Short Communication

Association of Aortic Atherosclerosis with Cerebral β -Amyloidosis and Learning Deficits in a Mouse Model of Alzheimer's Disease

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High fat/high cholesterol diets exacerbate β -amyloidosis in mouse models of Alzheimer's disease (AD). It has been impossible, however, to study the relationship between atherosclerosis and β -amyloidosis in those models because such mice were on atherosclerosis-resistant genetic backgrounds. Here we report the establishment of AD model mice, B6Tg2576, that are prone to atherosclerosis. B6Tg2576 mice were produced by back-crossing Tg2576 mice, an AD mouse model overexpressing human amyloid β -protein precursor with the Swedish double mutation, to C57BL/6 mice, a strain susceptible to diet-induced atherosclerosis. An atherogenic diet induced aortic atherosclerosis and exacerbated cerebral β -amyloidosis in B6Tg2576 mice. Compared with age-matched non-transgenic littermates, B6Tg2576 mice developed significantly more diet-induced aortic atherosclerosis. Unexpectedly, normal diet-fed B6Tg2576 mice also developed fatty streak lesions (early atherosclerosis) in the aorta. The aortic atherosclerotic lesion area positively correlated with cerebral β -amyloid deposits in B6Tg2576 mice on both atherogenic and normal diets. Furthermore, behavioral assessments demonstrated that B6Tg2576 mice fed an atherogenic diet had more spatial learning impairment than those fed a normal diet. Our results suggest that synergistic mechanisms may be involved in the pathogenesis of atherosclerosis and AD. These findings may have important implications in the prevention and treatment of cardiovascular diseases as well as AD. (*Am J Pathol* 2003, 163:2155–2164)

Growing evidence suggests that cardiovascular risk factors play important roles in the pathogenesis of Alzheimer's

disease (AD). An increase in prevalence of cerebral senile plaques is found in cognitively intact individuals with heart disease compared to age-matched controls with no heart disease.¹ The apolipoprotein (apo) E4 allele is a risk factor for AD as well as cardiovascular diseases.² Hypercholesterolemia is an independent risk factor for AD.³ Levels of atherogenic plasma lipoprotein components, low-density lipoprotein (LDL) cholesterol and apoB, are increased in AD patients and correlate with brain amyloid β -protein ($A\beta$) levels.^{4,5} Levels of atheroprotective plasma lipoprotein components, high-density lipoprotein (HDL) cholesterol and apoA-I, are decreased in AD patients.⁶ An apparent reduction of AD prevalence is found in people taking cholesterol-lowering drugs.^{7,8} Diet-induced hypercholesterolemia causes $A\beta$ deposits in the brain of rabbits⁹ and accelerates cerebral $A\beta$ deposition in amyloid β -protein precursor (APP) transgenic mice.^{10–12} However, the relationship between atherosclerosis and β -amyloidosis in experimental animals has not been investigated.

Mice do not develop atherosclerosis and β -amyloidosis even in old age without genetic and/or dietary manipulations.^{13,14} Indeed, Tg2576 mice, an AD mouse model, are resistant to dietary-induced atherosclerosis since the mice were maintained on an atherosclerosis-resistant genetic background.¹¹ A previous attempt to back-cross Tg2576 mice to C57BL/6 mice, a mouse strain susceptible to dietary-induced atherosclerosis, was unsuccessful due to significant declines in transgene transmission and survival after several generations of back-crossing.¹⁵ We report here the successful transfer of the APP transgene array of Tg2576 mice to the C57BL/6 background and the investigation of the effects

Supported in part by the Alzheimer's Association (NIRG-00-2281) and the National Institutes of Health (AG16582, AG12850, and NS43947).

Accepted for publication August 5, 2003.

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of an atherogenic diet on β -amyloidosis and behavioral functions.

