Mechanisms of Statin-mediated Inhibition of Small G-protein Function*

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3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have been reported to reduce the risk of Alzheimer disease. We have shown previously that statins inhibit a β-amyloid (Aβ)-mediated inflammatory response through mechanisms independent of cholesterol reduction. Specifically, statins exert anti-inflammatory actions through their ability to prevent the isoprenylation of members of the Rho family of small G-proteins, resulting in the functional inactivation of these G-proteins. We report that statin treatment of microglia results in perturbation of the cytoskeleton and morphological changes due to alteration in Rho family function. Statins also block Aβ-stimulated phagocytosis through inhibition of Rac action. Paradoxically, the statin-mediated inactivation of G-protein function was associated with increased GTP loading of Rac and RhoA, and this effect was observed in myeloid lineage cells and other cell types. Statin treatment disrupted the interaction of Rac with its negative regulator the Rho guanine nucleotide dissociation inhibitor (RhoGDI), an interaction that is dependent on protein isoprenylation. We propose that lack of negative regulation accounts for the increased GTP loading. Isoprenylation of Rac is also required for efficient interaction with the plasma membrane, and we report that statin treatment dramatically reduces the capacity of Rac to interact with membranes. These results suggest a mechanism by which statins inhibit the actions of Rho GTPases and attenuate Aβ-stimulated inflammation.

The primary action of statins is the inhibition of HMG-CoA reductase, blocking the de novo synthesis of cholesterol and resulting in lower plasma cholesterol levels (1). However, recent observations demonstrate that statins have pleiotropic actions that are not dependent on cholesterol reduction (2). Specifically, statins have been shown to inhibit vascular inflammation, enhance endothelial function, inhibit the proliferation of vascular smooth muscle, reduce platelet activation and aggregation, and increase atherosclerotic plaque stability. Many of these effects were postulated to depend on the disruption of Rho family gene-regulations, and this disruption, in turn, prevents various inflammatory responses. However, the specific effects statins have on G-protein regulation and localization have not been clearly delineated. We report that statin-mediated inhibition of isoprenylation prevented Rho family members from interacting with RhoGDI, resulting in increased levels of GTP-loaded G-proteins. Lack of isoprenylation also prevented translocation to the plasma membrane. These effects culminated in the functional inhibition of Rho-family G-proteins. We suggest that the salutary effects of statins in reducing the risk for AD may arise, in part, from inhibition of microglia-mediated inflammatory responses.

EXPERIMENTAL PROCEDURES

Materials and Reagents—β-Amyloid (Aβ) peptides Aβ-(25–35) and Aβ-(1–42), purchased from the American Peptide Company (Sunnyvale, CA), were dissolved in sterile water at a concentration of 2 mM and
then incubated for 5 days at 37 °C to induce fibril formation (17–19). Simvastatin and lovastatin were obtained from Calbiochem and prepared following the manufacturer's instructions. Briefly, the statin was converted to its active form by dissolving it in absolute ethanol (20 µl/µg statin to final concentration of 55.0% (v/v) ethanol) followed by addition of 1 N NaOH (to final concentration of 0.45 M). This solution was stored at −20 °C until use. Immediately prior to use the statin solution was neutralized (pH 7.2) with 1 N HCl. Mevalonic acid and cholesterol were purchased from Sigma-Aldrich and Calbiochem, respectively, and reconstituted in 100% ethanol. The GGpp transferase inhibitor GGTI-286 was purchased from Calbiochem and reconstituted in Me₃SO. Nile red Fluospheres (1 μm microspheres) and rhodamine phalloidin was purchased from Invitrogen. Geranylgeranyl pyrophosphate triammonium salt was purchased from Biomol (Plymouth Meeting, PA) and dissolved in 70% ethanol. PAK-GST and rhoetkin-GST fusion beads were purchased from Cytoskeleton (Denver, CO). Antibodies recognizing Rac (05–389) and flotillin-1 (610821) were purchased from Upstate (Waltham, MA), Calbiochem, Stressgen Biotechnologies (Victoria, British Columbia, Canada), and BD Biosciences, respectively. Antibodies to RhoA (SC-179) and RhoGDI (SC-360) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

