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Rosuvastatin reduces vascular inflammation and T cell and monocyte activation in HIV-infected subjects on antiretroviral therapy

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Abstract

Background—Despite suppressive antiretroviral therapy (ART), increased levels of immune activation persist in HIV-infected subjects. Statins have anti-inflammatory effects and may reduce immune activation in HIV disease.

Methods—SATURN-HIV is a randomized, double-blind, placebo-controlled trial assessing the effect of rosuvastatin (10mg/daily) on markers of cardiovascular risk and immune activation in ART-treated patients. T cell activation was measured by expression of CD38, HLA-DR, and PD1. Monocyte activation was measured with soluble markers (sCD14 and sCD163) and by enumeration of monocyte subpopulations and tissue factor (TF) expression. Markers of systemic and vascular inflammation and coagulation were also measured. SATURN-HIV is registered on clinicaltrials.gov, Identifier: NCT01218802

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Author Contributions

GM designed the study and obtained funding. N.T.F. J.R. B.C. S.J. B.F. performed experiments. N.S. D.L. M.L. and G.M. obtained patient samples. S.D. and Y.J. provided statistical support. All authors contributed to data analysis and writing of the manuscript.

Conflicts of Interest:

GAM served as a consultant, speaker, and has received research funding from Bristol-Myers Squibb, GlaxoSmithKline, Gilead, Merck, and Tibotec. GAM also chaired a DSMB for a Pfizer-funded study. All other authors declare no conflicts of interest.

Results—Rosuvastatin, compared to placebo, reduced sCD14 (−10.4% vs 0.5% $p=0.006$), Lp-PLA₂ (−12.2% vs −1.7% $p=0.0007$), and IP-10 (−27.5 vs −8.2%, $p=0.03$) levels after 48 weeks. The proportion of TF+ patrolling (CD14^{Dim}CD16+) monocytes was also reduced by rosuvastatin (−41.6%) compared to the placebo (−18.8%, $p=0.005$). There was also a greater decrease in the proportions of activated (CD38+HLA-DR+) T cells between the arms (−38.1% vs −17.8%, $p=0.009$ for CD4+ cells and −44.8% vs −27.4%, $p=0.003$ for CD8+ cells).

Conclusions—48 weeks of rosuvastatin treatment reduced significantly several markers of inflammation and lymphocyte and monocyte activation in ART-treated subjects.

Keywords

HIV-1; monocytes; T lymphocytes; inflammation; tissue factor; rosuvastatin

Introduction

Activation of the innate and adaptive immune system contributes to the progression of cardiovascular disease (CVD) in the general population^{1,2} and inflammation and immune activation are associated with mortality, including deaths related to CVD, in patients infected with the human immunodeficiency virus (HIV)^{3,4}. Immune activation may mediate HIV disease progression, vascular disease, diabetes^{3,5–8}, and an increased risk of both venous and arterial thrombosis^{9–20} in HIV-infected subjects. Lymphocyte activation, as measured by CD38 and HLA-DR expression on CD4+ and CD8+ T cells, is predictive of disease course in untreated HIV-infection²¹ and of CD4+ T cell reconstitution following initiation of antiretroviral therapy (ART)²². We have reported a direct relationship between the proportion of activated CD8+ T cells and mean common carotid artery (CCA) intima-media thickness (IMT) in HIV disease, and an increased proportion of activated CD8 + T cells in HIV-infected patients with coronary plaque (IMT>1.5cm) compared to these proportions in patients without plaque²³. In HIV-infected women, T cell activation was also associated with subclinical atherosclerosis^{24,25}, providing further evidence for a relationship between T cell activation in CVD risk in chronic HIV disease.

Several strategies to reduce chronic immune activation in treated HIV disease are underway, including this trial: Stopping Atherosclerosis and Treating Unhealthy bone with Rosuvastatin in HIV (SATURN-HIV). Statins, or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, have anti-inflammatory effects^{26,27}, and here, HIV-infected subjects receiving successful ART, and who had normal LDL-cholesterol levels, but elevated levels of immune activation, were randomized to receive rosuvastatin (10mg daily) or placebo. We have reported that 24 weeks of rosuvastatin treatment resulted in significant reductions in markers of monocyte subset activation²⁸ and in vascular inflammation (lipoprotein-associated phospholipase A 2, Lp-PLA₂).²⁹ Rosuvastatin had no effect on systemic inflammation or T cell activation;²⁸ results in discord with the findings of a small study where 8 week administration of high dose atorvastatin (80mg) reduced modestly the proportion of HLA-DR expressing CD8+ T cells³⁰ in patients not receiving ART. We hypothesized that rosuvastatin therapy may take longer to reduce T cell activation in treated subjects than in ART-naïve subjects, due to the lower levels of T cell activation generally reported in treated versus untreated HIV disease. Here, we present the results of a

pre-specified secondary analysis aimed at assessing the effects of statin administration on markers of immune activation and inflammation at 48 weeks.