Materials and Methods

Animals and Diets

Tg2576 mice, on C57BL/6XSJL F2 mixed background and overexpressing human APP with the Swedish double mutation,¹⁶ were back-crossed to C57BL/6J background for 10 to 11 generations to generate a line of APP transgenic mice designated as B6Tg2576 mice. Transgenic mice were identified by polymerase chain reaction (PCR) of tail genomic DNA using human APP-specific primers, 5'CGGAGGAGGATGACTCGGAT3' and 5'CAGCTGCTGTCTCTCGTTGG3'. Production of a DNA fragment of about 500 bp in length by PCR indicated the presence of the human APP transgene. At the age of 7 to 9 months, male and female B6Tg2576 mice and age-matched non-transgenic littermates ($n = 21$ and 25 , respectively) were divided randomly into two groups receiving either an atherogenic diet or a normal diet *ad libitum*. The atherogenic diet (TD 88051, Teklad, Madison, WI) contains 15.75% fat, 1.25% cholesterol, and 0.5% sodium cholate (w/w) as described by Paigen et al¹⁷. The mice were observed daily for morbidity and mortality and their body weight recorded weekly. After 4 months of diet feeding, these mice were sacrificed for analyses of plasma cholesterol profiles, aortic atherosclerotic lesions, and cerebral β -amyloidosis. Behavioral functions were assessed in a separate cohort of B6Tg2576 mice ($n = 10$) and non-transgenic littermates ($n = 11$) at age of 15 to 18 months after being fed the atherogenic diet for 4 months. All animal procedures used for this study were prospectively reviewed and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Determination of Plasma Total Cholesterol Concentrations and Lipoprotein Cholesterol Profiles

Blood samples were collected from anesthetized animals by retro-orbital bleeding or by cardiac puncture at the end of experiment. Plasma total cholesterol levels were determined colorimetrically by commercial reagents (Infinity cholesterol reagent; Sigma, St. Louis, MO). Plasma lipoprotein cholesterol profiles were analyzed by the chromatographic method developed in our laboratory.¹⁸

Quantification of Aortic Atherosclerosis

Atherosclerotic lesion areas were quantified by the method of Paigen et al¹⁹ with some modifications. Briefly, after mice were anesthetized and exsanguinated, they were perfused through the left ventricle with saline. The hearts were then fixed in 10% neutral buffered formalin for at least 1 week. After the lower two-thirds of the hearts were removed, the remaining tissue was washed thor-

oughly with distilled water and frozen in freezing medium (OCT Tissue-Tek; Miles Laboratories Ltd, Elkhart, IN), and sectioned in a cryostat at -20°C . Alternate 10- μm sections were saved on slides and observed for the beginning of the aortic root. Sections were then collected for additional 600 μm , or until the aortic cross-section was rounded and the valve cusps were no longer evident. Slides were stained with Oil Red O, and counterstained with hematoxylin. Stained lesion cross-sectional areas were measured in consecutive slides 80 μm apart by image analysis (SigmaScan Pro; SPSS Science, Chicago, IL), and the average lesion area was determined for each aortic sinus over the 400- μm length (five slides) providing the greatest mean lesion area.

Quantification of Cerebral β -Amyloidosis

Protocols for immunohistochemical analysis developed in our laboratory were used in this study.²⁰ Briefly, formalin-fixed and paraffin-embedded tissue sections were subjected to the avidin-biotin immunoperoxidase method to detect the antigens (eg, $\text{A}\beta$) using Vectastain ABC kit (Vector, Burlingame, CA). Primary antibodies used for assessing β -amyloidosis in the brain of mice included: 6E10 (a monoclonal antibody raised against amino acid 1–16 of $\text{A}\beta$, Signet, Dedham, MA), 4G8 (a monoclonal antibody raised against amino acid 17–24 of $\text{A}\beta$, Signet), and a polyclonal rabbit anti- $\text{A}\beta$ antibody (raised against a 30 amino acid synthetic peptide derived from full-length $\text{A}\beta$; Zymed, San Francisco, CA). Congo red and thioflavin S were used also to stain amyloid deposits.²¹ The amyloid burden in the cortex and hippocampus of mouse brain were quantified by the histomorphometry system consisting of a Leica DMR research microscope equipped for fluorescence, polarizer/analyzer, and bright-field microscopy, a SPOT RT Slider digital camera (Diagnostic Instruments, Sterling Heights, MI), and the Image Pro Plus v4 image analysis software (Media Cybernetics, Silver Spring, MD) capable of color segmentation and automation via programmable macros. Multiple images of 1 mm^2 each were captured and analyzed from five coronal brain sections at 500- μm intervals from each mouse using a 10 $\times$ objective and a 10 $\times$ eyepiece lens. A total area of 50 mm^2 giving the highest total $\text{A}\beta$ immunoreactivity was chosen to calculate the amyloid burden expressed as a percentage of total area covered by $\text{A}\beta$ immunoreactivity.

