Statins Reduce the Neurofibrillary Tangle Burden in a Mouse Model of Tauopathy

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Abstract
Statin treatment has been associated with a reduced risk of Alzheimer disease and decreased amyloid deposition in mouse models. No animal studies have reported effects of statins on tau aggregates and neurofibrillary tangles (NFTs), the pathological hallmarks of Alzheimer disease that correlate with dementia. We investigated the effect of statins on NFTs in a transgenic mouse tauopathy model and found the following: 1) 1-month treatment with the blood-brain barrier-permeable agent simvastatin in normocholesterolemic aged mice significantly reduced the NFT burden and decreased lectin-positive microglia; 2) simvastatin significantly decreased NFTs and improved T-maze performance in young animals treated for 8 months; 3) treatment of hypercholesterolemic mice for 5 months with blood-brain barrier-impermeable atorvastatin markedly reduced the NFT burden and decreased lectin-positive microglia; 4) nonstatin cholesterol-lowering strategies showed a modest NFT decrease compared with statin treatment; and 5) there was a positive correlation between microglial and NFT burden ($r = 0.8$). Together, these results suggest that statins reduce NFT burden irrespective of blood-brain barrier permeability at both early and late ages in long- and short-term treatment paradigms and under normocholesterolemic and hypercholesterolemic conditions. The decrease in microglia, coupled with the limited effect of nonstatin cholesterol lowering, suggests that the anti-NFT effect of statins may be related to their anti-inflammatory and not necessarily to their cholesterol-lowering properties. Statins may provide therapy against NFTs in tauopathies, particularly when NFTs are the major neuropathologic component.

Key Words: Alzheimer disease, Neurofibrillary tangles, Statins, Tau, Tauopathy.

INTRODUCTION
Statins are 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors that block the synthesis of mevalonate and downstream products, including cholesterol. Various epidemiologic studies have reported a lower risk of dementia including Alzheimer disease (AD) in patients treated with statins (1–6); others, however, including 2 large cohort studies, failed to show this relationship (7–10). Recently, there has been a surge of interest in the therapeutic potential of statins for AD; indeed, some evidence of their beneficial effects in mild to moderate AD has been reported (11). Statins have also been suggested to be beneficial in multiple sclerosis (12), cerebral ischemia (13), and Parkinson disease (14). In addition to their cholesterol-reducing effect, statins have anti-inflammatory and antioxidative effects (15, 16).

The effects of statins on the amyloid pathology in amyloid mice have been intensively studied; both protective (17, 18) and deleterious (19) effects have been reported. Recent human neuropathologic studies in the elderly have shown some lower amyloid burden in statin-treated subjects by Arvanitakis et al (8) but not by Li et al (7). The latter study showed a lower neurofibrillary tangle (NFT) burden, but this effect was not demonstrated in the former study. To the best of our knowledge, no study has been conducted, to date, to investigate the effects of statins on NFTs in an animal model. Investigation of the effect of statins on NFTs is of high priority because NFTs, which are composed of aggregates of the hyperphosphorylated microtubule-associated protein tau, correlate with dementia to a greater degree than amyloid plaques (20, 21). Neurofibrillary tangle pathology is also a key pathological feature in other tauopathies, such as frontotemporal lobar degenerations, corticobasal degeneration, and progressive supranuclear palsy; NFTs are present without amyloid plaques in these disorders. The controversies surrounding associations between statin therapy and the incidence of AD and AD-related neuropathologic abnormalities imply that the mechanisms involved are complex and not yet fully understood. One possible explanation could be that the effects of statin therapy on amyloid plaques and NFTs are not necessarily similar because these 2 histopathologic features reflect distinct independently regulated pathogenetic processes that react differently to various stimuli such as nicotine (22). Thus, investigating the effect of statins on each of these histopathologic features separately could lead to...
a better understanding of their effects on disease pathogenesis and to more efficient and specific therapeutic targeting. Because AD patients, and also to some degree older elderly people, may have both NFTs and amyloid deposits concomitantly and because amyloid mouse models have been reported to also contain some early-stage phosphorylated tau pathological conformers, it seems that the study of brains of tauopathy patients and especially of tauopathy animal models that have NFTs only (i.e. without amyloid deposits) may provide a more precise tool for examining effects of statins on NFT burden.

