High Dose Atorvastatin Decreases Cellular Markers of Immune Activation Without Affecting HIV-1 RNA Levels: Results of a Double-blind Randomized Placebo Controlled Clinical Trial

Anuradha Ganesan,[1,2] Nancy Crum-Cianflone,[2,3] Jeanette Higgins,[4] Jing Qin,[5] Catherine Rehm,[6] Julia Metcalf,[7] Carolyn Brandt,[3] Jean Vita,[1] Catherine F. Decker,[1] Peter Sklar,[8,9] Mary Bavaro,[3] Sybil Tasker,[10] Dean Follmann,[5] and Frank Maldarelli[11]

[1]National Naval Medical Center, Division of Infectious Diseases and [2]Infectious Disease Clinical Research Program, Uniformed Services University, Bethesda, Maryland; [3]Naval Medical Center San Diego, Division of Infectious Diseases, California; [4]AIDS Monitoring Laboratory, Clinical Services Program, Science Applications International Corporation–Frederick, Inc, National Cancer Institute-Frederick, [5]Biostatistics Research Branch, [6]Clinical Research Section, and [7]Biostatistics Research Branch, Clinical Research Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland; [8]Department of Medicine, Drexel University College of Medicine, Philadelphia, [9]Merck Research Laboratories, North Wales, Pennsylvania; [10]Pharmaceutical Product Development, Incorporated, Wilmington, Delaware; and [11]HIV Drug Resistance Program, National Cancer Institute, National Institutes of Health, Frederick, Maryland

(See the editorial commentary by Carr, on pages 751–2.)

Background. 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors (statins) exhibit antiviral activity against human immunodeficiency virus type 1 (HIV-1) in vitro and may modulate the immune response to HIV infection. Studies evaluating the antiviral activity of statins have yielded conflicting results.

Methods. We conducted a randomized, double-blind, placebo-controlled crossover trial to investigate the effect of atorvastatin on HIV-1 RNA (primary objective) and cellular markers of immune activation (secondary objective). HIV-infected individuals not receiving antiretroviral therapy were randomized to receive either 8 weeks of atorvastatin (80 mg) or placebo daily. After a 4–6 week washout phase, participants switched treatment assignments. The study had 80% power to detect a 0.3 $\log_{10}$ decrease in HIV-1 RNA level. Expression of CD38 and HLA-DR on CD4$^+$ and CD8$^+$ T cells was used to measure immune activation.

Results. Of 24 randomized participants, 22 completed the study. Although HIV-1 RNA level was unaffected by the intervention (–0.13 $\log_{10}$ copies/mL; $P = .85$), atorvastatin use resulted in reductions in circulating proportions of CD4$^+$ HLA-DR$^+$ (–2.5%; $P = .02$), CD8$^+$ HLA-DR$^+$ (–5%; $P = .006$), and CD8$^+$ HLA-DR$^+$ CD38$^+$ T cells (–3%; $P = .03$). Reductions in immune activation did not correlate with declines in serum levels of low-density lipoprotein cholesterol.

Conclusions. Short-term use of atorvastatin was associated with modest but statistically significant reductions in the proportion of activated T lymphocytes.

Antiretroviral therapy (ART) has transformed our clinical approach to human immunodeficiency virus (HIV) infection. Despite substantial advances in the management of HIV infection, concerns about transmitted drug resistance, ART-related toxic effects, and the consequences of chronic inflammation persist, emphasizing the need for ongoing research into alternate therapeutic targets and strategies to modulate the chronic immune activation/inflammation observed in this disease [1–3]. Strategies that block key interactions between the host and the virus, including those that

target lipid rafts, are an area of interest. Lipid rafts are plasma membrane microdomains rich in sphingolipids and cholesterol that play a critical role in the replication of HIV type 1 (HIV-1) [4, 5]. In vitro models suggest that disruption of lipid rafts with cholesterol-depleting agents, such as 3-hydroxy-3-methyl-glu-taryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), reduces HIV-1 particle production [5]. Furthermore, virions derived from cholesterol-depleted cells demonstrate reduced infectivity in vitro [5]. In addition, statins have demonstrated effects on protein prenylation and inhibit lymphocyte function antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1) interactions that influence viral entry and exit [5–9]. Statins exhibit anti-inflammatory effects [10] and may modify HIV pathogenesis by means of direct antiviral and indirect anti-inflammatory mechanisms.

To date, 4 prospective studies and 1 retrospective study have examined the effects of statins on HIV-1 RNA levels and have yielded conflicting results [6, 11–14]. Most studies have not included a comparator arm, and none were designed to investigate both virologic and cellular immune activation outcomes. We therefore conducted a double-blind, randomized, placebo-controlled crossover clinical trial with the primary objective of evaluating the effects of atorvastatin on HIV-1 RNA. Prespecified secondary objectives included a comprehensive evaluation of the effect of atorvastatin on cellular markers of immune activation.

METHODS

Study Population
HIV-infected adults not receiving antiretroviral therapy (ART) with a CD4$^+$ cell count >350 cells/μL, HIV-1 RNA >1000 copies/mL, serum low-density lipoprotein (LDL) cholesterol level <130 mg/dL, and serum alanine aminotransferase and aspartate aminotransferase levels <1.5 times the upper limit of normal were eligible to participate. To increase the precision of the baseline HIV-1 RNA, we required 3 baseline HIV-1 RNA measures that did not demonstrate substantial variation (<20% variation among the 3 baseline measures). Participants with known resistance to antiretroviral agents (on the basis of prior genotypes or a history of ongoing viremia while receiving ART) were excluded because of concerns that prior nonadherence may have resulted in sequence variations in the gag P6 region, potentially interfering with the effects of statins [15]. Additional exclusion criteria included pregnancy, a history of myositis, the receipt of lipid-lowering agents at the baseline visit, or use of therapeutic agents known to have substantial drug-drug interactions with statins. All participants were recruited at 1 of 3 sites: the National Naval Medical Center (NNMC), Bethesda, Maryland; the Naval Medical Center San Diego (NMCSD), San Diego, California; and the National Institutes of Health, Clinical Center, Bethesda, Maryland. The protocol was approved by the institutional review boards of the NNMC, the NMCSD, and the National Institute of Allergy and Infectious Diseases prior to its execution and was registered with the Clinical Trials network (registration ID, NCT00367458). All participants provided written informed consent before participating in the study.