Assessment of Behavioral Functions

Three AD-related behavioral functions, spatial learning and memory, exploration of environmental stimuli, and anxiety, were assessed in transgenic and non-transgenic mice after being fed the atherogenic diet or the normal control diet for 4 months. The testing schedule included exploration of the T-maze (days 1 to 10), the open-field (days 1 to 3), the elevated plus-maze (days 4 to 5), and spatial learning in the Morris water maze (days 6 to 11). All equipment and software were purchased from SD Instruments Inc., San Diego, CA.

All testing procedures were described previously.²² Briefly, spontaneous alternation was tested in a T-maze containing a central stem and two side arms. On the initial trial, the mice were placed in the stem with the right arm blocked (forced choice). After entering the available arm, the mice were kept in it for 60 seconds by closing the barrier behind them. The mice were then retrieved and after removing the barrier were immediately placed back in the stem for a free-choice trial. On each of the following 9 days, the same procedure was repeated, except that the blocked arm on the initial trial was changed alternatively from the right to the left. The number of alternations and the latencies before responding were recorded, with a cut-off period of 60 seconds per trial.

Motor activity was measured in an open-field, made of white acrylic with a 50 cm $\times$ 50 cm surface area and with each wall reaching 38 cm in height. The activity in the central and peripheral zones was recorded by an overhead video camera and analyzed by video-tracking SMART software (SD Instruments). The mice were placed in the open-field for a 5-minute session daily for 3 days. The distance traveled and the time spent in each zone were measured.

Anxiety was measured in elevated plus-maze, consisting of four arms in a cross-shaped form and a central region. Two of the arms were enclosed on three sides by walls, whereas the other two were not. The enclosed or open arms of the maze faced each other. The mice were placed in the central region and their behavior recorded for 5 minutes per session for 2 days. The number of entries and the time spent in either the enclosed or the open arms were measured.

Spatial orientation was evaluated in the Morris water maze consisting of a round basin (diameter, 112 cm) filled with water (22°C) to a height of 31 cm. The water was made opaque by mixing in dry milk to camouflage the escape platform (8 cm $\times$ 8 cm). The pool was placed in a room with abundant extra-maze visual cues. The acquisition of the spatial task consisted of placing the mice next to and facing the wall successively in north (N), east (E), south (S), and west (W) positions, with the escape platform hidden 1 cm beneath water level in the middle of the NE quadrant. In each trial the mouse was allowed to swim until it found the hidden platform, or until 60 seconds had elapsed, at which point the mouse was guided to the platform. The mouse was then allowed to sit on the platform for 10 seconds before being picked up. The escape latency and swim path length (distance) were recorded by the SMART system for four trials daily for 5 days. No pre-training was given before the mice were tested in the Morris water maze.

Statistical Analysis

Data were expressed as mean $\pm$ SE. Comparison of treatment groups was performed by two-tailed Student's *t*-test, Mann-Whitney rank sum test, and analysis of variance (ANOVA) with repeated measures. Correlations were determined by Pearson product moment correlation analysis. The SigmaStat software (SPSS Science,

Chicago, IL) was used for all statistical analyses. *P* < 0.05 was considered statistically significant.

Results

Age-Dependent Cerebral β -Amyloidosis in B6Tg2576 Mice

Tg2576 mice overexpress the human APP gene with the Swedish double mutation (KM670/671NL) and develop memory deficits and amyloid plaques in the cortex and hippocampus by 9 to 12 months of age.¹⁶ We back-crossed Tg2576 mice on a C57BL/6XSJL F2 background to C57BL/6 mice for more than 10 generations (B6.Tg2576 [N10 to N11]) and designated them as B6Tg2576 for simplicity. The rate of transgene transmission in B6Tg2576 mice was 36% at weaning (*n* = 112) and 70% of the transgenic mice survived up to 20 months of age to date.

To determine whether B6Tg2576 mice develop as much age-dependent cerebral β -amyloidosis as the parental Tg2576 mice, brain sections of B6Tg2576 mice at different ages were subjected to histochemical and immunohistochemical analyses. No A β immunoreactive deposit was detected before the age of 10 months. After 11 months of age, A β deposition in the hippocampus and cortex increased significantly with age (Figure 1, a and b). The β -amyloid load (percentage of area showing A β immunoreactivity) was quantified by morphometric analysis. The β -amyloid load ranged from 0.01% at 11 months to 2% at 18 months of age. These results are comparable to those reported for Tg2576 mice.²³ The A β deposits appeared mostly as apple-green birefringent amyloid in the neuropil (Figure 1, c and d) and vessel walls (Figure 1, e and f).