Methods

Study Design

SATURN-HIV is a randomized, double-blind, placebo-controlled study designed to measure the effect of rosuvastatin on markers of cardiovascular risk, skeletal health, and immune activation in HIV disease, and is registered on clinicaltrials.gov, Identifier: NCT01218802. The study was approved by the Institutional Review Board of University Hospitals Case Medical Center (Cleveland, OH) and all subjects signed a written consent prior to enrollment. Randomization was conducted by the Case investigational pharmacist at 1:1 to active rosuvastatin 10 mg daily versus matching placebo. Randomization was stratified by protease inhibitor (PI) use. Study drugs (active and placebo) were provided by Astra Zeneca.

All subjects were 18 years of age, without known coronary disease or diabetes, and on stable ART for at least 3 months and cumulative ART duration of at least 6 months, with HIV-1 RNA <1,000 copies/mL and fasting LDL-cholesterol (LDL-C) 130mg/dL and fasting triglycerides 500mg/dL. Subjects were excluded if they had a history of myocardial infarction, were pregnant or lactating, or had another active or controlled inflammatory condition. Additional entry criteria included evidence of either heightened T-cell activation, identified as the proportion of CD8+ T cells that expressed CD38+HLA-DR+ 19%, or levels of high sensitivity C-reactive protein (hs-CRP) 2mg/L. The T cell activation cutoff (>19%) was determined based on our prior data linking this level of immune activation to immunologic failure in treated HIV infection³¹. The CRP cutoff was selected based on criteria established in the JUPITER statin trial²⁷.

Study evaluations

At screening visit, self-reported demographics and medical history were obtained, along with a targeted physical exam including height and weight measurements. Blood was drawn after a 12-hour fast for lipoproteins, and hs-CRP. Percent CD8+ T cell activation was determined as described below. If enrollment criteria were met, subjects returned within 30 days for entry evaluations. At entry, a fasting blood draw was obtained for a lipid profile and for measurement of soluble markers of immune activation. HIV-1 RNA levels and CD4+ cell counts were obtained as part of routine care.

Blood/sample preparation

Whole blood samples were collected into EDTA containing tubes. Peripheral blood mononuclear cell (PBMC) samples were separated by centrifugation with Ficoll-Hypaque and were cryopreserved until analyzed in batch. Plasma was isolated by centrifugation for 10 minutes at 400xg and was frozen at -80°C until thawed once and analyzed in batch.

Flow Cytometry

Monocyte phenotyping—Fresh whole blood (300µl) samples were incubated for 15 minutes on ice with FACS Lyse buffer (BD Biosciences, San Diego, CA) and were then

washed in buffer (phosphate buffered saline with 1% bovine serum albumin and 0.1% sodium azide). Cells were then stained for 30 minutes in the dark, on ice, and then washed in buffer and fixed in 1% paraformaldehyde. Monocyte subsets were identified by size, granularity, and by expression of CD14 and CD16³². Isotype gating was used to identify positive expression of surface markers. Cell surface molecule expression was monitored by staining cells with fluorochrome-labeled antibodies: anti-Tissue Factor (fluorescein isothiocyanate, FITC, American Diagnostica, Stamford, CT), anti-CD14 (Pacific Blue), anti-CD16 (phycoerythrin, PE, both from BD Pharmingen, San Diego, CA).

T cell phenotyping—The proportion of activated T cells required to determine eligibility was measured by analyses of freshly collected whole blood samples, processed as above. T cells were identified by size and granularity and by positive expression of CD3 and CD4 or CD8. T cell activation was measured using anti-CD38 (PE), anti-HLA-DR (FITC), anti-CD3 (Peridinin Chlorophyll Protein Complex, PerCP) anti-CD8 (allophycocyanin-cy7, APC-cy7), anti-CD4 (allophycocyanin, APC, all from BD Biosciences).

Assessment of T cell activation for the baseline/entry, 24, and 48 week timepoints was performed by comparing expression of surface markers on cryopreserved PBMC samples from each patient. Samples were thawed and analyzed in batch, in order to maintain uniformity of cytometer settings among timepoints for each patient. In addition to the T cell markers described above, analysis of frozen PBMC samples also included a stain for cell viability (Live/Dead Violet, Pacific Blue) and an additional activation marker PD-1 (PE-Cy7, BD Pharmingen).

Monocytes were analyzed in real time, on fresh whole blood samples, as above using a Miltenyi MACS Quant flow cytometer (MiltenyiBiotec, BergischGladbach, Germany). MACS Quantify software (version 2.21031.1, MiltenyiBiotec) was used to analyze the data. Initial assessment of T cell activation for screening/enrollment was performed using an LSR II flow cytometer (Becton-Dickinson, San Jose, CA) and FACSDiva software version 6.1.1 (BD Biosciences). Longitudinal T-lymphocyte activation was analyzed on the MACS Quant.

