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Lovastatin Enhances Clearance of Apoptotic Cells (Efferocytosis) with Implications for Chronic Obstructive Pulmonary Disease 1

Konosuke Morimoto,* William J. Janssen,† Michael B. Fessler,† Kathleen A. McPhillips,‡ Valeria M. Borges,§ Russell P. Bowler,† Yi-Qun Xiao,‡ Jennifer A. Kench,‡ Peter M. Henson,‡ and R. William Vandivier2*

Statins are potent, cholesterol-lowering agents with newly recognized, broad anti-inflammatory properties (1). For example, statins suppress the innate immune response in vitro by inhibiting neutrophil migration (2), oxidative stress (3), NF-κB activation (4), proinflammatory mediator release (5, 6), expression of matrix metalloproteinases (7–9), and by increasing expression of constitutive NO synthase (10), peroxisome proliferator-activated receptor (PPAR)γ (11), PPARγ (4), and TGFβ1 (12). Statins also suppress the adaptive immune response by inhibiting IFN-γ-inducible MHC class II expression (13), decreasing expression of CD40/CD40L (14), and by direct blockade of LFA-1 (15). These pleiotropic, anti-inflammatory effects have important therapeutic implications, because 1) statins effectively treat animal models of sepsis, rheumatoid arthritis, acute lung injury, asthma, and emphysema (2, 16–19), and 2) clinically, statins have a promising therapeutic role in the acute coronary syndrome, stabilization of carotid artery plaques, sepsis syndrome, lung allograft rejection, and rheumatoid arthritis (7, 20–24). Therefore, statins appear to be emerging as a new class of immunomodulators, surpassing their originally envisaged role as cholesterol lowering drugs.

The immunomodulatory effects of statins are largely cholesterol independent; instead, they appear to depend upon the ability of statins to posttranslationally modify an extensive array of intracellular signaling molecules, including the Rho family of GTPases (1). Rho GTPases (e.g., RhoA, Rac-1, and Cdc42) are molecular switches, which for function, depend upon the covalent attachment of lipid adducts (prenylation) that direct membrane insertion, localization, and protein:protein interaction (25, 26). Statins regulate prenylation of Rho GTPases by blocking 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase-dependent manner. These findings indicate that statins enhance efferocytosis in vitro and in vivo, and suggest that they may play an important therapeutic role in diseases where efferocytosis is impaired and inflammation is dysregulated.

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because it is defective in systemic lupus erythematosus, rheumatoid arthritis, cystic fibrosis, bronchiectasis, asthma, and chronic obstructive pulmonary disease (COPD) (31–37). The regulation of efferocytosis is tightly controlled by the Rho family GTPTases, in that RhoA inhibits (38–40) and Rac-1/Cdc42/RhoG promotes (41–43) the process. Because statins robustly inhibit RhoA, a negative regulator of efferocytosis, we hypothesized that statins would enhance efferocytosis, and sought to determine whether statins might have therapeutic potential in COPD, a disease of impaired efferocytosis (31, 32).

Indeed, our data demonstrate that statins are potent inducers of efferocytosis in vitro and in vivo in an HMG CoA-reductase-dependent manner. Statins appear to exert their effect, not by suppressing cholesterol, but by disproportionately suppressing the pre-nylation and membrane localization of RhoA. Finally, these data may have therapeutic implications for the treatment of chronic inflammatory diseases of the lung, because statins augment efferocytosis in the naïve murine lung and in alveolar macrophages obtained from patients with COPD.

Materials and Methods

Human subjects

The study was approved by, and performed in accordance with, the ethical standards of the institutional review board on human experimentation at National Jewish Medical and Research Center. Written informed consent was obtained from each subject.

Experimental animals

Mice were housed and studied under institutional animal care and use committee-approved protocols in the animal facility of National Jewish Medical and Research Center. Experiments were performed on 8- to 12-wk-old, age-matched, female ICR mice (Harlan Sprague Dawley).