Exacerbation of Cerebral β -Amyloidosis in B6Tg2576 Mice by an Atherogenic Diet

To test whether an atherogenic diet affects cerebral β -amyloidosis, B6Tg2576 mice and age matched non-transgenic littermates were fed an atherogenic or a normal control diet for 4 months. Cerebral β -amyloid load in B6Tg2576 mice (12.3 $\pm$ 0.2 months old) fed an atherogenic diet (0.20 $\pm$ 0.05%, *n* = 12) was approximately twofold higher than that of B6Tg2576 mice (12.0 $\pm$ 0.3 months old) fed a normal diet (0.09 $\pm$ 0.02%, *n* = 9) (*P* < 0.05). Representative brain sections from control and atherogenic diet-fed mice are shown in Figure 1, g and h. Age-matched non-transgenic littermates of B6Tg2576 mice had no β -amyloid deposition in the brain regardless of which diet they were fed (data not shown).

Atherosclerosis in B6Tg2576 Mice

To test if B6Tg2576 mice are susceptible to diet-induced atherosclerosis, samples from B6Tg2576 mice and age matched non-transgenic littermates used for cerebral analyses above were studied for plasma lipoprotein cho-

Figure 1. Cerebral β -amyloidosis in B6Tg2576 mice. **a** and **b**: Brain sections were immunostained with anti- $A\beta$ antibody (6E10) and counterstained with hematoxylin as described in Materials and Methods. $A\beta$ -immunoreactive deposits are shown in the cortex and hippocampal regions of mice at 13 (**a**) and 18 (**b**) months of age on a normal diet. **c-f**: Congo red-stained brain sections showing an amyloid plaque (**arrow**) in hippocampus and amyloid deposition in a vessel wall viewed under the regular light (**c** and **e**) and the polarized light (**d** and **f**) demonstrating apple-green birefringence. **g** and **h**: Representative brain sections (immunostained with anti- $A\beta$ antibody (6E10) and counterstained with hematoxylin) of B6Tg2576 mice fed a normal diet (**g**) or an atherogenic diet (**h**) at 12 months of age, demonstrating about twofold increase of cerebral $A\beta$ deposition in atherogenic diet-fed mice. **Bars**, 200 μ m (**a** and **b**), 20 μ m (**c** and **d**), 65 μ m (**e** and **f**), and 80 μ m (**g** and **h**).

lesterol profiles and aortic atherosclerosis. The mice fed an atherogenic diet had a significant increase in total plasma cholesterol compared with mice fed a normal diet (168.3 ± 12.0 mg/dl ($n = 12$) vs. 47.8 ± 4.4 mg/dl ($n = 9$), $P < 0.001$). Consistent with previous reports,^{11,17} analysis of lipoprotein cholesterol profiles showed that an atherogenic diet increased the concentrations of atherogenic lipoproteins including very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL) and LDL, and decreased the concentration of atheroprotective

HDL (Figure 2a). As expected, the mice fed an atherogenic diet developed significant atherosclerotic lesions (Figure 2, b, c, f, i, and k). Interestingly, although atherogenic diet-fed B6Tg2576 mice and non-transgenic littermates had similar plasma lipoprotein cholesterol profiles (Figure 2a), B6Tg2576 mice developed significantly more aortic atherosclerotic lesions than did non-transgenic mice (Figure 2b). Unexpectedly, B6Tg2576 mice fed a normal diet also developed small but significant early atherosclerotic lesions in the aorta root (Figure 2, d and

