High-Density Lipoprotein-Mediated Cholesterol Efflux Capacity Is Improved by Treatment With Antiretroviral Therapy in Acute Human Immunodeficiency Virus Infection

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Background. Individuals infected with human immunodeficiency virus (HIV) have decreased high-density lipoprotein (HDL)-cholesterol and increased cardiovascular disease (CVD). Reverse cholesterol transport from macrophages may be inhibited by HIV and contribute to increased CVD. Human studies have not investigated longitudinal effects of HIV and antiretroviral therapy (ART) on cholesterol efflux.

Methods. Subjects with acute HIV infection were randomized to ART or not. Cholesterol efflux capacity was determined ex vivo after exposure of murine macrophages to apolipoprotein B-depleted patient sera obtained at baseline and after 12 weeks.

Results. After 12 weeks, HIV RNA decreased most in subjects randomized to ART. Available data on cholesterol demonstrated that efflux capacity from Abca1$^{+/+}$ macrophages was increased most by sera obtained from ART-treated subjects (20.5% ± 5.0% to 24.3 % ± 6.9%, baseline to 12 weeks, $P = .007$; ART group [$n = 6$] vs 18.0 % ± 3.9% to 19.1 % ± 2.9%, baseline to 12 weeks, $P = .30$; untreated group [$n = 6$] [$P = .04$ ART vs untreated group]). Change in HIV RNA was negatively associated with change in Abca1$^{+/+}$ macrophage cholesterol efflux ($r = -0.62$, $P = .03$), and this finding remained significant ($P = .03$) after controlling for changes in HDL-cholesterol, CD4$^+$ cells, and markers of monocyte or macrophage activation.

Conclusions. In subjects acutely infected with HIV, ATP-binding cassette transporter A1-mediated cholesterol efflux was stimulated to a greater degree over time by apolipoprotein B-depleted serum from subjects randomized to ART. The improvement in cholesterol efflux capacity is independently related to reduction in viral load.

Keywords. acute HIV infection; antiretroviral therapy; atherosclerosis; cholesterol efflux; inflammation.

Patients living with human immunodeficiency virus (HIV) infection are at increased risk of cardiovascular disease [1–5]. One of the potential mechanisms for atherosclerosis developing in patients infected with HIV may relate to the effects of the virus on reverse cholesterol transport. Human immunodeficiency virus infection may affect reverse cholesterol transport by multiple mechanisms including alterations in high-density lipoprotein (HDL)-cholesterol function, impairment of cellular cholesterol efflux by the virus or one of its proteins, or other mechanisms. In this regard, in vitro studies have demonstrated HIV Nef protein can impair cellular cholesterol efflux through down-regulation of ATP-binding cassette transporter A1 (ABCA1) [6]. Genetic mutations in the ABCA1 gene (*ABC1*) cause Tangier disease, a rare autosomal recessive disorder in which patients have HDL-cholesterol deficiency,

accumulation of cholesterol esters, and early atherosclerosis [7]. ATP-binding cassette transporter A1 plays a crucial role in stimulating cholesterol export from macrophages. Patients with HIV also have decreased HDL-cholesterol, which may be mediated in part via this mechanism [6, 8, 9]. We took advantage of a novel study, identifying patients with acute HIV infection for randomization to immediate antiretroviral therapy (ART) or no therapy, to assess the effects of reduction in viral load on cholesterol efflux capacity in a human model.

Acute HIV infection is defined by the time period from viral entry to completion of seroconversion and is characterized by high-level viremia, typically reaching levels of several million viral copies/mL plasma, higher than any other period in the natural history of HIV infection [10]. Whether to initiate ART for patients diagnosed with acute HIV infection remains controversial, and an emerging hypothesis is that the "seroconversion window" represents a unique opportunity for treatment and possible early modulation of the host's immune response to the virus [11]. This acute phase of HIV infection can also provide an insightful window into processes that may drive the development of atherosclerosis in patients infected with HIV. Thus, we chose this early stage of HIV infection to study the effects of virus on cholesterol efflux because this is the phase of infection when viremia is typically at its peak. The main hypothesis of this study is that the high magnitude of viremia characteristic of acute HIV infection impairs cholesterol efflux from macrophages that could be improved with suppression of viral replication by ART.

METHODS

Selection of Participants
Subjects were recruited from an ongoing, open-label, randomized trial of patients with acute HIV infection in which subjects were randomized at entry to 1 of 3 arms: (1) no therapy, (2) ART for 12 weeks, or (3) ART for 32 weeks in 2:1:1 ratio to assess effects of ART on HIV RNA and viral load set point. We received independent National Institutes of Health funding to perform an add-on study addressing a new endpoint of cholesterol efflux using this paradigm of acutely infected ART-naive patients. In this study, we assessed effects of ART on cholesterol efflux as well as fasting lipids, monocyte activation markers, and endothelial function over the first 12 weeks of randomized treatment. Eligible subjects who completed baseline and 12-week visits are included. Data from the 2 ART arms were collapsed into 1 group, because both of these groups received ART for the initial 12 weeks, compared with the group not randomized to ART. This design resulted in 2 analysis groups: ART-treated ($n = 8$) vs untreated ($n = 9$) (Figure 1).

