MAJOR ARTICLE

Plasma Levels of Soluble CD14 Independently Predict Mortality in HIV Infection

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Background. Chronic human immunodeficiency virus (HIV) infection is associated with intestinal permeability and microbial translocation that contributes to systemic immune activation, which is an independent predictor of HIV disease progression. The association of microbial translocation with clinical outcome remains unknown.

Methods. This nested case-control study included 74 subjects who died, 120 of whom developed cardiovascular disease and 81 of whom developed AIDS during the Strategies for Management of Anti-Retroviral Therapy (SMART) study with matched control subjects. Intestinal fatty acid binding protein (I-FABP), lipopolysaccharide (LPS), soluble CD14 (sCD14), endotoxin core antibody (EndoCAb), and 16S ribosomal DNA (rDNA) were measured in baseline plasma samples.

Results. Subjects with the highest quartile of sCD14 levels had a 6-fold higher risk of death than did those in the lowest quartile (95% confidence interval, 2.2–16.1; $P<.001$), with minimal change after adjustment for inflammatory markers, $CD4^+$ T cell count, and HIV RNA level. No other marker was significantly associated with clinical outcomes. I-FABP, LPS, and sCD14 were increased and EndoCAb was decreased in study subjects, compared with healthy volunteers. sCD14 level correlated with levels of IL-6, C-reactive protein, serum amyloid A and D-dimer.

Conclusions. sCD14, a marker of monocyte response to LPS, is an independent predictor of mortality in HIV infection. Therapeutic attenuation of innate immune activation may improve survival in patients with HIV infection.

Immune activation is a strong predictor of disease progression in human immunodeficiency virus (HIV) infection. Increases in T cell turnover [1], frequencies of activated T and B cells [2], and serum levels of proinflammatory cytokines and chemokines have been described [3]. A higher frequency or level of $CD38^+$ expression on $CD8^+$ T cells [4, 5] predicts a faster decrease of $CD4^+$ T cells, and higher frequencies of $CD38^+HLA\text{-}DR^+$ $CD4^+$ and $CD8^+$ T cells predict reduced recovery of $CD4^+$ T cells after initiating ART [6]. Higher $CD38^+$ expression on $CD4^+$ and $CD8^+$ T cells predicts shorter survival, independent of plasma HIV RNA levels [7]. Collectively, these observations indicate that immune activation is a critical component of HIV disease pathogenesis.

The causes of HIV-associated immune activation remain unclear but are likely multifactorial, including expansion of HIV-specific T cells, reactivity of innate immune cells to HIV-encoded Toll-like receptor (TLR)

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ligands, loss of immunoregulatory cells, and increased prevalence of other chronic infections [8]. Rapid depletion of $CD4^+$ T cells from the intestine [9, 10], decreased luminal immunoglobulin (Ig) A concentration [11], massive enterocyte apoptosis, and breakdown of enterocyte tight junctions [12, 13] result in a compromised intestinal mucosal barrier. Subsequent translocation of microbial products, such as lipopolysaccharide (LPS) and 16S ribosomal DNA (16S rDNA), contributes to immune activation. LPS, a component of the cell wall of gram-negative bacteria, binds membrane or soluble CD14 (sCD14) and the myeloid differentiation-2 (MD-2)-TLR4 complex, resulting in NF-κb activation and production of IL-6, IL-1β, TNF and type I interferons [14–16]. In HIV disease, both LPS and 16S rDNA correlate with systemic immune activation, increased frequencies of $CD38^+HLA\text{-}DR^+$ $CD8^+$ T cells, and elevated interferon α levels in the plasma [17, 18].

A potential causal link between HIV-associated inflammation and disease progression was shown in a randomized clinical trial of continuous versus intermittent antiretroviral therapy (the SMART study). HIV-infected persons were randomized to continuous antiretroviral therapy (ART) or $CD4^+$ T cell count–driven ART. Subjects randomized to the second arm had a higher risk of morbidity and mortality than did those in the first [19]. ART interruption resulted in a rapid increase in inflammation- and coagulation-associated biomarkers that were associated with increased risks of death, AIDS, and cardiovascular disease (CVD) [20–22]. The mechanism linking HIV replication to inflammation, coagulopathy, and disease progression remains largely undefined.

We hypothesized that markers of microbial translocation and its consequences would be associated with death, AIDS, and CVD. In this large cohort of HIV-infected subjects with high $CD4^+$ T cell counts and diverse treatment histories, we explored the relationship of enterocyte damage, microbial translocation, and LPS-mediated monocyte activation with these outcomes.

MATERIALS AND METHODS

Study Design

Details of the SMART trial have been previously described [19]. This randomized controlled trial assigned HIV-infected subjects >13 years old with $CD4^+$ T cell counts >350 cells/mm^3 to drug conservation (DC) or viral suppression (VS). In both arms, the goal of ART was to achieve maximal viral suppression. The trial and consent for stored samples was approved by the institutional review boards at the University of Minnesota and the enrolling sites.

Study Population

Of 5472 HIV-infected subjects enrolled in SMART, 85 died, 142 developed major CVD events, and 100 developed AIDS before protocol modification [19]. Seventy-four, 120, and 81 subjects, respectively, had specimens available. CVD events included myocardial infarction, stroke, coronary artery disease requiring surgery, congestive heart failure, peripheral vascular disease, drug treatment for CVD, deaths attributed to CVD, and unwitnessed deaths, which were likely sudden cardiac events [23]. Two control subjects were matched to each event. To make use of biomarkers already measured on stored samples [20–22], matching paralleled that of other nested case-control studies for this cohort, with subjects matched for age (within 5 years), sex, country, and date of enrollment (within 3 months) [20].

To establish reference values for these biomarkers, we recruited 35 HIV-uninfected volunteers from the National Institutes of Health and 32 individuals exposed to HIV but uninfected from the University of California–San Francisco. For the 63 samples with accessible data, the median age of subjects was 35 years (interquartile range [IQR], 27–43 years), and 52% were male. The individuals were deemed healthy on the basis of clinical histories, physical examinations, and laboratory tests.

