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Reducing Cerebral Microvascular Amyloid-β Protein Deposition Diminishes Regional Neuroinflammation in Vasculotropic Mutant Amyloid Precursor Protein Transgenic Mice

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Cerebral microvascular amyloid-β (Aβ) protein deposition is emerging as an important contributory factor to neuroinflammation and dementia in Alzheimer's disease and related familial cerebral amyloid angiopathy disorders. In particular, cerebral microvascular amyloid deposition, but not parenchymal amyloid, is more often correlated with dementia. Recently, we generated transgenic mice (*Tg-SwDI*) expressing the vasculotropic Dutch (E693Q)/Iowa (D694N) mutant human Aβ precursor protein in brain that accumulate abundant cerebral microvascular fibrillar amyloid deposits. In the present study, our aim was to assess how the presence or absence of fibrillar Aβ deposition in the cerebral microvasculature affects neuroinflammation in *Tg-SwDI* mice. Using *Tg-SwDI* mice bred onto an apolipoprotein E gene knock-out background, we found a strong reduction of fibrillar cerebral microvascular Aβ deposition, which was accompanied by a sharp decrease in microvascular-associated neuroinflammatory cells and interleukin-1β levels. Quantitative immunochemical measurements showed that this reduction of the neuroinflammation occurred in the absence of lowering the levels of total Aβ40/Aβ42 or soluble Aβ oligomers in brain. These findings suggest that specifically reducing cerebral microvascular fibrillar Aβ deposition, in the absence of lowering either the total amount of Aβ or soluble Aβ oligomers in brain, may be sufficient to ameliorate microvascular amyloid-associated neuroinflammation.

Key words: amyloid-β protein; transgenic mice; cerebral microvasculature; neuroinflammation; Alzheimer's disease; vasculotropic mutant

Introduction

Extracellular deposition of the amyloid-β (Aβ) protein in brain is a prominent pathological feature of Alzheimer's disease (AD) and related disorders (Selkoe, 2001). Aβ peptides are derived through sequential proteolytic processing of the Aβ precursor protein (AβPP) by β- and γ-secretase activities. Cerebral parenchymal Aβ deposition can occur as diffuse plaques, with little surrounding pathology, or as fibrillar plaques associated with dystrophic neurons and inflammation (Selkoe, 2001). Fibrillar Aβ deposition in the cerebral vasculature, a condition known as cerebral amyloid angiopathy (CAA), is also commonly found in Alzheimer's disease (Vinters, 1987; Jellinger, 2002; Rensink et al., 2003). Additionally, several familial forms of CAA exist that result from mutations that reside within the Aβ peptide sequence of

AβPP gene including the Dutch-type (E22Q) and Iowa-type (D23N), which cause early and severe cerebral vascular amyloid deposition (Levy et al., 1990; Van Broeckhoven et al., 1990; Grabowski et al., 2001). Recent studies have implicated cerebral microvascular Aβ deposition in promoting neuroinflammation and dementia in AD and related familial CAA disorders (Vinters, 2001; Atterns and Jellinger, 2004; Bailey et al., 2004; Greenberg et al., 2004). In particular, cerebral microvascular, but not parenchymal, amyloid deposition is more often correlated with dementia in individuals afflicted with Alzheimer's disease and CAA disorders (Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study, 2001; Thal et al., 2003; Atterns and Jellinger, 2004).

Recently, we generated transgenic mice that express human vasculotropic mutant human AβPP in brain (*Tg-SwDI*) and develop early-onset and robust cerebral deposition of Aβ, particularly in the cerebral microvasculature (Davis et al., 2004). The finding that *Tg-SwDI* mice accumulate extensive cerebral microvascular Aβ, despite low levels of transgene human AβPP expression and human Aβ production, appears to result from the ineffective clearance of the Dutch/Iowa mutant from brain across the capillary blood–brain barrier into the circulation (Davis et al., 2004; Deane et al., 2004).

In the present study, our aim was to assess how the presence or absence of fibrillar Aβ deposition in the cerebral microvasculature

affects neuroinflammation in *Tg-SwDI* mice. Here, we show that reducing fibrillar cerebral microvascular Aβ deposition in *Tg-SwDI* mice, through breeding onto an apolipoprotein E (apoE) knock-out background, significantly reduces the number of neuroinflammatory reactive astrocytes, activated microglia, and cerebral interleukin-1β (IL-1β) levels. Notably, these beneficial outcomes were achieved without lowering either the total amount of Aβ peptides in brain or the putatively pathogenic soluble Aβ oligomers, indicating that the neuroinflammation in the *Tg-SwDI* mice resulted from fibrillar microvascular Aβ deposition.

Materials and Methods

Transgenic mice. All work with animals followed National Institutes of Health guidelines and was approved by the Stony Brook University Institutional Animal Care and Use Committee (IACUC) or by the Duke University IACUC. *Tg-SwDI* mice were described recently (Davis et al., 2004), and *apoE*$^{-/-}$ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were on a pure C57BL/6 background. *Tg-SwDI/apoE*$^{-/-}$ mice were generated by successive breedings of heterozygous *Tg-SwDI* mice with the *apoE*$^{-/-}$ mice to yield mice that were *Tg-SwDI/apoE*$^{-/-}$. Eleven *Tg-SwDI/apoE*$^{+/+}$ and 10 *Tg-SwDI/apoE*$^{-/-}$ were examined at 12 months of age for the pathological studies.