Study Design
Previous in vitro studies revealed that substantial statin-induced decreases in cholesterol resulted in declines in HIV-1 production [5]. We therefore chose the highest Food and Drug Administration–approved dose of atorvastatin (80 mg daily) for maximal potential effect on lipid lowering. Using a crossover design, HIV-1 infected adults were enrolled and randomized in a double-blind fashion to receive either 8 weeks of an 80 mg dose of atorvastatin or placebo daily (phase A). Upon completion of phase A, participants underwent a 4–6 week washout phase, subsequently switching treatment assignments to complete an additional 8 weeks (phase B) in the opposite assignment. The duration of the washout phase ranged from 4 to 6 weeks to accommodate study windows and to allow for seasonal influenza vaccination. Study follow-up ended 4 weeks after completion of both phases.

Randomization and Blinding
Both placebo and drug were identical in appearance, and the assignment of the drug and placebo sequences was completed by a pharmacist who was not involved with the clinical conduct of the study. Study investigators remained blinded to lipid profile data until after completion of the study; instead, these results were reviewed in real time by independent medical monitors who ensured patient safety and protocol adherence.

Laboratory Methods
The HIV-1 RNA was quantitated in plasma by means of bDNA signal amplification–based hybridization (Siemens), and cellular markers of immune activation were analyzed at a single location (Science Applications International Corporation) to ensure consistency and to avoid operator-related variability [16]. Per protocol safety labs were performed at the individuals institutions and not centrally.

We used HLA-DR, CD38, and coexpression of CD38 and HLA-DR to identify activated CD4$^+$ and CD8$^+$ T cells. Immunophenotyping of peripheral blood drawn into ethyl-enediaminetetracetate was performed according to the manufacturer's instructions, by using a modification of the Centers for Disease Control and Prevention guidelines in a Clinical Laboratory Improvement Act–certified laboratory. Cells were lysed after staining with Optilyse C (Beckman Coulter), washed twice, and resuspended in 500 μL of phosphate-buffered saline (Cambrex). Samples were analyzed immediately on a Becton Dickinson FacsCanto flow cytometer (BD Biosciences).

Gating on $CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$ cells was used to determine the expression of the $HLA-DR^+$, $CD38^+$, and $HLA-DR^+$ $CD38^+$ cells.

Statistical Methods

The a priori calculated sample size for this study ($n = 22$) had 80% power to detect a 0.3 $\log_{10}$ decrease in HIV-1 RNA level and 90% power to detect a 0.4 $\log_{10}$ decrease in HIV-1 RNA level. We assessed changes in HIV-1 RNA levels, $CD4^+$ cell counts, markers of immune activation, and serum lipid levels after 8 weeks of treatment, comparing them with those measured at the start of treatment during both phases of the study. To assess the effects of treatment, we calculated the difference in changes, for all parameters, for each person using the formula (XFS – XIS) – (XFP – XIP), where X is the measure of the outcome (eg, $CD4^+$ cell count) at either the initial visit (I) (week 0) or the final visit (F) (week 8), while the participant was receiving either atorvastatin (S) or placebo (P). We tested the hypothesis that the median differences of these changes were zero using a paired Wilcoxon test. To adjust for the multiplicity of secondary objectives, we used a P value of $<.01$ to define significance [17]. The Spearman rank correlation was used to examine the associations between changes in cholesterol level, HIV-1 RNA level, and immune parameters; this test was used because it is robust to outliers. All analyses were performed using S-PLUS software, version 2.0 (Tibco).

RESULTS

Baseline Characteristics

We screened 34 HIV-infected persons and randomized 24 participants; 2 enrolled participants were withdrawn prior to study completion when they met prespecified termination criteria of a serum LDL cholesterol level of <40 mg/dL, chosen because of concerns regarding effects of intense lipid reduction [18]. Twenty-two participants completed the study. Table 1 describes the characteristics of the study participants at enrollment (baseline). Participants enrolled in this study were all male, relatively young (median age, 30 years [interquartile range {IQR}, 25–38 years]), and otherwise healthy. Self-reported ethnicity was Caucasian in 63%, African-American in 25%, and other in 13% of participants. The median baseline HIV-1 RNA level was 3.89 $\log_{10}$ copies/mL (IQR, 3.58–4.21 $\log_{10}$ copies/mL), and the median $CD4^+$ count was 568 cells/μL (IQR, 468–664 cells/μL). On average, participants had received a diagnosis of HIV infection 1.2 years prior to enrollment (IQR, 0.7–2.9 years). The median nadir $CD4^+$ cell count prior to randomization was 458 cells/μL (IQR, 362–601 cells/μL). A majority of the participants were ART naive (91%). The median serum total and LDL cholesterol levels at the baseline visit were 168 mg/dL (IQR, 144–172 mg/dL) and 97 mg/dL (IQR, 87–109 mg/dL), respectively. The median proportions of $CD4^+$ and $CD8^+$ T cells coexpressing the surface markers HLA-DR and CD38 at the baseline

Table 1. Baseline Demographic, Immunologic, and Virologic Characteristics of Participants Completing Both Phases of the Study

Characteristic	Value ($n = 22$)
Demographic characteristics	
Age, median years (IQR)	30 (25–38)
Race, no. (%)	
African-American	6 (25)
Caucasian	15 (63)
Other	3 (13)
Hispanic ethnicity, no. (%)	5 (21)
Male sex, no. (%)	22 (100)
HIV-specific variables	
Duration of infection, median years (IQR)	1.2 (0.7–2.9)
Nadir $CD4^+$ T cell count (cells/μL)	458 (362–601)
ART naive, no. (%)	20 (91%)
Baseline virologic characteristics, copies/mL	
$\log_{10}$ HIV-1 RNA level	3.89 (3.58–4.21)
Baseline immunologic characteristics, median cells/μL (IQR)	
Absolute $CD4^+$ T cell count	568 (468–664)
Absolute $CD8^+$ T cell count	829 (606–1137)
Immune activation parameters (cells/μL), %	
$CD4^+$ HLA DR$^+$	13 (9–17)
$CD4^+$ $CD38^+$	69 (65–72)
$CD4^+$ $CD38^+$ HLA DR$^+$	8 (6–14)
$CD8^+$ HLA DR$^+$	48 (42–61)
$CD8^+$ $CD38^+$	72 (67–81)
$CD8^+$ $CD38^+$ HLA DR$^+$	44 (34–56)
Serum lipid levels, mg/dL	
Total cholesterol	168 (144–172)
LDL cholesterol	97 (87–109)

NOTE. ART, antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; LDL, low-density lipoprotein.

visit were 8% (IQR, 6%–14%) and 44% (IQR, 34%–56%), respectively, and were higher than those typically detected in HIV-uninfected individuals [19].