To be included, subjects were identified to have acute HIV-1 infection defined by positive HIV viral load ($\geq$5000 copies RNA/mL plasma) and negative or indeterminate HIV-1 Western blot. Subjects were required to have the following: hemoglobin $\geq$7.0 g/dL; creatinine $\leq$3 × upper limit of normal (ULN);
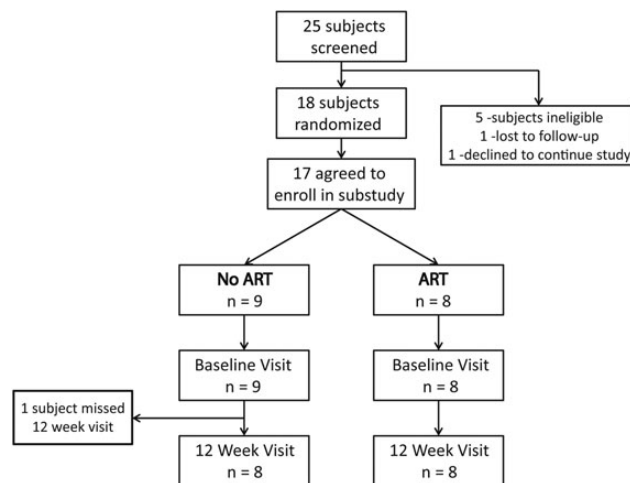

Figure 1. Overall schema for the current substudy is shown. The current substudy investigated cholesterol efflux capacity and other cardiovascular endpoints at baseline and week 12.

aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase <5 × ULN; and total bilirubin $\leq$2.5 × ULN. Exclusion criteria included opportunistic infections or acquired immune deficiency syndrome-defining illness not attributable to acute seroconversion illness, pregnancy or breastfeeding, immunosuppressive medications or immunomodulators, concomitant ART, anti-neoplastic agents, anti-tuberculosis prophylaxis or therapy, and serious illness other than acute HIV infection. All participants provided written informed consent, and the study was approved by Partners Healthcare Institutional Review Board. The trial is registered on ClinicalTrials.gov (NCT00705926).

Methods for Macrophage Cholesterol Efflux Assay Using Apolipoprotein B-Depleted Sera as an Acceptor
The cholesterol efflux assay was performed as follows: immortalized bone marrow macrophages were generated from $Abca1^{-/-}$ and $Abca1^{+/+}$ littermate mice (DBA/1-Abca1tm1 Jdm/J strain; Jackson Laboratories) as previously described [12]. All animal procedures were approved by the Massachusetts General Hospital (MGH) Subcommittee on Research Animal Care and were conducted in accordance with US Department of Agriculture Animal Welfare Act and Public Health Service Policy for Humane Care and Use of Laboratory Animals. After establishing single-cell clonal lines, 24-well plates were seeded at 100 000 cells/well in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 0.5 µg/mL penicillin/streptomycin and incubated for 48 h with liver X receptor (LXR) agonist (T0901317; Sigma-Aldrich) at final concentration of 1 µM. Cells were incubated in DMEM containing 10% FBS, 0.5 µg/mL penicillin/streptomycin, 1 µM LXR agonist, and 1.0 µCi/mL of [³H]-cholesterol for 24 h. Afterward, cells were washed with warm phosphate-buffered saline (PBS) and incubated at 37°C in 2 mg/mL fatty acid-free albumin

DMEM for 2 h. Cells were washed with PBS and treated with apolipoprotein (Apo)B-depleted sera from study subjects. In 24-well plate, 3 wells were left without treatment (media), 3 were used as positive control for ABCA1 only dependent efflux (10 μg/mL delipidated ApoA-1), and 3 wells ($n = 3$) for each serum sample were treated with 1.5% ApoB-depleted sera for 24 h. Media was clarified of cell debris by spinning for 5 min at 8000 rpm. A 200 μL aliquot of clarified media was mixed with 3 mL scintillation fluid. The NaOH (1 mL 0.1N) was added to each well, set on rocker for 30 minutes to lyse cells, then mixed with 3 mL scintillation fluid. Derived media and cell counts were used to calculate percent efflux (media counts)/(media counts + cell associated counts). Information on preparation of ApoB-depleted sera can be found in Supplementary Methods.

