Statin Therapy Reduces the *Mycobacterium tuberculosis* Burden in Human Macrophages and in Mice by Enhancing Autophagy and Phagosome Maturation

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**Background.** Statins are cholesterol-lowering drugs, targeting HMG-CoA reductase, thereby reducing the risk of coronary disorders and hypercholesterolemia. However, they also can influence immunologic responses.

**Methods.** Peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDMs) were isolated from patients with familial hypercholesterolemia (FH) during statin therapy. After infection of cells with *Mycobacterium tuberculosis*, bacterial burden was determined. In vivo, mice were treated with statins before aerosol-based infection with *M. tuberculosis* and were monitored for disease progression.

**Results.** PBMCs and MDMs from patients with FH receiving statin therapy were more resistant to *M. tuberculosis* infection, with reduced bacterial burdens, compared with those of healthy donors. Moreover, statin treatment in experimental murine *M. tuberculosis* infection studies increased host protection, with reduced lung burdens and improved histopathologic findings. Mechanistically, metabolic rescue experiments demonstrated that statins reduce membrane cholesterol levels, particularly by the mevalonate-isoprenoid arm of the sterol pathway. This promoted phagosomal maturation (EEA-1/Lamp-3) and autophagy (LC3-II), as shown by confocal microscopy and Western blot in macrophages. In addition, inhibitors of phagosome and autophagosome maturation reversed the beneficial effect of statins on bacterial growth.

**Conclusion.** These results suggest that statin-mediated reduction in cholesterol levels within phagosomal membranes counteract *M. tuberculosis*-induced inhibition of phagosomal maturation and promote host-induced autophagy, thereby augmenting host protection against tuberculosis.

**Keywords.** Macrophages; Cholesterol; *Mycobacterium tuberculosis*; Mice; Human.

Statins are a family of drugs widely used as inhibitors of cholesterol biosynthesis. It is therefore one of the best-selling drugs worldwide extensively prescribed to reduce morbidity and mortality in patients with coronary disorders and hypercholesterolemia [1]. Statins are competitive inhibitors of HMG-CoA reductase, a rate-limiting enzyme of the cholesterol biosynthesis pathway, which catalyzes conversion of HMG-CoA reductase into mevalonate [2]. It has been suggested that statins have pleiotropic effects, including broad-range immunomodulatory and antiinflammatory properties [3, 4]. Two unique observations that emerged were that statins reduced mortality in patients with bacteremia [5] and multiple organ dysfunction syndrome [6].

The ability of *Mycobacterium tuberculosis* to maintain persistent chronic infection is critically linked to its capacity to use host cholesterol. In addition,
cellular lipids found in foamy macrophages play a crucial role in reactivation of latent tuberculosis [7, 8]. We therefore hypothesized that cholesterol inhibition by statins could potentially alter protective immunity to M. tuberculosis infection and thus may alter disease outcome in the infected host.

During bacterial infection, statin administration in vivo has been reported to control the bacilli burden. For instance, growth of Salmonella enterica was reduced in a murine macrophage cell line and in experimental mice following treatment with lovastatin and atorvastatin, respectively [9]. In addition, mice administered with simvastatin had reduced growth of Chlamydia pneumoniae [10]. However, the mechanism behind the antimicrobial activity of statins remains inconclusive.

In the present study, we demonstrated that peripheral blood mononuclear cells (PBMCs) and monocyte-derived macrophages (MDMs) from patients with familial hypercholesterolemia (FH) who were receiving statin therapy presented increased resistance to M. tuberculosis infection, compared with the cells from healthy non–statin users. Human and murine macrophages subjected to statin treatment in vitro displayed a significant reduction in M. tuberculosis growth. Experimental tuberculosis in statin-treated mice resulted in increased protection against M. tuberculosis infection. Mechanistically, statins increased host-protective functions by countering Mycobacterium-induced inhibition of phagosomal maturation and autophagy.

METHODS

Human Subjects
Patients with FH were recruited by experienced specialists at a referral clinic (Groote Schuur Hospital, Cape Town) following provision of written informed consent. The diagnosis of heterozygous FH was based on clinical criteria (ie, severe low-density lipoprotein [LDL] hypercholesterolemia with tendon xanthomata) and, in all but one patient, was confirmed by genotyping for documented mutation(s) in the LDL receptor. All patients received high doses of atorvastatin for a minimum of 6 months. Patients with FH and healthy controls were sex matched. None of the patients were receiving tuberculosus treatment or had been treated. The experimental protocol and consent (HREC: 400/2009) was approved by the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town.

