Selective targeting of perivascular macrophages for clearance of β-amyloid in cerebral amyloid angiopathy

Cheryl A. Hawkes* and JoAnne McLaurin*,a,b,1

aCentre for Research in Neurodegenerative Diseases and bDepartment of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada M5S 3H2

Edited by Don W. Cleveland, University of California at San Diego, La Jolla, CA, and approved November 26, 2008 (received for review June 6, 2008)

Cerebral amyloid angiopathy (CAA), the deposition of β-amyloid (Aβ) peptides in leptomeningeal and cortical blood vessels, affects the majority of patients with Alzheimer’s disease (AD). Evidence suggests that vascular amyloid deposits may result from impaired clearance of neuronal Aβ along perivascular spaces. We investigated the role of perivascular macrophages in regulating CAA severity in the TgCRND8 mouse model of AD. Depletion of perivascular macrophages significantly increased the number of thioflavin S-positive cortical blood vessels. ELISA confirmed that this increase was underscored by elevations in total vascular Aβ1-42 levels. Conversely, stimulation of perivascular macrophage turnover reduced cerebral CAA load, an effect that was not mediated through clearance by microglio or astrocytes. These results highlight a function for the physiological role of perivascular macrophages in the regulation of CAA and suggest that selective targeting of perivascular macrophage activation might constitute a therapeutic strategy to clear vascular amyloid.

As many as 90% of all Alzheimer’s disease (AD) cases present with cerebral amyloid angiopathy (CAA), the deposition of β-amyloid (Aβ) in cortical and leptomeningeal blood vessels (1). The vascular Aβ deposits observed in AD have been shown in vitro to induce degeneration of human and murine cerebrovascular smooth muscle and endothelial cells and in vivo to inhibit angiogenesis, impair vascular tone, and decrease total cerebral blood flow (2, 3). Pathological examination of AD brains positive for CAA has revealed capillary fragmentation, thickening, and redundancy of vascular basement membranes and disruption of blood-brain barrier (BBB) permeability (4). More recently it has been demonstrated that external vessel diameter, vessel wall thickness, and luminal area were decreased by more than 50% in patients with AD disease duration exceeding 10 years compared with individuals diagnosed 5 years before autopsy (5). Clinically, the degree of CAA severity correlates with intracerebral hemorrhage, ischemic necrosis, and degree of dementia (6).

Impaired clearance of Aβ from the brain is thought to be one of the main causes of amyloid accumulation in sporadic AD. Several endogenous mechanisms exist for the removal of soluble Aβ from the central nervous system (CNS) to the periphery, including receptor-mediated clearance at the BBB and via bulk movement of interstitial fluid. In addition to putative problems with receptor-mediated Aβ transport across the BBB, it has been hypothesized that CAA might arise as a result of impaired clearance of cerebral Aβ along perivascular spaces (7). This suggestion is supported by histological studies of AD brains that have identified Aβ deposits in dilated perivascular spaces and within small intracortical vessels and arteries, a pattern consistent with drainage pathways nearest to the brain (7). Further, dextran and ovalbumin tracers injected into the interstitial fluid of the brain parenchyma, which distribute in patterns identical to those of vascular amyloid deposited in CAA, are taken up by perivascular macrophages within 3 to 24 h after injection (8). Interestingly, reports from both human and animal anti-Aβ immunization studies have demonstrated increased CAA load to be associated with cortical Aβ plaque removal (9).

The perivascular spaces are an extension of the subpial space and are bordered peripherally by the basement membrane of the glia limits and centrally by the outer surfaces of cerebral blood vessels (10). A heterogeneous population of cells reside within the perivascular spaces, including leptomeningeal mesothelial cells and macrophages, which, in combination with pericytes and astrocytic foot processes, contribute to the formation of the immune BBB (10). Perivascular macrophages are a group of innate immune cells that are distinguished from parenchymal microglia by their possession of acid phosphatase, nonspecific esterase activity, and expression of the hemoglobin-haptoglobin scavenger receptor CD163 and the mannose receptor CD206 (11, 12). Unlike parenchymal microglia, which exhibit very little turnover, perivascular macrophages are regularly replaced from the bone marrow at a rate of ~30% over 3 months (13). Although the full extent of the physiological role is not known, perivascular macrophages have been shown to act as antigen presenting cells, perform phagocytosis, and respond to transient CNS and peripheral inflammation (14, 15).

