Editorial: Idiopathic Hypercalciuria and Osteoporosis—Distinct Clinical Manifestations of Increased Cytokine-Induced Bone Resorption?

Idiopathic hypercalciuria (IH), a heterogeneous disorder first described by Albright et al. (1) is the most common abnormality in patients with nephrolithiasis, occurring in approximately 50% of the patients with recurrent stone formation.

Calcium (Ca) balance and calcium absorption studies have demonstrated that most patients with hypercalciuria have increased intestinal Ca absorption. The terms absorptive hypercalciuria and dietary hypercalciuria are frequently used to describe those patients who exhibit an abnormal response to an oral Ca load and who excrete normal amount of Ca after prolonged fasting and/or on a low Ca diet. The terms resorptive hypercalciuria or true idiopathic hypercalciuria are used to describe those stone formers who excrete increased amounts of Ca even when maintained on a low Ca diet or during the fasting state, pointing to the skeleton as an additional source of urinary Ca.

Studies have revealed that patients with fasting IH have decreased axial and peripheral bone density (2, 3) and increased bone resorption (4). Hypercalciuric stone formers are often advised to adhere to strict low Ca diets. Thus, chronic calcium deprivation may be a contributing factor. However, the finding of a more severe bone loss in patients with fasting IH strongly suggests that an abnormality in bone remodeling may be responsible, at least in part, for the excessive Ca excretion characteristic of these patients.

Although primary hyperparathyroidism or a renal “Ca leak” resulting in a tendency to hypocalcemia and secondary hyperparathyroidism may account for increased bone resorption and bone loss in some patients, PTH levels are usually normal or low in patients with IH. Increased levels of 1,25 (OH)2D3 are also frequently found in these patients. However, increased calcitriol production is unlikely to account for increased bone resorption because 1,25 (OH)2D3 levels are generally similar in patients with IH typically exhibit high bone turnover, the hallmark of postmenopausal osteoporosis and of that induced by androgen deficiency in men. High bone turnover has also been documented as a typical feature of a syndrome described by Perry et al., (5) characterized by the presence of hypercalciuria and osteoporosis in eugonadic men.

Postmenopausal osteoporosis (and its equivalents in men) is a heterogeneous disorder characterized by a progressive loss of bone tissue that begins after natural or surgical menopause and leads to fracture within 15–20 yr from the cessation of the ovarian function. The bone-sparing effect of sex steroids is mainly related to their ability to block bone resorption, although stimulation of bone formation is likely to play a contributory role. Sex steroid-dependent inhibition of bone resorption is, in turn, the result of both decreased osteoclastogenesis and a diminished resorptive activity of mature osteoclasts.

In recent years our understanding of the mechanism of sex steroid action in bone has grown considerably. This is mainly a result of the recognition that sex steroids regulate bone remodeling by modulating the production of cytokines and growth factors from bone marrow and bone cells (6, 7). Among these factors are IL-1α and β, IL-6, tumor necrosis factor α and β (TNF), M-CSF, and GM-CSF. In addition to osteoporosis, an increasing number of common clinical entities have been ascribed to an abnormal production of bone resorbing cytokine by bone and/or bone marrow cells. Among them are Paget’s disease, primary hyperparathyroidism, Ghoram-Stout’s disease, post-transplant osteoporosis, thyroid hormone induced bone loss, endometriosis, multiple myeloma, and rheumatoid arthritis.

Cytokines and Bone Remodeling

IL-1 and TNF are among the most powerful stimulators of bone resorption known and are well-recognized inhibitors of bone formation. These cytokines promote bone resorption in vitro and cause bone loss and hypercalcemia when infused in vivo. IL-1 and TNF activate mature osteoclasts indirectly via a primary effect on osteoblasts and inhibit osteoclast apoptosis. In addition, they markedly enhance osteoclast formation by stimulating osteoclast precursor proliferation both directly and by stimulating the pro-osteoclastogenic activity of stromal cells. A specific competitive inhibitor of IL-1, known as IL-1ra, has also been identified. IL-1ra, which has a 26% amino acid sequence homology with IL-1β, binds to cells expressing primarily the 87 kDa type I IL-1 receptor with nearly the same affinity as IL-1 and competes with either IL-1α or IL-1β on these cells without detectable IL-1 agonist effects. The type I IL-1 receptor is expressed in T cells, tissue macrophages, endothelial cells, and bone cells. IL-1ra also binds, although with a lower affinity, to the type II IL-1 receptor, which is expressed mainly in blood neutrophils and B cells. Since the binding of 5 molecules of IL-1 per cell is sufficient to induce a full biological response, a 50% IL-1 inhibition in bone cells requires amounts of IL-1ra up to 100 times in excess of the amounts of IL-1α or IL-1β present.