Isolation, Culture of Monocytes/Macrophages, and M. tuberculosis Infection
Human peripheral blood was collected in Vacutainer cell preparation tubes (BD, Franklin Lakes, NJ) from patients with FH and healthy donors. PBMCs or MDMs were isolated and subsequently infected with M. tuberculosis for 4 hours at 37°C as described in the Supplementary Materials.

Mice
C57BL/6 mice (age, 8–12 weeks) were maintained under specific-pathogen-free conditions in individual ventilated cages. All experiments were performed in accordance with the South African National Guidelines and University of Cape Town of practice for laboratory animal procedures. The protocol (AEC: 012/036) was approved by the Animal Ethics Committee, Faculty of Health Sciences, University of Cape Town.

Statin Treatment and M. tuberculosis Infection in Mice
Mice were treated every other day with 20 mg/kg of either simvastatin (Sigma-Aldrich) or rosuvastatin (AstraZeneca) and phosphate-buffered saline control for 6 weeks. Mice were infected with M. tuberculosis by aerosol exposure and euthanized at the indicated time points for bacterial burden and histopathologic analyses, as described elsewhere [11].

Simvastatin Treatment and M. tuberculosis Infection In Vitro
Bone marrow–derived macrophages (BMDMs) were generated as previously described [12]. A total of 5 × 10⁵ cells were treated with simvastatin (50 µM), with or without mevalonate (100 µM), 3-methyladenine (5 mM), bafilomycin A (10 nM), geranylgeranyl (20 µM), and squalene (20 µM; all from Sigma-Aldrich), for 24 hours. Cells were then infected with M. tuberculosis for 4 hours at 37°C (Supplementary Materials). At the indicated time points, bacterial growth was determined as previously described [13].

Cholesterol Content in Macrophages
Following statin treatment, macrophages were stained for detection of cholesterol, using filipin dye (Sigma-Aldrich) as previously described [14]. Quantification of filipin intensity was performed using LSM software. Alternatively, cholesterol content was analyzed in total macrophage cell lysates, using a cholesterol assay kit (Bioassay system) [15].

Cytotoxicity Assay
Following statin treatment, macrophages, drug-induced cytotoxicity was determined by measuring the reduction of yellow tetrazolium salt (MTT), as described elsewhere [16].

Fluorescence Confocal Microscopy
After statin treatment, macrophages were infected with green fluorescent protein (GFP)–expressing M. tuberculosis for 2 hours at 37°C. Cells were processed for immunofluorescence staining (Supplementary Materials) and images were analyzed using LSM and Matlab software (R2008a).

Western Blot Analysis
After statin treatment for 48 hours, macrophages were infected with M. tuberculosis for 2 hours at 37°C. Cells were washed with warm medium and lysed in ice-cold RIPA buffer containing protease inhibitors. Soluble lysate fractions were collected,
and blots were probed against EEA-1/LAMP-3/LC3-II as described in the Supplementary Materials.

Statistical Analysis
Data are presented as mean values ± standard error of the mean. Statistical analysis was performed using the unpaired Student t test or 1-way analysis of variance with the Dunnett post test, with a P value of ≤ 0.05 considered statistically significant.

RESULTS
Mononuclear Cells and Macrophages Isolated From Patients Receiving Statin Therapy Show Increased Resistance to M. tuberculosis Infection
Patients with FH and high blood cholesterol levels receive daily statin therapy. We therefore hypothesized that macrophages isolated from these patients may influence M. tuberculosis infection. To test this hypothesis, human mononuclear cells from patients with FH and healthy non–statin users were infected with M. tuberculosis. Growth of M. tuberculosis in PBMCs from statin-treated patients with FH had decreased significantly, by 2-fold, 3 days after infection, compared with controls (Figure 1A). Statins had no effect on cell viability (Figure 1B) and uptake (Figure 1C) during infection, excluding possible drug-induced cytotoxicity or variable initial uptake due to statin treatment. Initial M. tuberculosis uptake 4 hours after infection was similar in PBMCs from patients with FH and healthy non–statin users. Furthermore, infection of MDMs from patients with FH receiving statin therapy also resulted in a significant reduction of the M. tuberculosis burden, compared with MDMs from healthy controls (Figure 1D), with no difference in initial uptake 4 hours after infection (Figure 1E). Differentiation of PBMCs into MDMs was analyzed using flow cytometry (data not shown). Together, these results demonstrate that PBMCs and macrophages from patients with FH who are receiving statin therapy have acquired protective immune responses leading to reduced bacterial growth following infection with M. tuberculosis in vitro.

