J. Neurovirol. (2016) 22:584–596
DOI 10.1007/s13365-016-0433-8

Statin modulation of monocyte phenotype and function: implications for HIV-1-associated neurocognitive disorders

Anjana Yadav[1] · **Michael R. Betts**[2] · **Ronald G. Collman**[1,2]

Received: 24 September 2015 / Revised: 8 February 2016 / Accepted: 19 February 2016 / Published online: 28 March 2016

Abstract HIV-1-associated neurocognitive disorder (HAND) remains a persistent problem despite antiretroviral therapy (ART), largely a result of continued inflammation in the periphery and the brain and neurotoxin release from activated myeloid cells in the CNS. CD14+CD16+ inflammatory monocytes, expanded in HIV infection, play a central role in the pathogenesis of HAND and have parallels with monocyte-dependent inflammatory mechanisms in atherosclerosis. Statins, through their HMG-CoA reductase inhibitor activity, have pleiotropic immunomodulatory properties that contribute to their benefit in atherosclerosis beyond lipid lowering. Here, we investigated whether statins would modulate the monocyte phenotype and function associated with HIV-1 neuropathogenesis. Treatment ex vivo with simvastatin and atorvastatin reduced the proportion of CD16+ monocytes in peripheral blood mononuclear cells, as well as in purified monocytes, especially CD14++CD16+ "intermediate" monocytes most closely associated with neurocognitive disease. Statin treatment also markedly reduced expression of CD163, which is also linked to HAND pathogenesis. Finally, simvastatin inhibited production of monocyte chemoattractant protein-1 (MCP-1) and other inflammatory cytokines following LPS stimulation and reduced monocyte chemotaxis in response to MCP-1, a major driver of myeloid cell accumulation in the CNS in HAND. Together, these findings suggest that statin drugs may be useful to prevent or reduce HAND in HIV-1-infected subjects on ART with persistent monocyte activation and inflammation.

Keywords Monocyte · NeuroAIDS · HAND · Statin

Introduction

The introduction of antiretroviral therapy (ART) has resulted in a significant decline in HIV-1-related morbidity and mortality. However, neurological dysfunction, collectively referred to as HIV-associated neurocognitive disorder (HAND), has not declined as a whole, despite a sharp reduction in the incidence in the HIV-associated dementia (HAD), the most severe form of HAND (Mothobi and Brew 2012; Watkins and Treisman 2015). In ART-treated patients, HAND is seen mainly in people who began therapy at low CD4+ T cell counts (Munoz-Moreno et al. 2008; McCombe et al. 2013). Histopathological studies show a correlation between neurocognitive function and the extent of monocyte/macrophage (M/M) accumulation and activation in the brain, rather than the extent of viral antigen expression (Glass et al. 1995), supporting the concept that neuropathogenesis is due to the indirect effects of HIV-1 infection of the brain mediated through M/M and microglia (Yadav and Collman 2009; Chen et al. 2014).

The inflammatory CD14+CD16+ population of blood monocytes plays a particularly important role in the pathogenesis of HAND (Williams et al. 2012; Williams et al. 2014). This population is increased in chronic HIV-1 disease,

Electronic supplementary material The online version of this article (doi:10.1007/s13365-016-0433-8) contains supplementary material, which is available to authorized users.

✉ Ronald G. Collman
collmanr@mail.med.upenn.edu

[1] Department of Medicine, University of Pennsylvania Perelman School of Medicine, 36th and Hamilton Walk, Philadelphia, PA 19104, USA

[2] Department of Microbiology, University of Pennsylvania Perelman School of Medicine, 36th and Hamilton Walk, Philadelphia, PA 19104, USA

particularly in people with neurocognitive complications (Fischer-Smith et al. 2001; Williams et al. 2012), and has tissue-invasive properties (Fischer-Smith et al. 2001; Williams et al. 2013). Preferential infection of the CD16+ subset is thought to help virus traffic into tissues including brain (Ellery et al. 2007; Valcour et al. 2010). HIV encephalitis (HIVE), the pathological correlate of severe HAND, is associated with the accumulation in the brain of M/M cells expressing CD16 and CD163, and these cells are believed to be responsible for release of mediators that trigger neuronal injury (Fischer-Smith et al. 2001; Roberts et al. 2004; Borda et al. 2008; Fischer-Smith et al. 2008; Ndhlovu et al. 2014). Numerous CD163+ cells in HIVE and SIV encephalitis (SIVE) further suggest entry of monocytes into the CNS from the peripheral blood (Kim et al. 2006), since CD163 is not expressed by resident brain microglia and increased CD163+ CD16+ monocytes in the periphery is associated with increased frequency of CD163+CD16+ M/M in the CNS (Clay et al. 2007; Fischer-Smith et al. 2008). Elevated sCD163 in plasma, a result of surface CD163 shedding in response to pro-inflammatory stimuli, is also associated with neurocognitive impairment in HIV-1 infection (Moller 2012; Burdo et al. 2013; Liang et al. 2015; Wilson et al. 2014). These findings highlight the importance of CD16+ monocytes as a link between peripheral immune activation and CNS disease, as well as CD163+ cells.

One of the principal drivers of systemic immune activation in HIV-1 infection is disruption of the gut mucosal barrier, with translocation of microbial products including lipopolysaccaride (LPS) (Wallet et al. 2010; d'Ettorre et al. 2011; Shan and Siliciano 2014). Elevated LPS triggers monocyte activation, including upregulated CD16 and CD163 expression (Tippett et al. 2011; Vassallo et al. 2012), and enhances trafficking into the brain by compromising the integrity of the blood brain barrier (BBB) (Zhou et al. 2006; Wang et al. 2008). LPS also triggers release of inflammatory cytokines that further exacerbate immune activation in HIV infection. Among the cytokines upregulated by LPS is monocyte chemoattractant protein-1 (MCP-1), which is especially relevant to HIV neuropathogenesis as elevated MCP-1 is associated with increased risk of progression to neurocognitive disease (Eugenin et al. 2006; Dhillon et al. 2008; Zanin et al. 2012). Notably, the group of people with HIV infection who are most likely to show persistent microbial translocation and elevated LPS levels despite ART treatment are those who begin therapy at low CD4+ T cell counts (Jiang et al. 2009; Piconi et al. 2010), which is the same group at greatest risk for HAND despite ART treatment (Ellis et al. 2011; Wang et al. 2013). Thus, agents that modulate monocyte activation and/or function in response to LPS and MCP-1 may be particularly attractive as adjunctive therapy to treat or prevent HAND.

Statins are HMG-CoA reductase inhibitors that, in addition to their cholesterol-lowering activity, have pleiotropic immunomodulatory anti-inflammatory properties independent of their ability to reduce cholesterol levels (Jialal et al. 2001; Aboyans et al. 2006; Almuti et al. 2006). Accumulating evidence suggests that these immunomodulatory effects are a major component of their profound benefit in atherosclerosis (Everett et al. 2010). Importantly, HIV-associated immune activation and HAND have important parallels with atherosclerosis, including chronic monocyte activation, MCP-1-directed monocyte chemotaxis, and transendothelial monocyte migration (Schepers et al. 2006; Isoda et al. 2008; McKibben et al. 2015). Statins are known to impact monocyte phenotypes in atherosclerosis, although the extent to which cholesterol lowering versus pleiotropic mechanisms contribute is uncertain. However, both in vitro and animal studies have shown that statins can attenuate pro-inflammatory responses to LPS (Methe et al. 2005; Hodgkinson and Ye 2008). Atherosclerosis has emerged as a major problem in ART-treated HIV-infected individuals, and recent studies have begun to explore whether statins ameliorate markers of vascular and leukocyte inflammation linked to atherosclerosis in HIV-infected people (Niessner et al. 2006; De Wit et al. 2011; Ganesan et al. 2011; Funderburg et al. 2014; Funderburg et al. 2015), but neurocognitive disease as a target has received less attention. Therefore, we asked here whether statin treatment of peripheral blood cells ex vivo would impact the inflammatory monocyte population important in HAND pathogenesis, including CD16 and CD163 expression, chemokine and cytokine production, and chemotaxis. These data suggest a potential role as adjuvant treatment to prevent HAND through modulation of monocyte function, which warrants further investigation in vivo.

Materials and methods

Primary cell isolation and culture Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood, drawn from normal donors following informed consent, by density gradient centrifugation using Ficoll-Paque Plus (GE Healthcare). Cells were washed and suspended in complete RPMI-1640 (RPMI-1640 containing 1 % streptomycin, penicillin, and L-glutamine and supplemented with 10 % fetal bovine serum (FBS)). Purified monocytes were isolated from fresh PBMCs by negative selection using column purification (Human Monocyte Isolation Kit II, Miltenyi Biotec), per the manufacturer's instructions. The mean purity of monocyte preparations used in the study was 94 %.