Microbial Translocation and Enterocyte Damage Biomarkers

Intestinal fatty acid binding protein (I-FABP), a marker of enterocyte damage [24], and 4 markers associated with microbial translocation and the immune response to it (LPS, 16S rDNA, sCD14, and endotoxin core IgM antibody [EndoCAb]), were measured in baseline plasma samples from subjects who died, developed CVD, or developed AIDS and from their matched control subjects. All analyses were performed blinded to case and control status; each test was determined in duplicate, and the average of each marker was calculated.

Commercially available enzyme-linked immunosorbent assays were used according to the manufacturers' protocols for measuring sCD14 (R&D Systems), EndoCAb IgM (Cell Sciences), and I-FABP (Cell Sciences) on plasma diluted to 0.5%, 1%, and 50%, respectively. LPS levels were measured in plasma diluted to 10% using the Limulus assay (Lonza), as previously described [17]. 16S rDNA was measured by quantitative polymeras chain reaction as previously described [18], using forward primer (8F: 5′-AGAGTTTGATCCTGGCTCAG-3′), reverse primer (355R: 5′-CTGCTGCCTCCCGTAGGAGT-3′), and probe (63P: 5′-GCAGGCCTAACACATGCAAGTC-3′).

Statistical Methods

The primary objective of the study was to investigate the relationship between baseline levels of markers of microbial translocation with all-cause mortality, CVD, and AIDS. Conditional logistic regression analysis for matched case-control studies was used to assess associations of each baseline biomarker on subsequent development of each of these end points separately. Models with $\log_{10}$-transformed biomarker levels after adding 1.0 to each biomarker were considered, and estimated parameters were used to determine the increase in risk associated with its IQR. Analyses by quartiles of each biomarker were

also performed. The quartiles were calculated using the case patients and control subjects for each end point. Because many subjects had undetectable I-FABP levels, the lowest quartile for each condition contained >25% of the subjects. Odds ratios (ORs) along with 95% confidence intervals (CIs) and *P* for each of the 3 upper quartiles versus the lowest quartile (reference group) are shown. Results were presented as unadjusted, considering the matching factors, and 2 adjusted analyses. For the first, in addition to the matching variables, baseline characteristics considered were race, use of ART, HIV RNA, $CD4^+$ T cell count (nadir and baseline), treatment group (VS and DC), smoking status, body mass index (BMI), diabetes, use of blood pressure–lowering drugs, use of lipid lowering drugs, prior CVD, total cholesterol/high-density lipoprotein ratio, hepatitis B co-infection and hepatitis C co-infection. In the second adjusted analysis, the adjustment included baseline $\log_{10}$–transformed levels of IL-6, serum amyloid A (SAA), high-sensitivity C-reactive protein (hsCRP), and D-dimer.

We examined associations between baseline biomarker levels and clinical outcomes in the DC and VS arms separately and used an interaction term (product of $\log_{10}$-transformed marker and treatment group) to assess whether associations varied by treatment group.

We investigated the association among the biomarkers of microbial translocation levels (as listed above) with markers of inflammation and coagulation, baseline and nadir $CD4^+$ T cell counts, HIV RNA level, and ART status at the time of enrollment. Pairs of variables were compared using the Mann-Whitney *U* test, and correlations among variables were evaluated using Spearman's rank correlation. In exploratory analyses, untreated HIV-infected subjects, treated subjects with HIV RNA levels > 400 copies/mL, and treated subjects with HIV RNA levels ⩽ 400 copies/mL were compared using the Mann-Whitney *U* test. Cohorts of healthy volunteers were combined for comparison to HIV-infected subjects, and we adjusted for the age difference between the volunteers and the SMART participants using regression analysis.

All analyses were performed using SAS statistical software, version 9.2 (SAS).

Results

Baseline Characteristics

Despite matching with respect to age, the 74 subjects who died were a median of 2 years older than the control subjects ($P = .009$); those who died were more likely to be infected with hepatitis B/C ($P = .001$), smoke ($P = .002$), use antihypertensive drugs ($P = .04$), have a history of CVD ($P = .006$), and have a lower LDL cholesterol ($P = .04$) (Table 1). The median $CD4^+$ T cell counts of the control subjects who died were 551 and 619 cells/mm^3 ($P = .051$). Table 1 also summarizes differences between CVD and AIDS case patients and control subjects.

At baseline, most subjects were receiving ART. Both regimen and duration of ART varied, with a median of 4 years (IQR, 3-5 years) among all subjects [19]. Of those receiving ART at enrollment, most had an HIV RNA level ⩽400 copies/mL.

Association of Biomarker Levels with Clinical Outcomes

Baseline sCD14, produced by monocytes upon LPS stimulation [25, 26], was significantly higher in the plasma samples from subjects who died than in samples from matched control subjects (2.47 vs 2.23 × 10^6 pg/mL; $P < .001$; Table 1). Adjusted and unadjusted analyses were similar (Table 2). A strong risk gradient was evident with ORs for the fourth, third, and second quartiles versus the first quartile of 6.0 (95% CI, 2.2–16.1; $P < .001$), 3.3 (95% CI, 1.3–8.6; $P = .01$), and 2.1 (95% CI, 0.8–5.7, $P = .12$). The OR for mortality among subjects with the highest quartile of sCD14 levels was 8.0 (95% CI, 2.0–31.9; $P = .003$) after adjusting for mortality risk factors and 4.1 (95% CI, 1.2–13.9; $P = .02$) after adjusting for inflammatory markers, but subjects with levels in the second or third quartile no longer had a significantly increased risk of death. There was no significant difference in the OR of death among those who died in the first year of study enrollment (OR for fourth quartile versus first quartile, 4.3 [95% CI, 1.01–18.2]; $P = .049$), compared with that for those who died later (OR for fourth quartile vs first quartile of 6.6 [95% CI, 1.6–26.2]; $P = .008$). The OR associated with one IQR higher sCD14 level was 2.3 (95% CI, 1.5–3.5; $P < .001$). Subjects in the DC arm had a univariate OR of 3.5 for one IQR higher sCD14 level (95% CI, 1.5–8.3; $P = .004$), and those in the VS group had an OR of 2.0 (95% CI, 0.8–5.4; $P = .15$) ($P = .43$ for difference in DC and VS ORs; Table 3).