Plasma samples were collected at baseline, 24, and 48 weeks. Levels of soluble CD14 and CD163 were measured using Quantikine enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis MN). Levels of the proinflammatory cytokine IL-6 and soluble receptors of tumor necrosis factor α (sTNF-RI and sTNF-RII), interferon γ -inducible protein 10 (IP-10 or CXCL10), the cellular adhesion molecules soluble vascular cell adhesion molecule 1 (sVCAM-1) and soluble intercellular adhesion molecule 1 (sICAM-1), were determined by quantitative sandwich enzyme-linked immunosorbent assays (ELISAs) (R&D Systems, Minneapolis, MN). The D-dimer level was determined by immunoturbidimetric assay on a STA-R Coagulation Analyzer (Diagnostica-Stago, Parsippany, NJ). The fibrinogen level was determined by particle-enhanced immunonephelometric assay on a BNII nephelometer (Siemens). All interassay coefficients of variance were <12%, except for very low D-dimer values (<0.20 $\mu\text{g/mL}$), for which the interassay coefficient of variance was 21%. Intra assay coefficients of variance were not calculated for all but one marker because of the small number of samples in each run; for

IP-10, the interassay coefficient of variance was 3.3%. Plasma lipoprotein-associated phospholipase A2 (Lp-PLA2) concentrations were measured with an (ELISA; PLAC Test, diaDexus, South San Francisco, CA). Interassay and intra assay coefficients of variance were <5% and <9%, respectively. Calculated medians of the measurements were used in the analysis.

Statistical Methods

This was a pre-specified, pre-planned analysis to assess changes from baseline to 48-weeks in plasma inflammatory and coagulation indices and markers of lymphocyte and monocyte activation. All analyses were initially performed using intent-to-treat principles based on randomized treatment assignment which used all available data. Modifications to randomized treatment and missing values were ignored. As-treated analyses did not differ from intent-to-treat analyses; therefore, only the former data are presented.

Demographics, clinical characteristics, fasting metabolic parameters and inflammatory and coagulation markers are described by study group. Continuous measures are described by medians and interquartile ranges, and nominal variables are described with frequencies and percentages.

Nominal variables were compared using χ^2 analysis or Fisher's exact test. Continuous measures were tested for normality. For between-group comparisons (at baseline and changes from baseline to 48 weeks), normally-distributed variables were compared using t-tests, and non-normally-distributed variables were compared using Wilcoxon rank sum tests. For within-group changes from baseline to 48 weeks, normally-distributed variables were compared with a paired t-test, and non-normally-distributed variables were compared with Wilcoxon signed rank test. The non-parametric Spearman correlation was used to estimate correlations among changes of markers of inflammation and immune activation at week 48. Nominal p-values are presented throughout, with no correction for multiple comparisons.

All analyses were done using SAS 9.3. (SAS Institute Inc., Cary, NC, USA.)

Results

Baseline Characteristics

Two hundred and two patients were screened for enrollment; 55 patients were screen failures resulting in enrollment of 147 patients between March 2011 – August 2012 in the SATURN-HIV study (N=72 in the rosuvastatin arm, N=75 in the placebo arm, reported previously²⁸ and in Supplemental Figure 1). Demographic information and baseline characteristics are displayed in Table 1; indices were similar between groups. Overall, the median age of the patients was 47 years, 78% were male, and 70% were African American, 29% were Caucasian, and 1% was Hispanic. Baseline immune activation markers were also similar between groups, except for the proportion of CD14^{Dim}CD16⁺ monocytes that expressed TF (statin arm= 21.8% and placebo arm= 18.9%, p=0.047, Table 1); this difference was controlled for in the week 48 analysis. There was no difference in the number of subjects from each group on anti-hypertensive medication (statin: N=20; placebo: N=18,

P=0.60). Seven subjects had active hepatitis B (statin: N=3; placebo: N=4, P=0.74), and 12 subjects had active hepatitis C (statin: N=5; placebo: N=7, P=0.60).

Subject Disposition and safety data

Nineteen subjects (6 statin; 13 placebo) withdrew prior to the week 48 analysis: 16 were lost-to-follow-up. Two subjects withdrew due to Grade 2 myalgias with normal CPK levels; both were on placebo. One subject (also on placebo) was diagnosed with diabetes and dropped out of the study. One additional subject in the statin group stopped treatment at week 5 due to hospitalization for hydration to treat grade 3 myalgias without rhabdomyolysis or renal compromise, but continued to be followed on study, off study drug, and myalgia resolved soon after study drug was discontinued. Three subjects (all on placebo) changed ART regimens between baseline and 48 weeks: one was started on abacavir in place of didanosine, one switched truvada to epzicom, and another stopped lamivudine/zidovudine and started emtricitabine/tenofovir and maraviroc. Overall, 14 subjects (7 on statin and 7 on placebo) had HIV-1 RNA > 50 copies/mL at week 48. The patient flow chart is provided as Supplemental Figure 1. Neither HIV-1 RNA levels, CD4+ T cell counts, CD4/CD8 cell ratios, or their 48-week changes differed between the groups at 48 weeks.

