The Expression of Cholesterol Metabolism Genes in Monocytes From HIV-Infected Subjects Suggests Intracellular Cholesterol Accumulation

Eoin R. Feeney,1,2 Nuala McAuley,1 Jane A. O’Halloran,2 Clare Rock,2 Justin Low,2 Claudette S. Satchell,1 John S. Lambert,2 Gerald J. Sheehan,2 and Patrick W. G. Mallon1,2

1HIV Molecular Research Group, School of Medicine and Medical Science, University College Dublin, and 2Department of Infectious Diseases, Mater Misericordiae University Hospital, Dublin, Ireland

Background. Human immunodeficiency virus (HIV) infection is associated with increased cardiovascular risk and reduced high-density lipoprotein cholesterol (HDL-c). In vitro, HIV impairs monocyte-macrophage cholesterol efflux, a major determinant of circulating HDL-c, by increasing ABCA1 degradation, with compensatory upregulation of ABCA1 messenger RNA (mRNA).

Methods. We examined expression of genes involved in cholesterol uptake, metabolism, and efflux in monocytes from 22 HIV-positive subjects on antiretroviral therapy (ART-Treated), 30 untreated HIV-positive subjects (ART-Naive), and 22 HIV-negative controls (HIV-Neg).

Results. HDL-c was lower and expression of ABCA1 mRNA was higher in ART-Naive subjects than in both ART-Treated and HIV-Neg subjects (both \(P < .01\)), with HDL-c inversely correlated with HIV RNA (\(\rho = -0.52; P < .01\)). Expression of genes involved in cholesterol uptake (LDLR, CD36), synthesis (HMGCR), and regulation (SREBP2, LXRA) was significantly lower in both ART-Treated and ART-Naive subjects than in HIV-Neg controls.

Conclusions. In vivo, increased monocyte ABCA1 expression in untreated HIV-infected patients and normalization of ABCA1 expression with virological suppression by ART supports direct HIV-induced impairment of cholesterol efflux previously demonstrated in vitro. However, decreased expression of cholesterol sensing, uptake, and synthesis genes in both untreated and treated HIV infection suggests that both HIV and ART affect monocyte cholesterol metabolism in a pattern consistent with accumulation of intramonic cholesterol.

Keywords. ABCA1; cardiovascular disease; cholesterol; HDL; HIV; monocytes; reverse cholesterol transport.

Human immunodeficiency virus (HIV) infection is associated with an increased risk of cardiovascular disease (CVD) [1]. Untreated HIV-infected subjects have reduced high-density lipoprotein cholesterol (HDL-c) when compared with HIV-negative controls [2], and acquisition of HIV is associated with a decline in HDL-c [3]. Low HDL-c is an independent risk factor for CVD [4], and in HIV-infected patients, HDL-c is second only to age in relative contribution to calculated CVD risk [5]. HDL-c is involved in reverse cholesterol transport—the process whereby cholesterol is transported from peripheral tissues and atherosclerotic plaques back into the circulation and to the liver, where it can be recycled or eliminated [6]. Although the pathophysiology of low HDL-c in HIV infection remains unclear, higher HIV RNA is associated with lower HDL-c [7], and increased markers of inflammation (which directly correlate with ongoing viral replication [8]) are also associated with lower
HDLC [9], which suggests either a direct or indirect effect of the HIV virus on HDL metabolism.

Monocyte-macrophages play an important role in reverse cholesterol transport, cholesterol metabolism, and atherogenesis. Intracellular cholesterol accumulation in monocyte-macrophages leads to the formation of foam cells; cholesterol-laden macrophages, which are a characteristic feature of atherosclerotic plaques [10]. Intracellular cholesterol content depends on 3 major factors—cholesterol uptake into the cell, de novo cholesterol synthesis within the cell, and efflux of cholesterol out of cells. Monocytes can absorb or scavenge cholesterol (predominantly low-density lipoprotein [LDL] cholesterol) both in the circulation and in vessel walls (as macrophages) by means of the LDL receptor (LDLR) or CD36 receptor [11]. De novo intracellular cholesterol synthesis from 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) occurs by means of a pathway involving the HMG CoA reductase enzyme (HMGR) [12]. Monocytes efflux cholesterol by means of adenosine triphosphate (ATP)–binding cassette transporter A1 (ABCA1), a surface membrane protein that effluxes cholesterol from cells onto lipid-poor apolipoprotein A1 [6], forming nascent HDL-c particles. The membrane protein ATP-binding cassette transporter G1 (ABCG1) can also efflux cholesterol from cells, although onto lipidated lipoproteins created by ABCA1 [13].

