T-cell receptor activator of nuclear factor-kappa B ligand/osteoprotegerin imbalance is associated with HIV-induced bone loss in patients with higher CD4(+) T-cell counts

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T cell RANKL/OPG imbalance is associated with HIV-induced bone loss in patients with higher CD4+ T cell counts

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Abstract

Objective—Higher incidence of osteopenia and osteoporosis underlie increased rates of fragility fracture in HIV infection. B cells are a major source of Osteoprotegerin (OPG), an inhibitor of the key osteoclastogenic cytokine Receptor Activator of Nuclear Factor-κB Ligand (RANKL). We previously showed that higher B cell RANKL/OPG ratio contributes to HIV-induced bone loss. T cell OPG production in humans however remains undefined and the contribution of T cell OPG and RANKL to HIV-induced bone loss has not been explored.

Design—We investigated T cell OPG and RANKL production in ART-naïve HIV infected and uninfected individuals in relation to indices of bone loss in a cross-sectional study.

Methods—T cell RANKL and OPG production was determined by intracellular staining and flow cytometry, and plasma levels of bone resorption markers were determined by ELISA.

Results—We demonstrate for the first time *in vivo* human T cell OPG production, which was significantly lower in HIV-infected individuals and was coupled with moderately higher T cell RANKL production, resulting in a significantly higher T cell RANKL/OPG ratio. T cell RANKL/OPG ratio correlated significantly with BMD-derived Z-scores at the hip, lumbar spine and femur neck in HIV-infected individuals with CD4+ T cell counts ≥ 200 cells/μl but not in those with lower counts.

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Conclusions—Our data suggest that T cells may be a physiologically relevant source of OPG and T cell RANKL/OPG imbalance is associated with HIV-induced bone loss in CD4^{+} T cell-sufficient patients. Both B and T lymphocytes may thus contribute to HIV-induced bone loss.

Keywords

HIV; bone loss; OPG; RANKL; T cells; HIV-induced bone loss

Introduction

HIV infection is associated with significantly higher rates of osteopenia and osteoporosis which result in higher bone fracture rates in the HIV/AIDS population relative to the general population [1–3]. Bone loss is paradoxically exacerbated by antiretroviral therapy (ART) and with treated HIV-infected individuals living longer, there is heightened concern about the possibility of HIV/ART-associated bone loss compounding the effect of natural age-related bone loss. A better understanding of the mechanisms underlying HIV-induced bone loss would inform prevention and treatment measures to avert an epidemic of fragility fractures in this aging population.

Osteoporosis occurs when the rate of bone resorption by osteoclasts exceeds that of bone formation by osteoblasts. Osteoclast formation (osteoclastogenesis) is primarily regulated by the ratio of the key osteoclastogenic cytokine Receptor Activator of Nuclear factor-κB Ligand (RANKL), to that of its physiological inhibitor, osteoprotegerin (OPG).The immune system plays an important multifaceted role in bone turnover, with the RANKL/OPG pathway as a pivotal factor[4]; activated B and T cells are important sources of RANKL[5–7]and B cells are an important source of basal OPG that is regulated in part through costimulation by T cells[8, 9].We previously reported that B cell OPG production is significantly suppressed while RANKL production is significantly upregulated in both an animal model of HIV and in human HIV infection[5, 10].Furthermore, higher B cell RANKL/OPG ratio inversely correlated with BMD in the femoral neck and hip of ART-naïve HIV-infected people, suggesting a key role for B cells in HIV-induced bone loss[10].

Activated T cells and/or B cells secrete RANKL in several inflammatory conditions including rheumatoid arthritis (RA)[11], postmenopausal osteoporosis[12] and periodontal bone loss [7, 13], and peripheral T cell activation was associated with impaired BMD in HIV infection [14]. By contrast, T cells are not generally considered to be a source of OPG. In a previous study, human primary CD4^{+} T cells were stimulated into producing OPG *in vitro* in response to T cell receptor engagement using an anti-CD3 antibody in the presence of IL-4. Interestingly, addition of HIV gp120 envelope glycoprotein to the cultures decreased T cell OPG production[15] suggesting the same could happen in HIV-infected individuals. Direct T cell production of OPG in healthy and HIV-infected individuals and the physiological relevance of T cell OPG production *in vivo* has not been previously described and neither has a role for T cells in HIV-induced bone loss.

