AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 29, Number 6, 2013

DOI: 10.1089/aid.2012.0305

Perturbations of Circulating Levels of RANKL-Osteoprotegerin Axis in Relation to Lipids and Progression of Atherosclerosis in HIV-Infected and -Uninfected Adults: ACTG NWCS 332/A5078 Study

Theodoros Kelesidis,[1,2] Michelle A. Kendall,[3] Otto O. Yang,[1,2] Howard Hodis,[4] and Judith S. Currier[1]

Abstract

The receptor activator of the NF-κB ligand (RANKL)-osteoprotegerin (OPG) axis has been shown to play a role in the inflammatory process of atherogenesis and may be regulated by changes in levels of cholesterol. However, the interplay between HIV-1 infection, lipids, the RANKL-OPG axis, and atherosclerosis is poorly defined. Serum RANKL, OPG, and RANKL/OPG ratio were retrospectively assessed for 91 subjects from a 3-year study of carotid artery intima-media thickness (CIMT), which enrolled triads of risk factor-matched persons that were HIV-1 uninfected ($n=36$) or HIV-1$^+$ with ($n=29$) or without ($n=26$) continuous protease inhibitor (PI)-based therapy for ≥2 years. Associations of serum RANKL, OPG, and RANKL/OPG ratio to the primary outcomes of levels of circulating lipids and atherosclerosis progression were determined using multivariate regression models. Serum RANKL and RANKL/OPG ratio were significantly lower in HIV-infected versus HIV-uninfected subjects ($p<0.01$). Multivariate models for HIV-1$^+$ subjects, but not in uninfected controls, demonstrated that perturbations in serum cholesterol levels were significantly associated ($p<0.05$) with perturbations in serum levels of RANKL and OPG, and their ratio (RANKL/OPG). There were no significant associations of serum RANKL, OPG, and RANKL/OPG with progression of atherosclerosis in HIV-1$^+$ subjects. Our results suggest that HIV-1 infection is associated with reductions in both serum RANKL and the RANKL/OPG ratio, and perturbations in the circulating levels of RANKL and OPG are significantly associated with increases in cholesterol levels, but not with progression of atherosclerosis.

Introduction

Tumor necrosis factor (TNF) superfamily molecules, namely, the receptor activator of nuclear factor-κB ligand (RANKL), its receptor (RANK), and its soluble (decoy) receptor, osteoprotegerin (OPG), mediate interactions (RANKL-OPG axis) that exert pleiotropic effects on bone metabolism, endocrine functions, and the immune system.[1–3] RANK is expressed on the surface of hematopoietic precursor cells and mediates signaling that stimulates osteoclastogenesis.[1–3] Its ligand RANKL is expressed on osteoblast/stromal cell surfaces, whereas OPG (a secreted glycoprotein of the TNF receptor superfamily) acts as a decoy receptor that binds RANKL and prevents the activation of RANK.[1–3]

Atherosclerosis is an inflammatory process in which the RANKL-OPG axis is implicated. This axis typically is considered for its role in bone metabolism, but proinflammatory cytokines including interleukin (IL)-1b, IL-6, and TNF-α that are regulated by the RANKL-OPG axis in mediating bone resorption in osteoporosis[1–3] also play critical roles in the initiation and perpetuation of atherosclerosis.[4] Although reports of associations between circulating levels of OPG or RANKL with cardiovascular disease have been conflicting,[1,2,5] the most recent studies have shown that both

[1]Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine, University of California, Los Angeles, California.
[2]Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine, University of California, Los Angeles, California.
[3]Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, Massachusetts.
[4]Atherosclerosis Research Unit, Department of Medicine and Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California.

increased RANKL concentration and decreased OPG level are associated with vascular calcification, and increased RANKL/OPG ratio is significantly associated with coronary artery disease.[1,2,5] Moreover, atherosclerosis and osteoporosis share hyperlipidemia as an etiologic factor,[6,7] helper T cells can produce RANKL,[8,9] and oxidized lipids can directly increase the expression of RANKL from T cells through scavenger receptors.[8,9]

Human immunodeficiency virus type 1 (HIV-1) infection is characterized by a chronic state of systemic inflammation with increased levels of proinflammatory cytokines, hyperlipidemia, and helper T cell depletion,[10] and should therefore affect the RANKL-OPG axis, but these effects have not been defined. In view of reported positive associations of atherosclerosis, osteoporosis, and HIV-1 infection with proinflammatory activation of the RANKL-OPG axis and hyperlipidemia, and the stimulatory effects of oxidized lipids in production of RANKL from T cells,[8,9] we hypothesized that increased serum RANKL/OPG is independently associated with cholesterol levels and progression of atherosclerosis in HIV-1-infected subjects. Using blood specimens from a previously described matched cohort study, we determined associations of baseline characteristics, HIV-1 infection, protease inhibitors (PI) therapy, and the risk of development of subclinical atherosclerosis and its progression with the RANKL-OPG axis (serum soluble RANKL, OPG, and RANKL/OPG ratio) in HIV-1-infected and HIV-uninfected subjects.[11] Finally, we determined the associations of oxidized [low-density lipoprotein (LDL), non-high-density lipoprotein (non-HDL)] and nonoxidized (HDL and total cholesterol) lipids with circulating levels of the RANKL-OPG axis.

Materials and Methods

Study design

A5078 was a prospective, matched cohort study that was designed to investigate the role of PI therapy and HIV-1 infection on the risk for subclinical atherosclerosis and its progression; the study design and primary results have been previously published.[11] The current study is a subset analysis of samples obtained from subjects who were enrolled as risk factor (age, sex, race/ethnicity, smoking status, blood pressure, and menopause status)-matched triads of HIV-1-infected (HIV-1^+) individuals with viremia <500 RNA copies/ml with ($n=29$) or without ($n=26$) use of PI therapy, and HIV-1-uninfected (control) individuals ($n=36$) from 41 triads, of which 15 were complete. These subjects were recruited from eight academic medical centers in the United States and had overall low cardiovascular disease risk since the following clinical conditions were exclusionary: diabetes mellitus (DM) or current use of oral hypoglycemia agents and/or thiazolidinediones, family history of myocardial infarction, history of coronary heart disease or stroke, uncontrolled hypertension, untreated hypothyroid ism, or obesity.

Data collection

Metabolic syndrome was defined by National Cholesterol Education Program (NCEP) criteria.[12] Fasting glucose, insulin, lipids, homocysteine, high-sensitivity C-reactive protein (hs-CRP), cardiovascular disease-related measurements, $CD4^+$ T cell counts, and HIV-1 RNA levels were previously determined.[11] Carotid artery intima-media thickness (CIMT) of the far wall of the right common carotid artery was measured at baseline and longitudinally as previously published.[11] Using stored samples from this prospective study, serum total soluble RANKL and OPG were quantified by enzyme-linked immunosorbent assay (ELISA) in accordance with the manufacturer's protocol (OPG: R&D Systems, RANKL: Biovendor) on specimens from weeks 0 and 144 (or 96 if week 144 was missing).