Intracellular control of cholesterol content is controlled by 2 principal mechanisms (Figure 1). When intracellular sterol levels are low, the cholesterol sensor sterol regulatory element binding protein 2 (SREBP2) is upregulated [14]. This acts to increase cellular cholesterol by increasing cholesterol uptake and de novo synthesis through upregulation of LDLR [15] and HMGR [16] and decreasing efflux through downregulation of ABCA1 and ABCG1 [14, 17]. Conversely, when intracellular cholesterol is high, liver X receptor alpha (LXRA) is indirectly upregulated, probably by peroxisome proliferator-activated receptor gamma (PPARG) [18]. This acts to reduce intracellular cholesterol by increasing cholesterol efflux through increased ABCA1 and ABCG1 expression [12], increasing degradation of the LDL receptor [19], and increasing SREBP1c expression [20], which drives the cell toward fatty acid synthesis.

In vitro, HIV infection of monocytes directly affects intracellular cholesterol metabolism. Monocytes express the CCR5 coreceptor and may be infected with HIV [21] (although the number of infected cells is low [22]), and HIV-infected foam cells have been observed in atherosclerotic plaques from HIV-infected individuals [23]. It has been demonstrated in vitro in human macrophages that the HIV protein Nef binds to and increases degradation of ABCA1, leading to a reduction in cholesterol efflux from cells. This is accompanied by increased ABCA1 messenger RNA (mRNA) expression, which is thought to be a compensatory mechanism [23].

These findings have yet to be confirmed in vivo. We hypothesized that HIV infection would have effects not just on cholesterol efflux from cells but also on other genes involved in cholesterol uptake, synthesis, efflux, and regulation within circulating monocytes. We also questioned whether suppressive antiretroviral therapy (ART), which would reduce viral infection and therefore availability of HIV Nef protein, would have significant effects on these genes when compared with HIV-negative subjects. We therefore undertook to describe differences in gene expression in monocytes from HIV-infected subjects, HIV-negative controls, and virally suppressed HIV-infected subjects on ART.

METHODS

Subject Identification
Subjects were recruited from 3 groups; HIV-negative subjects confirmed by negative HIV antibody testing (HIV-Neg), HIV-infected subjects not on ART (ART-Naive), and HIV-infected subjects on suppressive ART with HIV RNA <50 copies/mL (ART-Treated). All subjects were required to be negative for the hepatitis C antibody and not on lipid-lowering therapy because these factors were expected to have separate and additional confounding effects on cholesterol metabolism [24]. The study was approved by the Mater Misericordiae University Hospital and Mater Private Hospital Research Ethics Committee, and all participants provided written, informed consent. HIV-infected subjects were recruited through the Department of Infectious Diseases at the Mater Misericordiae University Hospital, with the control group recruited from within the institution aligned with the department. Fasting blood samples (>10 hours overnight, water permitted) were obtained for clinical chemistry, lipids, lymphocyte T-cell subsets, monocyte isolation, and HIV RNA where applicable.

Monocyte Isolation
Monocytes were isolated using anti-CD14+–conjugated magnetic beads (Invitrogen). Buffy coats extracted from 20 mL of blood collected in EDTA were washed in a phosphate-buffered saline/0.1% bovine serum albumin and 0.6% sodium citrate solution and incubated on a rocker with beads for 30 minutes at 4°C. A magnet was used to separate bead-bound monocytes, which were washed 5 times with a phosphate-buffered saline/0.1% bovine serum albumin solution. After washing, TRIreagent (Ambion) was added, and the sample was snap frozen at −80°C. This method is expected to lead to at least an 80% depletion of monocytes from whole blood and <10% contamination with lymphocytes [25].