**Cell Culture**—THP-1 monocytes and BV-2 mouse microglial cell lines were employed because of their ability to respond to fibrillar Aβ (fAβ), (but not non-fibrillar forms of Aβ) through mechanisms that are identical to those in primary microglia (20–22). Human THP-1 monocytes were obtained from American Type Tissue Collection (Manassas, VA) and cultured in Roswell Park Memorial Institute (RPMI) 1640 (Whittaker Bioproducts, Walkersville, Maryland) at 37 °C and 5% CO₂. RPMI medium was supplemented with 10% heat-inactivated fetal bovine serum (FBS; catalog number SH30397.03, Hyclone, Logan, UT), 50 µM L-ascorbic acid, and 50 µM dipicolinic acid. The cells were then resuspended in relaxation buffer. Standard Western blotting procedures were used to separate and transfer samples to polyvinylidene difluoride membranes and then probed with antibodies recognizing Rac or RhoA. Aliquots of the cellular lysates were probed to monitor total cellular levels of the small G-proteins. Band intensities were quantified using a Bio-Rad VersaDoc image analysis hardware/software package.

**Phagocytosis Assay**—Microglial phagocytosis was assessed exactly as described by Koenigsknecht and Landreth (20) using BV-2 microglia, which faithfully reproduce the phagocytic actions of primary microglia. Briefly, BV-2 cells (1.5 x 10⁶) were plated onto 35-mm plates, the serum concentration was lowered to 2%, and the cells were treated with simvastatin or lovastatin overnight at 37 °C and 5% CO₂. The cells were then incubated for 30 min in the absence or presence of 60 µM fibrillar Aβ-(25–35) (63.6 µg/ml) peptide or 5 µM fAβ-(1–42) (22.6 µg/ml) peptide. Fluorescent microspheres were then added to the cells for 30 min after having been washed in PBS containing 1 mg/ml bovine serum albumin. The uptake of fluorescent microspheres was used as a marker of fluid phase phagocytosis. Cells were then fixed with 2% paraformaldehyde, and three random fields of cells (>100 cells) were counted on an inverted microscope.

**Phalloidin Staining**—BV-2 cells (1.5 x 10⁶) were collected, plated on coverslips resting in 24-well plates, and incubated in serum-free DMEM in the presence or absence of increasing concentrations of simvastatin and lovastatin overnight at 37 °C and 5% CO₂. The cells were rinsed with PBS before being fixed in 2% paraformaldehyde and washed again in PBS. The cells were then incubated at room temperature with 0.1% Triton X-100 buffer for 5 min and washed again in PBS. F-actin was visualized by incubation of the cells with rhodamine phalloidin (final concentration of 0.0017 units/µl) at room temperature and protected from light for 30 min. The coverslips were mounted on glass slides, and images were acquired at 40× or 20× magnification.

**Isolation of GTP-loaded Small G-proteins**—THP-1 cells (10 x 10⁶), BV-2 cells (7 x 10⁶), N2a cells (5 x 10⁶), or PC12 cells (6 x 10⁶) were plated into 6-well dishes. The cells were treated with simvastatin, lovastatin, or GGTI-286 with or without the addition of mevalonic acid, cholesterol, or GGpp overnight at 37 °C and 5% CO₂. The cells were lysed, and activated G-proteins were isolated by the ability of the active GTP-loaded species to interact with effector protein binding domains. Briefly, cells were washed with calcium/magnesium-free PBS and then lysed by the addition of lysis buffer followed by trituration. Cell lysates were cleared by centrifugation at 6000 x g for 5 min at 4 °C. Protein concentrations of the lysates were determined by the Bradford method (23). Activated Rac and RhoA were isolated by the incubation of 1 µg of protein sample with 20 µg of PAK-GST beads or Rhotekin-GST beads, respectively, for 1 h at 4 °C. The bead-protein complexes were washed with lysis buffer and resuspended in 10 µl of 1.5X Laemmli sample buffer but were not boiled. The samples were separated on 12% polyacrylamide gels and transferred to polyvinylidene difluoride membranes and then probed with antibodies recognizing Rac or RhoA. Aliquots of the cellular lysates were probed to monitor total cellular levels of the small G-proteins. Band intensities were quantified using a Bio-Rad VersaDoc image analysis hardware/software package.

**RhoGDI-Rac Interaction**—THP-1 cells (10 x 10⁶) were plated into 6-well plates. The cells were treated with simvastatin overnight. Cells were lysed with a 1% Triton lysis buffer followed by sonication on ice for 10 s. Lysates were cleared by centrifugation for 15 min at 16,000 x g at 4 °C, and protein concentrations were determined using the Bradford method (23). Aliquots of cellular lysates (1 mg) were combined with 30 µl of protein A beads and 2 µg of anti-RhoGDI antibody in a final volume of 500 µl. The samples were rotated for 2 h at 4 °C and then washed extensively with lysis buffer, resuspended in 1.5X Laemmli sample buffer, and boiled. Standard Western blotting protocols were used to separate and transfer samples to polyvinylidene difluoride membranes and then probed with anti-Rac antibodies to monitor the association of Rac with RhoGDI. Protein loading was evaluated by reprobing the blot with anti-RhoGDI antibodies. Band intensities were quantified using a Bio-Rad VersaDoc image analysis hardware/software package.