Figure 2. Plasma lipoprotein cholesterol profiles and aortic atherosclerosis. B6Tg2576 mice and age-matched non-transgenic (non-Tg) littermates were fed an atherogenic diet (A) or a normal control diet (C) for 4 months. **a**: Representative plasma lipoprotein cholesterol profiles. An atherogenic diet increased levels of atherogenic VLDL and IDL/LDL cholesterol and decreased atheroprotective HDL cholesterol in both B6Tg2576 and non-Tg mice. **b**: Aortic atherosclerotic lesions in atherogenic diet-fed non-Tg mice ($n = 15$) and B6Tg2576 mice ($n = 12$). *, $P < 0.05$. **c-e**: Representative frozen sections of hearts, stained by Oil Red O, from an atherogenic diet-fed B6Tg2576 mouse (**c**), demonstrating atherosclerotic lesions (**arrows**) in the aortic root region, from a normal diet-fed B6Tg2576 mouse (**d**), demonstrating small but significant lesions (**arrows**) in the aortic root, and from a normal diet-fed non-transgenic littermate of B6Tg2576 mice (**e**). No atherosclerotic lesion was found in normal diet-fed non-transgenic mice ($n = 10$). **f-h**: Atherosclerotic lesions in (**c-e**) at a higher magnification, respectively. **i-k**: Representative aortic atherosclerotic lesions (stained by Oil Red O) from an atherogenic diet-fed B6Tg2576 mouse (**i**), a normal diet-fed B6Tg2576 mouse (**j**), and an atherogenic diet-fed non-transgenic littermate of B6Tg2576 mice (**k**). **l-n**: Serial sections of (**i-k**), respectively, immunostained with anti- $A\beta$ antibody (6E10) and counterstained with hematoxylin, demonstrating co-localization of $A\beta$ immunoreactivity in atherosclerotic lesions in B6Tg2576 mice (**arrows** in **l** and **m**) but not in non-transgenic mice (**n**). **Bars**, 300 μ m (**c-e**) and 150 μ m (**f-n**).

Table 1. Cerebral and Aortic Lesions in Male and Female B6Tg2576 Mice

Lesions	Male (n)	Female (n)	P
Cerebral β -amyloidosis (%)			
Control diet	0.11 $\pm$ 0.02 (4)	0.07 $\pm$ 0.03 (5)	0.33
Atherogenic diet	0.18 $\pm$ 0.07 (5)	0.20 $\pm$ 0.06 (7)	0.82
Aortic atherosclerosis ($\mu\text{m}^2 \times 10^{-3}$)			
Control diet	1.77 $\pm$ 0.44 (4)	1.56 $\pm$ 0.88 (5)	0.85
Atherogenic diet	66.34 $\pm$ 26.32 (5)	102.04 $\pm$ 38.18 (7)	0.50

The ages of B6Tg2576 mice fed a control or an atherogenic diet were 12.0 ± 0.3 and 12.3 ± 0.2 months old, respectively.

g) although their lipoprotein cholesterol profiles were normal (Figure 2a). Non-transgenic littermates, however, showed no atherosclerosis on a normal diet (Figure 2, e and h). Immunohistochemical analyses showed the colocalization of β -amyloid immunoreactivity in aortic atherosclerotic lesions from B6Tg2576 mice, but not in lesions from non-transgenic mice (Figure 2, i–n). These results demonstrated that overexpression of a mutant form of APP initiates and/or accelerates the development of atherosclerosis in a susceptible mouse strain, suggesting a causative role of APP and/or its derivatives in the etiology of atherosclerosis.

Positive Correlation between Aortic Atherosclerosis and Cerebral β -Amyloidosis

Because no significant difference was observed in cerebral β -amyloidosis and aortic atherosclerosis regardless of gender (Table 1), the results from male and female mice were combined. The area of aortic atherosclerotic lesions was positively correlated with amyloid load in the brain of B6Tg2576 mice fed an atherogenic diet (Pearson correlation $r = 0.74$, $P < 0.05$, $n = 12$) (Figure 3a). Furthermore, a significant positive correlation between aortic atherosclerosis and cerebral β -amyloidosis was found in B6Tg2576 mice fed a normal diet (Pearson correlation $r = 0.79$, $P < 0.05$, $n = 9$) (Figure 3b).

Exacerbation of Learning Impairment in B6Tg2576 Mice Fed an Atherogenic Diet

To investigate whether an atherogenic diet affects cognitive functions, a separate cohort of B6Tg2576 mice and non-transgenic littermates fed an atherogenic diet or a normal diet were assessed for their abilities to acquire and process spatial information in the Morris water maze test (submerged platform) by using escape latencies (time needed to find the submerged platform) and path lengths (distances swum) as indicators of learning over 5 days. On a normal diet, B6Tg2576 mice showed learning impairment compared with non-transgenic littermates (Figure 4, a and b). While non-transgenic mice learned to find the hidden platform with the minimum escape latency by the second day of training, B6Tg2576 mice needed one more day to acquire the same task. When dietary effect was assessed in B6Tg2576 mice, the results showed that the escape latencies ($F_{1,9} = 18.04$, $P < 0.01$) were much longer in B6Tg2576 mice fed an atherogenic diet than the mice fed a normal diet (Figure 4c).