We investigated the effects of statins in a transgenic (tg) mouse model of NFTs, the double-mutant (DM) P301S/K257T that expresses tau protein under the regulation of the authentic tau promoter (DM-Tau-tg) (23). After statin treatment, we demonstrated a marked reduction in tau pathology/NFT burden assessed in independent studies; a decrease could be detected after short-term (i.e. 1 month) or long-term (i.e. 5 months) statin therapy using both blood-brain barrier (BBB)-permeable (simvastatin) and BBB-impermeable (atorvastatin) statins and in both normocholesterolemic and hypercholesterolemic mice. An improvement in memory deficits was also identified in young mice. Other cholesterol-lowering strategies resulted in only a limited anti-NFT effect, indicating that cholesterol lowering is not a major mechanism for reducing the NFT burden. A corresponding decrease in microglia burden in the CNS under statin therapy suggests that this process involves an anti-inflammatory activity, a possibility in accord with studies by us and others that indicate that induction of NFT pathology may involve immune-mediated mechanisms (24–27).

**MATERIALS AND METHODS**

**Animals**

The mouse model used was previously generated and described by us. These tg mice (DM-Tau-tg mice) express the human DM tau protein (P301S/K257T) regulated by the natural tau promoter. These mice exhibit a wide spectrum of features characteristic to tauopathy and AD, including NFTs in neurons as well as in glial cells, severely degenerated neurons, astrogliosis, plaquelike (i.e. amyloid-free) structures, anxiety, cognitive deficits, and in vivo long-term potentiation deficit (23). The DM-Tau-tg mice were crossed with CB6F1 mice to obtain tg offspring that were identified by polymerase chain reaction analysis of tail genomic DNA. All animal experiments were approved by the Animal Ethics Committee.

**Treatment**

The study design is summarized in the Table. In a pilot study (Experiment I), 9-month-old DM-Tau-tg mice, fed with standard mouse low-fat diet (4% fat; Koffolk, Tel Aviv, Israel), were treated daily with or without 20 mg/kg simvastatin (Teva, Jerusalem, Israel) intraperitoneally for 1 month (n = 6/group). In a second experiment (Experiment II), 4-month-old DM-Tau-tg mice were treated with or without 30 mg/kg simvastatin in drinking water for 8 months (n = 14–16/group) with the standard mouse low-fat diet.

In Experiment III, 4-month-old DM-Tau-tg mice were treated for 5 months with or without 0.01% atorvastatin (Pfizer, Morris Plains, NJ) (28) or with 0.005% cholesterol-lowering drug ezetimibe (Merck Sharp & Dohme, Cramlington, UK) (wt/wt) mixed in a high-fat chow diet (“western”: enriched with 21 g percent fat, 40 kcal percent butterfat, 0.15% [wt/wt] cholesterol diet [Research Diets, New Brunswick, NJ] (29); n = 6–8/group). In Experiment IV, 5-month-old DM-Tau-tg mice were fed for 5 months with the western or standard diet (n = 5–6/group).

**Examination of Cholesterol Levels**

Plasma samples were collected at the end of the studies, and total cholesterol levels were determined (30).

**Neuropathologic Examinations**

**Tissue Collection**

Animals were killed at the end of each experiment under deep anesthesia with diethyl ether and were quickly transcardially perfused with PBS followed by 4% paraformaldehyde in ice-cold PBS, pH 7.2. Brains and spinal cords were removed, postfixed for 20 hours in the same fixative and routinely processed for coronal or sagittal, respectively, paraffin sectioning at 6 µm. All chemicals used in this study were purchased from Sigma (St Louis, MO) unless specified otherwise.