Statin treatment and stimulations Simvastatin and atorvastatin (Sigma-Aldrich) were suspended in ethanol and DMSO, respectively, and used at 10 μM final concentration. Freshly isolated PBMCs were suspended at 1×10^6 cells/ml in complete RPMI-1640 and were incubated with or without 10 μM

(Methe et al. 2005; Pozo et al. 2006; Veillard et al. 2006) simvastatin or atorvastatin for 24 h. Control cultures were incubated with vehicle (ethanol or DMSO, respectively). Cultures were then stimulated for 24 h with LPS or MCP-1 in presence of the statin. Cells were then harvested for analysis (with any adherent cells gently removed with a plastic cell scraper), and culture supernatants were collected and stored at −80 °C for cytokine analyses. A similar experimental setup was followed for ex vivo treatment of purified monocytes.

Chemotaxis assay Chemotaxis of purified monocytes was evaluated using 6.5-mm diameter transwell inserts in 24-well plates containing polycarbonate membranes with 5 μm pores (Costar). The lower chambers containing 600 μl of media (RPMI + 0.1 % BSA) and MCP-1 or regulated on activation, normal T cell expressed and secreted (RANTES) (100 ng/ml; Peprotech) were equilibrated at 37 °C for 1 h, following which transwells containing monocytes (2×10^5 cells/100 μl) were inserted. After incubation at 37 °C for 3 h, inserts were removed, methanol fixed, stained with haematoxylin, and cells that had migrated to the underside of the insert were counted under a microscope. Data are presented as the chemotaxis index based on four separate experiments performed in duplicate. The chemotaxis index was calculated as the number of cells migrating to chemokines/number of cells migrating to medium alone.

FACS staining All antibodies were purchased from BD Biosciences unless noted otherwise. Antibodies for surface marker staining included CD14-Pacific Blue (clone M5E2), CD16-APC-Cy7 (clone 3GB), CD163-PE (clone GHI/61), CD4-PECy5.5 (clone OKT4) (Invitrogen), and CD8-PE Texas Red (clone 3B5) (Invitrogen). Cells were washed once with PBS then stained for viability with aqua amine-reactive dye (Invitrogen) for 15 min in the dark at room temperature. Cells were then washed with FACS staining buffer (PBS containing 2 % FBS), then stained by adding a master mix of the abovementioned antibodies. After incubation for 25 min at room temperature in the dark, cells were washed with FACS staining buffer and fixed with PBS containing 2 % paraformaldehyde. Fixed cells were stored at 4 °C in the dark until acquisition.

For each sample tube, 300,000 events were acquired on an LSRII flow cytometer (BD Immunocytometry Systems). Individual compensation tubes for each fluorochrome were prepared using antibody capture compensation beads (BD Biosciences). Data analysis was performed using FlowJo software (TreeStar). Gating on the monocyte population was first done on side scatter (SSC) versus forward scatter (FSC), followed by gating on viable cells. Subsequent gating was done on cells positively stained with anti-CD14 antibody before analyzing surface marker(s) expression on monocytes (Supplementary Fig. 1).

Cytokine and soluble marker assays MCP-1, MIP-1alpha, and IL-8 (CXCL8) were determined using the Luminex multiplex human cytokine assay kit (cat no. LHC0009, Invitrogen).

Statistical analysis Experiments were carried out on cells from independent donors ($n = 6$ for PBMC, $n = 3$ for purified monocytes). Statistical evaluations were carried out using paired two-tailed t test, and a p value ≤ 0.05 was considered to be significant. Results reported are expressed as mean ± standard deviation (SD) of independent experiments using different donors.

Results

Statin treatment decreases the CD14+CD16+ inflammatory monocyte subpopulation in vitro CD14+CD16+ inflammatory monocytes are important contributors to the development of CNS complications in HIV+ subjects. Two important factors contributing to this population of cells include chronic immune activation driven by persistent translocation of gut microbial products, including LPS, and production of chemokines within the CNS that recruits blood monocytes, particularly MCP-1. With this in mind, we investigated whether statin treatment would affect the inflammatory monocyte population following stimulation with LPS and MCP-1 ex vivo. PBMCs obtained from normal volunteers were incubated with simvastatin or atorvastatin, exposed to LPS or MCP-1, and phenotype of monocytes within the PBMC population assessed by FACS.

We first examined the phenotype of monocytes during a 48 h time course in the absence of any treatment or stimulation, to assess the relationship to features of circulating monocytes in vivo (Supplementary Fig. 1). This revealed an increase in monocyte CD16 expression, with consequent increase in the percentage of CD14++CD16+ subpopulation (also called "intermediate" monocytes) and "inflammatory" CD14+CD16+ monocytes relative to CD14++CD16− "classical" monocytes. There was also a reduction in CD14 expression in a subpopulation of monocytes, with a shift from CD14++ to CD14+. Most of the phenotypic change occurred during the first 24 h in vitro. Thus, monocytes maintained in these PBMC cultures resemble the phenotypic profile in HIV-1-infected individuals and allows us to evaluate the effects of statins on the inflammatory monocyte population.

We then tested the effect of statin treatment on monocyte populations. As shown in representative dot plots (Fig. 1a, b), simvastatin and atorvastatin both reduced the number of total CD14+CD16+ inflammatory monocytes at 48 h (Fig. 1c, d,

top panels). This reduction was significant in the LPS-stimulated cells following both simvastatin and atorvastatin treatment. More specifically, the effect was particularly prominent in the CD14++CD16+ intermediate subpopulation of the CD14+CD16+ monocytes (Fig. 1c, d, bottom panels). A decrease in CD16 mean fluorescence intensity (MFI) was also observed, which reached statistical significance in simvastatin-treated monocytes in the LPS group (Fig. 2a, b).

Statin treatment also downregulated surface CD14 expression on both resting and stimulated cells. This resulted in a reduced percentage of CD14++ monocytes (Fig. 1a, b, c lower panel, d lower panel, e), as well as a significant

