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Role of RANKL-RANK/Osteoprotegerin Pathway in Cardiovascular and Bone Disease Associated with HIV Infection

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Abstract

Patients with HIV-1 infection often develop multiple complications and comorbidities, including osteoporosis and atherosclerosis. The receptor activator of nuclear factor kappa-B/receptor activator of nuclear factor kappa-B ligand/osteoprotegerin axis has been identified as a possible common link between osteoporosis and vascular diseases. Since the discovery of this axis, much has been learned about its role in controlling skeletal biology and less about its role in the context of vascular biology. However, the exact role of the receptor activator of nuclear factor kappa-B ligand/osteoprotegerin axis in HIV infection is not completely understood. In this review we examine the mechanisms by which inflammation and immune dysregulation in HIV-1 infection may impact bone turnover and atherogenesis through perturbations in the receptor activator of nuclear factor kappa-B/receptor activator of nuclear factor kappa-B ligand/osteoprotegerin axis.

Keywords

Osteoprotegerin; RANK ligand; HIV; Bone; Immunity; Cardiovascular disease

Introduction

As life expectancy for HIV-1-infected patients continues to increase as a result of successful antiretroviral therapy (ART), morbidity and mortality from chronic complications of HIV-1 infection, such as osteoporosis and cardiovascular disease, will continue to rise[1]. However, the mechanisms that contribute to the increased incidence of these complications in HIV-1 infection remain to be elucidated[1].

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Supplementary Data

Supplementary data is available at AIDS Reviews journal online (http://www. aidsreviews.com). This data is provided by the author and published online to benefit the reader. The contents of all supplementary data are the sole responsibility of the authors.

The interplay between the immune and skeletal systems, often described as the “immuno-skeletal interface”, has been implicated in the pathogenesis of bone disease in the setting of HIV infection[2], a condition associated with a persistent state of immune activation and chronic inflammation[1]. Bone resorption is dependent on a cytokine known as receptor activator of the nuclear factor kappa B (NFκB) ligand (RANKL), a tumor necrosis factor (TNF) family member that is expressed on the surface of osteoblasts and is released by activated T-cells[3,4]. RANKL is also produced by osteoblasts, osteocytes, bone marrow stromal cells, and T-cells among others[3], it activates its receptor, receptor activator of the NFκB (RANK), which is largely expressed by osteoblasts, T- and B-cells, dendritic cells, and fibroblasts and has important immunological functions including the regulation of T-cell growth and dendritic cell functions[3,4]. Osteoprotegerin (OPG) does not bind RANKL exclusively, but also represents a decoy receptor even for TNF-related apoptosis-inducing ligand (TRAIL), a multifunctional cytokine involved in the regulation of cell survival and differentiation of several cell lineages and apoptosis in normal and malignant cells[5,6]. The complex regulation of both RANKL and OPG is summarized in supplementary tables 1 and 2. There is limited data on the role of the RANKL/OPG axis in HIV-1 infection and further understanding of the potential alterations in this system in metabolic disorders that develop during HIV-1 infection is needed. Herein, we review the available literature on the role of the RANKL/OPG axis specifically in HIV-1 infection and its complications, including osteoporosis and accelerated atherosclerosis.

RANKL/osteoprotegerin axis plays a critical role in the interplay between the immune system and bone and cardiovascular disease in patients not infected with HIV-1

The decisive roles played by the RANKL/OPG axis in regulating bone metabolism, the immune system, and cardiovascular disease in patients not infected with HIV-1 have been recently reviewed[7-10] and are also summarized in figure 1 and in supplementary table 3. Lymphocytes influence the basal production of bone-sparing OPG; however, under inflammatory conditions, activated lymphocytes become a significant additional source of RANKL[9]. Thus, elevated RANKL levels in chronic inflammatory conditions can be caused by more intensive synthesis of RANKL and/or a non-sufficient upregulation of OPG synthesis, which in turn leads to increased RANKL:OPG ratio. An increased RANKL:OPG ratio has been associated with bone resorption and atherogenesis[11].

RANKL/RANK/osteoprotegerin axis has an important role in HIV-1 infection

Cytokines produced by the host in response to HIV-1 infection play several critical roles in virus–host interactions, both in terms of regulation of viral replication and a variety of metabolic complications of HIV-1 and ART[1]. Alterations in T- and B-cell activation and cytokine production persist in the setting of HIV-1 infection, despite effective ART[1]. In the context of this inflammatory milieu, the mechanisms underlying the possible pathogenesis of bone and cardiovascular disease associated with HIV-1 infection have been previously described[12,13]. The pathophysiologic changes associated with HIV infection may affect bone metabolism and atherogenesis on multiple levels, including the direct disruption of B-cell

and T-cell functions, and immune activation[14]. Data from both *in vitro* and *in vivo* studies reviewed in this manuscript and also summarized in figure 2[15-28] provide evidence of the significant role of RANKL/OPG in HIV infection that may affect the immune system, and the risk for bone and cardiovascular disease.