No Effect of Short-Term High-Dose Atorvastatin Therapy on Level of HIV-1 RNA

To investigate the effects of statins on HIV-1 RNA levels, we compared levels during the statin and placebo phases, both for individual patients and for aggregate groups. During the statin phase of the study, the median change in HIV-1 RNA level was -0.03 $\log_{10}$ copies/mL (IQR, -0.35 to 0.04 $\log_{10}$ copies/mL), compared with -0.08 $\log_{10}$ copies/mL (IQR, -0.25 to 0.09 $\log_{10}$ copies/mL) during the placebo phase (Table 2). A washout period was used between phases, and an additional statistical test to assess drug carryover revealed no differential effect by period. Overall, atorvastatin use did not result in a statistically significant change in HIV-1 RNA levels, compared with placebo

Table 2. Comparison of Changes in Viral Load, Serum Lipid Levels, and Cellular Markers of Activation during the Statin and Placebo Phases of the Study

Parameter, serum level	Change during statin phase, median (IQR)	Change during placebo phase, median (IQR)	Treatment effect (change),[a] median (IQR)	P[b]
HIV-1 RNA, $\log_{10}$ copies/mL	−0.03 (−0.35 to 0.04)	−0.08 (−0.25 to 0.09)	−0.13 (−0.27 to 0.21)	.85
CD4$^+$ cell count	26 (2–71.5)	43 (−58.0 to 92.5)	37.5 (−49.25 to 121.8)	.31
CD8$^+$ cell count	−18 (−179.5 to 124.30)	22 (−80 to 126)	−51.5 (−200.3 to 167.8)	.57
CD4$^+$ HLA-DR$^+$, cells/µL, %	−2.0 (−4.0 to −0.25)	0.0 (−1.75 to 1.0)	−2.5 (−3.75 to 0)	.02
CD4$^+$ HLA-DR$^+$ CD38$^+$, cells/µL, %	−1.0 (−2.75 to 0.0)	0.0 (−1.0 to 1.0)	−0.5 (−3 to 0.75)	.15
CD4$^+$ CD38$^+$, cells/µL, %	1.0 (−3.5 to 3.75)	−0.5 (−4.0 to 2.75)	2.5 (0–6)	.20
CD8$^+$ HLA-DR$^+$, cells/µL, %	−2.5 (−7.5 to 0.0)	0.0 (−2.0 to 3.0)	−5 (−8.75 to 0)	.006
CD8$^+$ HLA-DR$^+$ CD38$^+$, cells/µL, %	−1.0 (−5.5 to 0.75)	1.0 (−2.75 to 1.75)	−3 (−6 to 0.5)	.03
CD8$^+$ CD38$^+$, cells/µL, %	1.0 (−5.75 to 3.0)	0.0 (−3.0 to 2.0)	−0.5 (−4 to 5)	.97
Total cholesterol, mg/dL	−60 (−69 to −50)	5.5 (−3.75 to 12.0)	−65 (−82 to −19)	<.001
LDL cholesterol, mg/dL	−52 (−59 to −32)	7 (−6.25 to 12.75)	−54 (−69 to −10)	<.001

NOTE. [a] To calculate treatment effect, we formed the difference in changes, for all parameters, for each person using the formula (XFS – XIS) – (XFP – XIP), where X is the measure of the outcome (eg, CD4+ cell count) at either the initial visit (I) (week 0) or the final visit (F) (week 8), while the participant was receiving either atorvastatin (S) or placebo (P). The paired Wilcoxon test was used to test the hypothesis that the median differences of the changes were zero.

[b] A P value of <.01 was considered to indicate a significant difference.

(−0.13 $\log_{10}$ copies/mL; $P = .85$) (Table 2). Interindividual variations in treatment response were observed. While receiving atorvastatin, 7 participants (32%) exhibited a >0.3 $\log_{10}$ copies/mL decline (range, −0.34 to −0.63 $\log_{10}$ copies/mL) in HIV-1 RNA, 3 participants (14%) had a >0.3 $\log_{10}$ copies/mL increase in HIV-1 RNA (range, 0.31–0.87 $\log_{10}$ copies/mL), and the rest exhibited no significant change. While receiving placebo, 4 participants (18%) exhibited a >0.3 $\log_{10}$ copies/mL decline in HIV-1 RNA (range, −0.39 to −1.01 $\log_{10}$ copies/mL), and 2 participants (9%) had an increase in HIV-1 RNA (range, 0.46–0.93 $\log_{10}$ copies/mL). There were no significant differences between the 2 phases in the proportions of individuals exhibiting a 0.3 $\log_{10}$ copies/mL decline in HIV-1 RNA level. On average, pill counts conducted during study visits revealed that >90% of study medications were taken by participants. Evaluation of serum lipid levels revealed significant reductions, corroborating the adherence data collected.
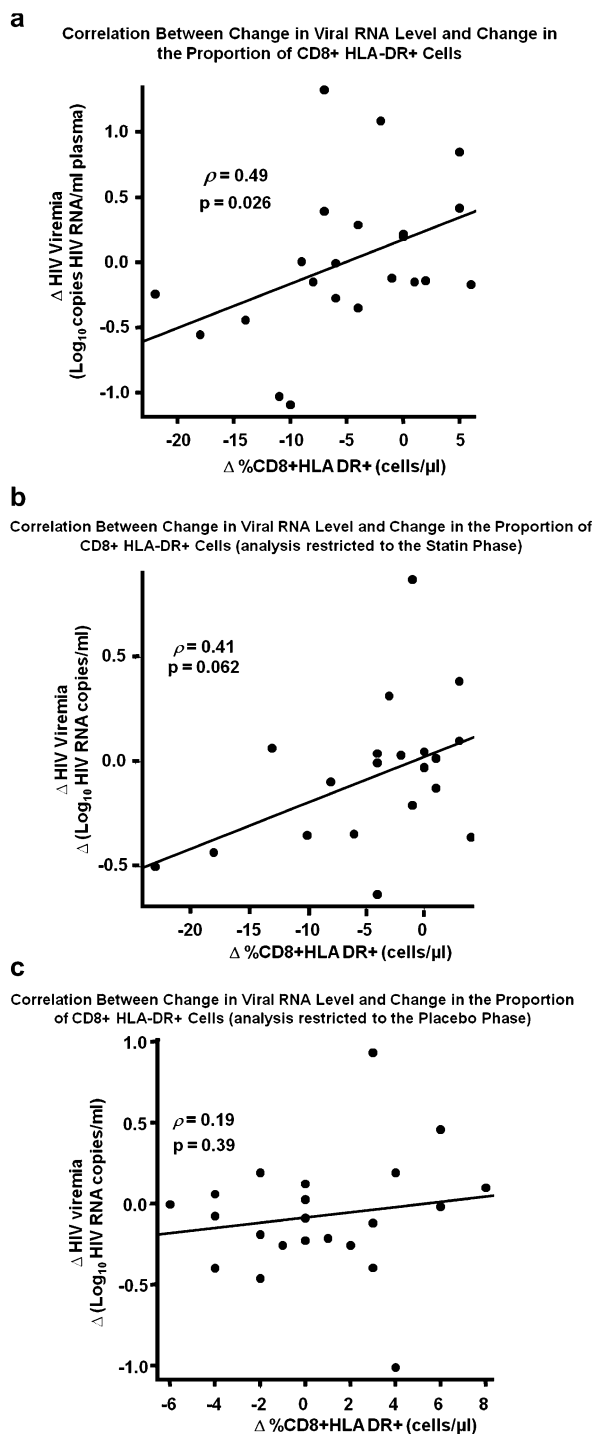
Short-Term Atorvastatin Therapy Decreased Cellular Markers of Immune Activation

