

Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells

(ovariectomy/interleukin 1/tumor necrosis factor α /granulocyte–macrophage colony-stimulating factor/osteoporosis)

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ABSTRACT To determine whether mononuclear cell secretory products contribute to the changes in bone turnover that characterize the development of postmenopausal osteoporosis, we evaluated the effects of oophorectomy and subsequent estrogen replacement on the spontaneous secretion of interleukin 1 (IL-1) and tumor necrosis factor α (TNF- α) and on the phytohemagglutinin A-induced secretion of granulocyte–macrophage colony-stimulating factor (GM-CSF) from peripheral blood mononuclear cells. In 15 healthy premenopausal women who underwent oophorectomy, increases in GM-CSF activity were observed as early as 1 week after surgery, whereas elevations in IL-1 and TNF- α and in hydroxyproline/creatinine and calcium/creatinine ratios, two urinary indices of bone resorption, were detectable 2 weeks after the surgical procedure. Six of the oophorectomized women received no estrogen therapy after surgery and in these subjects hydroxyproline/creatinine and calcium/creatinine ratios plateaued 6 weeks postoperatively, and all three cytokines reached the highest levels 8 weeks after oophorectomy, when the study ended. In the remaining 9 women, who were started on estrogen replacement therapy 4 weeks after oophorectomy, decreases in the indices of bone resorption paralleled decreases in the secretion of the cytokines, with lower levels detected after 2 weeks of therapy. In the women who did not receive estrogen therapy, circulating osteocalcin, a marker of bone formation, increased beyond preoperative levels 8 weeks after oophorectomy, whereas in the estrogen-treated subjects osteocalcin remained unchanged in the entire study period. In 9 female controls who underwent simple hysterectomy, cytokine release and biochemical indices of bone turnover did not change after surgery. These data indicate that changes in estrogen status *in vivo* are associated with the secretion of mononuclear cell immune factors *in vitro* and suggest that alterations in the local production of bone-acting cytokines may underlie changes in bone turnover caused by surgically induced menopause and estrogen replacement.

Postmenopausal osteoporosis, a common disorder characterized by a decreased bone mass and increased fracture risk (1), stems from an accelerated loss of bone that begins after natural or surgically induced menopause and progresses rapidly for 5 or 10 years thereafter (2, 3). That estrogen deficiency plays a major role in this condition is well supported by the higher prevalence of osteoporosis in women than in men (4), by the increase in the rate of bone mineral loss detectable by bone densitometry after artificial or natural menopause (5, 6), and by the protective effect of estrogen replacement with respect to both bone mass loss and fracture incidence (7, 8). Although the bone-sparing effect of estrogen

appears to be related to an inhibitory effect on bone resorption (9), the mechanism of the estrogen response remains unknown.

The discovery of estrogen receptors in osteoblasts (10–12) and osteoclasts (13) suggests that a direct mechanism(s) may be involved. Estrogen could modulate the response of bone cells to systemic hormones or the secretion by bone cells of local regulatory factors. Estrogen has indeed recently been found to decrease the release from human bone cells of interleukin 6 (14), a factor that promotes osteoclastic bone resorption (15). Estrogen, however, could also have indirect effects on bone, perhaps by modulating the production of one or more of the bone-regulating factors released locally by blood cells (16). Among these are interleukin 1 (IL-1), tumor necrosis factor α (TNF- α), and granulocyte–macrophage colony stimulating factor (GM-CSF), low molecular weight proteins produced by cells of the monocyte–macrophage line (17–20). Although IL-1 is best known for promoting lymphocyte proliferation (21), TNF- α is known for eliciting hemorrhagic necrosis of tumors (22), and GM-CSF is known for regulating hematopoiesis (23); all three factors are also recognized for their important effects on skeletal tissues. IL-1 and TNF- α induce osteoblasts to stimulate mature osteoclasts (24, 25), modulate bone cell proliferation (26, 27), and induce hypercalcemia and bone resorption *in vivo* (28, 29). In addition, IL-1, TNF- α , and GM-CSF appear to contribute to bone resorption by promoting osteoclast recruitment and differentiation from bone marrow precursors (30, 31).

In previous studies, we have shown that circulating mononuclear cells from subjects with “high-turnover” osteoporosis and from early postmenopausal women, in whom bone remodeling is typically elevated, secrete increased amounts of IL-1 that is blocked by estrogen/progesterone therapy (32, 33). Those results prompted the current study, which was designed to investigate the effects of surgically induced menopause and subsequent estrogen replacement on bone turnover and on mononuclear cell secretion of IL-1, TNF- α , and GM-CSF. Specifically, the aims of this study were to investigate (i) the possible association of surgical menopause with an increased secretion of mononuclear cell immune products other than IL-1, (ii) the effect of estrogen treatment (without progesterone) on the secretory activity of human mononuclear cells, and (iii) the temporal relationship between changes in bone turnover and cytokine secretion. We report here that oophorectomy is associated with a rapid increase in bone resorption and by progressive increments in

Abbreviations: IL-1, interleukin 1; TNF- α , tumor necrosis factor α ; GM-CSF, granulocyte–macrophage colony-stimulating factor; PHA, phytohemagglutinin A; BGP, osteocalcin; BMD, vertebral bone mineral density; LPS, lipopolysaccharide.