Inflammatory, Metabolic, Biochemical, Immunologic, and Virologic Parameters

Plasma soluble CD163 (sCD163), monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6, and sCD14 levels were quantified by enzyme-linked immunosorbent assay (ELISA) (Trillium Diagnostics [sCD163] and R&D Systems [high-sensitivity IL-6, MCP-1, and sCD14]). Endpoint *Limulus* amebocyte lysate assay (Associates of Cape Cod) was used to measure lipopolysaccharide levels. Total cholesterol, HDL, low-density lipoprotein, and triglycerides were determined using standard techniques. C-reactive protein was measured using ELISA. Human immunodeficiency virus RNA was measured in real time using clinically available reverse-transcription polymerase chain reaction assays. The main objective was to assess large reductions in extreme viremia for which clinically available assays were sufficient. Over the course of this clinical trial, methods to quantify viremia in the MGH Microbiology laboratory (Virology Quality Assurance certified) evolved as newer, more sensitive assays became available (see also Supplementary Methods). In an exploratory analysis, HIV Nef was quantified by ELISA (Immunodiagnostics). In the analysis of change in Nef, full data from baseline and 12 weeks were available from 6 subjects in the ART group and 6 subjects in the untreated group. Methods to assess effects of ART on efflux capacity and to assess endothelial function are included in Supplementary Methods.

Statistical Analysis

Data are presented as mean ± standard deviation or median (interquartile range), depending on normality of distribution. Comparisons between 2 groups (ART and no ART) were performed using the Student *t* test for normally distributed continuous variables and Wilcoxon rank-sum test if distribution was nonnormal. The primary endpoint for this analysis was change in cholesterol efflux between the treatment groups. To assess changes within each group, a paired *t* test was performed. Pearson correlation coefficients were used to assess correlations with change in cholesterol efflux. Linear regression modeling was used in adjusted analyses to assess relationships with change in cholesterol efflux. In this model, we controlled for changes in HDL-cholesterol, CD4+ count, and measures of innate immune activation (sCD163 and CXCL10) that changed significantly with ART. Two-tailed probability values are reported. Statistical significance was assumed when $P < .05$. All statistical analyses were performed using SAS JMP (SAS Institute).

RESULTS

Baseline Results

Baseline demographic characteristics, smoking rates, lipids, HIV disease-related parameters, and inflammatory markers were similar between the 2 treatment groups (Table 1). Stimulated cholesterol efflux capacity from murine Abca1+/+ and Abca1−/− macrophages was similar in response to ApoB-depleted sera obtained from subjects randomized to ART or not at baseline.

Antiretroviral Treatment

Nine subjects were randomized to no ART, and 8 subjects were randomized to ART (Figure 1). Regimens were chosen by each patient's individual HIV clinical care provider. Among those who received ART, 7 patients received protease inhibitor (PI)-based treatment regimens with nucleoside reverse-transcriptase inhibitors (NRTI) backbone and 1 patient received non-NRTI (NNRTI)-based regimen with NRTI backbone (Supplementary Table 1).

Changes in Primary Disease and Cholesterol Efflux Parameters Over 12 Weeks

Changes in Human Immunodeficiency Virus Disease Parameters

Twelve weeks after study entry, log HIV RNA decreased in both groups, but it decreased to a greater degree in the ART-treated group (Table 2). Reduction in HIV RNA in the untreated group was anticipated in the course of natural history of early HIV infection with the development of host HIV-specific immunity. As expected, CD4+ count increased in both groups, but the change was significantly greater within the ART-treated group. Human immunodeficiency virus Nef trended to decrease in both groups and to a greater degree in the ART-treated group ($-5.6 ± 10.0$ vs $-1.4 ± 2.3$ ng/mL), but it did not reach statistical significance.

Changes in Cholesterol Efflux

Over 12 weeks, cholesterol efflux capacity from Abca1+/+ macrophages was increased most by sera obtained from ART-treated subjects (20.5% ± 5.0% to 24.3% ± 6.9%, baseline to 12 weeks, $P = .007$ ART group vs 18.0% ± 3.9% to 19.1 ± 2.9%, baseline to 12 weeks, $P = .30$ untreated group) [$P = .04$ comparing change between ART-treated and untreated groups] (Table 2; Figure 2). The direction of change in Abca1+/+ efflux was positive in all subjects in the ART group, regardless of treatment with PI base

Table 1. Baseline Characteristics by Randomization Group[a]

