

COMMENTARIES

Is IFN- γ Involved in Bone Loss or Protection? Nothing Is Simple With Cytokines

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Commentary on: Gao Y, Grassi F, Ryan MR, Terauchi M, Page K, Yang X, Weitzmann MN, Pacifici R. IFN-gamma stimulates osteoclast formation and bone loss in vivo via antigen-driven T-cell activation. *J Clin Invest.* 2007 Jan;117(1):122-32.

Studies in the newly recognized field of osteoimmunology (or immunosteology) are flourishing, and debates concerning the role of T lymphocytes and IFN- γ in particular on bone remodeling have been quite confusing. IFN- γ , a major cytokine produced by activated T lymphocytes and NK cells, exemplifies the dual role of several cytokines on bone turnover, as it is capable either of activating or inhibiting osteoclastic bone resorption. In a new study by Gao *et al.* (1), the authors first confirm previous observations that IFN- γ dose-dependently decreased osteoclastogenesis in purified spleen and bone marrow macrophages exposed to RANKL and M-CSF, indicating direct inhibition at the level of the osteoclast precursor. They go on to describe contrary pro-osteoclastogenic properties of IFN- γ , via activation of T cells and T cell production of RANKL and TNF- α , two major osteoclastogenic factors. Through a nice set of experiments, the authors conclude that T cells are critical to the mechanism by which IFN- γ exerts net stimulatory effects on bone resorption and bone loss.

The development and differentiation of osteoblasts and osteoclasts is controlled by growth factors and cytokines produced by bone cells themselves, as well as by surrounding bone marrow cells, mostly monocytes and T cells (2). Bone indeed provides an ideal microenvironment for the development of hematopoietic stem cells, from which cells of the immune system derive. Crosstalk between bone and the immune system is now well documented, and a variety of cytokines are known to regulate osteoblast and osteoclast functions, which is particularly obvious when an immune response is triggered during inflammation (3;4). IL-1 β was the first cytokine to be described as capable of stimulating bone resorption (5). In addition to its ability to directly stimulate osteoclast formation and activity, IL-1 β is also an inhibitor of osteoblasts, thereby causing uncoupling between bone resorption and formation (5). Other cytokines that have been identified as main stimulators of bone resorption and/or as uncoupling agents include IL-6 and TNF- α (6;7). Transgenic animals overexpressing the soluble TNF receptor are protected against bone loss caused by OVX (7), whereas deletion of the IL-6 gene in mice protects them from OVX-

induced osteoporosis, with a mechanism involving prevention of osteoclast activation (8). Additional cytokines that have now been identified as stimulators of bone resorption include IL-7, IL-11, IL-15 and IL-17 (9-11). It is notable that IL-7, IL-15 and IL-17 are also major cytokines important for the development, maturation, proliferation and activation of T cells, and that both IL-15 and IL-17 are pro-inflammatory cytokines involved in a variety of autoimmune diseases (12;13). In addition, activated CD4⁺ T cells are a major source of RANKL, an indispensable activator of osteoclasts (14). On the other hand, IL-4, IL-10, IL-13, IL-18, GM-CSF and IFN- γ are all capable of inhibiting bone resorption. Hence, T lymphocytes are key players in the process of local and systemic bone loss associated with inflammation and autoimmune diseases. Whether T cell-mediated mechanisms similar to those implicated in graft rejection and inflammatory reactions prevail during bone resorption following estrogen deprivation remains unclear, however. Some studies report that T cell-deficient nude mice are protected against bone loss and the increase of bone turnover after estrogen withdrawal (15;16), whereas in other murine models of T cell-deficiency (such as RAG2KO and TCR α KO mice), analysis of bone structure by μ CT indicated that trabecular bone loss does occur after OVX (16). Nevertheless, the demonstration that T cell-deficient nude mice eventually lose bone after T cell reconstitution (17) clearly attributes to T lymphocytes a role in bone resorption after estrogen withdrawal.

IFN- γ has receptors on virtually all cell types, thereby exerting a multitude of biological effects. IFN- γ is the main activator of macrophages. Moreover, IFN- γ augments expression of MHC class II molecules in professional as well as nonprofessional antigen-presenting cells (APCs), mainly through the induction of CIITA (class II transactivator) (18), leading to increased T

cell activation by a feedback amplification loop. Despite its activating effects on macrophages and T cells, IFN- γ was initially described as a protective bone factor, as it directly inhibits osteoclast formation and activation *in vitro* (19;20). However, the role of IFN- γ *in vivo* is more complex as, on one hand, IFN- γ decreases osteoclastic bone resorption in nude mice (21), *i.e.*, in the absence of T cells, and on the other hand, it is positively correlated with bone resorption and bone loss in various inflammatory conditions in both mice and humans. Furthermore, administration of IFN- γ did not prevent bone loss in patients with rheumatoid arthritis (22). Could the complex effects of IFN- γ on bone resorption be due to both direct and indirect effects on osteoclasts?