Sample size

The primary objective evaluated two pairwise differences in yearly rates of change between groups, separately for each outcome (RANKL, OPG, and RANKL/OPG ratio). Based on sample availability from the parent study, we assumed 30 evaluable subjects per group. Using a two-sided, 0.05-level, two-sample *t*-test with two comparisons, we had the ability to detect a difference of 0.8 times the common standard deviation of the yearly rate of change in each outcome with 80% power.

Statistical methods

The yearly rate of change for each marker was based on two time points for each participant. Using a 48-week year, simple linear regression was used to calculate each participant's yearly rate of change (i.e., slope) in the marker (reported in marker unit/year). A matched analysis comparing the two HIV-1^+ groups (18 triad pairs) assessed the effect of PI therapy on the RANKL-OPG axis. Comparison of the HIV-1^+/non-PI and control groups (21 triad pairs; or combined HIV-1^+ group with the control group if there was no difference between the two HIV-1^+ groups) assessed the effect of HIV-1 infection on the RANKL-OPG axis. A variation on the Wilcoxon signed-rank test was used to compare the two HIV-1^+ subjects to the paired control subject within each triad.[13] Mixed models regression analyses with triad as a random effect evaluated whether baseline measurements of serum RANKL, serum OPG, serum RANKL/OPG, and CIMT were associated with baseline covariates. Progression of atherosclerosis was evaluated in two ways: (1) yearly rate of change in CIMT and (2) CIMT progression (defined a priori as yearly rate of change $\geq 12.2\,\mu$m/year as previously described[11]). Repeated measures regression analyses evaluated associations with yearly rates of change in the outcome. Conditional logistic regression modeling for matched pairs data stratified by triad evaluated associations with CIMT progression. Covariates significant in the univariate analysis ($p<0.05$) were examined together in multivariate analysis. SAS version 9.2 was used for the statistical analysis.

Results

HIV-1-infected subjects had more metabolic abnormalities than the HIV-1-uninfected subjects

Baseline anthropometric, metabolic, and inflammatory marker data on the 91 subjects in this study are summarized in Table 1. The subjects who did not remain in the study after week 96 did not differ in age and race/ethnicity from those who completed the week 144 visit. The two HIV$^+$ groups had comparable $CD4^+$ T cell blood counts, nadir $CD4^+$ T cell counts, plasma viremia, and usage of nucleoside reverse transcriptase inhibitors (NRTIs) at baseline. As reported

Table 1. Baseline Subject Variables by Group

Characteristic	*Total (N = 91)*	*HIV-1/PI (N = 29)*	*HIV-1/non-PI (N = 26)*	*Control (not HIV-1+) (N = 36)*	p-*value*[a]
Age (years), median (IQR)	41 (36–45)	41 (38–45)	41 (36–47)	40 (36–45)	0.86[b]
Sex, M	84 (92%)	28 (97%)	24 (92%)	32 (89%)	0.55[c]
Race/ethnicity					
White non-Hispanic	69 (76%)	23 (79%)	19 (73%)	27 (75%)	0.40[c]
Hispanic (regardless of Race)	17 (19%)	4 (14%)	5 (19%)	8 (22%)	
Other/unknown	5 (5%)	2 (7%)	2 (8%)	1 (3%)	
Body mass index (kg/m^2), median (IQR)	24.70 (23.40–27.60)	25.50 (23.60–27.60)	24.20 (22.00–26.60)	25.00 (23.75–27.95)	0.25[b]
Waist circumference (cm),[d] ≥90	40 (44%)	17 (59%)	7 (27%)	16 (44%)	0.05[c]
Waist/hip ratio,[d] median (IQR)	0.91 (0.86–0.94)	0.93 (0.92–0.96)	0.90 (0.86–0.94)	0.89 (0.83–0.92)	0.003[b]
Metabolic syndrome, present	11 (12%)	9 (31%)	1 (4%)	1 (3%)	0.001[c]
Lipid-lowering medications, present	12 (13%)	8 (28%)	4 (15%)	0 (0%)	0.001[c]
Metabolic parameters					
Fasting blood glucose (mg/dl), <126	91 (100%)	29 (100%)	26 (100%)	36 (100%)	
Total cholesterol (mg/dl), ≥200	43 (47%)	20 (69%)	9 (35%)	14 (39%)	0.02[c]
Direct LDL cholesterol (mg/dl),[e] ≥100	64 (70%)	22 (76%)	17 (65%)	25 (69%)	0.40[c]
HDL cholesterol (mg/dl), <35	20 (22%)	7 (24%)	6 (23%)	7 (19%)	0.90[c]
Triglycerides (mg/dl), ≥150	39 (43%)	18 (62%)	11 (42%)	10 (28%)	0.02[c]
Non-HDL cholesterol (mg/dl), median (IQR)	149 (128–187)	184 (134–206)	135 (118–170)	145 (125–171.5)	0.009[b]
Insulin (mU/liter),[f] median (IQR)	6.25 (5–8)	7.40 (6.20–14.50)	5.55 (4.55–7.15)	6 (5–7.60)	0.004[b]
Inflammatory parameters					
hs-CRP (mg/liter),[f] median (IQR)	1.10 (0.50–2.30)	1.40 (0.80–3.30)	1 (0.40–3.40)	1 (0.50–1.80)	0.26[b]
Homocysteine (μmol/liter),[f] median (IQR)	9.10 (7.40–11)	9.20 (7.90–13.80)	7.60 (6.35–8.80)	9.70 (8.70–11)	0.002[b]
HIV disease-related parameters	(*N* = 55)	(*N* = 29)	(*N* = 26)		
Baseline $CD4^+$ T cells ($cells/mm^3$), median (IQR)	488 (354–692)	535 (369–707)	477 (334–674)		0.56[b]
Nadir $CD4^+$ T cells ($cells/mm^3$),[g] ≤200	20 (38%)	11 (39%)	9 (35%)		0.78[c]
HIV-1 RNA (copies/ml), <50	46 (84%)	24 (83%)	22 (85%)		0.99[c]
Antiretroviral therapy (weeks), median (IQR)	156 (123–269)	250 (152–359)	117 (60–140)		<0.001[b]
PI use (weeks),[h] median (IQR)	113 (0–238)	190 (135–259)	0 (0–0)		<0.001[b]
Ritonavir use, any	9 (16%)	9 (31%)	0 (0%)		0.002[c]
NRTI use, any	52 (57.1%)	27 (93.1%)	25 (96.2%)		0.99[c]
NNRTI use, any	28 (30.8%)	8 (27.6%)	20 (76.9%)		<0.001[c]

[a]*p*-value for between group differences: HIV-1/PI versus HIV-1/non-PI versus control.
[b]Kruskal–Wallis test.
[c]Fisher's exact test.
[d]Due to missing data, the sample sizes are *N* = 90, *N* = 28, *N* = 26, and *N* = 36.
[e]Due to missing data, the sample sizes are *N* = 89, *N* = 27, *N* = 26, and *N* = 36.
[f]Due to missing data, the sample sizes are *N* = 84, *N* = 27, *N* = 24, and *N* = 33.
[g]Due to missing data, the sample sizes are *N* = 53, *N* = 27, and *N* = 26.
[h]Within the HIV-1/PI and HIV-1/non-PI groups, the choice of baseline antiretroviral therapy varied. In the PI group, 18 (62%) received a single PI (eight nelfinavir, eight indinavir, and two ritonavir), three received lopinavir/ritonavir, and eight received a dual PI combination (one did not include ritonavir). Overall, 12 (41%) of the subjects within the PI group were receiving ritonavir as part of their therapy. In the non-PI group, 20 (77%) were receiving a combination of nonnucleoside reverse transcriptase inhibitors and nucleoside analogues, five (19%) were receiving nucleoside analogues only, and one (4%) was receiving no antiretroviral therapy.