Primary cell isolation and culture

Human monocyte-derived macrophages (HMDMs) and neutrophils were isolated and prepared from normal blood, as previously described (44). HMDMs were cultured in X-vivo medium (Cambrex BioScience) with 10% pooled human serum at 37°C in 10% CO2 for 7 days before use. HMDMs were pretreated with 0–10 μM lovastatin for 4–12 h before experimentation. Phagocytosis assays were then performed in the presence and absence of the following reagents at the indicated concentrations: PI, as described (49). Each condition was tested in duplicate and a minimum of 400 HMDMs were counted per condition. In all cases, during analysis, the reader was blinded to the sample identification.

HMDMs were pretreated with 0–10 μM lovastatin for 4–12 h before experimentation. Phagocytosis assays were then performed in the presence and absence of the following reagents at the indicated concentrations: PI, as described (49). Each condition was tested in duplicate and a minimum of 400 HMDMs were counted per condition. In all cases, during analysis, the reader was blinded to the sample identification.

In vitro phagocytosis assays

Phagocytic assays were performed on day 7 HMDMs, as previously described (31). Briefly, apoptotic human neutrophils were added to HMDMs at a 5:1 ratio (apoptotic cell to HMDM) and incubated at 37°C in 10% CO2 for 40 min in 500 μl of X-Vivo medium. HMDMs were washed gently with cold PBS to remove unengested cells, fixed, and stained with a modified Wright-Giemsa (Fisher Scientific) Phagocytosis was determined by visual inspection of samples and was expressed as the phagocytic index (PI), as described (49). Each condition was tested in duplicate and a minimum of 400 HMDMs were counted per condition. In all cases, during analysis, the reader was blinded to the sample identification.

Lovastatin activation

Lovastatin (Sigma-Aldrich) was converted to its active form by dissolving 25 mg of the lactone form in 500 μl of 100% ethanol, heated to 50°C, alkalized by adding 250 μl of 0.6 M NaOH, and incubated at 50°C for 2 h. After incubation, the solution was neutralized with 0.4 M HCl at pH 7.5. Aliquots of stock solution were stored frozen at −20°C until used (47, 48).

In vivo phagocytosis assays

To test the effect of lovastatin and mevalonate on uptake of apoptotic cells, mice were divided into four groups and treated as follows: 1) control group, treated with vehicle (0.5% carboxymethylcellulose sodium, 0.9% sodium chloride, 0.4% polysorbate 80, 0.9% benzyl alcohol in deionized water) by gavage and PBS by i.p. injection; 2) lovastatin group, treated with lovastatin (10 mg/kg) in vehicle by gavage and PBS by i.p. injection; 3) mevalonate group, treated with vehicle by gavage and 10 mg/kg mevalonate by i.p. injection; 4) lovastatin/mevalonate group, treated with both of lovastatin (10 mg/kg) in vehicle by gavage and mevalonate (10 mg/kg) by i.p. injection. Mice were treated three times, spaced within 30 h, before the time of experimentation.

Apoptotic thymocytes were instilled intratracheally as previously described (45). Briefly, mice were anesthetized with Avertin, following which 10 × 106 Cell Tracker Red-stained (Molecular Probes) apoptotic thymocytes, suspended in 50 μl of PBS, were instilled intratracheally using a modified animal feeding needle (Fisher Scientific). Forty minutes later, whole lung bronchoalveolar lavage was performed with a total of 5 ml of ice-cold PBS. Lavage cells were fixed and stained with modified Wright-Giemsa (Fisher Scientific), Phagocytosis was determined by visual inspection of samples (see Fig. 5A), as previously described (45, 50), and was expressed as a PI. A minimum of 400 alveolar macrophages were counted blindly.

Recovered apoptotic thymocytes were determined as follows. Total lavage cells (including erythrocytes) were counted using a hemacytometer. The percentage of free thymocytes in the lavage was determined by FACS analysis (Fig. 5B). Macrophages were excluded, based upon macrophage forward-side scatter characteristics, F4/80 staining, and autofluorescence. Total recovered thymocytes were calculated by multiplying total lavage cells by the percentage of Cell Tracker Red-positive cells.

