The Evolution of Aβ Peptide Burden in the APP23 Transgenic Mice: Implications for Aβ Deposition in Alzheimer Disease

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Abstract

Background: High levels of Aβ in the cerebral cortex distinguish demented Alzheimer’s disease (AD) from nondemented elderly individuals, suggesting that decreased amyloid-beta (Aβ) peptide clearance from the brain is a key precipitating factor in AD.

Materials and Methods: The levels of Aβ in brain and plasma as well as apolipoprotein E (ApoE) in brain were investigated by enzyme-linked immunosorbent assay (ELISA) and Western blotting at various times during the life span of the APP23 transgenic (Tg) and control mice. Histochemistry and immunocytochemistry were used to assess the morphologic characteristics of the brain parenchymal and cerebrovascular amyloid deposits and the intracellular amyloid precursor protein (APP) deposits in the APP23 Tg mice.

Results: No significant differences were found in the plasma levels of Aβ between the APP23 Tg and control mice from 2–20 months of age. In contrast, soluble Aβ levels in the brain were continually elevated, increasing 4-fold at 2 months and 33-fold in the APP23 Tg mice at 20 months of age when compared to the control mice. Soluble Aβ42 was about 60% higher than Aβ40. In the APP23 Tg mice, insoluble Aβ40 remained at basal levels in the brain until 9 months and then rose to 680 µg/g cortex by 20 months. Insoluble Aβ40 was negligible in non-Tg mice at all ages. Insoluble Aβ42 in APP23 Tg mice rose to 60 µg/g cortex at 20 months, representing 24 times the control Aβ42 levels. Elevated levels of ApoE in the brain were observed in the APP23 Tg mice at 2 months of age, becoming substantially higher by 20 months. ApoE colocalized with Aβ in the plaques. Beta-amyloid precursor protein (βAPP) deposits were detected within the neuronal cytoplasm from 4 months of age onward. Amyloid angiopathy in the APP23 Tg mice increased markedly with age, being by far more severe than in the Tg2576 mice.

Conclusions: We suggest that the APP23 Tg mouse may develop an earlier blockage in Aβ clearance than the Tg2576 mice, resulting in a more severe accumulation of Aβ in the perivascular drainage pathways and in the brain. Both Tg mice reflect decreased Aβ elimination and as models for the amyloid cascade they are useful to study AD pathophysiology and therapy.

Introduction

Alzheimer's disease (AD) has a multifactorial pathogenesis in which one of the major features is the deposition of amyloid-beta (Aβ) peptides as plaques in brain parenchyma and in vessel walls as cerebral amyloid angiopathy (CAA). Despite the prominence of insoluble deposits of Aβ in the AD brains, recent observations suggest that these brains can be distinguished from those of nondemented elderly, not only by the amount of insoluble Aβ in plaques in the brain but also by the high levels of soluble Aβ (1,2). This suggests that failure of soluble Aβ to clear from the extracellular space of the cortex may play a major role in the induction of dementia in AD. The questions therefore arising are: how does Aβ normally drain from the brain, how is this drainage impeded, and how can drainage of Aβ be improved?

Tracer studies and physiologic experiments in rodents have shown that drainage of interstitial fluid and proteins from the extracellular space of the brain depends on (1) the size and charge of the molecules, (2) the dimensions of the extracellular space of the cortex, and (3) the presence of flow channels around capillaries and arteries (3–6). The pattern of distribution of a number of different amyloids such as Aβ (7), cystatin (8), PrP (9), and Aβ (10,11) in their...
respective cerebral amyloid angiopathies suggests that amyloid peptides are deposited in perivascular drainage pathways, both within the cortex and in the leptomeninges (12). Defining the biochemical factors and physiologic influences that govern drainage of all these amyloid peptides from the human brain will offer therapeutic opportunities for the reduction in amyloid load in the brain and thus possibly amelioration of the clinical features of AD and other dementias.

