Published in final edited form as:

J Neurovirol. 2016 October ; 22(5): 584–596. doi:10.1007/s13365-016-0433-8.

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

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Abstract

HIV-1 associated neurocognitive disorders (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 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 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

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Conflict of Interest:

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

referred to as HIV-associated neurocognitive disorders (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, Fumaz et al. 2008; McCombe, Vivithanaporn 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, Fedor 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, Gill et al. 2014).

The inflammatory CD14+CD16+ population of blood monocytes plays a particularly important role in the pathogenesis of HAND (Williams, Eugenin et al. 2012; Williams, Veenstra et al. 2014). This population is increased in chronic HIV-1 disease, particularly in people with neurocognitive complications (Fischer-Smith, Croul et al. 2001; Williams, Eugenin et al. 2012), and has tissue-invasive properties (Fischer-Smith, Croul et al. 2001; Williams, Calderon et al. 2013). Preferential infection of the CD16+ subset is thought to help virus traffic into tissues including brain (Ellery, Tippet et al. 2007; Valcour, Shiramizu 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, Croul et al. 2001; Roberts, Masliah et al. 2004; Borda, Alvarez et al. 2008; Fischer-Smith, Bell et al. 2008; Ndhlovu, Umaki 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, Alvarez 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, Rodrigues et al. 2007; Fischer-Smith, Bell 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, Weiffenbach et al. 2013; Liang, Duan et al. 2014; Wilson, Singh 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 lipopolysaccharide (LPS) (Wallet, Rodriguez et al. 2010; d'Ettorre, Paiardini et al. 2011; Shan and Siliciano 2014). Elevated LPS triggers monocyte activation, including upregulated CD16 and CD163 expression (Tippett, Cheng et al. 2011; Vassallo, Mercie et al. 2012), and enhances trafficking into the brain by compromising the integrity of the blood brain barrier (BBB) (Zhou, Lapointe et al. 2006; Wang, Sun 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 MCP-1, which is especially relevant to HIV neuropathogenesis as elevated MCP-1 is associated with increased risk of progression to neurocognitive disease (Eugenin, Osiecki et al. 2006; Dhillon, Williams et al. 2008; Zanin, Delbue 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, Lederman et al. 2009; Piconi, Trabatttoni et al. 2010), which is the same group at greatest risk for HAND despite ART treatment (Ellis, Badiie et al. 2011; Wang, Zheng 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, Stein et al. 2001; Aboyans, Labrousse et al. 2006; Almuti, Rimawi et al. 2006). Accumulating evidence suggests that these immunomodulatory effects are a major component of their profound benefit in atherosclerosis (Everett, Glynn 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, Eefting et al. 2006; Isoda, Folco et al. 2008; McKibben, Margolick et al. 2015). Statins are known to impact monocyte phenotypes in atherosclerosis, although the extent to which cholesterol lowering versus pleiotropic mechanisms contribute are uncertain. However, both in vitro and animal studies have shown that statins can attenuate pro-inflammatory responses to LPS (Methe, Kim 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, Steiner et al. 2006; De Wit, Delforge et al. 2011; Ganesan, Crum-Cianflone et al. 2011; Funderburg, Jiang et al. 2014; Funderburg, Jiang et al. 2015), but neurocognitive disease as a target has received less attention. Therefore, we asked here whether statins 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 warrant 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 μ M final concentration. Freshly isolated PBMCs were suspended at 1 $\times$ 10⁶ cells/ml in complete RPMI-1640 and were incubated with or without 10 μ M (Methe, Kim et al. 2005; Pozo, de Nicolas et al. 2006; Veillard, Braunersreuther et al. 2006) simvastatin or atorvastatin for 24 hours. Control cultures were incubated with vehicle (ethanol or DMSO respectively). Cultures were then stimulated for 24h 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 6.5mm diameter transwell inserts in 24 well plates containing polycarbonate membranes with 5 μ m pores (Costar). The lower chambers containing 600 μ l of media (RPMI + 0.1% BSA) and MCP-1 or RANTES (100 ng/ml; Peprotech) were equilibrated at 37°C for 1hr, following which transwells containing monocytes (2 $\times$ 10⁵ cells/100 μ l) were inserted. After incubation at 37°C for 3hr, 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 above mentioned 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-1 α 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 2 tailed t-test and a p value ≤ 0.05 was considered to be significant. Results reported are expressed as mean $\pm$ standard deviation (SD) of independent experiments using different donors.

Results

Statin treatment decreases the CD14⁺CD16⁺ inflammatory monocyte sub-population 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 48h 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⁺ sub-population (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 24h 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 and 1b), simvastatin and atorvastatin both reduced the number of total CD14⁺CD16⁺ inflammatory monocytes at 48 hours (Fig. 1c and 1d, 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” sub-population of the CD14⁺CD16⁺ monocytes (Fig. 1c and 1d, 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 and 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 and d lower panels; e), as well as a significant 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 towards 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, Graversen et al. 2001; Schaer, Vallelleian et al. 2007) that is significantly up-regulated on the CD14⁺⁺CD16⁺ monocytes (Tippett, Cheng et al. 2011) and has been suggested as a biomarker for HIV-1 disease progression and neurocognitive disease (Roberts, Masliah et al. 2004; Tippett, Cheng et al. 2011). Therefore, we evaluated the effects of statin treatment on monocyte CD163 expression. As shown in Fig. 3a and 3b, 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, Cheng et al. 2011), indicating statin-induced reduction in CD16⁺/CD163⁺ monocytes, a key population implicated in progression of HAND (Roberts, Masliah et al. 2004; Tippett, Cheng 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, Liu 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, Liu 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-1 α and a strong trend towards reduction in IL-8 production ($p < 0.09$) (Fig. 4b & 4c) (MCP-1 stimulation did not induce MIP-1 α production, and IL-8 induction was minimal; data not shown). Both MIP-1 α 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, Giovannetti et al. 2002; Letendre, Zheng et al. 2011; Bastard, Soulie et al. 2012; Mamik and Ghorpade 2014; Wada, Jacobson et al. 2015). Additionally, CSF IL-8 correlates with HIV-associated neurocognitive disorders (Kamat, Lyons et al. 2012; Yuan, Qiao et al. 2013; Yuan, Liu et al. 2015).