FACS analysis

FACS analysis was done as previously described (31). Briefly, HMDMs were suspended in HBBS containing 2% FCS (Gemini Bio-Products), blocked with human serum, except cells for FcγR staining, incubated with 5 μg of the primary Ab for 30 min on ice, washed twice, then incubated with the secondary Ab (1/50 dilution) on ice for 30 min. Washed macrophages were analyzed on a FACSscan cytometer using CellQuest Pro (BD Biosciences) and FloJo (Tree Star) software.

Abs used in FACS analysis were as follows: BD Biosciences/BD Pharmingen Abs include mouse monoclonal anti-human CD36 IgM, anti-human integrin β2/CD11b, and human integrin α7/CD107 (FcγRIIIa) IgG2b, and mouse monoclonal IgG1, IgG2a, IgG2b, IgM, κ isotype controls (Chemicon International). Abs include mouse monoclonal anti-human integrin αvß6 IgG1 (Affinity BioReagent) Abs include chicken

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polyclonal anti-human calreticulin IgY (American Diagnostica) Abs in-clude mouse monoclonal anti-human α-chain CD91 IgG1. Mouse mono-
clonal anti-human PS recognition structure IgM (217) was prepared in this 
laboratory as previously described (27). Jackson ImmunoResearch Labor-
atories Abs include chicken IgY isotype control, Cy-3 goat IgG anti-
mouse IgM, and Cy-3 goat IgG anti-mouse IgG, and Cy-3 goat anti-chicken IgY.

Western blotting
HMDM membrane fractions were resuspended and separated on SDS-PAGE as described previously (51, 52). Briefly, HMDMs plated on 10-cm tissue-
culture dish were harvested and resuspended in PBS. Pelleted cells were 
lysed by Reporter Lysis Buffer (Promega) and by repeated freeze-thaw 
cycles. Cell lysates were spun down at 3,000 rpm for 10 m. Supernatants 
were collected and spun-down at 10,000 × g for 45 m. The pellet was 
soyribilized in lysate buffer (20 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM 
DTT, 1% Triton X-100). Samples were run on a 7.5% SDS-PAGE gel, 
transferred to nitrocellulose, blocked with 3% milk, and sequentially in-
cubated with primary and secondary Abs, Total protein was measured by 
the BCA Protein Assay kit (Pierce) and equivalent amounts were loaded 
into gels. Immunoblotting was performed using mAbs against RhoA, 
RhoB, and Rac-1 (clone 23A8, 1 μg/ml; Upstate Biotechnology) and an immunode-
tection was accomplished using a mouse anti-mouse HRP-conjugated secondary Ab 
(1/10,000). Membranes were developed using Amersham ECL system 
(Amersham Biosciences).

Statistics
The means were analyzed using ANOVA for multiple comparisons; when 
ANOVA indicated significance, the Dunnett’s method was used to com-
pare groups with an internal control. For all other experiments in which two 
conditions were being compared, a Student’s t test assuming equal variance 
was used. All data were analyzed using JMP (version 3) Statistical Soft-
ware for Macintosh (SAS Institute) and are presented ± SEM.

Results
Lovastatin increases uptake of apoptotic cells (effectorcytosis)
We tested the effect of statins on effectorcytosis by incubating 
HMDMs with lovastatin for 6–48 h. Lovastatin (5 μM) increased 
effectorcytosis as early as 6 h (Fig. 1B). However, at 24 h, lovastatin 
increased effectorcytosis in a dose-dependent fashion, exerting a 
maximum effect at 10 μM. In contrast, lovastatin had no effect on 
binding of apoptotic cells to HMDMs (Fig. 1C). Lovastatin (1 μM) 
also increased effectorcytosis in HMDMs after 5 days of treatment 
compared with untreated cells, suggesting that the effect was pro-
longed (Fig. 1D).