Figure 1. Reduced Mycobacterium tuberculosis growth in human mononuclear cells and macrophages derived from statin-treated patients with familial hypercholesterolemia (FH). A, Mononuclear cells from patients with FH showed decreased growth of M. tuberculosis, compared with healthy donors (*P < .05, by the Student t test; n = 10–15 donors for each group). B, Both groups had no effect on cell viability (n = 8–9 donors for each group). C, Uptake of M. tuberculosis (n = 11 donors for each group). D and E, Macrophages from patients with FH showed reduced growth (*P < .05, by the Student t test; n = 6 donors for each group) and has no effect on uptake of M. tuberculosis (n = 3 donors for each group). Abbreviations: CFU, colony-forming unit; MDM, monocyte-derived macrophage; PBMC, peripheral blood mononuclear cell.
Statins Mediate Increased Host Protection Against M. tuberculosis Infection in Mice

Because statin therapy decreased bacterial growth in human PBMCs and macrophages (Figure 1), we investigated M. tuberculosis infection in a statin-treated experimental mice model. Mice were treated intraperitoneally with simvastatin or rosvastatin (20 mg/kg) and vehicle control every second day for 2 weeks. This was followed by low-dose aerosol-based M. tuberculosis infection to mimic natural human infection. Statin treatment was continued up to 4 weeks after infection, as outlined in Figure 2A. Because of the increased activity of HMG-Co reductase in rodents, the therapeutic dose used to block cholesterol biosynthesis needs to be higher than that in humans (0.1–1 mg/kg) [17]. Both statins showed a protective response, with up to a 10-fold reduction in bacilli burden in the spleens, livers, and lungs of infected mice, compared with untreated control animals, 4 and 8 weeks after infection (Figure 2B). This resulted in significant smaller microabscesses in the lung, as shown by pulmonary histopathologic analysis and quantification of the lesion sizes (Figure 2C). The observed stronger effect of rosuvastatin may be due to the longer half-life (20 hours) compared to simvastatin (2 hours) [18]. Taken together, these results suggest that treatment of M. tuberculosis–infected mice with statins is beneficial, with a decreased bacterial burden in infected organs accompanied by a reduced histopathology.

Reduced Growth of M. tuberculosis in Statin-Treated Murine Macrophages

We next investigated whether treatment of murine macrophages with statins has an effect on the growth of M. tuberculosis that is similar to that shown with human host cells (Figure 1A and 1D). Murine BMDMs were treated with simvastatin and then infected with M. tuberculosis, and intracellular bacterial growth was determined during the course of infection. Treatment resulted in a significant reduction in bacterial numbers, which was observable 1 day after infection and resulted in a 3-fold decrease during the next 2 days, compared with untreated cells (Figure 3A). As observed in human macrophages, simvastatin had no influence on cell viability (Figure 3B) and M. tuberculosis uptake (Figure 3C) in murine macrophages.

To determine whether the effect on bacterial growth was a direct consequence of inhibiting the cholesterol biosynthetic pathway, exogenous mevalonate, a precursor in the biosynthetic pathway of cholesterol downstream of HMG-CoA reductase, was
added to simvastatin-treated cells. Supplementation of mevalonate completely abrogated the simvastatin-mediated decrease in bacterial growth (Figure 3D). This indicated that antimycobacterial growth was due to the inhibition of HMG-CoA reductase, as reflected in the involvement of the mevalonate arm of the sterol pathway. Next, we investigated which specific downstream branch of the mevalonate pathway was involved in antimycobacterial activity during simvastatin treatment. Metabolic rescue experiments were performed that involved downstream metabolites, such as geranylgeraniol, a precursor of the isoprenoid pathway, and squalene, a precursor of the cholesterol pathway. Interestingly, the antimycobacterial activity of simvastatin was partially rescued by the addition of geranylgeraniol or squalene (Figure 3E). Together, these results demonstrate that statins are able to decrease both membrane and total cholesterol in macrophages.