sCD14 was insignificantly higher in those who developed CVD, compared with their matched control subjects (median sCD14 level, 2.44 vs 2.33 × 10^6 pg/mL; $P = .11$; and OR for fourth vs first quartile of 1.7 [95% CI, 0.9–3.2]; $P = .09$; Tables 1 and 4). The OR (fourth vs first quartile) remained 1.7 (95% CI, 0.9–3.1; $P = .10$) for the 70 individuals with cases of myocardial infarction, stroke, surgery for coronary artery disease, or death from CVD. The association between sCD14 level and AIDS was weak and not significant (Tables 1 and 5).

Subjects who died had insignificantly higher I-FABP levels (median level, 174.4 vs 72.3 pg/mL; $P = .10$; and OR for fourth vs first quartile, 1.8 [95% CI, 0.9–3.7]; $P = .10$). No other differences in the biomarkers were found between case patients and matched control subjects.

Comparison of Levels of Biomarkers in SMART Subjects and Healthy Individuals

To contextualize these data, we measured the biomarkers in healthy volunteers (median age, 35 years). Because these cohorts were not matched, the comparisons must be interpreted cautiously. Median LPS levels were greater in the SMART subjects than in the healthy volunteers (32.5 and 25.0 pg/mL, respectively) (Figure 1A). Similarly, the median sCD14 level was

Table 1. Baseline Characteristics of Study Subjects

Category	Individuals who died (n=74)	Control subjects (n=148)	P	CVD events (n=120)	Control subjects (n=238)	P	AIDS events (n=81)	Control subjects (n=162)	P
Demographic characteristic									
Age, years (25th, 75th percentile)	50 (42, 55)	48 (43, 55)	.009	49 (44, 56)	48 (42, 55)	<.0001	46 (40, 53)	45 (39, 52)	.04
Female sex, %	21.6	21.6	N/A	19.2	19.3	N/A	28.4	28.4	N/A
White/other race, %	50	60	(Ref)	58.3	61.8	(Ref)	69.1	63.6	(Ref)
Black race, %	50	40	.16	41.7	38.2	.51	30.9	36.4	.36
$CD4^+$ cell count, cells/mm^3									
Nadir (25th, 75th percentile)	249 (152, 360)	245 (118, 351)	.90	209 (107, 328)	241 (133, 350)	.44	225 (140, 368)	243 (135, 350)	.97
Baseline (25th, 75th percentile)	551 (410, 713)	619 (476, 838)	.051	607 (463, 841)	638 (496, 816)	.88	588 (465, 729)	577 (488, 764)	.88
Prior AIDS, %	28.4	27.7	.91	39.2	26.1	.02	37.4	19.1	.001
United States, %	96.0	96.0	N/A	90.0	90.8	N/A	85.2	85.2	N/A
ART/HIV RNA level									
No ART	21.6	17.8	(Ref)	14.2	13.9	(Ref)	27.2	20.3	(Ref)
ART, HIV RNA level ≤400 copies/mL, %	54.1	64.2	.32	65.8	58.8	.76	50.6	61.7	.13
ART, HIV RNA level >400 copies/mL, %	24.3	18.2	.87	20.0	27.3	.38	22.2	18.0	.81
Hepatitis B/C, %	46.0	23.0	.001	25.8	18.9	.12	19.8	21.0	.81
Other characteristics									
Current smoker, %	56.8	33.8	.002	55.0	37.4	.002	45.7	37.0	.18
BMI (25th, 75th percentile)	24.8 (21.6, 29.9)	25.6 (23.2, 29.4)	.45	25.2 (22.4, 28.7)	25.6 (23.2, 29.6)	.15	25.3 (22.1, 28.3)	25.4 (23.0, 28.8)	.67
Diabetes, %	23.0	13.5	.08	19.2	9.2	.01	9.9	10.5	.88
Blood pressure–lowering drugs, %	37.8	24.3	.04	43.3	32.4	.04	28.4	20.4	.16
Lipid-lowering drugs, %	16.2	23.0	.22	27.5	24.4	.50	21.0	16.7	.42
Prior CVD, %	14.9	3.4	.006	13.3	5.5	.01	8.6	3.1	.08
Total cholesterol (mg/dL)/HDL cholesterol (mg/dL) (25th, 75th percentile)	4.5 (3.5, 6.1)	4.8 (3.6, 5.8)	.58	5.1 (3.9, 6.8)	4.5 (3.5, 5.6)	.01	4.9 (3.7, 6.6)	4.7 (3.5, 5.9)	.10
LDL cholesterol (mg/dL) (25th, 75th percentile)	100 (72, 132)	107 (88, 139)	.04	108 (83, 150)	111 (93, 136)	.73	106 (88, 129)	112 (90, 137)	.56
Triglycerides (mg/dL) (25th, 75th percentile)	169 (110, 305)	200 (120, 300)	.51	193 (140, 305)	180 (124, 289)	.68	196 (128, 272)	165 (128, 262)	.19
Drug conservation arm, %	62.2	58.8	.63	62.5	47.5	.01	79.0	47.5	<.001

Table 1. (*Continued*)