Evidence from *in vitro* studies that HIV-1 upregulates the RANKL/osteoprotegerin ratio

A positive feedback loop exists between RANKL production and HIV-1 replication, which may be relevant to both the pathophysiology of HIV-1-linked osteopenia and control of HIV-1 replication[29]. This pathway appears distinct from those of other cytokine activators of HIV-1, is enhanced synergistically by TNF-α, and suppressed by interferon (IFN)-γ[29] and is summarized in table 1. In addition, viral proteins such as gp120, Vpr, and Tat can upregulate the RANKL expression on osteoclastic progenitor cells, enhancing osteoclastic activity[15,16]. The HIV-1 envelope protein gp120 also induces RANKL secretion from lymphocytes and macrophages[16] and several HIV-1 proteins inhibit the osteoblast activity[17] with a derangement of osteoblasts and osteoclast homeostasis (Table 1). In addition, TRAIL, which binds OPG, is activated by HIV-1 Tat protein in monocytes[27,28]. Although the prospect of direct HIV-1 infection of osteoblasts is still controversial[22], studies based on osteoblast cultures from bone marrow biopsies of HIV-infected patients and control patients suggest that HIV-1 can modify the bone matrix through changes in the cytokine microenvironment rather than a direct impairment of osteoblast activity[25]. Overall, these *in vitro* studies suggest that HIV-1 *per se* can directly upregulate expression of RANKL in osteoclasts and their progenitors and in immune cells (macrophages, lymphocytes), while it can downregulate expression of OPG in osteoblasts.

Changes in the immune system during HIV-1 infection can directly affect the RANKL/osteoprotegerin axis

HIV-1 infection may affect both the cellular and humoral immune responses[30] (Table 1). The interactions between RANKL, TRAIL, OPG, and immune cells have been previously described[16,31] and are summarized in figure 1 and supplementary table 3. As the RANKL/OPG axis has a major role in regulation of the immune system (both adaptive and innate immunity) and *vice versa*, immune dysregulation during HIV-1 infection can also lead to dysregulation in the RANKL/OPG axis. A hallmark of HIV infection is loss of $CD4^+$ T-lymphocytes, which is accompanied by increased immune activation, affecting all major cell populations of the immune system[32]. The HIV-1 infection can lead to B-cell and T-cell exhaustion, resulting in a dysfunctional memory B-cell compartment[32]. In addition to this direct disruption of the immune cells, chronic immune activation is a recognized feature of HIV infection and a strong predictor of disease progression[33]. This loss of T-cells early in the course of HIV infection is thought to partially result in gastrointestinal mucosal damage, which in turn leads to systemic translocation of bacterial cell wall products including lipopolysaccharide (LPS). These bacterial cell wall products are capable of activating both the innate and adaptive immune systems[33]. *In vitro* studies have shown that LPS can stimulate osteoclast production by promoting osteoblast production of RANKL[34]. On the

other hand, recent *in vitro* studies on HIV-1 pathogenesis point to increased susceptibility of $CD4^+$ T-cells to TRAIL-mediated apoptosis[5,6]. TRAIL binds OPG and antagonizes its effects. In addition, viral replication requires activation-induced cellular transcription factors to drive viral RNA transcription, and increased immune activation would favor enhanced HIV-1 replication[35]. Thus, the *in vitro* data outlined in table 1 regarding the interplay between HIV-1, the RANKL/OPG axis, and the immune system suggest, overall, that HIV-1, through its interaction with the immune system, can upregulate the RANKL:OPG ratio, which in turn may increase immune activation and HIV-1 infectivity.

The impact of HIV infection on the immuno-skeletal interface was recently evaluated *in vivo* using the HIV-1 transgenic rat model, which has been demonstrated to recapitulate many of the immunologic and clinical abnormalities seen with human HIV infection[2]. In HIV-1 transgenic rats, osteoclastogenesis was associated with altered B-cell function, leading to a significant decline in production of bone-sparing OPG and an increased expression of the osteoclastogenic cytokine RANKL[2], leading to an elevated RANKL:OPG ratio. However, in humans, the complex interactions between HIV-1, the immune system, and the RANKL/OPG axis are largely unknown and may lead to variable RANKL:OPG ratios, depending on the degree of immune activation and dysregulation during the course of HIV-1 infection. Further studies in human HIV infection will broaden our understanding of these complex interactions.

