Cytokines and Estrogen in Bone: Anti-Osteoporotic Effects

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Deficiency of the female hormone 17β-estradiol (E_2), caused by either menopause or removal of the ovaries, results in accelerated bone loss. As a consequence, bone mass declines after menopause (Fig. 1); this decline is the major factor contributing to the high rate of disabling bone fractures in postmenopausal women. Indeed, about one-third of all postmenopausal Caucasian women will experience at least one osteoporotic fracture during their lives, and 300,000 new cases of osteoporotic hip fractures are reported annually in the United States (*1*). In fact, postmenopausal osteoporosis affects 1.5 million people each year, making this disease a major health care problem. The pathologic bone loss underlying this condition can largely be prevented by early estrogen replacement therapy, but the mechanism by which estrogens exert their bone sparing effect has been unclear. Recent advances suggest that E_2 regulates the circuitry of cytokine action that controls bone remodeling, potentially providing a more precise understanding of how E_2 exerts its action in bone.

Bone remodeling is the process by which the catabolic effects (bone resorption) of one cell type of bone, osteoclasts, are balanced by the anabolic effects (bone formation) of a second cell type, osteoblasts. Normal bone remodeling proceeds in a highly regulated cycle in which osteoclasts adhere to bone and subsequently remove it by acidification and proteolytic digestion. Once osteoclasts leave the removal site, osteoblasts enter and secrete osteoid (a matrix of collagen and other proteins), which is calcified into new bone. Osteoclast-mediated resorption can be influenced by two processes: activation, in which the resorptive function of mature osteoclasts is increased, and recruitment, in which osteoclast progenitors are stimulated to yield more mature cells (Fig. 2). Activation occurs when inducers of bone resorption, such as parathyroid hormone (PTH), interleukin-1 (IL-1), or tumor necrosis factor (TNF) (*2*), stimulate osteoblasts to secrete a specific set of cytokines, and then some of these cytokines act directly on osteoclasts to cause bone resorption. Recruitment is more complex: Osteoclasts are derived from progenitor cells that also give rise to circulating monocytes and their analogs in tissue macrophages. For maturation, these progenitors require cytokines, such as macrophage–colony stimulating factor (M-CSF) or granulocyte-macrophage—colony stimulating factor (GM-CSF) and interleukin-6 (IL-6). Activated osteoblasts secrete M-CSF, GM-CSF, and IL-6 and, therefore, can recruit new osteoclasts by the secretion of these cytokines (*3*). Thus, a factor capable of altering osteoclast activity or osteoblast cytokine secretion could have a profound effect on bone remodeling. E_2 appears to be one such factor.

Research on the role of E_2 in bone remodeling has focused on three major questions: (i) What is the target cell or cells for E_2 action? (ii) How does E_2 alter cytokine secretion (or cytokine receptor expression) by bone cells? (iii) Does that alteration in cytokine biochemistry actually affect bone remodeling? One of the first observations linking cytokines to osteoporosis was that peripheral blood monocytes (PBM) from both men and women with osteoporosis secreted larger amounts of IL-1 than PBM from unaffected individuals (*4*). That E_2 could regulate cytokines was shown by the fact that PBM from either untreated premenopausal or estrogen- and progesterone-treated–postmenopausal women with osteoporosis secreted less IL-1 than untreated postmenopausal women (nonosteoporotic and osteoporotic) (*5*). Thus, the loss of E_2 that accompanies menopause allows PBM to secrete more IL-1, and E_2 inhibits IL-1 secretion. IL-1 is one of the most potent indirect inducers of bone resorption in vitro, and so it is likely to exacerbate osteoporosis in vivo. Because there is no apparent circulating IL-1 in postmenopausal women, the IL-1 that actually increases bone resorption likely originates from cells in the bone—either PBM that have homed to bone or estrogen-sensitive cells of the monocyte-macrophage lineage that reside in bone. Whether osteoclasts or their precursors secrete elevated concentrations of IL-1 in response to the loss of E_2 has not yet been determined, although macrophages and osteoclasts do express E_2 receptors (*6*).