Since MCP-1 is involved in the trafficking of monocytes to the CNS (Williams, Calderon 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 μ m 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 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, Burger 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 and c; representative dot plots in Supplementary Fig. 3a and b), with a trend towards 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 and 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 sub-population, and 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, Croul 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, Gill 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, Lederman et al. 2009; Pilakka-Kanthikeel, Kris et al. 2014). Similarly, MCP-1 is elevated in HIV infection and, in particular, recruits monocytes to tissues (Williams, Calderon 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, Eugenin 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, Cheng et al. 2011). CD163 is a scavenger receptor for haptoglobin-hemoglobin complexes (Graversen, Madsen et al. 2002), which also serves as a receptor for bacterial products (Fabriek, van Bruggen et al. 2009). The CD16⁺CD163⁺ monocyte subset is also preferentially infected by HIV-1 (Jaworowski, Kamwendo 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, Autissier et al. 2006; Ancuta, Kunstman et al. 2006; Ancuta, Wang et al. 2006). We found that statin treatment particularly impacts CD14⁺CD16⁺ sub-population 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, Rogacev et al. 2011; Wilson, Singh et al. 2014), has recently been linked to inflammation and monocyte activation (Sulicka, Surdacki et al. 2013), and are the main producer of reactive oxygen species (a cause of oxidative stress), among the monocyte subsets (Zawada, Rogacev et al. 2011). Of note, at the time point analyzed (after 48h 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 (Figs 4a, b and 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, Cheng et al. 2001; Han, Ryu et al. 2005; Wang, Zou et al. 2006; Rallidis, Hamodraka et al. 2008). MCP-1 is believed to be a principal driver of monocyte trafficking into the CNS (Eugenin, Osiecki et al. 2006; Dhillon, Williams 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 leukocytes transmigration across the BBB in HIV-1 disease (Lane, Lore et al. 2001; Kamat, Lyons et al. 2012). These findings are consistent with prior reports (Bruegel, Teupser et al. 2006; Marino, Maresca 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, Vago et al. 1998; Park, Wang et al. 2001; Namiki, Kawashima et al. 2002; Ragin, Wu et al. 2006; Rantapaa-Dahlqvist, Boman 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, Crum-Cianflone 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, Spudich 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, Jiang et al. 2014; Funderburg, Jiang 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, Hileman 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, Marquie-Beck 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, Gill 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 a outcome of statin immunomodulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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).

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Fig. 1. Statin treatment decreases the number of CD14+CD16+ inflammatory monocytes in vitro Freshly isolated PBMCs were treated with simvastatin or atorvastatin (10 μ M) for 24hr, or vehicle alone, following which cells were stimulated with LPS (100ng) or MCP-1 (100ng) for 24hrs. Monocyte surface expression of CD16 was analyzed by FACS 24hr 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 $\pm$ SD of 6 independent experiments using cells from different donors (* p <0.05 and ** p <0.001; 2 tailed t-test). (**e**) Representative histograms showing decrease in monocyte CD14++ expression following simvastatin and atorvastatin treatment.

Fig. 2. Statin treatment differentially affects monocyte surface markers

Mean fluorescence intensity (MFI) of CD14, CD16 and CD163 on resting and stimulated monocytes in presence and absence of (a) simvastatin or (b) atorvastatin (10 μ M). Data represent mean $\pm$ SD of 6 independent experiments using cells from different donors (* p <0.05, ** p <0.001 and *** p <0.0001 as compared to untreated group; 2 tailed t-test).

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 μ M). **(b)** Quantitated data from 6 independent experiments each for simvastatin and atorvastatin using cells from different donors (mean $\pm$ SD). **(c)** Representative dot plot showing effect of simvastatin on monocytes co-expression of CD16 and CD163. Data represent mean $\pm$ SD of 6 independent experiments (* p <0.05 and ** p <0.001 as compared to untreated group; 2 tailed t-test).

Fig. 4. Simvastatin inhibits production of inflammatory chemokines by PBMC

PBMCs were treated with simvastatin, stimulated with LPS (100ng), and supernatant levels of MCP-1 (a), MIP-1α (b) and IL-8 (c) were determined by Luminex assay. Results expressed as mean $\pm$ SD from 4 separate experiments using cells from different donors (*p<0.05 as compared to untreated group; 2 tailed t-test).

Fig. 5. Simvastatin inhibits MCP-1 dependent monocyte chemotaxis

Monocytes were treated with simvastatin (10 μ M) for 24hr, added to transwell inserts, and exposed to lower chambers containing MCP-1 or RANTES (100 ng/ml). Migrating cells were counted 3 hours later as described in Materials and Methods. Results are expressed as chemotactic index and reflect mean $\pm$ SD from 4 separate experiments using cells from different donors, performed in duplicate (* p <0.05; 2 tailed t-test).

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 μ M) for 24hr, or vehicle alone, following which cells were stimulated with LPS (100ng) or MCP-1 (100ng) for 24hrs. Monocyte surface expression of CD16 and CD163 was analyzed by FACS 24hr 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 $\pm$ SD of 3 independent experiments using cells from different donors (*p < 0.05; 2 tailed t-test).