Recently, Fiala et al. (16) reported that blood-derived macrophages from patients with AD were less effective at phagocytosing Aβ1-42 than those from nondemented individuals. Given that perivascular macrophages are constitutively phagocytic, and in light of their localization within perivascular spaces and proximity to vascular amyloid, we hypothesized that perivascular macrophages play a role in regulating the deposition of vascular Aβ. To test this hypothesis, we examined the effect of perivascular macrophage depletion and turnover on CAA severity in the TgCRND8 mouse model of AD (17). The pattern of vascular amyloid deposition in leptomeningeal and small cortical blood vessels observed in these mice mirrors that typically found in human CAA.

Results

Depletion of Perivascular Macrophages Increases CAA Severity. The use of liposome-encapsulated clodronate, an intracellular toxin, has been well characterized to study the effects of peripheral macrophage depletion and has more recently been adapted to examine the innate immune response to CNS injury (18, 19). To assess the effect of selective perivascular macrophage depletion on CAA severity, we injected clodronate- or vehicle-encapsulated liposomes into the left lateral ventricle of 4-month-old TgCRND8 mice expressing a mild degree of CAA (clodronate, n = 32; vehicle, n = 30). Clodronate administration significantly reduced the number of perivascular macrophages throughout both ipsi- and contralateral brain regions, including cortical and hippocampal areas, as dem-
Administration of liposome-encapsulated clodronate depletes perivascular macrophages. (A and B) TgCRND8 mice injected with PBS liposomes (A) showed more CD163-positive perivascular macrophages (green) associated with GLUT-1 immunoreactive (red) blood vessels in the caudate putamen than those that received clodronate-containing liposomes (B). (C) Immunoblotting of right brain homogenates (30 μg/lanes) demonstrated a significant reduction in CD206 levels (P = 0.002, n = 5) in mice receiving clodronate. (D and E) Photomicrographs showing that CD206 immunoreactivity is expressed by perivascular macrophages (red) but not by GFAP-immunoreactive astrocytes (D, green) or Iba1-positive microglia (E, green) in TgCRND8 mice. (Scale bars: A and B, 10 μm; D and E, 20 μm.)

Fig. 1. Administration of liposome-encapsulated clodronate depletes perivascular macrophages. (A) CD206; (B) GAPDH; (C) PBS; (D) Clodronate.

onstrated by the relative absence of macrophage-specific marker CD163 immunoreactivity in clodronate-treated animals compared with controls [Fig. 1 A and B and supporting information (SI) Fig. S1 A and B]. This reduction was quantitatively confirmed by a 56% ± 4.5% reduction in levels of the macrophage-specific protein CD206 between animals administered clodronate versus those receiving vehicle (P = 0.002; Fig. 1C).

Although macrophages are known to express the CD206 receptor, there are conflicting reports on cell-specific expression within the adult CNS (11, 20). To confirm the localization of CD206 expression to perivascular macrophages in our mouse model, we performed double labeling immunocytochemistry with anti-CD206 receptor and anti-glial fibrillary acidic protein (GFAP) or anti-ionized calcium-binding adaptor molecule-1 (Iba1) antisera to rule out astrocyte and microglia expression, respectively. Under our conditions, CD206 receptor expression was expressly localized to perivascular macrophages, whereas no co-localization was noted between CD206 receptor expression and GFAP-positive astrocytes (Fig. 1D). Iba1-immunoreactive processes were stained around blood vessels (Fig. S1C), but did not co-localize with CD206-positive macrophages within the perivascular space (Fig. 1E and Fig. S1D).