The concurrent longer path length ($F_{1,9} = 6.05$, $P < 0.05$) displayed by the atherogenic-diet fed B6Tg2576 mice indicates a spatial learning deficit as opposed to a retarded swimming speed (Figure 4d). The dietary effect, however, was not significant in non-transgenic mice (escape latency: $F_{1,8} = 0.28$, $P = 0.61$; path length: $F_{1,8} = 0.21$, $P = 0.66$) (Figure 4, e and f), indicating that diet affects learning ability through mutant APP transgene expression.

No Significant Diet Effect on Exploration of Environment and Anxiety of B6Tg2576 Mice

Before the Morris water maze test, the same cohort of B6Tg2576 mice was assessed for their willingness to explore the environment in a T-maze, motor activity in an open field, and anxiety in an elevated plus-maze. The results showed no significant differences between atherogenic diet-fed and normal diet-fed B6Tg2576 mice in their performances in these behavioral functions (Table 2). These data further indicated that increased learning deficit of atherogenic diet-fed B6Tg2576 mice in the Morris water maze was not due to unwillingness to explore or abnormal motor activity and anxiety.

Discussion

Diet-induced hypercholesterolemia accelerates A β deposition in the brain of APP transgenic mice.^{10–12} The correlation between atherosclerosis and cerebral β -amyloidosis has not been investigated in experimental animals because the model mice used in previous studies were genetically resistant to atherosclerosis, with the exception of APP transgenic mice deficient in apoE. As the apoE-deficient mice possess fundamentally altered lipid metabolism and marked reduction in A β deposition in the brain,²⁴ they are not suitable for studying such a relationship. A cholate-containing high fat/high cholesterol atherogenic diet induces hypercholesterolemia and atherosclerosis in susceptible strains of mice including C57BL/6.¹⁷ We have successfully established a transgenic mouse line, B6Tg2576, by back-crossing Tg2576 mice to C57BL/6 mice. B6Tg2576 mice developed both atherosclerosis and cerebral β -amyloidosis without reduction in amyloid load. An atherogenic diet increased atherosclerosis and β -amyloidosis and also impaired learning in the B6Tg2576 mice. To our knowledge, this study is the first to demonstrate a positive correlation

Figure 3. Correlation of aortic atherosclerosis and cerebral β -amyloidosis. Quantitative morphometric analyses of aortic atherosclerosis and cerebral β -amyloidosis were performed as described in Materials and Methods. **a:** Correlation in atherogenic diet-fed B6Tg2576 mice ($n = 12$). Pearson correlation $r = 0.74$, $P < 0.05$. **b:** Correlation in normal control diet-fed B6Tg2576 mice ($n = 9$). Pearson correlation $r = 0.79$, $P < 0.05$.

between cerebral β -amyloidosis and aortic atherosclerosis in experimental animals.

Atherosclerosis is associated with AD or cerebral amyloid angiopathy in humans.^{25,26} Aortic atherosclerosis and brain microvasculopathies correlate in patients with hereditary cerebral hemorrhage with amyloidosis, Dutch type.²⁷ The mechanisms by which atherosclerosis and β -amyloidosis are connected have not been fully understood. The role of cholesterol in atherosclerosis is well established but its role in β -amyloidosis is not fully understood. Several lines of evidence have suggested that cholesterol may affect β -amyloidosis by modulation of proteolytic processing of APP and/or subsequent amyloid formation and deposition. The secretion of neuroprotective α APPs (the soluble N-terminal derivative of APP following α -secretase cleavage) from cultured cells has been shown to decrease following an increase in the cholesterol content of the cells.²⁸ When cellular cholesterol levels are reduced with a cholesterol-lowering drug, the production of $A\beta$ is inhibited by an increase in α -secretase activity^{29,30} and a decrease in both β - and