The effect of lovastatin on effectorcytosis is dependent on 
HMG-CoA reductase
Most, but not all, statin effects are related to their ability to com-
petitively block HMG-CoA reductase, and thereby decrease pro-
duction of multiple intermediates in the cholesterol biosynthetic 
pathway. Mevalonate is the initial product of HMG-CoA reductase 
(Fig. 1A) and mevalonate levels are decreased by statin therapy. 
We performed “rescue” experiments with mevalonate to determine 
whether lovastatin was acting through an HMG-CoA reductase-
dependent pathway (Fig. 1E). Mevalonate reversed the ability of 
lovastatin to potentiate effectorcytosis, confirming HMG-CoA re-
ductase dependency. In contrast, lovastatin suppressed phagocy-
tosis of IgG-opsonized erythrocytes through the FcγR, as has re-
cently been shown (53, 54), and this also appeared to be dependent 
on HMG-CoA reductase (Fig. 1F).

Lovastatin does not affect expression of effectorcytosis receptors
We considered that lovastatin may exert its positive effect on ef-

cectorcytosis by increasing expression of key uptake receptors, es-

specially because statins were recently reported to increase the ex-

pression of HMDM effectorcytosis receptors (i.e., α5β3, CD36, CD44, CD14) or 
the FcγRIIa (Fig. 2). Lovastatin also had no effect on staining by mAb 217. The protein 
target of mAb 217 was originally thought to be the phosphatidylyl-
sine receptor, but is now not known.

Lovastatin-enhanced effectorcytosis is dependent on prenylation
Statins regulate the posttranslational modification of hundreds of 
proteins by controlling the production of key substrates of protein 
prenylation, such as FPP and GGPP. Because mevalonate is an 
upstream precursor of both FPP and GGPP, statins ultimately de-
crease their levels as well. We performed dose-response rescue 
experiments with GGPP and FPP, and found that both reversed 
lovastatin-enhanced effectorcytosis, albeit with different potencies 
(Fig. 3, A and B). Even though these experiments suggested a role 
for both farnesylation and geranylgeranylation, they did not rule 
out the possibility that the effect of FPP was due to its conversion 
to GGPP and augmentation of geranylgeranylation. This is an im-
portant issue because the known Rho GTPase regulators of ef-

cectorcytosis are only geranylgeranylated. Incomplete inhibition of 
HMG-CoA reductase could potentially allow low level synthesis 
of the 5-carbon isopentenyl pyrophosphate (IPP), which normally 
condenses with 15-carbon FPP to produce 20-carbon GGPP (Fig. 1A), 
thereby creating a pathway for FPP repletion to influence effectorcytosis.

Specific inhibitors of geranylgeranyltransferase I (GGT-2133; 
IC50 = 38 nM) and farnesyltransferase (FTI-276; IC50 = 0.5 nM) 
were used to further examine the role of these separate prenylation 
pathways on effectorcytosis. Both inhibitors enhanced effectorcytosis, 
but the effect of FTI-276 appeared to be more modest than GGTI-
2133 (Fig. 3, C and D). It is important to note that farnesyltrans-
ferase inhibitors may increase the production of geranylgera-
lated proteins, presumably by shunting excess FPP to GGPP (56). 
This suggests that farnesyltransferase inhibitors may not solely act 
by decreasing farnesylated proteins, but instead they may also 
change the balance in favor of geranylgeranylated proteins. Consis-
tent with this possibility, combinations of GGTI-2133 and FTI-
276 did not enhance effectorcytosis more than lovastatin or GGTI-
2133 alone (data not shown). Therefore, effectorcytosis appears to be 
regulated by intracellular signaling molecules that are geranylgera-
ylated, and possibly by those that are farnesylated.

Lovastatin suppresses membrane-bound RhoA greater than 
Rac-1
Statins inhibit prenylation and membrane localization of a variety 
of Rho GTPases, including positive (Rac-1) and negative (RhoA) 
regulators of effectorcytosis. Yet, in HMDMs, lovastatin consist-
tently enhanced effectorcytosis, implying that lovastatin may exert a 
prolonged, disproportionate effect on the prenylation and mem-
brane localization of RhoA. To address this hypothesis, HMDMs 
were treated with and without lovastatin for 24 h, and membrane 
fractions were assessed for RhoA and Rac-1 staining by Western 
blot. Lovastatin decreased membrane-bound RhoA greater than 
Rac-1 (Fig. 4, A and B), suggesting a mechanism for the positive 
effect of lovastatin on effectorcytosis.