Tissue preparation. Mice were killed with an overdose of 2.5% avertin, and the brains were immediately removed and bisected in the midsagittal plane. One hemisphere was snap-frozen and used for the protein analyses. The other hemisphere was placed in 70% ethanol, followed by xylene treatment and embedding in paraffin for immunohistochemical and histological analyses.

Quantitative immunoblot analysis for cerebral human AβPP. The levels of human AβPP in *Tg-SwDI/apoE*$^{+/+}$ and *Tg-SwDI/apoE*$^{-/-}$ mouse forebrain was determined by quantitative immunoblotting using the human AβPP-specific monoclonal antibody P2–1 as described previously (Davis et al., 2004).

Immunochemical analysis of cerebral Aβ peptides. Soluble pools of Aβ40 and Aβ42 were determined by using a specific ELISA of carbonate-extracted mouse forebrain tissue, and, subsequently, the insoluble Aβ40 and Aβ42 levels were determined by ELISA of guanidine lysates of the insoluble pellets resulting from the carbonate-extracted brain tissue (Johnson-Wood et al., 1997; DeMattos et al., 2002). Total Aβ40 and Aβ42 levels were determined by combining the soluble and insoluble levels of each form. Affinity-purified rabbit polyclonal antibody highly selective for soluble Aβ oligomers was prepared following protocols as described previously (Lambert et al., 2001). Soluble Aβ oligomers were analyzed in TBS-soluble forebrain fractions using dot-blot analysis following previously described protocols (Chang et al., 2003) and quantitated using Bio-Rad (Hercules, CA) VersaDoc system and the Quantity One software.

Immunohistochemical analysis. Immunohistochemistry and histology were performed as reported previously (Davis et al., 2004). Briefly, sections were cut in the sagittal plane at 10 μm thickness using a microtome, deparaffinated, and rehydrated. Antigen retrieval was performed by treatment with proteinase K (0.2 mg/ml) for 10 min at 22°C for Aβ and collagen staining and by 10 mM sodium citrate solution, pH 9.0, for 30 min at 90°C in a water bath for activated microglia staining. Primary antibodies were detected with horseradish peroxidase-conjugated or alkaline phosphatase-conjugated secondary antibodies and visualized either with a stable diaminobenzidine solution (Invitrogen, Carlsbad, CA)
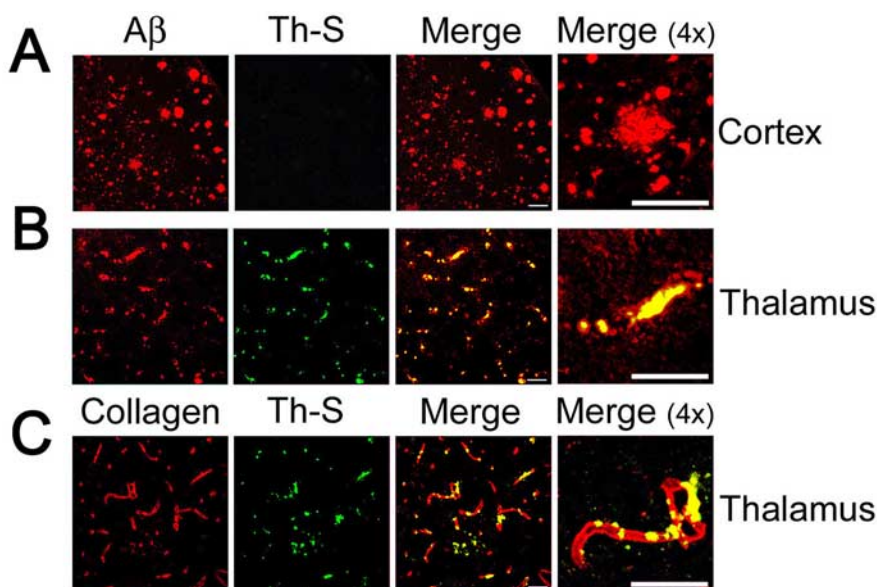

Figure 1. *Tg-SwDI* mice develop fibrillar Aβ deposits exclusively in the cerebral microvasculature. *A*, Abundant Aβ immunostaining, but lack of thioflavin-S (Th-S) amyloid staining, of diffuse parenchymal deposits in the neocortex of *Tg-SwDI* mice. *B*, Colocalization of Aβ immunostaining and thioflavin-S amyloid staining in the thalamus of *Tg-SwDI* mice is predominantly localized in microvasculature structures. *C*, Colocalization of vascular collagen IV immunostaining and thioflavin-S amyloid staining in the thalamus of *Tg-SwDI* mice. Scale bars, 50 μm.

or with the fast red substrate system (Spring Bioscience, Fremont, CA), respectively, as substrate. Sections were counterstained with hematoxylin. Thioflavin-S staining for fibrillar amyloid was performed as described previously (Dickson et al., 1990). The following antibodies were used for immunohistochemical analysis: mouse monoclonal antibody 66.1, which recognizes residues 1–5 of human Aβ (1:200) (Deane et al., 2003), rabbit polyclonal antibody to collagen type IV (1:100; Research Diagnostics, Flanders, NJ), mouse monoclonal antibody to glial fibrillary acidic protein (GFAP) for the detection of astrocytes (1:300; Chemicon, Temecula, CA), mouse monoclonal anti-keratan sulfate antibody for the detection of activated microglia (clone, 5D4; 1:200; Seikagaku Corporation, Tokyo, Japan).

