IL-7 Drives T Cell-Mediated Bone Loss Following Ovariectomy

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ABSTRACT: Ovariectomy-induced bone loss stems in large measure from a realignment of adaptive immune responses leading to the activation and expansion of tumor necrosis factor (TNF)-producing T cells. The mechanisms driving this T cell expansion are complex but we have recently reported that the pro-osteoclastogenic cytokine interleukin (IL)-7 plays a critical role in this process. The mechanisms of IL-7 action are intricate and poorly defined. We present herein an overview of our current understanding of IL-7 action on bone turnover and the role of IL-7 in ovariectomy-induced bone loss.

KEYWORDS: IL-7; T cells; estrogen; osteoclast; osteoblast

IL-7 AND OSTEOCLASTOGENESIS

IL-7 has long been known to stimulate osteoclastic bone loss when injected into mice *in vivo*.[1] However, the mechanisms of IL-7 action *in vivo* are complex and both the B cell and T cell lineages are strongly impacted by this cytokine. As IL-7 administration *in vivo* mimics both the bone destruction and the expansion of B lineage cells observed during ovariectomy, it was originally proposed that IL-7 may stimulate bone destruction by a mechanism involving B cells.[1] However, in an *in vitro* model of human osteoclastogenesis, we discovered that IL-7 stimulated osteoclast formation in the complete absence of B lineage cells. In contrast, T cells were an absolute requirement. Additional investigations determined that IL-7 stimulated osteoclastogenesis *in vitro* by inducing the production of RANKL by T lymphocytes.[2] To investigate the role of T cells in IL-7 mediated bone loss *in vivo*, we injected IL-7 into T cell-replete heterozygous nude mice and T cell-deficient nude mice. While the injection of IL-7 into T cell-replete mice induced significant bone

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loss, T cell-deficient nude mice were completely protected. Reconstitution of T cells into nude mice by means of adoptive transfer rescued the capacity of IL-7 to induce bone destruction. Furthermore, equal numbers of splenocytes derived from T cell-replete mice were found to secrete both RANKL and tumor necrosis factor (TNF)-α *ex vivo* following IL-7 administration *in vivo*. In contrast, T cell-deficient splenocytes from nude mice failed to produce RANKL or TNF-α but T cell reconstitution of nude mice again rescued the capacity of IL-7 to elicit cytokine production from splenocytes.[3]

IL-7 may in fact have two different competing actions on osteoclastogenesis. Addition of IL-7 to bone marrow cultures stimulated by exogenous RANKL and M-CSF inhibits osteoclast formation.[4] The mechanism of this direct suppressive effect of IL-7 is not clear; however, it is reported that early B cell precursors of the $B220^+$ lineage, which populate the bone marrow and are known to expand during estrogen deficiency,[5] may be capable of acting as an additional pool of osteoclast precursors *in vitro* as these precursor cells have the capacity to differentiate into bone-resorbing osteoclasts.[3,4,6] IL-7 may suppress the differentiation of this osteoclast precursor population, possibly by stimulating their differentiation toward mature B cells. While the relevance of $B220^+$ cells in ovariectomy-induced bone destruction *in vivo* remains to be investigated, the net effect of IL-7 *in vivo* appears to be pro-osteoclastogenic.

IL-7 AND BONE LOSS IN ESTROGEN DEFICIENCY

To address the issue of whether IL-7 plays a role in the bone destruction stimulated by ovariectomy, we measured IL-7 levels in sham operated and ovariectomized mice.[7] Significant elevations in IL-7 protein and mRNA were detected in bone marrow from ovariectomized mice. Importantly, injection of a neutralizing antibody against IL-7 completely prevented bone destruction following ovariectomy. Indeed, IL-7 has potent actions on the entire T cell lineage, and preliminary studies now suggest that IL-7 acts during ovariectomy to induce the expansion of hematopoietic stem cells in the bone marrow giving rise to enhanced numbers of T cell precursors. In addition, IL-7 appears to stimulate the process of thymic export as well as play a role in peripheral T cell expansion.[8] IL-7 consequently acts at multiple sites to expand T cell numbers and to stimulate T cell osteoclastogenic cytokine production.

Further investigations revealed that IL-7 neutralization *in vivo* prevented the rise in biochemical indices of bone turnover while causing a significant upregulation in indices of bone formation. This data suggested that IL-7 not only stimulates bone resorption but also suppresses bone formation. To verify the suppressive effect of IL-7 on bone formation, we treated neonatal mouse calvaria with IL-7 in organ cultures. IL-7 suppressed new bone formation in calvarial cultures, in addition to suppressing bone morphogenetic protein

(BMP)-2 stimulated new bone formation. Finally, IL-7 administration in mice dramatically suppressed bone formation *in vivo* as quantitated by double calcein labeling. To investigate the mechanism of IL-7 action on bone formation we evaluated the effects of IL-7 on the endogenous transcription of Runx2 (Cbfa-1) using a heterologous Runx2-driven promotor transfected into ROS 17/2.8 osteoblastic cells. In addition, we quantitated runx2 transcription in response to IL-7 using a reporter driven by the distal osteoblast-specific runx2 promotor. In both cases IL-7 suppressed reporter transcription by approximately 50%.

These data suggested that in ovariectomy conditions elevated concentrations of IL-7 not only upregulate bone resorption but simultaneously reduce the magnitude of the natural compensatory increase in bone formation, thus

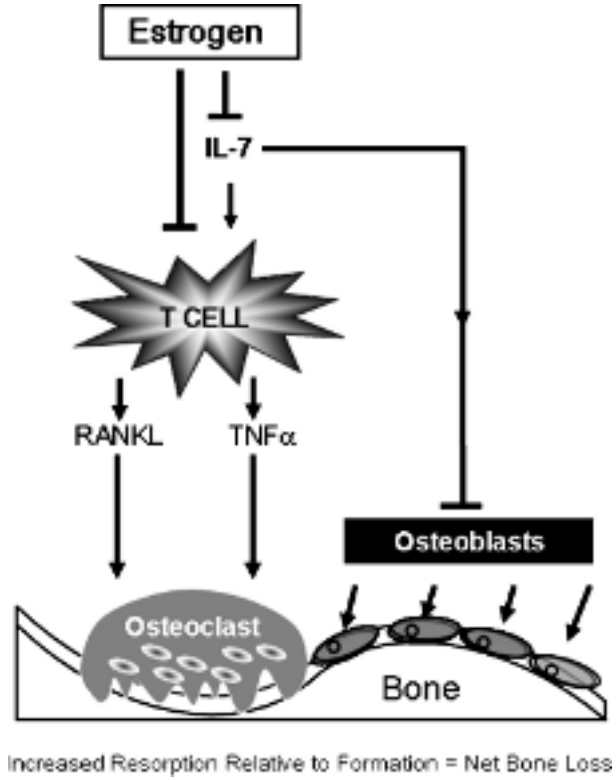

FIGURE 1. Mechanism of IL-7 action in estrogen deficiency bone loss. During estrogen deficiency, upregulated levels of IL-7 stimulate expansion of T cell populations leading to enhanced TNF- and RANKL-driven osteoclastogenesis. In addition, IL-7 suppresses the differentiation of osteoblasts limiting the magnitude of the compensatory increase in bone formation exacerbating bone loss.

preventing the reacquisition of bone homeostasis. The overall effects of IL-7 on bone mass are summarized diagrammatically in FIGURE 1.

CONCLUSION

Taken together, a growing body of evidence now suggests that the suppression of IL-7 by estrogen is a key mechanism by which estrogen protects bone mass, and that ovariectomy leads to an IL-7-driven wave of bone destruction.

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