The development of beta-amyloid precursor protein (APP) transgenic (Tg) mice carrying mutations that enhance the production of the Aβ peptides has opened new avenues for testing the hypothesis that failure of perivascular drainage of Aβ is a major event in the pathogenesis of AD. In this dementia, the amount of insoluble Aβ n-40 and Aβ n-42 accumulating in the brain parenchyma and cerebrovascular walls appears to be intimately correlated with the apolipoprotein E (ApoE) genotype. In the brain, ApoE plays a major role in the transport of cholesterol and triglycerides, and in the transport and clearance of Aβ peptides, and is synthesized by astroglial and microglial cells. In humans there are three allelic forms of ApoE recognized as e2, e3, and e4, which code for three ApoE polypeptides: ApoE2, ApoE3, and ApoE4. There is a definitive association between the number of copies an individual has of the ApoE4 form and the risk of developing early-onset AD as well as between ApoE4 and the CAA in AD. Those carrying the ApoE e4/e4 genotype have an overwhelming amount of amyloid deposited in the brain parenchyma and in cortical and leptomeningeal vessel walls.

The APP Tg mice also provide an opportunity for tracking amyloid deposition and drainage. Thus, throughout the life span of these rodents, the level of water-soluble and water-insoluble Aβ peptides and ApoE can be measured in the brain and correlated with levels of soluble Aβ in the plasma. From a chemical viewpoint, the Aβ peptides can be structurally analyzed and equated with the type of amyloid deposits observed morphologically. Furthermore, the differences between strains of Tg mice can be determined, thus offering different facets of the spectrum of amyloid deposition and drainage. Most importantly, however, the Tg animal models would be ideal for assessing the efficacy of drugs or antibodies destined to increase clearance of Aβ, reduce the amyloid burden, and enhance cognitive ability.

The present study compares levels of Aβ in blood and brain throughout the life span of APP23 Tg mice. In this strain, the βAPP gene expression is driven by the neuronal Thy-1 promoter. Amyloid deposition is first visible microscopically in the brain parenchyma and in arterial walls as CAA at about 7–9 months of age (13). Aβ levels in the brain and plasma are compared between APP23 Tg and control mice from 2–20 months of age. Because the age of onset of sporadic AD and the metabolism of Aβ appear to be intimately associated with ApoE (14), we also determined the levels of ApoE in the mouse brain tissue. Finally, the pattern of Aβ accumulation in the brain in APP23 Tg mice is compared to that of another strain of APP Tg mouse, the Tg2576, that we characterized previously (15). We hypothesize that a progressive failure in the clearance of Aβ from the brain to the blood in the βAPP 23 Tg mice results in the characteristic accumulation pattern of both soluble and insoluble Aβ in the brains of these animals.

**Materials and Methods**

**Animals and Tissues**

The levels of Aβ40 and Aβ42 were estimated in the blood and in the brains of the APP23 Tg mice and in non-Tg control mice. In the brain, the Aβ peptides were quantified in the water-soluble and water-insoluble tissue fractions. A total of 72 mice were used in these experiments: 36 were Tg for the human βAPP751 carrying the Swedish mutations (K670N and M671L) under the neuronal murine Thy-1 promoter fragment, and 36 were wild-type littersmates that acted as controls. At 2, 4, 9, 12, 14, and 20 months of age, six Tg mice and six controls were selected with equal numbers of male and female mice. All Tg mice used in this study were hemizygous.

**Blood**

Approximately 1 ml of blood was obtained by cardiac puncture and drained into a 1-ml syringe primed with 25 μl of ethylenediaminetetraacetic acid (EDTA) as anticoagulant. The plasma was separated from the blood and in arterial walls as CAA at approximately 80°C until used. Mouse brains were removed immediately post mortem; the left cerebral hemispheres were rapidly frozen in liquid nitrogen and stored at −80°C and the right hemispheres were fixed in 4% buffered paraformaldehyde.