**Histology and Immunohistochemistry**

Brain and spinal cord paraffin sections were routinely silver-impregnated using the Gallyas silver method, which stains tangles and nerve cell processes (i.e. fine fibrils containing the abnormal tau protein in AD and tauopathies) (23).

For NFT immunohistochemistry (IHC), the AT8 (1:50) and AT180 (1:50) mouse monoclonal antibodies (Innogenetics, Ghent, Belgium), which recognize tau phosphorylated at 202/205 and 231, respectively, were used as previously described (23). Briefly, paraffin sections were deparaffinized and rehydrated in graded alcohols; antigen retrieval was performed with citrate buffer, pH 6, in microwave for 10 minutes. Immunostaining was performed using the Mouse-on-Mouse system (Vector Laboratories, Burlingame, CA). Sections were incubated overnight at 4°C with the primary antibodies AT8 and AT180, and immunostaining was visualized using the avidin-biotin complex (Vectastain; Vector Laboratories, Burlingame, CA) with 3,3`-diaminobenzidine tetrahydrochloride (Dako Cytomation, Glostrup, Denmark) as chromogen. Sections were counterstained with hematoxylin.

Glial reactions were detected using biotinylated lectin (Lycopersicon esuletum tomato, dilution 3 µg/mL) and glial fibrillary acidic protein (GFAP) rabbit polyclonal antibody (1:800; Dako) for microglia and reactive astrocytes, respectively. Briefly, paraffin sections were deparaffinized, rehydrated in graded alcohols, and endogenous peroxidase was blocked with H2O2 in methanol for 10 minutes. For lectin IHC, sections were incubated in 0.3% Triton X for 15 minutes; GFAP sections were incubated in fetal bovine serum for 30 minutes. Primary reagents were applied for 2 hours at room temperature.
temperature, and immunoreactions were visualized using the labeled streptavidin method or the Rabbit EnVision System (DAKO) with 3,3'-diaminobenzidine tetrahydrochloride as chromogen and counterstained as previously described.

**Neuropathologic Evaluation**

Brains and spinal cords were studied with optical microscopy by 2 blinded estimators. Generally, 3 different brain sections per staining, at least 100 μm apart, were used for each animal, and the areas of interest (cortex, hippocampus, cerebellum, and brainstem) were examined. Spinal cords were additionally studied using 10 sections per animal per staining. All sections were studied under 20× optical fields, additionally using high-power (40×) observation to clarify any possible doubts where necessary. A total of about 50 microscopic fields per animal were evaluated. The preselected areas of brains and the spinal cords were studied using different scales, depending on the staining. The brain and spinal cord sections were silver-impregnated by a modified Gallyas silver method that stains tangles, and nerve cell Gallyas-positive cells were identified and counted under high-power fields as shrunken dark cells, as previously described (31, 32). The adjacent serial sections were used for counting of AT8- and AT180-positive cells, as well as for lectin-positive microglia; we defined and counted as “positive” cells that were 3,3'-diaminobenzidine tetrahydrochloride brown in each IHC and had a clearly identified nucleus. In addition, the burden of GFAP-positive astrocytes was semi-quantitatively assessed using the following scale: 0 for 0 to 1 GFAP-positive cells per 20× field, 1+ for 2 to 10 cells, 2+ for 11 to 30 cells, and 3+ for more than 30 cells (23).