RANKL, osteoprotegerin, and TRAIL in untreated HIV-1 infection

In vitro studies often offer conflicting results regarding the interactions between HIV replication and the levels of RANKL, OPG, and TRAIL (Table 2). This is due in part to the failure to consider the potential involvement of soluble HIV-1 gene products, HIV-1-associated cytokines, and physiologic regulators of osteoclast formation such as IFN-γ on these parameters. Thus, *in vitro* studies may not be able to adequately describe the possible interaction between HIV-1-related inflammatory cytokines and RANKL/OPG synthesis.

Previous studies have shown that HIV-1-positive ART-naive subjects display an increase in plasma RANKL compared to healthy controls[36-38], consistent with *in vitro* data where the increase of RANKL synthesis was related to HIV-1 gp120 and vpr treatment[15,16,38]. In one study, both RANKL and OPG were significantly increased in the plasma of ART-naive HIV-1-positive patients compared to a healthy control group, but there was no correlation between OPG plasma levels and HIV-1-RNA viral load[36]. In contrast in this same study, higher RANKL and TRAIL levels were directly related to plasma HIV-1-RNA viral load[36]. This direct correlation suggests a possible linkage between RANKL and TRAIL increases and viral replication. Although plasma levels of TRAIL are increased in HIV-1-infected patients[36,39] and enhancement of TRAIL-mediated apoptosis of $CD4^+$ T-cells[36] has been described in these patients, no correlation was found between higher TRAIL levels and lower bone mineral density[36]. However, further studies are needed to elucidate the role of TRAIL in HIV-1 infection and the RANKL/OPG axis. The higher plasma RANKL and OPG concentrations in ART-naive individuals compared to healthy subjects suggests a role of HIV-1 infection in this cytokine deregulation, probably through derangement of the immune system[36]. In another study it was also suggested that the increase in RANKL in untreated

HIV-1-infected subjects may be a reflection of increased immune activation and subsequent expression and production of RANKL by activated T-cells associated with untreated HIV infection[40].

Antiretroviral therapy is a major determinant of the RANKL/ osteoprotegerin axis in HIV-1 infection

In vitro studies suggest that antiretroviral therapy can directly affect the RANKL/ osteoprotegerin axis

The etiology and causes of ART-associated bone loss are likely multifactorial[41]. Antiretrovirals may have both direct and indirect effects as they suppress HIV replication. The ability of certain antiretrovirals to interfere with IFN-γ function and the RANKL/OPG in bone physiology may be particularly damaging in HIV-1 infection[42]. *In vitro* experiments with osteoblast and osteoclast-like cell lines suggest that specific antiretrovirals are involved in the perturbation of the RANKL/OPG axis, and different doses of the same drug may have different effects on the RANKL pathway and activate or inhibit specific intracellular pathways, interfering with expression of RANKL[43]. The majority of data in this area involves the protease inhibitor class of drugs, where individual drugs within this class appear to have differential effects on the RANKL/OPG axis. The *in vitro* effects of ART on the RANKL/OPG axis are summarized in table 2.

HIV-1-positive patients on antiretroviral therapy may have variable circulating RANKL levels

Studies examining the relationship between ART exposure and RANKL levels have yielded conflicting results[37,44,45]. Supplementary table 4 describes the details of 14 studies that have examined this association. Although in some studies, treatment with protease inhibitors (PI) tended to increase[45] blood levels of RANKL, whereas treatment with nucleoside reverse transcriptase inhibitors (NRTI)[37,44] and nonnucleoside reverse transcriptase inhibitors (NNRTI)[37] tended to reduce RANKL, in most studies the relative contribution of specific antiretrovirals on systemic levels of RANKL could not be determined[46,47]. In one prospective study, circulating RANKL and the RANKL:OPG ratio increased in the HIV-1 infected patients who stopped ART and this increase was associated with increased bone mass density and reduced markers of bone resorption[40]. The authors suggested that ART may increase bone turnover through a RANKL/OPG independent pathway and that the increase in RANKL on discontinuation of ART may be a compensatory response to the reduction in markers of bone turnover[40].

The lower levels of total serum RANKL compared to HIV-1-uninfected patients found in some studies of ART-treated patients[48,49] may reflect suppressed bone remodeling and unfavorable bone micro architecture[50]. Increased RANKL tissue expression in HIV-1-positive patients may also lead to negative feedback loop and lower circulating levels of RANKL. It has previously been suggested that serum levels of RANKL may be very different from local tissue expression and activity[51]. It may be possible that with increased local RANKL activity there is less production of soluble RANKL[51]. Consistent with this hypothesis, circulating RANKL levels were inversely associated with local bone RANKL

mRNA levels[52]. In HIV infection, *in vitro* data suggest that RANKL activity is increased at the tissue level, whether via HIV infection itself[53] or secondary to medication effects[53]. Thus, increased local RANKL activity in HIV-infected patients may be associated with lower circulating RANKL levels. Further studies are needed to directly measure RANKL activity within the arteries, bone, and lymphocytes of HIV-infected individuals.

