Published in final edited form as:

AIDS. 2014 November 13; 28(17): 2627–2631. doi:10.1097/QAD.0000000000000475.

Effects of Atorvastatin and Pravastatin on Immune Activation and T-Cell Function in ART-suppressed HIV-1 Infected Patients

Edgar Turner Overton^{#1}, Sarah Sterrett^{#1}, Andrew O. Westfall¹, Shannon M. Kahan², Greer Burkholder¹, Allan J. Zajac², Paul A. Goepfert^{1,2}, and Anju Bansal¹

¹ Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, 35294 USA

² Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL, 35294 USA

These authors contributed equally to this work.

Abstract

This retrospective study was designed to assess statin effects on T-cell activation from HIV-infected individuals. PBMC from ART-suppressed HIV-infected individuals receiving atorvastatin or pravastatin were evaluated for T-cell activation, exhaustion and function. Atorvastatin was associated with a significant reduction in CD8 T-cell activation (HLA-DR, CD38/HLA-DR) and exhaustion (TIM-3, TIM-3/PD-1) while pravastatin had no effect. In contrast, pravastatin increased antigen specific IFN- γ production. These results suggest a differential effect of statins on immune activation and function.

Keywords

HIV-1; Atorvastatin; Pravastatin; ART; immune activation; exhaustion; T-cells

RESEARCH LETTER

Immune activation contributes to HIV pathogenesis [1-10]. Even with suppressive antiretroviral therapy (ART), immune activation persists and remains elevated compared to HIV-uninfected individuals [11-15]. While statins primarily serve as lipid-lowering therapy [16, 17], they also reduce T-cell surface signaling molecules and decrease T-cell activation [18-21]. Recent studies demonstrate that statins reduce T-cell activation and are associated with decreased morbidity and mortality among HIV-infected persons [22-24]. Specifically, atorvastatin significantly reduced T-cell activation in viremic HIV-infected individuals [25].

CORRESPONDING AUTHOR: Anju Bansal, Department of Medicine, University of Alabama at Birmingham, 908, 20th Street South, CCB 334, Birmingham, AL 35294, Phone: 205-996-2214, Fax 205-975-6027, abansal@uab.edu.

CONFLICT OF INTEREST:

All authors declare no financial conflict of interest for this study.

AUTHOR CONTRIBUTIONS

E.T.O., A.J.Z., P.A.G., and A.B. contributed to the conception and design of the experiments; S.S. and S.M.K. performed the experiments; A.B. and S.S. analyzed the data; A.O.W. performed the statistical analysis of the data; E.T.O., G.B., and P.A.G. provided clinical care for the patients; G.B., helped identify patients for the study. E.T.O., and A.B. wrote the manuscript. A.B. supervised the entire project.

Here, we evaluated the effects of pravastatin and atorvastatin on T- cell activation and exhaustion in ART-suppressed individuals.

We used cryopreserved samples from 21 HIV-infected persons who were virologically suppressed (<50 copies/ml) with ART for >12 months (range, 20-137) and remained suppressed throughout follow-up [26]. Seventeen received adjunctive statin therapy for >6 months (range, 3-32): seven subjects received 10mg atorvastatin; 10 received pravastatin (5 with 20 mg; 5 with 40 mg). A median viability of 97% and recovery at 87% was observed for PBMC samples used in this study. As a control group, we identified four ART-suppressed individuals (median CD4 T-cell count: 498 c/mm³; median duration of ART: 119 weeks) not receiving statins. The study was approved by the IRB at UAB.

To assess statin effects on T-cell proliferation, we performed *in-vitro* assays as previously described [27] using cryopreserved PBMC from four statin-naïve individuals. We compared *invitro* effects of three statins on T-cell proliferation in response to SEB using cells from four ART-suppressed controls (no statin). Compared to pravastatin, both atorvastatin and rosuvastatin significantly suppressed SEB mediated CD4 and CD8 T-cell proliferation which was partially restored with exogenous mevalonate (Figure 1A-B).

Ex-vivo analyses of expression levels of activation (CD38, HLA-DR), exhaustion (PD-1, TIM-3), and memory (CD27, CD45RO) markers were performed on cryopreserved PBMC from the 17 subjects receiving adjuvant statins before and during statin therapy and the 4 controls using surface staining with fluorochrome conjugated antibodies for specific cell surface markers [27]. Clinical and demographic features were not significantly different between groups (data not shown). Hyperlipidemia was the indication for statin use in all subjects; none were diabetic. In addition, baseline levels of immune activation and exhaustion were similar between the two groups. CD8 T-cell activation (HLA-DR and HLA-DR/CD38) and exhaustion (TIM-3) markers were significantly reduced following atorvastatin but not pravastatin treatment (Figure 1C-D). A significant reduction of CD4 T-cell exhaustion markers (TIM-3 and PD-1) was also observed with atorvastatin ($p<0.05$). We did not identify any sex-specific differences in activation or exhaustion marker expression. To evaluate whether ART alone explained these changes, we measured changes in expression levels on PBMC from four virologically suppressed and statin naïve individuals. We identified no changes in activation (CD38, HLA-DR) or exhaustion markers (TIM-3, PD1), suggesting that ART therapy alone did not dictate our findings.

