Supporting Information

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SI Methods

Materials. Anti–TNF-α (AF-510-NA), rmTNF-α, rmM-CSF, and rhRANKL were from R&D Systems. All other reagents were purchased from Sigma Chemical unless indicated.

HIV-1 Transgenic Rats. Animal procedures were approved by the IACUCs of Emory University and the Atlanta Veterans Affairs Medical Center. Male hemizygous NL4-3Δgag/pol Fischer 344 rats (HIV-1 Tg; Harlan) have been previously described in detail (1) and were bred in the animal facility at the Atlanta Veterans Affairs Medical Center under a 12-h/12-h light/dark cycle with food and water provided ad libitum as described (2). Rats were analyzed for bone endpoints between 8 and 14 mo of age as indicated.

Bone Mineral Density. Ex vivo BMD measurements of lumbar spine, femurs, and tibias were performed for each rat at 14 mo of age by DXA using a PIXImus2 bone densitometer (GE Medical Systems) and associated software. Briefly, bones were excised and skeletal muscle removed by gently dissection. Bones were placed in the center of the scanner pallet and scanned individually. Using a single fixed rat femur scanned and repositioned five times the short term coefficient of variation of the system was found to be 0.17%. Repeated scanning of the same bone at weekly intervals for 5 mo revealed a long-term interassay coefficient of variation of 0.65%.

Cell Purification for RANKL and OPG Quantification. Splenocytes were recovered by maceration and BM was extracted from long bones by centrifugation at 13,500 × *g* for 60 s, and erythrocytes removed by osmotic lysis as previously described (3). B cells were immunomagnetically purified using antirat IgM-conjugated microbeads according to the manufacturer's protocol (Miltenyi Biotech).

Flow Cytometry. Flow cytometry was performed on an Accuri flow cytometer with analysis using C-flow plus software (Accuri Cytometers). B cells were stained using anti–rat-CD45R-PE (eBioscience) and IgD-FITC (Santa Cruz Biotechnology), monocytes by CD11b-FITC (Antigenex America), and RANK by staining for RANK-PE (Imgenex). Appropriate isotype- and fluorochrome-matched controls were used for all antibodies to control for nonspecific binding and to set gates.

In Vitro Osteoclastogenesis Assays. Osteoclastogenesis assays were performed as follows. Whole BM depleted of erythrocytes by osmotic lysis were plated at 1×10^5 cells/well in 96-well plates in 200 μL αMEM supplemented with 10% FBS (Invitrogen) and treated with rmM-CSF (30 μg/mL) and rhRANKL at subsaturating doses (15 ng/mL) or saturating doses (50 ng/mL). RANKL was preincubated for 5 min with 5 μg/mL cross-linking anti–poly-His-antibody, per the manufacturer's instructions. Some cultures received rmTNF-α (2.5 ng/mL) or neutralizing TNF-α antibody (20 μg/mL). Cultures were fed after 3 d by replacing half the medium and cytokines/antibodies. After 7 to 10 d, cultures were fixed and stained for TRAP using a leukocyte acid phosphatase kit (Sigma). $TRAP^+$ multinucleated cells (≥3 nuclei) were quantified under light microscopy and normalized for size based on number of nuclei.

μCT. μCT was performed in rats 8 to 9 mo of age using a μCT 40 scanner (Scanco) by an operator blind to the grouping of animals. Briefly, femurs were excised from killed rats and skeletal muscle removed by gently dissection. Bones were fixed in 10% neutral buffered formalin for 48 h and stored thereafter in 70% ethanol at 4 °C until analyzed. The trabecular bone compartment was scanned at the right distal femoral metaphysis at a resolution of 12 μm (70 KV and 114 μA). Seventy tomographic slices were taken covering a total area of 840 μm. Cortical bone was quantified at the mid-diathesis by taking 100 tomographic slices at a resolution of 12 μm. For each group, one representative sample was selected for 3D reconstruction to generate a visual representation at 12 μm or 6 μm as indicated in the legend to Fig. 1 in the main text.

Trabecular structural parameters were calculated by using internal software with manual contouring, and included the trabecular indices trabecular total volume, trabecular bone volume, trabecular thickness, trabecular space, trabecular number, and trabecular connectivity density. Cortical indices included cortical thickness and cortical bone volume.

Real-Time RT-PCR. Real-time RT-PCR was performed on an ABI Prism 7000 instrument (Applied Biosystems) as described (3) using commercial (Applied Biosystems) Master Mix. Rat-specific primer sets for OPG, RANKL, and PGK were designed using Primer-BLAST (National Center for Biotechnology Information), selecting primers that spanned major introns. Primers generated a single product as assessed by dissociation analysis. Primers were synthesized by Integrated DNA Technologies and are as follows. RANKL forward primer was 5′-TTT GCT CAC CTC ACC ATC AA; reverse primer was 5′-TCC GTT GCT TAA CGT CAT GT; OPG forward primer was 5′- TCC GGA AAC AGA GAA GCA AC; reverse primer was 5′-TGT CCA CCA GAA CAC TCA GC; and PGK forward primer was 5′-TGC CTG TTG ACT TTG TCA CTG; reverse primer was 5′-GCG GTG TCT CCG CCT C. M-CSF was normalized to β-actin and analyzed using commercial Applied Biosystems primer/probe sets for M-CSF (Rn00576849_m1) and β-actin (4352931E). Relative changes in gene expression between WT and HIV-1 Tg samples were calculated using the $2^{-\Delta\Delta CT}$ method with normalization to PGK or β-actin (4).

1. Reid W, et al. (2001) An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. *Proc Natl Acad Sci USA* 98:9271–9276.
2. Lassiter C, et al. (2009) HIV-1 transgene expression in rats causes oxidant stress and alveolar epithelial barrier dysfunction. *AIDS Res Ther* 6:1.
3. Li Y, et al. (2007) B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass in vivo. *Blood* 109:3839–3848.
4. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.