To determine the effect of perivascular macrophage depletion on cortical CAA severity, brain sections were stained with thioflavin S (thioS) to detect Aβ. ThioS is one of the two accepted dyes used to visualize pathologically relevant amyloid plaques, but does not bind monomeric Aβ. No differences in the number or intensity of thioS-positive blood vessels were detected between untreated animals and those that received vehicle-encapsulated liposomes (Fig. 2A and B), indicating that the surgery itself did not alter amyloid deposition. Administration of clodronate liposomes and the subsequent loss of perivascular macrophages resulted in a 5-fold increase in the percentage of total cortical area covered by thioS-positive blood vessels (vehicle, 0.05% ± 0.005%; clodronate, 0.28% ± 0.05%; P = 0.009; Fig. 2 C and D). The degree of vessel staining was also increased in clodronate-treated mice. Expression of endothelial basement membrane proteins such as perlecan and fibronectin have previously been shown to be up-regulated in association with increased CAA and may also bind to thioS (21). To determine whether basement membrane protein expression was affected by clodronate treatment and to confirm the specificity of thioS binding to amyloid deposits, we performed double labeling of thioS staining in conjunction with perlecan or fibronectin. No changes were noted in the immunoreactivity of perlecan (Fig. 2 E and F) or fibronectin (data not shown) between vehicle- (Fig. 2E) and clodronate-treated (Fig. 2F) animals. Further, double labeling experiments demonstrated that, although thioS and perlecan were expressed within the same blood vessel, no co-localization was observed. These results suggest that the clodronate-induced increase in thioS-positive cortical blood vessels was caused by an up-regulation of vascular amyloid deposition.

Perivascular Macrophage Depletion Increases Vascular Levels of Aβ42.

To confirm that vascular amyloid was specifically increased, we performed double labeling immunocytochemistry of brain tissues with anti-Aβ1-42 (clone 6F/3D) and anti-a-smooth muscle actin. Similar to the thioS staining, clodronate-treated mice showed a greater number of Aβ-positive blood vessels throughout the cortex compared with vehicle-injected animals (Fig. S2 A–D). We next examined whether Aβ40 or Aβ42 was preferentially deposited following clodronate treatment. No appreciable differences in the amount or intensity of vascular Aβ40 immunoreactivity were noted between vehicle- (Fig. 2G) and clodronate-treated tissue sections (Fig. 2H). These histological results were confirmed by sandwich ELISA, in which no differences were detected in total Aβ40 levels in isolated blood vessels (vehicle, 23.8 ± 1.6 ng/g; clodronate, 21.5 ± 2.4 ng/g; P = 0.21), vessel-depleted cortex (vehicle, 18.4 ± 1.0 ng/g; clodronate 17.6 ± 1.5 ng/g; P = 0.32) or plasma (vehicle, 3,462 ± 84.5 pg/mL; clodronate, 3,569 ± 185.8 pg/mL; P = 0.29) from the two treatment groups. By contrast, brain sections from clodronate-treated TgCRND8 mice exhibited an increase in the number of cortical vessels positive for anti-Aβ42 immunoreactivity compared with vehicle-injected animals (Fig. 2 I and J). An increase in the intensity of anti-Aβ42 staining was also noted in clodronate-treated animals. Similarly, ELISA results showed a significant increase in total Aβ42 levels in blood vessels isolated from clodronate-treated mice versus controls (P = 0.03; Fig. 2K). Interestingly, Aβ42 levels were decreased in the corresponding cortical samples (P = 0.04; Fig. 2L), whereas plasma concentrations were not different between the two treatment groups (P = 0.29; Fig. 2M). Quantification of Aβ1-42-positive plaques confirmed the significant decrease in cortical plaque load in clodronate-treated mice versus controls (vehicle, 0.004% ± 0.001% of cortex covered; clodronate, 0.002% ± 0.0003%; P = 0.04). These data would thereby suggest that perivascular macrophage deposition in TgCRND8 mice induces a preferential vascular deposition of Aβ42 peptides, whereas Aβ40 levels are relatively unaffected. Although human CAA consists predominantly of Aβ40, these results are not surprising given the preferential expression and amyloidogenic properties of Aβ42 in the TgCRND8 mouse.