Characteristic	No Treatment (Mean ± SD)	ART (Mean ± SD)	P Value
Demographics	(n = 9)	(n = 8)	
Age, years	34.4 ± 10.3	38.3 ± 10.0	.45
Gender, % male	78	100	.16[b]
Current smoker, %	11	13	.93[b]
Cholesterol efflux Capacity	(n = 7)	(n = 6)	
Abca1$^{+/+}$ efflux, %	18.0 ± 3.9	20.5 ± 5.0	.33
Abca1$^{-/-}$ efflux, %	17.4 ± 5.5	20.7 ± 4.9	.29
Fasting lipids	(n = 7)	(n = 8)	
LDL-cholesterol, mg/dL	83 ± 27	77 ± 29	.65
HDL-cholesterol, mg/dL, median (IQR)	39 (26, 62)	30 (25, 35)	.13[c]
Total cholesterol, mg/dL	145 ± 34	131 ± 29	.37
Triglycerides, mg/dL	90 ± 21	123 ± 69	.25
Endothelial function	(n = 7)	(n = 8)	
RH-PAT index, median (IQR)	2.2 (1.4, 2.8)	2.0 (1.7, 2.6)	.86[c]
HIV disease-related Parameters	(n = 9)	(n = 8)	
CD4$^+$ T-lymphocytes, cells/mm^3	581 ± 236	408 ± 174	.11
Log HIV RNA, copies/mL	5.48 ± 1.16	5.74 ± 0.46	.56
Markers of monocyte/macrophage Activation and microbial translocation	(n = 8)	(n = 7)	
sCD163, ng/mL	1985 ± 924	1867 ± 800	.80
CXCL10, pg/mL, median (IQR)	769 (243, 950)	987 (514, 1488)	.27[c]
MCP-1, pg/mL	291 ± 148	336 ± 234	.65
sCD14, ng/mL	2023 ± 542	2392 ± 1091	.41
LPS, EU/mL, median (IQR)	0.3 (0.1, 1.1)	0.3 (0.2, 0.7)	0.95[c]
Markers of inflammation	(n = 8)	(n = 7)	
hsCRP, mg/L, median (IQR)	2.3 (0.3, 3.9)[d]	0.9 (0.3, 1.4)	.61[c]
hsIL-6, pg/mL, median (IQR)	1.2 (1.0, 1.3)	0.9 (0.7, 2.3)	.86[c]

Abbreviations: ART, antiretroviral therapy; CRP, C-reactive protein; EU, endotoxin units; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; hs, high-sensitivity; IL, interleukin; IQR, interquartile range; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; RH-PAT, reactive hyperemia peripheral arterial tonometry; sCD163, soluble CD163; SD, standard deviation.

[a] All P values were calculated using Student's t test unless noted otherwise.

[b] Pearson's χ^2 test.

[c] Wilcoxon rank-sum test.

[d] n = 7.

or NNRTI base (Figure 3). In the patient not receiving a PI, the change in efflux capacity was 6%, consistent with the change in the entire ART-treated group. In contrast, efflux capacity from Abca1$^{-/-}$ macrophages tended to increase, but it was not statistically significant (Table 2). Among those not receiving ART, a mixed response was seen in Abca1$^{+/+}$ efflux (Figure 3).

Association Between Changes in Human Immunodeficiency Virus Disease-Related and Immune Activation Parameters With Cholesterol Efflux Capacity

Change in log HIV RNA was significantly negatively correlated with change in cholesterol efflux (r = − 0.62, P = .03) (Table 3; Figure 4) among all subjects. In an exploratory analysis, %change in Nef trended to be negatively correlated with change in cholesterol efflux among ART-treated patients, but it did not reach statistical significance (r = − 0.41). In contrast, no apparent

relationship was seen between change in Nef and cholesterol efflux in the non-ART-treated group (r = − 0.04) (Supplementary Figure 2). Other factors were not significantly related to change in efflux capacity.

Changes in Secondary Endpoints Over 12 Weeks
Changes in Lipids

In general, HDL-cholesterol increased in both groups, without significant differences between groups (Table 2). Increase in HDL-cholesterol was of comparable magnitude in each group, but increase in HDL-cholesterol was significant within the ART group.

Changes in Markers of Immune Activation, Inflammation, Microbial Translocation, and Endothelial Function

In terms of markers of monocyte activation, sCD163 and CXCL10 significantly decreased in the ART-treated group

Table 2. Changes From Baseline to 12 Weeks in Both Randomization Groups (Untreated vs Antiretroviral Treatment Group)[a]