Figure 4. Morris water maze acquisition. B6Tg2576 mice ($n = 11$) and non-transgenic littermates ($n = 10$) at age of 15 to 18 months were assessed for their abilities to acquire and process spatial information in the submerged platform version of the Morris water maze after being fed a control normal diet (C) or an atherogenic diet (A) for 4 months. **a** and **b:** Spatial learning impairment of B6Tg2576 mice on a normal diet. While non-transgenic mice (non-Tg) learned to find the hidden platform with the minimum escape latency (**a**) and path length (**b**) by the second day of training, B6Tg2576 mice needed one more day to acquire the same task. **c** and **d:** Diet effect on spatial learning of B6Tg2576 mice. The atherogenic diet was associated with more impaired learning indicated by the increase of escape latencies (**c**) in the maze acquisition. In agreement with increased escape latencies, the atherogenic diet was associated with a concurrent increase of swimming path length (**d**) in B6Tg2576 mice, indicating the directional nature of the learning deficit in atherogenic diet-fed mice as opposed to the retarded swimming speed. **e** and **f:** Diet effect on escape latencies (**e**) and path lengths (**f**) of non-transgenic littermates of B6Tg2576 mice. No significant diet effect was observed in the performance of these non-transgenic mice in the water maze. *, $P < 0.05$; **, $P < 0.01$.

γ -secretase activity,^{31,32} activities that are involved in the proteolytic processing of APP. In this study, we did not investigate whether atherogenic diet affects cholesterol metabolism in the brain of B6Tg2576 mice. Several studies have provided evidence that plasma cholesterol is transported across the blood-brain barrier. Significant increases in brain cholesterol have been observed in mice fed high cholesterol diets.^{10,33} Diet-induced hypercholesterolemia is associated with an increase in amyloidogenic processing of APP and subsequent amyloid deposition in the brain of PSAPP (presenilin1 and APP double transgenic) mice.¹⁰ Furthermore, transport of LDL across the blood-brain barrier has been shown to be mediated by the LDL receptor and has been proposed to be a critical mechanism by which essential lipids, includ-

Table 2. Exploration of Environment and Anxiety of B6Tg2576 Mice

Tests	Control diet	Atherogenic diet	P
Spontaneous alternation (T-maze)			
Rate (%)	67.0 ± 6.0	60.0 ± 4.0	0.39
Latency (s)	27.8 ± 1.6	57.0 ± 14.0	0.26
Motor activity (open field)			
Path length (cm)	2969.6 ± 375.5	2182.2 ± 300.3	0.15
% time in the central zone	7.2 ± 1.9	5.7 ± 1.7	0.59
Anxiety (elevated plus-maze)			
Entries to open arms			
Day 1	13.7 ± 2.8	8.8 ± 2.7	0.24
Day 2	9.2 ± 1.7	6.0 ± 1.1	0.17
% time in open arms			
Day 1	68.2 ± 11.3	57.3 ± 15.9	0.58
Day 2	45.1 ± 10.2	43.3 ± 16.8	0.93

B6Tg2576 mice at age of 15–18 months after being fed an atherogenic (n = 5) or a normal control diet (n = 6) for 4 months were tested. The results indicate that the mice fed the atherogenic diet do not differ significantly in exploration of environment, motor activity, and anxiety from mice fed the control diet.

ing cholesterol, are delivered to brain cells.³⁴ In addition, cholesterol-lowering agents can decrease levels of cerebral A β in the brain of guinea pigs and PSAPP mice.^{31,35} In summary, these studies support that increased plasma and/or cellular levels of cholesterol may be involved in the etiology of β -amyloidosis as well as atherosclerosis.

On the other hand, the clearance of A β may be impaired under the condition of dyslipidemia. In addition to increasing plasma total cholesterol levels, the atherogenic diet used in this study altered the distribution of plasma lipoproteins: atherogenic lipoproteins (VLDL, IDL, and LDL) were increased and the atheroprotective lipoprotein (HDL) was decreased (Figure 2a). The anti-atherogenic properties of HDL relate partly to its role in reverse cholesterol transport, the removal of cholesterol from peripheral tissues for transport to the liver for excretion.³⁶ In the plasma and brain, A β is, at least in part, carried on an HDL-like lipoprotein.^{37,38} HDL increases the degradation of A β by microglia *in vitro*.³⁹ An inverse relationship between plasma HDL levels and the cerebral β -amyloid load is found in Tg2576 mice fed an atherogenic diet.¹¹ Cultured smooth muscle cells internalize A β via a receptor-mediated lipoprotein pathway.⁴⁰ Thus, the uptake and degradation of A β may be lipoprotein-dependent and A β shares its clearance pathway with cholesterol.