Lovastatin reverses impaired effectorcytosis by lysophosphatidic 
acid (LPA), a potent RhoA activator
We next used the potent RhoA activator, LPA to address whether 
lovastatin could reverse impaired effectorcytosis in vitro (57). LPA-
suppressed effectorcytosis by HMDMs, and this suppression was 
preserved by lovastatin (Fig. 4C), suggesting that lovastatin, or for 
that matter other Rho pathway inhibitors, could play a therapeutic

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role in diseases where suppression of efferocytosis contributes to disease pathogenesis.

Lovastatin increases efferocytosis in vivo

We tested whether statins could enhance efferocytosis by treating mice with lovastatin (10 mg/kg) three times over 30 h. Ten million, Cell Tracker Red-labeled apoptotic murine thymocytes were then instilled intratracheally and clearance was assessed. This model has previously been used to evaluate efferocytosis by macrophages and epithelial cells in vivo (45, 50). Defective efferocytosis is suggested by either decreased uptake into alveolar macrophages (i.e., decreased PI; Fig. 5A), or by increased recovery of apoptotic cells in the bronchoalveolar lavage (Fig. 5B). Lovastatin modestly increased efferocytosis in the naive murine lung as measured by an increase in the PI (Fig. 5C) and by a decrease in the recovery of apoptotic thymocytes (Fig. 5D).

To examine whether the action of lovastatin on efferocytosis in vivo was HMG-CoA reductase dependent, mice were treated with
Lovastatin (10 mg/kg), three times over 30 h, in the presence or absence of rescue mevalonate, and clearance of apoptotic thymocytes by alveolar macrophages was assessed. Lovastatin again increased efferocytosis by alveolar macrophages in vivo, and this effect was prevented by mevalonate (Fig. 5E). Together, these results indicate thatLovastatin enhances efferocytosis in the naive murine lung in an HMG-CoA reductase-dependent manner, thus confirming in vitro results.

**Lovastatin enhances efferocytosis by human alveolar macrophages taken from patients with COPD**

Accumulating evidence suggests that efferocytosis is dysregulated in chronic inflammatory lung diseases, such as COPD, and may contribute to disease pathogenesis. For example, several animal models of COPD are associated with increased accumulation (58–61) and impaired removal (45) of apoptotic cells. Likewise, apoptotic cells are increased in COPD lungs (31, 62–65) and efferocytosis is defective in COPD alveolar macrophages ex vivo (32). Therefore, we tested the effect ofLovastatin on efferocytosis by alveolar macrophages isolated from GOLD stage 2 (66) COPD patients (Table I). Lovastatin enhanced efferocytosis in these alveolar macrophages in an HMG-CoA reductase-dependent fashion (Fig. 6). Taken together, these data suggest that statins may have therapeutic potential in diseases, such as COPD, where efferocytosis is suppressed and inflammation is dysregulated.

**Discussion**

Apoptotic cell clearance defects are increasingly recognized in diseases of chronic inflammation (31, 32) and autoimmunity (33, 34, 67, 68), suggesting that effective efferocytosis may be necessary for the maintenance of homeostasis. This concept is based on the role efferocytosis plays in suppressing both the innate (69, 70) and adaptive (68) immune response and in removing autoantigens (71). Interestingly, this concept is not new. Over 100 years ago, Metchnikoff (72) recognized this removal process and its importance to homeostasis, which he termed “physiologic inflammation.” Therefore, therapies that enhance efferocytosis may offer a unique therapeutic benefit, especially if impaired efferocytosis is a determinant of disease pathogenesis. Our findings indicate thatLovastatin enhances efferocytosis in vitro and in vivo. Lovastatin’s effect on efferocytosis depends on its ability to inhibit HMG-CoA reductase, decrease prenylation substrates, and to alter the balance of Rho GTPases. Lovastatin may have therapeutic potential in chronic inflammatory diseases with impaired efferocytosis because it reverses impaired efferocytosis in vitro and in vivo, and enhances efferocytosis by alveolar macrophages from patients with COPD.