The interpretation of the biological effects of IL-1 is further complicated by the fact that the binding of IL-1 to the type I receptor is antagonized not only by IL-1ra, but also by soluble type I (sIL-1 RI) and type II IL-1 receptor (sIL-1 RII) anti IL-1α autoantibodies and IL-1β binding proteins. Moreover, while sIL-1 RII antagonizes the effects of IL-1ra, sIL-1 RII binds IL-1β, but does not bind IL-1α. Thus, sIL-1 RII can compete with cell-associated receptors for IL-1β and potentiate the inhibitory action of IL-1ra. Recognition that the biological effects of IL-1 are not only a function of net concentration of IL-1 molecules, but rather of the fine balance between agonist and antagonist molecules, may
facilitate the interpretation of contrasting data obtained measuring IL-1 activity by bioassay and IL-1 concentrations by enzyme linked immunoassay or immunoradiometric assays. For example, an association between estrogen deficiency and increased IL-1 activity was demonstrated in studies conducted by measuring IL-1 activity by bioassay. Conversely, this association was not observed when IL-1 was measured by enzyme linked immunosorbent assay or immunoradiometric assay.

Another cytokine that has received considerable attention for its proosteoclastogenic effects is IL-6. This factor exerts its effects via a cell surface receptor that consists of a ligand binding chain (IL-6R) and a signal transducing chain known as gp130. Although IL-6 alone does not stimulate osteoclast formation, when bound to soluble IL-6R, IL-6 stimulates the early stages of osteoclastogenesis in human and murine cultures, presumably by forming a complex with gp130 expressed on either stromal cell or osteoblasts. Interestingly, corticosteroids have been found to increase the stromal cell expression of IL-6R, suggesting the possibility that steroid-induced bone loss may be caused, at least in part, by an increase in stromal cell responsiveness to IL-6. IL-6 increases in vitro bone resorption in systems rich in osteoclast precursors, such as the mouse fetal metacarpal assay, whereas it has no effect in organ cultures where more mature cells predominate, such as murine fetal radii. This suggests that IL-6 is more potent in increasing the formation of osteoclasts from hemopoietic precursors than in activating mature osteoclasts. Nevertheless, the effects of IL-6 on bone resorption in vivo remain controversial, because blocking of IL-6 does not decrease in vivo bone resorption, because IL-6 levels do not correlate with indices of bone turnover in postmenopausal women, and because osteoporosis is not a feature of transgenic mice overexpressing IL-6 or IL-6R. However, this cytokine does cause hypercalcemia in nude mice.

The formation of osteoclasts in bone marrow cultures is also increased by GM-CSF. This factor stimulates the early stages of osteoclastogenesis in cooperation with IL-3. In humans, GM-CSF is indeed the most potent proosteoclastogenic factor among the known growth factors and cytokines. Thus, although in the mouse osteoclast formation is completely blocked by anti-M-CSF but not anti-GM-CSF antibodies, GM-CSF is critical for the proliferation and differentiation of human osteoclast precursors.

Although most bone cell-targeting cytokines are produced by either bone and bone marrow cells, mononuclear cells of the monocyte/macrophage lineage are recognized as the major source of IL-1 and TNF. In contrast, proosteoclastogenic “downstream” cytokines are mainly produced by stromal cells and osteoblasts. Thus, osteoclastogenesis requires the hierarchical interaction of mononuclear cells, stromal cells, and/or osteoblasts and hematopoietic osteoclast precursors, as well as the conditioning effect of numerous cytokines.

Cytokines and idiopathic hypercalciuria

In this issue of the JCEM Ghazzali and colleagues (8) report that unstimulated peripheral blood monocytes from stone formers with IH secrete larger amounts of IL-1, TNF, and GM-CSF than healthy controls. Monocytes from subjects with dietary hypercalciuria produce cytokine levels similar to those of healthy controls. LPS-stimulated monocytes produce higher IL-1 and GM-CSF levels than unstimulated cells although they fail to release larger amounts of TNF. Ghazzali et al. propose a selective activation of specific monocyte populations as an explanation for the differential response to LPS observed in the study. Although this is a likely possibility, other mechanisms should be considered. For example, LPS is known to stimulate the shedding of TNF receptors, thus resulting in the binding and blocking of free TNF. Moreover, in many experimental models the increase in TNF messenger RNA expression induced by LPS is not accompanied by an increased translation of TNF protein. Of note also is the fact that LPS treated cells from the three groups of patients produced equal amounts of IL-1, TNF, and GM-CSF, presumably because the cells were maximally stimulated.