In addition to IL-1, other cytokines that act on bone can also be detected when E_2 concentrations are reduced. PBM from ovariectomized premenopausal women, who have reduced serum E_2 levels, constitutively secrete more TNF-α, as well as more IL-1 and can be induced to secrete more GM-CSF than age-matched controls (*7*). TNF-α, like IL-1, is a potent inducer of bone resorption, requires the presence of osteoblasts for its resorptive activity, and stimulates osteoblasts to secrete factors like GM-CSF and IL-6, which induce formation of osteoclasts from precursors (Fig. 2). Thus, the loss of estrogen results in an increase of cytokines in the bone remodeling circuitry. Are the effects of estrogen on cytokines responsible for estrogen's protective role against osteoporosis? Correlative evidence suggests that this is the case: Some ovariectomized women on estrogen replacement therapy show a decrease in cytokine production with a concomitant reduction in bone resorption. In contrast, amounts of cytokines produced by PBM from ovariectomized women who received no estrogen therapy steadily increased, while women who underwent a hysterectomy without ovariectomy showed no change in either cytokine secretion or biochemical parameters of bone resorption.

A separate line of investigation has determined the effects of cytokines on further cytokine secretion from osteoblasts, their precursors, and stromal cells. Murine stromal cells, human bone cells, and rat and mouse

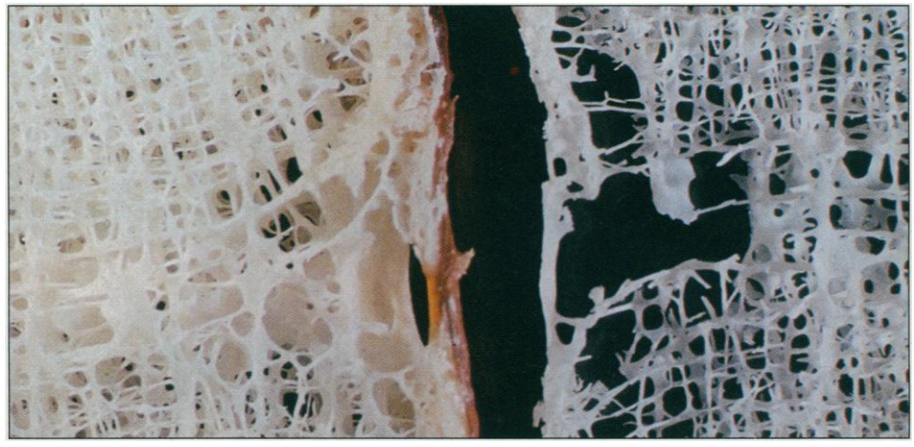

Fig. 1. Fragile bones. Normal bone (**left**) can become osteoporotic (**right**) when the body is deprived of estrogen after menopause. [Michael Kline/Peter Arnold, Inc.]

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osteoblast-like cell lines activated with IL-1 and TNF-α all secrete IL-6 (8). The secretion of IL-6 induced in this way can be inhibited by treating the cells in vitro with E_2 and, to a lesser extent, with testosterone and progesterone. Thus, cytokine secretion by authentic bone cells can be down-regulated by the addition of E_2 directly to osteoblasts and stromal cells, a finding that is consistent with the expression of E_2 receptors by osteoblasts (9). Furthermore, these data predict that one target for E_2 is either osteoblasts or their precursors, possibly stromal cells, in contrast to the data described above, which suggest that the monocyte is the target. However, some investigators have been unsuccessful in reproducing the inhibitory effect of E_2 on IL-6 secretion in vitro (*10*), possibly due to differences among cell preparations or culture conditions. The observation that other steroid hormones also inhibit cytokine secretion suggest the presence of specific steroid DNA binding sites (steroid response elements), which confer hormone sensitivity to target genes. However, no steroid response elements, including those for E_2, are apparent in the IL-6 promotor. It is possible that other transcription regulators may interact with various steroid receptors and that nonsteroid hormone response elements are involved.

The IL-6 made by these stromal cells and osteoblasts is crucial in the recruitment arm of the bone remodeling circuit, that is, in causing the differentiation of osteoclast precursors to mature osteoclasts. Indeed, the stimulatory effect of TNF-α on osteoclast formation was inhibited by either E_2 or a neutralizing antibody to IL-6. These findings and the observation that IL-6 has colony-stimulating activity reinforce the importance of IL-6 in osteoclast differentiation (*11*). Evidence from experiments in whole animals is also consistent with this conclusion. The absence of E_2 in ovariectomized mice leads to an increase in colony-forming units for granulocytes and macrophages, enhanced osteoclast development in vitro in bone marrow cell cultures, and an increased number of osteoclasts in vivo (*12*). These changes were inhibited by E_2 or an antibody to IL-6. Thus, osteoblast precursors, possibly stromal cells, respond to the loss of E_2 by secreting IL-6, which then induces osteoclastogenesis (Fig. 2).