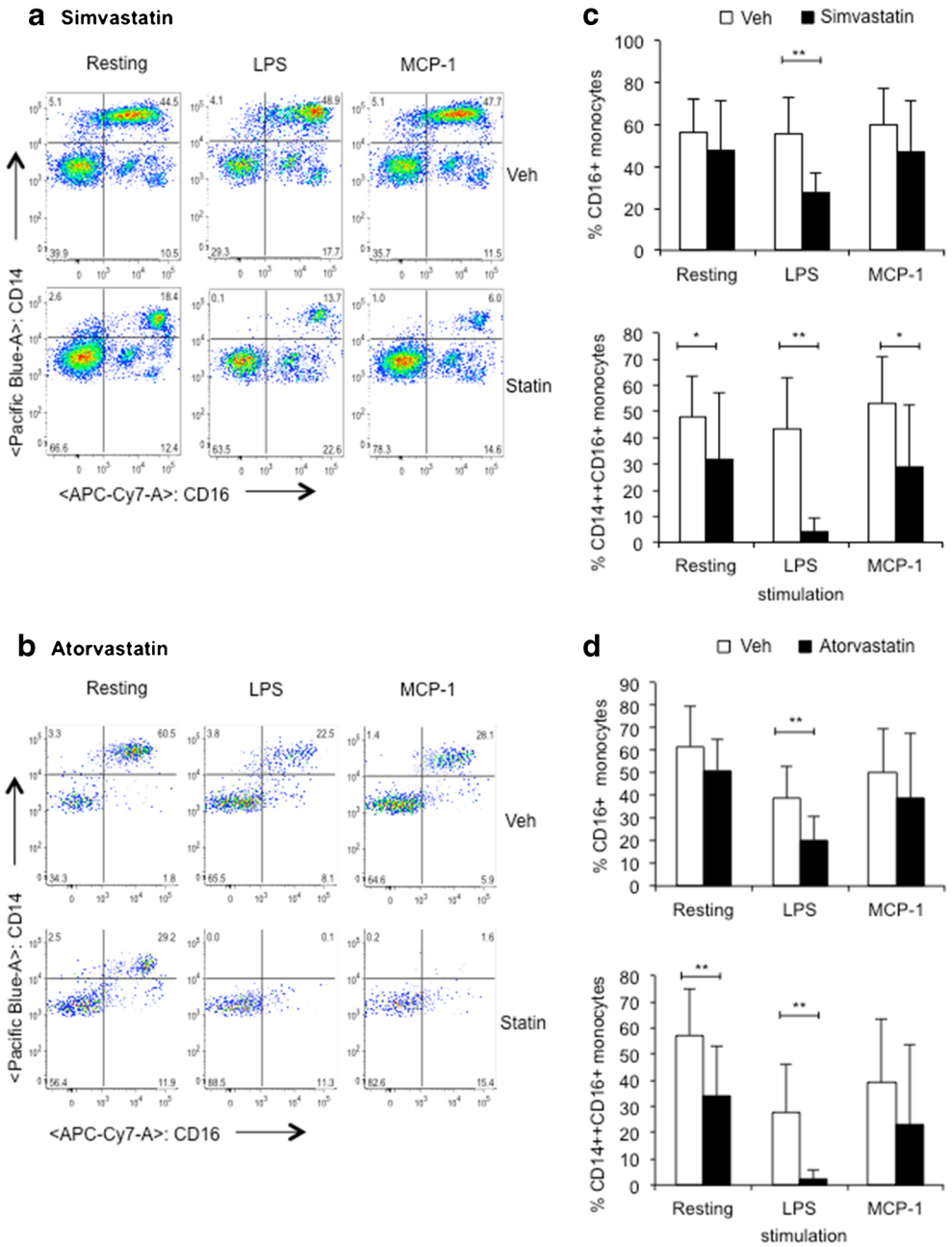

Fig. 1 Statin treatment decreases the number of CD14+CD16+ inflammatory monocytes in vitro. Freshly isolated PBMCs were treated with simvastatin or atorvastatin (10 μM) for 24 h, or vehicle alone, following which cells were stimulated with LPS (100 ng) or MCP-1 (100 ng) for 24 h. Monocyte surface expression of CD16 was analyzed by FACS 24 h later. Representative dot plots showing effect of simvastatin **(a)** and atorvastatin **(b)** on CD14 and CD16 expression. **c** Proportion of total CD14+ monocytes that were CD16+ (*top*) or CD14++CD16+ (*bottom*) following simvastatin treatment. **d** Proportion of total CD14+ monocytes that were CD16+ (*top*) or CD14++CD16+ (*bottom*) following atorvastatin treatment. Results are mean ± SD of six independent experiments using cells from different donors (*$p < 0.05$ and **$p < 0.001$; two-tailed *t* test). **e** Representative histograms showing decrease in monocyte CD14++ expression following simvastatin and atorvastatin treatment

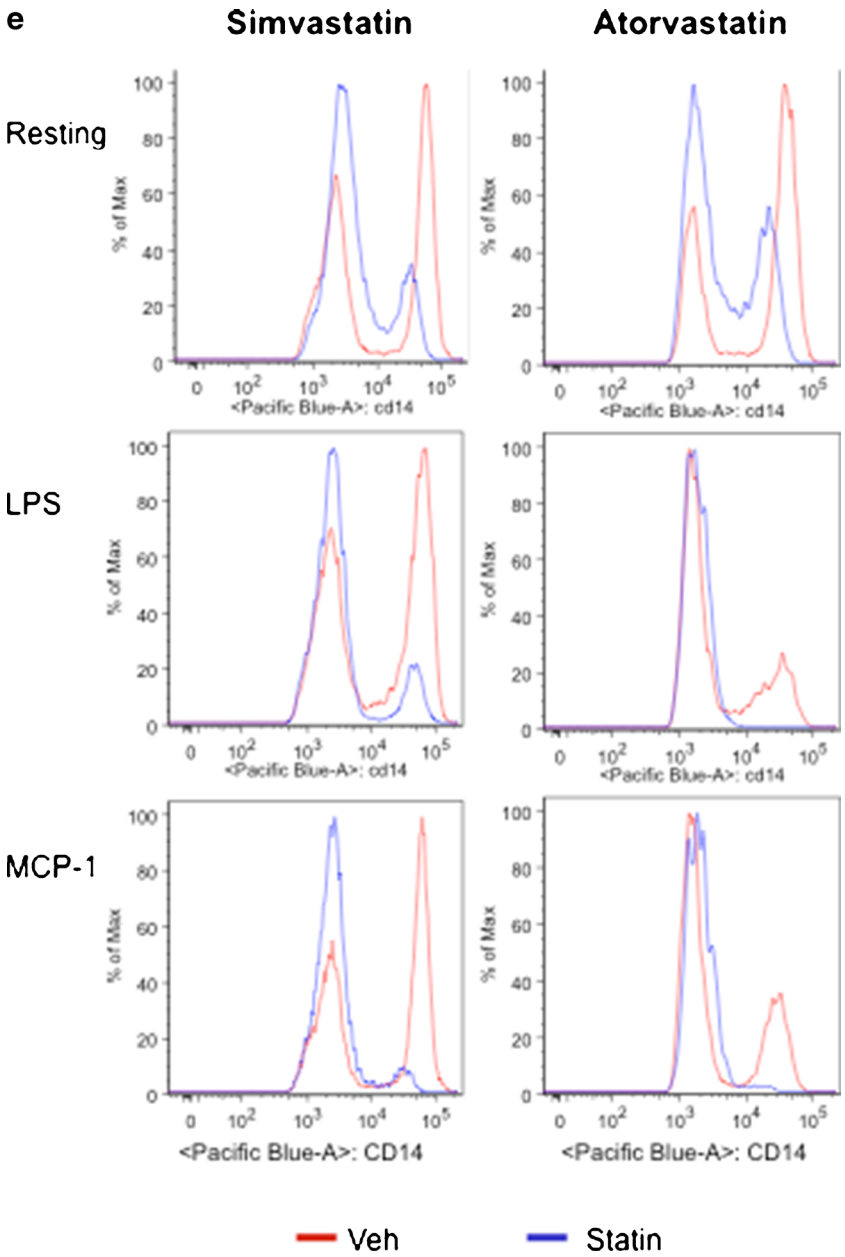

Fig. 1 (continued)

reduction in the overall CD14 MFI in the monocyte population following both simvastatin and atorvastatin treatment (Fig. 2a, b). Therefore, statin treatment modulates the monocyte population by causing a phenotypic shift from a CD14++CD16+ to a CD14+CD16− phenotype (Fig. 1a, b), impacting both surface CD14 and well as CD16. These observations suggest a shift toward a less inflammatory phenotype.

Statin treatment decreases the CD163+ monocyte population in vitro CD163 is a haptoglobin-hemoglobin scavenger receptor expressed by monocytes and macrophages (Kristiansen et al. 2001; Schaer et al. 2007) that is significantly upregulated on the CD14++CD16+ monocytes (Tippett et al. 2011) and has been suggested as a biomarker for HIV-1 disease progression and neurocognitive disease (Roberts et al. 2004; Tippett et al. 2011). Therefore, we evaluated the effects of statin treatment on monocyte CD163 expression. As shown in Fig. 3a, b, both simvastatin and atorvastatin reduced the percentage of CD163+ monocytes under resting and stimulated conditions. We also observed significant decrease in the CD163 MFI in cells treated with simvastatin (LPS and MCP-1 stimulated) and with atorvastatin (LPS stimulated) (Fig. 2a, b). As shown in Fig. 3c, CD163+ monocytes are also CD16+, consistent with previous reports (Tippett et al. 2011), indicating statin-induced reduction in CD16+/CD163+