To gain further insight into whether T cells are a relevant source of OPG and RANKL in humans, particularly in HIV infection, and whether HIV infection is associated to T cell RANKL/OPG imbalance and therefore enhanced bone loss, we studied T cell OPG and

RANKL production in uninfected and ART-naïve HIV-infected individuals. Given that CD4^{+} T cell impairment is a hallmark of progressive HIV infection [16–18] and CD4^{+} T cell-expressed RANKL in particular was shown to play an important role in bone and joint destruction in RA [11], we hypothesized that HIV infection-associated CD4^{+}impairment could further adversely affect bone by altering RANKL/OPG balance. We demonstrate, for the first time, T cell production of OPG in healthy and HIV-infected individuals. We also show that as with B cells, HIV infection was associated with lower T cell OPG expression concurrent with higher T cell RANKL expression, resulting in T cell RANKL/OPG imbalance. This imbalance was significantly associated with T cell activation, and correlated significantly with quantitative measures of BMD in various fracture-prone anatomical sites in moderately but not severely CD4^{+} T lymphopenic HIV-infected patients. Our data suggest that T cells do indeed produce OPG *in vivo*, and that T cells may be a physiologically relevant source of OPG. We also show that similar to B cells, T cell RANKL/OPG imbalance contributes to HIV-induced bone loss, paradoxically in CD4^{+} T cell-sufficient patients.

Materials and methods

Study Population

This was a sub-analysis of a cross-sectional study of 62 ART-naïve HIV-infected and 58 HIV-negative (referred family and friends of the HIV-infected group) male and female volunteers recruited from an urban outpatient clinic (the Grady Infectious Diseases Program, Atlanta, Georgia, USA) between November 2010 and December 2011[10]. Clinical, laboratory and demographic data including smoking and medication history, and familial history of fracture were collected at enrollment and subjects ages <30 and >50 years were excluded to eliminate the confounding effects of age-related bone remodeling and turnover (Table S1). All individuals provided written informed consent before undergoing any study procedures, and the study was designed according to the World Health Organization (WHO) ethical guidelines for human studies and approved by the Emory University Institutional Review Board (IRB). At enrollment, demographic information, detailed medication history, clinical and laboratory data, including complete blood counts with platelets and differential and full chemistry profiles, were collected (Table S1).

Study Procedures and clinical assays

Negative HIV sero-status was determined in healthy volunteers by rapid enzyme-linked immunosorbent assay (ELISA) after pre-test counseling. In HIV-seropositive individuals, documentation of infection by ELISA, western blot and/or plasma HIV RNA was recorded. Baseline plasma HIV RNA was measured with the Abbott Real Time HIV PCR assay (Abbott Molecular, Des Plaines, IL, USA) and CD4^{+} T cell counts with percentage were obtained for the HIV-seropositive group.

Bone densitometry by Dual energy x-ray absorptiometry (DXA)

BMD was assessed using the same DXA machine (Lunar prodigy scanner [GE Lunar, Madison, WI.]) and the same software (Encore Software, v.2010 13.31) for all participants at Emory University Hospital. Rates of osteoporosis and osteopenia were assessed by

comparing the subject's BMD to that of a reference database reflecting peak BMD values, adjusted for gender and ethnicity. We further computed Z-scores, comparing subject BMD to an age matched reference database adjusted for gender and ethnicity (Table S2).

Cell and plasma collection and storage

Peripheral blood mononuclear cells (PBMC) and plasma were isolated from cell processing tubes (CPT, BD Vacutainer) after centrifugation. Plasma was stored at 80°C and PBMCs were stored in liquid N2 (181°C) until use. Lymphocytes from HIV-negative and HIV-infected individuals were found to be between 95%–97% and 90%–93% viable respectively, upon thawing. After 18 hr at 37°C, viability of PBMC from HIV-negative and infected individuals declined to 93% and 78% respectively. Dead cells were excluded from all flow cytometry analyses by gating.