We measured CD27 and CD45RO expression to assess changes in memory T-cell subsets: naïve (CD27+CD45RO+), intermediate (CD27+CD45 RO-), late (CD27-CD45RO+) and terminally differentiated (CD27-CD45RO-) phenotypes [28]. A shift to an earlier stage of differentiation and a reduction in terminally differentiated subset was noted with atorvastatin but no change with pravastatin (Figure 1E-F). Atorvastatin related reduction of activation marker expression was restricted to terminally differentiated (CD27-CD45RO-) subset of CD8 T-cells (HLA-DR (44% decline, $p=0.063$) and CD38/HLA-DR (55% decline, $p=0.031$); data not shown. These differences were not explained by different proportions of terminally differentiated CD8 T-cells between the two groups at the pre or on statin time points ($p=0.13$, Figure 1 E-F). By contrast, CD4 T-cell activation (HLA-DR and CD38/

HLA-DR) increased with pravastatin in the early differentiated subset (CD27+CD45RO+ ; $p = 0.0312$), but this change was not reflected in the total CD4 T-cell population (data not shown).

Since atorvastatin was able to reduce markers associated with chronic T cell activation and exhaustion, we next evaluated whether statin treatments improved T-cell functionality. When PBMC were antigenically stimulated with HIV (gag) or CMV (pp65) peptide pools, increased proliferation and IFN- γ production by CMV specific CD4 and CD8 T cell subsets was observed only for the pravastatin group (data not shown). No functional changes for HIV were observed in either statin group.

Our study demonstrates a differential effect of atorvastatin and pravastatin on T-cell activation, exhaustion and function. Atorvastatin significantly reduced markers of activation and exhaustion on T-cell populations; an effect most pronounced on terminally differentiated effector memory CD8 T-cells. Given that this T-cell subset contributes to the detrimental inflammatory state of chronic viral infections, these changes may be very important in the context of HIV infection, even in persons with suppressed viremia [29, 30]. We focused on virologically suppressed individuals given persistent immune activation. Similar to data from viremic subjects, we found reduced CD8+ T cell activation markers with atorvastatin [25]. This appears to be statin-specific, as pravastatin had no effect on these markers.

Atorvastatin, but not pravastatin, reduced markers of exhaustion (TIM-3 and TIM-3/PD1) on CD8 T-cells. PD-1 is upregulated on both CD4 and CD8 T-cells during chronic HIV infection even after ART administration and has been correlated with functional T-cell exhaustion in chronic viral infections [31]. Reducing PD-1 and TIM-3 expression may restore T-cell function [32, 33]. Whether the reduction of these inhibitory pathways by statins will yield improved immune responses needs further evaluation.

Limitations of our study include its small sample, non-randomized approach, and baseline CD4 count differences. Speculatively, since the pravastatin group had higher CD4 pre-and on-statin CD4 count, this group might exhibit lower levels of immune activation prior to statin initiation. However, baseline expression of markers was not different between groups (data not shown). In addition, a comparison of activation markers in patients (CD4 $<500\text{c/mm}^3$) showed similar patterns as shown in figure 1C-D which suggests that a higher CD4 count in pravastatin was not responsible for a lack of detectable impact on the activation/exhaustion markers.

In conclusion, atorvastatin reduced markers of T cell activation and exhaustion in virologically suppressed HIV-infected individuals. Given the anti-inflammatory effects of statins and the recent ACC/AHA guidelines suggesting the benefits of statins extend beyond lipid-lowering, future research will assess which statin provides the greatest benefit for HIV-infected persons.

ACKNOWLEDGEMENTS

We are indebted to the participants enrolled in the CNICS studies at UAB. We are also very grateful to Marion Spell and the UAB CFAR Flow core for technical support and also to Tiffanie Mann for excellent technical assistance.

FINANCIAL SUPPORT:

This work was supported by the NIH-funded CNICS (R24 AI067039). Flow cytometry was performed, in part, in the UAB Center for AIDS Research Flow Cytometry Core, which is funded by NIH grant P30 AI027767. AZ was supported by AI099867 and SK was supported in part by training grant AI007051.