RNA, cDNA Preparation, and Quantitative Polymerase Chain Reaction
RNA was extracted from samples stored in TRIreagent as per manufacturer’s instructions and transcribed to complementary DNA (cDNA) as previously described [26]. Primers for each
gene were designed using Primer 3 [27] (http://frodo.wi.mit.edu/primer3/) unless referenced from previous publications and are listed in Table 1. Polymerase chain reaction (PCR) products were purified using the QIAquick PCR Purification Kit (Qiagen), sequenced (GATC Biotech) and entered into a BLAST search (http://blast.ncbi.nlm.nih.gov) to ensure primer specificity. Gene expression was determined on 2 µL aliquots of cDNA by real-time quantitative PCR using a 96-well, 480 Lightcycler platform (Roche Diagnostics) against standard curves of known concentration, derived from serial dilutions of cDNA generated using site-specific primers, quantified using SYBR Green, and sequenced as above. All samples were performed in duplicate, with internal positive and negative controls. When within-sample values were >0.5 log difference, the PCR reaction was repeated. Gene expression was normalized to the housekeeping gene ACTINB.

Statistical Analysis

Data are reported as median and interquartile range (IQR) unless otherwise stated. Between-group differences were compared using an unpaired $t$ test and Mann–Whitney $U$ test for parametric and nonparametric data, respectively (as determined by Q-Q plot). Correlations were performed using Spearman rank correlation. Linear regression was used to correct for significant differences between groups where applicable. After differences in the groups were realized (Table 2), differences between the HIV-Neg and ART-Naive groups were corrected by repeating the analysis limited to whites only and by correcting for baseline sex. Where differences between the ART-Naive and ART-Treated groups were observed, these were corrected for baseline age and sex, and differences between the HIV-Neg and ART-Treated groups were corrected by limiting the analysis to whites only.

Based on previous studies of monocyte gene expression that used the same protocol as this study [25], we estimated that with a mean gene expression ratio (gene of interest: ACTINB expression) of 1.14 with a standard deviation of 0.76, a sample size of 20 subjects per group would provide 80% power to determine a difference of 60.5% between groups at a significance of .05.

RESULTS

Twenty-two HIV-Neg, 30 ART-Naive, and 22 ART-Treated persons were recruited. Subject characteristics are listed in
Table 1. Primer Sequences

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene ID</th>
<th>Reference Sequence</th>
<th>Forward</th>
<th>Reverse</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>ACTINB [25]</td>
<td>NM_001010</td>
<td>5'-CTCTTCCAGCCTCTCCTCCT-3'</td>
<td>5'-AGACCTGCTTGGCCGTACAG-3'</td>
<td>116</td>
</tr>
<tr>
<td>ATP-binding cassette transporter A1</td>
<td>ABCA1</td>
<td>NM_005502</td>
<td>5'-CCAACACTCAGGAAGCAT-3'</td>
<td>5'-GAGCCGCTCATCATCTCAT-3'</td>
<td>137</td>
</tr>
<tr>
<td>ATP-binding cassette transporter G1</td>
<td>ABCG1</td>
<td>NM_004915</td>
<td>5'-ACTCGGGCTCCTCTCCTCCT-3'</td>
<td>5'-CATGGCTTTGAGCGGTTAGT-3'</td>
<td>152</td>
</tr>
<tr>
<td>Peroxisome proliferator-activated receptor γ</td>
<td>PPARG[25]</td>
<td>NM_015869</td>
<td>5'-TGCAGGGATCAAGGAGCAG-3'</td>
<td>5'-GAAGAAGGGAAATGTGGCA-3'</td>
<td>115</td>
</tr>
<tr>
<td>Sterol regulatory ele-</td>
<td>SREBF2</td>
<td>NM_004599</td>
<td>5'-TGGCTTCTCCCTCCTCCTCA-3'</td>
<td>5'-GAGAGGCACAGGAAAGTGAG-3'</td>
<td>153</td>
</tr>
<tr>
<td>mentary element</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver X receptor</td>
<td>NR1H3</td>
<td>NM_001130102</td>
<td>5'-CGGGATTCACACTAATGTT-3'</td>
<td>5'-TCAGGGATCTTGTCTCCT-3'</td>
<td>213</td>
</tr>
<tr>
<td>HMG CoA reductase</td>
<td>HMGCR</td>
<td>NM_000859</td>
<td>5'-GTCATTCCAAGCCAAGTTGT-3'</td>
<td>5'-CATCTCTGTCATCCTGT-3'</td>
<td>184</td>
</tr>
<tr>
<td>CD36</td>
<td>CD36</td>
<td>NM_001001547</td>
<td>5'-ATGGAAGTGAGCAGCCTA-3'</td>
<td>5'-GCCATTGAGTGAAGAAA-3'</td>
<td>157</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>LDLR</td>
<td>NM_000527</td>
<td>5'-GCTTGTCTGACCTCGAAGA-3'</td>
<td>5'-AATGCGCAGAGATGCATTT</td>
<td>190</td>
</tr>
</tbody>
</table>