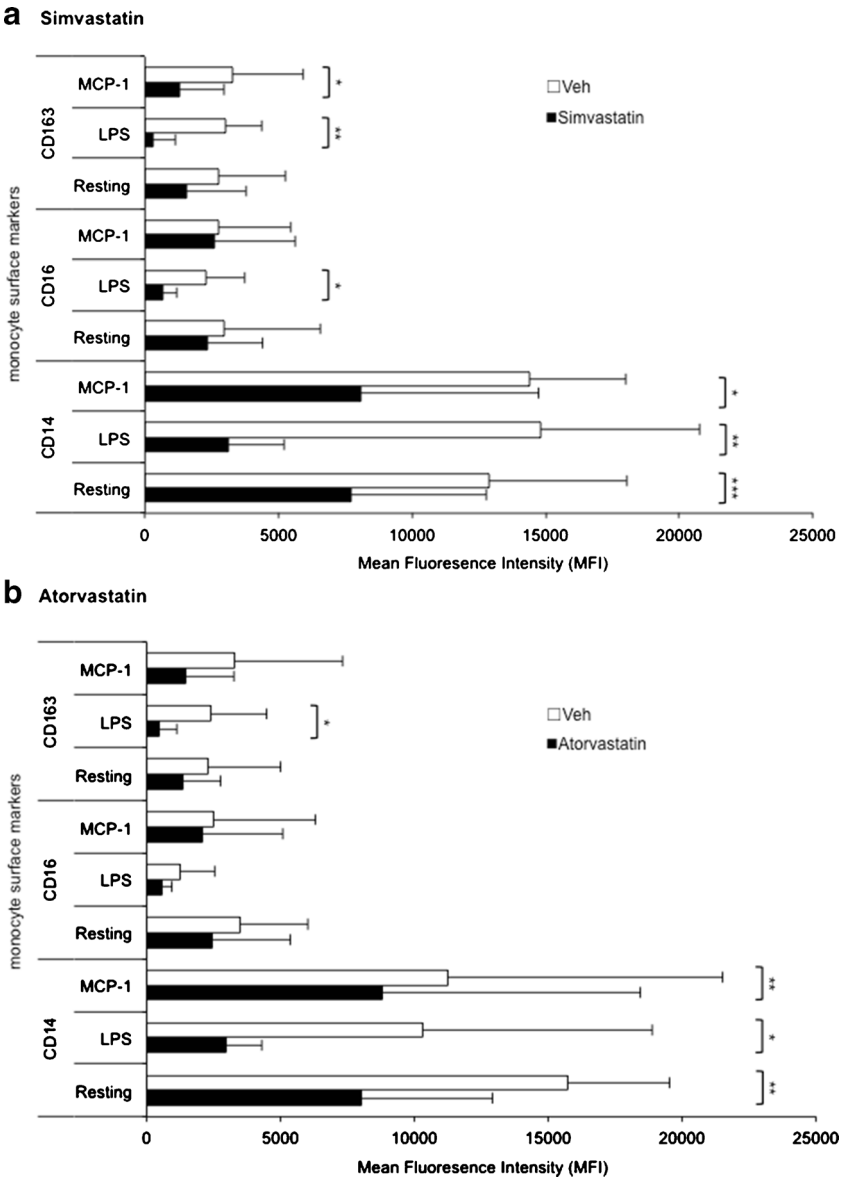

Fig. 2 Statin treatment differentially affects monocyte surface markers. Mean fluorescence intensity (MFI) of CD14, CD16, and CD163 on resting and stimulated monocytes in the presence and absence of **a** simvastatin or **b** atorvastatin (10 μM). Data represent mean ± SD of six independent experiments using cells from different donors (*$p < 0.05$, **$p < 0.001$, and ***$p < 0.0001$ as compared to untreated group; two-tailed *t* test)

monocytes, a key population implicated in progression of HAND (Roberts et al. 2004; Tippett et al. 2011).

Simvastatin suppresses MCP-1 production by PBMCs and inhibits monocyte chemotaxis in vitro Given the important role of MCP-1 in HAND pathogenesis (Yuan et al. 2015), we assessed the effects of statin treatment on MCP-1 production ex vivo by LPS-stimulated PBMC, recognizing that monocytes are a major source of MCP-1 production in response to LPS (Wang et al. 2000). As shown in Fig. 4a, resting PBMC produced no detectable MCP-1, while LPS induced marked production. Treatment with simvastatin reduced MCP-1 production by PBMCs by >90 %. Simvastatin also showed significant suppression of LPS-induced MIP-1α and a strong trend toward reduction in IL-8 production ($p < 0.09$) (Fig. 4b, c) (MCP-1 stimulation did not induce MIP-1α production, and IL-8 induction was minimal; data not shown). Both MIP-1α and IL-8 are markers of inflammation in HIV infection, and IL-8 has specifically been associated with ongoing inflammation in HIV+ individuals on HAART (Pierdominici et al. 2002; Letendre et al. 2011; Bastard et al. 2012; Mamik and Ghorpade 2014; Wada et al. 2015). Additionally, CSF IL-8 correlates with HIV-associated neurocognitive disorders (Kamat et al. 2012; Yuan et al. 2013; Yuan et al. 2015).

Since MCP-1 is involved in the trafficking of monocytes to the CNS (Williams et al. 2013), we investigated the effect of simvastatin on monocyte chemotaxis in response to MCP-1, using transwell chambers with polycarbonate membranes and 5 μm pores. As shown in Fig. 5, migration of monocytes was significantly suppressed by simvastatin. We also saw a reduction in migration to the chemokine RANTES. There were no

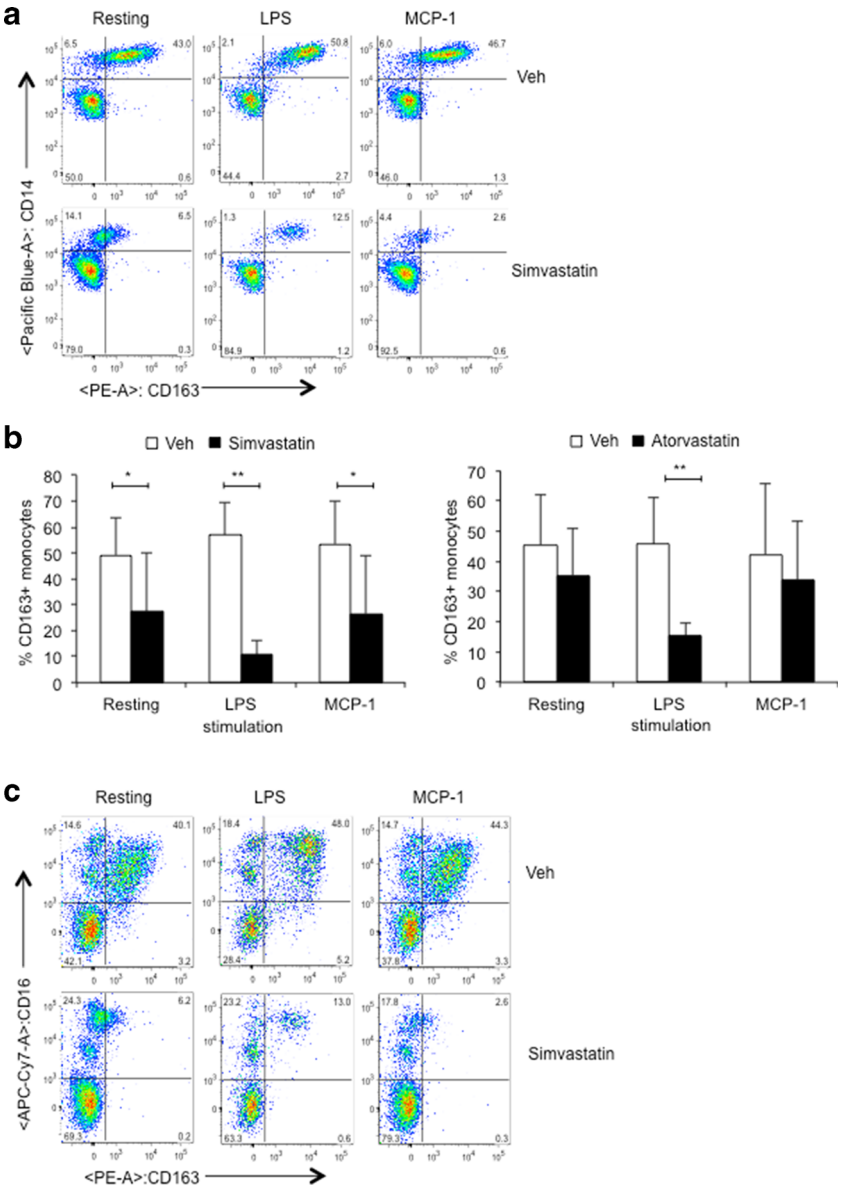

Fig. 3 Statin treatment downregulates monocyte CD163 expression in vitro. PBMCs were treated with simvastatin or atorvastatin and then subject to stimulation as in Fig 1 and analyzed for monocyte expression of CD163. **a** Representative dot plot showing monocyte CD163 expression under resting and stimulated conditions, with and without treatment with simvastatin (10 μM). **b** Quantitated data from six independent experiments each for simvastatin and atorvastatin using cells from different donors (mean ± SD). **c** Representative dot plot showing effect of simvastatin on monocytes co-expression of CD16 and CD163. Data represent mean ± SD of six independent experiments (*$p < 0.05$ and **$p < 0.001$ as compared to untreated group; two-tailed *t* test)

observable toxic effects of simvastatin, indicating that chemotaxis inhibition was not due to nonspecific effects on cell viability (Supplementary Fig. 2). These findings are consistent with prior reports that statins block monocyte chemotaxis (Montecucco et al. 2009) and extend them to a variety of phenotypic and functional features relevant to HIV neurocognitive disease.