PI, protease inhibitor; IQR; interquartile range; LDL, low-density lipoprotein; HDL, low-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor.

Table 2. Summary of RANKL, Osteoprotegerin, RANKL/Osteoprotegerin Results by Group

	Total (N = *91*)	*HIV-1* (N = *55*)	*HIV-1/PI* (N = *29*)	*HIV-1/non-PI* (N = *26*)	*Control* (N = *36*)	p-*value*
RANKL [pg/ml, median (IQR)]						
Baseline RANKL (pg/ml)	22,124 (10,352, 44,003)	15,059 (7,764, 26,537)	15,750 (12,655, 26,270)	10,946 (5,175, 26,537)	42,829 (17,249, 70,496)	<0.001[a]; <0.001[b]
Week 96/144 RANKL (pg/ml)	22,048 (12,508, 52,074)	17,632 (10,990, 34,314)	16,749 (8,606, 27,071)	18,633 (11,209, 51,565)	35,339 (19,989, 100,835)	0.002[a]; 0.008[b]
Yearly rate of change in RANKL (pg/ml/year)	1,531 (−1,715, 6,516)	1,771 (−1,978, 5,842)	−483 (−3,311, 2,336)	4,034 (402, 7,544)	1,476 (−1,576, 11,576)	0.81; 0.08; 0.10; 0.96[c]
p-value for yearly rate of change in RANKL[d]		0.04	0.86	0.0006	0.06	
OPG [pg/ml, median (IQR)]						
Baseline OPG (pg/ml)	1,054 (781, 1,535)	1,087 (781, 1,601)	1,204 (860, 1,687)	1,035 (781, 1,392)	1,013 (772, 1,289)	0.52[a]; 0.48[b]
Week 96/144 OPG (pg/ml)	1,152 (825, 1,580)	1,206 (763, 1,590)	1,313 (1,054, 1,640)	1,122 (680, 1,540)	1,107 (848, 1,509)	0.68[a]; 0.39[b]
Yearly rate of change in OPG (pg/ml/year)	9 (−79, 106)	6 (−88, 106)	5 (−87, 105)	36 (−88, 120)	19 (−48, 104)	0.79; 0.44; 0.83; 0.32[c]
p-value for yearly rate of change in OPG[d]		0.92	0.86	0.93	0.19	
RANKL/OPG [median (IQR)]						
Baseline RANKL/OPG	18.23 (9.98, 48.81)	13.32 (6.72, 27.80)	13.69 (6.72, 27.80)	12.04 (7.35, 18.26)	37.68 (18.16, 75.54)	<0.001[a]; <0.001[b]
Week 96/144 RANKL/OPG	19.83 (10.14, 54.27)	16.16 (7.98, 41.23)	11.55 (7.40, 29.38)	20.56 (9.71, 45.34)	28.44 (15.97, 84.90)	0.006[a]; 0.01[b]
Yearly rate of change in RANKL/OPG (per year)	0.68 (−2.77, 6.69)	1.18 (−2.01, 6.56)	0.56 (−3.43, 2.18)	3.02 (0.30, 6.96)	0.50 (−5.19, 7.88)	0.44; 0.23; 0.19; 0.33[c]
p-value for yearly rate of change in RANKL/OPG[4]		0.04	0.99	0.002	0.53	

[a]Wilcoxon test for *between* group differences: HIV-1 versus control.

[b]Kruskal–Wallis test for *between* group differences: HIV-1/PI versus HIV-1/non-PI versus control.

[c]Wilcoxon *p*-value for *between* matched group differences within each visit week: pairings assessed were HIV-1 versus control ($N = 32$ matched pairings), HIV-1/PI versus HIV-1/non-PI ($N = 18$ matched pairings), HIV-1/PI versus control ($N = 26$ matched pairings), and HIV-1/non-PI versus control ($N = 21$ matched pairings).

[d]Wilcoxon test for nonzero yearly rate of change *within* each group, where the yearly rate of change for each participant was calculated based on the two specimen dates using simple linear regression (i.e., slope = yearly rate of change).

OPG, osteoprotegerin;

Table 3. Univariate Associations of RANKL, Osteoprotegerin Levels or RANKL/Osteoprotegerin Ratio with Baseline Variables