Quantitative analysis of regional Aβ deposition and microvascular CAA. Total amyloid burden in the regions of the subiculum, dentate gyrus, thalamus, and frontotemporal cortex was respectively quantified on the same set of systematically sampled Aβ-immunostained sections using NIH ImageJ 1.32 software. The percentage of Aβ-associated blood vessels in the same fields as above was determined using stereological principles, as described previously (Long et al., 1998).

Quantitative analysis of reactive astrocyte and activated microglia cell densities. Total numbers of astrocytes and activated microglia in the subiculum, dentate gyrus, thalamus, and frontotemporal cortex regions were estimated using a computerized stereology system (Stereologer; Systems Planning and Analysis, Alexandria, VA). Every 10th section was selected and generated 10–15 sections per reference space in a systematic-random manner. Immunopositive cells were counted using the optical-fractionator method with the dissector principle and unbiased counting rules (Long et al., 1998).

Measurement of cerebral IL-1β levels. Hemibrains isolated from 12-month-old *Tg-SwDI/apoE*$^{+/+}$ and *Tg-SwDI/apoE*$^{-/-}$ mice were homogenized in 10 vol of 50 mM Tris-HCl and 150 mM NaCl, pH 7.5, containing protease inhibitor cocktail (SM Biotech, Huntington Station, NY) at 4°C. The samples were centrifuged at 14,000 × *g* for 50 min at 4°C. The supernatants were collected, and the protein concentrations were determined using the BCA kit (Pierce, Rockford, IL). The levels of IL-1β in the samples were determined using a mouse IL-1β immunoassay kit (Biosource International, Camarillo, CA).

Statistical analysis. Data were analyzed using Student's *t* test at a significance level *p* = 0.05.
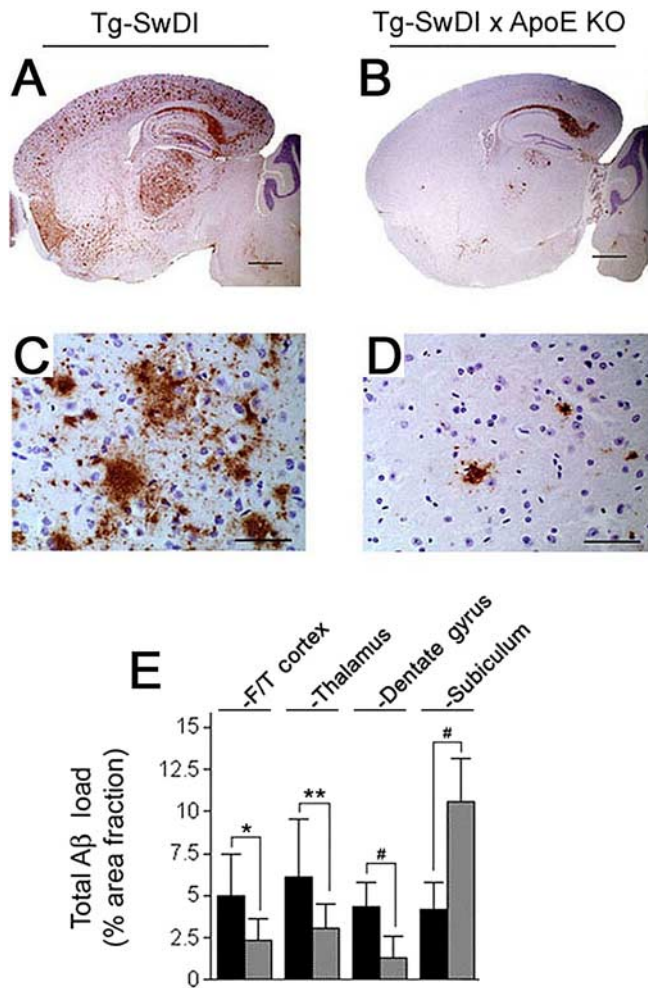
Figure 2. Lack of endogenous mouse apoE lowers cerebral Aβ deposition in *Tg-SwDI* mice. ***A–D***, Deposition of Aβ in forebrain (***A***, ***B***) and frontotemporal cortex (***C***, ***D***) in *Tg-SwDI* in the absence or presence, respectively, of endogenous mouse apoE. Scale bars: ***A***, ***B***, 1 mm; ***C***, ***D***, 50 μm, respectively. ***E***, Quantitative image analysis of regional Aβ deposition in *Tg-SwDI* mice in the presence (black bars) or absence (gray bars) of endogenous mouse apoE. Data shown are mean ± SD ($n = 10$). *$p < 0.01$; **$p < 0.02$; #$p < 0.001$. F/T, Frontotemporal; KO, knock-out.