For the purpose of examining direct and indirect effects of IFN- γ on osteoclasts, the authors utilized three transgenic murine models: a T cell-deficient mouse (nude mouse), an IFN- γ null mouse (*IFN- γ (-/-)*) and a newly generated transgenic mouse combining a deficit in TGF- β signaling (*CD4dnTGF β IIIR*) and a knockdown of IFN- γ (*CD4dnTGF β IIIR/IFN- γ (-/-)*). In the first model, the authors confirmed that administration of recombinant IFN- γ had no effect in T cell-deficient nude mice, while it resulted in significant bone loss in wild type and T cell-replete nude mice. Hence, the bone resorptive effect of IFN- γ requires the presence of T cells. These authors previously reported that bone marrow levels of TGF- β , a major T cell homeostatic agent, decrease with estrogen deficiency, leading to uncontrolled proliferation and activation of T cells and thereby increasing IFN- γ production, a trigger for bone loss (17). In keeping with these findings, the present study shows that bone loss after OVX was markedly reduced in *IFN- γ (-/-)* mice compared to wild type animals. In this experiment, however, neither spine BMD nor

trabecular BV/TV at the distal femur were significantly higher in OVX *IFN- γ* ^{-/-} mice compared to OVX WT mice, because baseline BMD is reduced in *IFN- γ* ^{-/-} mice compared to WT mice. In contrast to OVX mice, bone loss occurred normally in *IFN- γ* ^{-/-} mice when LPS was injected. LPS in this case leads to an increase in the activity of APCs that is IFN- γ -independent, but Toll-like receptor-dependent. In turn, APCs activate T cells via their enhanced MHC class II expression, as explained above, mimicking the mechanisms of bone resorption observed in inflammatory diseases. On one hand, the experiments indicate that the upstream mechanisms of T cell activation may differ between OVX- and inflammation-induced bone loss. On the other, the results suggest that OVX *IFN- γ* ^{-/-} mice could be protected against bone loss because APCs are not activated in the absence of IFN- γ .

At this stage, the ultimate demonstration of the crucial role of APCs on bone loss would require OVX experiments in MHC class II-deficient mice or other models of APC deficiency (23). Instead, the authors performed a somewhat redundant third set of experiments to evaluate the role of the T cell activator (TGF- β)-effector (IFN γ) system on the skeleton. In this system, *CD4dnTGF β IIIR* mice represent a model of increased T cell activation and proliferation (by lack of TGF- β , as seen in estrogen-deficiency, see above) and TGF β /IFN- γ double-deficient mice (*CD4dnTGF β IIIR/IFN- γ* ^{-/-}) a model of T cell activation blockade (by inhibition of the APC-dependent loop of T cell amplification). Accordingly, *CD4dnTGF β IIIR* mice had a low bone mass phenotype compared to WT animals, with a failure to accumulate spine BMD between 8 and 16 weeks of age, which was partially rescued in *CD4dnTGF β IIIR/IFN- γ* ^{-/-} mice. The latter was accompanied by a net decrease in T cell activation and proliferation, itself subordinated to a

significant decrease in macrophage activity and capacity to present antigen, as expected. Finally, transfer of T cells from *CD4dnTGF β IIIR* and *CD4dnTGF β IIIR/IFN- γ* ^{-/-} mice into nude mice both resulted in higher bone loss compared to transferred WT T cells, confirming that spontaneously activated T cells are a trigger for bone resorption. Therefore, the authors conclude that T cells are critical to the mechanism by which IFN- γ exerts net stimulatory effects on bone resorption and bone loss.

In conclusion, what is the role of T cells, and of IFN- γ in particular, on bone turnover? First, there is no doubt that activated T cells, as seen during inflammation, are central to osteoclast activation and bone loss, mainly through RANKL production, which does not necessarily require IFN- γ . Second, T cell activation follows estrogen deficiency, increasing the production of IFN- γ , whose predominant effect is to activate osteoclastogenesis and bone loss through T cell amplification and RANKL production. This overcomes direct inhibitory effects of IFN- γ on osteoclast activation. Whether T cell-dependent mechanisms predominate or modulate direct osteoclast activation by estrogen deficiency remains somewhat unclear. In another circumstance, inhibition of osteoclastogenesis by IFN- γ could be the predominant mechanism of action of this cytokine under physiological conditions of bone turnover, such as bone modeling and remodeling during growth, explaining why BMD and/or trabecular bone density are actually lower (rather than higher) in nude mice and *IFN- γ* ^{-/-} mice compared to WT animals. Altogether, these observations suggest different thresholds for dual effects of IFN- γ on osteoclasts and T cells. Nothing is simple with cytokines.

Conflict of Interest: The authors report that no conflicts of interest exist.