Data on circulating osteoprotegerin levels in HIV-1-infected patients on antiretroviral therapy are conflicting

Data from *in vivo* studies in HIV-1-infected subjects regarding circulating OPG levels are conflicting and are summarized in supplementary table 5. Concentrations of circulating OPG were found to be significantly increased in HIV-1-infected subjects compared to age-matched controls in three studies[36,37,54]. It seems that OPG is dependent on HIV-1 infection, but independent of viral load[36]. In addition, elevated OPG levels in HIV-1-infected patients were significantly correlated with high TNF-α levels[54] and this may reflect enhanced immune activation in these patients. These data suggest that HIV-1 or ART may act on the osteoblast and stromal cells that are producers of OPG. Raised OPG levels may also be a parameter of enhanced activity in the OPG system, possibly correlated to enhanced activity of other members of the TNF family such as RANKL[36,37,54].

In contrast, in one cross-sectional study, plasma OPG levels were greatly diminished in HIV-1-infected subjects compared to age-matched controls[55]. A decrease in serum OPG levels might reflect an increase in OPG binding to RANKL[56] or may also be partially explained through HIV-1-induced immune dysregulation. The synthesis of OPG by T-cells is a compensatory mechanism to counterbalance the expression of RANKL and TRAIL by these activated cells[55] and this autoregulatory mechanism might be overridden in chronic disease conditions[57]. Inversely, the decrease of OPG production by T-cells induced by HIV-1 gp120 proteins and PIs could reflect abnormal local immune responses[55]. Thus, diseases directly related to the $CD4^+$ subset of T lymphocytes, such as HIV-1, could lead to abnormal tissue responses that include diminished OPG production[55].

Antiretrovirals such as drugs within the PI[55] or NRTI classes[58] were associated with a reduction in OPG levels *in vivo*[59]. Protease inhibitors decreased the spontaneous production of OPG by T-cells and were associated with an increased incidence of osteoporosis in HIV-1 patients that in turn can also contribute to lower levels of OPG[60,61]. Finally, in a recent study in HIV-1-infected subjects, both RANKL and OPG decreased significantly with ART initiation, while their ratio remained constant[59]. It was suggested that the reduction of inflammation that accompanies treatment of HIV infection may indirectly reduce OPG levels and that this could contribute to the early loss of bone mineral density observed after the initiation of ART[59].

However, in other studies, ART use did not affect[62] or even increase levels of OPG[63]. In one study, all patients achieved undetectable viremia, the main factor that drives systemic inflammation, and thus the authors concluded that other factors except for inflammation may be implicated in regulation of circulating OPG levels in HIV-1-infected subjects[63]. Thus, the significance of circulating OPG levels in HIV-1 infection remains unknown because these levels may not reflect local tissue expression.

Data on circulating RANKL/ osteoprotegerin levels in HIV-1-infected patients on antiretroviral therapy are limited

Different types of OPG with varying potencies to neutralize soluble RANKL (sRANKL) can be present in different diseases[64], but the potency of OPG to neutralize RANKL in HIV-1 infection remains unknown. In one study there was no difference in the RANKL:OPG ratio between HIV-1-infected patients without osteopenia and their osteopenic counterparts, suggesting that osteoclastogenesis was not influenced by HIV-1 infection or ART[65]. However, in most studies examining osteopenia in HIV-1-infected patients, the RANKL:OPG ratio was not determined, or if it was, the measurements were only assessed at a single time point. Investigation of the specific effect of ART on the RANK/RANKL/OPG axis may best be examined in HIV-uninfected persons receiving ART for the prevention of HIV infection or in virologically suppressed, ART-treated, HIV-infected persons switching ART regimens. In this way, the specific effect of the ART component might be evaluated independently of its effect on immune function, as seen during the early stages of HIV treatment.

Explanation for discordant results regarding blood levels of osteoprotegerin and RANKL in studies of HIV-1-infected subjects is multifactorial

Several hypotheses can be formulated to explain conflicting results in the measurement of levels of RANKL/OPG in human studies.

First, discrepancies in determination of blood levels of RANKL/OPG between different studies might be explained by the fact that the RANKL/OPG axis plays a crucial role in immune regulation[66]. It remains unclear whether the contribution of the immune system, mainly immune activation, on the production of RANKL could be considered constant or even absent if patients have excellent control of viral replication and immune response[37,46]. Thus, it is possible that differences in the state of immune activation between different groups of HIV-1-infected patients could explain discrepancies in circulating levels of RANKL/OPG between different studies.