Abbreviations: ATP, adenosine triphosphate; CoA, coenzyme A.

Table 2. Subjects in the ART-Treated group tended to be older than those in the other 2 groups, were slightly more males in the ART-Naive group than in the other 2 groups, and there were more white subjects in the HIV-Neg group than in the other 2 groups. All ART-Treated subjects had an undetectable HIV RNA (<50 copies/mL); the majority (n = 21, 95%) were treated with the nucleoside and nucleotide reverse-transcriptase combination tenofovir/emtricitabine as an antiretroviral backbone, with 12 on the protease inhibitor lopinavir/ritonavir and 10 on the nonnucleoside reverse-transcriptase inhibitor efavirenz as a third agent. Sufficient RNA for analysis could be extracted from all subjects except for 2 HIV-infected subjects, 1 in the ART-Naive group and 1 in the ART-Treated group. There was no between-group difference in expression of the housekeeping gene (ACTINB) between arms (data not shown).

Fasting Lipid Parameters

Compared with subjects in the other 2 groups, ART-Naive subjects had significantly lower total cholesterol (ART-Naive, 4.3 [3.8–4.8] mmol/L vs HIV-Neg, 5.3 [4.7–5.7] mmol/L and ART-Treated 4.9 [4.4–5.5] mmol/L; both P < .01) and HDL-c (ART-Naive, 0.9 [0.8–1.3] mmol/L vs HIV-Neg, 1.4 [1.2–1.7] mmol/L and ART-Treated 1.3 [1.1–1.8] mmol/L; both P < .01). There was a trend toward lower LDL cholesterol in ART-Naive subjects compared with HIV-Neg controls (2.8 [2.6–3.2] vs 3.3 [2.6–3.6] mmol/L; P = .054; Figure 2A–C). These differences remained significant after correction. There was no substantial difference in any fasting parameter between HIV-Neg and ART-Treated subjects. Lower HDL-c correlated with higher HIV RNA regardless of whether all HIV-positive subjects were analyzed as a whole (ρ = −0.57; P < .001) or whether the analysis was restricted to untreated HIV-infected subjects (Figure 2D; ρ = −0.52; P < .001).