**Isolation and Quantification of Aβ Peptides**

**Blood** One hundred microliters of the rodent plasma was diluted with 400 μl of 98% formic acid (Fluka Chemie AG, Buchs, Switzerland; glass distilled in our laboratory) and loaded onto a size-exclusion Superose 12-column equilibrated with 80% glass distilled formic acid (GDF). Plasma fractionation was carried out on a Fast Performance Liquid Chromatography apparatus (FPLC, Pharmacia Biotech, Uppsala, Sweden) at a flow rate of 15 ml per hr at room temperature (RT) and monitored at 280 nm. Fractions containing the 3- to 8-kDa peptides were collected in polypropylene tubes, pooled, and, after the addition of 5 μl of 2% betaine, the acid was eliminated by vacuum centrifugation. The dried specimens were dissolved in 50 μl of 80% GDF, then diluted with 250 μl of 10 X TTBS (0.5 M Tris-HCl, pH 8.0, 1.37 M NaCl, 27 mM KCl, 0.5%
Tween 20). Volumes were then adjusted to 1 ml with distilled water (DW), the pH adjusted to 7.4 with 10 N NaOH using a pH meter equipped with a microelectrode and each specimen’s volume adjusted to 2.5 ml with DW. Samples were immediately submitted to Aβ immunoassay: 50 μl of the capture antibody were plated either with the R163 antibody raised against Aβ residues 34–40 or with the R165 antibody raised against Aβ residues 36–42, at concentrations of 10 μg/ml in 10 mM sodium carbonate, pH 9.6. After the addition of 100 μl of blocking solution (1% bovine serum albumin in TTBS), the plates were incubated for 1 hr at RT. Either 100 μl of the test specimens or of the Aβ40 or Aβ42 standards was added in triplicate and the plates incubated for 2 hr at RT on a rocking apparatus. Unbound materials were removed by 4 TTBS automatic washes (Tecan, Salzburg, Austria). After addition of 50 μl of the reporting antibody (4G8, raised against residues 17–24 of Aβ) labeled with europium according to the manufacturer’s specifications (Wallac Inc., Gaithersburg, MD, USA), the specimens were incubated for 1 hr at RT and washed four times with TTBS and three times with DW. Following addition of 50 μl of the Enhancement Solution (Wallac Inc.), the plates were read in a fluorimeter (Wallac Inc.) at an excitation of 340 nm and emission of 615 nm. The concentrations of Aβ in the samples were calculated using the standard curves generated by each plate (range 25–1000 pg/ml) as a frame of reference and the final values adjusted to ng/ml considering the experimental dilution factors.

**Brain** The amounts of water-soluble and water-insoluble Aβ peptides were related to the amount of brain tissue (in mg) in the sample. Left cerebral hemispheres from each mouse were minced and thoroughly disrupted with a Dounce homogenizer in 4 ml of 50 mM Tris HCl, pH 7.4 buffer containing a mixture of protease inhibitors (1 mM EDTA, 500 μg/l leupeptin, 700 μg/l pepstatin, 35 mg/l phenylmethylsulfonyl fluoride, 100 mg/l o-phenanthroline, and 100 mg/l benzamidine). The specimens were centrifuged at 100,000 ×g in a Sorvall AH-650 rotor at 4°C for 1 hr. One hundred microliters of the supernatant was applied to the microtiter plates for europium immunoassay of water-soluble Aβ peptides. The insoluble pellets were homogenized in 1 ml of 2% SDS. Five hundred microliters of the homogenate was mixed with 2 ml of 90% GDFA. Fifty microliters of the acid lysate was neutralized as mentioned for the plasma samples with the exception that the final volume was brought up to 5 ml instead of 2.5 ml and submitted to europium immunoassay. The final yields of soluble and insoluble Aβ were adjusted to μg per g of cerebral cortex.

**Protein Quantification and Western Blotting**

Protein determinations were performed on the aqueous supernatants and water-insoluble pellets of brain tissue. The pellets were homogenized in 1 ml of 2% SDS in 20 mM Tris HCl, pH 8.0, and diluted 500 times prior to application to the microtiter plates. The protein values were determined using Micro BCA Protein Assay reagent kit (Pierce, Rockford, IL, USA).

Western blotting was used to determine the levels of ApoE in the mouse brains. For this purpose we used a goat anti-rat polyclonal antibody (see below). This antibody was raised against rat ApoE purified by the method of Holmquist and Carlson (16), its purity assessed by SDS-PAGE (17), and its specificity confirmed by two-dimensional crossed immunoelectrophoresis (18). The Western blots were developed with the Supersignal Chemiluminescent Substrate (Pierce) and the relative intensity of the bands measured on a scanning ChemiImager (Alpha Innotech Corp., San Leandro, CA, USA).