Figure 3. Lack of endogenous mouse apoE eliminates cerebral microvascular Aβ deposition in *Tg-SwDI* mice. ***A–D***, Fibrillar microvascular amyloid deposition in *Tg-SwDI* mouse thalamus revealed by immunostaining for Aβ (brown) and collagen type IV (red; ***A***, ***B***) and thioflavin-S fluorescence staining (***C***, ***D***) in the presence (***A***, ***C***) or absence (***B***, ***D***) of endogenous mouse apoE. Scale bars, 50 μm. ***E***, Quantitative stereological estimation of microvascular amyloid deposition in the presence (black bars) or absence (gray bars) of endogenous apoE. Data shown are mean ± SD ($n = 10$). *$p < 0.01$; **$p < 0.0001$. F/T, Frontotemporal; KO, knock-out.

Results

Accumulation of fibrillar Aβ exclusively in the cerebral microvasculature of *Tg-SwDI* mice

We reported recently that *Tg-SwDI* mice develop early-onset and robust accumulation of cerebral Aβ deposits, particularly on the cerebral microvasculature (Davis et al., 2004). *Tg-SwDI* mice exhibit an age-dependent increase in cerebral microvascular Aβ accumulation starting at several months of age that becomes very extensive, affecting ≈50% of microvessels in certain regions by 12 months (Davis et al., 2004). To characterize the nature of the cerebral parenchymal and microvascular Aβ deposits in brain sections from 12-month-old *Tg-SwDI* mice, we performed double labeling and confocal microscopy for Aβ by immunolabeling and for fibrillar amyloid by thioflavin-S staining. Abundant diffuse Aβ deposits were observed in the cortex that did not stain with thioflavin-S (Fig. 1*A*). In contrast, in the thalamic region, for example, numerous microvascular Aβ deposits were seen that strongly stained with thioflavin-S, indicating their fibrillar nature (Fig. 1*B*). To confirm that the fibrillar Aβ was localized to the cerebral microvasculature, we performed double labeling and confocal microscopy for microvessels by immunolabeling for
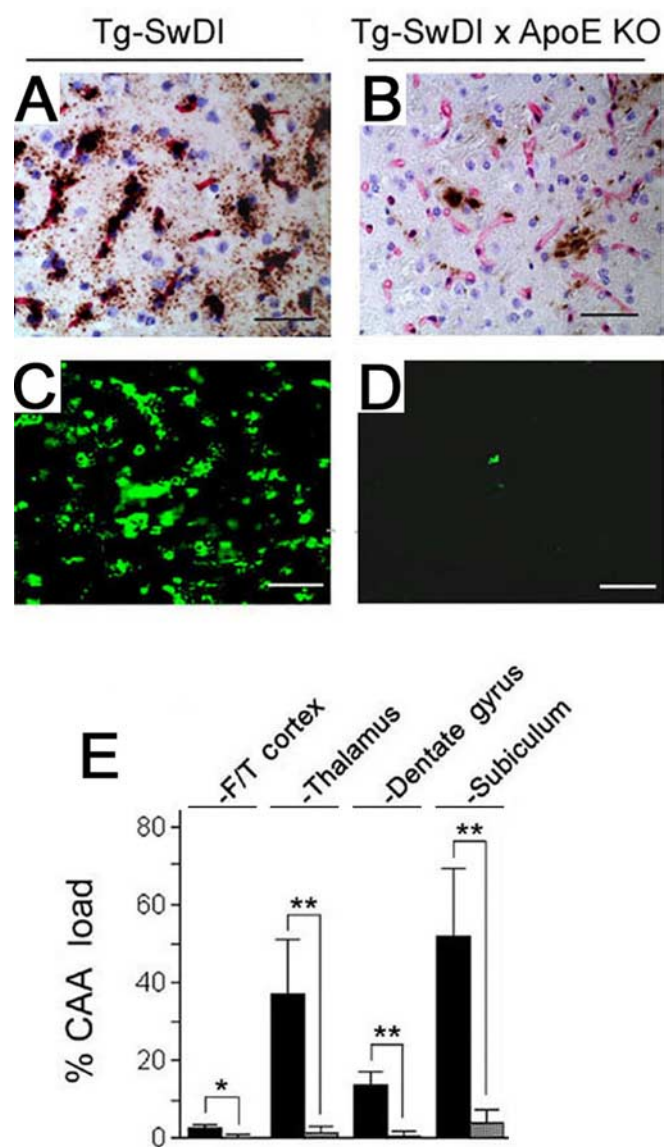
collagen type IV in conjunction with thioflavin-S staining (Fig. 1*C*). These results indicate that, in *Tg-SwDI* mice, fibrillar Aβ accumulation is restricted to the cerebral microvasculature in contrast to the diffuse parenchymal deposits.