Changes in Fasting Lipid levels

Lipid profiles in the rosuvastatin group changed by week 48, with a significant decrease in low-density lipoprotein levels (LDL, -23.4%, $p<0.0001$) and a non-significant increase in high-density lipoprotein (HDL, relative increase from baseline =0.7%, $p=0.76$) and triglyceride (5.5%, $p=0.47$). Patients receiving placebo did not have significant changes in these indices (HDL= 3.7%, $p=0.33$, LDL= 7.5%, $p=0.053$, and triglycerides= 4.2%, $p=0.51$). The difference between groups was significant only for the changes in LDL-C ($p<0.0001$ between groups).

Changes in markers of immune activation

There were several significant differences between treatment arms when comparing levels of cellular and plasma markers of immune activation at week 48 (Table 2), extending our findings from the 24 week timepoint^{28,29}. Rosuvastatin administration reduced levels of sCD14 by 10.4%, relative to baseline levels, while subjects receiving placebo had a 0.5% increase in sCD14 levels ($p=0.006$, Figure 1A). Subjects receiving rosuvastatin also had a greater reduction in the proportion of TF+ CD14^{Dim}CD16+ (patrolling) monocytes compared to the reduction seen in subjects receiving placebo (-41.6% vs -18.8%, $p=0.005$, Figure 1B). Among the patients receiving rosuvastatin, there were reductions from baseline levels in the proportions of TF expressing cells among all monocyte subsets (TF+ CD14+CD16-, -52.1%, $p=0.0002$, TF+ CD14+CD16+, -33.7%, $p<0.0001$, TF+ CD14^{Dim}CD16+, -41.6%, $p<0.0001$, Table 2). Proportions of all monocytes that expressed TF tended to decrease more in patients receiving a statin (-43.2%) compared to the decrease measured in patients receiving the placebo (-27.2%, $P=0.087$). Among patients in the active group, we did not see a significant relationship between changes in relative LDL levels and changes in the proportions of monocyte subsets that expressed tissue factor or the soluble markers of monocyte activation, sCD14 and sCD163 (Table 3).

Plasma levels of vascular inflammation (Lp-Pla₂) were reduced significantly from baseline in the statin arm (−12.2%) compared to the placebo arm (−1.7%, $p=0.0007$, Figure 1C). There was a modest relationship between relative changes in LDL and Lp-Pla₂ ($r=0.25$, $p=0.05$) among patients receiving rosuvastatin. There were no differences in the relative changes of several markers between the groups at week 48; however, many markers were reduced relative to baseline within the statin group, including levels of sCD163 (−12.3%, $p=0.001$), sTNF-RI (−0.7%, $p=0.03$), and fibrinogen (−6.0%, $p=0.014$). Also among the active group, the relative changes in VCAM-1, TNF-RII, and fibrinogen were all related (Table 3) and none of these changes were related to changes in LDL.

Plasma levels of interferon gamma-induced protein 10 (IP-10) were reduced significantly, relative to baseline levels, in patients receiving rosuvastatin (−27.5%), compared to levels in patients receiving placebo (−8.2%, $p=0.03$, Figure 3D). Relative changes in IP-10/CXCL10 levels among the statin group were related to relative changes in plasma D-dimer ($r=0.37$, $p=0.012$), VCAM-1 ($r=0.61$, $p<0.0001$), fibrinogen ($r=0.45$, $p=0.002$) and sCD163 ($r=0.34$, $p=0.022$) levels (Table 3).

We also assessed markers of T cell activation; proportions of both CD4+ and CD8+ T cells that express CD38 and HLA-DR were reduced significantly by statin treatment (−38.1% and −44.8%, respectively) compared to placebo (−7.8%, $p=0.0009$, and −27.4%, $p=0.0035$, Figure 2B,C). Proportions of cells that express CD38, HLA-DR and the T cell exhaustion marker PD-1 are also reduced by statin treatment compared to placebo, among CD4+ (−42.5% vs −24.1%, $p=0.0019$) and CD8+ T cells (−45.5% vs −26.3%, $p=0.0016$, Table 2). Relative changes in activation marker expression among CD4+ and CD8+ T cells in the active group were not related to changes in LDL or changes in viremia, but were directly related to changes in both IP-10 and VCAM-1 levels (Table 3).

Discussion

The SATURN-HIV study is the first double blind, randomized, placebo-controlled, clinical trial of a statin as an immunomodulatory therapy in HIV-infected subjects on suppressive ART. Overall, changes from baseline in monocyte and vascular activation markers at week 24 of treatment were similar at week 48^{28,29}. Rosuvastatin treatment, compared to placebo, reduced significantly plasma levels of sCD14, a marker that independently predicts morbidity in HIV disease⁶, by week 24, and this decline plateaued between weeks 24 and 48. Statin treatment also decreased levels of sCD163, but this decrease was not significantly different from the one measured in the placebo arm; further studies aimed at defining the potentially divergent mechanisms behind sCD14 and sCD163 induction may prove valuable. Rosuvastatin treatment reduced the proportion of circulating TF+ patrolling (CD14^{Dim}CD16+) monocytes by week 24, and this decline continued through 48 weeks. Expression of tissue factor can initiate the extrinsic clotting pathway³³, and we have shown previously that TF levels on monocytes are related to plasma levels of D-dimer³⁴, potentially linking monocyte activation to coagulation in HIV-infected subjects. Statin treatment reduced the proportion of TF positive monocytes in all subsets, but this reduction was not significantly different from reductions seen in placebo recipients among the traditional (CD14+CD16−) and inflammatory (CD14+ CD16+) subsets. The procoagulant