Characteristic	Change in Untreated Group (Mean ± SD)	Within Group P Value	Change in ART Group (Mean ± SD)	Within Group P Value	Between Group P Value
Cholesterol efflux Capacity	(n = 6)		(n = 6)		
Abca1$^{+/+}$ efflux, %	1.0 ± 2.1	.30	3.8 ± 2.1	.007	.04
Abca1$^{-/-}$ efflux, %	2.0 ± 2.4	.09	3.5 ± 4.2	.10	.46
Fasting lipids	(n = 6)		(n = 8)		
LDL-cholesterol, mg/dL	−3 ± 28	.82	17 ± 30	.16	.25
HDL-cholesterol, mg/dL	8 ± 14	.22	6 ± 6	.02	.74
Total cholesterol, mg/dL	3 ± 37	.86	28 ± 31	.04	.19
Triglycerides, mg/dL, median (IQR)	−16 (−25, 5)	.14	0.5 (−34, 53)	.40	.40[b]
Endothelial function	(n = 6)		(n = 8)		
RH-PAT index	−0.10 ± 0.64	.72	0.12 ± 1.64	.84	.77
HIV disease-related parameters	(n = 8)		(n = 8)		
CD4$^+$ T-lymphocytes (cells/mm^3)	104 ± 350	.43	174 ± 134	.008	.61
Log HIV RNA (copies/mL)	−1.59 ± 1.04	.004	−3.54 ± 0.43	<.0001	.0002
HIV Nef (ng/mL)	−1.4 ± 2.3[c]	.21	−5.6 ± 10.0[c]	.19	.33
Markers of monocyte/macrophage Activation and microbial Translocation	(n = 6)		(n = 7)		
sCD163, ng/mL	16 ± 1002	.97	−536 ± 523	.04	.23
CXCL10, pg/mL, median (IQR)	−205 (−1312, −97)	.18	−775 (−1224, −443)	.002	.13[b]
MCP-1, pg/mL, median (IQR)	−31 (−292, 3)	.14	−67 (−109, −2)	.14	.83[b]
sCD14, ng/mL	−388 ± 332	.04	−281 ± 739	.35	.75
LPS, EU/mL, median (IQR)	−0.13 (−0.70, 0.15)	.24	−0.02 (−0.14, 0.12)	.36	.83[b]
Markers of inflammation	(n = 6)		(n = 7)		
hs-CRP, mg/L, median (IQR)	−0.2 (−1.1, 0.2)	.37	0 (−0.5, 0.6)	.99	.62[b]
hsIL-6, pg/mL, median (IQR)	−0.5 (−0.9, 0.3)	.016	−0.2 (−1.4, 0.1)	.30	.83[b]

Abbreviations: ART, antiretroviral therapy; CRP, C-reactive protein; EU, endotoxin units; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; hs, high-sensitivity; IL, interleukin; IQR, interquartile range; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; RH-PAT, reactive hyperemia peripheral arterial tonometry; sCD163, soluble CD163; SD, standard deviation.

[a] All P values were calculated using Student's t test unless noted otherwise.
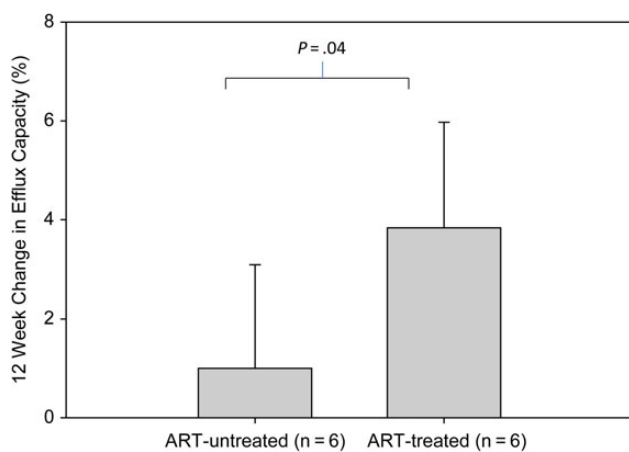
[b] Wilcoxon rank-sum test.

[c] n = 6.

Figure 2. The graphic shows a comparison of 12-week change in cholesterol efflux capacity by apolipoprotein B-depleted sera from antiretroviral therapy (ART) vs untreated patients with acute infection using Abca1$^{+/+}$ macrophages. Change in the ART-treated group is greater than change in the untreated group (P = .04).

after 12 weeks (Table 2). The magnitude of the changes was greater in the ART-treated group, although these changes were not significantly different between the ART-treated and ART-untreated randomization groups (Table 2). In contrast, sCD14 and high-sensitivity IL-6 decreased significantly only within the non-ART group, although again changes between groups were not significantly different. C-reactive protein, a marker of generalized inflammation, did not change significantly in either group (Table 2). Significant changes in reactive hyperemia peripheral arterial tonometry (RH-PAT) indices were not seen (Table 2).

Multivariate Analysis for Change in Cholesterol Efflux Capacity from Abca1$^{+/+}$ Macrophages

In multivariate analysis among all subjects, in a model including change in HDL-cholesterol, change in log viral load, change in CD4$^+$ cells, change in sCD163, and change in CXCL10, change in log viral load remained significantly associated with change in cholesterol efflux (P = .03) (Table 4).