Lack of apoE strongly reduces cerebral microvascular Aβ deposition in *Tg-SwDI* mice

The major goal of the present study was to assess how the presence or absence of fibrillar Aβ deposition in the cerebral microvasculature affects neuroinflammation in *Tg-SwDI* mice. The lipid transport protein apoE has been shown to facilitate Aβ deposition in brain parenchyma and cerebral vessels in humans as well as in human AβPP transgenic mouse models of Aβ deposition (Schmechel et al., 1993; Bales et al., 1999; Holtzman, 2001; Chalmers et al., 2003; Fryer et al., 2003; Holtzman, 2004). There-

fore, to modify Aβ levels, heterozygous *Tg-SwDI* mice were bred onto an apoE knock-out background, and the resulting *Tg-SwDI/apoE*$^{-/-}$ mice were compared with *Tg-SwDI/apoE*$^{+/+}$ mice littermates at 12 months of age. There was a significant decrease ($p < 0.01$) in the extent of total Aβ deposition by ≈50% in the regions of the hippocampus, thalamus, and neocortex in *Tg-SwDI/apoE*$^{-/-}$ mice compared with *Tg-SwDI/apoE*$^{+/+}$ mice (Fig. 2*A,B*). The one regional exception was the subiculum, which presented a surprising, more than twofold increase in parenchymal Aβ deposition (Fig. 2*B*). These results are consistent with findings that endogenous mouse apoE facilitates cerebral Aβ deposition in human AβPP transgenic mice (Bales et al., 1999; Holtzman, 2001; Fryer et al., 2003; Holtzman, 2004). However, the lack of apoE only reduced, but did not prevent, diffuse parenchymal Aβ deposition in *Tg-SwDI* mice.

In contrast to the parenchyma, a more striking decrease in the amount of cerebral microvascular Aβ was observed with a nearly complete loss of cerebral microvascular amyloid deposits in the *Tg-SwDI/apoE*$^{-/-}$ mice (Fig. 3). Quantitative stereological measurements confirmed the virtually complete elimination of cerebral microvascular Aβ load in animals lacking apoE compared with those expressing apoE (Fig. 3*A,B*). Even within the subiculum, in which there was a pronounced increase in parenchymal Aβ deposition ($p < 0.001$) (Fig. 2*B*), there was also a highly significant abolition of cerebral microvascular Aβ deposition ($p < 0.001$) (Fig. 3*B*). These data indicate that apoE is involved with facilitating diffuse Aβ deposition in the parenchyma of most brain regions but is more essential for promoting robust fibrillar cerebral microvascular deposition in *Tg-SwDI* mice.

Absence of apoE and reduction of cerebral Aβ deposition does not lower total Aβ levels or soluble Aβ oligomers in brains of *Tg-SwDI* mice

Quantitative immunoblotting measurements showed that the absence of apoE had no observable effect on levels of transgene-encoded human AβPP levels in *Tg-SwDI* mouse brains (Fig. 4*A,B*). Likewise, quantitative ELISA analysis showed that *Tg-SwDI/apoE*$^{-/-}$ mice have similar levels of forebrain total Aβ40 and Aβ42 compared with *Tg-SwDI/apoE*$^{+/+}$ mice (Fig. 4*C*). The ratio of Aβ40:Aβ42 was found to be very similar, at ~10:1, both in isolated cerebral microvessels and vascular-depleted parenchymal forebrain fractions. To understand why there was a reduction in cerebral Aβ deposition without a lowering of total Aβ levels in *Tg-SwDI/apoE*$^{-/-}$ mice, we next compared the amounts of insoluble Aβ obtained from the guanidine fraction with soluble Aβ obtained from the carbonate fraction from brains of these mice. Lower levels of insoluble Aβ and higher levels of soluble Aβ were detected in the *Tg-SwDI/apoE*$^{-/-}$ mice, resulting in a ≈50% reduction in this ratio ($p < 0.001$) (Fig. 5*A*), which is consistent with the observed decrease in cerebral Aβ deposition (Fig. 2). Despite the decrease of insoluble deposited Aβ, quantitative dot-blot analysis using a polyclonal antibody specific for soluble Aβ oligomers (Lambert et al., 2001; Chang et al., 2003) revealed an approximate twofold increase in these forms ($p < 0.001$) (Fig. 5*B,C*). These findings demonstrate that, although the absence of apoE in *Tg-SwDI* mice markedly affected deposition of insoluble Aβ, particularly on the cerebral microvascula-
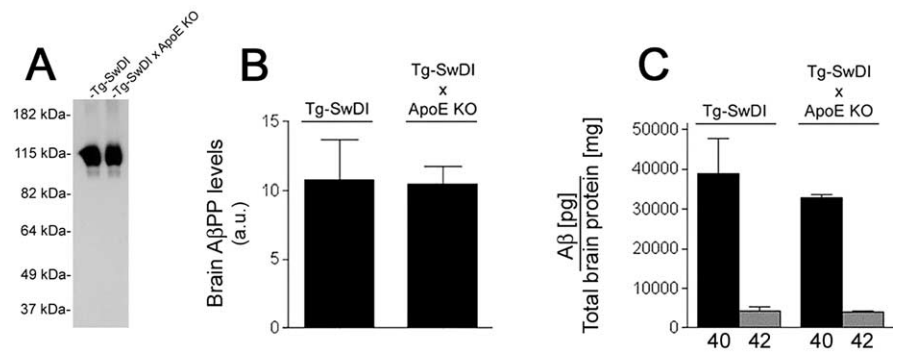