![FIGURE 1.](image)

**TABLE. Treatment Design Summary**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age at Treatment, Months</th>
<th>Duration of Treatment, Months</th>
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<th>Treatment</th>
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<td>Simvastatin</td>
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<td>Western</td>
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<td>IV</td>
<td>5</td>
<td>5</td>
<td>Western</td>
<td>- (control)</td>
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**FIGURE 1.** Short-term simvastatin treatment of double-mutant-Tau-transgenic (tg) mice results in a decrease of neurofibrillary tangle burden (assessed by Gallyas method). (A) A decrease in Gallyas-positive neurons (arrows and inserts) in cerebral cortex (B1), hippocampus (B2), and brainstem (B3) of treated mice compared with control tg mice (A1–A3) (Scale bars = 100 μm). (B) Assessment of dark neurons per high-power field (*p = 0.018, Student t-test).
Statins and Neurofibrillary Tangles

A
Cortex  Hippocampus  Brain stem

Tg control

A1  A2  A3

Tg + simvastatin

B1  B2  B3

C1  C2  C3

Tg control

D1  D2  D3

Tg + simvastatin

B

a

AT8 IHC (% of control)

Tg control  Tg + simvastatin

100  80  60  40  20  0

b

A1100 IHC (% of control)

Tg control  Tg + simvastatin

100  80  60  40  20  0
Isolation of Sarcosyl-Insoluble Tau

Sarcosyl-insoluble tau was prepared from brains of treated and untreated DM-Ta u-tg mice homogenized in 10 mmol/L Tris-HCl (pH 7.4), 0.15 mol/L NaCl, 1 mmol/L PMSF, 2 mmol/L EGTA, 10 mmol/L NaF, and spun for 15 minutes at 10,000 × g. The supernatant obtained was brought to 1% N-lauroylsarcosinate and incubated for 1 hour at room temperature. After a 35-minute spin at 100,000 × g, the sarcosyl-insoluble pellets were collected and used for immunoblotting with human tau Ab Tau-13 (kindly provided by Prof. L.I. Binder, Northwestern University) (1:5000) (23).

T-Maze

We used the T-maze test for assessing the spatial short-term memory and alternation behavior, analyzing the animals’ ability to recognize and differentiate between a new unknown and a familiar compartment. The performance of the treated DM-Ta u-tg was compared with that of the nontreated DM-Ta u-tg and to non-tg mice (n = 10) (23).

Statistical Analysis

All data are presented as mean ± SEM, and statistical analysis was performed using the SPSS 14.0 software. The unpaired Student t-test or the nonparametric Mann-Whitney U test was used for the comparison of quantitative variables between 2 groups, where appropriate. The Spearman rank correlation was calculated between pairs of quantitative variables. For the T-maze analysis, the unpaired t-test was used for comparison between the 2 groups (23).

RESULTS

Simvastatin Treatment Decreased NFT Burden and Microglial Lectin Positivity in Brains of Normocholesterolemic DM-Ta u-tg Mice

We first treated DM-Ta u-tg mice, under standard diet conditions, with the widely used BBB-permeable statin, simvastatin, to achieve maximum accessibility of the drug to the brain. This treatment was administered for 1 month starting when the mice were 9 months of age, when NFT deposition is already established in this model (which begins at 6 months). There was a significant decrease of tau pathology/NFT burden, as indicated by the significant decrease in Gallyas-positive cells in the cerebral cortex, hippocampus, and brainstem (Fig. 1; p = 0.018). This decrease in NFTs was further confirmed by IHC with AT8 and AT180, which revealed a significant decrease of 31% and 25%, respectively, compared with nontreated controls (Fig. 2; p = 0.005 and p = 0.02, respectively). A significant reduction (28.7%) in microglial burden in brain assessed by lectin staining was also observed (Fig. 3; p < 0.0001). Plasma cholesterol levels were comparable between treated and untreated mice (2.72 ± 0.22 mmol/L and 2.9 ± 0.16 mmol/L [mean ± SEM], respectively). No difference was noticed in reactive astrocytes by GFAP staining (data not shown).