Second, the majority of RANKL is cell bound and is not detectable in the circulation, and thus circulating RANKL is only a small part of total RANKL[56,67]. In addition, only small amounts of locally acting cytokines are present in systemic circulation, and thus serum OPG and RANKL may not reflect the levels of these cytokines at the bone tissue level[68]. Both RANKL and OPG are also expressed in metabolically active tissues such as the liver[56], but the contributions of these tissues to the blood levels of RANKL remains unknown, especially in HIV-1 infection. Thus, differences in metabolic activity of different tissues between different groups of HIV-1-infected patients with different comorbidities (e.g. accelerated atherosclerosis, osteopenia) and on different antiretrovirals may also explain discordant results regarding measurement of serum RANKL/ OPG levels in HIV-1-infected patients.

Third, current commercially available methods detect total levels of RANKL and OPG and not exclusively the biologically active forms[69]. Unfortunately, many studies measuring RANKL do not described which form of RANKL is detected (free, bound, or total form). It is possible that in chronic inflammatory conditions, a higher part of free sRANKL (potentially effective RANKL) exists, whereas in healthy control patients the part of inactivated form (OPG bound) of sRANKL may be higher[11]. This remains to be shown for patients with HIV-1 infection.

Finally, RANKL/OPG was measured in serum in most studies and in plasma in others and this can cause discordant results. Serum concentrations of cytokines such as RANKL and OPG can be different between serum and plasma[70] and such differences could be, at least in part, related to clotting necessary to recover serum[71]. Thus, differences between serum and plasma measurements[71], use of different antiretrovirals between different studies[54], the variety of different assays used, a lack of standardization of commercially available kits, and measurement of RANKL/OPG levels at different time points during the treatment of HIV-1 could explain discrepancies between different studies.

Changes in levels of the RANKL/ osteoprotegerin axis have been associated with bone disease in HIV-infected subjects

Changes in the RANKL/OPG axis could lead to an impaired balance in functions of osteoblasts and osteoclasts that may explain bone disease in HIV-1 infection[36,37,45]. Antiretrovirals can reduce the bone mass in HIV-1-infected patients that may be associated with a reduction in total systemic levels of RANKL (Table 5, Fig. 1). It is speculated that low serum RANKL may reflect suppressed bone remodeling and unfavorable bone micro architecture[50]. Increased levels of OPG in HIV-infected patients[36,37,54] may reflect a compensatory mechanism to downregulate increased bone resorption, as seen in prior studies of menopausal women[72]. On the other hand, a decrease in serum OPG levels in HIV-infected subjects observed in certain studies might reflect an increase in OPG binding to RANKL, which may have a beneficial effect on bone[56]. In a prospective study of HIV-1-infected subjects on intermittent ART, increases in RANKL and the RANKL:OPG ratio predicted increases in bone mineral dinsity over time[40]. However, as noted previously, circulating RANKL/OPG concentrations may not reflect their concentrations in the bone micro-environment and soluble levels of RANKL may not correlate with the activity of membrane RANKL, which has been shown to be more potent in stimulating osteoclastogenesis *in vitro*[73]. Thus, further studies in HIV-1-infected subjects are needed to elucidate the role of the RANKL/OPG axis in bone disease in these patients.

There is limited data on the role of the RANKL/osteoprotegerin axis in cardiovascular disease in HIV-infected subjects

Although reports of associations between circulating levels of OPG or RANKL with cardiovascular disease in HIV-1-uninfected subjects have been conflicting[8,74], the most recent studies have shown that increased RANKL concentration and decreased OPG level each are associated with vascular calcification, and an increased RANKL:OPG ratio is

significantly associated with coronary artery disease[8,74]. However, there is limited data regarding the role of the RANKL/OPG axis in cardiovascular disease in HIV-infected subjects. Elevated OPG levels in HIV-1-infected patients were found to be associated with high ankle-brachial indices[75] and with markers of endothelial dysfunction[58]. A recent study found that serum RANKL was lower in HIV-infected individuals on ART compared to controls, and was negatively associated with the number of coronary segments with plaque and Agatston coronary artery calcium score in HIV-infected individuals, even after adjusting for traditional cardiovascular risk factors[51]. However, in a smaller study there were no significant associations of serum RANKL, OPG, and RANKL/OPG with progression of atherosclerosis in HIV-1-positive subjects on ART[49]. Further studies are necessary to understand the physiological regulation and potential effects of RANKL on coronary atherosclerosis development in the HIV population during effective ART.