Covariate	*All subjects*		*HIV-1$^+$ subjects*		*Control subjects*	
	Parameter estimate (95% CI)	p-*value*	*Parameter estimate (95% CI)*	p-*value*	*Parameter estimate (95% CI)*	p-*value*
	Baseline RANKL (per 1,000 pg/ml))					
HIV$^+$ versus control	−36.41 (−57.15, −15.68)	0.001	(−)	(−)	(−)	(−)
Waist/hip Ratio (per 0.1 unit)	−19.24 (−35.88, −2.60)	0.02	(−)	(−)	(−)	(−)
Higher total cholesterol (i.e., ≥200 mg/dl) versus lower	(−)	(−)	15.05 (−1.55, 31.65)	0.07	(−)	(−)
Baseline OPG (per 100 pg/ml)	−0.07 (−1.49, 1.36)	0.93	−0.08 (−1.28, 1.13)	0.90	0.24 (−2.55, 3.03)	0.86
	Yearly Rate of Change in RANKL (per 1,000 pg/ml/year)					
Female sex	25.48 (4.14, 46.82)	0.02	(−)	(−)	30.26 (4.71, 55.81)	0.02
HIV$^+$ versus control	−7.56 (−13.57, −1.55)	0.01	(−)	(−)	(−)	(−)
Body mass index (kg/m^2)	−2.01 (−3.29, −0.74)	0.002	(−)	(−)	−5.40 (−8.65, −2.15)	0.002
Larger waist circumference (i.e., ≥90 cm) versus smaller	(−)	(−)	4.14 (−0.53, 8.81)	0.08	−21.29 (−36.87, −5.71)	0.01
Waist-to-hip ratio (per 0.1 units)	−9.88 (−15.81, −3.95)	0.001	(−)	(−)	−21.52 (−34.94, −8.11)	0.002
Higher total cholesterol (i.e., ≥200 mg/dl) versus lower	(−)	(−)	−6.35 (−12.39, −0.31)	0.04	(−)	(−)
Higher HDL cholesterol (i.e., ≥35 mg/dl) versus lower	(−)	(−)	−8.99 (−15.21, −2.76)	0.005	(−)	(−)
Non-HDL cholesterol (per 10 mg/dl)	−0.99 (−1.87, −0.12)	0.03	(−)	(−)	(−)	(−)
Homocysteine (per 10 μmol/liter)	(−)	(−)	(−)	(−)	−37.69 (−81.37, 6.00)	0.09
Higher reported nadir CD4 (i.e., >200 cells/mm^3) versus lower	(−)	(−)	6.62 (1.08, 12.16)	0.02	(−)	(−)
Years of PI exposure (per 1 year)	(−)	(−)	−1.73 (−2.47, −1.00)	<0.001	(−)	(−)
Baseline OPG (per 100 pg/ml)	0.15 (−0.39, 0.70)	0.58	0.05 (−0.34, 0.43)	0.81	0.71 (−0.60, 2.01)	0.28
	Baseline OPG (per 100 pg/ml)					
Female sex	8.99 (1.86, 16.13)	0.01	(−)	(−)	12.10 (3.96, 20.25)	0.005
Waist-to-hip ratio (per 0.1 units)	(−)	(−)	(−)	(−)	−4.85 (−9.53, −0.17)	0.04
Higher total cholesterol (i.e., ≥200 mg/dl) versus lower	−2.80 (−6.01, 0.41)	0.09	−4.65 (−8.57, −0.73)	0.02	(−)	(−)
Higher HDL cholesterol (i.e., ≥35 mg/dl) versus lower	3.97 (0.26, 7.68)	0.04	(−)	(−)	(−)	(−)
Insulin (per 10 mU/liter)	4.22 (1.51, 6.94)	0.003	4.97 (1.93, 8.00)	0.003	(−)	(−)
hs-CRP (per 10 mg/liter)	3.02 (0.06, 5.97)	0.05	4.99 (1.28, 8.71)	0.01	(−)	(−)
Baseline RANKL (per 1,000 pg/ml)	−0.0002 (−0.03, 0.03)	0.99	−0.0002 (−0.07, 0.07)	0.99	0.004 (−0.040,0.047)	0.86

(*continued*)

Table 3. (Continued)

Covariate	*All subjects*		*HIV-1$^+$ subjects*		*Control subjects*	
	Parameter estimate (95% CI)	p-*value*	*Parameter estimate (95% CI)*	p-*value*	*Parameter estimate (95% CI)*	p-*value*
	Baseline RANKL/OPG (per 10 units)					
HIV$^+$ versus control	−4.00 (−6.14, −1.86)	<0.001	(−)	(−)	(−)	(−)
Waist/hip ratio (per 0.1 unit)	−1.75 (−3.50, −0.004)	0.05	(−)	(−)	(−)	(−)
Higher total cholesterol (i.e., ≥200 mg/dl) versus lower	(−)	(−)	1.68 (0.21, 3.16)	0.03	(−)	(−)
Larger waist circumference (i.e., ≥90 cm) versus smaller	(−)	(−)	(−)	(−)	−4.40 (−9.11, 0.32)	0.07
	Yearly Rate of Change in RANKL/OPG (per 10 units/year)					
HIV$^+$ versus control	−0.61 (−1.14, −0.08)	0.03	(−)	(−)	(−)	(−)
Age (per 10 years)	(−)	(−)	(−)	(−)	1.05 (−0.4, 2.15)	0.06
Body mass index (kg/m^2)	−0.20 (−0.31, −0.09)	<0.001	−0.08 (−0.16, −0.001)	0.05	−0.45 (−0.76, −0.14)	0.005
Waist/hip ratio (per 0.1 unit)	−0.46 (−1.00, 0.08)	0.09	(−)	(−)	(−)	(−)
Larger waist circumference (i.e., ≥90 cm) versus smaller	(−)	(−)	(−)	(−)	−1.91 (−3.37, −0.45)	0.01
Higher total cholesterol (i.e., ≥200 mg/dl) versus lower	−0.59 (−1.26, 0.08)	0.09	−0.81 (−1.29, −0.34)	0.001	(−)	(−)
Higher direct LDL cholesterol (i.e., ≥100 mg/dl) versus lower	(−)	(−)	−0.60 (−1.13, −0.06)	0.03	(−)	(−)
Higher HDL cholesterol (i.e., ≥35 mg/dl) versus lower	(−)	(−)	(−)	(−)	−1.71 (−3.52, 0.09)	0.06
Higher triglycerides (i.e., ≥150 mg/dl) versus lower	−0.81 (−1.43, −0.19)	0.01	(−)	(−)	−1.76 (−3.33, −0.18)	0.03
Non-HDL cholesterol (per 10 mg/dl)	−0.07 (−0.14, 0.01)	0.09	−0.06 (−0.11, −0.02)	0.01	(−)	(−)
Insulin (per 10 mU/liter)	−0.60 (−1.19, −0.02)	0.04	(−)	(−)	(−)	(−)
Homocysteine (per 10 μmol/liter)	(−)	(−)	(−)	(−)	−3.66 (−7.75, 0.42)	0.08
Higher reported nadir CD4 (i.e., >200 cells/mm^3) versus lower	(−)	(−)	0.40 (−0.08, 0.87)	0.099	(−)	(−)
Years of PI exposure (per 1 year)	(−)	(−)	−0.17 (−0.23, −0.10)	<0.001	(−)	(−)

The baseline variables considered for all subjects were age, gender, race, fasting lipid measurements [total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol, triglycerides, and non-HDL cholesterol], use of lipid-lowering drugs, fasting glucose, body mass index, waist circumference, waist/hip ratio, insulin, high-sensitivity C-reactive protein, homocysteine, CIMT, serum RANKL, and serum OPG. For the HIV-1-infected subjects, additional variables included years of PI use, CD4$^+$ T cell count, and nadir CD4$^+$ T cell count. The associations of these baseline variables with baseline serum levels of RANKL, OPG, and RANKL/OPG were assessed. In addition, since only serum RANKL and RANKL/OPG significantly changed over time (Table 2), the associations of the above baseline variables with the yearly rate of change in serum levels of RANKL and RANKL/OPG were also assessed. Only statistically significant ($p < 0.1$) associations and the associations between baseline serum RANKL and baseline serum OPG are shown.