PRESENTATION OF WORK:

This work was presented at the Immune Activation in HIV Infection: Basic Mechanisms and Clinical Implications meeting (poster # 1008) held at Breckenridge, Colorado, April 3 -8th, 2013.

REFERENCES

1. Ascher A, M. S. Sheppard HW. AIDS as immune system activation: a model for pathogenesis. Clin Exp Immunol. 1988; 73:165–167. [PubMed: 3263225]
2. Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, Jacobson LP, 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; 179:859–870. [PubMed: 10068581]
3. Plaeger SF, Collins BS, Musib R, Deeks SG, Read S, Embry A. Immune activation in the pathogenesis of treated chronic HIV disease: a workshop summary. AIDS Res Hum Retroviruses. 2012; 28:469–477. [PubMed: 21854232]
4. Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RM. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. J Immunol. 2002; 169:3400–3406. [PubMed: 12218162]
5. Hearps AC, Maisa A, Cheng WJ, Angelovich TA, Lichtfuss GF, Palmer CS, et al. HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy. AIDS. 2012; 26:843–853. [PubMed: 22313961]
6. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, et al. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. J Infect Dis. 2009; 199:1177–1185. [PubMed: 19265479]
7. Lederman MM, Calabrese L, Funderburg NT, Clagett B, Medvik K, Bonilla H, et al. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. J Infect Dis. 2011; 204:1217–1226. [PubMed: 21917895]
8. Burdo TH, Lo J, Abbara S, Wei J, DeLelys ME, Preffer F, et al. Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. J Infect Dis. 2011; 204:1227–1236. [PubMed: 21917896]
9. Ledwaba L, Tavel JA, Khabe P, Maja P, Qin J, Sangweni P, et al. Pre-ART levels of inflammation and coagulation markers are strong predictors of death in a South African cohort with advanced HIV disease. PLoS One. 2012; 7:e24243. [PubMed: 22448211]
10. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, et al. Plasma levels of soluble CD14 independently predict mortality in HIV infection. J Infect Dis. 2011; 203:780–790. [PubMed: 21252259]
11. French MA, King MS, Tschampa JM, da Silva BA, Landay AL. Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T cells. J Infect Dis. 2009; 200:1212–1215. [PubMed: 19728788]
12. Gandhi RT, Spritzler J, Chan E, Asmuth DM, Rodriguez B, Merigan TC, et al. Effect of baseline- and treatment-related factors on immunologic recovery after initiation of antiretroviral therapy in