**Rac Membrane Localization**—THP-1 cells (10 x 10⁶) were plated into 6-well plates and then treated with simvastatin overnight at 37 °C and 5% CO₂. We adapted the cellular fractionation protocol from Zhao et al. (24). Briefly, following statin treatment for 18 h the cells were lysed by incubation in relaxation buffer (100 mM KCl, 3 mM NaCl, 3.5 mM MgCl₂, 1.25 mM EGTA, and 10 mM PIPES, pH 7.3) on ice for 15 min followed by 10 s of sonication. Cells were cleared by centrifugation at 500 x g for 5 min at 4 °C. The resulting supernatant was loaded into plastic ultracentrifuge tubes and centrifuged for 1 h at 110,000 x g at 4 °C in a Beckman Coulter SW50.1 rotor. The resulting supernatant was removed and saved as the “cytosolic” fraction, and the membrane pellet was then resuspended in relaxation buffer. Standard Western blotting procedures were used to separate the fractions and transfer them to polyvinylidene difluoride membranes where they were probed with anti-Rac antibodies to determine the relative amount of Rac in each compartment. Membrane fractionation was assessed by stripping the blots and reprobing with the membrane marker flotillin. Band intensities were quantified using a Bio-Rad VersaDoc image analysis hardware/software package.
Statins modulate BV-2 cell morphology. A, BV-2 cells (1 × 10⁶) were treated with increasing concentrations of simvastatin (Sim) or lovastatin (Lov) for 18 h. Cells were then fixed with 4% paraformaldehyde and labeled with rhodamine phalloidin. Images were acquired at 40× magnification. These results are representative of three separate experiments. B, BV-2 cells (1 × 10⁶) were treated with simvastatin in the presence or absence of geranylgeranyl pyrophosphate for 18 h. Cells were visualized as described above, and images were acquired at 20× magnification. The lower sections are enlargements of parts of the upper sections. These results are representative of two separate experiments.

ties were quantified using a Bio-Rad VersaDoc image analysis hardware/software package. Mean values ± S.E. were calculated, and statistical differences were determined using a one-way analysis of variance. A Tukey-Kramer post test was used to determine p values.

RESULTS

Statins Act to Inhibit Small G-Protein Actions

Statin Treatment Alters Cell Morphology—We observed that BV-2 microglia exposed to statins exhibit altered cellular morphology. To explore these changes, BV-2 microglia were treated with simvastatin or lovastatin for 18 h. Following this treatment period the cells were fixed and stained with phalloidin to visualize actin filaments. At statin concentrations as low as 250 nM the BV-2 cells begin to lose their processes and become rounded (Fig. 1A). Co-treatment of BV-2 cells with simvastatin and increasing concentrations of the isoprenoid GGpp prevented the retraction of cellular processes (Fig. 1B). These results suggest that the morphological changes are due to the statin-mediated inhibition of isoprenylation. The actin-based cytoskeleton is regulated through action of the Rho family of small G-proteins and, thus, statin-mediated change in cellular morphology is likely due to the impaired Rho family function necessary to maintain cytoskeletal processes and extensions (4).

Statins Inhibit Aβ-induced Phagocytosis—The capacity of statin treatment to alter cellular morphology led us to investigate the biological effects of statins on microglial function. Exposure of microglia to fAβ results in the induction of phagocytosis through a Rac-dependent mechanism and thus should be inhibited by statin treatment through inhibition of this GTPase (20). BV-2 microglial cells were incubated with increasing concentration of statins for ~18 h. Following pretreatment with statin, cells were exposed to 5 μM fAβ-(1–42) or 60 μM fAβ-(25–35) for 30 min. The percentage of phagocytic cells was then determined. Both simvastatin and lovastatin reduced fAβ-(1–42)-stimulated phagocytosis to basal levels at concentrations as low as 250 nM (Fig. 2, A and B). Similar results were observed when Aβ-(25–35) was used to drive phagocytosis (Fig. 2, C and D). We detected inhibition of fAβ-induced phagocytosis at concentration of simvastatin as low as 100 nM (Fig. 2G).