Up to 2% of expressed cellular proteins are prenylated and over 150 prenylated proteins have been identified (73, 74), suggesting that the effect of statins on efferocytosis is likely to be complex. Our findings indicate thatLovastatin enhanced efferocytosis in vitro, in part, by altering the membrane balance of RhoA and Rac-1, two key regulators of efferocytosis. Geranylgeranyltransferase I prenylates both RhoA and Rac-1, yetLovastatin suppressed membrane localization of RhoA to a greater extent than Rac-1. The reason(s) for this disproportionate effect is unclear, but it suggests that enzyme kinetics favor prenylation of Rac-1 over RhoA. BecauseLovastatin increased efferocytosis after 5 days of treatment, the effect appears to be sustained. Possible explanations for this
Lovastatin may enhance efferocytosis by suppressing membrane-bound RhoA. A and B, HMDMs were treated with and without lovastatin for 24 h and assessed for membrane-bound RhoA and Rac-1 by Western blot and densitometry. A, Representative Western blots from five separate experiments are shown. CD71 was used as a membrane marker and control for equal loading. B, Lovastatin significantly decreased membrane-bound RhoA greater then Rac-1 ($p < 0.05$). C, LPA (10 $\mu$M), a potent RhoA activator, decreased ingestion of apoptotic Jurkat T cells by HMDMs in the absence, but not the presence, of lovastatin. Jurkat T cells were used in these experiments instead of neutrophils because they are ingested more avidly by HMDMs. The mean PI as a percentage of control ± SEM is shown for six replicates per group. Control mean PI: 42.2 ± 10.3. *, Significantly different from control ($p < 0.05$). †, Different from control ($p = 0.051$).

Prolonged effect include: 1) geranylgeranyltransferase I may prenylate Rac-1 more efficiently than RhoA, or 2) prenylation of Rac-1 may be less substrate dependent. Even during lovastatin treatment, prenylation substrates (GGPP or FPP) would be expected to be present in small quantities due to incomplete blockade of HMG-CoA reductase, or due to salvage pathway activity (75). Alternatively, 3) the half-life of prenylated Rac-1 may be longer than prenylated RhoA. This possibility is less likely, because lovastatin enhanced efferocytosis as long as 5 days after treatment. We also noted that the ability of lovastatin to increase efferocytosis by HMDMs waned during days 3 and 4 of treatment, but increased again at day 5. We do not have a clear explanation for this observation, but it was consistent across both concentrations tested.

Our data suggest that farnesylated proteins might negatively regulate efferocytosis, because inhibition of farnesyltransferase modestly increased efferocytosis, and FPP repletion reversed lovastatin-enhanced efferocytosis. RhoB is an attractive candidate for this effect, because it is both farnesylated and geranylgeranylated (76) and it plays a known role in the phagocytosis of Pneumocystis (77). Whether farnesyltransferase inhibitors also shift the balance toward production of other geranylgeranylated proteins that could enhance efferocytosis, like Rac-1, Cdc42, or RhoG (43, 78), remains to be determined.

Growing evidence from animal models indicates that statins may have a role in the treatment of inflammatory lung diseases, including acute lung injury (2), asthma (18), and emphysema (19). Our data supports this notion, because lovastatin increased efferocytosis in the lungs of naive mice. In vivo, lovastatin may enhance efferocytosis by altering the balance of membrane-bound RhoA and Rac-1, as was demonstrated in vitro. Alternatively, in vivo and especially in an inflammatory environment, lovastatin may enhance efferocytosis by suppressing oxidative stress, because oxidative stress inhibits efferocytosis by activating RhoA (K. A. McPhillips, manuscript in preparation). Lovastatin also suppresses matrix metalloproteinase-9 (8), which inhibits efferocytosis in vitro (R. W. Vandivier, unpublished data), and is an important component of lung inflammation. Finally, statins increase PPARγ, which has also been shown to increase efferocytosis (79).