Category	Individuals who died (n=74)	Control subjects (n=148)	P	CVD events (n=120)	Control subjects (n=238)	P	AIDS events (n=81)	Control subjects (n=162)	P
Biomarkers									
LPS, pg/mL (25^{th}, 75^{th} percentile)	32.7 (24.7, 42.9)	32.6 (24.2, 47.9)	.76	32.7 (23.7, 47.4)	34.0 (25.8, 45.0)	.62	35.9 (22.4, 52.3)	31.2 (23.3, 43.6)	.40
16S rDNA level, copies/μL (25^{th}, 75^{th} percentile)	7.70 (2.6, 34.3)	7.62 (3.9, 12.7)	.56	7.45 (2.65, 14.15)	8.00 (4.06, 13.88)	.33	9.72 (3.97, 16.91)	7.85 (3.45, 14.0)	.15
sCD14 level, $\times 10^6$ pg/mL (25^{th}, 75^{th} percentile)	2.47 (2.19, 2.91)	2.23 (2.01, 2.63)	<.001	2.44 (2.10, 2.79)	2.33 (2.01, 2.67)	.11	2.38 (2.14, 2.70)	2.31 (2.05, 2.68)	.43
EndoCAb level, MMU/mL (25^{th}, 75^{th} percentile)	128.1 (56.1, 177.2)	115.1 (54.0, 168.6)	.54	104.6 (37.0, 160.7)	118.9 (50.4, 171.2)	.19	112.5 (71.9, 180.7)	134.0 (79.6, 190.3)	.48
I-FABP level, pg/mL (25^{th}, 75^{th} percentile)	174.4 (20.0, 520.7)	72.3 (20.0, 345.4)	.10	149.7 (20.0, 447.2)	140.7 (20.0, 405.8)	.53	175.3 (20.0, 396.0)	113.5 (20.0, 478.0)	.55

NOTE. Median values are reported, unless otherwise indicated. ART, antiretroviral therapy; BMI, body mass index, calculated as weight in kilograms divided by the square of height in meters; CVD, cardiovascular disease; EndoCAb, endotoxin core antibody; HIV, human immunodeficiency virus; I-FABP, intestinal fatty acid binding protein; LDL, low-density lipoprotein; rDNA, ribosomal DNA; Ref, reference; sCD14, soluble CD14.

2.34×10^6 pg/mL in the SMART subjects, compared with 1.66×10^6 pg/mL in healthy individuals (Figure 1B). EndoCAb levels were lower in the SMART subjects than in the healthy volunteers (median EndoCAb level, 121 MMU/mL vs 197.3 MMU/mL; Figure 1C). sCD14 levels were higher in ART-treated subjects with HIV RNA levels ⩽ 400 copies/mL than in untreated subjects, and EndoCAb levels were lower in treated than in untreated subjects. There were no differences in 16S rDNA levels between subjects and healthy volunteers (data not shown).

I-FABP was higher and more frequently detectable (>20 pg/mL) in the SMART subjects than in the healthy individuals (median I-FABP level, 139.8 pg/mL vs 20.0 pg/mL; 61.8% vs 26.8%; Figure 1D) even when limiting the analysis to treated subjects with HIV RNA levels ⩽ 400 copies/mL. Adjusting for age did not substantially alter the differences observed in any of these biomarkers between the healthy volunteers and the SMART subjects.

Rank Correlations among Markers of Microbial Translocation and LPS Bioactivity, Markers of HIV Infection, and Inflammatory Markers

Because of the association of microbial translocation with immune activation, which is a strong predictor of disease progression [7,27], we explored the relationship of biomarkers measured here with inflammatory markers. Because many subjects had undetectable HIV RNA, we examined these relationships separately among subjects with HIV RNA levels ⩽400 copies/mL and those with HIV RNA levels >400 copies/mL. In subjects with HIV RNA levels ⩽400 copies/mL, sCD14 levels correlated positively with IL-6 level ($r = .18$; $P < .001$; Table 6), SAA ($r = .16$; $P < .001$), hsCRP ($r = .10$; $P = .04$), and D-dimer ($r = .11$; $P = .03$). In contrast, among those subjects with detectable HIV RNA levels, sCD14 correlated with IL-6 ($r = .26$; $P < .001$; Table 6), SAA ($r = .18$; $P = .004$), and hsCRP ($r = .18$; $P = .005$), whereas HIV RNA levels correlated with IL-6 levels ($r = .23$; $P < .001$; Table 6), and D-dimer ($r = .42$; $P < .001$).

Because the effects of ART on intestinal permeability and the immunological response to microbial products have not been well characterized, we examined the association between markers of microbial translocation and inflammation in the untreated subset. Among these 117 subjects, sCD14 levels correlated with IL-6 level ($r = .35$; $P < .001$), and D-dimer ($r = .26$; $P = .006$). Plasma HIV RNA levels correlated with levels of IL-6 ($r = .21$; $P = .03$), D-dimer ($r = .46$, $P < .001$), sCD14 ($r = .26$; $P = .005$), LPS ($r = .19$; $P = .04$), and EndoCAb ($r = .28$, $P = .002$), although these findings should be interpreted cautiously, because these subjects did not comprise a random sample of those enrolled in the study.

Association of Enterocyte Damage as Measured by I-FABP Level and Baseline and Nadir $CD4^+$ T Cell Count

Enterocyte damage facilitates microbial translocation, which has been shown to be inversely related to $CD4^+$ T cell recovery after

Table 2. Increased Mortality in Human Immunodeficiency Virus–Infected Subjects with High Baseline sCD14 Levels