Other roles of the RANKL/osteoprotegerin axis in HIV-infected subjects

It was recently shown that higher OPG circulating levels were significantly associated with the presence of microalbuminuria in untreated HIV-1-infected patients, but not in HIV-1-infected subjects on ART, further suggesting a role for systemic inflammation in the pathogenesis of glomerular injury in HIV-1 infection[62]. However, interpretation of the role of the RANKL/OPG axis in HIV-associated diseases should be done after understanding the limitations of determining levels of RANKL/OPG in HIV-infected subjects.

Future studies

Limitations of studies measuring RANKL in HIV-1-infected patients include the lack of an appropriate healthy control population[41], lack of measurement in all studies of other important cytokines that can affect RANKL levels, such as OPG/TRAIL, small number of patients, and large variability in RANKL/OPG blood levels between different studies. In addition, the contribution of the immune system, mainly immune activation, and of antiretrovirals on the production of RANKL/ OPG was not assessed in most of the studies.

Further studies addressing the aforementioned limitations are needed to elucidate the role of the RANKL/OPG axis in HIV-1 infection. These studies should take into consideration the effect of different antiretroviral agents, immune activation, and expression of RANKL/OPG at different tissues. It may be necessary to investigate mRNA expression of OPG and RANKL in the tissues of interest and correlate these results with the individual serum levels of the patients in order to know how representative the measurable amount of OPG and RANKL in serum of HIV-infected patients is. Finally, since concentrations of circulating OPG evaluated at one time point can be variable as a compensatory response to variable abnormal osteoclastic activity[68], an appropriate profile of circulating RANKL/OPG concentrations in HIV-1-infected subjects will require a longitudinal measurement of circulating RANKL and OPG over several months.

Conclusion

Interest in the role of the RANKL/OPG axis during the course of HIV disease has grown in recent years. To date, studies on the role of the RANKL/OPG axis in HIV-1 infection have not produced conclusive results. Discrepant clinical conclusions are possibly due to differences in the selection criteria of HIV-1-infected patients and the complexity of various ART regimens. Serum measurements of OPG and RANKL are likely to be influenced by a variety of ongoing processes, and are unlikely to reflect what is ongoing in the tissue (for example the bone) itself. Elucidating the RANKL/OPG axis in HIV-1 infection may open a window to novel strategies to forestall an epidemic of future bone or cardiovascular disease in this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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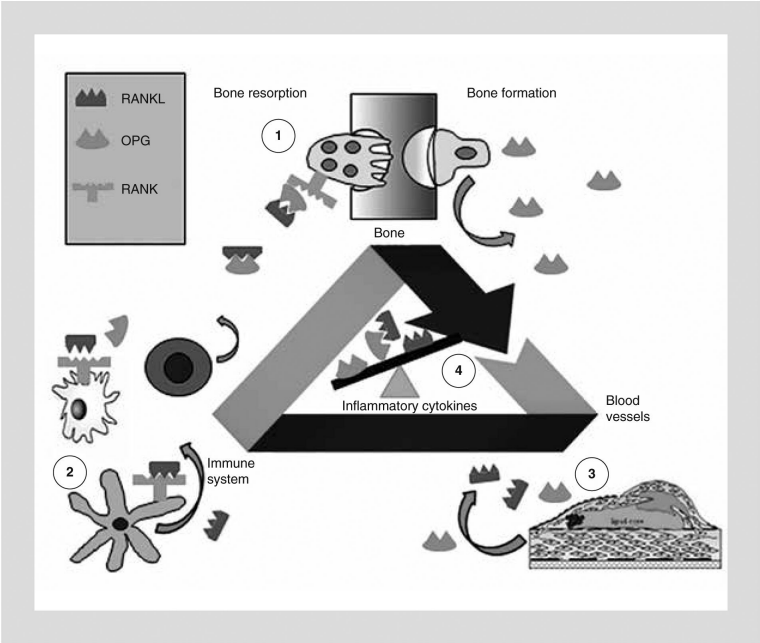

Figure 1.

The RANKL/OPG system plays a critical role in regulating bone metabolism, the immune system, and cardiovascular disease. (1) Activation of the RANKL/RANK pathway is essential for normal osteoclast differentiation and activation, whereas OPG has also been identified as a regulator of bone formation. (2) On the other hand, changes in the RANKL/OPG system have also been associated with changes in macrophages, dendritic cells, lymphocytes (reviewed in table 3), which are involved in systemic inflammation, osteoclastogenesis, and atherogenesis. (3) RANKL and OPG are found not only in bone, but also in the blood vasculature and increased RANKL:OPG ratio increases activity of matrix metalloproteinases leading to increased atherogenesis. (4) Increased ratio of circulating RANKL:OPG in the setting of systemic inflammation promotes osteoclastogenesis and atherogenesis. OPG: osteoprotegerin; RANK: receptor activator of nuclear factor kappa-B; RANKL: receptor activator of nuclear factor kappa-B ligand.

