amyloid beta protein in CAA. In: Verbeek, MM.; de Waal, RMW.; Vinters, HV., editors. *Cerebral Amyloid Angiopathy in Alzheimer's Disease and Related Disorders*. Dordrecht: Kluwer Academic Publishers; 2000. p. 157-177.
45. Weller RO, Massey A, Newman TA, Hutchings M, Kuo YM, Roher AE. Cerebral amyloid angiopathy: amyloid beta accumulates in putative interstitial fluid drainage pathways in Alzheimer's disease. *Am J Pathol* 1998;153:725–733. [PubMed: 9736023]

46. Weller RO, Massey A, Kuo YM, Roher AE. Cerebral amyloid angiopathy: accumulation of A beta in interstitial fluid drainage pathways in Alzheimer's disease. *Ann N Y Acad Sci* 2000;903:110–117. [PubMed: 10818495]
47. Roher AE, Kuo YM, Esh C, Knebel C, Weiss N, Kalback W, et al. Cortical and leptomeningeal cerebrovascular amyloid and white matter pathology in Alzheimer's disease. *Mol Med* 2003;9:112–122. [PubMed: 12865947]
48. Roher AE, Weiss N, Kokjohn TA, Kuo YM, Kalback W, Anthony J, et al. Increased Abeta peptides and reduced cholesterol and myelin proteins characterize white matter degeneration in Alzheimer's disease. *Biochemistry* 2002;41:11080–11090. [PubMed: 12220172]
49. Kalback W, Esh C, Castano EM, Rahman A, Kokjohn T, Luehrs DC, et al. Atherosclerosis, vascular amyloidosis and brain hypoperfusion in the pathogenesis of sporadic Alzheimer's disease. *Neurol Res* 2004;26:525–539. [PubMed: 15265270]
50. Wilcock DM, Jantzen PT, Li Q, Morgan D, Gordon MN. Amyloid-beta vaccination, but not nitro-nonsteroidal anti-inflammatory drug treatment, increases vascular amyloid and microhemorrhage while both reduce parenchymal amyloid. *Neuroscience* 2007;144:950–960. [PubMed: 17137722]
51. Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. Neuropathology of human Alzheimer disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003;9:448–452. [PubMed: 12640446]
52. Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 1999;400:173–177. [PubMed: 10408445]
53. Morgan D, Diamond DM, Gottschall PE, Ugen KE, Dickey C, Hardy J, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 2000;408:982–985. [PubMed: 11140686]
54. Racke MM, Boone LI, Hepburn DL, Parsadainian M, Bryan MT, Ness DK, et al. Exacerbation of cerebral amyloid angiopathy-associated microhemorrhage in amyloid precursor protein transgenic mice by immunotherapy is dependent on antibody recognition of deposited forms of amyloid beta. *J Neurosci* 2005;25:629–636. [PubMed: 15659599]
55. Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M, et al. Cerebral hemorrhage after passive anti-Abeta immunotherapy. *Science* 2002;298:1379. [PubMed: 12434053]
56. Wilcock DM, Rojiani A, Rosenthal A, Subbarao S, Freeman MJ, Gordon MN, et al. Passive immunotherapy against Abeta in aged APP-transgenic mice reverses cognitive deficits and depletes parenchymal amyloid deposits in spite of increased vascular amyloid and microhemorrhage. *J Neuroinflammation* 2004;1:24. [PubMed: 15588287]
57. Schroeter S, Khan K, Barbour R, Doan M, Chen M, Guido T, et al. Immunotherapy reduces vascular amyloid-beta in PDAPP mice. *J Neurosci* 2008;28:6787–6793. [PubMed: 18596154]
58. Roher AE, Esh CL, Kokjohn TA, Castano EM, Van Vickle GD, Kalback WM, et al. Amyloid beta peptides in human plasma and tissues and their significance for Alzheimer's disease. *Alzheimers Dement* 2009;5:18–29. [PubMed: 19118806]
59. Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, et al. Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model. *Nat Neurosci* 2002;5:452–457. [PubMed: 11941374]
60. Janus C, Pearson J, McLaurin J, Mathews PM, Jiang Y, Schmidt SD, et al. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 2000;408:979–982. [PubMed: 11140685]
61. Morgan D. Mechanisms of A beta plaque clearance following passive A beta immunization. *Neurodegener Dis* 2005;2:261–266. [PubMed: 16909007]
62. Ferrer I, Boada RM, Sanchez Guerra ML, Rey MJ, Costa-Jussa F. Neuropathology and pathogenesis of encephalitis following amyloid-beta immunization in Alzheimer's disease. *Brain Pathol* 2004;14:11–20. [PubMed: 14997933]
63. Masliah E, Hansen L, Adame A, Crews L, Bard F, Lee C, et al. Abeta vaccination effects on plaque pathology in the absence of encephalitis in Alzheimer disease. *Neurology* 2005;64:129–131. [PubMed: 15642916]