**Histochemical and Immunohistochemical Analyses** The right hemispheres from the Tg and non-Tg mice were fixed for 48 hr in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, the tissue dehydrated with alcohols and xylene, and embedded in paraffin wax. Parasagittal 5-μm thick sections of whole-hemisphere sections were stained with modified Cambell-Switzer, hematoxylin and eosin, thioflavin S, and by immunohistochemistry (19) for Aβ, βAPP, and ApoE. The primary antibodies used for Aβ were 6E10 (Senetec, Maryland Heights, MO, USA) against the Aβ amino acid sequence 1–17, and R163 and R165 against the C-terminal portions of the Aβ peptide ending at amino acids 40 and 42 (20), respectively (all three were used at a dilution of 1:1000). Frozen sections (40-μm thick) from an additional set of two APP23 Tg animals and two non-Tg animals for each age point in this study were used for immunohistochemical staining for ApoE (anti-rat ApoE, kindly provided by Dr. C. Bisgaier, Esperion Therapeutics, Inc. and by Dr. P. Dolphin, Dalhousie University) at a dilution of 1:100, and for heparan sulfate proteoglycan (Chemicon, Temecula, CA, USA). For βAPP, the 22C11 antibody (Boehringer-Mannheim, Indianapolis, IN, USA) against the N-terminus and the R37 antibody (21) against the C-terminus were used at dilutions of 1:50 and 1:5000, respectively. Sections stained for Aβ were pretreated for 5 min with 90% formic acid while those stained for βAPP with the 22C11 antibody were pretreated for 1 min. Sections stained for ApoE or βAPP with the R37 antibody were first boiled for 10 min in 0.1 M sodium citrate buffer, pH 7.4. Secondary antibodies and avidin-biotin peroxidase complex were obtained from Vector (Burlingame, CA, USA). 3,3’-diaminobenzidine (Sigma, St. Louis, MO, USA) with 1% nickel ammonium sulfate was used as the chromogen. Some sections were counterstained with neutral red. Stained sections were viewed with bright-field light microscopy (immunoperoxidase stains) or fluorescence microscopy and confocal scanning laser microscopy (thioflavine S-stained sections).
Preparation of Whole-Mounts of Cerebral Vessels

The cerebrums from 20-month-old APP23 Tg mice (n = 4), and Tg2576 mice (n = 4), were each sectioned to separate the hemispheres and coronally cut into two slices. The brain tissue was lysed with two changes of 50 ml each of 2% SDS, 2 mM EDTA, 50 mM Tris-HCl, pH 7.4. The tissue was very gently stirred with a magnetic bar for a period of 36 hr, after which the vascular tufts were recovered by filtration through a 40-μm nylon mesh fitted at the end of a 2.5 × 10 cm Plexiglass cylinder. The nylon mesh was immediately shaken into 50 ml of DW and the vessels recovered by centrifugation at 1500 × G for 15 min and washed once more with 50 ml of DW. The resulting SDS-insoluble vascular extracellular matrix and basal lamina with the attached amyloid were spread out on a microscope slide, air dried at 60°C for 3 hr, fixed with absolute ethanol for 30 min, and rinsed with DW. The specimens were stained with 0.2 μm filtered 1% aqueous thioflavine S for 10 min, rinsed with 70% ethanol to remove unbound stain, and mounted under glass cover slips with a gel medium.

Results

Aβ Levels in Tg Mouse Plasma

There was an initial elevation of the plasma levels of Aβ40 and Aβ42 from 2–9 months of age in the APP23 Tg mice (Fig. 1AB). By 14 months, levels of Aβ had fallen, followed by a more moderate decrease by 20 months. A similar pattern was observed in the non-Tg mouse population, with no statistically significant differences between the Tg and non-Tg mice at any age for both Aβ40 and Aβ42 (Fig. 1C). At 9 months of age, the average values for Aβ40 and Aβ42 in the Tg mice were 13 ng/ml and 19 ng/ml, respectively, which were close to the values for the non-Tg mice: 8 ng/ml and 16 ng/ml, respectively. By 20 months of age, the plasma levels of Aβ40 and Aβ42 in the Tg mice decreased to an average of 4 ng/ml and 3.8 ng/ml, respectively. In the non-Tg mice, these values were lower: 1 ng/ml and 0.4 ng/ml, respectively (Fig. 1AB). However, the differences between Aβ40 levels in Tg and non-Tg mice at 14 and 20 months were not significant (p = 0.051 and p = 0.113, respectively).