phenotype of monocyte subsets we report in HIV disease mirrors that of uninfected patients who have recently experienced an acute coronary event³², and proportions of CD16+ monocytes in HIV-infected and uninfected subjects have been linked to morbidity^{35,36}; linking monocyte activation to CVD progression in HIV disease.

Rosuvastatin treatment, compared to placebo treatment, also reduced significantly levels of Lp-Pla₂, at week 24, and this decline persisted through week 48. Lipoprotein-associated phospholipase A 2 is a specific marker of vascular inflammation and several studies have shown that increases in Lp-PLA₂ predict cardiovascular events in the general population^{37–39}. By reducing both the procoagulant phenotype of monocytes and inflammation of the vascular endothelium, which may act as a substrate for clot formation, one could speculate that rosuvastatin treatment may reduce coagulation risk in HIV disease.

Plasma levels of IP-10/CXCL10 were reduced from baseline by 48 weeks in the statin group compared to the placebo group; a result that was not seen at 24 weeks. Increased IP-10 levels have been associated with rapid disease progression in acute HIV-infection⁴⁰ and with immunologic treatment failure following ART initiation⁴¹.

Markers of T cell activation were reduced by 48 weeks of rosuvastatin treatment; this is a new result, as 24 weeks of statin treatment had little effect on these indices. Expression of CD38 and HLA-DR on CD8+ T cells is directly related to disease course in untreated HIV disease²¹, to plasma levels of LPS in HIV-infected patients^{32,42}, to proportions of CD16+ monocytes³², and inversely to CD4+ T cell reconstitution following initiation of ART²². Our previous work reported a direct relationship between the proportion of activated CD8+ T cells and mean CCA-IMT in HIV disease, and an increased proportion of activated CD8+ T cells among HIV-infected patients with coronary plaque (IMT>1.5cm) compared to those without plaque²³. Some T cells are capable of homing to vascular endothelium^{43,44}, and up to 50% of lymphocytes in human carotid artery plaques are activated CD8+ T cells⁴⁵. Ganesan et al reported a modest decrease (–3%) in the proportion of CD8+CD38+HLA-DR+ cells after ART-naïve patients received high dose (80mg) atorvastatin treatment³⁰. Here, for the first time, we show rosuvastatin treatment has beneficial effects on T cell activation (both CD4+ and CD8+) in subjects on ART. Another notable finding in our study is that rosuvastatin also resulted in significant reductions in the proportions of T cells that express PD-1, a marker reflective of cellular exhaustion and impaired proliferative capacity after T cell receptor engagement⁴⁶. By reducing PD-1 expression, statin treatment may enhance the functionality of T cells in HIV-infected patients.

Forty-eight weeks of statin treatment reduced cellular markers of T cell activation, monocyte activation, and circulating markers of immune activation, many of which have been linked to disease progression and cardiovascular risk in HIV disease. The decrease in levels of these markers was independent of changes in LDL-C levels, suggesting an anti-inflammatory effect of statins that is not related to an effect on lipid levels. While we cannot report the exact mechanism by which rosuvastatin treatment is reducing T cell, monocyte, and endothelial cell activation, one could speculate that rosuvastatin may be reducing the inflammatory response to microbial translocation by reducing Toll-like receptor 4 levels⁴⁷ or by increasing expression of Kruppel-like Factor 2, which can be modulated by statin

treatment and has anti-inflammatory effects in several cell types, including T cells⁴⁸, endothelial cells⁴⁹ and myeloid cells⁵⁰. The temporal relationship between the reduction in “innate immune activation” at 24 weeks (sCD14, monocyte activation) and the reduction in “adaptive immune activation” (T cell activation) at 48 weeks must also be investigated further. Mechanistic studies assessing the effects of rosuvastatin treatment on regulators of innate immune signaling and inflammation are planned; perhaps early, sustained alterations in innate activation provide beneficial effects to the immune system that enable T cell activation markers to decrease (CD38, HLA-DR) and T cell responsiveness to improve (lower PD-1 expression).