We evaluated the effects of statin administration on cellular markers of activation by comparing T cell subsets during the statin and placebo phases. As shown in Table 2, atorvastatin use was not associated with significant changes in either the absolute numbers of CD4$^+$ (median change, +37.5 cells/µL; $P = .31$) or CD8$^+$ T lymphocytes (median change, −51.5 cells/µL; $P = .57$), in comparison with placebo. Atorvastatin use did, however, result in significant declines in the proportion of HLA-DR$^+$ CD8$^+$ T cells (median change, −5.0%; $P = .006$) and resulted in marginal reduction in the proportion of HLA-DR$^+$ CD4$^+$ T cells (median change, −2.5%; $P = .02$) and HLA-DR$^+$ CD38$^+$ CD8$^+$ T cells (median change, −3%; $P = .03$). After 8 weeks of

treatment with atorvastatin, a majority of the participants (14 [64%]) exhibited declines in the proportion of HLA-DR$^+$ CD8$^+$ T cells. In comparison, treatment with placebo resulted in declines in the proportion of HLA-DR$^+$ CD8$^+$ T cells in 8 participants (36%) (Figure 1). Atorvastatin use was associated with marginal declines in the numbers of proliferating CD4$^+$ T lymphocytes as measured by Ki-67 expression (median change, −1%; $P = .03$); proliferation in the CD8$^+$ compartment was unaffected (median change, −1.2%; $P = .19$). We compared the proportions of HLA-DR$^+$ CD8$^+$ T cells prior to atorvastatin initiation with levels after completion of washout among participants who received atorvastatin first followed by placebo, and a difference was not observed ($P = .72$). We conducted the
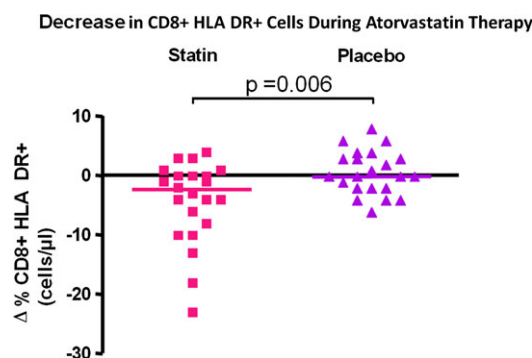
Figure 1. Decreases in CD8$^+$ HLA-DR$^+$ T lymphocytes during atorvastatin therapy. Each square and triangle represents changes in the proportion of circulating CD8$^+$ HLA-DR$^+$ T cells in an individual patient (week 8 − week 0) during the atorvastatin and placebo phases of the study, respectively, while the line represents the median change for the group. Response to atorvastatin was heterogeneous, with most participants (15 of 22) exhibiting a decline. In comparison, participants receiving placebo demonstrate a clustering around the median.

a

Correlation Between Change in Viral RNA Level and Change in the Proportion of CD8+ HLA-DR+ Cells

$\rho = 0.49$
$p = 0.026$

Y-axis: Δ HIV Viremia (Log$_{10}$ copies HIV RNA/ml plasma)
X-axis: Δ %CD8+HLA DR+ (cells/µl)

b

Correlation Between Change in Viral RNA Level and Change in the Proportion of CD8+ HLA-DR+ Cells (analysis restricted to the Statin Phase)

$\rho = 0.41$
$p = 0.062$

Y-axis: Δ HIV Viremia Δ (Log$_{10}$ HIV RNA copies/ml)
X-axis: Δ %CD8+HLA DR+ (cells/µl)

c

Correlation Between Change in Viral RNA Level and Change in the Proportion of CD8+ HLA-DR+ Cells (analysis restricted to the Placebo Phase)

$\rho = 0.19$
$p = 0.39$

Y-axis: Δ HIV viremia Δ (Log$_{10}$ HIV RNA copies/ml)
X-axis: Δ %CD8+HLA DR+ (cells/µl)