Aβ Levels in APP23 Tg Mouse Brains

The water-soluble fractions of Aβ40 and Aβ42 peptides in APP23 Tg mouse brain were substantially and constantly elevated at all ages when compared to the levels in non-Tg mice (Fig. 2AB). Throughout the life span of the Tg mice, the average levels of the Aβ42 were higher than those of the Aβ40 (34.4 μg/g of cortex versus 21.6 μg/g). In contrast, the average values for the rodent endogenous Aβ40 and Aβ42 were similar in the non-Tg mice (3.9 μg/g and 4.1 μg/g, respectively). At 2 months of age, the mean value for the total soluble Aβ in the brains of APP23 Tg mice was already four times greater than in the non-Tg mice. At 20 months of age, this difference was far more pronounced, amounting to 33 times more total Aβ in the APP23 Tg mouse brains than in the non-Tg mice (Fig. 2C). The relatively high values of total soluble Aβ at 2 months of age in the non-Tg mice may result from the higher values of endogenous βAPP normally observed during fetal development.

The water-insoluble fractions of Aβ40 remained at a basal level during the first 9 months after birth in the brains of the APP23 Tg mice. This was followed by a moderate increase until 14 months of age and then a sharp elevation, to 680 μg/g of cortex, by 20 months (Fig. 3A). As expected, the levels of endogenous insoluble Aβ40 remained negligible throughout the life span of the non-Tg mice (Fig. 3A). The levels of the water-insoluble Aβ42 in the APP23 Tg mice remained at a basal endogenous level until the age of 14 months but then increased.

Fig. 1. The levels of Aβ in the plasma of the APP23 Tg mice and control littermates. The measurements of these peptides were made in the 3- to 8-kDa FPLC formic acid-separated fractions at 2, 4, 9, 12, 14, and 20 months of age. Solid and broken lines correspond to the Tg mice and control mice, respectively. (A) Aβ40. (B) Aβ42. (C) The sum of Aβ40 and Aβ42. There were no significant differences between the Tg and non-Tg mice.
by 20 months of age to about 60 μg/g of cortex (Fig. 3B). The average value for the non-Tg water-insoluble Aβ42 at 20 months of age was about 24-fold lower (2.5 μg/g of cortex) than in the APP23 Tg mice and considered to be the basal endogenous value (Fig. 3B).

When the levels of total soluble and insoluble Aβ40, as measured by europium immunoassay in the APP23 Tg mice at 20 months of age, were added and averaged, they amounted to 700 μg/g of brain. By the same age, the water-soluble and -insoluble Aβ42 yielded a mean value of 91.5 μg/g of brain (Figs. 2 and 3). There was a 7.7-fold greater abundance of Aβ40 over Aβ42. The Aβ40:Aβ42 ratio yielded by the acid extraction and subsequent chromatographic separation on a high performance liquid chromatography reverse-phase 5RPC column, in a previous study, however, showed only a 3- to 4-fold greater abundance of Aβ40 over Aβ42 (13). The discrepancy may be due to the use of 22-month-old Tg mice in the previous study rather than the younger 20-month-old Tg mice in the present study. Furthermore, during the water extraction for the EuIA some of the water-soluble Aβ42 may be bound to other molecules which hinders immunoreactive epitopes (22).

Levels of ApoE in Tg Mouse Brains

The values of ApoE in the brains of the APP23 Tg mice were elevated relative to those obtained for the non-Tg littermates (Fig. 4). This elevation was already noticeable at 2 months of age and became significant (p < 0.020) in the Tg mice at 14 and 20 months of age (Fig. 4).

Histologic Evolution of Amyloid Deposits

In the present study, deposits of Aβ are first detected by histology in the brain at 9 months of age in the APP23 Tg mice. However, in a more extensive study, amyloid deposits have been observed in animals as young as 6.7 months of age (M. Staufenbiel, unpublished observations). Such deposits are isolated or widely scattered and reside mainly in the frontal cortex (Fig. 5A). With increasing age, plaques increase in number and in size, ranging up to 100 μm in diameter at 12 months (Fig. 5B), 180 μm at 14 months (Fig. 5C), and 200 μm at 20 months (Fig. 5D).
Amyloid plaque core morphology also changes with age. At 9 months, plaques consist mostly of small dense bodies about 10–20 μm in diameter (Fig. 5E). Beginning at 12 months, star-like forms are more common, with the arms of the stars composed of radially arranged bundles of filaments (Fig. 5FG). Cerebral amyloid angiopathy is first apparent at 9 months of age in APP23 Tg mice and, like the Aβ plaques, becomes increasingly more common in older animals. The thickness of the vascular amyloid coat also increases in older animals (Fig. 5D), with some vessels in 14- and 20-month-old animals exhibiting prominent nodular deposits of amyloid (Fig. 5H).