The SATURN-HIV study has generated several novel observations, but it has limitations. The study population was very specific: HIV+ subjects with stable ART and low or undetectable plasma HIV-1 RNA levels, normal LDL-cholesterol, and heightened baseline immune activation. These patients are also mostly African American men; these factors may potentially limit the generalizability of this result to the HIV-infected population as a whole. By reducing vascular inflammation, the procoagulant phenotype of monocytes, and markers of T cell activation, we would predict that statin treatment would reduce thrombotic risk, but this is planned for longer studies with clinical events as study endpoints. SATURN-HIV does provide though several potential directions for further mechanistic studies and provides rationale for a longer term, clinical endpoint study, exploring the effects of statin treatment on morbidity and mortality in chronic treated HIV disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Rosuvastatin treatment reduces levels of vascular inflammation and immune activation

Plasma samples were thawed and levels of soluble CD14, lipoprotein-associated phospholipase A2 (Lp-PLA₂), and interferon γ -inducible protein 10 (IP-10/CXCL10) were measured by ELISA. A) The monocyte activation marker sCD14 was also decreased in the statin arm (10.4%), while patients receiving placebo had an increase in sCD14 levels (0.5%) and this difference was significant between groups ($p=0.018$). B) Tissue factor expression on patrolling (CD14^{Dim}CD16⁺) monocytes was also reduced by statin treatment (-41.6%) and this change was significantly different from the change measured in the placebo arm (-18.8%, $p=0.005$). C) Patients receiving statin treatment had a relative reduction in Lp-PLA₂ levels from baseline (-12.2%) and this reduction was significantly greater than was the decrease measured in the placebo arm (-1.7%, $p=0.0007$). D) Patients receiving statin treatment had a relative reduction in IP-10 levels from baseline (-27.5%) and this reduction was significantly greater than was the decrease measured in the placebo arm (-8.2%, $p=0.027$).

Figure 2. Rosuvastatin treatment reduces markers of T cell activation

Measurement of T cell activation was performed by comparing expression of surface markers (CD38, HLA-DR, and PD-1) on cryopreserved PBMC samples from baseline, week 24 and 48 for each patient. Samples were thawed and analyzed in batch. The proportions of CD38+HLA-DR+ cells among A) CD4+ and B) CD8+ Tcells were reduced by rosuvastatin treatment (−38.1% and −44.8%) and these changes were statistically significantly different from the changes measured in the placebo arm (−17.8%, $p=0.0009$, and −27.4%, $p=0.004$).

Table 1
Baseline Demographic, Virologic, and Immunologic Indices

Median values and interquartile ranges are reported when appropriate. Of note, the median percentages of CD8+CD38+HLA-DR+ cells reported in this table (on study) are based on results from thawed PBMC samples; percentages used for screening were generated from fresh whole blood samples. Values generated from thawed samples are notoriously lower than those from fresh samples; this explains why the median values displayed here are less than the screen failure cutoff of 19%.

	Statin n=72	Placebo n=75
Age (yrs)	45.6 (41.1, 51.4)	46.9 (39.2, 53.6)
Male sex	81%	76%
African American race	71%	69%
Body Mass Index (kg/m ²)	26.6 (23.4, 30.0)	27.2 (23.5, 30.5)
Current Smoking	60%	72%
LDL cholesterol mg/dL	96 (76–107)	97 (77–121)
HDL cholesterol mg/dL	47 (38–58)	46 (37–57)
Hepatitis B + (%)	4%	5%
Hepatitis C+ (%)	7%	9%
Systolic blood pressure, mmHg	122 (112–136)	120 (110–132)
Diastolic blood pressure, mm Hg	79 (73–85)	80 (72–83)
Currently on anti-hypertensive medicine (%)	28%	24%
CD4+ count (cells/μl)	607.5 (439.5, 847.5)	627.0 (398.0, 853.0)
Nadir CD4+ count (cells/μl)	172.5 (83.5, 312.0)	189.5 (89.0, 281.0)
HIV-1 RNA <50 copies/mL	78%	77%
HIV duration (months)	133 (75, 199)	145 (73, 232)
Duration of ART(months)	63 (37, 119)	71 (39, 116)
On PI at entry	50%	48%
Duration of PI (months)	47 (13, 106)	39 (2, 80)
hsCRP (μg/mL)	1.6 (0.8, 4.9)	2.0 (0.7, 5.2)
IL-6 (pg/mL)	2.9 (1.9, 4.1)	2.7 (2.0, 5.3)
sTNF-RI (ng/mL)	1.59 (1.32, 2.15)	1.50 (1.23, 2.44)
sTNF-RII (ng/mL)	2.45 (1.78, 3.01)	2.16 (1.61, 2.62)
Lp-PLA2 (ng/mL)	165 (134, 199)	169, (142, 206)
IP-10/CXCL10 (pg/mL)	224.6 (169, 353)	219.4 (144, 344)
Fibrinogen (mg/dL)	380 (338, 512)	391 (331, 477)
ICAM (ng/mL)	223 (170, 318)	245 (172, 305)
VCAM (ng/mL)	697 (574, 827)	640 (533, 727)
D-dimer (μg/mL)	0.19 (0.13, 0.33)	0.18 (0.09, 0.29)
CD8+CD38+HLA-DR+ (%)	13.3 (9.0, 19.1)	11.5 (8.0, 16.5)
CD4+CD38+HLA-DR+ (%)	5.3 (3.7, 6.8)	5.1 (3.5, 6.3)
sCD14 (ng/mL)	2178 (1783, 2497)	2138 (1611, 2455)
sCD163 (ng/mL)	645 (501, 822)	651 (475, 901)