CI, confidence interval; OPG, osteoprotegerin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PI, protease inhibitor; hs-CRP, high-sensitivity C-reactive protein; CIMT, carotid artery intima-media thickness.

previously in the primary analysis of this cohort, the HIV-1$^+$ subjects tended to have more metabolic abnormalities than the control subjects.[14]

HIV-1 infection was associated with significantly lower serum RANKL and RANKL/OPG ratio

In view of the limited data regarding circulating levels of RANKL and OPG in HIV-1 infection,[15–17] we first determined the possible associations between treated HIV-1 infection and changes in serum levels of RANKL, OPG, and their serum ratio. As shown in Table 2, at baseline and week 96/144, serum RANKL levels and the RANKL/OPG serum ratio were significantly lower in both HIV-1$^+$ groups (with and without PI treatment) and in the combined HIV-1$^+$ group compared to the control group ($p \leq 0.01$). Both the combined HIV-1$^+$ and HIV/non-PI groups had significantly greater serum RANKL and RANKL/OPG levels by 96/144 weeks compared to baseline ($p \leq 0.04$) in contrast to the control group ($p = 0.06$). There were no significant differences in serum OPG when comparing the two HIV-1$^+$ groups and the combined HIV-1$^+$ group to the control group at both time points ($p > 0.3$). In a matched analysis, there were no significant differences in the yearly rates of change in serum levels of RANKL, OPG, and RANKL/OPG between the combined HIV-1 and control groups ($p > 0.4$). Thus, HIV-1 infection was associated with lower baseline serum levels of RANKL and RANKL/OPG, but not with changes in these biomarkers over time.

Antiretroviral therapy with PI did not affect the changes in levels of RANKL and RANKL/OPG in HIV-infected subjects

Given the numerous *in vitro* effects of antiretroviral therapy (ART) with PI on OPG/RANKL regulation in osteoblasts,[18] we then explored the association between therapeutic regimens with versus without PIs and alterations in circulating levels RANKL, OPG, and their ratio *in vivo* in HIV-infected subjects. There were no significant differences between both HIV-1$^+$ groups in serum RANKL, OPG, RANKL/OPG levels at both time points ($p > 0.2$; data not shown), and the yearly rates of change in serum RANKL, OPG, and RANKL/OPG (matched analysis $p > 0.4$) (Table 2). Thus, we were not able to detect a statistically significant relationship between PI exposure and markers of the RANKL-OPG axis in HIV-1$^+$ subjects.

Higher baseline cholesterol levels were associated with lower baseline serum OPG and higher RANKL/OPG serum ratio in HIV-infected subjects

To confirm our hypothesis that hyperlipidemia is independently associated with circulating levels of RANKL and/or OPG in HIV-1 infection and that the interplay of lipids with the RANKL-OPG axis may be important for the progression of atherosclerosis in HIV-1 infection, we investigated factors significantly associated with baseline serum RANKL, OPG, and RANKL/OPG, as identified by univariate and multivariate models. Factors associated with lower baseline serum RANKL and RANKL/OPG included HIV-1 infection ($p \leq 0.001$) and larger baseline waist-to-hip ratio (WHR) ($p \leq 0.05$) in univariate analysis in all study subjects (Table 3). In a multivariate analysis that included all study subjects, lower baseline serum OPG was associated with being male (parameter estimate -9.43; 95% CI: -17.73, -1.14; $p = 0.03$), lower baseline HDL (parameter estimate -3.97; 95% CI: -7.54, -0.40; $p = 0.03$), and lower baseline insulin (parameter estimate -4.13; 95% CI: -6.62, -1.64; $p = 0.002$). In the HIV-1$^+$ subjects only, univariate analysis found that higher baseline total cholesterol was associated with higher baseline serum RANKL and RANKL/OPG and lower baseline serum OPG ($p \leq 0.07$). In multivariate analysis in the HIV-1$^+$ subjects, lower baseline serum OPG was associated with higher baseline total cholesterol (parameter estimate -4.69; 95% CI: -8.47, -0.92; $p = 0.02$), lower baseline insulin (parameter estimate -4.35; 95% CI: -7.35, -1.35; $p = 0.008$), and lower baseline hs-CRP (parameter estimate -4.22; 95% CI: -7.59, -0.86; $p = 0.02$). However, in the control subjects, there were no associations of baseline levels of lipids with baseline serum levels of the RANKL-OPG axis ($p > 0.3$; data not shown).

Serum levels of lipids were also associated with changes over time in serum levels of RANKL and RANKL/OPG serum ratio in HIV-infected subjects

We then investigated whether the observed associations of baseline serum levels of lipids with changes in serum levels RANKL, OPG, and their serum ratio were also observed over time. Statistically significant increases in RANKL and RANKL/OPG serum ratio were observed during the period of follow-up within the HIV-1 combined ($p = 0.04$) (Table 2). We investigated factors that were associated with the yearly rates of change in RANKL and RANKL/OPG (Table 3).

In multivariate analysis including all study subjects, negative yearly rates of change in RANKL (data not shown) and in RANKL/OPG were associated with having HIV-1 (parameter estimate -0.62; 95% CI: -1.13, -0.11; $p = 0.02$) and higher baseline body mass index (BMI) (parameter estimate -0.20, 95% CI: -0.31, -0.09; $p < 0.001$) (Table 3). In univariate analysis in the HIV-1$^+$ subjects, a negative yearly rate of change in RANKL was associated with higher baseline HDL ($p = 0.005$) and higher baseline total cholesterol ($p = 0.04$) and a negative yearly rate of change in RANKL/OPG was associated with higher baseline total cholesterol ($p = 0.001$) and higher baseline LDL ($p = 0.03$). In multivariate analysis in the HIV-1$^+$ subjects, with nadir CD4$^+$ cell count and years of PI use in the model, only higher baseline HDL (parameter estimate -7.51, 95% CI: -13.76, -1.26; $p = 0.02$) remained significantly associated with a negative yearly rate of change in RANKL (Table 3). However, these associations were not found in the control subjects. The different univariate associations of cholesterol with serum RANKL/OPG over time in HIV-1$^+$ versus controls are illustrated in Table 3.

The serum levels of the RANKL, OPG, and the RANKL/OPG serum ratio were not associated with changes in CIMT over time in HIV-infected subjects

We then examined the possible relationship of serum levels of RANKL, OPG, and their serum ratio with progression of atherosclerosis in HIV-1$^+$ subjects considering the previously described interplay between the RANKL-OPG axis and atherosclerosis in HIV-1-uninfected subjects. In a separate analysis of this study, we found no significant differences in baseline CIMT between the combined HIV-1$^+$ and control groups ($p = 0.81$).[14] Baseline serum RANKL and RANKL/OPG were

not associated with baseline CIMT, yearly rate of change in CIMT, and progression of CIMT ($p > 0.3$) (Table 4). Although baseline serum OPG was associated with progression of CIMT ($p = 0.04$) in univariate analysis in all subjects, this association was not found in the HIV-1$^+$ subjects ($p = 0.19$).

Discussion

In this analysis of stored serum samples from a prospective 3-year study, we found that HIV-1-infected subjects on ART had significantly lower levels of RANKL and RANKL/OPG ratio, but similar OPG levels, compared to control subjects. In HIV-1-infected subjects, cholesterol was significantly associated with baseline serum levels and/or changes over time in the RANKL-OPG axis. These findings underscore the complicated interactions between ART, HIV-1 infection, and the RANKL-OPG axis.