When this therapy was administered at an early age (i.e. 4 months), before onset of NFTs, and for a longer 8-month duration to achieve a maximal effect (Experiment II), there was again a robust decrease in treated versus control NFTs, as assessed by Gallyas staining (30%, p = 0.005).
**Immunoblotting of sarcosyl-insoluble brain fractions with the human tau Ab Tau-13 revealed a similar trend: there was a decrease of 24.8% in the ratio of sarcosyl-insoluble (pellet) to soluble (supernatant) human tau in the simvastatin-treated compared with tg-controls (0.266 ± 0.06 and 0.20 ± 0.01, respectively); this did not achieve statistical significance. The treated animals showed also an improvement in memory deficits, as indicated by the improved T-maze performance (i.e. a longer time spent in the unfamiliar arm compared with nontreated mice; p = 0.049) (Fig. 4).**

**Atorvastatin Treatment Decreased NFT Burden and Microglial Staining in Brains of Hypercholesterolemic DM-Tau-tg Mice**

We next explored the anti-NFT effect of statins under conditions that may have therapeutic and applicable relevance, as follows: 1) feeding the mice with a high-fat diet (western diet, characteristic to western countries) to induce hypercholesterolemia, which is considered a risk factor for AD (33); and 2) using the third-generation statin, atorvastatin, which does not cross the BBB, thereby potentially eliminating possible CNS-related adverse effects of statins (33, 34). Accordingly, 4-month-old hypercholesterolemic DM-Tau-tg mice fed with a western diet were treated with atorvastatin for 5 months. There was a 13% reduction in plasma cholesterol levels in the atorvastatin-treated animals compared with the nontreated controls (3.8 ± 0.13 mmol/L and 4.3 ± 0.25 mmol/L, respectively). As shown in Figure 5, this regimen resulted in a substantial reduction in NFT burden, indicated by a 59% decrease in immunoreactivity against the phosphorylated residues 202/205 (with AT8 antibody) and of 53% against residue 231 (AT180 antibody; p < 0.0001). In addition, there was a 20.3% reduction in microglia burden, as assessed by lectin staining (p < 0.0001; Fig. 6). No difference was detected in reactive astrocytes, as assessed with GFAP staining (data not shown). These results show that the BBB-impermeable statin atorvastatin was also effective in reducing the NFT burden.

**The Cholesterol Absorbance Inhibitor, Ezetimibe, Reduced the NFT Burden but to a Lesser Extent than Atorvastatin**

To determine whether the anti-NFT effect of statins is related to cholesterol-lowering effects, we compared the

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**FIGURE 4.** Long-term simvastatin treatment of double-mutant (DM)–Tau-transgenic (tg) mice resulted in a decrease of neurofibrillary tangle burden and improved T-maze performance. **(A)**. Semiquantitative assessment shows a significant decrease in Gallyas-positive cells in brains (cortex, hippocampus, and brainstem) of the treated mice compared with control tg mice (*p < 0.0001, nonparametric Mann-Whitney U test). **(B)** Sarcosyl-insoluble (pellet) versus sarcosyl-soluble (supernatant) human tau in brain homogenates. Quantitative assessment shows a trend of the smaller sarcosyl-insoluble fraction in the treated DM-Tau-tg mice relative to nontreated tg controls; this did not reach statistical significance. Examples demonstrate brain fractions immunoblotted with tau 13 Ab. **(C)** Performance in the T-maze. Simvastatin-treated DM-Tau-tg mice spent significantly more time in the unfamiliar arm relative to the nontreated DM-Tau-tg controls (*p = 0.049). For comparison, the better performance of non-tg mouse controls compared with nontreated DM-Tau-tg mice is shown (p = 0.01, unpaired t-test).
FIGURE 5. Atorvastatin treatment of double-mutant Tau-transgenic (tg) mice resulted in a marked decrease of neurofibrillary tangle (NFT) burden assessed by immunohistochemistry (IHC) for NFT-related phosphorylated tau. (A) There is a significant decrease in mouse monoclonal antibody AT8- and AT180-positive neurons (black arrows) in cerebral cortex (B1, D1), hippocampus (B2, D2), and brainstem (B3, D3) of treated compared with control tg mice (A1-3, C1-3), respectively (Scale bars = 100 μm). (B) Semiquantitative assessment of AT8 (a) and AT180 (b) staining (*p < 0.0001 and *p < 0.0001, respectively, nonparametric Mann-Whitney U test).