Figure 4. Effects of endogenous mouse apoE on cerebral human AβPP and Aβ levels in *Tg-SwDI* mice. *A*, Immunoblot analysis of transgene-encoded human AβPP levels in mouse forebrain extracts. *B*, Quantitation of human AβPP immunoblots ($n = 4$) of mouse forebrain extracts. *C*, ELISA measurements of total Aβ40 (black bars) and Aβ42 (gray bars) in mouse forebrain tissue. ELISA data shown are mean ± SD ($n = 12–16$). a.u., Arbitrary units; KO, knock-out.
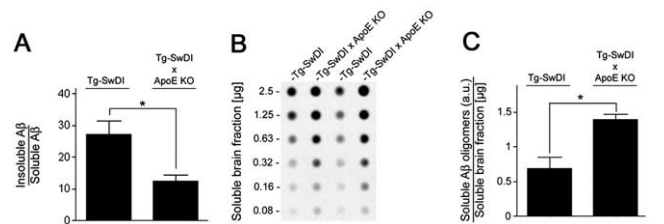

Figure 5. Effects of endogenous mouse apoE on cerebral soluble and insoluble Aβ levels in *Tg-SwDI* mice. *A*, Ratio of total insoluble Aβ/soluble Aβ. *B*, Representative dot-blot analysis of Aβ oligomers in soluble mouse forebrain extracts. *C*, Quantitation of soluble Aβ oligomers from dot blots of *Tg-SwDI* and *Tg-SwDI/apoE*$^{-/-}$ mice. Data shown are mean ± SD ($n = 5$). *$p < 0.001$. KO, Knock-out.

ture, it had no observable influence on the levels of total brain Aβ and, notably, elevated the levels of soluble Aβ oligomers.

Reduction of cerebral microvascular amyloid decreases associated neuroinflammatory cells and IL-1β levels in *Tg-SwDI* mice

Recent studies indicate that neuroinflammation is associated with cerebral microvascular Aβ deposition, where it may contribute to the progressive dementia of AD and related familial CAA disorders (Grabowski et al., 2001; Atterns and Jellinger, 2004; Eng et al., 2004; Harkness et al., 2004; Maat-Schieman et al., 2004). Specifically, accumulation of reactive astrocytes and activated microglia is found in proximity to fibrillar cerebral microvascular Aβ deposits (Grabowski et al., 2001; Eng et al., 2004). Because *Tg-SwDI* mice develop fibrillar Aβ deposition exclusively on the microvasculature (Figs. 1, 3), they provide a unique model to investigate neuroinflammation driven by microvascular amyloid in the absence of parenchymal fibrillar amyloid. Large numbers of astrocytes exhibiting a reactive ramified appearance were tightly associated with microvessels in *Tg-SwDI/apoE*$^{+/+}$ mice (Fig. 6*A,C*), whereas the number of these cells was significantly reduced in the *Tg-SwDI/apoE*$^{-/-}$ mice that lack appreciable cerebral microvascular amyloid (Fig. 6*B,D*). Quantitative stereological measurement of astrocyte densities showed that the highest numbers of these neuroinflammatory cells were found in regions with the most abundant fibrillar cerebral microvascular Aβ deposition (i.e., thalamus and subiculum) (Fig. 3*E*).

Similarly, abundant microvascular-associated activated microglia were found in the *Tg-SwDI/apoE*$^{+/+}$ mice (Fig. 7*A,C*), whereas the numbers of these cells was clearly reduced in the absence of microvascular amyloid (Fig. 7*B,D*). Quantitative ste-
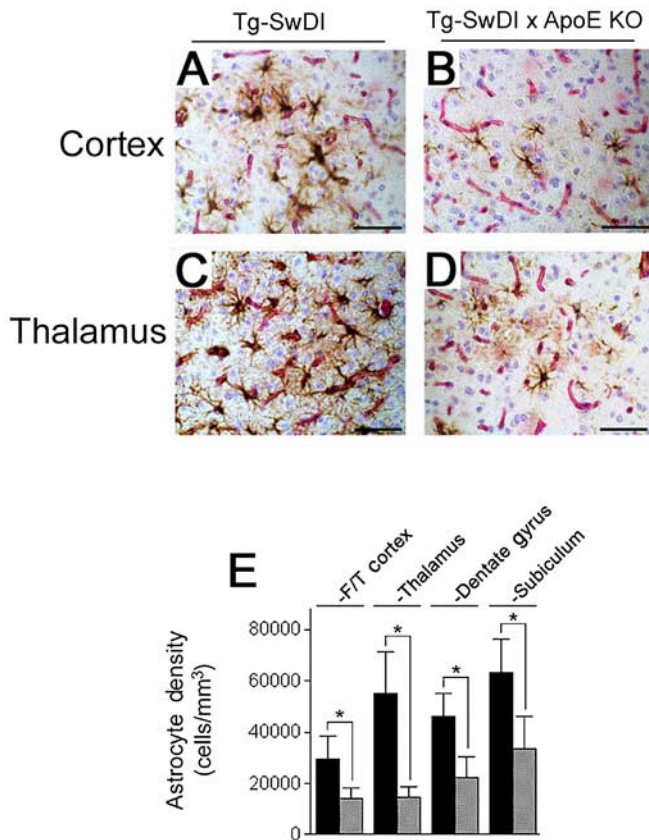
Figure 6. Reduced cerebral microvascular amyloid decreases reactive astrocytes in *Tg-SwDI* mice. *A–D*, Microvascular-associated reactive astrocytes revealed by GFAP-positive immunostaining (brown) and collagen type IV (red) in *Tg-SwDI* mouse frontotemporal cortex (*A*, *B*) or thalamus (*C*, *D*) in the presence (*A*, *C*) or absence (*B*, *D*) of endogenous mouse apoE. Scale bars, 50 μm. *E*, Quantitative stereological estimation of reactive astrocyte densities in brain regions of *Tg-SwDI* mice in the presence (black bars) or absence (gray bars) of endogenous mouse apoE. Data shown are mean ± SD ($n = 10$). *$p < 0.001$. F/T, Frontotemporal; KO, knock-out.