64. Nicoll JA, Barton E, Boche D, Neal JW, Ferrer I, Thompson P, et al. Abeta species removal after abeta42 immunization. *J Neuropathol Exp Neurol* 2006;65:1040–1048. [PubMed: 17086100]
65. Meyer-Luehmann M, Spires-Jones TL, Prada C, Garcia-Alloza M, de Calignon A, Rozkalne A, et al. Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. *Nature* 2008;451:720–724. [PubMed: 18256671]
66. Gervais F, Paquette J, Morissette C, Krzywkowski P, Yu M, Azzi M, et al. Targeting soluble Abeta peptide with Tramiprosate for the treatment of brain amyloidosis. *Neurobiol Aging* 2007;28:537–547. [PubMed: 16675063]
67. Kukar T, Prescott S, Eriksen JL, Holloway V, Murphy MP, Koo EH, et al. Chronic administration of R-flurbiprofen attenuates learning impairments in transgenic amyloid precursor protein mice. *BMC Neurosci* 2007;8:54. [PubMed: 17650315]
68. McGeer PL, McGeer EG. NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies. *Neurobiol Aging* 2007;28:639–647. [PubMed: 16697488]
69. Augustin S, Rimbach G, Augustin K, Schliebs R, Wolfram S, Cermak R. Effect of a short- and long-term treatment with Ginkgo biloba extract on Amyloid Precursor Protein Levels in a transgenic mouse model relevant to Alzheimer's disease. *Arch Biochem Biophys*. 2008
70. Chauhan NB, Siegel GJ, Feinstein DL. Effects of lovastatin and pravastatin on amyloid processing and inflammatory response in TgCRND8 brain. *Neurochem Res* 2004;29:1897–1911. [PubMed: 15532546]
71. Heininger K. A unifying hypothesis of Alzheimer's disease. IV. Causation and sequence of events. *Rev Neurosci* 2000;11(Spec No):213–328. [PubMed: 11065271]
72. Mattson MP. Pathways towards and away from Alzheimer's disease. *Nature* 2004;430:631–639. [PubMed: 15295589]
73. Muller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. *Prog Neurobiol* 2008;85:393–406. [PubMed: 18603345]
74. Turner PR, O'Connor K, Tate WP, Abraham WC. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. *Prog Neurobiol* 2003;70:1–32. [PubMed: 12927332]
75. Liu SJ, Zhang AH, Li HL, Wang Q, Deng HM, Netzer WJ, et al. Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads to hyperphosphorylation of tau and impairment of spatial memory. *J Neurochem* 2003;87:1333–1344. [PubMed: 14713290]
76. Engel T, Hernandez F, Avila J, Lucas JJ. Full reversal of Alzheimer's disease-like phenotype in a mouse model with conditional overexpression of glycogen synthase kinase-3. *J Neurosci* 2006;26:5083–5090. [PubMed: 16687499]
77. Heininger K. A Unifying Hypothesis of Alzheimer's Disease. I. Ageing Sets the Stage. *Hum Psychopharmacol* 1999;14:363–414.
78. Heininger K. A Unifying Hypothesis of Alzheimer's Disease. II. Pathophysiological Processes. *Hum Psychopharmacol* 1999;14:525–581.
79. Heininger K. A unifying hypothesis of Alzheimer's disease. III. Risk factors. *Hum Psychopharmacol* 2000;15:1–70. [PubMed: 12404343]
80. Roher AE, Kokjohn TA, Beach TG. An Association with Great Implications: Vascular Pathology and Alzheimer Disease. *Alzheimer Disease & Associated Disorders* 2006;20:73–75. [PubMed: 16493240]
81. Baloyannis SJ. Mitochondrial alterations in Alzheimer's disease. *J Alzheimers Dis* 2006;9:119–126. [PubMed: 16873959]
82. Devi L, Prabhu BM, Galati DF, Avadhani NG, Anandatheerthavarada HK. Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J Neurosci* 2006;26:9057–9068. [PubMed: 16943564]
83. Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH. Mitochondria are a direct site of A beta accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum Mol Genet* 2006;15:1437–1449. [PubMed: 16551656]

84. Chen JX, Yan SD. Amyloid-beta-induced mitochondrial dysfunction. *J Alzheimers Dis* 2007;12:177–184. [PubMed: 17917162]