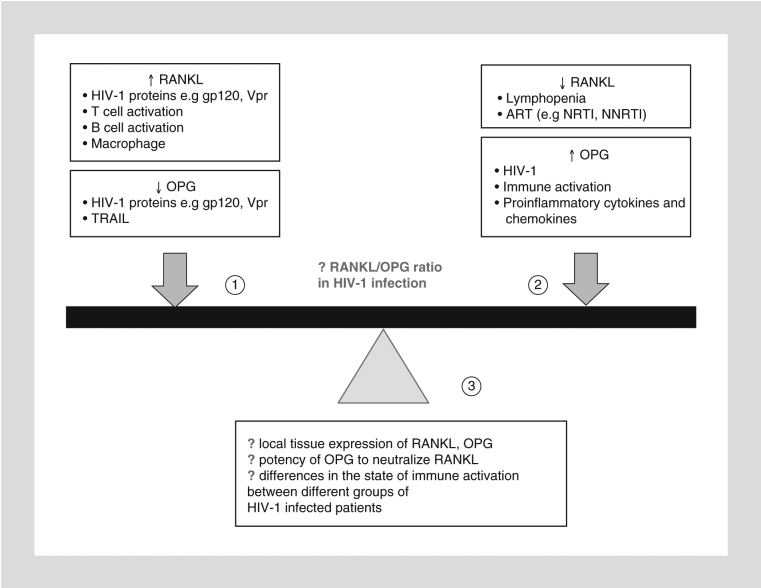

Figure 2.

HIV-1 infection can affect the RANKL/OPG system through multiple mechanisms. The relative balance of mechanisms in HIV-1 infection that upregulate (1) or downregulate (2) the ratio of RANKL:OPG (Tables 3, 4) in combination with unknown factors (3) may lead to a variable circulating RANKL:OPG ratio between different HIV-1-infected subjects that is associated with cardiovascular and bone disease and immune dysregulation. Arrows indicate observed direct effects based on in vitro studies and/or observations based on in vivo studies and do not necessarily imply causation. ART: antiretroviral therapy; IFN-γ: interferon-gamma; LPS: lipopolysaccharide; NNRTI: nonnucleoside reverse transcriptase inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; OPG: osteoprotegerin; PI: protease inhibitor; RANKL: receptor activator of nuclear factor kappa-B ligand; TNF-α*: tumor necrosis factor* α*; TRAIL: TNF-related apoptosis-inducing ligand.*

Table 1

Summary of interplay between RANKL, OPG, and immune system in HIV-1 infection

Direct interaction between HIV-1 and the RANKL/OPG system
- Gp120, Vpr, and Tat can upregulate RANKL expression, enhancing osteoclastic activity (HIV ↑ RANKL → ↑ binding to RANK → ↑ TRAF6 and MKKs → ↑ osteoclast gene differentiation and amplification)[16,17]
- Vpr enhances production of RANKL by acting in synergy with glucocorticoid[17,18]
- HIV envelope protein gp120 induces RANKL secretion[16,17]
- RANKL also upregulates HIV replication[16,17]
- HIV and ART can cause osteopenia, bone matrix effects, change bone metabolism, and elicit bone structure alterations → abnormal RANKL, OPG production[17-25]
- HIV can impair bone homeostasis by gp120/cell membrane interaction, inducing apoptosis by paracrine/autocrine TNF-α activation → abnormal RANKL, OPG production[15-17,21,23]
- Possible direct HIV-1 infection of osteoblasts → abnormal RANKL, OPG production[17-25]
- HIV tat protein activates TRAIL which binds OPG[26-28]
Indirect interaction between HIV-1-RANKL/OPG system through innate immunity
- HIV-induced gastrointestinal mucosal damage leads to systemic bacterial translocation and ↑ LPS that activates both the innate and adaptive immune systems. LPS ↑ osteoclast production by ↑osteoblast production of RANKL, IL-1, and TNF-α[34]
- PBMC from HIV-1-seropositive patients spontaneously overproduced biologically active RANKL[47]
- RANKL induces greater osteoclastic activity in adherent PBMCs of HIV^+ women than those of HIV- women[9,47]
Indirect interaction between HIV-1 and the RANKL/OPG system through adaptive immunity
- RANKL may increase activation and killing of virus-specific cytotoxic $CD8^+$ T-cells[9,29,76,77]
- HIV infection ↓ T-cell costimulation → ↓ OPG[2,9,30,78]
- HIV infection ↑ activated mature B-cells → ↑ RANKL[2,9,30,78]
- HIV infection ↓ resting memory B-cells → ↓ OPG production[2,9,30,78]
- HIV-1 infection causes severe B-cell exhaustion→ ↓ OPG → HIV-induced osteoclastic bone loss[2,9,30,78]
- HIV ↑ secretion of M-CSF by macrophages → M-CSF ↑ CD4/CCR5 receptors and virus gene expression in macrophages → M-CSF ↑ sensitivity of macrophages to HIV, the differentiation of osteoclasts, ↑ RANKL activity, and ↓ osteoprotegerin levels → enhanced osteoclastogenesis[9,16]
- A block in the transition of monocytes to macrophages in HIV-1 Tg rats may lead to a pooling of monocytes and osteoclast precursors → ↑ HIV-induced bone loss[9,16]
- RANKL enhances cross-presentation by DC of HIV antigens and anti-HIV memory responses[79]