Reports on dietary effects on cognitive functions in humans are not conclusive: vascular risk factors including hypercholesterolemia are associated with cognitive impairment^{41,42} while dietary fat and cholesterol intake do not increase the risk for dementia.⁴³ Very recently, in a biracial community study, a high intake of saturated or *trans*-unsaturated fat has been shown to increase the risk of AD.⁴⁴ In rats, diets high in saturated fat also are associated with cognitive impairment.⁴⁵ Although several studies have reported dietary effects on β -amyloidosis in transgenic mice,^{10–12} behaviors of those mice have not been reported. We demonstrate here that B6Tg2576 mice fed an atherogenic diet are severely impaired in spatial learning as indicated by longer escape latencies during the acquisition of the Morris water maze (Figure 4c). The concurrent longer path length displayed by the atherogenic-diet fed mice indicates a spatial learning

deficit as opposed to a retarded swimming speed (Figure 4d). In addition, the atherogenic diet-fed B6Tg2576 mice performed similarly to the normal diet-fed B6Tg2576 mice in a T-maze, an open field, and an elevated plus-maze (Table 2), further indicating that increased learning deficit of atherogenic diet-fed B6Tg2576 mice in the Morris water maze was not due to unwillingness to explore or to abnormal motor activity and anxiety. As all mice tested displayed similar physical activity and swimming ability, no animals were excluded from the analysis. Notably, dietary effect on spatial learning was specifically associated with the mutant APP transgene because the atherogenic diet-fed non-transgenic littermates performed similarly in the Morris water maze to the normal diet-fed mice (Figure 4, e and f). These results suggest that exacerbation of β -amyloidosis by an atherogenic diet may be one potential mechanism for its detrimental effect on learning.

While much attention has been focused on the effects of cardiovascular risk factors on the development of AD, little attention has been paid to the role(s) of A β and/or APP in the etiology of atherosclerosis. In human atherosclerotic plaques, A β produced from platelet-derived APP may be involved in macrophage activation.⁴⁶ In our study, B6Tg2576 mice fed a normal diet developed small but significant fatty streak aortic lesions that were positively correlated with cerebral β -amyloid load (Figure 3b). Moreover, B6Tg2576 mice fed an atherogenic diet developed more atherosclerotic lesions than non-transgenic littermates fed the same diet (Figure 2b). These differences cannot be fully explained by changes in cholesterol metabolism as plasma lipoprotein cholesterol profiles were similar in B6Tg2576 and non-transgenic mice fed the same diet (Figure 2a). In B6Tg2576 mice, because the human APP transgene is under the control of a brain-specific prion promoter, A β /APP immunoreactivity was only detected in the brain (Figure 1) and not in peripheral tissues (data not shown). The plasma A β level, however, is increased in Tg2576 mice.¹⁶ As A β can be taken up by vascular smooth muscle cells,⁴⁰ the development of atherosclerotic lesions could be related to the action of A β on the vessel walls. Indeed, immunohistochemical analysis of the aorta showed that A β /APP immunoreactivity was co-localized in the atherosclerotic le-

sions in B6Tg2576 mice (Figure 2, l and m). Here, we demonstrated that overexpression of a mutant form of APP initiates and/or promotes the development of atherosclerosis in a susceptible mouse strain, suggesting a causative role of APP and/or its derivatives in the etiology of atherosclerosis. These findings warrant further investigations of mechanisms by which β -amyloidosis and atherosclerosis are connected, in particular, investigation of the roles of A β and APP in atherogenesis.

In conclusion, by establishing a mouse model that is prone to both atherosclerosis and β -amyloidosis, we have shown for the first time that aortic atherosclerosis correlates positively with cerebral β -amyloidosis and that an atherogenic diet is associated with exacerbated learning impairment in APP transgenic mice. Our study supports the concept that anti-atherogenic therapies, including dietary regimens, may be effective in prevention and treatment of AD.

Acknowledgments

We thank Dr. Karen Hsiao Ashe at University of Minnesota for providing Tg2576 mice. We also thank Patti Hall and Terry Lewis for technical assistance, Dr. Robert Lalonde at University of Rouen in France for sharing his expertise in behavioral testing, and Drs. Michael Wyss, G. M. Anantharamiah, and Jere P. Segrest for helpful discussions.

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