Gene Expression

Cholesterol Efflux

In ART-Naive subjects, ABCA1 expression was significantly higher than in subjects in the HIV-Neg (P < .01) or ART-Treated groups (P = 0.04), consistent with in vitro data that showed a Nef-induced block of cholesterol efflux was associated with upregulated ABCA1 expression (Figure 3A). Among all HIV-infected subjects, higher ABCA1 expression correlated with higher HIV RNA (ρ = 0.49; P < .01) and trended toward correlation with lower HDL-c (ρ = −0.27; P = .06), all of which supports a scenario where impairment in cholesterol efflux and reverse cholesterol transport lead to the lower HDL-c seen in HIV infection. Although there was no significant difference in ABCG1 expression between groups (Figure 1B, P = .06 and P = .18), there was a trend toward lower ABCG1 expression in the ART-Naive group compared with the other 2 groups. Between-group differences in ABCA1 expression were not altered by correction for differences in baseline demographics.
Cholesterol Uptake

Expression of both cholesterol uptake genes \( CD36 \) and \( LDLR \) were significantly lower in the ART-Naive group than in the HIV-Neg group (Figure 3C and 3D, \( P < .001 \) and \( P < .01 \), respectively). However, unlike changes in cholesterol efflux genes, expression of \( CD36 \) and \( LDLR \) remained lower in those in the ART-Treated group, with differences again remaining unchanged after correction for baseline differences between groups.

Abbreviations: ABCA1, adenosine triphosphate–binding cassette transporter A1; ABCG1, adenosine triphosphate–binding cassette transporter G1; ACTINB, β-actin; ART, antiretroviral therapy; EFV, efavirenz; LDLR, low-density lipoprotein receptor; HMGCR, HMG coenzyme A reductase; LPVr, lopinavir/ritonavir; LXRα, liver X receptor alpha; PPARG, peroxisome proliferator-activated receptor gamma; SREBP2, sterol regulator element binding protein 2.
groups. Within the ART-Treated group, expression of CD36 and LDLR was higher among those subjects on efavirenz than those on lopinavir, with the between-group difference in expression of CD36 reaching statistical significance ($P = .01$). These findings suggest that, in untreated HIV-positive subjects, there is a downregulation of cholesterol uptake in circulating monocytes and this downregulation persists in those on effective ART, changes which may reflect excess availability of intracellular cholesterol within cells, with cells attempting to further reduce cholesterol uptake as a result.

**Cholesterol Synthesis and Regulation**

Further supporting this view, expression of the cholesterol synthesis gene HMGCR was markedly lower in both HIV-positive groups when compared with the HIV-Neg group (Figure 3E), and remained significant after correction for between group differences. Within the ART-Treated group, there was no difference in expression regardless of the use of efavirenz or lopinavir, suggesting reduced new cholesterol formation within monocytes, which is again consistent with increased availability of intracellular cholesterol.

Similarly, expression of the sterol-sensing gene SREBP2 was significantly lower in the ART-Naive group than in the HIV-Neg group ($P = .03$), and was significantly lower again in the ART-Treated group ($P = .001$), with a trend toward lower expression in those on lopinavir-based therapy ($P = .07$) (Figure 3G). Again, these differences were not substantially changed after correction for differences in baseline demographics. SREBP2 expression is downregulated in the setting of increased intracellular sterols, so these data are again consistent with increased intracellular cholesterol. In a similar fashion, expression of the nuclear cofactor LXRA was lower in the ART-Naive group than in the HIV-Neg group, and much lower among ART-Treated subjects, with no difference between those treated with efavirenz or lopinavir (Figure 3H). After correction for differences in ethnicity, the difference in LXRA expression between HIV-Neg subjects and ART-Naive subjects was no longer significant ($P = .054$), although correcting for other differences in baseline demographics had no effect on significance. There was no difference in PPARG expression between any of the 3 groups (Figure 3F).

**DISCUSSION**

This is the first study to examine the in vivo expression of cholesterol metabolism genes in monocytes of HIV-infected patients. Our findings support previous in vitro data that suggest a direct effect of HIV infection on control of cholesterol efflux. In addition, we have observed differences in expression of genes involved in cholesterol uptake, synthesis, control, and efflux in monocytes from both untreated and treated HIV-infected subjects that point to a profile consistent with an excess of available intracellular cholesterol in monocytes of HIV-infected patients. This pattern of changes would be concerning for accelerated atherosclerosis given the role of intracellular cholesterol accumulation in monocyte-macrophages in the development of atherosclerosis.