Flow Cytometry

The following anti-human monoclonal antibodies (clones) from eBiosciences, San Diego CA, were used for surface staining of PBMCs for total T cells, subsets and activation: CD3 (HIT3a), CD4 (SK3), CD8 (SK1) and CD69 (FN50) and Ki-67 (20Raj1) was used for intracellular staining to assess turnover. Intracellular expression of OPG and RANKL in T cells, was performed as previously described [10]. Briefly, cells were first stained for surface markers and then fixed and permeabilized using the BD Cytofix/Cytoperm Fixation/permeabliziation kit (BD Biosciences). To quantify intracellular OPG, cells were incubated with 5 μg/ml of anti-human OPG-biotin (Leinco Technologies) for 30 minutes at 4°C,followed by incubation with Streptavidin-PE. RANKL was quantified by incubation with 5 μg/ml recombinant OPG-Fc (R&D systems) for 30 minutes at 4°C as previously described [12], followed by anti-human IgG Fc-PE (BD Biosciences) for another 30 minutes at 4°C. All cells were fixed in 4% paraformaldehyde, data acquired on an Accuri flow cytometer (BD Immunocytometry Systems, San Jose, CA) and analyzed using FlowJo software version 9.7 (Ashland, OR).

ELISA for bone turnover markers

Commercial ELISAs were used to measure plasma CTx and osteocalcin (Immunodiagnostic Systems, Scottsdale, AZ), and OPG and total soluble RANKL (Alpco Diagnostics, Salem, NH) according to the manufacturers' instructions.

Statistical Analyses

Prior to the statistical analyses, normal distribution and homogeneity of the variances were tested. To analyze the differences in subset distribution between HIV-negative and HIV-infected groups, simple comparisons were done using Student's *t* test (for parametric data) or Wilcoxon rank sum test (for non-parametric data) for each of the subsets. Demographic and clinical characteristics were compared between the HIV- and HIV+ groups with the two-sample t-test for continuous variables and with a Chi-square test for proportions (Table S1).

We quantified the relationships between T cell RANKL/OPG ratio and CD69 and Ki-67 expression of CD4$^+$ and CD8$^+$ T cells, and with BMD (density, T-score and Z-score) from hip, femur neck and lumbar spine as outcomes and T cell RANKL/OPG ratio as the

predictor. Left/right BMD measurements from hip and femur neck were averaged. We quantified the relationships between outcome and predictor variables with a non-parametric (Spearman's rank correlation) univariable method.

Because bone density can be impacted by multiple factors, we performed a covariate-adjusted analysis including HIV status, enrollment age, gender, and BMI, as potential predictors of T cell OPG, RANKL and RANKL/OPG ratio in multiple linear regression (SAS PROC GLM; Tables 1 and S3). The multivariable results are summarized with adjusted means and mean differences, and 95% confidence intervals (CIs). Analyses were performed using GraphPad Prism for Mac OS X software (La Jolla, CA) and SAS software (Cary, NC).All statistical tests were 2-sided and P-values   0.05 were considered statistically significant.

Results

Demographic and clinical data

The demographics and clinical characteristics of the study population have been described previously [10]. Briefly, the uninfected and HIV-infected groups were comparable with regards to race, age, and other osteoporosis risk factors. However, 69% percent of the HIV-infected group were men as compared with 48% of the HIV-negative group (P = 0.02) reflecting the demographics of the HIV/AIDS epidemic in the U.S. Furthermore, body mass index (BMI) was significantly lower in the HIV-infected group (P = 0.0003) as expected. As previously reported[10, 19], we also determined the levels of the bone turnover markers C-terminal Telopeptide of Collagen Type I (CTx), a marker of bone resorption, and osteocalcin (OCN) a marker of bone formation and found a significantly elevated rate of bone resorption (CTx) and a diminished rate of bone formation (OCN), which showed a trend towards statistical significance, in HIV-infected subjects (Data not shown).

HIV infection is associated with lower T cell OPG and higher T cell RANKL production

In vitro T cell OPG production was demonstrated in a previous study only after extensive manipulation and activation [15]but spontaneous T cell OPG production by human primary T cells has not been demonstrated. Using multicolor flow cytometry, we quantified the intracellular expression of OPG and RANKL in total T cells (CD3$^+$) from uninfected (n = 58) and HIV-infected (n = 62) individuals as an assessment of their ability to produce these cytokines. We were able to detect both OPG and RANKL production in T cells form healthy uninfected and HIV-infected individuals, with significantly fewer OPG$^+$ and more RANKL$^+$ T cells in the HIV-infected patients (Figure 2A), although the difference in RANKL-producing T cells showed a trend towards statistical significance (Figure 2A). These changes in T cell OPG and RANKL production resulted in a 146% (p = 0.003) higher T cell RANKL/OPG ratio (%) in the HIV$^+$ group (median ratio = 0.4800) relative to the uninfected group (median ratio = 0.1950)(Figure 2A).In multivariable analyses (Table 1), after adjusting for known osteoporosis risk factors including age, sex and BMI, T cell OPG and T cell RANKL/OPG ratio did not differ with any other covariates.