To determine whether macrophage depletion had an effect on microglial activation, Iba1 expression was assessed in clodronate-injected TgCRND8 mice. Iba1-positive activated microglia were identified throughout the parenchyma and surrounding the vasculature in the brains of vehicle- and clodronate-injected mice (Fig. 2 N and O). However, no quantitative difference in Iba1 levels was found between mice treated with vehicle- or clodronate-containing
Fig. 2. Depletion of perivascular macrophages increases CAA severity. (A–C) Naive TgCRND8 mice (A) and those treated with PBS solution (B) show fewer thioS-positive cortical blood vessels than clodronate-treated mice (C). (D) Total cortical area covered in thioS-positive blood vessels was increased 5-fold in clodronate-treated animals \((P = 0.01, n = 10)\). (E and F) No colocalization was found between thioS (green) and anti-perlecan (red) staining in PBS solution- (E) or clodronate-treated mice (F). (G and H) No differences in A\(/\beta_{42}\)-positive staining were noted between vehicle- (G) and clodronate-treated animals (H). (I and J) The number and intensity of A\(/\beta_{42}\)-positive cortical blood vessels was increased in mice treated with clodronate (J) versus control animals (I). (K–M) Total human A\(/\beta_{42}\) levels in cortical blood vessels isolated from clodronate-treated mice (K) were significantly increased \((P = 0.03, n = 12)\) compared with PBS solution-injected animals. A\(/\beta_{42}\) levels were significantly decreased in the cortical samples \((L, P = 0.04, n = 12)\) of clodronate-treated animals, but were not altered in plasma samples \((M, P = 0.29)\). (N–P) Iba-1-positive microglia were noted throughout the cortex of vehicle- (N) and clodronate-treated mice (O). No differences were noted in Iba-1 levels \((P = 0.54, n = 4)\) between treatment groups. Values represent mean ± SEM of samples analyzed in triplicate; \(*P < 0.05\) and \(**P < 0.01\). (Scale bars: A–C, 75 μm; D and F, 10 μm; G–J, 20 μm; N and M, 70 μm.)
literals (Fig. 2P; P = 0.54). These data indicate that perivascular macrophage depletion did not significantly alter microglial activity.

Increases in CAA severity have been associated with increases in micro-hemorrhages in human studies and animal models of AD (9, 22). To investigate the impact of increased Aβ42 deposition on cerebral “microbleeds,” we examined brain tissues for the hemoglobin breakdown product hemosiderin by Prussian blue staining (9). Despite the significant increase in vascular amyloid following clodronate treatment, we were unable to detect Prussian blue labeling of blood vessels in vehicle- or clodronate-treated animals. Staining was evident only along the tract mark at the site of injection and could be distinctly identified within microglial cells (Fig. S2E and F). As such, no differences in Prussian blue staining were noted between clodronate- and vehicle-injected animals, suggesting that increased CAA did not compromise vascular integrity.

**Chitin Administration Stimulates Turnover of Perivascular Macrophages.** Given our findings that perivascular macrophage depletion increased CAA severity, we examined the effects of perivascular macrophage turnover on vascular amyloid load. At 5 months of age, TgCRND8 mice deposit significant CAA and thus allowed for detection of treatment-induced changes. To stimulate perivascular macrophage turnover, we used chitin, a naturally occurring biopolymer of N-acetyl-β-D-glucosamine expressed in the cell walls of fungi, crustaceans, insects, and worms (chitin, n = 21; vehicle, n = 20). Chitin uptake in peripheral macrophages is believed to occur via binding to the CD206 receptor (23).

It has previously been shown that perivascular macrophages phagocytose fluorescently conjugated dextran dyes injected into the lateral ventricles of the mouse brain (15). This method can thus be used to visualize macrophage turnover following the sequential administration of red and green dyes, by determining the ratio of singly and doubly labeled cells. Although chitin is known to stimulate peripheral macrophages, its effect on perivascular macrophages has not been determined. To examine the effect of chitin administration on perivascular macrophage turnover in the presence of significant CAA load, we injected 5 µg of chitin or PBS solution plus dextran conjugated fluorescein into the left lateral ventricle of 5-month-old TgCRND8 mice, followed 2 weeks later by a second injection of chitin or PBS plus dextran-conjugated Alexa Fluor 594. Dextran dyes were selectively taken up by macrophages and could be distinctly identified within microglial cells (Fig. S2C). Staining was evident only along the tract mark at the site of injection and Fig. 3C and D) showing that clearance of vascular amyloid was mediated by perivascular macrophages, we examined brain sections from chitin-treated animals for colocalization of Aβ with perivascular macrophages, reactive astrocytes, or activated microglia. No colocalization was observed between GFAP-positive astrocytes or their end feet projections (Fig. 4A). Similarly, although activated, Iba1-immunoreactive microglia were clearly found to be associated with parenchymal plaques (Fig. 4B, arrows), no such association was observed between microglia and vascular amyloid. By contrast, CD163-positive macrophages demonstrated positive co-localization with thioS-stained amyloid in cortical and leptomeningeal vessels (Fig. 4C). These results suggest that chitin-induced reduction of CAA load was mediated via uptake by perivascular macrophages, rather than by activated microglia or reactive astrocytes.