Our data confirm that untreated HIV-infected subjects have lower HDL-c compared with both treated and untreated controls and that higher HIV RNA correlates with lower HDL-c [7]. This fits with a direct effect of HIV on HDL metabolism. The cholesterol concentration of the HIV lipid envelope is approximately 2.5 times that of normal cell membranes [28], and HIV preferentially assembles and buds from the site of cholesterol-rich lipid rafts on the cell membrane [29]. It therefore benefits the virus to increase intracellular cholesterol concentrations, a scenario supported by the observed gene profile.

Evidence currently points to a role for HIV Nef binding ABCA1 and increasing its degradation, which leads to a reduction in cholesterol efflux from monocytes [23]. Even though HIV infects few, if any, monocytes, circulating Nef is detectable in the plasma of HIV-infected individuals [30] and can be taken up by uninfected cells [31]. In animal models, plasma from simian immunodeficiency virus–infected macaques blocks ABCA1-mediated cholesterol efflux, an effect that can be reversed by the addition of anti-Nef antibodies [32]. These data suggest that HIV Nef may affect cells without the cells being directly infected by virus. This proposed mechanism of impairment of cholesterol efflux from cells is supported by our findings of higher ABCA1 expression in untreated HIV-infected patients than in uninfected controls, presumably reflecting compensatory upregulation of ABCA1 in response to Nef-mediated impairment of cholesterol efflux previously described in vitro [23] and the fact that higher ABCA1 expression correlates with both higher HIV RNA and lower HDL-c. Our data also support the hypothesis that low HDL-c in HIV infection is due to impairment in cholesterol efflux and reverse cholesterol transport through a direct impairment of ABCA1 function. This is further supported by the pattern of fasting lipids observed in untreated HIV-infection (low HDL, LDL, and total cholesterol), which is similar to (but less severe than) the pattern seen in homozygous Tangier disease, where an inherited mutation in ABCA1 leads to a nonfunctioning protein unable to efflux cholesterol from cells, which is clinically characterized by accelerated atherosclerosis and premature cardiovascular disease [33, 34]. Indeed, the potential role for HIV in reducing HDL in vivo is further supported from our observations in treated HIV-infected subjects, in whom ABCA1 and ABCG1 expression were observed to be similar to that in HIV-Neg controls. That HDL-c levels in those on ART are also similar to those in HIV-negative controls supports a viral effect on ABCA1 that causes the reduction in HDL-c observed in untreated HIV infection and that is removed with suppressive ART.
We also demonstrate the novel finding that expression of genes involved in cholesterol uptake (CD36 and LDLR) and cholesterol synthesis (HMGCR) are lower in untreated HIV infection, in conjunction with a lower expression of the cholesterol sensor SREBP2. Because SREBP2 is directly downregulated in the setting of increased intracellular cholesterol and both cholesterol uptake and synthesis would be expected to be reduced in the setting of excess intracellular cholesterol, these changes are consistent with an overall picture of excess intramonoocyte cholesterol and defective efflux in untreated HIV-positive subjects. We also demonstrate that LXRA expression is lower in untreated HIV-infected subjects than in HIV-negative controls. This finding is unexpected because expression of LXRA would be expected to be the opposite of the expression of SREBP2. Unlike SREBP2, LXRA expression is not directly affected by intracellular cholesterol concentrations but is controlled through an indirect pathway involving PPARG, the expression of which was not different between groups. Therefore, there may be other, as yet unreported interactions or pathways between LXRA and other genes not examined here, which may have given rise to these findings.

However, despite the observations of restored ABCA1 expression and HDL in those on effective, suppressive ART, expression of cholesterol uptake and synthesis genes remained lower in treated subjects than in HIV-negative subjects. In addition, both SREBP2 and LXRA expression were also lower in treated subjects than in HIV-negative or untreated HIV-infected subjects. These data suggest that the lower SREBP2 observed is due to ongoing cholesterol accumulation within these cells. Therefore, despite successful control of viral replication through ART, antiretrovirals may have further effects on intracellular cholesterol metabolism that result in a gene expression profile that suggests persistent excess intracellular cholesterol, an area that requires further research.