Given that HIV infection is characterized by drastic changes in T cell homeostasis, lymphopenia and marked alterations in T cell subsets, we also determined the absolute counts of T cells producing OPG and RANKL. The absolute numbers of T cells expressing OPG remained significantly lower in HIV+ patients compared to uninfected controls whereas the absolute counts of RANKL+ T cells were statistically comparable between both groups. As with T cell percentage ratio, these differences translated into a significantly higher total T cell RANKL/OPG ratio (Figure 2B). Multivariable analyses adjusted for age, sex and BMI confirmed that the difference in numbers of absolute T cells expressing OPG and T cell RANKL/OPG ratio were attributable to HIV infection and not to the other covariates/confounders (Table S3).

T cell subset production of OPG and RANKL

We next set out to determine which of the major T cell subsets are responsible for OPG and RANKL production and whether HIV infection was associated with T cell subset production of OPG and RANKL. We stained samples from a subset of randomly selected samples (10 HIV- and 10 HIV+) forCD4+ and CD8+T cell OPG and RANKL production. OPG production was comparable between CD4+ and CD8+ T cells both in the uninfected controls (84% vs. 78% respectively) and HIV+ individuals (97% vs. 92% respectively), albeit lower in the HIV+ group for both subsets, but this difference only attained statistical significance for the CD8+ T cell subset(p = 0.04)(Figure 2C, left panel). RANKL production was also comparable between CD4+ and CD8+ T cells in uninfected controls (15% vs. 13% respectively)and HIV+ individuals (34% vs. 30% respectively) but overall, both RANKL-producing CD4+ and CD8+T cells were significantly higher in the HIV-infected group(p =0.003 and p = 0.001)respectively (Figure 2C, right panel).

Higher levels of T cell activation and turnover correlate with T cell RANKL and OPG changes in all HIV-infected individuals

We [20] and others [21] have previously reported that RANKL is secreted by activated T cells, but the relationship between T cell activation and OPG/RANKL production in HIV infection is not clear. To assess the role of T cell activation in RANKL and OPG production in HIV infection, we stained T cell subsets for surface expression of CD69, an activation marker rapidly expressed by T cells upon TCR engagement [22], and for intracellular expression of Ki-67, a marker of proliferation and cell turnover[23].Both CD69-expressing (Figure 3A) and Ki-67-expressing (Figure 3B) CD4+ and CD8+ T cells were expanded in HIV-infected individuals. Peripheral T cell activation was previously shown to be associated with impaired BMD in HIV infection[14]and we found that T cell RANKL/OPG ratio was significantly associated with CD69 (r = 0.26, P = 0.05) and Ki-67 expression (r = 0.32, P = 0.04) in CD8+ T cells but not in CD4+ T cells (r = 0.21, P = 0.12 and r = 0.08, P = 0.61 for CD69 and Ki-67 respectively). Multivariable analyses of T cell expression of CD69 and Ki-67 adjusted for HIV status, age, sex and BMI also confirmed that these differences were not due to confounding factors (Table S6).

T cell RANKL/OPG imbalance correlates with BMD-derived Z-scores in moderately lymphopenic but not in severely lymphopenic HIV positive individuals

We previously showed strong associations between B cell RANKL/OPG and hip BMD, T- and Z-scores[10].To assess a similar contribution of the higher T cell RANKL/OPG ratio to the HIV-induced decline in bone mass, we performed correlation analyses between the T cell RANKL/OPG ratio in all HIV-infected individuals and BMD indices at the hip, femur neck and lumbar spine. The T cell RANKL/OPG ratio was not significantly associated with total BMD (or with BMD-derived T- or Z-Scores) at the Hip, Femur neck, or Lumbar spine when all HIV-infected patients were analyzed in aggregate (data not shown).