Supplementing the medium of statin-treated cells with mevalonic acid, the product of HMG-CoA reductase, reversed the statin-mediated inhibition of phagocytosis (Fig. 2E). Furthermore, prevention of isoprenylation with the geranylgeranyl transferase inhibitor GTI-286 mimicked the anti-phagocytic actions of statins (Fig. 2F). These data demonstrate that statins disrupt Rac function via perturbation of isoprenylation, inhibiting Aβ-stimulated phagocytosis.

Statins Increase GTP Loading of Small G-proteins

The statin-mediated inhibition of phagocytosis led us to directly test their effects on Rac activation in THP-1 cells. The THP-1 cells have proven valuable in examining intracellular signaling mechanisms, as they are non-adherent and thus have low background levels of integrin-mediated signaling. Rac is an important regulator of the actin-based cytoskeleton, and these non-adherent cells exhibit low basal levels of Rac activity, allowing facile detection of statin-mediated changes. We tested whether simvastatin altered GTP loading by using PAK-GST beads to isolate active, GTP-loaded Rac. THP-1 cells were treated with statins for 18 h followed by isolation of GTP-Rac. Surprisingly, we found that treatment with both simvastatin and lovastatin dramatically increased the GTP loading of Rac (Fig. 3A). Following statin treatment, Rac was observed as a doublet by Western blot. The lower mobility band corresponded to non-prenylated Rac species (25–27). We were also able to demonstrate that the GTP loading of RhoA was increased by treatment with statins (Fig. 3B). We observed a similar statin-stimulated increase in Rac GTP loading in BV-2 microglia (Fig. 3C). This response is not unique to myeloid lineage cells, as statin-induced Rac GTP loading was also detected in N2a mouse neuroblastoma cells (Fig. 3D) and PC12 pheochromocytoma cells (Fig. 3E). Thus, the ability of statin treatment to provoke the conversion of Rac to its GTP-bound form is common to several cell types, including both inflammatory and non-inflammatory cell systems.

In a number of experiments we found that statin treatment resulted in elevation of total cellular Rac levels, which is most evident in N2a cells. This is likely to be the result of impaired degradation of the unmodified proteins as reported by Stamatakis et al. (25).

We next investigated the effects of supplementing the medium with various members of the cholesterol biosynthetic pathway to establish whether this effect was a consequence of the reduction of cholesterol or other intermediates in its biosynthetic pathway on statin-mediated increase in Rac GTP loading. THP-1 cells were treated with simvastatin with or without the addition of increasing concentrations of cholesterol, and the statin-induced GTP loading of Rac was found to be unchanged (Fig. 4A). Mevalonic acid supplementation of simvastatin-treated THP-1 cells was found to be associated with decreased levels of GTP-loaded Rac (Fig. 4B). Mevalonic acid supplementation also eliminated the slower migrating non-prenylated Rac species, indicating that it relieved the inhibition on isoprenylation. Adding GGpp, the isoprenoid employed for the modification of Rac (3), to the medium of statin-treated cells prevented Rac GTP loading in response to statin treatment.
Finally, prevention of GGpp isoprenylation by treatment with the geranylgeranyl transferase inhibitor GGTI-286 increased GTP-loaded Rac, reproducing the effects of statins (Fig. 4C). These data establish that statin-mediated blockade of isoprenylation paradoxically leads to increased Rac GTP loading.

Statins Inhibit the Interactions between Rac and Its Negative Regulator RhoGDI

To elucidate the mechanisms underlying the statin-mediated increase in GTP-loaded Rac, we investigated the effects of statins on the interaction between Rac and its regulator RhoGDI. Rho family G-pro-
teins such as Rac are subject to negative regulation by the cytosolic inhibitory protein RhoGDI (28). Under resting conditions, GDP-loaded Rac localized in the cytosol bound to RhoGDI, which prevents GDP dissociation and therefore suppresses activity. Upon stimulation the Rac-RhoGDI complex dissociates, allowing Rac to interact with its guanine nucleotide exchange factors, become GTP-loaded (active), and translocate to the plasma membrane to interact with its effectors. Importantly, the interaction between Rac and RhoGDI is mediated by both protein-protein interactions and protein-lipid interactions (29). The C-terminal geranylgeranyl isoprenyl moiety of Rac binds to a hydrophobic region on RhoGDI, and lack of the isoprenyl modification prevents this interaction (30). We hypothesized that statin prevention of Rac isoprenylation would disrupt the interaction with RhoGDI and account for the enhanced GTP loading. We tested this hypothesis by monitoring the interaction between Rac and RhoGDI. Following 18 h of statin treatment, RhoGDI was immunoprecipitated from THP-1 cells, and the resulting Western blots were probed with anti-Rac antibodies. Statin treatment inhibited the association of Rac with RhoGDI (Fig. 5). This result is representative of three independent experiments that, on average, lowered the amount of Rac isolated in with RhoGDI by at least 40%.