	Statin n=72	Placebo n=75
CD14+CD16+ monocytes (%)	22.9 (18.1, 34.0)	23.4 (18.6, 35.9)
CD14dimCD16+ monocytes (%)	12.7 (8.8, 15.5)	10.0 (7.5, 14.3)
CD14+CD16+TF+ (%)	13.4 (7.9, 17.4)	10.9 (7.7, 15.9)
CD14dimCD16+TF+ (%)	21.8(15.6, 29.6)	18.9 (12.6, 25.3)

Table 2

Summary data of changes in plasma and cellular markers of activation and inflammation from baseline to week 48

Plasma samples were thawed and levels of inflammatory markers and immune activation markers were measured by ELISA. Monocyte subsets were identified in fresh whole blood samples using size, granularity, and by expression of CD14 and CD16. Monocyte tissue factor expression was measured concurrently. Measurement of T cell activation was performed by comparing expression of surface markers (CD38, HLA-DR, and PD-1) on cryopreserved PBMC samples from baseline, week 24 and 48 for each patient. Samples were thawed and analyzed in batch. Median values and interquartile ranges are reported.

	Statin n=72		Placebo n=75		P value Between two groups
	Median (Q1, Q3)	Within group p value	Median (Q1, Q3)	Within group p value	
Inflammatory, Adhesion, and Coagulation molecules					
<i>hsCRP</i> (mg/L)	1.7% (-57.3, 69.4)	0.0256	28.6% (-30.2, 105.3)	0.0012	0.2018
<i>IL-6</i> (pg/mL)	-12.3% (-44.6, 24.1)	0.3144	-3.7% (-31.6, 21.4)	0.0980	0.4444
<i>sTNF-RI</i> (ng/mL)	-0.7% (-33.3, 16.5)	0.0331	3.8% (-32.1, 14.7)	0.0703	0.6764
<i>sTNF-RII</i> (ng/mL)	25.5% (-3.6, 54.1)	<0.0001	34.8% (15.6, 55.8)	<0.0001	0.0908
<i>IP-10/CXCL10</i> (pg/mL)	-27.5% (-40.9, -3.2)	0.0208	-8.2% (-28.4, 23.5)	0.7811	0.0268
<i>Lp-PLA2</i> (ng/mL)	-12.2% (-25.0, -4.9)	<0.0001	-1.7% (-13.5, 7.5)	0.5921	0.0007
<i>ICAM</i> (ng/mL)	4.9% (-6.3, 13.9)	0.0751	3.1% (-10.1, 14.6)	0.3816	0.8300
<i>VCAM</i> (ng/mL)	3.1% (-10.1, 15.0)	0.1073	8.3% (-10.0, 24.2)	0.0024	0.1968
<i>D-dimer</i> (µg/mL)	19.7% (-16.7, 120)	0.3305	37.5% (-28.9, 84.6)	0.0089	0.9923
<i>Fibrinogen</i> (mg/dL)	-6.0% (-16.6, 4.7)	0.0147	-2.6% (-7.6, 8.1)	0.9919	0.0643
Lymphocyte Activation					
<i>CD8+CD38+HLA-DR+</i> (%)	-44.8% (-56.9, -21.1)	<0.0001	-27.4% (-45.5, -1.6)	0.0188	0.0035
<i>CD8+CD38+</i> (%)	-29.8% (-41.7, -19.0)	<0.0001	-19.5% (-34.7, -7.0)	<0.0001	0.0178
<i>CD8+CD38+HLA-DR+PDI+</i> (%)	-45.5% (-58.3, -23.4)	<0.0001	-26.3% (-47.6, 28.8)	0.9325	0.0016
<i>CD4+CD38+HLA-DR+</i> (%)	-38.1% (-53.0, -15.8)	<0.0001	-17.8% (-39.50)	0.0037	0.0009
<i>CD4+CD38+</i> (%)	-15.3% (-22.2, -6.1)	0.0012	-13.6% (-23.4 -6.8)	<0.0001	0.8337
<i>CD4+CD38+HLA-DR+PDI+</i> (%)	-42.5% (-57.2, -22.7)	0.0020	-24.1% (-43.0, 17.5)	0.3502	0.0019
Monocyte Activation					
<i>sCD14</i> (ng/mL)	-10.4% (-27.9, 8.7)	0.0179	0.5% (-7.7, 20.1)	0.0882	0.0056
<i>sCD163</i> (ng/mL)	-12.3% (-29.5, 0.8)	0.0099	-8.6% (-26.6, 9.9)	0.1566	0.6863