Although the RANKL-OPG axis has not been studied extensively in HIV-1 infection, *in vitro*[15,16] and *in vivo* studies[17] suggest that HIV-1 infection has numerous effects on the RANKL-OPG axis. *In vitro* studies suggest that HIV-1 infection per se,[16] immune dysregulation,[19] and changes in blood levels of TNF-related apoptosis-inducing ligand (TRAIL), a basic member of the TNF superfamily,[20] during HIV-1 infection as well as ART[18] can affect the RANKL-OPG axis. However, most of these studies offer conflicting results as to whether HIV-1 infection is associated with a higher or lower RANKL/OPG ratio and do not consider the potential involvement of changes in cytokine levels that occur during HIV-1 infection *in vivo*.

Limited data from *in vivo* studies on levels of circulating RANKL[21–23] and OPG [22, 23] in untreated and treated HIV-1 infection are conflicting. A recent study found that serum RANKL was lower in HIV-infected individuals than controls and was negatively associated with the number of coronary segments with plaque and Agatston coronary artery calcium score in HIV-1-infected individuals even after adjusting for traditional cardiovascular risk factors.[24] Similar to these findings and contrary to the increased RANKL/OPG ratio found in other systemic inflammatory conditions,[25] we found that HIV-1-infected subjects on either NNRTI- or PI-based ART had significantly lower levels of total serum RANKL and RANKL/OPG ratio compared to control subjects at both baseline and after 2–3 years of follow-up. However, few studies have previously determined the RANKL/OPG ratio in HIV-1 infection and suggested that HIV-1 infection[26] and ART[21] increased[22] or did not affect the RANKL/OPG ratio. Without longitudinal data on patients initiating different ART regimens, it is not possible to define the role of ART on RANKL levels. Differences in the assays used,[27] the potency of OPG to neutralize RANKL,[28] the metabolic activity of different tissues that express the majority of RANKL and OPG,[28] the state of immune activation,[22] and the use of different antiretrovirals[29] between different groups of HIV-1-infected subjects could all explain discrepancies between different studies.

The interplay between T lymphocytes, lipids, and bone may also explain the lower circulating levels of RANKL in HIV-1-infected compared to HIV-uninfected subjects. Increased RANKL expression in T cells and reduced OPG expression in T cells have previously been shown to be associated with reduced bone density and hyperlipidemia.[9] HIV-1 infection is associated with T cell lymphopenia and reduced bone density.[30] Thus, it is possible that reduced systemic levels of RANKL in HIV-1-infected subjects may reflect reduced T cell-derived RANKL. Moreover, the nadir CD4$^+$ T cell count, a marker of HIV-1 disease severity, was associated with the yearly rate of change in RANKL and RANKL/OPG, and this is consistent with previous *in vitro* studies that have shown that HIV-1 can induce changes in the production of RANKL and RANKL/OPG in CD4$^+$ T cells[19] and recent *in vivo* studies that serum RANKL was positively associated with CD4$^+$ counts in HIV-1 infection and that patients with lower CD4$^+$ T lymphocyte counts had lower serum RANKL levels.[24] Further studies are needed to investigate the contribution of T cell dysfunction to RANKL production in HIV-1-infected patients.

Another explanation for our unexpected findings that HIV-1-infected patients had lower serum levels compared to the controls may be that increased RANKL tissue expression in HIV-1 infection may lead to a negative feedback loop and lower circulating levels of RANKL. Indeed, serum levels of RANKL may be very different from local tissue expression and activity.[24] RANKL mRNA levels have been found to be higher in human atherosclerotic plaques than in normal vessels,[19] and an inverse relationship between serum RANKL levels and measures of coronary artery disease has been reported.[31,32] It may be possible that with increased local RANKL activity there is less release of cell surface RANKL to soluble RANKL.[24] Consistent with this hypothesis, circulating RANKL levels were inversely associated with local tissue RANKL mRNA levels.[33] In HIV-1 infection, *in vitro* data suggest that RANKL activity is increased at the tissue level whether via HIV-1 infection itself [34] or secondary to medication effects.[34] This raises an intriguing possibility that these factors increase local RANKL activity in HIV-1-infected patients that may be associated with lower circulating RANKL levels. Our findings highlight the need for further studies to evaluate local RANKL activity within the tissues of HIV-1-infected individuals.

Despite the aforementioned effects of ART on the RANKL/OPG ratio, we did not find a significant effect of PI therapy on the RANKL-OPG system in our small study. A larger study found that PI use is associated with lower serum RANKL levels in HIV-1-infected patients.[24] Data from *in vitro* experiments suggest that only specific PIs are involved in the perturbation of the RANKL-OPG system and different doses of the same PI may have different effects on the RANKL-OPG pathway.[18] Thus, it is possible that the PI drugs used in our cohort have less impact on the RANKL-OPG axis compared to other PI drugs, such as ritonavir,[35] which was used in only 30% of the subjects in our PI group. Our study was not designed to detect changes within the PI class; however, this issue will be important to examine in future studies.

We also found that lower baseline serum OPG was significantly associated with higher baseline total cholesterol. Our findings are consistent with previous studies that indicate that the molecular mechanisms of osteoclastogenesis and RANKL signaling are highly dependent on cholesterol[36,37] and that cholesterol levels can be a significant predictor for serum RANKL/OPG level.[38] Higher levels of oxidized LDL may increase serum levels of RANKL.[8,9] However, in our study we did not find significant associations of the circulating levels of oxidized fractions of cholesterol (LDL and non-HDL) with serum levels of RANKL and OPG. The RANKL-OPG-LDL

Table 4. Univariate Associations of RANKL or Osteoprotegerin with Carotid Artery Intima-Media Thickness

	All subjects		*HIV-1$^+$ subjects*		*Control subjects*	
Covariate	*Parameter estimate (95% CI)*	p-*value*	*Parameter estimate (95% CI)*	p-*value*	*Parameter estimate (95% CI)*	p-*value*
Baseline CIMT (μm)						
Baseline RANKL (per 1,000 pg/ml)	−0.14 (−0.47, 0.19)	0.40	−0.17 (−1.03, 0.69)	0.69	−0.07 (−0.46, 0.31)	0.70
Baseline OPG (per 100 pg/ml)	0.29 (−2.09, 2.68)	0.81	0.35 (−3.47, 4.18)	0.85	0.42 (−2.68, 3.52)	0.78
Baseline RANKL/OPG (per 10 units)	−1.45 (−4.64, 1.73)	0.36	−1.90 (−11.50, 7.69)	0.68	−0.58 (−4.21, 3.04)	0.75
Yearly rate of change in CIMT (per 1 μm/year)						
Baseline RANKL (per 1,000 pg/ml)	−0.01 (−0.06, 0.03)	0.61	0.01 (−0.09, 0.11)	0.86	−0.03 (−0.08, 0.03)	0.34
Baseline OPG (per 100 pg/ml)	0.06 (−0.26, 0.38)	0.72	0.31 (−0.17, 0.79)	0.20	−0.29 (−0.71, 0.12)	0.16
Baseline RANKL/OPG (per 10 units)	−0.03 (−0.49, 0.42)	0.89	−0.05(−1.17, 1.08)	0.94	−0.02 (−0.51, 0.47)	0.93
Progression of CIMT						
	Odds ratio estimate (95% CI)	p-*value*	*Odds ratio estimate (95% CI)*	p-*value*	*Odds ratio estimate (95% CI)*	*p-value*
Baseline RANKL (per 1,000 pg/ml)	1.00 (0.99, 1.01)	0.84	1.01 (0.99, 1.02)	0.63	0.99 (0.98, 1.01)	0.38
Baseline OPG (per 100 pg/ml)	1.17 (1.01, 1.35)	0.04	1.01 (0.97, 1.14)	0.19	0.98 (0.89, 1.07)	0.64
Baseline RANKL/OPG (per 10 units)	0.98 (0.90, 1.08)	0.70	0.99 (0.81, 1.22)	0.96	0.95 (0.84, 1.06)	0.35

OPG, osteoprotegerin; CIMT, carotid artery intima-media thickness.

cholesterol interplay may be more evident in those patients with a higher underlying cardiovascular risk[1,2] and our small study may be underpowered to demonstrate these associations in subjects who were otherwise at low risk for atherosclerosis.