Figure 2. Correlation between the changes in plasma human immunodeficiency virus type 1 (HIV-1) RNA levels and change in the proportion of circulating CD8$^+$ HLA-DR$^+$ cells. Changes in panel A reflect the difference of the changes (week 8 – week 0) during the statin phase and (week 8 – week 0) during the placebo phase. Change was calculated for both HIV-1 RNA and proportion of CD8$^+$ HLA-DR$^+$ T cells using the formula (XFS – XIS) – (XFP – XIP), where X is the measure of the outcome (ie, percentage of CD8$^+$ HLA-DR$^+$ T cells or HIV-1 RNA level) at either the initial visit (I) (week 0) or the final visit (F) (week 8), while the participant was receiving either atorvastatin (S) or placebo (P). *P* values are calculated using the Spearman rank correlation. *B,* Correlation between the changes in plasma HIV-1 RNA levels and change in the proportion of circulating CD8$^+$ HLA-DR$^+$ cells (analysis restricted to the statin phase). Changes in Panel B reflect changes restricted to the atorvastatin (week 8 – week 0) phase of the study. *P* values are calculated using the Spearman rank correlation. *C,* Correlation between the changes in plasma HIV-1 RNA levels and change in the proportion of circulating CD8$^+$ HLA-DR$^+$ cells. Changes in Panel C reflect changes restricted to the placebo (week 8 – week 0) phase of the study. *P* values are calculated using the Spearman rank correlation.

same analysis in all participants, irrespective of their randomization sequence (atorvastatin followed by placebo or placebo followed by atorvastatin), and observed no significant difference ($P = .28$), suggesting that there was no residual effect on immune activation parameters 4–6 weeks after completion of atorvastatin therapy.

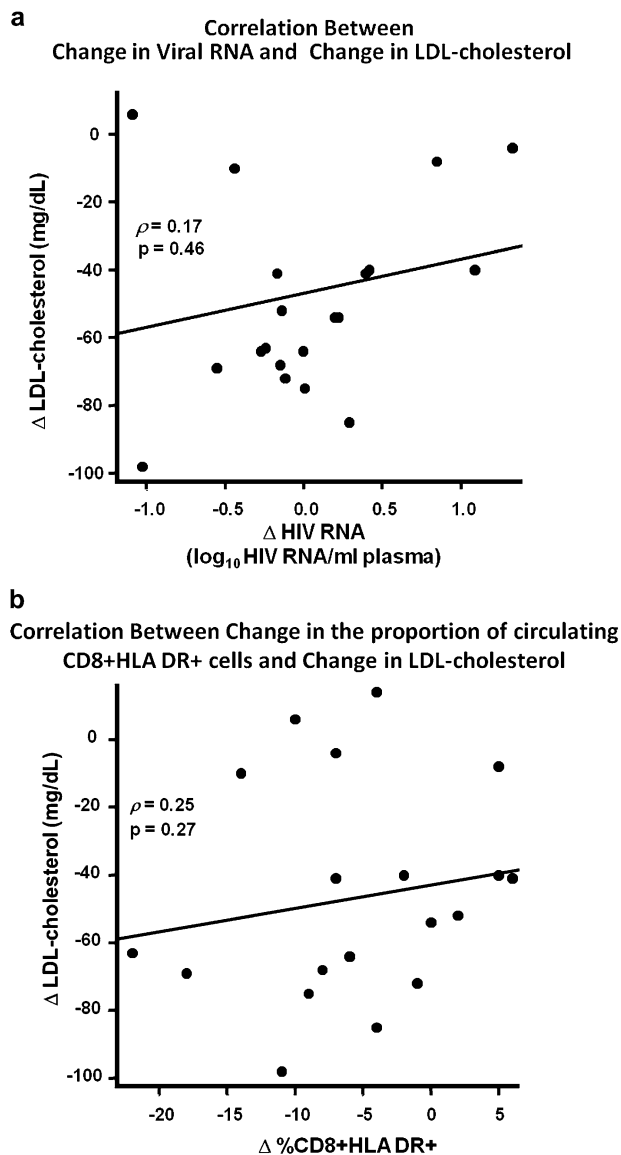### Reductions in Activated T Cells Correlate with Reductions in HIV-1 RNA

Although an overall effect on HIV-1 RNA levels was not observed, atorvastatin use resulted in statistically significant reductions in the proportion of HLA DR$^+$ CD8$^+$ T cells. Because cellular immune activation may have an effect on viral RNA levels, [20] we investigated whether there were correlations between the changes in HLA DR$^+$ CD8$^+$ T cells and changes in HIV-1 RNA. Despite the absence of an overall effect on HIV-1 RNA, a correlation was observed between the difference of changes in the proportion of HLA-DR$^+$ CD8$^+$ T cells and the difference of changes in HIV-1 RNA ($\rho = .49$; $P = .03$) (Figure 2A). Examinations of the changes by phase revealed a marginal correlation while participants were receiving atorvastatin ($\rho = .41$; $P = .06$) and the absence of a correlation while they were receiving placebo ($\rho = .19$; $P = .39$). (Figures 2B and 2C).

Reductions in Serum LDL Cholesterol Seem not to Correlate with Observed Reductions in Proportion of Activated T Cells and HIV-1 RNA

After completion of 8 weeks of treatment with atorvastatin, the median changes in serum total and LDL cholesterol levels were –60 mg/dL (IQR, –69 to –50 mg/dL) and –52 mg/dL (IQR, –59 to –32 mg/dL), respectively. In comparison, after 8 weeks of treatment with placebo, the median total and LDL cholesterol levels increased by 5.5 mg/dL (IQR, –3.75 to 12.0 mg/dL) and 7 mg/dL (IQR, –6.25 to 12.75 mg/dL), respectively. Among participants who received atorvastatin first followed by placebo, comparison of serum lipid levels before atorvastatin and after washout revealed no significant differences in serum levels of LDL cholesterol ($P = .86$), total cholesterol ($P > .99$), and high-density lipoprotein cholesterol ($P = .77$).