Statin treatment of purified monocytes decreases the CD16+ and CD163+ cell populations In order to determine whether the statins affected monocytes directly or was mediated mainly through other cell types in the PBMC population, we carried out similar experiments on purified monocytes isolated by negative selection. Consistent with PBMC treatment results, both simvastatin and atorvastatin significantly reduced the proportion of CD14+CD16+, CD14++CD16+, and CD14+CD163+ monocytes following LPS stimulation (Fig. 6a, c; representative dot plots in Supplementary Fig. 3a and b), with a trend toward decrease in other conditions. A reduction in mean fluorescence intensity was also observed for all three surface markers and reached statistical significance for CD14 (LPS and MCP-1 stimulated) and for CD163 (LPS stimulated) (Fig. 6b, d). These observations indicate, therefore, that statins impact monocytes directly.

Discussion

In this study, we demonstrated that simvastatin and atorvastatin impact CD14+CD16+ monocytes, especially the CD14++CD16+CD163+-activated monocyte subpopulation, and

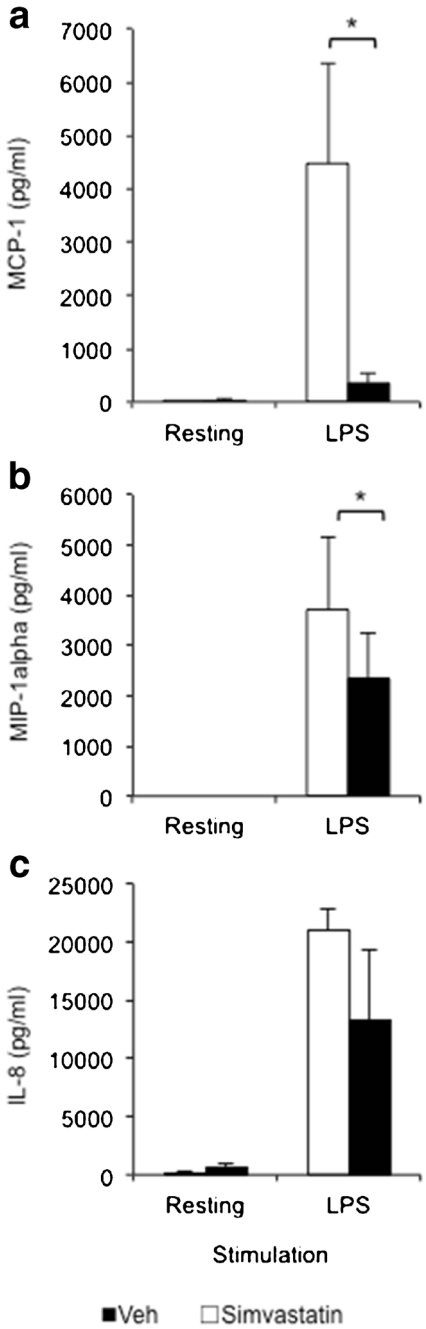

Fig. 4 Simvastatin inhibits production of inflammatory chemokines by PBMC. PBMCs were treated with simvastatin, stimulated with LPS (100 ng), and supernatant levels of MCP-1 **(a)**, MIP-1α **(b)**, and IL-8 **(c)** were determined by Luminex assay. Results expressed as mean ± SD from four separate experiments using cells from different donors (*$p < 0.05$ as compared to untreated group; two-tailed *t* test)

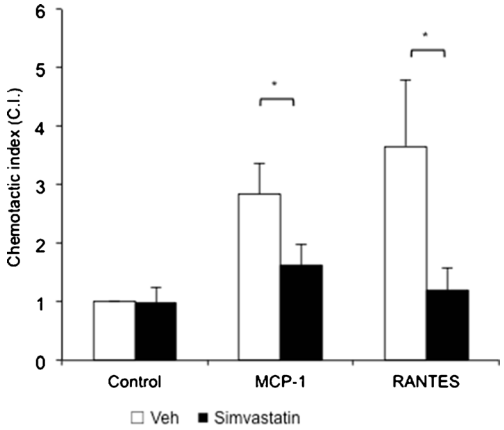

Fig. 5 Simvastatin inhibits MCP-1-dependent monocyte chemotaxis. Monocytes were treated with simvastatin (10 μM) for 24 h, added to transwell inserts, and exposed to lower chambers containing MCP-1 or RANTES (100 ng/ml). Migrating cells were counted 3 h later as described in Materials and methods. Results are expressed as chemotactic index and reflect mean ± SD from four separate experiments using cells from different donors, performed in duplicate (*$p < 0.05$; two-tailed *t* test)

reduce the proportion of cells with this inflammatory phenotype. This effect is seen both on monocytes in mixed PBMC culture and purified monocytes. Statins also decrease the chemotaxis of monocytes in response to MCP-1 and reduce the production of several inflammatory cytokines. These findings are of significance as they correlate with key steps in the pathogenesis of HAND.

A principal finding of our study was that both simvastatin and atorvastatin reduced the proportion of monocytes exhibiting the CD14+/CD16+ inflammatory phenotype in PBMC cultures ex vivo. This population of cells, expanded in HIV infection, is believed to have neuroinvasive properties and contribute to the accumulation of myeloid cells in the brain with consequent neurological injury (Fischer-Smith et al. 2001). We saw this effect both in unstimulated monocytes and in cells activated with two stimuli implicated in HAND pathogenesis, LPS and MCP-1. Residual inflammation in ART-treated individuals, believed to be a major contributor to HAND (Yadav and Collman 2009; Chen et al. 2014), is partially driven by persistent translocation of microbial products such as LPS, due to incomplete restoration of damaged gut immune barrier (Jiang et al. 2009; Pilakka-Kanthikeel et al. 2014). Similarly, MCP-1 is elevated in HIV infection and, in particular, recruits monocytes to tissues (Williams et al. 2013). Thus, modulation of monocyte phenotype in response to LPS and MCP-1 may be particularly relevant to HAND.

Statin-induced reduction of activated CD16+ monocytes is important, as CD16+ monocytes are greatly expanded in HAND and their migration into the CNS is critical for HIV neuropathogenesis (Williams et al. 2012). Furthermore, in contrast to HIV-1-uninfected individuals, where CD163 is primarily expressed by the CD14++CD16− classical monocytes, in HIV-1-positive individuals, increased CD163 expression is seen on CD14++CD16+ intermediate monocyte subset (Tippett et al. 2011). CD163 is a scavenger receptor for haptoglobin-hemoglobin complexes (Graversen et al. 2002), which also serves as a receptor for bacterial products (Fabriek et al. 2009). The CD16+CD163+ monocyte subset is also