Moreover, we found that lower HDL cholesterol was independently associated with positive changes over time in serum levels of RANKL in HIV-1-infected subjects. Lower HDL levels are inversely associated with higher levels of oxidized LDL and increased HDL redox activity in HIV-1-uninfected subjects.[39,40] HIV-1-infected patients have lower HDL levels[41] and HDL with higher redox activity[39,42] compared to HIV-uninfected patients. Previous *in vitro* studies have shown that oxidized lipoproteins can directly upregulate production of RANKL from T cells through scavenger receptors[8,9] and that oxidized HDL can also upregulate the NF-κB pathway.[43] Consistent with these findings in a separate analysis of the current cohort, we have recently shown that increased HDL redox activity was independently associated with higher serum levels of RANKL/OPG in HIV-1-infected subjects (but not in HIV-uninfected subjects).[44] Thus, both total levels of cholesterol as well as oxidized lipoproteins/cholesterol may be associated with regulation of the RANKL-OPG pathway in HIV-1-infected patients and further mechanistic studies need to elucidate the interplay between lipids and the RANKL-OPG pathway in HIV-1 infection.

Although the RANKL-OPG system has been suggested to play an important role in cardiovascular disease in HIV-1-uninfected subjects,[2] there are limited data regarding this role in HIV-1-infected subjects.[17] Unlike baseline RANKL and RANKL/OPG, higher baseline OPG was associated with progression of CIMT in univariate analysis, similar to other studies.[2,45] However, this association was not found in the HIV-1-infected subjects.

Several factors could explain the lack of association of the RANKL-OPG axis with progression of atherosclerosis. Our study focused on determining the isolated effect of HIV-1 infection and PI therapy on progression of atherosclerosis and the subjects enrolled in our study had a low cardiovascular risk profile. The risk for progression of atherosclerosis associated with the RANKL-OPG axis may be more evident in those patients with a higher underlying cardiovascular risk[1,2] and our small study may be underpowered to demonstrate this risk in subjects who were otherwise at low risk for atherosclerosis. Moreover, the endpoints used for progression of atherosclerosis vary across different studies[11] and different factors may influence different measures of atherosclerosis, such as the presence of plaque, coronary calcification, or carotid IMT.

Despite the above limitations, our study provides some of the first information on the RANKL-OPG axis in HIV-1-infected subjects in the setting of a prospective study that includes measures of progression of atherosclerosis. We found that serum levels of the RANKL and OPG in HIV-1-infected subjects on ART are perturbed compared to HIV-uninfected individuals, and that cholesterol levels correlate significantly with the RANKL-OPG axis in HIV-1 infection. It is clear that there are complex interplays between HIV-1 infection, ART, inflammation, immune system, cardiovascular disease, and the RANKL-OPG axis. Further studies need to better define the role of the RANKL-OPG axis in HIV-1 infection and measure both blood and tissue levels of RANKL and OPG.

Acknowledgments

The authors wish to thank the patients for their participation in this study. This work was supported by the National Institute of Allergy and Infectious Diseases (AI068634 and AI056933) and UCLA AIDS Institute and the UCLA Center for AIDS Research (AI28697). Partial funding for laboratory work was provided by the University of Washington's CVD and Metabolic Complications of HIV/AIDS Data Coordinating Center (5R01HL095126).

Author Disclosure Statement

No competing financial interests exist.

References

1. D'Amelio P, Isaia G, and Isaia GC: The osteoprotegerin/RANK/RANKL system: A bone key to vascular disease. J Endocrinol Invest 2009;32:6–9.
2. Venuraju SM, Yerramasu A, Corder R, and Lahiri A: Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. J Am Coll Cardiol 2010;55:2049–2061.
3. Haynes DR, Barg E, Crotti TN, *et al.:* Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology (Oxford) 2003;42:123–134.
4. Kleemann R, Zadelaar S, and Kooistra T: Cytokines and atherosclerosis: A comprehensive review of studies in mice. Cardiovasc Res 2008;79:360–376.
5. Mohammadpour AH, Shamsara J, Nazemi S, Ghadirzadeh S, Shahsavand S, and Ramezani M: Evaluation of RANKL/OPG serum concentration ratio as a new biomarker for coronary artery calcification: A pilot study. Thrombosis 2012;2012:306263.
6. Parhami F, Garfinkel A, and Demer LL: Role of lipids in osteoporosis. Arterioscler Thromb Vasc Biol 2000;20:2346–2348.
7. Nabel EG: Cardiovascular disease. N Engl J Med 2003;349:60–72.
8. Graham LS, Parhami F, Tintut Y, Kitchen CM, Demer LL, and Effros RB: Oxidized lipids enhance RANKL production by T lymphocytes: Implications for lipid-induced bone loss. Clin Immunol 2009;133:265–275.
9. Graham LS, Tintut Y, Parhami F, *et al.:* Bone density and hyperlipidemia: The T-lymphocyte connection. J Bone Miner Res 2010;25:2460–2469.
10. Currier JS: Update on cardiovascular complications in HIV infection. Top HIV Med 2009;17:98–103.
11. Currier JS, Kendall MA, Henry WK, *et al.:* Progression of carotid artery intima-media thickening in HIV-infected and uninfected adults. AIDS 2007;21:1137–1145.
12. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–2497.
13. Lehmann E: *Nonparametrics: Statistical Methods based on Ranks*. Holden-Day, San Francisco, 1975.
14. Kelesidis T, Kendall MA, Yang OO, Hodis HN, and Currier JS: Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. J Infect Dis 2012;206:1558–1567.