Figure 7. Reduced cerebral microvascular amyloid decreases activated microglia in *Tg-SwDI* mice. *A–D*, Microvascular-associated activated microglia revealed by 5D4-positive immunostaining (brown) and collagen type IV (red) in *Tg-SwDI* mouse frontotemporal cortex (*A*, *B*) or thalamus (*C*, *D*) in the presence (*A*, *C*) or absence (*B*, *D*) of endogenous mouse apoE. Scale bars, 50 μm. *E*, Quantitative stereological estimation of activated microglial densities in brain regions of *Tg-SwDI* mice in the presence (black bars) or absence (gray bars) of endogenous mouse apoE. Data shown are mean ± SD ($n = 10$). *$p < 0.0001$; **$p < 0.01$. F/T, Frontotemporal; KO, knock-out.

reological measurement of activated microglial densities also showed that the numbers of these neuroinflammatory cells were, again, the highest in regions with the most abundant fibrillar cerebral microvascular Aβ deposition (i.e., thalamus and subiculum) (Fig. 7E). Nevertheless, their presence was significantly decreased in all regions of *Tg-SwDI/apoE*$^{-/-}$ mice compared with *Tg-SwDI/apoE*$^{+/+}$ mice ($p < 0.01$) (Fig. 7E).

The neuroinflammatory cytokine IL-1β has been shown to be elevated in brain and, notably, in cerebral microvessels from patients with AD (Grammas and Ovase, 2001, 2002). Similarly, we found markedly elevated levels of IL-1β in *Tg-SwDI/apoE*$^{+/+}$ mice (Fig. 8). However, there was a significant decrease in IL-1β levels in *Tg-SwDI/apoE*$^{-/-}$ mice. Together, these findings indicate that the lowering of cerebral microvascular amyloid in *Tg-SwDI* mice resulted in a pronounced reduction of associated neuroinflammation.

Discussion

The present findings show that the strong reduction of fibrillar cerebral microvascular Aβ deposition in *Tg-SwDI* mice decreases microvascular amyloid-induced neuroinflammation. The *Tg-SwDI* mouse is a unique model in that it exclusively develops fibrillar amyloid deposits in the cerebral vasculature, primarily on the microvasculature, whereas Aβ deposits in the brain parenchyma are primarily diffuse. In *Tg-SwDI* mice, cerebral microvascular amyloid starts to deposit at several months and exten-
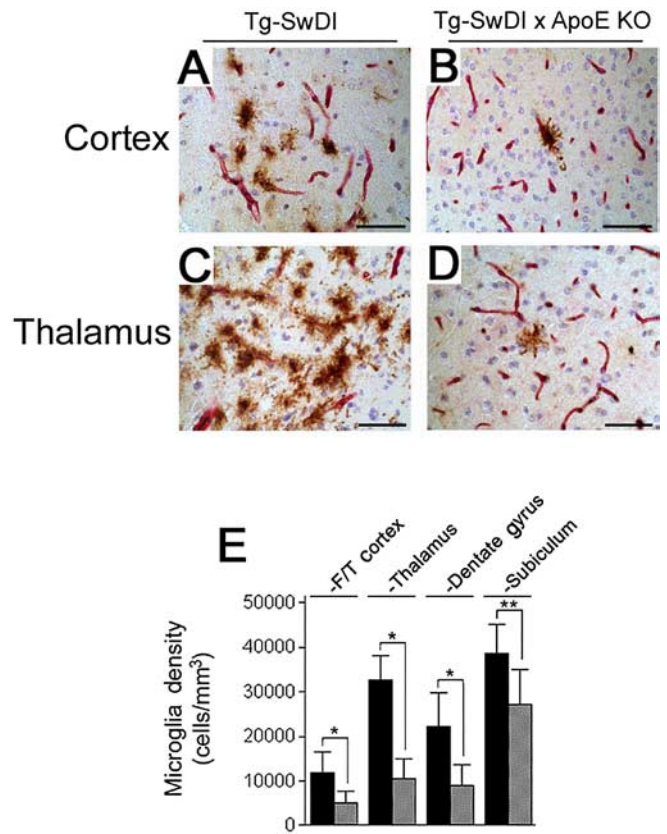
sively accumulates as the mice age (Davis et al., 2004). Although cerebral microvascular amyloid accumulation is extensive in this model, associated microhemorrhage is not a robust event. The restriction of fibrillar amyloid solely to the cerebral vasculature accurately reflects the site of fibrillar Aβ deposition and vascular amyloid-associated neuroinflammation in patients with the Dutch-type or Iowa-type familial CAA disorders (Grabowski et al., 2001; Maat-Schieman et al., 2004).