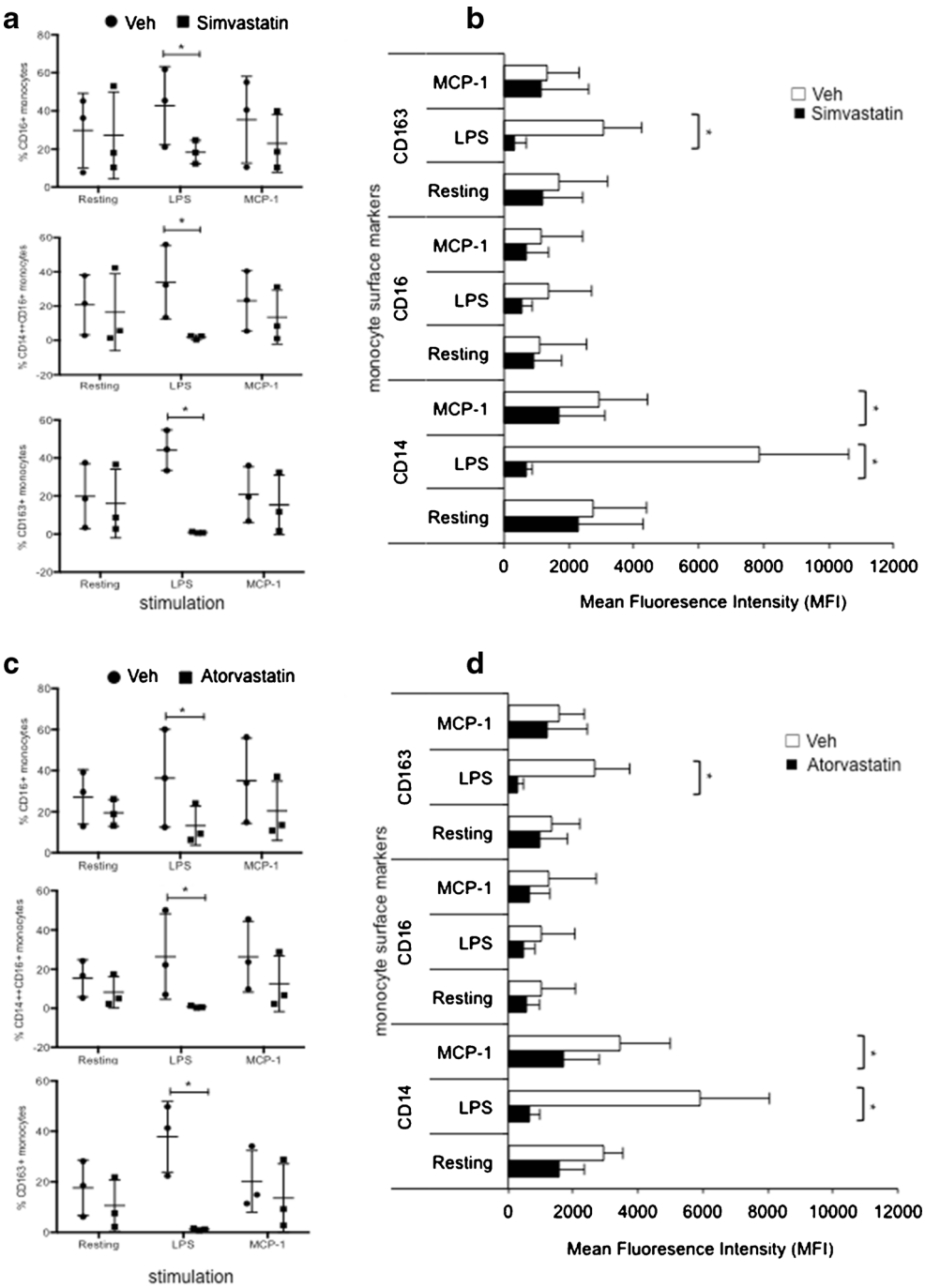

Fig. 6 Statin treatment of purified monocytes decreases the number of CD16 and CD163 expressing cells. Monocytes isolated by negative selection were treated with simvastatin (**a**, **b**) or atorvastatin (**c**, **d**) (10 μM) for 24 h, or vehicle alone, following which cells were stimulated with LPS (100 ng) or MCP-1 (100 ng) for 24 h. Monocyte surface expression of CD16 and CD163 was analyzed by FACS 24 h later. Data show effect of simvastatin and atorvastatin on the proportion of monocytes that were CD16+, CD14++CD16+, or CD163+ (**a**, **c**) and mean fluorescence intensity of CD14, CD16, and CD163 (**b**, **d**). Results are mean ± SD of three independent experiments using cells from different donors (*$p < 0.05$; two-tailed *t* test)

preferentially infected by HIV-1 (Jaworowski et al. 2007); is a source of inflammatory soluble factors like TNF-alpha, IL-1beta, and IL-6; and upon differentiation into macrophages, promotes viral replication and activates T cells for HIV infection (Ancuta et al. 2006a, b, c; Wang et al. 2006). We found that statin treatment particularly impacts CD14++CD16+ subpopulation of monocytes, which is also CD163+. This effect is associated with a proportional increase in CD14+CD16−CD163− monocytes. Since we did not observe increased cell death following drug treatment (Supplementary Fig. 2), this suggests that there is phenotypic modulation leading to a shift in the relative proportion of pro- and anti-inflammatory monocytes. These observations are of significance as CD14++CD16+ intermediate monocytes have been associated with disease progression in chronic HIV infection (Zawada et al. 2011; Wilson et al. 2014), have recently been linked to inflammation and monocyte activation (Sulicka et al. 2013), and are the main producer of reactive oxygen species (a cause of oxidative stress), among the monocyte subsets (Zawada et al. 2011). Of note, at the time point analyzed (after 48 h ex vivo),

resting monocytes have acquired a surface phenotype more similar to the activated profile seen in HIV-infected people, with CD16 expression even in unstimulated cells (Supplementary Fig. 1). As a consequence, we see few of the classical (CD14++CD16−) monocyte subset. Nevertheless, even though the resting cells are CD16+, they do not spontaneously secrete cytokines (Fig. 4a–c), and thus, stimulation-elicited responses can also be assessed.

Simvastatin strongly inhibited monocyte chemotaxis in response to MCP-1 (Fig. 5), which is consistent with other reports (Pasceri et al. 2001; Han et al. 2005; Wang et al. 2006; Rallidis et al. 2008). MCP-1 is believed to be a principal driver of monocyte trafficking into the CNS (Eugenin et al. 2006; Dhillon et al. 2008). Production of MCP-1 by LPS-stimulated PBMC was also strongly inhibited as well as more modest reductions in IL-8 and MIP-1alpha (Fig. 4), which are additional chemokines involved in inflammation and leukocyte transmigration across the BBB in HIV-1 disease (Lane et al. 2001; Kamat et al. 2012). These findings are consistent with prior reports (Bruegel et al. 2006; Marino et al. 2014). Since CD14+CD16+ monocytes migrate to the CNS during HIV infection and contribute to elevated MCP-1 levels in the brain (thereby being involved in further recruitment of peripheral monocytes), the ability of statins to inhibit monocyte MCP-1 production is of relevance to M/M activation and trafficking, which contributes to HAND and other inflammatory conditions (Cinque et al. 1998; Park et al. 2001; Namiki et al. 2002; Ragin et al. 2006; Rantapaa-Dahlqvist et al. 2007). Thus, statins may not only modulate the activated monocyte population in the blood but may also directly impact their entry into the CNS.

In HIV-infected people, statins have been used widely for treatment of lipid disorders. More recently, much interest has been focused on potential immunomodulatory effects. An early study of atorvastatin in untreated individuals showed a small but statistically significant reduction in T cell activation markers, without an effect on viral load (Ganesan et al. 2011). Another study of atorvastatin in untreated individuals examined the CNS and found no effect on cerebrospinal fluid viral load or levels of neopterin, a marker of intrathecal immune activation (Probasco et al. 2008). However, it is likely that suppression of viral replication and cessation of further immune cell destruction would be necessary before any adjunctive immunomodulation. Very recently, a large study of rosuvastatin in ART-suppressed individuals, focused on cardiovascular risk factors, demonstrated decreased sCD14 and sCD163 plasma levels and a reduction in the CD14+CD16+ monocyte population (Funderburg et al. 2014; Funderburg et al. 2015), which is consistent with our findings. That study also reported reductions on T cell activation markers. Rosuvastatin also slowed the decline in kidney function, which is another target organ linked to systemic inflammation (Longenecker et al. 2014), and suggests that benefit is not restricted just to atherosclerotic outcomes. A retrospective analysis of subjects in the CHARTER cohort did not find an association between statin use and neurocognitive performance, but those subjects were prescribed statins for established clinical indications, which are themselves associated with neurological consequences (Letendre et al. 2007). Thus, the potential role of statins in vivo to prevent neurocognitive disease has yet to be determined.

We studied atorvastatin and simvastatin because these drugs have two properties important for potential use as adjunctive HAND treatment. In contrast to rosuvastatin, both atorvastatin and simvastatin are lipophilic and enter the CNS. As a result, they may have impact not only on blood monocytes, activation of which contributes to neuroinvasion and CNS accumulation, but may also impact myeloid lineage cells and immune activation within the CNS (Yadav and Collman 2009; Chen et al. 2014). In addition, both drugs are off-patent and thus economically more feasible for resource-limited areas of the world that have the greatest burden of infected people at risk for HAND.

In summary, we demonstrate monocyte-specific immunomodulatory effects of the lipophilic statins atorvastatin and simvastatin, suggesting potential utility as adjunctive therapy of HAND. Given the high prevalence of neurocognitive disease in ART-treated individuals, combined with the increasing numbers of long-term ART-treated individuals in high HIV prevalence areas of the world, neurological outcomes warrant focused investigation as an outcome of statin immunomodulation.

Acknowledgments We thank D. Carnathan, G. Makedonas, C. Pombo, and K. Demers for assistance with flow cytometry and members of the Collman lab for critical input and advice. This work was supported by NIH grant MH061139 to R.G.C, and we acknowledge assistance from multiple Cores of the Penn Center for AIDS Research (P30-AI045008).

Compliance with ethical standards

Conflict of interest The authors A. Yadav, M.R. Betts, and R.G. Collman declare that they have no conflict of interest.