To confirm that increased perivascular macrophage turnover specifically cleared vascular amyloid, brain sections were stained for anti-Aβ42 and anti-α-smooth muscle actin. Chitin-treated mice showed a decrease in the number of Aβ-positive blood vessels throughout the cortex compared with vehicle-injected animals (Fig. 4D). Given the preferential effect of perivascular macrophage depletion on Aβ42, we next investigated whether an Aβ42 effect was also observed following perivascular macrophage turnover. Consistent with results from the clodronate experiments, no differences in Aβ40-immunoreactivity were observed between chitin- (Fig. 4E) and vehicle-treated mice (Fig. 4D). These findings are in contrast to anti-Aβ42 staining, which revealed a notable
Figure 4. Perivascular macrophages clear CAA. (A–C) Chitin-treated mice showed no colocalization between thioS (A–C, green) and GFAP-positive astrocytes (A, red), nor with Iba1-positive microglia (B, red), which were however associated with parenchymal amyloid plaques (B, arrows). However, CD163-immunoreactive macrophages (C, red) colocalized with thioS-labeled vascular amyloid (C, green) in these mice. (D–G) No differences were noted between vehicle- (D) and chitin-treated animals (E) in brain tissue sections processed for anti-Aβ40 staining. A significant reduction in Aβ42-positive staining (F and G) was noted in chitin-treated animals (G) compared with controls (F). (H–J) Total human Aβ42 levels were significantly decreased in blood vessels (H, P = 0.04) and plasma samples (I, P = 0.04, n = 6) isolated from chitin-treated mice, but not in vessel-depleted cortical samples (I, P = 0.16). Values represent mean ± SEM of samples analyzed in triplicate; *P < 0.05. (Scale bars: A and B, 5 μm; C, 25 μm; D–G, 20 μm.)

Discussion

The role of vascular dysregulation in the etiology of AD dementia is increasingly becoming a topic of research. Aβ peptides have been shown to cause vasoconstriction and intraluminal thickening and induce smooth muscle cell death, thereby increasing the risk of cerebral hemorrhage and stroke (2, 4, 5). However, the pathogenesis of CAA has not been fully addressed experimentally. We sought to examine the role of perivascular macrophages in the clearance of CAA from cortical blood vessels of the TgCRND8 mouse model of AD. We report that CAA severity is exacerbated following selective perivascular macrophage depletion and that the more toxic Aβ42 peptide is predominantly altered by this treatment. Further, stimulation of perivascular macrophage turnover results in the clearance of Aβ42-immunoreactive and thioS-positive vascular amyloid deposits via CD163-positive macrophages. These data provide additional evidence to support a perivascular pathway for amyloid clearance and suggest that, under normal conditions, macrophages within the perivascular spaces may function to eliminate Aβ peptides from the cerebral vasculature.

Differences in Iba1 levels were found between mice treated with vehicle or chitin (P = 0.91; Fig. S4C). These results are consistent with a role of perivascular macrophage-mediated clearance of Aβ from the cerebral vasculature.
Depletion of Perivascular Macrophages. Perivascular macrophage depletion was carried out according to the protocol adapted by Poftilet al. (19) (see SI Methods). Four-month-old TgCRND8 mice were anesthetized with isoflurane and stereotactically injected with 10 μL of PBS solution or clodronate-containing liposomes into the left lateral ventricle (coordinates from Bregma: anteroposterior, −0.2 mm; mediolateral, 1.2 mm; dorsoventral, 2.3 mm; n = 30 per group). Animals were killed 1 month later and brains were processed for further analysis.

Stimulation of Perivascular Macrophage Turnover. Five-month-old TgCRND8 mice received a 5-μL injection of either PBS solution or cloniton (1 mg/mL) into the left lateral ventricle, which was repeated 14 days later (n = 20 per group). A subset of animals received a combination of cloniton or PBS solution plus 5 μL dextran fluorescein dye, followed 2 weeks later by an injection of cliton or PBS solution plus 5 μL dextran Alexa Fluor 594 dye (n = 4 per group). Animals were killed 14 days after the last injection and brains were processed for further analysis.