**Figure 3.** Simvastatin (Sim) pretreatment reduces *Mycobacterium tuberculosis* growth in murine macrophages. A, Pretreatment with simvastatin resulted in decreased *M. tuberculosis* growth in macrophages over the course of infection (*P* < .05, **P** < .01, by the Student t test; *n* = 3 experiments). B, Simvastatin has no adverse effect on cell viability, compared with control macrophages. C, Simvastatin has no effect on initial uptake of bacteria 4 hours after infection. D, Effect of simvastatin on *M. tuberculosis* growth was reversed by supplementation of exogenous mevalonate in macrophages. E, Metabolic rescue experiments revealed that simvastatin-mediated decrease in *M. tuberculosis* growth was partially rescued by addition of the intermediates such as squalene (SQ) and geranylgeraniol (GG; ***P*** < .001, by the Student t test; *n* = 3 experiments). Abbreviation: CFU, colony-forming unit.

**Statins Decrease Cholesterol Levels Without Affecting the Phagocytic Capacity of Macrophages**

To further investigate the host-protective mechanism of statins, macrophages were stained with filipin, a fluorescent dye that specifically binds to cell membrane cholesterol. Treatment of macrophages with simvastatin significantly decreased filipin intensity, whereas supplementation of mevalonate during simvastatin treatment restored filipin intensity (Figure 4A). This was confirmed by biochemical extraction of lipids from cell lysates, which was significantly reduced during simvastatin treatment but restored by adding mevalonate (Figure 4B). Together, these results demonstrate that simvastatin is able to decrease both membrane and total cholesterol in macrophages.

Phagocytic uptake by macrophages is a key process during *M. tuberculosis* infection, and changes in membrane cholesterol level could potentially influence this active cellular process. To test this possibility, infection studies using GFP-expressing
M. tuberculosis revealed no differences in uptake between simvastatin-treated and untreated control macrophages (Figure 4C). We observed no effect on the extracellular growth of M. tuberculosis in the presence of simvastatin in culture broth medium (Figure 4D), excluding the possibility that the observed reduction in intracellular growth of M. tuberculosis was due to the direct effect of statins on the pathogen. Taken together, these results suggest that simvastatin is able to decrease cholesterol biosynthesis without influencing phagocytosis or extracellular growth of M. tuberculosis.

**Figure 4.** Simvastatin (Sim) decreases intracellular cholesterol levels but has no effect on the phagocytic ability of macrophages. A, Macrophages stained with the cholesterol-binding dye filipin, showing that mevalonate restored the simvastatin-mediated decrease in cholesterol levels (original magnification x1000). Fluorescent intensity (arbitrary units) per cell was quantified (**P < .01, by the Student t test; n = 50–100 cells/group). B, Cholesterol content was decreased in simvastatin-treated macrophages, with the effect of simvastatin abrogated in presence of mevalonate (Meva; n = 2). C, Simvastatin has no effect on the uptake of Mycobacterium tuberculosis in macrophages (n = 3). D, Growth of M. tuberculosis in culture broth supplemented with simvastatin has no direct bactericidal effect on bacterium (n = 3).

**M. tuberculosis** revealed no differences in uptake between simvastatin-treated and untreated control macrophages (Figure 4C). We observed no effect on the extracellular growth of M. tuberculosis in the presence of simvastatin in culture broth medium (Figure 4D), excluding the possibility that the observed reduction in intracellular growth of M. tuberculosis was due to the direct effect of statins on the pathogen. Taken together, these results suggest that simvastatin is able to decrease cholesterol biosynthesis without influencing phagocytosis or extracellular growth of M. tuberculosis.

**Statins Promote Maturation of Phagosomes in M. tuberculosis–Infected Macrophages**

During internalization of M. tuberculosis, the bacteria associate with cholesterol-rich membrane domains [19]. This is reflected by the accumulation of lipid-rich bodies within phagosomes in the periphery of the bacterium [20], which thereby allows the bacilli to evade host-protective functions. We investigated the possibility that the decrease in intracellular cholesterol levels caused by statins was able to reverse this Mycobacterium-induced evasion mechanism. Simvastatin-treated or untreated BMDMs were infected with GFP-expressing M. tuberculosis. Two hours later, phagosomal maturation markers were labeled with fluorescent antibodies, and colocalization studies were performed using confocal microscopy. As soon as a mycobacterium is internalized, early endosomal antigen 1 (EEA-1) is recruited to the membrane of phagosomes [21, 22]. This is followed by the recruitment of a late marker, lysosome-associated membrane protein (LAMP), which fuses with lysosomes [23], as seen in the untreated control cells in Figure 5A. Of importance, simvastatin-treated macrophages showed significantly increased colocalization of M. tuberculosis with EEA-1, as well as with LAMP-3, in quantitative analysis (Figure 5A). Increased protein production was confirmed by Western blot (Figure 5B) and densitometric analysis of bands (Figure 5C). These results suggest that statins influence host immune responses by promoting phagosome maturation.