69% of the HIV-infected patients in our cohort were severely CD4+ lymphocytopenic, with CD4+ T cell counts <200 cells/μl blood, while the remaining 31% were more moderately cytopenic, with CD4+ T cell counts 200 cells/μl blood and corresponding one log lower viral loads in the moderate group(Figure S1). In a bid to explore the possibility that severely lymphopenic HIV-infected individuals would present with more severe bone changes, we stratified the HIV+ patients by CD4 counts into two groups: CD4 count <200 and 200 cells/μl. Both groups were otherwise clinically and demographically comparable (Table S4). There were also no differences in DXA measurements, bone turnover markers (CTx and OCN) and T cell OPG, RANKL and RANKL/OPG ratio (Table S5). Interestingly, we found strong inverse associations between T cell RANKL/OPG and BMD, T- and Z-scores at the hip, lumbar spine and femur neck in the moderately but not in the severely CD4+lymphocytopenic group (Table 2). Despite relatively strong inverse correlations for BMD and T-scores, particularly hip BMD, only the associations with Z-scores (a comparison of subject BMD to uninfected age-matched peers)were statistically significant.

Discussion

Although human B cells are established OPG producers[24]and we reported that murine B-lineage cells account for up to 64% of total OPG in the bone microenvironment, [9],T cells are not generally considered to be a significant source of OPG.A previous report demonstrated that CD4+ but not CD8+T cells could be coaxed into producing OPG *in vitro* when stimulated with anti-CD3 antibody or cultured for 6–9 days with either IL-1β, GM-CSF, TNF-α or Vitamin D3 [15]. By contrast, another study reported that activated CD8+ but not CD4+ T cells, express a substantial amount of OPGin vitro [11, 25].We present here an important extension of these studies and suggest that both CD8+andCD4+T cells have the capacity to make this bone-protective factor in humans, and HIV infection significantly diminishes T cell OPG production. To our knowledge this is the first demonstration of human T cell OPG production in the absence of *in vitro* T cell stimulation and the first report showing that both the proportion (%) and absolute numbers of T cells producing OPG are significantly lower in HIV-infected individuals. We also demonstrate that T cell RANKL/OPG imbalance may contribute to HIV-induced bone loss in patients with moderate but not severe CD4+lymphocytopenia.

Activated T cells secrete RANKL *in vitro*[20, 21] and in a previous study, peripheral T cell activation was associated with impaired BMD in HIV infection [14]. Activated T cells and/or B cells also secrete RANKL in several inflammatory pathological contexts associated with

bone loss, including rheumatoid arthritis (RA)[11], postmenopausal osteoporosis[12] and periodontal infection[7, 13]. T cell activation and turnover, as measured by CD69 and Ki-67 expression, were higher in HIV-infected individuals as expected, and interestingly, T cell RANKL/OPG correlated with the expression of CD69 and Ki-67 on CD8^{+} T cells but not on CD4^{+} T cells. In contrast to the chronic activation markers HLA-DR and CD38 which are upregulated later in the cell cycle[26], surface CD69 expression peaks within 24 hours of activation and declines rapidly if the stimulus is withdrawn, and is thus a good indicator of ongoing activation[22]. Our results thus suggest that actively cycling T cells may play a role in bone loss in HIV infection.

We also found a strong trend towards a larger proportion of RANKL-producing T cells in HIV infection. Importantly, the higher RANKL coupled with a significantly lower OPG resulted in an overall significantly higher T cell RANKL/OPG ratio in the HIV-infected group. As we previously reported for B cell RANKL/OPG imbalance [10],we expected this T cell RANKL/OPG imbalance to favor more bone loss and interestingly, we found strong inverse correlations between T cell RANKL/OPG ratio and BMD as well as BMD-derived T- and Z-scores, although only Z-scores reached statistical significance. Importantly, strong correlations and significant data were only obtained in the moderately, and not in the severely, CD4^{+}lymphocytopenic subset of our HIV-infected cohort, despite comparable BMD measures in both groups. Given that the differences in T cell OPG production are more robust than RANKL differences, T cell OPG may be the main driving driving force behind the observed T cell RANKL/OPG imbalance.

BMD-derived T-score which compares BMD of a subject to that of a young healthy adult at peak BMD, is the index used to make clinical assessments of bone status and indeed osteopenia and osteoporosis are clinical definitions based on specific T-score ranges. However, our data suggest that in young HIV-infected individuals the Z-score, which reflects BMD normalized for chronological age, may be a more appropriate measure of T cell RANKL and OPG effects on bone turnover. The reason is presently unclear but may be related to the age-adjusted normalization of the Z-score compared to the absolute BMD and T-scores. Indeed, OPG production is reported to significantly increase with age in humans[27, 28]and guidelines for the treatment and management of bone disease in HIV infection recommend the use of Z-scores for interpretation of DXA scan data in subjects <50 years of age[29].