Immunocytochemistry. Mice were deeply anesthetized with sodium pentobarbital and perfused with 0.1 M PBS solution (pH 7.4) and 10% formalin or snap-frozen. Sections (20 μm) were incubated overnight with anti-CD163 (1:500), anti-Iba1 (1:1,000), anti-Aβ42 (1:500), or anti-Aβ40 (1:70) and developed by using the glucose oxidase-DAB-nickel enhancement method (see SI Methods for antibody sources). For thioS staining, sections were incubated with 1% thiosulfate in 70% EtOH, washed in PBS solution. For this plus GFAP/CD163/perlecan double labeling, sections were incubated overnight with anti-GFAP (1:250), anti-Iba1 (1:250), anti-CD163 (1:100), or anti-perlecan (1:100); exposed to Alexa Fluor 594-conjugated anti-rabbit/mouse (1:200); and then processed for thioS staining (see SI Methods for additional staining procedures). Photomicrographs of visualized sections were captured with a Zeiss Axioscope 2 Plus microscope and exported to Adobe Photoshop CS.

Methods

Animals. TgCRND8 mice overexpressing the human Swedish (KM670/671NL) and Indianaan (V717F) APP mutations under a hamster prion protein promoter were maintained on an outbred C3H/C57BL6 background (17). Mice were age- and sex-matched and allowed food and water ad libitum. All experiments were carried out according to the guidelines stipulated by the University of Toronto and Canadian Council for Animal Care.

ACKNOWLEDGMENTS. The authors thank Mary Brown, Kevin DaSilva, Daniela Fenili, and Dr. Teresa DeLuca for their technical assistance. This work was supported by grants from the Canadian Institutes for Health Research (C.H., J.M.), the Natural Sciences and Engineering Research Council (J.M.), the Alzheimer’s Society of Canada (C.H.), and the Ontario Alzheimer’s Society (J.M.).
Supporting Information

Hawkes and McLaurin 10.1073/pnas.0805453106

SI Methods

Materials. Phosphatidylcholine, cholesterol, p-amino-phenyl-α-D-mannopyranoside, dichloromethylenediphosphonic acid di-sodium salt (clodronate), chitin (from crab shell), and thioflavin S were purchased from Sigma-Aldrich. Anti-CD163 and anti-CD206 antibodies were obtained from AbDSerotec, anti-human Aβ40 and anti-human Aβ42 antibodies were from Chemicon, anti-GAPDH was from Meridian Life Science, anti-Iba1 was purchased from Wako Pure Chemicals, and anti-GFAP antibodies, horseradish peroxidase-conjugated secondary antibodies, and the enhanced chemiluminescence kit were purchased from Dako. Fluorescently conjugated secondary antibodies and polyacrylamide electrophoresis gels (4%–20%) were purchased from Invitrogen. Human Aβ40 and Aβ42 ELISA kits were obtained from BioSource.

Depletion of Perivascular Macrophages. Multilaminar mannosylated liposomes were prepared by dissolving 178 mg of phosphatidylcholine and 27 mg of cholesterol in 8 mL of chloroform, to which 9.25 mg of p-amino-phenyl-α-D-mannopyranoside in 5 mL methanol was added in a round-bottomed flask. After evaporation, the lipid film was dispersed in 10 mL of PBS solution (0.1 M, pH 7.4) alone or containing 2.5 g of clodronate. Both preparations were kept under a nitrogen stream for 2 h at room temperature, sonicated for 3 min, and resuspended in 4 mL of sterile PBS solution.

Cortical Blood Vessel Isolation. The brains of PBS-perfused mice were rapidly removed and dissected for cortices, which were homogenized in 1.5 mL of blood vessel isolation buffer (0.1 M NH₄CO₃, 5 mM EDTA, 0.01% sodium azide and protease inhibitor mixture) by using a Dounce homogenizer (6 strokes). Homogenates were centrifuged (100,000 g, 1 h, 4 °C) and pellets resuspended in 500 μL of 0.1M NH₄CO₃ plus 7% SDS (plus protease inhibitor mixture) and stirred for ~4 h. Tissues were then sequentially filtered through 100-μm and 40-μm mesh filters to isolate blood vessel tufts from cortical filtrate.