Table 3. Correlation Between Change in CV Risk Factors, HIV Disease-related Parameters, and Markers of Monocyte/ Macrophage Activation and Inflammation to the Change in Abca1$^{+/+}$ Efflux Capacity Among All Subjects (ART and Non-ART Treated)

	Pearson Correlation Coefficient ($n = 12$)	P Value
Traditional CV risk factors		
LDL-cholesterol	0.52	.08
HDL-cholesterol	0.34	.28
Total cholesterol	0.54	.07
Triglycerides	0.09	.78
HIV disease-related parameters		
CD4$^+$ T-lymphocytes	−0.02	.95
Log HIV RNA level	−0.62	.03
Markers of monocyte/macrophage activation and microbial translocation		
sCD163	−0.39	.21
sCD14	0.13	.69
LPS	0.04	.90
CXCL10	0.08	.80
MCP-1	−0.06	.86
Marker of generalized inflammation		
hsCRP	0.05	.87
hsIL-6	0.15	.65

Abbreviations: ART, antiretroviral therapy; CRP, C-reactive protein; CV, cardiovascular; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; hs, high-sensitivity; IL, interleukin; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; sCD163, soluble CD163.
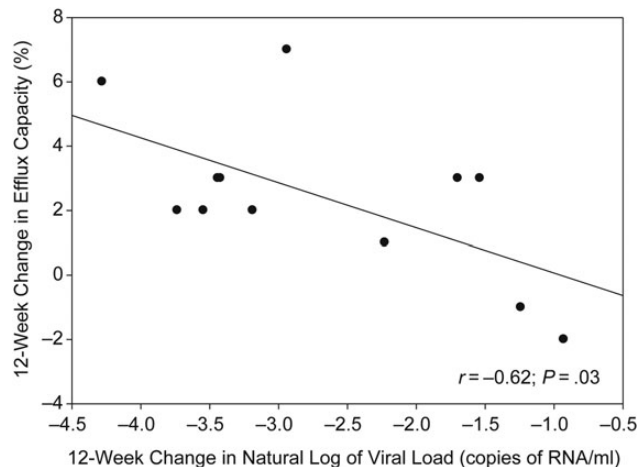

Figure 4. The graphic shows the relationship of change in ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux capacity with change in log human immunodeficiency virus RNA. Linear regression is used to assess relationship. Pearson correlation coefficient = −0.62; $P = .03$.

Assessment of Antiviral Drug Effects on Efflux

Antiretroviral compounds used to treat the patients were spiked into uninfected control sera at their reported C_{max} concentrations either alone or in combinations used in the treatment group. Apolipoprotein B-depleted supernatants were prepared

from the spiked sera samples and used in the macrophage efflux assay. Supplementary Figure S1A shows no significant differences in efflux for sera spiked with water-soluble antiretroviral compounds relative to the vehicle-treated control sera. For compounds soluble in dimethyl sulfoxide (DMSO), there was a small but significant ($P < .05$) reduction in efflux for samples spiked with tenofovir or ritonavir-boosted atazanavir (Supplementary Figure S1B). However, in the drug combinations, with or without ritonavir, no significant changes were observed relative to water or DMSO vehicle-treated sera (Supplementary Figure 1C). As expected, treating the macrophages with lipid-poor ApoA-I induced a significant increase in efflux (Supplementary Figure 1D). This control demonstrates the macrophage
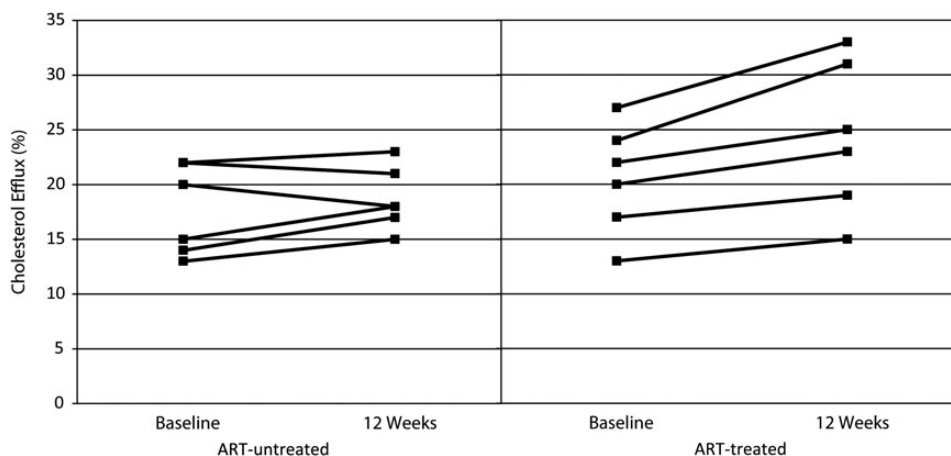

Figure 3. Changes in cholesterol efflux capacity from baseline to 12 weeks are shown for each individual subject in the antiretroviral therapy-treated and untreated groups.