References

1. Gao Y, Grassi F, Ryan MR, Terauchi M, Page K, Yang X, Weitzmann MN, Pacifici R. IFN-gamma stimulates osteoclast formation and bone loss in vivo via antigen-driven T cell activation. *J Clin Invest*. 2007 Jan;117(1):122-32.
2. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. *N Engl J Med*. 1995 Feb 2;332(5):305-11.
3. Klaus J, Armbrecht G, Steinkamp M, Bruckel J, Rieber A, Adler G, Reinshagen M, Felsenberg D, von Tirpitz C. High prevalence of osteoporotic vertebral fractures in patients with Crohn's disease. *Gut*. 2002 Nov;51(5):654-8.
4. Walsh NC, Crotti TN, Goldring SR, Gravalles EM. Rheumatic diseases: the effects of inflammation on bone. *Immunol Rev*. 2005 Dec;208:228-51.
5. Dewhirst FE, Stashenko PP, Mole JE, Tsurumachi T. Purification and partial sequence of human osteoclast-activating factor: identity with interleukin 1 beta. *J Immunol*. 1985 Oct;135(4):2562-8.
6. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. *Nature*. 1986 Feb 6-12;319(6053):516-8.
7. Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, Yamaguchi A, Yoshiki S, Matsuda T, Hirano T, et al. IL-6 is produced by osteoblasts and induces bone resorption. *J Immunol*. 1990 Nov 15;145(10):3297-303.
8. Ammann P, Rizzoli R, Bonjour JP, Bourrin S, Meyer JM, Vassalli P, Garcia I. Transgenic mice expressing soluble tumor necrosis factor-receptor are protected against bone loss caused by estrogen deficiency. *J Clin Invest*. 1997 Apr 1;99(7):1699-703.
9. Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, Ciliberto G, Rodan GA, Costantini F. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO J*. 1994 Mar 1;13(5):1189-96.
10. Weitzmann MN, Cenci S, Rifas L, Brown C, Pacifici R. Interleukin-7 stimulates osteoclast formation by up-regulating the T-cell production of soluble osteoclastogenic cytokines. *Blood*. 2000 Sep 1;96(5):1873-8.
11. Ogata Y, Kukita A, Kukita T, Komine M, Miyahara A, Miyazaki S, Kohashi O. A novel role of IL-15 in the development of osteoclasts: inability to replace its activity with IL-2. *J Immunol*. 1999 Mar 1;162(5):2754-60.
12. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, Tanaka S, Kodama T, Akira S, Iwakura Y, Cua DJ, Takayanagi H. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J Exp Med*. 2006 Nov 27;203(12):2673-82.
13. Kotake S, Kamatani N. The role of IL-17 in joint destruction. *Drug News Perspect*. 2002 Jan;15(1):17-23.
14. McInnes IB, Liew FY. Interleukin 15: a proinflammatory role in rheumatoid arthritis synovitis. *Immunology Today*. 1998 Feb;19(2):75-9.

15. Kong YY, Feige U, Sarosi I, Bolob B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature*. 1999 Nov 18;402(6759):304-9.
16. Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. *J Clin Invest*. 2000 Nov;106(10):1229-37.
17. Lee SK, Kadono Y, Okada F, Jacquin C, Koczon-Jaremko B, Gronowicz G, Adams DJ, Aguila HL, Choi Y, Lorenzo JA. T lymphocyte-deficient mice lose trabecular bone mass with ovariectomy. *J Bone Miner Res*. 2006 Nov;21(11):1704-12.
18. Gao Y, Qian WP, Dark K, Toraldo G, Lin AS, Guldborg RE, Flavell RA, Weitzmann MN, Pacifici R. Estrogen prevents bone loss through transforming growth factor beta signaling in T cells. *Proc Natl Acad Sci U S A*. 2004 Nov 23;101(47):16618-23.
19. Cenci S, Toraldo G, Weitzmann MN, Roggia C, Gao Y, Qian WP, Sierra O, Pacifici R. Estrogen deficiency induces bone loss by increasing T cell proliferation and lifespan through IFN-gamma-induced class II transactivator. *Proc Natl Acad Sci U S A*. 2003 Sep 2;100(18):10405-10.
20. Kamolmatyakul S, Chen W, Li YP. Interferon-gamma down-regulates gene expression of cathepsin K in osteoclasts and inhibits osteoclast formation. *J Dent Res*. 2001 Jan;80(1):351-5.
21. Tohkin M, Kakudo S, Kasai H, Arita H. Comparative study of inhibitory effects by murine interferon gamma and a new bisphosphonate (alendronate) in hypercalcemic, nude mice bearing human tumor (LJC-1-JCK). *Cancer Immunol Immunother*. 1994 Sep;39(3):155-60.
22. Cannon GW, Pincus SH, Emkey RD, Denes A, Cohen SA, Wolfe F, Saway PA, Jaffer AM, Weaver AL, Cogen L, et al. Double-blind trial of recombinant gamma-interferon versus placebo in the treatment of rheumatoid arthritis. *Arthritis Rheum*. 1989 Aug;32(8):964-73.
23. Clausen BE, Waldburger JM, Schwenk F, Barras E, Mach B, Rajewsky K, Forster I, Reith W. Residual MHC class II expression on mature dendritic cells and activated B cells in RFX5-deficient mice. *Immunity*. 1998 Feb;8(2):143-55.