Lovastatin did not enhance efferocytosis by altering apoptotic cell binding or by increasing expression of efferocytosis receptors. In contrast, Ruiz-Velasco et al. (55) found that lovastatin treatment (10 $\mu$M) increased CD36 surface expression and mRNA in human monocytes at 24 h and THP-1 cells at 48 h. The disparity between our findings may relate to intrinsic differences between human monocytes and HMDMs.

Lovastatin suppressed FcyR-mediated phagocytosis in an HMG-CoA reductase-dependent manner, confirming recent reports (53, 54). Interestingly, these authors all concluded that statins suppress FcyR-mediated phagocytosis by inhibiting cholesterol...
biosynthesis, and not by inhibiting prenylation (53, 54). Like effec-
trocytosis, prenylated proteins like Rac-1, Cdc42, and Rab11 are
required for FcγR-mediated phagocytosis (80, 81), and a role for
RhoA has been suggested (82). Therefore, we propose that under
certain conditions statins might also influence FcγR phagocytosis
through modulation of Rho GTPases. The effect of statins on FcγR-mediated phagocytosis is concerning because of its potential
to impair host defense, but ultimately, its importance remains to be
demonstrated in vivo. In a Klebsiella pneumoniae mouse model of
pneumonia, lovastatin delayed bacterial clearance and enhanced
dissemination (2). In contrast, statins have consistently improved
survival in animal and human bacterial sepsis (16, 22, 83), imply-
ing that lovastatin may not augment efferocytosis when used in
specific diseases. For example, cystic fibrosis, bronchiectasis, and
COPD are attractive targets for statin therapy because they are all
associated with accumulation, and defective clearance, of apo-
totic cells (31, 32). Indeed, our data provides “proof of principle” for
this approach because lovastatin increased efferocytosis by human
alveolar macrophages taken from patients with COPD. Lee et al. (19)
have also shown that statin treatment inhibits the development of
cigarette smoke-induced emphysema in rats. However, we
would not suggest that potential therapeutic targets be limited to
these chronic inflammatory lung diseases, because accumulation of
apootic cells and failed efferocytosis has also been implicated in
systemic inflammatory diseases, like glomerulonephritis (34),
rheumatoid arthritis (36), and systemic lupus erythematosus (35),

The ability of statins to enhance efferocytosis suggests a new
mechanism by which statins may modulate acute and chronic in-
flammatory diseases, and may help direct statin clinical trials to
specific diseases. For example, cystic fibrosis, bronchiectasis, and
COPD are attractive targets for statin therapy because they are all
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totic cells (31, 32). Indeed, our data provides “proof of principle” for
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The authors have no financial conflict of interest.

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Disclosures
The authors have no financial conflict of interest.

Table I. Subject demographics

<table>
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<td>n</td>
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<td>Age (years)</td>
<td>57 ± 2.1</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>63.3 ± 8.2</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>80.4 ± 9.9</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>60.2 ± 0.3</td>
</tr>
<tr>
<td>Smoking history (pack-years)</td>
<td>47 ± 15.9</td>
</tr>
</tbody>
</table>

*FEV, Forced expiratory volume in 1 s; FVC, forced vital capacity.

FIGURE 6. Lovastatin reverses impaired efferocytosis in COPD alveo-
lar macrophages ex vivo. Human alveolar macrophages were isolated from
patients with GOLD stage 2 COPD, then incubated with or without love-
statin or mevalonate for 24 h before coculture with apoptotic human neu-
rophils. Lovastatin (Lova; 10 μM) treatment enhanced efferocytosis by
GOLD stage 2 alveolar macrophages (n = 3), but not in the presence of
mevalonate (l-Meva; 50 μM). Control mean PI: 5.5 ± 1.9, *Significantly
different from control (p < 0.05).