Staining patterns for Aβ immunocytochemistry vary in the 14- and 20-month-old APP23 Tg mouse brains, depending on the antibody used. With the 6E10 antibody (Fig. 5J), plaque cores are intensely stained and diffuse plaque-like staining is also present, whereas the R163 antibody, against Aβ40, stains plaque cores intensely (Fig. 5J). The R165 antibody, against Aβ42, stained fewer plaque cores with less intensity (Fig. 5K), but also resulted in faint, focal staining of the neuropil, similar to diffuse plaques seen in the human brains. Sections stained with the antibody against ApoE showed intense staining of plaques (Fig. 5L) from their first appearance at 9 months of age. Control sections for the ApoE staining was not present, however, with either the R163 antibody, against Aβ, or R165 antibodies (data not shown).

Immunocytochemistry for Aβ and βAPP revealed cytoplasmic staining of neurons. The 6E10 antibody against Aβ showed intense, punctate staining within the cytoplasm of cortical (mostly layer III, Fig. 5M) and hippocampal (CA1 region) pyramidal neurons (data not shown). This was first apparent in 4-month-old animals when only a few neurons per section were positive. Both the number of positive neurons and the number and intensity of intracellular punctae increased progressively with age so that by 20 months positively stained neurons were very common in the cortex. Such intracellular staining was not present, however, with either the R163 (Fig. 5N) or R165 antibodies (data not shown) against Aβ ending at amino acid 40 or 42, respectively. Staining with the antibodies 22C11 (Fig. 5O) and R37 (Fig. 5P) against the N- and C-terminal...
portions of APP, respectively, showed frequent and profuse intraneuronal granular deposits within the majority of cortical neurons.

None of the control sections were stained when antibodies directed at Aβ were used. This included sections of brain from APP23 Tg mice stained as indicated, but with omission of the primary antibody, as well as sections from control, non-Tg littermates. For APP immunocytochemistry, non-Tg littermates showed light neuropil staining but lacked intraneuronal staining. Control sections of brain prepared by omitting the primary antibody were devoid of staining in Tg and non-Tg mouse brain.

**Vascular Amyloid Load**

Whole-mount preparations of the vascular tree from 22-month-old APP23 Tg mouse cerebrum, stained with thioflavine S, revealed an extensive and heavy load of amyloid in the walls of arteries and arterioles in the brain parenchyma and in the leptomeninges (Fig. 6B). Notably, this contrasted sharply with the light and scanty load of vascular amyloid observed in another strain of Tg rodent, the Tg2675 mice, also at 22 months of age (Fig. 6A).

**Discussion**

We demonstrated that Aβ40 and Aβ42 levels in the plasma of APP23 Tg mice were not significantly elevated above those of non-Tg mice at any point between 2 and 20 months of age. In contrast, water-soluble and water-insoluble Aβ in the brain were elevated at 2 and 9 months, respectively, and increased considerably with advancing age. The rise of Aβ in the brain was accompanied by a significant increase in ApoE, especially between 14 and 20 months of age. Histologic and immunocytochemical analyses revealed morphologic changes in the APP23 Tg mice that reflected the increased brain Aβ levels. Amyloid plaques increased progressively in size and number from 9 to 20 months in the Tg mice, and a substantial amount of Aβ was deposited in vessel walls as cerebral amyloid angiopathy was histologically evident from the age of 9 months onward. Immunocytochemistry showed colocalization of ApoE, Aβ40, and Aβ42 in plaques, and also revealed APP within neuronal cytoplasm of the APP23 Tg mice.

The origin of Aβ in AD is uncertain, even though it is established that APP is the Aβ precursor molecule. Immunocytochemical analysis of APP23 Tg mice revealed APP localization to neuronal cell bodies. The possibility of Aβ in the neurons was also raised as they were stained with the 6E10 antibody, which initially suggests the presence of intraneuronal Aβ. However, the lack of immunoreactivity with the R163 and R165 antibodies, which specifically recognize the Aβ peptide C-termini, revealed that the 6E10 antibody is actually recognizing the Aβ sequence within the APP. The abundant intraneuronal staining with antibodies against the N- and C-termini of the APP molecule confirmed the presence of large amounts of full-length APP within these cells as early as 4 months of age. Therefore, it appears that intracellular Aβ concentrations remain at low, histochemically
undetectable levels in these animals despite marked overexpression of mutant APP. This finding may have negative implications for recently emerging hypotheses regarding the importance of intraneuronal Aβ in AD pathogenesis (22,23). Our immunocytochemical studies have shown that a large part of the neuronal cytoplasm is packed with APP that could cause severe neuronal damage at the extreme level expressed in the Tg mice. This may, per se, create an abnormal condition, which may not be part of the typical human AD pathogenesis.