We investigated whether the relative effects of statins on lipid parameters were associated with relative changes in immune

a

Correlation Between Change in Viral RNA and Change in LDL-cholesterol

$\rho = 0.17$
$p = 0.46$

Y-axis: Δ LDL-cholesterol (mg/dL)
X-axis: Δ HIV RNA (log_{10} HIV RNA/ml plasma)

b

Correlation Between Change in the proportion of circulating CD8+HLA DR+ cells and Change in LDL-cholesterol

$\rho = 0.25$
$p = 0.27$

Y-axis: Δ LDL-cholesterol (mg/dL)
X-axis: Δ %CD8+HLA DR+

Figure 3. *A,* Correlation between change in plasma human immuno-deficiency virus type 1 (HIV-1) RNA levels and change in serum low-density liproprotein (LDL) cholesterol levels. Change in Panel A reflects the difference of the changes during the atorvastatin phase and those during the placebo phase. Change was calculated for both HIV-1 RNA and serum LDL cholesterol levels using the formula (XFS − XIS) − (XFP − XIP), where X is the measure of the outcome (ie, LDL cholesterol or HIV-1 RNA level) at either the initial visit (I) (week 0) or the final visit (F) (week 8), while the participant was receiving either atorvastatin (S) or placebo (P). P values are calculated using Spearman rank correlation. *B,* Correlation between the changes in the proportion of circulating CD8^{+} HLA-DR^{+} cells and change in serum LDL cholesterol level. Change in this panel reflects the difference of the changes during the atorvastatin phase and the placebo phase. Change was calculated for both serum LDL cholesterol and proportion of CD8^{+} HLA-DR^{+} T cells using the formula (XFS − XIS) − (XFP − XIP), where X is the measure of the outcome (ie, CD8^{+} HLA-DR^{+} T cells or serum LDL cholesterol level) at either the initial visit (I) (week 0) or the final visit (F) (week 8), while the participant was receiving either atorvastatin (S) or placebo (P). P values are calculated using the Spearman rank correlation.

activation or viral RNA levels. As shown in Figures 3A and 3B, there was no association between the reductions in serum LDL cholesterol level and viral RNA or proportions of CD8^{+} HLA-DR^{+} T lymphocytes.

Safety and Tolerability

Atorvastatin at 80 mg daily was well tolerated. No grade 3 or 4 elevations in liver-associated enzymes were observed during the study. Three participants had grade 3 elevations in creatinine phosphokinase (CPK) levels. All 3 participants reported myalgias and noted a temporal increase in their physical exercise regimens. Cessation of their altered exercise schedule, without discontinuing treatment assignments, resulted in resolution of the CPK elevations and symptoms.

DISCUSSION

Statin compounds exhibit anti-inflammatory and antiviral effects in vitro and therefore represent an attractive potential therapy for HIV-infected patients [5, 6, 8–10]. Prior in vivo studies evaluating the effects of statins on HIV-1 RNA levels have yielded conflicting results. In a single nonrandomized study, HIV-infected participants exhibited a significant decline with lovastatin therapy [6]. However, other prospective studies that evaluated pravastatin, simvastatin, and atorvastatin failed to demonstrate an effect on HIV-1 RNA [11, 12, 14]. The ability to discern a difference in HIV-1 RNA level after treatment with a statin in these studies may have been limited by the small sample size, as well as the nonrandomized, open-label, and observational nature of most of these studies. We therefore undertook a study to evaluate the effects of atorvastatin on HIV-1 RNA and its effects on cellular markers of activation.

Our study used a rigorous double-blind study design and was adequately powered to reveal a 0.3 log_{10} difference in HIV-1 RNA levels with use of atorvastatin; no consistent inhibitory effect on viremia was observed. Ongoing studies of host and viral factors may reveal possible sources for the heterogeneity of response to atorvastatin observed in our study. Nevertheless, our observations with statin therapy suggest that consistent and potent inhibition of late steps in virus replication will require more specific targeting.

It is unlikely that the choice of atorvastatin rather than lovastatin influenced the results of our study. Lovastatin is the only statin compound with demonstrated effects on HIV-1 RNA both in vivo and in vitro. Although the in vitro effects of lovastatin are mediated through several different mechanisms, including the inhibition of LFA-1 and ICAM-1 interactions, a property unique to lovastatin, the antiviral effects of lovastatin in vivo have been primarily attributed to the compound's effects on protein prenylation [6, 8, 21]. Atorvastatin was chosen for this study because it is a potent inhibitor of HMG-CoA reductase, with demonstrated effects on protein prenylation [22, 23].

The pharmacokinetic properties of atorvastatin might in part explain the observed discrepancy between the observed in vitro effects and lack of in vivo effects [23, 24]. Following the administration of an 80 mg dose of atorvastatin, maximum plasma concentrations are 12.5 ± 7.5 ng/mL [25]. In contrast, the concentrations used in vitro to disrupt lipid rafts are higher and in the micromolar range [5]. Extrapolation and direct comparisons of peak plasma levels and those used in vitro to disrupt lipid rafts have limitations, especially given the presence of metabolites (eg, 2 and 4 hydroxy atorvastatin) that retain similar bioequivalency and contribute to nearly 70% of the inhibitory activity observed [23, 24]. Nevertheless, the concentrations achieved in vivo with an 80 mg dose of atorvastatin are probably lower than those achieved in vitro and insufficient to consistently result in the disruption of these integral plasma membrane microdomains.

An additional limitation of our study is that we examined the effects of atorvastatin in viremic individuals. Given the recognized benefits of ART and the improved access to ART, it is likely that in the future a majority of patients will receive ART. Thus, studies designed to evaluate the adjunctive effects of statins in ART-treated individuals with viral suppression are likely to have clinical relevance.

Several studies have emphasized the importance of immune activation in the pathogenesis of HIV infection and its complications. Levels of inflammatory cytokines and cellular markers of activation independently correlate with disease progression in HIV-infected participants [26–29]. Chronic persistent immune activation contributes to the observed CD4 depletion in both untreated and successfully treated patients and plays a role in the pathogenesis of non-AIDS–related complications, such as chronic kidney and coronary artery disease (CAD) [19, 27, 30, 31]. Therefore, novel approaches designed to modulate the chronic persistent immune activation observed in both ART-treated and untreated individuals may be important. Because statins influence the chronic inflammation/activation observed in other disease states, we examined the effects of statins on cellular markers of activation [10, 32, 33]. In our study, atorvastatin treatment resulted in modest reductions in the proportion of HLA-DR$^+$ CD8$^+$ T cells. The selective effects of statins on major histocompatibility complex (MHC) class II expression, observed in our study, are consistent with prior results [33]. Because MHC class II expression is required for antigen presentation and T cell activation, our results suggest that the use of atorvastatin may influence the chronic immune activation observed in HIV-infected patients.

Statins influence multiple aspects of immune function, by influencing endothelial activation, leukocyte adhesion, and monocyte/macrophage and natural killer cell activation; statins may modulate the innate arm of the immune system and through their effects on T cell activation and regulatory T cell function can influence the adaptive arm of the immune system [33–40]. The clinical consequences of shifts in the immune parameters that we observed are not known; the immunopathogenesis of HIV involves a delicate and complex interplay between the innate and adaptive arms of the immune system. Additional large, adequately powered studies are necessary to address this question directly and to distinguish potential immune benefits from benefits of decreasing serum lipid levels.