**DISCUSSION**

The primary action of HMG-CoA reductase inhibitors is the inhibition of cholesterol synthesis, which leads to reduction of plasma cholesterol levels (1). Recently these compounds have been shown to have pleiotropic effects that are unrelated to cholesterol reduction (2). Specifically, statins have been shown to have robust anti-inflammatory actions. These effects have been attributed to the prevention of isoprenylation and the subsequent disruption of small G-protein functions (2). Although the pleiotropic effects of statins have been demonstrated to include attenuation of inflammation in the vasculature, their mechanisms of action in the central nervous system are just beginning to be explored (2, 31, 32). We and others have reported the ability of statins to down-regulate inflammatory reactions in various models of central nervous system diseases including AD and multiple sclerosis (9, 16, 33, 34). Indeed, epidemiological studies suggest that statin treatment reduces the risk of developing AD by as much as 70% (12–14); however, a definitive linkage will only be established through ongoing clinical trials of the statins in AD patients. In addition, recent prospective studies have demonstrated statin-mediated reductions in soluble Aβ levels and improvements in cognitive function (15, 35–38). However, recently statins have been reported to increase the microglial activation in hippocampal slice cultures (39). These results demonstrate the complex
actions of these drugs and indicate the critical need to better understand their mechanisms of action.

The AD brain is characterized by the deposition of Aβ-containing plaques accompanied by a robust microglia-mediated inflammatory response that exacerbates and accelerates the primary disease process (40). Furthermore, microglia-derived cytokines have been shown to contribute to the pathogenesis of the disease, and the plaque burden correlates with cytokine levels (22, 41, 42). We have postulated that the protection statins provided to patient populations at risk for AD may arise, in part, from their capacity to inhibit inflammation in the brain (16). We demonstrated that statin treatment inhibited the ability of microglia to mount a pro-inflammatory response following Aβ stimulation by inhibiting cytokine production, thereby blocking inducible nitric oxide synthase induction and the activation of NADPH oxidase and preventing the release of reactive oxygen species. These effects were shown to be a consequence of reduced isoprenoid levels and secondary to disruption of small G-protein function. We have extended these observations by demonstrating that statin treatment, via depletion of isoprenoid precursors, inhibits phagocytosis in response to fibrillar Aβ. We propose that the inhibition of cytokine release due to statin treatment is beneficial, but the effect of statin-mediated blockade of phagocytosis requires careful interpretation. Microglial phagocytic activity has been suggested to mediate the normal clearance of as well as that observed in immunization models of AD (43–45), and, therefore, its inhibition may have detrimental consequences. Although epidemiological studies support the overall beneficial actions of statins, the specific in vivo effects that these drugs have on inflammation and phagocytosis need to be clarified.

Our findings are consistent with other reports demonstrating Rho GTPase dysfunction following statin treatment, but few have addressed the underlying mechanism of action (46–49). One of the most striking findings in this study was the recognition that statin treatment strongly stimulated the GTP loading of Rac and RhoA. This is paradoxical, as GTP loading is classically taken as a measure of GTPase activation, yet these species are functionally inactive. It should be noted that this effect differs from two reports showing that statins negatively regulate the GTP loading of RhoA in monocytes (50) and MT-2 cells (51), but it is consistent with a brief report by Vecchione and Brandes (52). We have now extended these findings by exploring the mechanistic basis of this phenomenon and found that statin-mediated inhibition of isoprenylation prevented Rac from interacting with its primary cellular regulator.
protein, RhoGDI. RhoGDI negatively regulates the actions of Rho family members by sequestering them in the cytosol and preventing the release of GDP (28). GDIs interact with Rho proteins via both protein-protein and protein-lipid interactions (29). Furthermore, interactions between the C-terminal isoprenyl modification of Rho and a hydrophobic region of RhoGDI have been reported as the dominant link between the two proteins (30). We report that statin treatment and the subsequent inhibition of isoprenylation negatively affects Rho family function by inhibiting the interactions with RhoGDI. We also contend that prevention of this interaction may account for increased amounts of GTP-loaded Rac. These effects were not only observed in BV-2 microglia and THP-1 monocytes but also in N2a neuroblastoma cells and PC12 pheochromocytoma cells, demonstrating a common effect in multiple cell types.