Our experiments revealed that substantial differences exist in Aβ accumulation kinetics and patterns in different Tg mouse strains that overproduce human amyloid through enhanced expression of APP. The amount of Aβ in the brain and plasma of the Tg2576 mice and control littermates was measured at intervals between 2–20 months (15). The levels of total Aβ in plasma increased up to 12 months of age in the Tg2576 mice, relative to controls, and then fell to almost endogenous Aβ levels (Fig. 7).

In contrast, while both water-soluble and water-insoluble forms of Aβ remained at basal levels in the brain until 9 months of age, Aβ increased exponentially from 12–20 months of age. Amyloid deposits in the brain parenchyma are first discernible at 12 months of age in Tg2576 mice.

The dramatic differences between the APP23 and Tg2576 mice in the distribution of amyloid may be due in part to the cDNA construct used. The APP23 Tg mouse contains the APP751 cDNA construct and includes the Kunitz protease inhibitor domain, which is absent in the APP695 cDNA construct used in the Tg2576 Tg mice. Although both the Tg mouse APPs carry the Swedish mutations to enhance the generation of Aβ, the promoters are different, with the Thy-1 in the APP23 Tg mouse and the hamster prion in the Tg2576 Tg mouse. At least as surprising as the difference between the two Tg mice is the difference between the control strains. This may be due to the different backgrounds of the two Tg mice. The APP23 Tg mice are in the C57BL/6J (Black 6), which have been successfully back-crossed with the same strain of mice, while the Tg2576 mice were in the C57B6/SJL, which have been back-crossed to the wild-type B6SJL mice from Jackson Laboratories, Bar Harbor, Maine, USA.

The acute overexpression of proteins in transgenic mice may result in induction of compensatory physiologic responses designed to eliminate the abundant alien protein. Several mechanisms, alone or in concert, include extracellular proteolysis, microglial, and/or astroglial phagocytosis with lysosomal degradation, sequestration of Aβ by binding species such as ApoE or β2-macroglobulin, followed by receptor-mediated endocytosis by brain or vascular cells expressing LDL or LRP receptors. Moreover, a portion of the Aβ could be eliminated through the arachnoid granulations that remove cerebrospinal fluid (CSF) into the venous circulation.

In addition to direct mechanisms of Aβ elimination, levels of brain protein may be controlled by allowing transport into the blood. There are several situations in which molecules are constantly produced within brain tissue but the absence of disposal mechanisms leads to excessive accumulation either within cells or extracellular spaces. Thus, different amyloid polypeptides, like Aβ, cystatin, Aβi, and prions accumulate in the extracellular spaces of the cerebral cortex. The pattern of Aβ accumulation in the walls of intracerebral and leptomeningeal arteries in CAA suggests that it is deposited in the periarterial interstitial fluid drainage channels through which, as suggested, Aβ is normally eliminated from the human brain (12,24). In CAA, Aβ accumulates in the walls of cortical capillaries, arterioles, and small arteries and in leptomeningeal arteries to such a degree that it is stained in histologic preparations. Biochemical studies, however, have detected the presence of Aβ in the walls of arteries as large as the middle cerebral artery and basilar artery in young adults in their third decade. Levels of Aβ in these arteries are significantly increased in AD, but no Aβ accumulation has been detected in extracranial arteries (25). These observations support the hypothesis that soluble Aβ, possibly bound to ApoE, is eliminated from the human brain along periarterial interstitial fluid drainage pathways (26) that are homologous with those described in rodents (5,6,27). Soluble and particulate tracers injected into the central gray matter of the rodent brain pass along periarterial pathways into regional lymph nodes in the neck (6,28,29). Experimental studies strongly suggest that these pathways play a significant role in B-cell immunity and T-cell immune reactions in the rodent brain (6,28–30).

The pattern of Aβ abundance in the Tg2576 mouse is in marked contrast to that in the APP23 Tg mouse. High levels of water-soluble Aβ in the brains of APP23 Tg mice from 2–20 months of age imply that clearance of Aβ may not be as efficient as in Tg2576.