Our current data support a significant contribution of T cells to HIV-induced bone loss but only in subjects with relatively higher numbers of CD4^{+}T cells. While all subjects may have a high baseline of bone resorption stemming from other factors such as B cell RANKL/OPG disruption, subjects with relatively higher CD4^{+}T cell numbers may be protected from additional T cell-associated bone loss. However, ART initiation paradoxically worsens bone loss, inducing rates of bone loss as high as 6% in the first year[30–32]. In a recent study using a mouse model of immune reconstitution, we demonstrated that CD4^{+} T cell restoration by ART likely significantly contributes to the pathogenesis of ART-associated bone loss [32]. We have also reported that bone resorption is positively associated with the magnitude of immune reconstitution following ART in humans[33], while others have reported that low baseline CD4^{+}count leads to greater loss of BMD with ART[34]. We[33] and others[34] have

previously reported that patients with the lowest CD4 counts undergo the most extensive bone loss following ART initiation. We speculate that this is a consequence of inflammatory events associated with immune recovery (including CD4^{+} T cell reconstitution) under ART. Indeed, we recently reported that T cell repopulation in immunodeficient mice leads to enhanced bone resorption and loss of BMD due to T cell homeostatic expansion and the revival of adaptive immune responses[32](Weitzmann, M.N., et al. (2017). "Homeostatic expansion of CD4+ T cells promotes cortical and trabecular bone loss while CD8+ T cells induce trabecular bone loss only". *Journal of Infectious Diseases*, In press). Our new data suggest that the most lymphopenic patients, who were spared the brunt of T cell-driven bone loss during HIV infection are the population most predisposed to undergo the most aggressive bone loss in the setting of ART.

In conclusion, our present study evaluates the role of T cells in HIV-induced bone loss, and we demonstrate that human T cells produce OPG *in vivo* and OPG-producing T cells are depleted in HIV infection concurrent with a trend toward higher RANKL-producing T cells. Furthermore, we showed that T cell RANKL/OPG ratio was significantly higher in HIV infection and was associated with diminished Z-scores at multiple fracture-prone anatomic sites in moderately CD4^{+}lymphocytopenic but not in severely CD4^{+}lymphocytopenia HIV-infected individuals. These findings broaden the current understanding of the pathogenesis of HIV-induced bone loss, and establish for the first time that the contribution of T cells to bone homeostasis may be mediated through the expression of both OPG and RANKL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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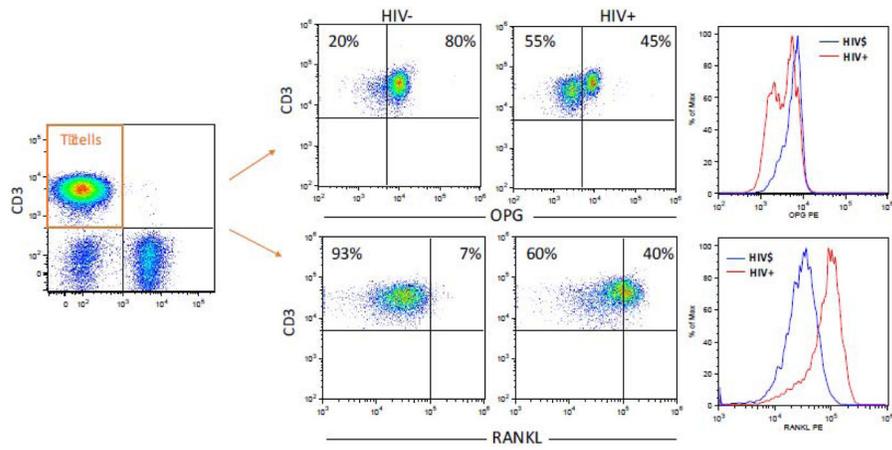
Figure 1. T cell OPG and RANKL Expression-Gating