- HIV-1-positive subjects: results from ACTG 384. *J Acquir Immune Defic Syndr*. 2006; 42:426–434. [PubMed: 16810109]
13. Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, Lampiris H, 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; 187:1534–1543. [PubMed: 12721933]
 14. Robbins GK, Spritzler JG, Chan ES, Asmuth DM, Gandhi RT, Rodriguez BA, et al. Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 384. *Clin Infect Dis*. 2009; 48:350–361. [PubMed: 19123865]
 15. Valdez H, Connick E, Smith KY, Lederman MM, Bosch RJ, Kim RS, et al. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. *AIDS*. 2002; 16:1859–1866. [PubMed: 12351945]
 16. Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA*. 2001; 285:2486–2497. [PubMed: 11368702]
 17. Stone NJ, Robinson J, Lichtenstein AH, Merz CN, Blum CB, Eckel RH, et al. 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2013
 18. Chung HK, Lee IK, Kang H, Suh JM, Kim H, Park KC, et al. Statin inhibits interferon-gamma-induced expression of intercellular adhesion molecule-1 (ICAM-1) in vascular endothelial and smooth muscle cells. *Exp Mol Med*. 2002; 34:451–461. [PubMed: 12526087]
 19. Li D, Chen H, Romeo F, Sawamura T, Saldeen T, Mehta JL. Statins modulate oxidized low-density lipoprotein-mediated adhesion molecule expression in human coronary artery endothelial cells: role of LOX-1. *J Pharmacol Exp Ther*. 2002; 302:601–605. [PubMed: 12130721]
 20. Omi H, Okayama N, Shimizu M, Fukutomi T, Imaeda K, Okouchi M, et al. Statins inhibit high glucose-mediated neutrophil-endothelial cell adhesion through decreasing surface expression of endothelial adhesion molecules by stimulating production of endothelial nitric oxide. *Microvasc Res*. 2003; 65:118–124. [PubMed: 12686169]
 21. Greenwood J, Mason JC. Statins and the vascular endothelial inflammatory response. *Trends Immunol*. 2007; 28:88–98. [PubMed: 17197237]
 22. Chao C, Xu L, Abrams DI, Towner WJ, Horberg MA, Leyden WA, et al. HMG-CoA reductase inhibitors (statins) use and risk of non-Hodgkin lymphoma in HIV-positive persons. *AIDS*. 2011; 25:1771–1777. [PubMed: 21681055]
 23. Moore RD, Bartlett JG, Gallant JE. Association between use of HMG CoA reductase inhibitors and mortality in HIV-infected patients. *PLoS One*. 2011; 6:e21843. [PubMed: 21765919]
 24. Overton ET, Kitch D, Benson CA, Hunt PW, Stein JH, Smurzynski M, et al. Effect of statin therapy in reducing the risk of serious non-AIDS-defining events and nonaccidental death. *Clin Infect Dis*. 2013; 56:1471–1479. [PubMed: 23386631]
 25. Ganesan A, Crum-Cianflone N, Higgins J, Qin J, Rehm C, Metcalf J, et al. 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. *J Infect Dis*. 2011; 203:756–764. [PubMed: 21325137]
 26. Kitahata MM, Rodriguez B, Haubrich R, Boswell S, Mathews WC, Lederman MM, et al. Cohort profile: the Centers for AIDS Research Network of Integrated Clinical Systems. *Int J Epidemiol*. 2008; 37:948–955. [PubMed: 18263650]
 27. Bansal A, Jackson B, West K, Wang S, Lu S, Kennedy JS, et al. Multifunctional T-cell characteristics induced by a polyvalent DNA prime/protein boost human immunodeficiency virus type 1 vaccine regimen given to healthy adults are dependent on the route and dose of administration. *J Virol*. 2008; 82:6458–6469. [PubMed: 18448544]
 28. Riou C, Treurnicht F, Abrahams MR, Mlisana K, Liu MK, Goonetilleke N, et al. Increased memory differentiation is associated with decreased polyfunctionality for HIV but not for cytomegalovirus-specific CD8+ T cells. *J Immunol*. 2012; 189:3838–3847. [PubMed: 22966086]

29. Desai S, Landay A. Early immune senescence in HIV disease. *Curr HIV/AIDS Rep.* 2010; 7:4–10. [PubMed: 20425052]
30. Fuller MJ, Khanolkar A, Tebo AE, Zajac AJ. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. *J Immunol.* 2004; 172:4204–4214. [PubMed: 15034033]
31. Breton G, Chomont N, Takata H, Fromentin R, Ahlers J, Filali-Mouhim A, et al. Programmed death-1 is a marker for abnormal distribution of naive/memory T cell subsets in HIV-1 infection. *J Immunol.* 2013; 191:2194–2204. [PubMed: 23918986]
32. Velu V, Titanji K, Zhu B, Husain S, Pladevega A, Lai L, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature.* 2009; 458:206–210. [PubMed: 19078956]
33. Sakhdari A, Mujib S, Vali B, Yue FY, MacParland S, Clayton K, et al. Tim-3 negatively regulates cytotoxicity in exhausted CD8+ T cells in HIV infection. *PLoS One.* 2012; 7:e40146. [PubMed: 22792231]

Figure 1. A-B. *In-vitro* effects of statins on T-cell proliferation

For *in-vitro* experiments, PBMC from 4 HIV infected individuals with undetectable viral load on ART were CFSE labelled and stimulated with SEB for 5 days in the presence of the indicated statins, with or without mevalonic acid (MA, +/-). Experiments were performed in triplicate and data are expressed as a % of SEB only stimulated proliferation in CD4 (A) and CD8 (B) T-cell subsets. Proliferation in the presence of atorvastatin and rosuvastatin (without mevalonic acid) was significantly different from the pravastatin group in a Wilcoxon Rank Sum test (** $p < 0.01$). C-F. *In-vivo* effects of statins on markers of immune

activation and exhaustion in T-cells. The *in-vivo* effect of atorvastatin and pravastatin on markers of T cell activation and immune exhaustion are shown in panels **C-D**. The expression of CD38 and HLA-DR (**C**) and TIM3 and PD1 (**D**) is shown as percentage change in marker expression relative to pre-statin i.e. baseline (mean+ SEM). The two statin groups were compared by the Mann-Whitney U test (*p 0.05). The proportion of the four CD8 T cell subsets i.e. naive (CD27+CD45RO+), intermediate (CD27+CD45RO-), late (CD27-CD45RO+), and terminally (CD27-CD45RO-) differentiated subsets before (pre) and during (on) pravastatin and atorvastatin treatment are shown as pie charts (**E** and **F** respectively).