FIGURE 6. Atorvastatin treatment results in a decreased microglial burden. (A) There is a significant decrease in lectin staining of microglia (arrows) and processes (arrowheads) in cerebral cortex (B1), hippocampus (B2), and brainstem (B3) of treated compared with control transgenic (tg) mice (A1-3) (Scale bars = 100 μm). (B) Semiquantitative assessment (*p < 0.0001, nonparametric Mann-Whitney U test).
NFT-reducing effect of statin in hypercholesterolemic DM-Tau-tg mice with that of ezetimibe, a nonstatin cholesterol-reducing agent that selectively inhibits intestinal cholesterol absorption (35). There was a 16% reduction in cholesterol level in plasma of the ezetimibe-treated animals compared with the nontreated controls (3.6 ± 0.13 mmol/L and 4.3 ± 0.25 mmol/L, respectively). As shown in Figure 7, 5 months of treatment with ezetimibe starting at 4 months of age did not cause a significant change in mouse monoclonal antibody AT8 immunoreactivity compared with nontreated animals (A). Although ezetimibe treatment reduced AT180 immunoreactivity (**p = 0.001), the reduction was less than that observed with atorvastatin treatment compared with nontreated animals (B) (**p = 0.05, nonparametric Mann-Whitney U test). IHC, immunohistochemistry; tg, transgenic.

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Low-Fat (Standard) Diet Caused Only a Slight Decrease in NFT Burden Relative to Mice Fed a Western Diet

To investigate the effect of cholesterol lowering per se on NFT pathology, the benefit of a lipid-lowering diet compared with a high-fat western diet on the NFT burden was evaluated. After 5 months of feeding starting at 5 months of age, the plasma cholesterol levels in the standard diet–fed mice were much lower than those of mice fed the western diet: 2.5 ± 0.2 mmol/L versus 4 ± 0.15 mmol/L, respectively; a decrease of 37%. Interestingly, the normocholesterolemic mice showed only a slight and nonsignificant decrease in the NFT burden compared with hypercholesterolemic mice: a decrease of 11.6% (p = 0.49) and 10.7% (p = 0.78) with AT8 and AT180 immunostaining, respectively (Fig. 8). Similarly, there was a nonsignificant decrease in Gallyas-positive cells in brain and spinal cords (p = 0.43). A microglia burden decrease was also detected in the standard diet–fed mice (12.3%; p < 0.0001) that was smaller than with the statin treatments (yet smaller than with the statin treatment; Figs. 3, 6), and there was no change in GFAP staining (data not shown). These results suggest that a lipid-lowering...
diet exhibits only a small nonsignificant benefit in reducing the NFT burden that is much smaller than the effects of atorvastatin treatment (Fig. 5), whereas the decrease in plasma cholesterol was much greater with the low-fat diet than with atorvastatin. The small anti-NFT effect of the low-fat diet may also suggest that hypercholesterolemia does not seem to be a significant risk factor for accelerating NFT pathology: an 82% increase in plasma cholesterol resulted in a nonsignificant increase of 13% and 12% for AT8 and AT180 IHC, respectively.

**Microglial Burden Assessed by Lectin Staining Correlates With NFT Burden**

To assess the possibility that a decreased NFT burden may be attributed, at least partially, to a decrease in microglia,