Interestingly, IL-6 levels were not significantly increased in subjects with IH. This finding is not unexpected because IL-6 does not stimulate in vitro bone resorption, and inhibition of IL-6 activity with anti-IL-6 antibody does not prevent ovariectomy-induced bone loss (9). Thus, information obtained in other experimental models is consistent with the notion that IL-6 does not moderate the increase in bone resorption of IH patients.

Ghazzali and colleagues (8) also reported that bone density was significantly lower in IH patients than in age-matched controls. Interestingly, the study also revealed the lack of a correlation between bone density and spontaneous cytokine production, although an inverse relationship was found between bone density and LPS-induced IL-6 secretion, and a positive correlation was noted between bone density and LPS-induced GM-CSF levels. Several factors should be taken into consideration in interpreting the apparent complex relationship between cytokine levels and bone mass. First, cytokine levels reflect the rate of bone remodeling at the time of sample collection, whereas bone density measurements provide values reflecting all past and current events capable of influencing skeleton development and involution. Thus, cross-sectional studies are rarely informative with respect to the effects of cytokines on bone mass. Second, the secretion of some cytokines, such as IL-6, increases with age (10), whereas the production of other factors, such as IL-1 and TNF does not. Thus, the opposite effects of aging on IL-6 production and bone mass could account for the existence of a correlation between these two variables independently of the effects of IL-6 in bone.

The interesting study by Ghazzali et al. (6) confirms and extends previous reports on a possible role for cytokines in the pathogenesis of IH (4, 11). Studies by us had demonstrated that patients with fasting IH have decreased bone mass and an increased monocytic production of IL-1 and increased bone resorption (4). However, we did not examine the production of other critical bone resorbing factors. In a subsequent study Weisanger et al. (11) demonstrated that monocytes from patients with IH have an increased expression of IL-1α mRNA and produce larger amounts of IL-1α. They also confirmed the existence of a correlation between age-normalized bone density values and IL-1α levels. The study of Ghazzali et al. (8) not only confirmed these observations, but also provides insights on the contribution of TNF and GM-CSF.

However, it should be recognized that none of these three studies demonstrate the existence of a cause/effect relationship between increased production of cytokines, bone loss, and hypercalciuria. The possibility that increased amounts of cytokines are produced in response to increased bone resorption should be considered. Collagen and other matrix proteins released into the bone microenvironment during bone resorption are, in fact, known to bind to integrin receptors expressed in monocytes and stimulate cytokine production (12).

Although a direct link between increased cytokine production and IH remains to be demonstrated, an increased monocytic production of IL-1 and TNF have been shown to play an important causal role in postmenopausal osteoporosis (6). Studies conducted with specific inhibitors of IL-1 and TNF have, in fact, demonstrated that the functional block of these cytokines prevent bone loss and block osteoclast formation and in vivo bone resorption in ovariectomized rats and mice (13, 14). Thus, it is tempting to speculate (Fig. 1) that both IH and postmenopausal bone
loss may result from a primary increase in bone resorption induced by an overproduction of critical cytokines such as IL-1, TNF, and GM-CSF by bone marrow cells. The different outcome of these two clinical entities (stone formation vs. spontaneous fractures) may be conditioned by factors such as intestinal absorption of calcium or calcitriol serum levels, which are both high in IH and low in postmenopausal subjects. It should also be noted that, while sex steroids have been demonstrated to directly regulate cytokine production via an effect on cytokine gene expression, the mechanism by which cytokine production is upregulated in IH remains to be determined.

Further studies in humans and in animal models will be important to define the contribution of cytokine-mediated bone resorption in the pathogenesis of IH. This appears particularly relevant in view of the recent discovery of oral agents capable of preventing bone loss in ovariectomized rats via inhibition of IL-1 and TNF production (15, 16). Such cytokine inhibitors may lay the foundation for a novel strategy for the treatment of IH, osteoporosis, and similar disorders caused by a cytokine-mediated stimulation of bone resorption.

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