ART: antiretroviral therapy; BMD: bone mineral density; CTL: cytotoxic T lymphocyte; DC: dendritic cell; FN-**y** interferon-gamma; MAPK: mitogen-activated protein kinase M-CSF: macrophage colony-stimulating factor; NFκB: nuclear factor kappa-B; OPG: osteoprotegerin; PPAR-y: peroxisome proliferator activated receptor; PBMC: peripheral blood mononuclear cell; PI: protease inhibitor; RANKL: receptor activator o nuclear factor kappa-B ligand; TRAF6: tumor necrosis factor receptor-associated protein 6

Table 2

Direct and indirect effects of antiretroviral therapy on the RANK/OPG system

Medication	
PI class effects[37,80]	- Cause direct bone loss (demineralization) - HIV PIs have differential effects on osteoblast and osteoclast maturation and function, despite targeting the same catalytic site of the retroviral protease - May interfere with IFN-γ function in bone physiology - Switching to an antiretroviral regimen containing tenofovir and efavirenz, and excluding PI and stavudine restores the RANKL/OPG equilibrium
Atazanavir[43]	- No effect on RANKL or OPG content in cell culture supernatants of osteoblasts
Darunavir[43]	- ↑ Blood levels of RANKL
Fosamprenavir[43]	- ↑ Blood levels of OPG - ↓ Blood levels of RANKL
Indinavir[16,80]	- No impact on the RANKL system - No effect on RANKL or OPG content in cell culture supernatants of osteoblasts
Lopinavir[80]	- ↓ Osteogenesis, calcium accumulation, and OPG expression in the osteoblasts - No impact on the RANKL system
Nelfinavir[16,80]	- ↓ Osteogenesis, calcium accumulation, and OPG expression in the osteoblasts - No impact on the RANKL system
Ritonavir[16,80]	- ↓ IFN-γ, which normally ↓ TRAF6, RANKL activity, MKKs - ↓ RANKL-induced and IL-4-induced Akt and NFκB activation - Modulates osteoclast differentiation through Wnt/β-catenin pathway - Can modify the response of PBMCs to RANKL and MCSF in HIV^+ subjects - Adherent PBMCs from HIV^+ women receiving RTV-boosted PI regimens exhibit greater induction of osteoclast-like cells after exposure to autologous serum compared to women on regimens that do not contain ritonavir - ↓ RANKL serum concentration and RANKL:OPG ratio (not seen with other PIs)
Saquinavir[16,80]	- ↑ Bone resorption activity - ↓ IFN-γ, which normally inhibits TRAF6 and RANKL activity MKKs - No effect on RANKL or OPG content in cell culture supernatants of osteoblasts
Tipranavir[43]	- ↑ Blood levels of OPG - ↓ Blood levels of RANKL
Nucleoside reverse transcriptase inhibitor	
NRTI[81]	- ↓ Bone mineral content in AIDS patients
Zidovudine[81]	- ↑ Osteoclast differentiation *in vitro* through stimulation of the TRAP promoter, mediated through the transcription factor, NFκB, in a RANKL-dependent manner

Medication	
	- ↑ The sensitivity of osteoclast precursors to RANKL

AP: alkaline phosphatase; AZT: zidovudine; BMD: bone mineral density; IFN-γ: interferon-gamma; MAPK: mitogen-activated protein kinase; M-CSF: macrophage colony-stimulating factor; MKK: mitogen-activated protein kinase kinase; NFκB: nuclear factor kappa-B; NRTI: nucleoside reverse transcriptase inhibitor; OPG: osteoprotegerin; PBMC: peripheral blood mononuclear cell; PI: protease inhibitor; RANKL: receptor activator of nuclear factor kappa-B ligand; TRAF6: tumor necrosis factor receptor-associated protein 6; TRAP: tartrate-resistant acid phosphatase.