		25th – 49th Percentile		50th – 74th Percentile		⩾74th Percentile	
Biomarker	<25th Percentile(Reference)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
sCD14 ($\times 10^6$ pg/mL)							
N (case patients/control subjects)	10/46	16/39		21/35		27/28	
Univariate	1.0	2.1 (0.8–5.7)	.12	3.3 (1.3– 8.6)	.01	6.0 (2.2–16.1)	<.001
Adjusted—risk factors[a]	1.0	2.8 (0.8–10.0)	.10	2.7 (.8–9.0)	.11	8.0 (2.0–31.9)	.003
Adjusted—inflammation[b]	1.0	2.3 (0.7–8.1)	.18	2.9 (.9–9.4)	.07	4.1 (1.2–13.9)	.02
LPS, pg/mL							
N (case patients/control subjects)	0	20/35		22/34		15/40	
Univariate	1.0	1.5 (0.6–3.5)	.39	1.6 (.7–3.7)	.25	0.9 (0.4–1.9)	.76
Adjusted—risk factors[a]	1.0	1.1 (0.4–3.1)	.82	1.3 (.5–3.5)	.63	0.4 (0.2–1.2)	.11
Adjusted—inflammation[b]	1.0	1.5 (0.5–4.7)	.45	1.4 (.5–4.4)	.55	1.2 (0.4–3.2)	.78
I-FABP, pg/mL							
N (case patients/control subjects)	23/59	9/20		19/36		23/32	
Univariate	1.0	1.1 (0.5–2.7)	.79	1.4 (.6–2.9)	.42	1.8 (0.9–3.7)	.10
Adjusted—risk factors[a]	1.0	1.2 (0.4–3.6)	.80	2.2 (.8–5.9)	.12	1.8 (0.7–4.4)	.20
Adjusted—inflammation[b]	1.0	1.5 (0.5–4.5)	.44	1.7 (.6–4.7)	.29	1.5 (0.6–3.9)	.38
16S rDNA, copies/μL							
N (case patients/control subjects)	24/32	13/42		16/39		21/34	
Univariate	1.0	0.4 (0.2–0.9)	.03	0.5 (.2–1.2)	.12	0.7 (0.3–1.7)	.45
Adjusted—risk factors[a]	1.0	0.2 (0.1–0.7)	.01	0.4 (.1–1.3)	.12	0.4 (0.1–1.2)	.10
Adjusted—inflammation[b]	1.0	0.4 (0.1–1.3)	.14	0.5 (.1–1.8)	.28	1.3 (0.4–4.1)	.60
EndoCAb, MMU/mL							
N (case patients/control subjects)	18/38	15/40		20/35		21/34	
Univariate	1.0	0.8 (0.3–1.9)	.60	1.2 (.5– 2.6)	.67	1.2 (0.5–2.8)	.61
Adjusted—risk factors[a]	1.0	0.8 (0.2–2.4)	.64	1.7 (.6–4.8)	.29	1.3 (0.4–4.2)	.62
Adjusted—inflammation[b]	1.0	1.1 (0.4–3.3)	.82	0.8 (.3–2.1)	.65	1.4 (0.5–4.0)	.48

NOTE. Median values are reported, unless otherwise indicated. Biomarkers other than sCD14 level did not significantly increase the risk of death. Percentile cutoff points are LPS: <24.2, 24.2–32.5, 32.6–45.0, >45.0; sCD14: <2.10, 2.10–2.31, 2.31–2.72, >2.72; I-FABP: <22.7, 22.7–107.8, 107.9–377.0, >378.0; 16S rDNA: <3.4, 3.4–7.6, 7.7–13.2, >13.2; EndoCAb: <56.0, 56.0–123.0, 124–170.0, >170.0. EndoCAb, endotoxin core antibody; hsCRP, high-sensitivity C-reactive protein; I-FABP, intestinal fatty acid binding protein; IL, interleukin; LPS, lipopolysaccharide; OR, odds ratio; rDNA, ribosomal DNA; SAA, serum amyloid A; sCD14, soluble CD14.

[a] Risk factors include age, race (black vs other), use of ART and human immunodeficiency virus RNA level (no ART, ART and ⩽400 copies/mL, ART and >400 copies/mL), CD4+ cell count, smoking status, body mass index, prior cardiovascular disease, diabetes, use of blood pressure medication, use of lipid lowering medication, total/high-density lipoprotein cholesterol ratio, hepatitis B virus or hepatitis C virus co-infection, and treatment group (viral suppression or drug conservation).

[b] Markers of inflammation (and coagulation) include IL-6, hsCRP, SAA and D-dimer.

initiation of ART [18, 28]. Therefore, in exploratory analyses, we assessed whether there was a relationship between $CD4^+$ T cell counts and enterocyte damage as represented by I-FABP levels. We found that the 425 subjects with detectable I-FABP levels had lower baseline $CD4^+$ T cell counts (579 vs 659 cells/mm^3; $P = .002$; data not shown) and prior nadir $CD4^+$ T cell counts

Table 3. The Increased Risk for Mortality Conferred by Higher sCD14 Levels Does Not Differ between Treatment Arms

	Drug conservation arm		Viral suppression arm		
Biomarker	OR (95% CI)[a]	*P*	OR (95% CI)	*P*	*P* for interaction
sCD14 level, $\times 10^6$ pg/mL	3.5 (1.5–8.3)	.004	2.0 (0.8–5.4)	.15	.43
LPS, pg/mL	1.0 (0.6–1.7)	.96	0.7 (0.3–1.7)	.40	.63
I-FABP, pg/mL	0.9 (0.4–2.1)	.84	2.3 (0.6–8.8)	.19	.58
16S rDNA, copies/μL	0.9 (0.4–2.2)	.90	0.5 (0.2–1.4)	.21	.26
EndoCAb, MMU/mL	1.1 (0.8–1.6)	.49	0.9 (0.6–1.4)	.66	.57

NOTE. No biomarker other than sCD14 was associated with mortality in either treatment arm. CI, confidence interval; EndoCAb, endotoxin core antibody; I-FABP, intestinal fatty acid binding protein; LPS, lipopolysaccharide; OR, odds ratio; rDNA, ribosomal DNA; sCD14, soluble CD14.

[a] OR based on univariate analysis derived from conditional logistic model, associated with a one interquartile range higher level of biomarker after $\log_{10}$ transformation.