![Graph A](image1.png)  
**FIGURE 9.** Microglial burden correlated with neurofibrillary tangle burden in double-mutant Tau-transgenic mice. There was a positive correlation between mouse monoclonal antibody AT8 (A) immunohistochemistry (IHC) and AT180 (B) IHC and lectin staining for microglia ($r = 0.706$, $*p < 0.0001$; and $r = 0.819$, $*p < 0.0001$, respectively; Spearman rank correlation was calculated between pairs of quantitative variables).
we determined whether there was a correlation between NFT and microglial staining in the brains of the DM-Tau-tg mice, as was suggested in the brains of patients with progressive supranuclear palsy and corticobasal degeneration tauopathies (36). As shown in Figure 9, there was a positive significant correlation between AT8 IHC as well as AT180 IHC assessed with lectin staining for microglia ($r = 0.706, p < 0.0001$; and $r = 0.819, p < 0.0001$, respectively). Similar results were demonstrated when assessing each experiment separately, but this was less significant because of the limitations of smaller sample sizes.

**DISCUSSION**

We set out to establish the effect of statin treatment on the NFT pathology in a tg mouse model for tauopathy with NFTs only and to determine whether such an effect is related to blood cholesterol levels. We found that normocholesterolemic mice receiving the BBB-permeable simvastatin both for a long duration before NFT onset and in aged mice for a short duration showed a significant decrease in NFT burden. We conclude that a BBB-permeable statins given even for a short-term when NFTs are already present can markedly reduce the NFT burden. An anti-NFT effect was also detected in hypercholesterolemic younger mice that received a long-term course of the BBB-impermeable atorvastatin, thus indicating that a BBB-nonpermeable statins are also effective in reducing NFT burden. Using nonstatin dietary and pharmacological cholesterol-lowering strategies to assess the effect of cholesterol lowering on NFT reduction revealed only limited effects on reducing NFT, suggesting that statins may act in ways not only attributable to their cholesterol-lowering effects. In addition, these results suggest that the cholesterol-elevating conditions only modestly (if at all) exacerbate NFT pathology.

Effective mechanisms maintaining free cholesterol homeostasis, endogenous biosynthesis, and exogenous cholesterol import in neurons have been demonstrated (37). Therefore, the BBB-permeable simvastatin may have a limited effect on neuronal cholesterol levels, especially when low levels of simvastatin (20–30 mg/kg) were administered. This also points to the possibility that cholesterol lowering in neurons does not account for the marked anti-NFT effect induced by the statins used in this study (i.e. not by low-dose simvastatin and not likely by the BBB-impermeable atorvastatin). Although we detected no association between the decrease in NFT burden and plasma cholesterol levels, our results point to a possible association between the decrease in NFT burden and microglia. This was indicated by the marked decrease in microglial activation under both statin treatments with simvastatin or atorvastatin, along with marked decreases in NFT burden, by the lack of microglial decrease under ezetimibe treatment despite a partial decrease in NFT burden, and by the smaller microglial decrease with the low-fat diet with a very limited decrease in NFT burden. These observations led us to consider that microglial regulation is a possible pathway involved in the anti-NFT processes mediated by statins.

Microglia are the major resident immunocompetent cells in the brain and consequently play a particular role in immunomodulation (38). The possibility that inhibition of microglia is involved in the decrease of NFT pathology seems plausible in light of the study of Ishizawa and Dickson (36), which suggests a specific link between microglia and NFTs. These authors found that there were more activated microglia in postmortem brain tissue from corticobasal degeneration and progressive supranuclear palsy patients than in normal controls, and that the microglial burden correlated with tau burden in most of the pathologically affected areas (39). A relationship between NFT pathology and microglia is further supported by our results showing a positive correlation between microglial and NFT burden in the DM-Tau-tg mice (Fig. 9). In accordance with this notion, statins have been shown to reduce microglial activation in cell lines and primary cultures, suppressing CD40 and interleukin 6 secretion and reducing cell viability (40–42). In animals affected by experimental autoimmune encephalomyelitis, it has been shown that atorvastatin treatment promoted differentiation of naïve T cells to Th2 cells, with the characteristic increase in interleukin 4 concentration, and that this process was accompanied by a decrease in microglial activation and an amelioration of disease (12). In addition, atorvastatin attenuates amyloid-induced increases in the cell surface markers of activated microglia and chemokines in rats (43). The possibility that the anti-NFT effect of statins is attributed to the anti-inflammatory properties of statins may be further supported by findings indicating that the NFT pathology is indeed susceptible to and can be regulated by immune-mediated processes. For example, Li et al (24) showed that interleukin 1 mediates pathological effects of microglia on tau phosphorylation in cortical neurons, and Schneider et al (25) reported that experimental autoimmune encephalomyelitis–affected rats exhibited NFT pathology. We recently reported that immunizing mice with tau protein induced a CNS inflammatory response accompanied by robust NFT pathology (27). Moreover, NFT pathology was further accelerated by lipopolysaccharide injection in an AD-tg model (26).