15. Fakruddin JM and Laurence J: Interactions among human immunodeficiency virus (HIV)-1, interferon-gamma and receptor of activated NF-kappa B ligand (RANKL): Implications for HIV pathogenesis. Clin Exp Immunol 2004;137: 538–545.
16. Fakruddin JM and Laurence J: HIV-1 Vpr enhances production of receptor of activated NF-kappaB ligand (RANKL) via potentiation of glucocorticoid receptor activity. Arch Virol 2005;150:67–78.
17. Jang JJ, Schwarcz AI, Amaez DA, *et al.:* Elevated osteoprotegerin is associated with abnormal ankle brachial indices in patients infected with HIV: A cross-sectional study. J Int AIDS Soc 2010;13:12.
18. Gibellini D, Borderi M, de CE, *et al.:* Analysis of the effects of specific protease inhibitors on OPG/RANKL regulation in an osteoblast-like cell line. New Microbiol 2010;33:109–115.
19. Fakruddin JM and Laurence J: HIV envelope gp120-mediated regulation of osteoclastogenesis via receptor activator of nuclear factor kappa B ligand (RANKL) secretion and its modulation by certain HIV protease inhibitors through interferon-gamma/RANKL cross-talk. J Biol Chem 2003;278: 48251–48258.
20. Gibellini D, Re MC, Ponti C, *et al.:* HIV-1 Tat protein concomitantly down-regulates apical caspase-10 and up-regulates c-FLIP in lymphoid T cells: A potential molecular mechanism to escape TRAIL cytotoxicity. J Cell Physiol 2005;203:547–556.
21. Brown TT, Ross AC, Storer N, Labbato D, and McComsey GA: Bone turnover, osteoprotegerin/RANKL and inflammation with antiretroviral initiation: Tenofovir versus non-tenofovir regimens. Antivir Ther 2011;16:1063–1072.
22. Mora S, Zamproni I, Cafarelli L, *et al.:* Alterations in circulating osteoimmune factors may be responsible for high bone resorption rate in HIV-infected children and adolescents. AIDS 2007;21:1129–1135.
23. Gibellini D, Borderi M, de CE, *et al.:* RANKL/OPG/TRAIL plasma levels and bone mass loss evaluation in antiretroviral naive HIV-1-positive men. J Med Virol 2007;79: 1446–1454.
24. Hwang JJ, Wei J, Abbara S, Grinspoon S, and Lo J: Receptor activator of nuclear factor kappa B ligand (RANKL) and its relationship to coronary atherosclerosis in HIV patients. J Acquir Immune Defic Syndr 2012;61(3):359–363.
25. D'Amelio P, Isaia G, and Isaia GC: The osteoprotegerin/RANK/RANKL system: A bone key to vascular disease. J Endocrinol Invest 2009;32:6–9.
26. Seminari E, Castagna A, Soldarini A, *et al.:* Osteoprotegerin and bone turnover markers in heavily pretreated HIV-infected patients. HIV Med 2005;6:145–150.
27. Chan BY, Buckley KA, Durham BH, Gallagher JA, and Fraser WD: Effect of anticoagulants and storage temperature on the stability of receptor activator for nuclear factor-kappa B ligand and osteoprotegerin in plasma and serum. Clin Chem 2003;49:2083–2085.
28. Wagner D and Fahrleitner-Pammer A: Levels of osteoprotegerin (OPG) and receptor activator for nuclear factor kappa B ligand (RANKL) in serum: Are they of any help? Wien Med Wochenschr 2010;160:452–457.
29. Ueland T, Bollerslev J, Godang K, Muller F, Froland SS, and Aukrust P: Increased serum osteoprotegerin in disorders characterized by persistent immune activation or glucocorticoid excess—possible role in bone homeostasis. Eur J Endocrinol 2001;145:685–690.
30. Currier JS and Havlir DV: Complications of HIV disease and antiretroviral therapy. Top HIV Med 2009;17:57–67.
31. Schoppet M, Schaefer JR, and Hofbauer LC: Low serum levels of soluble RANK ligand are associated with the presence of coronary artery disease in men. Circulation 2003;107:e76.
32. Crisafulli A, Micari A, Altavilla D, *et al.:* Serum levels of osteoprotegerin and RANKL in patients with ST elevation acute myocardial infarction. Clin Sci (Lond) 2005;109:389–395.
33. Findlay D, Chehade M, Tsangari H, *et al.:* Circulating RANKL is inversely related to RANKL mRNA levels in bone in osteoarthritic males. Arthritis Res Ther 2008;10:R2.
34. Fakruddin JM and Laurence J: HIV envelope gp120-mediated regulation of osteoclastogenesis via receptor activator of nuclear factor kappa B ligand (RANKL) secretion and its modulation by certain HIV protease inhibitors through interferon-gamma/RANKL cross-talk. J Biol Chem 2003;278: 48251–48258.
35. Yin MT, Modarresi R, Shane E, *et al.:* Effects of HIV infection and antiretroviral therapy with ritonavir on induction of osteoclast-like cells in postmenopausal women. Osteoporos Int 2011;22:1459–1468.
36. Luegmayr E, Glantschnig H, Wesolowski GA, *et al.:* Osteoclast formation, survival and morphology are highly dependent on exogenous cholesterol/lipoproteins. Cell Death Differ 2004;11:S108–S118.
37. Hada N, Okayasu M, Ito J, *et al.:* Receptor activator of NF-kappaB ligand-dependent expression of caveolin-1 in osteoclast precursors, and high dependency of osteoclastogenesis on exogenous lipoprotein. Bone 2012;50:226–236.
38. Oh ES, Rhee EJ, Oh KW, *et al.:* Circulating osteoprotegerin levels are associated with age, waist-to-hip ratio, serum total cholesterol, and low-density lipoprotein cholesterol levels in healthy Korean women. Metabolism 2005;54:49–54.
39. Kelesidis T, Currier JS, Huynh D, *et al.:* A biochemical fluorometric method for assessing the oxidative properties of HDL. J Lipid Res 2011;52:2341–2351.
40. Patel S, Drew BG, Nakhla S, *et al.:* Reconstituted high-density lipoprotein increases plasma high-density lipoprotein anti-inflammatory properties and cholesterol efflux capacity in patients with type 2 diabetes. J Am Coll Cardiol 2009; 53:962–971.
41. Currier JS: Update on cardiovascular complications in HIV infection. Top HIV Med 2009;17:98–103.
42. Kelesidis T, Reddy ST, Huynh D, *et al.:* Effects of lipid-probe interactions in biochemical fluorometric methods that assess HDL redox activity. Lipids Health Dis 2012;11:87.
43. Zhang M, Gao X, Wu J, *et al.:* Oxidized high-density lipoprotein enhances inflammatory activity in rat mesangial cells. Diabetes Metab Res Rev 2010;26:455–463.
44. The interplay of the osteoprotegerin/RANKL axis and dysfunctional HDL in HIV-positive adults: ACTG NWCS 332/A5078 study. Presented at the XIX International AIDS Conference 2012, Washington, DC, June 2012 [Abstract THAB0201].
45. Van CA and Golledge J: Osteoprotegerin, vascular calcification and atherosclerosis. Atherosclerosis 2009;204(2): 321–329.

Address correspondence to:
Judith S. Currier
Center for Clinical AIDS Research and Education
David Geffen School of Medicine, UCLA
9911 West Pico, Suite 980
Los Angeles, California 90035

E-mail: jscurrier@mednet.ucla.edu