Gating strategy to determine intracellular expression of OPG and RANKL in T cells (CD3^{+}) in HIV- and HIV+ individuals.
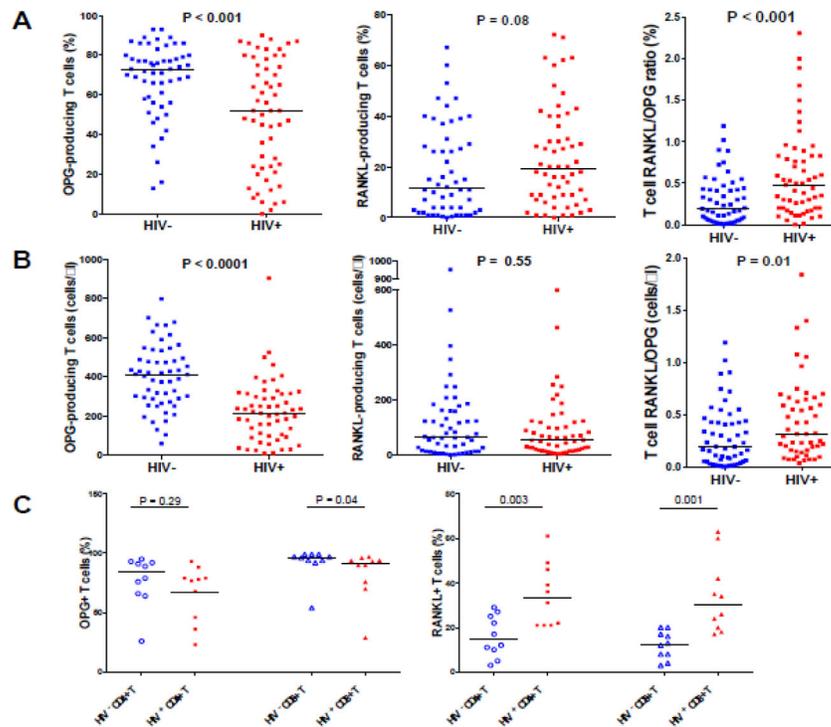
Figure 2. T cell OPG and RANKL expression in uninfected (HIV⁻) and HIV-infected (HIV⁺) Individuals

(**A**) Higher percentage of OPG⁺ T cells and lower percentage of RANKL⁺ T cells results in increased T cell RANKL/OPG ratio (percentage) and(**B**) Lower absolute counts of OPG⁺ T cells and unchanged absolute numbers of RANKL⁺ T cells results in increased T cell RANKL/OPG ratio (absolute counts)in HIV-infected individuals (n = 62) compared to HIV-control subjects (n = 58).(C) **OPG and RANKL production in CD4⁺ and CD8⁺ T cells.** Intracellular OPG and RANKL production in CD4⁺ and CD8⁺ T cells in HIV⁻ (n = 10) and HIV⁺ (n = 10) individuals. HIV serostatus groups were compared using Wilcoxon rank-sum test and horizontal lines represent medians.

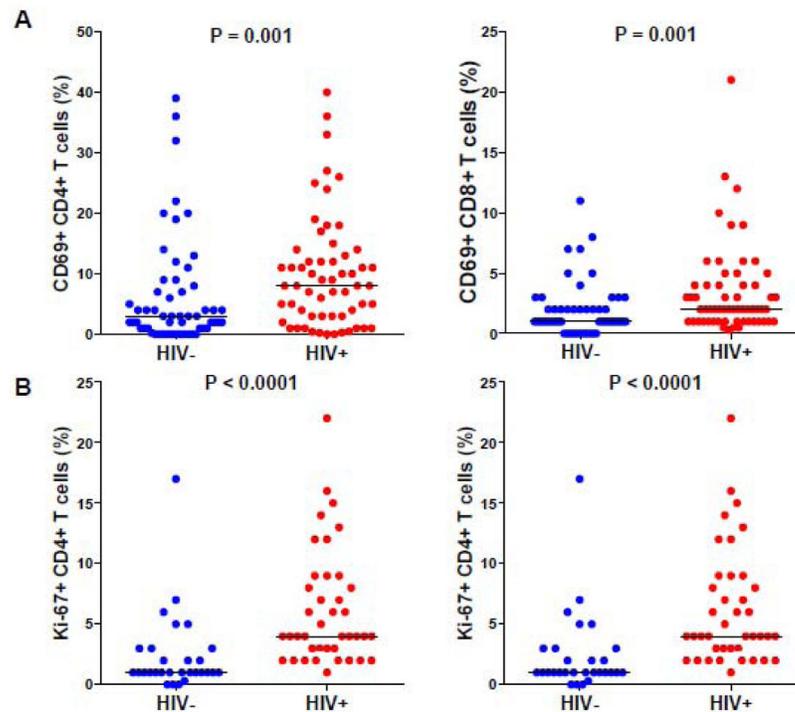**Figure 3. HIV-induced T cell activation and turnover**

Higher T cell (**A**) activation (CD69 expression on CD4^{+} T cells (left panel) and CD8^{+} T cells (right panel)) and (**B**) turnover (Ki-67 expression on CD4^{+} T cells (left panel) and CD8^{+} T cells (right panel)) in uninfected and HIV-infected patients. HIV serostatus groups were compared using Wilcoxon rank-sum test and horizontal lines represent medians.