These findings are consistent with other studies investigating the activities of Rac1 and its splice variant Rac1b that have demonstrated the regulatory role of RhoGDI (53). Rac1b makes up a small fraction of total Rac in colorectal tumor cells; however, the amount of activated Rac1b can exceed that of Rac1. The principal molecular difference that accounts for this observation is the ability of Rac to readily bind and dissociate with RhoGDI, whereas Rac1b does not. Thus, the inability to interact with RhoGDI results in elevated levels of GTP-loaded Rac1b. We argue that statin-induced lack of isoprenylation leads to Rac proteins that, like Rac1b, are incapable of interacting with RhoGDI, which results in elevated levels of GTP-loaded Rac. It is unclear whether the non-prenylated G-proteins spontaneously exchange GDP for GTP in the absence of their interaction with RhoGDI or if they also require the catalytic activity of guanine nucleotide exchange factors. These findings also demonstrate that the unprenylated species accumulates in the cells, and this may account for our observation that elevated levels of Rac were frequently observed following statin treatment.

Although we propose that the lack of interaction with RhoGDI explains the increased levels of activated GTPases, the inhibition of Rho family-mediated functions arises from the prevention of their translocation to the plasma membrane. Work by Laufs and Liao (54) has demonstrated that the lack of isoprenylation prevents RhoA and RhoB translocation to the plasma membrane in endothelial cells. Our experiments demonstrate a similar effect in microglia with Rac. Translocation to the membrane is critical for activation of downstream effectors including the NADPH oxidase, PAK, the Wiskott-Aldrich syndrome protein, and POR1 (30, 55, 56).

These findings provide an alternative mechanism for the statin-mediated reduction in AD risk. It was initially argued that the protective actions were a consequence of reducing neuronal membrane cholesterol levels with a subsequent inhibition of amyloidogenic Aβ production (57–60). Depletion of cholesterol from cellular membranes decreases production of Aβ mediated by the β- and γ-secretases while increasing the non-amyloidogenic α-secretase-mediated processing of APP (61–64). Others have reported the statins modulate APP processing by distinct cholesterol-dependent and -independent mechanisms (65, 66). Specifically, reduction of isoprenoids has recently been shown to disrupt Aβ production and increase α-secretase activity through the inhibition of Rho kinase. We propose that, in addition to these beneficial effects on neuronal APP processing, statins provide protection by blocking the microglia-mediated inflammatory response. Statin treatment blocks inflammation by preventing the isoprenylation of Rho GTPases. Although we report that this increases the levels of GTP-loaded Rac and Rho, ultimately the lack of the isoprenyl modification prevents translo-

**Figure 5.** Statins prevent RhoGDI-Rac interactions. A, THP-1 cells (2 × 10⁶) were treated with increasing concentrations of simvastatin for 18 h. Cells were lysed, and RhoGDI-Rac complexes were immunoprecipitated with anti-RhoGDI antibodies. Isolated complexes were separated by Western blot (top row). Protein loading was evaluated by stripping the blots and reprobing with anti-RhoGDI antibodies (bottom row). These results are representative of three separate experiments. B, quantification of Rac band intensities was performed by densitometric analysis of three independent experiments, and the average ± S.E. of the values are shown. Rac levels were normalized to RhoGDI levels in each lane. Veh, vehicle.

**Figure 6.** Statins prevent Rac translocation to the plasma membrane. A, THP-1 cells (2 × 10⁶) were treated with increasing concentrations of simvastatin for 18 h. Using ultracentrifugation, cell lysates were fractionated by into membrane and cytosolic fractions. These fractions were separated by Western blot and probed with anti-Rac antibodies. Blots were stripped and reprobed with the membrane marker flotillin to assess the efficiency of the fractionation. These results are representative of three separate experiments. B, quantification of membrane-associated Rac band intensities was performed by densitometric analysis of three independent experiments, and the average ± S.E. of the values are shown. Membrane-associated Rac levels were normalized to flotillin levels in each lane. *, p < 0.05; Veh, vehicle.
cation to the plasma membrane and results in inhibition of GTPase functions. Statins provide protection from AD by two distinct mechanisms. Although they down-regulate neuronal APP processing and Aβ production, they simultaneously attenuate microglia-mediated inflammation.

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