Table 4. Multivariate Model to Assess Relationships to Change in Abca1$^{+/+}$ Cholesterol Efflux (%)

Variable	β-Coefficient	P Value
Log HIV RNA, copies/mL	−1.81	.03
HDL-cholesterol, mg/dL	0.090	.25
CD4$^+$ T-lymphocytes, cells/mm^3	−0.0068	.17
sCD163, ng/mL	−0.00036	.75
CXCL10, pg/mL	−0.0010	.52

Abbreviations: HDL, high-density lipoprotein; HIV, human immunodeficiency virus; sCD163, soluble CD163.

cell line used in this assay has robust ABCA1-dependent efflux activity.

CONCLUSIONS

We report here the first longitudinal human study in HIV-infected patients on cholesterol efflux capacity measured using the ability of apolipoprotein B-depleted sera from HIV-infected patients to promote cholesterol efflux from macrophages. These data were obtained in the context of a study of acute HIV infection and subsequent randomization to ART or no treatment. Cholesterol efflux is an important component of the reverse cholesterol transport pathway relevant to protection from atherosclerosis development. We selected this model of acute HIV infection that is characterized by extreme levels of viremia. In the current study, ART treatment for 12 weeks significantly increased HDL-cholesterol, increased CD4 count, reduced HIV RNA, and decreased markers of monocyte/macrophage activation. Importantly, we also found the capability of Apo B-depleted sera from patients during acute HIV infection to induce cholesterol efflux from Abca1$^{+/+}$ wild-type macrophages with preserved ABCA1 function was significantly increased in ART-treated subjects. The significant relationship between reduction in viral load and increased cholesterol efflux capacity was independent of changes in HDL-cholesterol, CD4$^+$ T cells, or change in monocyte activation markers, providing further evidence that the virus and/or related proteins may be responsible for altering efflux capacity during acute HIV infection. Moreover, the increase in efflux seen with ART was not likely due to ART itself, because we show no effects of ART to increase cholesterol efflux in a set of in vitro studies.

Reverse cholesterol transport has a very important role in atheroprotection and in the general population, HDL-mediated cholesterol efflux capacity has been demonstrated to be a predictor of coronary artery disease [13]. Within the arterial wall, excess cholesterol stored in macrophages contributes to atherosclerosis. Reverse cholesterol transport reduces cholesterol in macrophages by transporting cholesterol from macrophages to the liver where it is excreted in bile. ATP-binding cassette transporter A1 facilitates efflux of cellular cholesterol inside macrophages to lipid-poor ApoA1 to form nascent pre-β-HDL. Mature α-HDL particles can also accept free cholesterol through efflux mechanisms mediated by ABCG1 and scavenger receptor class B type 1 (SR-B1), as well as through the process of passive diffusion from the macrophage cell membrane.

The cholesterol efflux process is a transport mechanism requiring active transporters including ABCA1 as well as acceptor lipoprotein particles. The differences in efflux capacity secondary to ART among subjects acutely infected with HIV may be due to increased acceptor capacity of the sera via HDL-cholesterol or other acceptors or due to effects on the cellular side of cholesterol efflux mediated by changes in viral proteins or other factors. Acute HIV infection is associated with the appearance of the acute phase response [14] and increased inflammatory cytokines [15]. During the acute phase response, reverse cholesterol transport and HDL-cholesterol can be altered [16, 17]. The functional capability of HDL-cholesterol can be modified by infection and inflammation [18], and, thus, the acceptor capacity of HDL could be affected during acute HIV infection.

In vitro studies by Mujawar et al [6] were the first to demonstrate that HIV inhibits cholesterol efflux from macrophages via Nef-mediated down-regulation of ABCA1. Subsequent studies in animal models also support the effect of HIV Nef protein on suppressing cholesterol efflux [8, 9]. However, a cross-sectional study had previously shown ABCA1 gene expression in monocytes was higher in untreated HIV-infected patients than HIV-negative individuals or ART-treated HIV-infected patients. These authors had postulated the increased expression to be compensatory in response to Nef-mediated impairment of cholesterol efflux [19]. Within our study, in an exploratory analysis, we observed a trend towards an inverse relationship between changes in Nef concentration and cholesterol efflux capacity longitudinally among patients acutely infected with HIV, suggesting that Nef may affect cholesterol efflux consistent with prior in vitro studies [6]. Further studies with larger numbers of patients will be needed to confirm and extend these findings.