Table 4. No Significantly Increased Risk of CVD-Associated Events in HIV-Infected Subjects Conferred by Levels of Markers of Enterocyte Damage, Microbial Translocation, or the Immune Response to Microbial Translocation at Baseline

		25th–49th Percentile		50th–74th Percentile		>75th Percentile	
Biomarker	<25th Percentile (Ref)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
sCD14 level, $\times 10^6$ pg/mL							
N (case patients/control subjects)	25/65	28/61		31/58		36/54	
Univariate OR	1.0	1.1 (0.6–2.1)	.66	1.4 (0.7–2.6)	.32	1.7 (0.9–3.2)	.09
LPS level, pg/mL							
N (case patients/control subjects)	33/56	30/60		26/64		31/58	
Univariate OR	1.0	0.9 (0.5–1.6)	.62	0.7 (0.4– 1.3)	.26	0.9 (0.5–1.7)	.79
I-FABP level, pg/mL							
N (case patients/control subjects)	39/91	20/28		28/61		33/56	
Univariate OR	1.0	1.6 (0.8–3.3)	.15	1.1 (0.6–1.9)	.81	1.3 (0.8–2.4)	.31
16S rDNA level, copies/μL							
N (case patients/control subjects)	34/55	28/62		27/62		31/59	
Univariate OR	1.0	0.7 (0.4–1.3)	.24	0.6 (0.3–1.2)	.16	0.8 (0.4–1.5)	.51
EndoCAb level, MMU/mL							
N (case patients/control subjects)	32/57	34/56		31/58		23/67	
Univariate OR	1.0	1.1 (0.6–2.0)	.73	1.0 (0.5–1.8)	.90	0.6 (0.3–1.2)	.13

NOTE. Percentile cutoff points are LPS: <25.0, 25.0–33.5, 33.5–45.0, >45.0; soluble CD14: <2.02, 2.02–2.35, 2.36–2.71, >2.71; I-FABP: <.91, .91–142.19, 142.20–414.59, >414.59; 16S ribosomal DNA: <3.4, 3.4–7.8, 7.9–13.9, >13.9; EndoCAb: <43.0, 43.0–115.0, 116.0–169.0, >169.0. CI, confidence interval; CVD, cardiovascular disease; EndoCAb, endotoxin core antibody; HIV, human immunodeficiency virus; I-FABP, intestinal fatty acid binding protein; LPS, lipopolysaccharide; OR, odds ratio; rDNA, ribosomal DNA; Ref, reference; sCD14, soluble CD14.

(219 vs 258 cells/mm^3; $P = .002$; data not shown). In the 254 viremic patients, $CD4^+$ percentage correlated inversely with I-FABP levels ($r = -.25$; $P < .001$; data not shown). Thus, ongoing enterocyte damage is associated with lower $CD4^+$ T cell counts.

DISCUSSION

Although markers of microbial translocation have been associated with immune activation in HIV disease, the clinical significance of these relationships is poorly defined [17, 27]. We

Table 5. No Significantly Increased Risk of AIDS events in HIV-Infected subjects conferred by markers of enterocyte damage, microbial translocation, or the immune response to microbial translocation at baseline.

		25th – 49th Percentile		50th – 74th Percentile		⩾74th Percentile	
Biomarker	<25th Percentile (Ref)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
sCD14 level, $\times 10^6$ pg/mL)							
N (case patients/control subjects)	17/43	20/41		23/39		21/39	
Univariate OR	1.0	1.2 (0.6–2.5)	.65	1.5 (0.7–3.3)	0.28	1.4 (0.6–3.2)	.39
LPS level, pg/mL							
N (case patients/control subjects)	22/38	13/48		24/38		22/38	
Univariate OR	1.0	0.5 (0.2–1.1)	.08	1.1 (0.5–2.4)	0.83	1.0 (0.5–2.2)	>.99
I-FABP level, pg/mL							
N (case patients/control subjects)	24/65	13/18		25/35		18/42	
Univariate OR	1.0	2.0 (0.8–4.7)	.13	1.8 (0.9–3.7)	0.09	1.2 (0.6–2.5)	.68
16S rDNA level, copies/μL							
N (case patients/control subjects)	18/41	18/43		18/42		26/35	
Univariate OR	1.0	0.9 (0.4–2.1)	.88	0.9 (0.4–2.2)	0.90	1.7 (0.7–3.8)	.22
EndoCAb level, MMU/mL							
N (case patients/control subjects)	21/39	24/37		18/43		18/43	
Univariate OR	1.0	1.2 (0.6–2.5)	.60	0.8 (0.3–1.6)	0.47	0.8 (0.4–1.6)	.50

NOTE. Percentile cutoff points are LPS: <22.8, 22.8–32.1, 32.2–47.0, >47.0; soluble CD14: <2.06, 2.06–2.32, 2.33–2.68, >2.68; I-FABP: <2.70, 2.70–149.57, 149.57–468.00, >468.00; 16S ribosomal DNA: <3.8, 3.8–8.5, 8.6–15.0, >15.0; EndoCAb: <76.0, 76.0–128.0, 128.1–186.0, >186.0. CI, confidence interval; CVD, cardiovascular disease; EndoCAb, endotoxin core antibody; HIV, human immunodeficiency virus; I-FABP, intestinal fatty acid binding protein; LPS, lipopolysaccharide; OR, odds ratio; rDNA, ribosomal DNA; Ref, reference; sCD14, soluble CD14.