Because protease inhibitors have been shown to impair both SREBP and PPARG expression in adipose tissue [35] and both these genes play important roles in the regulation of cholesterol metabolism, we examined the difference in expression of these genes depending on whether patients were treated with ART comprising the protease inhibitor lopinavir or the non-nucleoside reverse transcriptase inhibitor efavirenz. Although we observed a trend toward lower PPARG and SREBP2 expression in lopinavir-treated subjects (which would be expected), in conjunction with a lower expression of cholesterol uptake and synthesis genes, these differences did not reach statistical significance, perhaps reflecting a lack of statistical power secondary to low numbers in each arm.

Our data differ from that in some previous studies. In vitro data has demonstrated that protease inhibitor exposure (such as ritonavir) can upregulate CD36, LXRa, and PPARG mRNA in human macrophages in vitro [36] and that ritonavir exposure increases CD36 expression in murine macrophages [37].

Another large cross-sectional study found increased CD36 expression as measured by flow cytometry on CD14+ monocytes isolated from both untreated and treated HIV-infected subjects compared with HIV-negative controls [38]. These previous in vitro data are in contrast to our findings and warrant further investigation. We observed lower CD36 and a trend toward lower PPARG expression in lopinavir-/ritonavir-treated subjects compared with those treated with efavirenz, with expression of both well below that observed in HIV-negative controls. In vitro studies on isolated cells are unable to consider the significant effects of HIV infection on cholesterol metabolism in vivo, and any effects of antiretrovirals need to be considered alongside potential effects of HIV on cholesterol homeostasis. In addition, differences in gene expression may not always correlate with differences in protein levels or cell-surface protein expression.

In vitro, it has been demonstrated that viral infection of murine macrophages results in reduced cholesterol synthesis through an interferon γ–driven downregulation of SREBP2 and HMGCR [39]. This has been postulated as an innate immune response to viral infection designed to reduce the cholesterol supply available to viruses. This could explain some of the downregulation of SREBP2 and HMGCR expression observed in ART-Naive subjects, but it does not explain the lower expression observed in virally suppressed ART-Treated subjects, suggesting a potential additive effect of ART exposure on the decreased expression of these genes.

Our study does have limitations, not least its cross-sectional design, which limited our ability to directly assess causality. There are some demographic differences between groups, which we have corrected for through statistical methods. In addition we did not examine protein expression, and because no subjects have experienced clinical cardiovascular disease, we have no data on the clinical consequences of the observed derangements in gene expression on cardiovascular endpoints. Although measurement of both plasma and intracellular HIV Nef would potentially reinforce our data, such measurements, alongside assays of monocyte activation, were beyond the scope of this study. The role of monocyte activation in particular will require further, preferably longitudinal, studies, some of which are already under way. Nonetheless, these data are consistent with previous reports and will help guide prospective studies to further examine the effect of both HIV infection and ART on gene expression, intracellular cholesterol, and cholesterol metabolism in monocytes in vivo.

In summary, we confirm that ABCA1 expression in monocytes is altered in vivo in HIV infection, and we demonstrate the novel finding of derangements in expression of numerous genes involved in cholesterol metabolism in monocytes of untreated HIV-infected subjects that points to excess intracellular cholesterol availability compared with HIV-negative controls. Although ABCA1 expression in those on effective ART returns
to levels similar to that of HIV-negative controls, appreciable differences in the expression of cholesterol synthesis, uptake, and regulatory genes remain. These data in treated HIV-infected subjects suggest that monocyte cholesterol accumulation may be occurring despite effective ART, point to potential mechanisms that may contribute to the elevated risk of cardiovascular disease observed in both treated and untreated HIV-infected patients, and serve to highlight the importance of defects in reverse cholesterol transport and HDL-c metabolism in this vulnerable patient group. Further prospective studies examining the effect of both HIV infection and ART on these genes, as well as on intracellular cholesterol and their clinical effects, are required.

Notes

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