References

Aboyans V, Labrousse L et al (2006) Predictive factors of stroke in patients undergoing coronary bypass grafting: statins are protective. Eur J Cardiothorac Surg 30(2):300–304

Almuti K, Rimawi R et al (2006) Effects of statins beyond lipid lowering: potential for clinical benefits. Int J Cardiol 109(1):7–15

Ancuta P, Autissier P et al (2006a) CD16+ monocyte-derived macrophages activate resting T cells for HIV infection by producing CCR3 and CCR4 ligands. J Immunol 176(10):5760–5771

Ancuta P, Kunstman KJ et al (2006b) CD16+ monocytes exposed to HIV promote highly efficient viral replication upon differentiation into macrophages and interaction with T cells. Virology 344(2):267–276

Ancuta P, Wang J et al (2006c) CD16+ monocytes produce IL-6, CCL2, and matrix metalloproteinase-9 upon interaction with CX3CL1-expressing endothelial cells. J Leukoc Biol 80(5):1156–1164

Bastard JP, Soulie C et al (2012) Circulating interleukin-6 levels correlate with residual HIV viraemia and markers of immune dysfunction in treatment-controlled HIV-infected patients. Antivir Ther 17(5):915–919

Borda JT, Alvarez X et al (2008) CD163, a marker of perivascular macrophages, is up-regulated by microglia in simian immunodeficiency virus encephalitis after haptoglobin-hemoglobin complex stimulation and is suggestive of breakdown of the blood-brain barrier. Am J Pathol 172(3):725–737

Bruegel M, Teupser D et al (2006) Statins reduce macrophage inflammatory protein-1alpha expression in human activated monocytes. Clin Exp Pharmacol Physiol 33(12):1144–1149

Burdo TH, Weiffenbach A et al (2013) Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. AIDS 27(9):1387–1395

Chen MF, Gill AJ et al (2014) Neuropathogenesis of HIV-associated neurocognitive disorders: roles for immune activation, HIV blipping and viral tropism. Curr Opin HIV AIDS 9(6):559–564

Cinque P, Vago L et al (1998) Elevated cerebrospinal fluid levels of monocyte chemotactic protein-1 correlate with HIV-1 encephalitis and local viral replication. AIDS 12(11):1327–1332

Clay CC, Rodrigues DS et al (2007) Neuroinvasion of fluorescein-positive monocytes in acute simian immunodeficiency virus infection. J Virol 81(21):12040–12048

De Wit S, Delforge M et al (2011) Downregulation of CD38 activation markers by atorvastatin in HIV patients with undetectable viral load. Aids 25(10):1332–1333

d'Ettorre G, Paiardini M et al (2011) HIV persistence in the gut mucosa of HIV-infected subjects undergoing antiretroviral therapy correlates with immune activation and increased levels of LPS. Curr HIV Res 9(3):148–153

Dhillon NK, Williams R et al (2008) Roles of MCP-1 in development of HIV-dementia. Front Biosci 13:3913–3918

Ellery PJ, Tippett E et al (2007) The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. J Immunol 178(10):6581–6589

Ellis RJ, Badiee J et al (2011) CD4 nadir is a predictor of HIV neurocognitive impairment in the era of combination antiretroviral therapy. AIDS 25(14):1747–1751

Eugenin EA, Osiecki K et al (2006) CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood-brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. J Neurosci 26(4):1098–1106

Everett BM, Glynn RJ et al (2010) Rosuvastatin in the prevention of stroke among men and women with elevated levels of C-reactive protein: justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER). Circulation 121(1):143–150

Fabriek BO, van Bruggen R et al (2009) The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. Blood 113(4):887–892

Fischer-Smith T, Croul S et al (2001) CNS invasion by CD14+/CD16+ peripheral blood-derived monocytes in HIV dementia: perivascular accumulation and reservoir of HIV infection. J Neurovirol 7(6):528–541

Fischer-Smith T, Bell C et al (2008) Monocyte/macrophage trafficking in acquired immunodeficiency syndrome encephalitis: lessons from human and nonhuman primate studies. J Neurovirol 14(4):318–326

Funderburg NT, Jiang Y et al (2014) Rosuvastatin treatment reduces markers of monocyte activation in HIV-infected subjects on antiretroviral therapy. Clin Infect Dis 58(4):588–595

Funderburg NT, Jiang Y et al (2015) Rosuvastatin reduces vascular inflammation and T-cell and monocyte activation in HIV-infected subjects on antiretroviral therapy. J Acquir Immune Defic Syndr 68(4):396–404

Ganesan A, Crum-Cianflone N et al (2011) 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 203(6):756–764

Glass JD, Fedor H et al (1995) Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. Ann Neurol 38(5):755–762

Graversen JH, Madsen M et al (2002) CD163: a signal receptor scavenging haptoglobin-hemoglobin complexes from plasma. Int J Biochem Cell Biol 34(4):309–314

Han KH, Ryu J et al (2005) HMG-CoA reductase inhibition reduces monocyte CC chemokine receptor 2 expression and monocyte chemoattractant protein-1-mediated monocyte recruitment in vivo. Circulation 111(11):1439–1447

Hodgkinson CP, Ye S (2008) Statins inhibit toll-like receptor 4-mediated lipopolysaccharide signaling and cytokine expression. Pharmacogenet Genomics 18(9):803–813

Isoda K, Folco E et al (2008) Glycated LDL increases monocyte CC chemokine receptor 2 expression and monocyte chemoattractant protein-1-mediated chemotaxis. Atherosclerosis 198(2):307–312

Jaworowski A, Kamwendo DD et al (2007) CD16+ monocyte subset preferentially harbors HIV-1 and is expanded in pregnant Malawian women with Plasmodium falciparum malaria and HIV-1 infection. J Infect Dis 196(1):38–42

Jialal I, Stein D et al (2001) Effect of hydroxymethyl glutaryl coenzyme a reductase inhibitor therapy on high sensitive C-reactive protein levels. Circulation 103(15):1933–1935

Jiang W, Lederman MM et al (2009) 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 199(8):1177–1185

Kamat A, Lyons JL et al (2012) Monocyte activation markers in cerebrospinal fluid associated with impaired neurocognitive testing in advanced HIV infection. J Acquir Immune Defic Syndr 60(3):234–243

Kim WK, Alvarez X et al (2006) CD163 identifies perivascular macrophages in normal and viral encephalitic brains and potential precursors to perivascular macrophages in blood. Am J Pathol 168(3):822–834

Kristiansen M, Graversen JH et al (2001) Identification of the haemoglobin scavenger receptor. Nature 409(6817):198–201

Lane BR, Lore K et al (2001) Interleukin-8 stimulates human immunodeficiency virus type 1 replication and is a potential new target for antiretroviral therapy. J Virol 75(17):8195–8202

Letendre SL, Marquie-Beck J et al (2007) The role of cohort studies in drug development: clinical evidence of antiviral activity of serotonin reuptake inhibitors and HMG-CoA reductase inhibitors in the central nervous system. J Neuroimmune Pharmacol 2(1):120–127

Letendre SL, Zheng JC et al (2011) Chemokines in cerebrospinal fluid correlate with cerebral metabolite patterns in HIV-infected individuals. J Neurovirol 17(1):63–69

Liang H, Duan Z, et al (2015) Higher levels of circulating monocyte-platelet aggregates are correlated with viremia and increased sCD163 levels in HIV-1 infection. Cell Mol Immunol 12(4):435–443

Longenecker CT, Hileman CO et al (2014) Rosuvastatin preserves renal function and lowers cystatin C in HIV-infected subjects on antiretroviral therapy: the SATURN-HIV trial. Clin Infect Dis 59(8):1148–1156

Mamik MK, Ghorpade A (2014) Chemokine CXCL8 promotes HIV-1 replication in human monocyte-derived macrophages and primary microglia via nuclear factor-kappaB pathway. PLoS One 9(3):e92145

Marino F, Maresca AM et al (2014) Simvastatin down-regulates the production of Interleukin-8 by neutrophil leukocytes from dyslipidemic patients. BMC Cardiovasc Disord 14:37

McCombe JA, Vivithanaporn P et al (2013) Predictors of symptomatic HIV-associated neurocognitive disorders in universal health care. HIV Med 14(2):99–107

McKibben RA, Margolick JB et al (2015) Elevated levels of monocyte activation markers are associated with subclinical atherosclerosis in men with and those without HIV infection. J Infect Dis 211(8): 1219–1228

Methe H, Kim JO et al (2005) Statins decrease Toll-like receptor 4 expression and downstream signaling in human CD14+ monocytes. Arterioscler Thromb Vasc Biol 25(7):1439–1445