Table 1

Multivariable analyses of T cell OPG and RANKL expression adjusted for HIV status, age, sex and BMI

Outcome	Predictor		n	Adjusted Mean (95% CI)	P	Mean Difference(95% CI)
T cell OPG (%)	**HIV status**	HIV Negative	57	68.2 [61.8, 74.7]	**<0.001**	20.2 [11.0, 29.5]
		HIV Positive	61	48.0 [41.3, 54.7]		
	Age, years	30–40	62	56.4 [50.1, 62.7]	0.45	
		41–50	56	59.8 [53.2, 66.5]		
	Sex	Female	47	55.5 [48.0, 63.0]	0.31	
		Male	71	60.7 [54.6, 66.9]		
	BMI, kg/m²	<25.0	50	60.4 [53.0, 67.9]	0.55	
		25.0–29.9	31	59.7 [50.9, 68.4]		
		30	37	54.2 [45.9, 62.6]		
T cell RANKL (%)	**HIV status**	HIV Negative	57	19.4 [14.0, 24.7]	0.43	–3.1 [–10.8, 4.6]
		HIV Positive	61	22.5 [16.9, 28.0]		
	Age, years	30–40	62	21.0 [15.8, 26.2]	0.96	
		41–50	56	20.8 [15.2, 26.4]		
	Sex	Female	47	21.1 [14.9, 27.4]	0.92	
		Male	71	20.7 [15.6, 25.8]		
	BMI, kg/m²	<25.0	50	25.9 [19.7, 32.1]	0.18	
		25.0–29.9	31	17.9 [10.6, 25.2]		
		30	37	18.9 [12.0, 25.9]		
T cell RANKL / OPG ratio	**HIV status**	HIV Negative	57	0.29 [0.18, 0.40]	**<0.001**	–0.32 [–0.48,–0.15]
		HIV Positive	61	0.61 [0.49, 0.73]		
	Age, years	30–40	62	0.43 [0.32, 0.54]	0.69	
		41–50	56	0.47 [0.35, 0.58]		
	Sex	Female	47	0.53 [0.40, 0.67]	0.07	
		Male	71	0.37 [0.26, 0.48]		
	BMI, kg/m²	<25.0	50	0.53 [0.40, 0.66]	0.27	
		25.0–29.9	31	0.37 [0.21, 0.52]		
		30	37	0.45 [0.30, 0.60]		

Table 2

Correlations between T cell RANKL/OPG and BMD measures in moderately (CD4^{+} T cell count 200) and severely (CD4^{+} T cell count < 200) CD4^{+}lymphocytopenic (CD4^{+} T cell count 200) HIV positive patients

CD4 count 200	N	Spearman Correlation Coefficient	P
Hip BMD (g/cm³) [*]	16	−0.45	0.08
Hip T-score [*]	16	−0.37	0.16
Hip Z-score [*]	16	−0.59	**0.02**
Femur neck BMD (g/cm³) [*]	17	−0.35	0.17
Femur neck T-score [*]	17	−0.27	0.29
Femur neck Z-score [*]	17	−0.56	**0.02**
Lumbar spine BMD (g/cm³)	17	−0.42	0.10
Lumbar spine T-score	17	−0.38	0.13
Lumbar spine Z-score	17	−0.50	**0.04**

CD4 count <200	N	Spearman Correlation Coefficient	P
Hip BMD (g/cm³) [*]	39	−0.14	0.41
Hip T-score [*]	39	−0.11	0.52
Hip Z-score [*]	39	−0.10	0.53
Femur neck BMD (g/cm³) [*]	40	−0.04	0.82
Femur neck T-score [*]	40	−0.11	0.49
Femur neck Z-score [*]	40	−0.13	0.43
Lumbar spine BMD (g/cm³)	39	0.14	0.38
Lumbar spine T-score	39	0.19	0.26
Lumbar spine Z-score	39	0.27	0.09

[*] Averaged across left and right sides