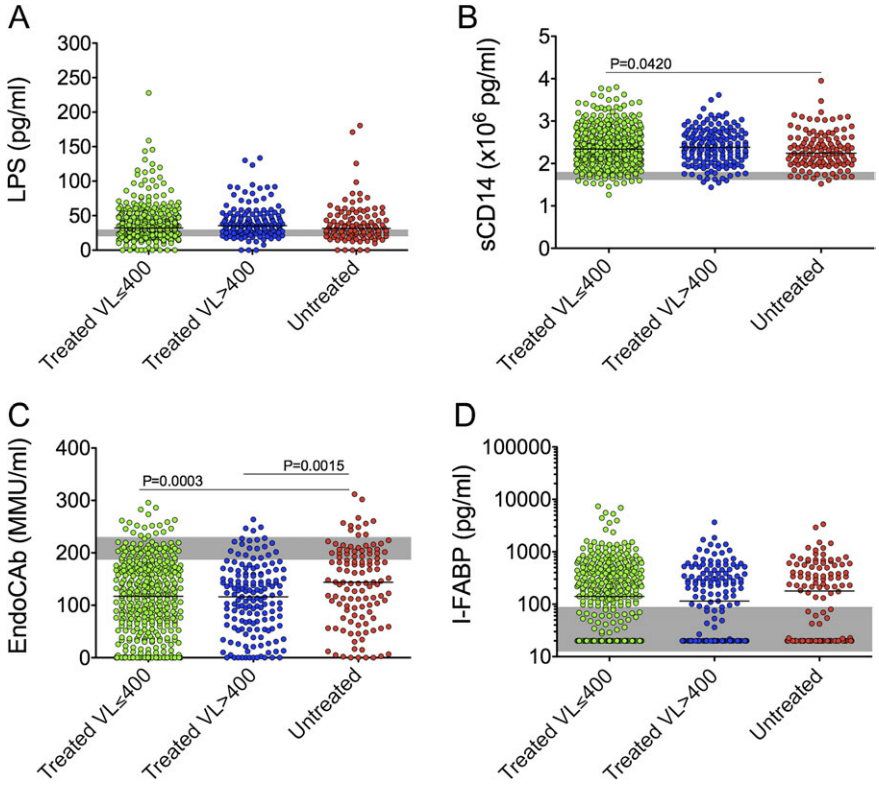

Figure 1. Increased enterocyte damage and microbial translocation in Strategies for Management of Anti-Retroviral Therapy (SMART) study subjects. Shaded areas represent the 95% confidence interval (CI) for levels observed in healthy, human immunodeficiency virus (HIV)–uninfected volunteers. Some tests were not performed for healthy volunteers because of insufficient plasma volumes. *A*, lipopolysaccharide (LPS) levels are higher in most SMART subjects, compared with the reference range; $n = 420$ for HIV-infected, treated subjects with HIV RNA levels ⩽ 400 copies/mL; $n = 155$ for HIV-infected, treated subjects with HIV RNA levels >400 copies/mL; $n = 117$ for HIV-infected, untreated subjects; $n = 67$ for HIV-uninfected volunteers. *B*, soluble CD14 (sCD14) levels are higher than the reference range in most SMART subjects, but treated subjects with HIV suppression have higher sCD14 levels than do untreated subjects; $n = 420$ for HIV-infected, treated subjects with HIV RNA levels ⩽400 copies/mL; $n = 155$ for HIV-infected, treated subjects with HIV RNA levels >400 copies/mL; $n = 117$ for HIV-infected, untreated subjects; $n = 65$ for HIV-uninfected volunteers. *C*, endotoxin core antibody (EndoCAb) levels are lower in most SMART subjects and are lower in treated subjects than in untreated subjects; $n = 419$ for HIV-infected, treated subjects with HIV RNA levels ⩽400 copies/mL; $n = 155$ for HIV-infected, treated subjects with HIV RNA levels >400 copies/mL; $n = 117$ for HIV-infected, untreated subjects; $n = 60$ for HIV-uninfected volunteers. *D*, intestinal fatty acid binding protein (I-FABP) levels are increased in most SMART subjects regardless of treatment status. A value of 20 pg/mL, which is the limit of detection, was assigned to subjects and volunteers with I-FABP levels below the limit of detection; $n = 417$ for HIV-infected, treated subjects with HIV RNA levels <400 copies/mL (61.5% with detectable levels); $n = 155$ for HIV-infected, treated subjects with HIV RNA levels >400 copies/mL (60.9% with detectable levels); $n = 116$ for HIV-infected, untreated subjects (62.4% detectable with detectable levels); $n = 41$ for HIV-uninfected volunteers (26.8% detectable). VL, viral load.

measured markers of microbial translocation (LPS), humoral immune response (EndoCAb), LPS bioactivity (sCD14), and enterocyte damage (I-FABP) among participants in a study of continuous versus intermittent ART. Using a case-control study design, we found that (1) high sCD14 levels were associated with an increased risk of all-cause mortality; (2) the association of sCD14 level with mortality persisted after adjustment for baseline $CD4^+$ T cell count and HIV RNA level and for markers of

Table 6. Correlation of sCD14 and Human Immunodeficiency Virus (HIV) RNA Levels with Inflammatory Markers

		IL-6	Serum amyloid A	hsCRP	D-dimer
HIV RNA level ⩽400 copies/mL	sCD14	r=.18 *P*<.001	r=.16 *P*<.001	r=.10 *P*=.04	r=.11 *P*=.03
HIV RNA level >400 copies/mL	sCD14	r=.26 *P*<.001	r=.18 *P*=.004	r=.18 *P*=.005	r=.09 *P*=.16
	HIV RNA	r=.23 *P*<.001	r=.00 *P*=.93	r=.04 *P*=.55	r=.42 *P*<.001

NOTE. For subjects with HIV RNA ⩽ 400 copies/mL, sCD14 levels correlate with levels of the inflammatory markers interleukin (IL)-6, serum amyloid A, C-reactive protein, and D-dimer, a marker of activation of the coagulation pathways, whereas for subjects with HIV RNA >400 copies/mL, sCD14 levels correlate with IL-6, serum amyloid A and CRP. HIV RNA level only correlates with IL-6 and D-dimer. HIV, human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; sCD14, soluble CD14.

inflammation and was evident for subjects in both the DC and VS groups; (3) sCD14 levels correlated with levels of IL-6, hsCRP, SAA, and D-dimer; (4) I-FABP, a specific indicator of enterocyte death, was detectable in many SMART subjects; and (5) detectable I-FABP levels were associated with lower nadir and baseline $CD4^+$ T cell counts. Collectively, these observations are consistent with a model in which HIV infection causes ongoing damage to the gut mucosa, leading to increased microbial translocation, increased systemic inflammation, and increased mortality.