Moller HJ (2012) Soluble CD163. Scand J Clin Lab Invest 72(1):1–13

Montecucco F, Burger F et al (2009) Statins inhibit C-reactive protein-induced chemokine secretion, ICAM-1 upregulation and chemotaxis in adherent human monocytes. Rheumatology (Oxford) 48(3): 233–242

Mothobi NZ, Brew BJ (2012) Neurocognitive dysfunction in the highly active antiretroviral therapy era. Curr Opin Infect Dis 25(1):4–9

Munoz-Moreno JA, Fumaz CR et al (2008) Nadir CD4 cell count predicts neurocognitive impairment in HIV-infected patients. AIDS Res Hum Retroviruses 24(10):1301–1307

Namiki M, Kawashima S et al (2002) Local overexpression of monocyte chemoattractant protein-1 at vessel wall induces infiltration of macrophages and formation of atherosclerotic lesion: synergism with hypercholesterolemia. Arterioscler Thromb Vasc Biol 22(1):115–120

Ndhlovu LC, Umaki T et al (2014) Treatment intensification with maraviroc (CCR5 antagonist) leads to declines in CD16-expressing monocytes in cART-suppressed chronic HIV-infected subjects and is associated with improvements in neurocognitive test performance: implications for HIV-associated neurocognitive disease (HAND). J Neurovirol 20(6):571–582

Niessner A, Steiner S et al (2006) Simvastatin suppresses endotoxin-induced upregulation of toll-like receptors 4 and 2 in vivo. Atherosclerosis 189(2):408–413

Park IW, Wang JF et al (2001) HIV-1 Tat promotes monocyte chemoattractant protein-1 secretion followed by transmigration of monocytes. Blood 97(2):352–358

Pasceri V, Cheng JS et al (2001) Modulation of C-reactive protein-mediated monocyte chemoattractant protein-1 induction in human endothelial cells by anti-atherosclerosis drugs. Circulation 103(21): 2531–2534

Piconi S, Trabattoni D et al (2010) Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy. AIDS 24(13):1991–2000

Pierdominici M, Giovannetti A et al (2002) Changes in CCR5 and CXCR4 expression and beta-chemokine production in HIV-1-infected patients treated with highly active antiretroviral therapy. J Acquir Immune Defic Syndr 29(2):122–131

Pilakka-Kanthikeel S, Kris A et al (2014) Immune activation is associated with increased gut microbial translocation in treatment-naive, HIV-infected children in a resource-limited setting. J Acquir Immune Defic Syndr 66(1):16–24

Pozo M, de Nicolas R et al (2006) Simvastatin inhibits the migration and adhesion of monocytic cells and disorganizes the cytoskeleton of activated endothelial cells. Eur J Pharmacol 548(1-3):53–63

Probasco JC, Spudich SS et al (2008) Failure of atorvastatin to modulate CSF HIV-1 infection: results of a pilot study. Neurology 71(7):521–524

Ragin AB, Wu Y et al (2006) Monocyte chemoattractant protein-1 correlates with subcortical brain injury in HIV infection. Neurology 66(8):1255–1257

Rallidis LS, Hamodraka ES et al (2008) Simvastatin exerts its anti-inflammatory effect in hypercholesterolaemic patients by decreasing the serum levels of monocyte chemoattractant protein-1. Int J Cardiol 124(2):271–272

Rantapaa-Dahlqvist S, Boman K et al (2007) Up regulation of monocyte chemoattractant protein-1 expression in anti-citrulline antibody and immunoglobulin M rheumatoid factor positive subjects precedes onset of inflammatory response and development of overt rheumatoid arthritis. Ann Rheum Dis 66(1):121–123

Roberts ES, Masliah E et al (2004) CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE). J Neuropathol Exp Neurol 63(12):1255–1264

Schaer CA, Vallelian F et al (2007) CD163-expressing monocytes constitute an endotoxin-sensitive Hb clearance compartment within the vascular system. J Leukoc Biol 82(1):106–110

Schepers A, Eefting D et al (2006) Anti-MCP-1 gene therapy inhibits vascular smooth muscle cells proliferation and attenuates vein graft thickening both in vitro and in vivo. Arterioscler Thromb Vasc Biol 26(9):2063–2069

Shan L, Siliciano RF (2014) Unraveling the relationship between microbial translocation and systemic immune activation in HIV infection. J Clin Invest 124(6):2368–2371

Sulicka J, Surdacki A et al (2013) Elevated markers of inflammation and endothelial activation and increased counts of intermediate monocytes in adult survivors of childhood acute lymphoblastic leukemia. Immunobiology 218(5):810–816

Tippett E, Cheng WJ et al (2011) Differential expression of CD163 on monocyte subsets in healthy and HIV-1 infected individuals. PLoS One 6(5):e19968

Valcour VG, Shiramizu BT et al (2010) HIV DNA in circulating monocytes as a mechanism to dementia and other HIV complications. J Leukoc Biol 87(4):621–626

Vassallo M, Mercie P et al (2012) The role of lipopolysaccharide as a marker of immune activation in HIV-1 infected patients: a systematic literature review. Virol J 9:174

Veillard NR, Braunersreuther V et al (2006) Simvastatin modulates chemokine and chemokine receptor expression by geranylgeranyl isoprenoid pathway in human endothelial cells and macrophages. Atherosclerosis 188(1):51–58

Wada NI, Jacobson LP et al (2015) The effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation. AIDS 29(4):463–471

Wallet MA, Rodriguez CA et al (2010) Microbial translocation induces persistent macrophage activation unrelated to HIV-1 levels or T-cell activation following therapy. AIDS 24(9):1281–1290

Wang ZM, Liu C et al (2000) Chemokines are the main proinflammatory mediators in human monocytes activated by Staphylococcus aureus, peptidoglycan, and endotoxin. J Biol Chem 275(27):20260–20267

Wang J, Zou H et al (2006) The expression of monocyte chemoattractant protein-1 and C-C chemokine receptor 2 in post-kidney transplant patients and the influence of simvastatin treatment. Clin Chim Acta 373(1-2):44–48

Wang H, Sun J et al (2008) Human immunodeficiency virus type 1 infection increases the in vivo capacity of peripheral monocytes to cross the blood-brain barrier into the brain and the in vivo sensitivity of the blood-brain barrier to disruption by lipopolysaccharide. J Virol 82(15):7591–7600

Wang Z, Zheng Y et al (2013) High prevalence of HIV-associated neurocognitive disorder in HIV-infected patients with a baseline CD4 count </= 350 cells/muL in Shanghai, China. Biosci Trends 7(6):284–289

Watkins CC, Treisman GJ (2015) Cognitive impairment in patients with AIDS - prevalence and severity. HIV AIDS (Auckl) 7:35–47

Williams DW, Veenstra M et al (2014) Monocytes mediate HIV neuropathogenesis: mechanisms that contribute to HIV associated neurocognitive disorders. Curr HIV Res 12(2):85–96

Williams DW, Eugenin EA et al (2012) Monocyte maturation, HIV susceptibility, and transmigration across the blood brain barrier are critical in HIV neuropathogenesis. J Leukoc Biol 91(3): 401–415

Williams DW, Calderon TM et al (2013) Mechanisms of HIV entry into the CNS: increased sensitivity of HIV infected CD14 + CD16+ monocytes to CCL2 and key roles of CCR2, JAM-A, and ALCAM in diapedesis. PLoS One 8(7):e69270

Wilson EM, Singh A et al (2014) Monocyte-activation phenotypes are associated with biomarkers of inflammation and coagulation in chronic HIV infection. J Infect Dis 210(9):1396–1406

Yadav A, Collman RG (2009) CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. J Neuroimmune Pharmacol 4(4):430–447

Yuan L, Qiao L et al (2013) Cytokines in CSF correlate with HIV-associated neurocognitive disorders in the post-HAART era in China. J Neurovirol 19(2):144–149

Yuan L, Liu A et al (2015) The relationship of CSF and plasma cytokine levels in HIV infected patients with neurocognitive impairment. Biomed Res Int 2015:506872

Zanin V, Delbue S et al (2012) Specific protein profile in cerebrospinal fluid from HIV-1-positive cART-treated patients affected by neurological disorders. J Neurovirol 18(5):416–422

Zawada AM, Rogacev KS et al (2011) SuperSAGE evidence for CD14++ CD16+ monocytes as a third monocyte subset. Blood 118(12):e50–e61

Zhou H, Lapointe BM et al (2006) A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide. J Immunol 177(11):8103–8110