**Statins Induce Autophagy in M. tuberculosis–Infected Macrophages**

As a survival mechanism, infected macrophages are able to induce autophagy to bypass M. tuberculosis–mediated inhibition
of phagolysosomal maturation and subsequent persistence [13]. Induction of autophagy leads to the incorporation of light chain 3-II (LC3-II) protein into the autophagic membrane. This facilitates fusion of the autophagosome with lysosomes [24, 25]. Interestingly, statin treatment before \textit{M. tuberculosis} infection significantly increased colocalization of \textit{M. tuberculosis} bacilli with LC3-II in BMDMs (Figure 5A). The increase in the quantity of LC3-II was further confirmed by Western blot analysis (Figure 5B and C). These results indicate that statin is able to augment autophagy in macrophages.

To further validate the role of phagolysosome maturation and autophagy, we blocked phagosomal acidification through selective inhibition of proton-translocating V-type ATPases, such as bafilomycin A1 [26]. 3-methyladenine was used to inhibit autophagy by blocking autophagosome formation via the inhibition of type III phosphatidylinositol 3 kinases [27]. Macrophages treated with simvastatin upon addition of 3-methyladenine or bafilomycin A1 (Figure 6A) completely abrogated the simvastatin-mediated reduced growth of \textit{M. tuberculosis}, with chloroquine used as positive control for the induction of autophagy. These results further suggest that statins augment host-protective autophagy in infected macrophages. Moreover, induction of autophagy was also analyzed using FACS, which was reversed upon 3-methyladenine and bafilomycin A1 supplementation (Figure 6B). We further investigated whether uninfected macrophages would also show an increase in phagosomal and autophagy markers, as observed in \textit{M. tuberculosis}-infected macrophages shown in Figure 5A. Indeed, simvastatin treatment alone was able to significantly increase the expression of EEA-1, LAMP-3, and LC3 II (data not shown). As expected, addition of bafilomycin A1 reversed the induction of autophagy by simvastatin (data not shown), indicating that the simvastatin-mediated...

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\caption{Simvastatin enhances phagosomal maturation and autophagy. A, Confocal images of murine macrophages treated with simvastatin showed increased colocalization of green fluorescent protein (GFP)–expressing \textit{Mycobacterium tuberculosis} (green) and EEA-1, LAMP-3, and LC3-II (red). Insets represent individual color channels (original magnification ×40). Quantitative colocalization of GFP expressing \textit{M. tuberculosis} with phagosomal or autophagy markers revealed a significant increase following simvastatin treatment (*\textit{P} < .05, by the Student \textit{t} test; \textit{n} = 600 cells/sample). B and C, Western blot \(B\) and densitometry \(C\) showed significant increase in phagosomal and autophagy markers in total macrophage cell lysates following \textit{M. tuberculosis} infection (*\textit{P} < .05, **\textit{P} < .01, by the Student \textit{t} test; \textit{n} = 3). Abbreviation: CFU, colony-forming units.}
\end{figure}
effect on autophagy markers was independent of \textit{M. tuberculosis} infection. Together, these results suggest that statins induce host-protective autophagy during \textit{M. tuberculosis} infection.

\section*{DISCUSSION}

Statins are drugs commonly used for the treatment of patients with familial hypercholesterolemia. These patients have high blood cholesterol due to a genetic disorder in 1 or both alleles of the LDL receptor \[28\]. Even though the liver preferentially metabolizes statins, it has been reported that statins can inhibit accumulation of cholesterol esters in human MDMs \[29\]. Here, we report that PBMCs and MDMs from patients with FH showed early significant reduced mycobacterial growth with no major effect on cellular viability in the presence of statin. These findings emphasize that statin therapy in patients can induce immunomodulatory properties in PBMCs and MDMs, resulting in increased protection against \textit{M. tuberculosis} infection.

To uncover the underlying host-protective mechanisms of statins, we further investigated the effect of statins in an experimental murine model for human tuberculosis, using aerosol-based infection to mimic human exposure. Simvastatin therapy in mice led to a significant (up to 10-fold) reduction in bacterial burdens in peripheral organs, with dissemination of \textit{M. tuberculosis} effectively reduced in the liver and spleen of infected mice. The lungs of infected animals showed a 1.5-fold reduction in bacterial burdens, which could be further improved to a 3-fold reduction when rosuvastatin with an extended half-life was used. An important consequence of statin treatment was the reduced pulmonary pathology in \textit{M. tuberculosis}–infected mice observed 4 weeks after infection. Increased host-protective immune responses as a result of statin treatment is not solely restricted to \textit{Mycobacterium} infection, since statin-treated mice also showed a 2-fold reduction in \textit{S. enterica} bacterial burden \[9\], but mechanistically it might be different.