![Fig. 7. Time-dependent comparison between the amount of plasma levels of total Aβ between two strains of Tg mice (APP23 versus Tg2576) and their respective control littermates. Tg, transgenic; C, non-transgenic controls. All measurements were carried out by EuIA and adjusted to ng/ml of plasma.](image-url)
mice below 12 months of age. This argument is supported by the low values of Aβ found in the plasma of the APP23 Tg mice relative to those observed in the Tg2576 mice (Fig. 7). The less efficient clearance of Aβ is reflected in the earlier histologic detection of amyloid in the brains of the APP23 Tg mice at 7–9 months compared to the Tg2576 mice at approximately 10–12 months. The vascular amyloid deposition pattern characteristic of APP23 Tg mice and AD, in which an inverse gradient is evident, initiating in the arterioles and small arteries with decreased deposition detectable in larger diameter vessels is consistent with a failure to clear the high levels of soluble amyloid into circulation. In AD, the deposition of vascular amyloid starts at the most peripheral layer of the tunica media, which is in direct contact with periartrial spaces, suggesting that the soluble Aβ is entrapped by the extracellular matrix as it moves into systemic circulation. Such severe changes in the integrity of the cerebral vessels, with a loss of vascular myocytes, results in compromised cerebral blood flow and ischemia. The vast amount of vascular amyloid in the APP23 Tg mice results in a prominent pathology leading to infarcts, hemorrhagic strokes, aneurisms, hematomas, vascular obstructions, and vasculitis (31,32). These pathologic changes have not been described for the Tg2576 mice in which the amount of vascular amyloid in comparison is moderate.

APP23 Tg mice resemble AD patients who carry the ApoE4/E4 phenotype, which results in an earlier onset of AD with a preponderance of Aβ40 in the brain and an overwhelming parenchymal and leptomeningeal CAA. In contrast, the suggested efficient Aβ elimination from the brain to the blood during early life of the Tg2576 mice may be analogous to human AD patients with phenotypes E3/E3 or E3/E2 in which there is a lesser amount of CAA. If the ApoE phenotype plays an important role in determining the load of vascular Aβ, then potential differences in the ApoE primary structure between the strains of Tg mice may give further clues as to how ApoE is involved in Aβ transport and elimination.

Although CAA occurs in both the APP23 Tg mice and Tg2576 mice, it is only histologically discernible after Aβ40 and Aβ42 have accumulated in the brain and drainage of Aβ to the blood has apparently ceased in the Tg2576 mice. As in human cerebral vessels, biochemically detectable amyloid (33) may be deposited prior to its appearance as histologically detectable CAA. A measurable amount of Aβ, estimated at about 20 ng/mg of protein, is needed for the visual detection of amyloid by histologic techniques (K. Bales, personal communication). Insoluble Aβ42 may impede the drainage of the more soluble Aβ40 and provide the initial template for soluble Aβ40 polymerization into fibrils. This appears to be the case in human AD in which Aβ42 accumulates in vessel walls in CAA before the more soluble Aβ40, but thereafter Aβ40 levels rise both within the brain and in vessel walls. The incomplete separation of interstitial fluid drainage pathways from the CSF compartment in rodents (5,27) is reflected in the very high levels of Aβ amounting to approximately 40 ng/ml in the APP23 Tg mice CSF (31). These levels are well above those seen in human AD CSF. Furthermore, CSF Aβ42 and Aβ40 levels are decreased in patients with AD relative to nondemented controls. This probably reflects the greater separation of Interstitial Fluid (ISF) drainage from the CSF in the human brain compared with rodents (6). In humans, the CSF and periartrial space are segregated by the pia mater, whereas in rodents, the separation between these compartments is more primitive and the periartrial space directly communicates with the CSF subarachnoidal space. This may be an additional factor in the higher incidence of Aβ accumulation in aged humans compared to aged animals.

The heterogeneity exhibited in almost every aspect of AD patients has complicated attempts to define risk factors and predict the course of the disease. Consideration of the incomplete analogy between AD and the Tg mouse models has led others to conclude that the more exact AD modeling may require the “humanizing” of Tg systems (34). However, Tg mice may be used to investigate separate facets of AD, even if these models fail to completely mimic the entire AD complex. We have confirmed that the APP23 and the Tg2576 Tg mice can be used to investigate the critical elimination of Aβ from the brain. These transgenic model systems clearly will enable productive evaluations of novel therapeutic interventions directed toward decreasing amyloid formation and deposition.

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