In several ways, the present study suggests a significant role for cerebral microvascular amyloid deposition in promoting neuroinflammation in amyloid-depositing diseases. First, this study provides novel evidence demonstrating that cerebral microvascular amyloid deposition can solely induce local neuroinflammation in human AβPP transgenic mice in the absence of parenchymal plaque fibrillar amyloid deposition. The presence of endogenous mouse apoE has been shown to be instrumental in promoting amyloid deposition in other human AβPP transgenic mouse models (Bales et al., 1999; Holtzman, 2001, 2004; Fryer et al., 2003). Accordingly, using the *apoE*$^{-/-}$ background as a tool to reduce microvascular amyloid deposition, we show a marked decrease in microvascular-associated neuroinflammatory cells and a significant lowering of neuroinflammatory cytokine IL-1β levels. The present microvascular amyloid findings are consistent with numerous genetic epidemiological as well as *in vitro* and *in vivo* studies implicating a strong association between apoE and
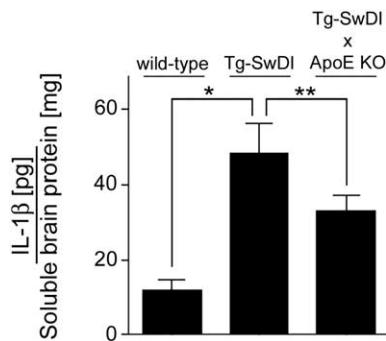
Figure 8. Reduced cerebral microvascular amyloid lowers the elevated IL-1β levels found in *Tg-SwDI* mice. The levels of IL-1β were measured in soluble forebrain extracts of 12-month-old wild-type, *Tg-SwDI/apoE* $^{+/+}$, and *Tg-SwDI/apoE* $^{-/-}$ mice by ELISA analysis. Data shown are mean ± SD (n = 5). *p < 0.0002; **p < 0.01. KO, Knock-out.

fibrillar Aβ deposition in brain (Schmechel et al., 1993; Holtzman, 2001; Chalmers et al., 2003; Ashford, 2004; Holtzman, 2004).

Second, our findings indicate that it is the deposited fibrillar forms of Aβ in the brain insoluble fraction that are strongly associated with inducing neuroinflammation in the *Tg-SwDI* model. In particular, the neuroinflammatory effects are the most robust in the regions that exhibit the highest amounts of cerebral microvascular amyloid deposition. In this regard, it is notable that elevated levels of inflammatory cytokines, including IL-1β, were reported in microvessels isolated from AD patients (Grammas and Ovase, 2001, 2002). Moreover, Aβ treatment was shown to stimulate production of IL-1β in cultured endothelial cells (Suo et al., 1998).

Third, *Tg-SwDI* mice are markedly deficient in their ability to clear Dutch/Iowa mutant Aβ peptides from brain across the blood–brain barrier into the circulation (Davis et al., 2004; Deane et al., 2004). This finding is further supported by the absence of detectable human Aβ in plasma obtained from *Tg-SwDI* mice on either an *apoE* $^{+/+}$ or *apoE* $^{-/-}$ background (data not shown). Therefore, the *Tg-SwDI* model provided a unique opportunity to investigate the effects of modulating Aβ deposition without altering the total pool of Aβ in brain via clearance into the circulation. Indeed, the lack of apoE drastically reduced Aβ deposition, particularly on the cerebral microvasculature, without significant influence on the levels of total Aβ in brain. Soluble Aβ oligomers have been proposed as potentially key pathogenic mediators of neuronal dysfunction in AD (Gong et al., 2003; Kayed et al., 2003; Cleary et al., 2004). However, despite a marked increase in the amount of soluble Aβ oligomers when cerebrovascular amyloid was reduced on the *apoE* $^{-/-}$ background, there was a clear reduction of neuroinflammation. Although these findings do not diminish the potential importance of soluble Aβ oligomers in the pathogenesis of neurodegenerative diseases, they indicate that cerebral microvascular fibrillar Aβ deposition can promote neuroinflammation on its own. The significance of pathogenic, vascular-mediated Aβ fibrillar assembly and deposition is also supported by several previous *in vitro* results using primary cerebrovascular cell cultures (Van Nostrand et al., 1997; Verbeek et al., 1997; Melchor and Van Nostrand, 2000).

Finally, these data show that fibrillar Aβ deposition is a viable target for the treatment of cerebral microvascular amyloid-induced neuroinflammation. In fact, recent studies have suggested that treatments aimed at reducing CAA-induced inflammation in afflicted individuals have improved the dementia

associated with this particular pathology (Eng et al., 2004; Harkness et al., 2004). Because CAA pathology is commonly found in AD, this target may have additional implications in combined treatment strategies for this neurodegenerative condition and its related disorder. As the present findings suggest, the interaction between Aβ and apoE that facilitates cerebral microvascular amyloid deposition leading to neuroinflammation represents one such target. Furthermore, these results indicate that disruption of pathogenic fibrillar amyloid remains an attractive therapeutic goal, and *Tg-SwDI* mice provide a unique model to test such approaches.

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