The inhibition of microglial burden under statins, which seem to be cholesterol independent, may involve inhibition of synthesis of isoprenoid intermediates in the mevalonate pathway, specifically the geranylgeranlypyrophosphate, which has been reported to regulate microglial activation in cultures (44). Inhibition of geranylgeranlypyrophosphate synthesis resulted in attenuation of the microglial inflammatory responses (42). Our finding that both BBB-permeable and -impermeable statins reduce the NFT burden may indicate that this statin-related anti-NFT effect occurs irrespective of BBB permeability (45); that it is in part a peripheral rather than a local CNS effect. Although reduction in microglia induced by a BBB-permeable statin can more easily be explained, reduction of microglia by a BBB-impermeable statin might be attributed to reduced monocyte migration across the BBB and reduced differentiation into microglia in the brain parenchyma (46, 47); this suggests an explanation for the effects of a BBB-impermeable statin on brain lesions. The use of BBB-impermeable statins has been suggested to be advantageous based on the CNS side effects reported under BBB-permeable statin therapy (34, 48).
Of special interest is the rapid anti-NFT effect detected after 1 month of simvastatin treatment. Even more interesting is that this effect was noticed when treatment was started at 9 months of age (3 months after the onset of the NFT accumulations). This points to the substantial anti-NFT potential effect of statins and suggests possible beneficial therapeutic potential within a short scale of treatments; this may be effective also at a later age when disease has already started. This robust effect of statins may point to the possibility that statins also exert some direct disease-modifying effect on NFTs, such as affecting the kinases/phosphatases involved in NFT formation, possibly in a similar manner to the effect reported for statins and nonsteroidal anti-inflammatory drugs on amyloid-related secretases (49, 50).

In addition to the decrease in NFTs in the mice treated with simvastatin, we also observed an improvement in memory. This has also been recently reported in statin-treated amyloid tg mice, but without a decrease in amyloid burden (51). Perhaps a decrease in early-stage phosphorylated tau conformers, characterizing some amyloid mice, is involved in the improvement of memory because tau pathology correlates best with dementia, even better than the amyloid pathology. Yet, the finding that non-tg mice also showed improved memory with statins points to the involvement of additional factors (51).

In conclusion, the robust anti-NFT beneficial effect of statins presented here in a set of independent experiments using a model of tauopathy alludes to distinct beneficial effects of statins on NFTs, possibly mediated at least in part by a reduction of activated microglia. Because activated microglia contribute to amyloid plaque clearance (52), their reduction might interfere with elimination of amyloid. And conversely, therapeutic manipulations aimed to activate microglia for amyloid clearance may have the undesirable effect of exacerbating the formation of NFTs. This paradoxical modus operandi may provide at least a partial explanation for the persistent controversy on the effect of statins in AD, where amyloid pathology is also present, whereas it may point to the therapeutic potential of statins in tauopathies with “NFTs only.”

Our study opens new horizons for the study of statins and other anti-inflammatory or antimicrobial agents against NFT pathology. Further investigations to identify the target proteins that are involved in reducing NFT burden are warranted. This may shed more light on the mechanisms involved in statin-mediated AD risk reduction and explore its therapeutic potential for other tauopathies, in which NFTs are the only or the major pathological feature.

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