Statins reduce both total and membrane cholesterol levels in host macrophages. As a result, we have shown that these processes lead to an increase in phagosomal maturation, which is known to provide better defense against \textit{M. tuberculosis} in host cells. This was reflected in reduced bacterial burdens in macrophages and, subsequently, in improved protection against \textit{M. tuberculosis} in infected mice. During \textit{Salmonella} infection, a similar mechanism seems to take place, in which during statin treatment, the association between cathepsin D and \textit{Salmonella}-containing vacuoles (SCVs) was enhanced. The association of cathepsin D to SCVs is critical for phagosomal maturation and subsequent degradation of the bacterium \[9, 30\].

Autophagy is a self-destructive mechanism in host cells, leading to the degradation of cellular proteins. This critical process is important in host innate immunity since it is responsible for the elimination of infectious agents, including bacteria \[13, 31, 32\], viruses \[33\], and parasites \[34\]. Evidence suggests that autophagy plays a crucial role in antimycobacterial resistance by acting as an alternative mechanism to control \textit{M. tuberculosis} infection in macrophages, as well as to defend and counteract \textit{M. tuberculosis} evasion strategies. Recently, it has been shown that \textit{M. tuberculosis} uses an effector protein of the ESAT-6 secretion system 1 (ESX-1)/type VII secretion system to arrest autophagosome-lysosome fusion in human dendritic cells. On the other hand, \textit{M. tuberculosis} strains such as Bacille-Calmette Guerin and H37Ra, which lack functional ESAT-6, were unable to block autophagosomal maturation \[35\]. However, the role of the bacterial ESX-1 system is double edged as it also promotes permeabilization of \textit{M. tuberculosis}–containing phagosomes, thereby exposing bacterial surface–bound DNA to the host DNA-sensing pathway that targets \textit{M. tuberculosis}.

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\caption{Simvastatin (Sim)–mediated decrease in growth of \textit{Mycobacterium tuberculosis} is reversed by addition of autophagy inhibitors. \textit{A}, Effect of simvastatin on bacterial growth was reversed by addition of an autophagy inhibitors (3-methyladenine [3-MA] or bafilomycin A1 [Baf]). Chloroquine served as a positive control for the induction of autophagy (*\(P<.05\), **\(P<.01\) vs control, by the Student t test; n = 3). \textit{B}, Simvastatin resulted in an increase in the percentage of LC-3 II–positive macrophages, which was abrogated in presence of 3-MA and bafilomycin A1 (*\(P<.05\) vs control, by the Student t test; n = 2). Abbreviation: CFU, colony-forming units.}
\end{figure}
for selective ubiquitin-mediated autophagy [36]. Our study expands upon these findings, as we have shown that statin treatment restored autophagy in BMDMs infected with M. tuberculosis H37Rv (in which autophagy is usually impaired), which could be reversed with the inhibitors of autophagosome maturation.

Moreover, we demonstrated that both squalene (cholesterol arm) and geranylgeraniol (isoprenoid arm) of the mevalonate pathway were able to partially rescue statin-mediated antimycobacterial activity. This observation is consistent with recent findings in which statin-induced autophagy was shown to be mediated via inhibition of geranylgeranyl biosynthesis in a prostate cancer cell line, PC3 [37]. The beneficial role of statins in the defense against M. tuberculosis and other pathogens is intriguing, with future investigations being beneficial to identify specific downstream metabolites of the mevalonate pathway for use as potential host-directed drug targets [38]. A sequential approach of inhibiting metabolites of the intermediate arms of the mevalonate pathway should potentially uncover pathogen-specific metabolites responsible for infection and persistence.

In summary, our findings reveal a beneficial role for statins during tuberculosis in both a human and mouse model. Mechanistically, we showed some evidence that inhibition of the host mevalonate pathway by statins could induce host protection against tuberculosis by enhancing phagosomal maturation and autophagy. Thus, statins and drugs inhibiting the downstream cholesterol biosynthesis pathway may be interesting targets for host-directed drug targeting against tuberculosis, supplementing current bacterial drug therapies.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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