We did not observe an overall effect on HIV-1 RNA levels; however, the participants who exhibited the greatest reductions in immune activation exhibited greater reductions in viremia. One potential explanation for this observation could be that, in some individuals, atorvastatin reduces the pool of activated T cells sufficiently to result in reductions in HIV-1 RNA [20, 41]. Alternatively, atorvastatin might have altered the balance between pro- and anti-inflammatory cytokines, favoring an anti-inflammatory milieu that favor reductions in viral replication [42, 43]. Although random variation in HIV-1 RNA is a potential explanation for this effect during the atorvastatin phase, the presence of a biological effect of atorvastatin in selected patients cannot be ruled out.

While a growing body of literature points to the immunomodulating effects of statins in HIV-uninfected patients [10], to our knowledge, our study is the first to reveal statin-induced changes in activated T cell subsets in HIV-infected participants. Although similar, and sometimes greater, reductions in immune activation have been observed with other agents [44–46], the safety and tolerability profile of the statin group of compounds remains an advantage.

In our study, we did not observe a correlation between the reductions in HLA-DR expression on CD8$^+$ T cells and the degree of decline in serum lipid levels. Our observations suggest that the effects on cellular activation are perhaps less dependent on the extent of cholesterol depletion, but rather depend on the nonlipid effect of statins. This is not entirely unexpected; anti-inflammatory effects of statins have been observed as early as 3 days after initiation of statin therapy, at a time when maximal benefits of lipid lowering would not be expected [47]. In fact, among participants with underlying CAD and those at high risk for CAD, the clinical benefits of statins extend beyond those ascribed to their lipid lowering affects alone [48, 49]. The statin group of compounds exert their immunomodulatory effects through multiple lipid-independent mechanisms, such as influencing protein prenylation and the functioning of the Rho and Rac family of GTPases and altering MHC class II expression and costimulatory molecule expression [32]. Larger and appropriately powered studies that specifically examine effects of lower doses of statins, that would not have a substantial effect on lipid levels but may affect immune parameters, are needed to evaluate this hypothesis.

In summary, although atorvastatin had no overall effect on HIV-1 RNA levels in our study, its use resulted in significant reductions in the proportion of activated CD8$^+$ T lymphocytes

as evidenced by a decrease in the proportion of HLA-DR$^+$, CD8$^+$ T cells. Our pilot study was not designed to demonstrate clinical benefit; large studies with longer durations of follow-up that are designed to evaluate the potential effects of statins are needed to investigate any clinical benefit of this drug for HIV-infected patients. The availability of generic formulations and the results of recent studies revealing a benefit to statin use in the primary prevention of CAD, a common complication in HIV-infected patients, make the continued study of statins as disease-modifying agents in HIV-infected individuals particularly attractive [48].

References

1. Johnson JA, Li JF, Wei X, et al. Minority HIV-1 drug resistance mutations are present in antiretroviral treatment-naive populations and associate with reduced treatment efficacy. PLoS Med 2008; 5:e158.
2. Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. Top HIV Med 2009; 17:118–23.
3. Friis-Moller N, Reiss P, Sabin CA, et al. Class of antiretroviral drugs and the risk of myocardial infarction. N Engl J Med 2007; 356:1723–35.
4. Pike LJ. Rafts defined: a report on the Keystone Symposium on Lipid Rafts and Cell Function. J Lipid Res 2006; 47:1597–8.
5. Ono A, Freed EO. Plasma membrane rafts play a critical role in HIV-1 assembly and release. Proc Natl Acad Sci U S A 2001; 98:13925–30.
6. del Real G, Jimenez-Baranda S, Mira E, et al. Statins inhibit HIV-1 infection by down-regulating Rho activity. J Exp Med 2004; 200:541–7.
7. Iyengar S, Hildreth JE, Schwartz DH. Actin-dependent receptor colocalization required for human immunodeficiency virus entry into host cells. J Virol 1998; 72:5251–5.
8. Giguere JF, Tremblay MJ. Statin compounds reduce human immunodeficiency virus type 1 replication by preventing the interaction between virion-associated host intercellular adhesion molecule 1 and its natural cell surface ligand LFA-1. J Virol 2004; 78:12062–5.
9. Gilbert C, Bergeron M, Methot S, Giguere JF, Tremblay MJ. Statins could be used to control replication of some viruses, including HIV-1. Viral Immunol 2005; 18:474–89.
10. Jain MK, Ridker PM. Anti-inflammatory effects of statins: clinical evidence and basic mechanisms. Nat Rev Drug Discov 2005; 4:977–87.
11. Probasco JC, Spudich SS, Critchfield J, et al. Failure of atorvastatin to modulate CSF HIV-1 infection: results of a pilot study. Neurology 2008; 71:521–4.
12. Sklar PA, Masur H, Grubb JR, et al. Pravastatin does not have a consistent antiviral effect in chronically HIV-infected individuals on antiretroviral therapy. AIDS 2005; 19:1109–11.
13. Waters L, Stebbing J, Jones R, et al. The effect of statins on HIV rebound and blips. J Acquir Immune Defic Syndr 2005; 39:637–8.
14. Moncunill G, Negredo E, Bosch L, et al. Evaluation of the anti-HIV activity of statins. AIDS 2005; 19:1697–700.
15. Ojesina AI, Chaplin B, Sankale JL, et al. Interplay of reverse transcriptase inhibitor therapy and gag p6 diversity in HIV type 1 subtype G and CRF02_AG. AIDS Res Hum Retroviruses 2008; 24:1167–74.
16. Dewar RL, Highbarger HC, Sarmiento MD, et al. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. J Infect Dis 1994; 170:1172–9.
17. Friedman LM, Furberg CD, Demets DL. Fundamentals of clinical trials. Littleon, MA: John Wright PSG Inc, 1983.
18. Berry PH, MacDonald JS, Alberts AW, et al. Brain and optic system pathology in hypocholesterolemic dogs treated with a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Am J Pathol 1988; 132:427–43.
19. Hunt PW, Martin JN, Sinclair E, et al. T cell activation is associated with lower CD4$^+$ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis 2003; 187:1534–43.
20. Margolick JB, Volkman DJ, Folks TM, Fauci AS. Amplification of HTLV-III/LAV infection by antigen-induced activation of T cells and direct suppression by virus of lymphocyte blastogenic responses. J Immunol 1987; 138:1719–23.
21. Weitz-Schmidt G. Lymphocyte function-associated antigen-1 blockade by statins: molecular basis and biological relevance. Endothelium 2003; 10:43–7.
22. Guijarro C, Blanco-Colio LM, Ortego M, et al. 3-Hydroxy-3-methylglutaryl coenzyme A reductase and isoprenylation inhibitors induce apoptosis of vascular smooth muscle cells in culture. Circ Res 1998; 83:490–500.
23. Poli A. Atorvastatin: pharmacological characteristics and lipid-lowering effects. Drugs 2007; 67(suppl 1):3–15.
24. Pfizer. Lipitor (atorvastatin calcium) tablets for oral administration prescribing information. www.pfizer.com/files/products/uspi_lipitor.pdf. Accessed 5 January 2011.
25. Borek-Dohalsky V, Huclova J, Barrett B, Nemec B, Ulc I, Jelinek I. Validated HPLC-MS-MS method for simultaneous determination of atorvastatin and 2-hydroxyatorvastatin in human plasma-pharmacokinetic study. Anal Bioanal Chem 2006; 386:275–85.
26. Giorgi JV, Hultin LE, McKeating JA, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. J Infect Dis 1999; 179:859–70.
27. Hunt PW, Brenchley J, Sinclair E, et al. Relationship between T cell activation and CD4$^+$ T cell count in HIV-seropositive individuals with undetectable plasma HIV RNA levels in the absence of therapy. J Infect Dis 2008; 197:126–33.
28. Ganesan A, Chattopadhyay PK, Brodie TM, et al. Immunologic and virologic events in early HIV infection predict subsequent rate of progression. J Infect Dis 201:272–84.
29. Rodger AJ, Fox Z, Lundgren JD, et al. Activation and coagulation biomarkers are independent predictors of the development of opportunistic disease in patients with HIV infection. J Infect Dis 2009; 200:973–83.