Double Labeling and Fluorescent Immunocytochemistry. For CD163 and GLUT-1/CD31, free-floating brain tissue sections were washed in PBS solution, blocked for 15 min with 15% normal goat serum, and incubated overnight with anti-CD163 (1:100) or anti-α-smooth muscle actin (1:250), developed with the appropriate fluorescently conjugated secondary antibody (1:200), treated for 2 min with 70% formic acid, and washed in PBS solution before being incubated overnight with anti-Aβ40 or anti-Aβ42 (1:100). Sections were rinsed in PBS solution, developed with the corresponding fluorescently conjugated anti-rabbit/mouse secondary antibody (1:200), and cover-slipped by using DakoShield Mounting Media (Dako). For anti-CD206 immunostaining, frozen brain sections were treated for 10 min in absolute EtOH, incubated overnight with anti-CD206 (1:100), and treated for 2 h with Alexa Fluor 594-conjugated anti-rat (1:200). The same sections were incubated overnight with either anti-Iba1 (1:200) or anti-GFAP (1:250) and developed with Alexa Fluor 488-conjugated secondary antibodies. Fluorescent photomicrographs were captured using a confocal microscope (Zeiss) and exported to Adobe Photoshop CS.

Statistical Analysis. Images from singly labeled thioS, Ab8-17, and dextran-positive perivascular macrophages were serially quantified (3 sections per mouse) by using Openlab 4.0.2’ imaging software (Improvision) by an examiner blinded to experimental treatment. ELISA measurements were done in triplicate for plasma, isolated blood vessels, and vessel-depleted cortical samples. For all treatment groups, mean values ±/− SEM were used to construct histograms and analyzed by a one-way or two-way Student’s t test with significance set at P < 0.05.
Fig. S1. Perivascular macrophages and juxtavascular microglia localize to cortical blood vessels. (A and B) Immunofluorescent photomicrographs depicting the specific localization of CD163-positive perivascular macrophages (green) to CD31-immunoreactive (red) cerebral blood vessels. Fewer perivascular macrophages were localized to perivascular spaces in the cortex of clodronate-treated (B) animals compared with vehicle-injected (A) TgCRND8 mice. (C) Iba1-positive staining of juxta-vascular microglial processes abutting a cortical blood vessel (arrows). (D) Photomicrographs showing that CD206-immunoreactivity is expressed by perivascular macrophages (red), but not by Iba1-positive microglial cell bodies (green). (Scale bars: A and B, 35 μm; C and D, 20 μm.)
Fig. S2. Depletion of perivascular macrophages increases CAA load, but does not induce cerebral micro-hemorrhages. (A–D) Immunofluorescent photomicrographs showing double labeling of brain tissue sections from vehicle- (A and C) and clodronate-injected (B and D) animals stained for Aβ (green) and α-smooth muscle actin (red). Note that the number and intensity of Aβ-positive cortical blood vessels was increased in mice treated with clodronate. (E and F) Tissue sections from the brains of mice treated with PBS- (E) or clodronate-containing liposomes (F) stained with Prussian blue to detect the presence of hemosiderin did not exhibit CAA-related micro-hemorrhages. (Scale bars: A and B, 5 μm; C–F, 20 μm.)
Fig. S3. Stimulation of perivascular macrophage turnover reduces Ab42 deposition in cortical blood vessels and leptomeninges. (A) Photomicrograph depicting the localization of dextran-positive perivascular macrophages (red and yellow) to the perivascular spaces of GLUT-1-immunoreactive cortical blood vessels (blue) in tissues sections from chitin-treated animals. (B) Quantification of CD206 levels in the right hemisphere of chitin-treated TgCRND8 mice did not differ significantly from vehicle-injected controls. (C and D) Immunofluorescent photomicrographs showing double labeling of brain tissue sections from vehicle- (C) and chitin-injected (D) animals stained for Aβ42 (green) and α-smooth muscle actin (red). Note that the intensity of Aβ-labeling positive cortical blood vessels was decreased in mice treated with chitin. (E) CD163-immunoreactive macrophages (red) colocalized with Aβ42-specific vascular amyloid (green) in chitin-injected mice.
Fig. S4. Chitin treatment does not affect microglial activation. (A–C) Iba-1-positive microglia were noted throughout the cortex of vehicle- (A) and chitin-treated (B) mice. No differences were noted in Iba-1 levels (C) between treatment groups (p = 0.91, n = 4). Values represent mean ± SEM of samples analyzed in triplicate. (Scale bar, 70 μm.)