In addition, to further explore whether the effects on cholesterol efflux are mediated through ABCA1 or other components of the efflux transport system, we also examined the cholesterol efflux capacity in macrophages with no ABCA1 function to test the stimulatory capacity of serum via pathways that are independent of ABCA1. We saw a trend towards an increase in cholesterol efflux capacity in Abca1$^{-/-}$ macrophages (although not statistically significant), suggesting that ART may also help restore cholesterol efflux mediated by processes dependent upon ABCG1, SR-B1, or passive diffusion. Taken together, our data suggest that ABCA1 may play a more significant role in cholesterol efflux, but do not rule out the possibility of other mechanisms contributing to increased efflux capacity with viral suppression.

Our data suggest that improved cholesterol efflux capacity may result from an effect of ART to suppress viremia, as we show in our multivariate analysis. However, to further assess whether there may be a direct effect of ART to stimulate efflux, we performed in vitro testing exposing macrophages to relevant concentration of ART agents used by the subjects in this study. Our in vitro experiments assessing the effects of ART on cholesterol efflux did not show effects of these agents to stimulate cholesterol efflux, again pointing to suppression of viremia as a major contributor to improving efflux capacity.

Patients with chronic HIV infection have been shown to have higher levels of monocyte/macrophage activation. In this study, we extend our understanding of immune activation levels during the continuum of HIV infection, showing very high levels of innate immune activation markers sCD163, CXCL10, MCP-1, and sCD14 during the acute phase of HIV infection. Indeed, these levels appear much higher than seen among chronically HIV-infected patients and HIV-negative controls using a similar assay [20, 21]. Prior studies have shown that not only are lymphoid cells affected early in HIV infection, but myeloid cells are also altered in acute HIV infection, and CD16^{+} proinflammatory monocytes have been observed to be increased in acute HIV infection [22]. This points to the multifaceted host response to viremia, which may also affect HDL-mediated cholesterol efflux capacity.

In the current randomized study, we demonstrate a significant decline in markers of monocyte/macrophage activation, including sCD163 and CXCL10, with ART among subjects acutely infected with HIV. The data from the current study extend prior findings of elevated sCD163 that decreased with ART in a small study of patients acutely infected with HIV [23]. We have also previously shown that sCD163 is elevated in patients infected with HIV and is associated with noncalcified coronary plaque that is more vulnerable to rupture [24]. CXCL10 has been found to be higher in patients infected with HIV compared with HIV-negative controls [21, 25, 26]. CXCL10 has also been implicated in the inflammatory pathways of coronary artery disease [27–29]. This salutatory effect on proatherogenic immune activation by ART together with the improvement in HDL-mediated cholesterol efflux may contribute to reduced cardiovascular risk.

In the current study, endothelial function assessed by RH-PAT index did not change in the 12-week period after initial acute HIV infection. However, the baseline RH-PAT indices in both groups were not pathologically low to begin with, and, thus, we may not have seen an effect as patients started with indices in a range not considered to be high risk [30].

Strengths of our study include the longitudinal design with randomization to an interventional arm and an untreated control arm. The study population of patients acutely infected with HIV is a patient population that is difficult to capture. The diagnosis of acute HIV is often not recognized because the associated symptoms may be nonspecific. In this study, such patients were enrolled based on rigorous criteria and were identified before seroconversion, with high levels of viremia. The acute HIV patient group is also unique because there are no confounding effects of prior or concomitant ART.

The study also has some limitations. Our data suggest that HIV viral infection and degree of viremia are important determinants of effects on cholesterol efflux. However, we cannot determine definitively whether suppression of specific components of the viral particle contributes to increased cholesterol efflux. In contrast to our results, in a cross-sectional study by Rose et al [31] using plasma from patients infected with HIV, there was no difference in cholesterol efflux between treated and untreated subjects. However, the 2 studies investigated HIV infection at different stages (acute vs chronic infection), and, in our randomized interventional trial, we examined changes in cholesterol efflux over time within the same cohort and not just a cross-sectional comparison.

In this study, we show for the first time in humans that ART may help to restore HDL-mediated cholesterol efflux capacity when administered during acute HIV infection, most likely mediated through the suppression of viremia. Furthermore, ART also improved HDL-cholesterol and reduced proatherogenic innate immune activation markers sCD163 and CXCL10. Data from this study and others suggest that HIV may adversely impact host reverse cholesterol transport, leading to impairment of cholesterol efflux from macrophages. This effect may predispose to development of foam cells and atherosclerotic disease in the host. The main findings of the current study may have implications for the clinical care of patients infected with HIV, providing additional evidence for the benefit of suppressing viremia to reduce atherosclerotic risk even in the very early stage of acute HIV infection, especially because newer treatments with less metabolically untoward effects are increasingly available.

the editors consider relevant to the content of the manuscript have been disclosed.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

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