30. Gupta SK, Komarow L, Gulick RM, et al. Proteinuria, creatinine clearance, and immune activation in antiretroviral-naive HIV-infected subjects. J Infect Dis 2009; 200:614–8.

31. Crowe SM, Westhorpe CL, Mukhamedova N, Jaworowski A, Sviridov D, Bukrinsky M. The macrophage: the intersection between HIV infection and atherosclerosis. J Leukoc Biol 87:589–98.

32. Greenwood J, Steinman L, Zamvil SS. Statin therapy and autoimmune disease: from protein prenylation to immunomodulation. Nat Rev Immunol 2006; 6:358–70.

33. Kwak B, Mulhaupt F, Myit S, Mach F. Statins as a newly recognized type of immunomodulator. Nat Med 2000; 6:1399–402.

34. Weitz-Schmidt G, Welzenbach K, Brinkmann V, et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nat Med 2001; 7:687–92.

35. Mausner-Fainberg K, Luboshits G, Mor A, et al. The effect of HMG-CoA reductase inhibitors on naturally occurring CD4$^+$CD25$^+$ T cells. Atherosclerosis 2008; 197:829–39.

36. Methe H, Kim JO, Kofler S, Nabauer M, Weis M. Statins decrease Toll-like receptor 4 expression and downstream signaling in human CD14$^+$ monocytes. Arterioscler Thromb Vasc Biol 2005; 25:1439–45.

37. Niessner A, Steiner S, Speidl WS, et al. Simvastatin suppresses endotoxin-induced upregulation of Toll-like receptors 4 and 2 in vivo. Atherosclerosis 2006; 189:408–13.

38. Mulhaupt F, Matter CM, Kwak BR, et al. Statins (HMG-CoA reductase inhibitors) reduce CD40 expression in human vascular cells. Cardiovasc Res 2003; 59:755–66.

39. Yoshida M, Sawada T, Ishii H, et al. Hmg-CoA reductase inhibitor modulates monocyte-endothelial cell interaction under physiological flow conditions in vitro: involvement of Rho GTPase-dependent mechanism. Arterioscler Thromb Vasc Biol 2001; 21:1165–71.

40. Schonbeck U, Libby P. Inflammation, immunity, and HMG-CoA reductase inhibitors: statins as antiinflammatory agents? Circulation 2004; 109:II18–26.

41. Tong-Starksen SE, Luciw PA, Peterlin BM. Human immunodeficiency virus long terminal repeat responds to T cell activation signals. Proc Natl Acad Sci U S A 1987; 84:6845–9.

42. Diomede L, Albani D, Sottocorno M, et al. In vivo anti-inflammatory effect of statins is mediated by nonsterol mevalonate products. Arterioscler Thromb Vasc Biol 2001; 21:1327–32.

43. Chun TW, Engel D, Mizell SB, Ehler LA, Fauci AS. Induction of HIV-1 replication in latently infected CD4$^+$ T cells using a combination of cytokines. J Exp Med 1998; 188:83–91.

44. Rizzardi GP, Harari A, Capiluppi B, et al. Treatment of primary HIV-1 infection with cyclosporin A coupled with highly active antiretroviral therapy. J Clin Invest 2002; 109:681–8.

45. Vermeulen JN, Prins JM, Bunnik E, et al. Intravenous immunoglobulin (IVIG) treatment for modulation of immune activation in human immunodeficiency virus type 1 infected therapy-naive individuals. AIDS Res Hum Retroviruses 2007; 23:1348–53.

46. Lederman MM, Smeaton L, Smith KY, et al. Cyclosporin A provides no sustained immunologic benefit to persons with chronic HIV-1 infection starting suppressive antiretroviral therapy: results of a randomized, controlled trial of the AIDS Clinical Trials Group A5138. J Infect Dis 2006; 194:1677–85.

47. Link A, Ayadhi T, Bohm M, Nickenig G. Rapid immunomodulation by rosuvastatin in patients with acute coronary syndrome. Eur Heart J 2006; 27:2945–55.

48. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008; 359:2195–207.

49. Ray KK, Cannon CP, McCabe CH, et al. Early and late benefits of high-dose atorvastatin in patients with acute coronary syndromes: results from the PROVE IT-TIMI 22 trial. J Am Coll Cardiol 2005; 46:1405–10.