	Statin n=72		Placebo n=75		P value Between two groups
	Median (Q1, Q3)	Within group p value	Median (Q1, Q3)	Within group p value	
CD14+CD16+ monocytes (%)	-1.9% (-41.6, 29.4)	0.3081	-6.0% (-25.9, 24.1)	0.3701	0.9814
CD14dimCD16+ monocytes (%)	-7.5% (-35.1, 35.8)	0.1810	3.4% (-25.8, 43.6)	0.0387	0.2985
CD14+CD16-TF+ (%)	-52.1% (-64.1, -11.2)	0.0002	-31.2% (-67.3, 36.6)	0.5900	0.1739
CD14+CD16+TF+ (%)	-33.7% (-62.3, -7.8)	<0.0001	-29.9% (-57.6, 24.8)	0.4812	0.1978
CD14dimCD16+TF+ (%)	-41.6% (-56.7, 3.1)	<0.0001	-18.8% (-42.5, 21.0)	0.7998	0.0049

Table 3
Correlations between relative changes of markers of inflammation and immune activation, at week 48

Associations between relative changes among plasma and cellular markers were assessed using Spearman's correlations. Correlations that were significant ($p < 0.05$) are highlighted in grey.

	sTNF-RI (ng/mL)	sTNF-RII (ng/mL)	IP-10/CXCL10 (pg/mL)	Lp-PLA2 (ng/mL)	VCAM (ng/mL)	D-dimer (μg/mL)	Fibrinogen (mg/dL)	CD4+CD38+ HLA-DR+ (%)	CD8+CD38+ HLA-DR+ (%)	sCD14 (ng/mL)	sCD163 (ng/mL)	CD14dim CD16+TF+ (%)	LDL (mg/dL)
sTNF-RI (ng/mL)													
sTNF-RII (ng/mL)	$r = -0.09, p = 0.47$												
IP-10/CXCL10 (pg/mL)	$r = 0.13, p = 0.39$	$r = 0.27, p = 0.07$											
Lp-PLA2 (ng/mL)	$r = -0.20, p = 0.1$	$r = -0.05, p = 0.68$	$r = 0.08, p = 0.59$										
VCAM (ng/mL)	$r = 0.05, p = 0.67$	$r = 0.4, p < 0.001$	$r = 0.64, p < 0.001$	$r = 0.17, p = 0.17$									
D-dimer (μg/mL)	$r = -0.11, p = 0.37$	$r = 0.08, p = 0.51$	$r = 0.37, p = 0.01$	$r = 0.19, p = 0.13$	$r = 0.19, p = 0.13$								
Fibrinogen (mg/dL)	$r = -0.09, p = 0.45$	$r = 0.42, p < 0.001$	$r = 0.45, p < 0.01$	$r = 0.06, p = 0.64$	$r = 0.37, p < 0.01$	$r = 0.06, p = 0.64$							
CD4+CD38+ HLA-DR+ (%)	$r = 0.24, p = 0.06$	$r = 0.13, p = 0.32$	$r = 0.31, p = 0.04$	$r = 0.04, p = 0.78$	$r = 0.27, p = 0.03$	$r = 0.21, p = 0.10$	$r = 0.11, p = 0.42$						
CD8+CD38+ HLA-DR+ (%)	$r = 0.16, p = 0.21$	$r = 0.20, p = 0.12$	$r = 0.43, p < 0.01$	$r = 0.12, p = 0.36$	$r = 0.34, p < 0.01$	$r = 0.17, p = 0.19$	$r = 0.25, p = 0.06$	$r = 0.76, p < 0.001$					
sCD14 (ng/mL)	$r = 0.25, p = 0.1$	$r = 0.09, p = 0.57$	$r = 0.05, p = 0.76$	$r = 0.27, p = 0.07$	$r = 0.18, p = 0.22$	$r = -0.08, p = 0.60$	$r = 0.06, p = 0.7$	$r = 0.08, p = 0.60$	$r = 0.01, p = 0.97$				
sCD163 (ng/mL)	$r = 0.40, p = 0.01$	$r = 0.04, p = 0.81$	$r = 0.34, p = 0.02$	$r = 0.13, p = 0.38$	$r = 0.27, p = 0.07$	$r = -0.01, p = 0.97$	$r = 0.33, p = 0.02$	$r = 0.12, p = 0.43$	$r = 0.05, p = 0.75$	$r = 0.15, p = 0.34$			
CD14dim CD16+TF+ (%)	$r = 0.04, p = 0.75$	$r = 0.03, p = 0.80$	$r = -0.04, p = 0.79$	$r = -0.00, p = 0.97$	$r = -0.04, p = 0.78$	$r = -0.22, p = 0.08$	$r = 0.08, p = 0.54$	$r = 0.16, p = 0.21$	$r = -0.02, p = 0.85$	$r = 0.07, p = 0.67$	$r = 0.05, p = 0.77$		
LDL (mg/dL)	$r = -0.15, p = 0.21$	$r = 0.10, p = 0.44$	$r = 0.14, p = 0.35$	$r = 0.25, p = 0.05$	$r = 0.23, p = 0.07$	$r = 0.00, p = 0.97$	$r = 0.23, p = 0.07$	$r = -0.08, p = 0.53$	$r = 0.16, p = 0.21$	$r = -0.05, p = 0.74$	$r = 0.02, p = 0.90$	$r = 0.03, p = 0.81$	