Although the studies with isolated cells suggest that a primary effect of E_2 is to act on osteoblasts and stromal cells to modulate IL-6 secretion, they do not tell the whole story. Clearly, there are also effects of E_2 on the secretion of IL-1 and the other cytokines by PBM or similar cells (described above). These two separate effects of estrogen can be reconciled: An increase in IL-6 secretion from osteoblasts could result from a primary effect of E_2 on IL-1 or TNF-α production by PBM, since each of these factors potently induce IL-6. Or E_2 could affect both PBM (monocyte-macrophage cells) and the osteoblast, each of which responds independently.

The important implications of these investigations are that the anti-osteoporotic effect of E_2 may be explained by its fundamental ability to interact with bone cells and regulate the cytokine circuitry that controls bone remodeling. In the E_2 replete state, the hormone functions as a governor to reduce cytokine production and thereby temper the rate or extent of osteoclast formation and activity. This represents normal remodeling. In the E_2-deficient state, the governor is lost, allowing for increased cytokine secretion, leading to more osteoclast formation and increased bone resorption. Although the precise target cells, the order of interactions, and the specific cytokines affected remain controversial, a new line of experimentation has been opened. In addition, evaluation of cytokine levels could possibly be used as a diagnostic tool to monitor osteoporosis. And drugs that interfere with cytokine action (for example, IL-1 receptor antagonists) could provide new therapies that would allow for the effective treatment of a debilitating disease.

References and Notes

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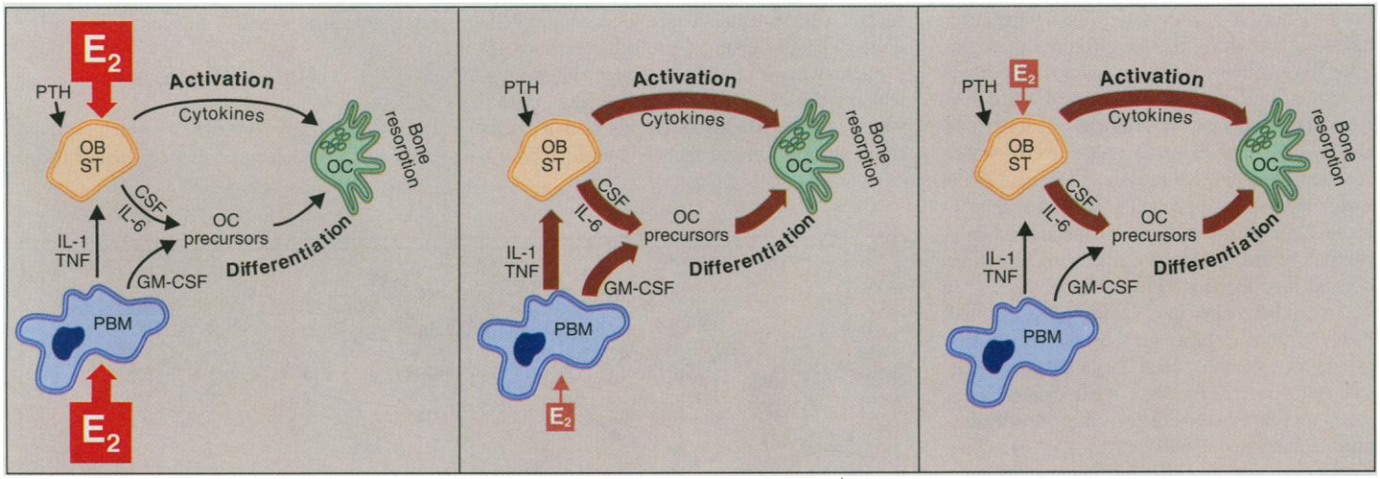

Fig. 2. Estrogen regulates bone cell cytokines. (**Left**) Normal bone remodeling. With E_2 acting as an inhibitor, peripheral blood monocytes (PBM) serve as a source of limited quantities of IL-1, TNF, and GM-CSF; and the stromal cells or osteoblasts secrete small amounts of IL-6 and CSF. (**Middle**) Estrogen deficiency: the role of PBM. Absence of E_2 action on PBM causes increased secretion of IL-1, TNF, and GM-CSF; consequently, osteoclast differentiation and activation are increased. (**Right**) Estrogen deficiency: the role of stromal cells and osteoblasts. In the E_2-deficient situation, osteoblasts and stromal cells secrete more IL-6, causing increased osteoclast differentiation and also secrete more cytokines that directly activate osteoclasts. OB, osteoblasts; ST, stromal cells; OC, osteoclast; and CSF, colony stimulating factors.