Subjects with the highest sCD14 levels, reflecting the greatest monocyte activation, had a 6-fold higher rate of death than did those with the lowest levels of sCD14. The likelihood of type I error is increased, given comparisons of 5 variables for 3 outcomes, but the *P* value of $<.001$ makes it unlikely that this finding was attributable to chance. sCD14 level has been associated with disease progression in chronic viral hepatitis (N.G.S., unpublished data) and mortality in other clinical situations characterized by endotoxemia, including hemodialysis [29]. High sCD14 levels could lead to increased CD38 expression on $CD4^+$ or $CD8^+$ T cells, which is a predictor of $CD4^+$ T cell loss and death [30,31,6]. In this study, the causes of death included CVD, AIDS, hepatic and renal disease, malignancy, and others [19]. Although higher levels of sCD14 were not associated with increased risk of CVD or AIDS, subjects may not have been followed up long enough to detect the impact that sCD14 could have on progression to these particular end points. Nonetheless, high levels of LPS-induced monocyte activation may point to a common mechanistic pathway that underlies the different pathological conditions that culminate in death in HIV-infected individuals.

sCD14 is unlikely to be merely a marker of an acute phase response. The LPS binding site on CD14 is well-described [32]. Endotoxin challenge in humans upregulates CD14 expression 2.5-fold [33], and LPS stimulation induces sCD14 secretion by monocytes [25, 26]. sCD14 increases macrophage and neutrophil responses to LPS 50–100-fold, compared with LPS alone [34]. sCD14 is elevated in other diseases characterized or exacerbated by endotoxemia, such as hepatitis [35], rheumatoid arthritis, and systemic lupus erythematosus [36], but not in all situations with elevated acute phase reactants, including viral infections or after cardiac surgery [37, 38]. The weak correlation between sCD14 and IL-6 levels suggests an indirect relationship in which the factors that cause increased sCD14 levels–namely, microbial products–also cause increased levels of inflammatory markers.

Adjusting for inflammatory markers, $CD4^+$ T cell count, or HIV RNA level did not reduce the association of sCD14 levels with death, suggesting that sCD14 independently predicts mortality. Although the increased risk of death in the VS arm was not significant (likely because it was underpowered), the lack of interaction between the DC arm and the VS arm suggests that the effect of sCD14 is independent of treatment interruption, intermittent high-level viremia, and low $CD4^+$ T cell counts. Surprisingly, sCD14 levels were higher in subjects receiving ART with HIV RNA level $\leq$ 400 copies/mL than they were in untreated individuals. This could reflect an effect of treatment on sCD14 levels, but it contrasts with published data showing no change in sCD14 level after 48 weeks of ART [17] and a decrease in sCD14 levels after >1 year of ART [39, 40]. Because the *P* for this comparison was .04, this finding could be a chance occurrence, or it could reflect differences in disease severity between these unmatched groups of subjects. Similarly, lower EndoCAb levels in treated patients and the lack of difference in LPS levels between patients receiving ART and those not receiving ART may reflect these confounding factors.

Contrary to what we hypothesized, neither LPS nor 16S rDNA was directly associated with clinical end points. 16S rDNA degrades easily after freeze-thaw cycles, which may have affected our results [41]. The lack of association between LPS and sCD14 levels may be related to the biology of LPS and the host response to it. LPS is cleared rapidly from the blood by numerous mechanisms, including transfer to high-density lipoprotein, formation of LBP–LPS complexes [42], scavenging by EndoCAb [43], and clearance by sCD14 [44]. LBP, EndoCAb, and HDL and other plasma proteins inhibit LPS measurement [45], and plasma turbidity and interference from triglycerides can complicate LPS measurement [46]. Alternatively, a given level of LPS may result in differing levels of monocyte activation in different individuals. There may be a genetic component to the response to LPS, akin to the polymorphism in the peptidoglycan receptor NOD2 in Crohn disease [47]. Indeed, polymorphisms in both NOD2 and TLR4 have been associated with bacteremia and mortality [47]. Furthermore, a polymorphism in the promoter region of the gene encoding CD14 (-159C/T) has been associated with higher sCD14 levels in heavy alcohol drinkers [48] and in those with hepatitis C infection [49]. Thus, when considering the consequences of microbial translocation, measures of the host response to the microbial product (bioreactivity), rather than measures of the microbial product itself, may be more accurate and relevant.

Histological analysis of intestinal biopsy specimens from HIV-infected patients shows enterocyte damage [50]. Because only dying enterocytes release I-FABP [24], the elevated I-FABP levels detected in most subjects may reflect ongoing structural damage to the gastrointestinal tract. Indeed, the association of lower $CD4^+$ T cell counts with higher I-FABP levels also supports the hypothesis that damage to the gut, immune activation, and $CD4^+$ T cell loss are intimately linked. Because our HIV-negative volunteer group was not matched for factors that could affect enterocyte death, such as liver disease and age, further investigation with longitudinal samples in a matched, diverse patient population is needed to confirm these findings.

Taken together, our data show that high plasma levels of the monocyte-expressed LPS receptor sCD14 are associated with an increased risk of all-cause mortality in HIV-infected subjects in the SMART study. The diversity of the patients enrolled in this study, who were from virtually every continent, and many of whom had hepatitis co-infection and other co-morbidities and were receiving various ART regimens (or none at all) [19], makes the results relevant to many patient populations. The markers of inflammation that confer an increased risk of death in this patient population correlate with the level of monocyte stimulation by LPS. In addition, we show that I-FABP, a marker of enterocyte death, is increased in these HIV-infected subjects and that detectable levels are associated with lower baseline and nadir $CD4^+$ T cell counts. These results suggest that attenuation of the inflammation induced by microbial products, in addition to viral suppression, may be necessary to maximally reduce mortality and improve clinical outcomes.

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