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mononuclear cell GM-CSF, IL-1, and TNF- α secretion. We also show that estrogen replacement therapy normalizes bone turnover and restores preoperative cytokine levels.

METHODS

Subjects. The study population consisted of 15 healthy Caucasian premenopausal women, 41.9 ± 2.4 years old (mean \pm SE), undergoing total hysterectomy with bilateral oophorectomy, and 9 healthy control premenopausal Caucasian women, 39.8 ± 2.3 years old, undergoing total abdominal hysterectomy without oophorectomy. In all cases, surgery was performed for either uterine myomas or uncontrollable nonneoplastic bleeding. All subjects had normal vertebral bone mineral density (BMD), as assessed by dual energy radiography (34, 35), and had no history of fractures, back pain, or loss of height. All gave their informed consent and were ambulatory and in good health. Patients with a history of endometriosis, endometrial cancer, or other malignancies were excluded as were those with documented high alcohol intake, autoimmune disease, or conditions that could affect bone remodeling or mononuclear cell function. Previous or concurrent treatments with agents influencing bone metabolism were also exclusionary. None of the patients developed complications or medical problems requiring treatment with drugs that interfere with calcium metabolism or mononuclear cell function. None of the patients was taking calcium supplements or following a low-calcium diet before or during the study. All patients resumed their customary diets within 4 days of surgery and reported no changes in their average calcium intake during the rest of the study.

Study Protocol. In all women, a blood and a 2-hr urine sample were obtained after an overnight fast before surgery and again 1, 2, and 4 weeks after surgery. Women who underwent simple hysterectomy were not evaluated beyond the 4th week after the operation. Women who underwent hysterectomy with bilateral oophorectomy were divided into two groups and were followed for 4 more weeks. The first group included nine subjects, 41.8 ± 5.9 years old, who were started on estrogen replacement with oral conjugated estrogen (Premarin) at 0.625 mg/day 4 weeks after surgery. The second group included six women, 42.0 ± 5.0 years old, who either refused or were advised by their physicians not to take estrogen treatment and therefore remained untreated for the remaining time of the study. Both groups of patients were evaluated by the use of additional urine and blood sample analyses 5, 6, and 8 weeks after surgery. Each urine sample was measured for calcium, creatinine, and total hydroxyproline. Results are expressed as calcium/creatinine and hydroxyproline/creatinine ratios. These indices were measured in 12-hr fasting samples because hydroxyproline excretion after a 12-hr fast is not influenced by the previous day's diet (36). Blood samples were assayed for osteocalcin (BGP) and 17β -estradiol. IL-1, TNF- α , and GM-CSF activities were measured in the 48-hr culture media of, respectively, unstimulated and phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells obtained from each blood sample (see below).

Laboratory Methods. Mononuclear cell cultures. To assay IL-1, TNF- α , and GM-CSF, mononuclear cell cultures were prepared as described (32, 33). The mononuclear cells were allowed to adhere to a 24-well tissue culture plate for 2 hr. After incubation, the nonadherent cells (enriched lymphocytes) were removed from the wells, and the adherent population (enriched monocytes) was washed twice to remove any remaining nonadherent cells. The adherent cell population was then incubated for 48 hr with or without PHA (1 μ g/ml). The supernatant fractions were collected 48 hr later and then passed through 0.22- μ m filters and stored at

-20°C until assayed. The adherent population was found to be >95% monocytes. Monocytes comprised $19.5\% \pm 4.5\%$ of the entire original mononuclear population isolated by Ficoll density-gradient centrifugation. The number of monocytes obtained from each patient did not change throughout the study. The interpatient variability for monocyte number was 11%. To normalize the results for the number of monocytes per culture well GM-CSF and TNF- α activities are expressed as pg per 10^6 adherent cells and IL-1 is expressed as units per 10^6 adherent cells.

GM-CSF assay. GM-CSF activity in the culture media of unstimulated and PHA-stimulated mononuclear cells was measured with a two-site ELISA specific for human GM-CSF (37). The sensitivity of the method was 8 pg/ml. GM-CSF activity was undetectable in all culture media from unstimulated mononuclear cells, whereas measurable levels were found in the media of PHA-stimulated cells. Thus, data are presented only for PHA-stimulated cells.

TNF- α assay. TNF- α activity in the culture media of unstimulated mononuclear cells was assayed with a radioimmunoassay (Genzyme) that utilizes an antibody that does not cross-react with TNF- β , IL-1, IL-2, IL-6, and interferons (α , β , and τ) (38). The sensitivity of the method was 100 pg/ml.

IL-1 assay. The unstimulated mononuclear cell conditioned media were assayed for total IL-1 activity (IL-1 α and - β) by assessing the increment in mitogen-induced proliferation of the helper T cell D10.G4.1 (D10 cells) as described (32, 33). D10 cell proliferation was measured by the colorimetric method with 3-(4,5-dimethylthiazol-2-Y1)-2,5-diphenyl tetrazolium bromide as described (39).

Since agents in addition to IL-1 are comitogenic in the T-cell assay (40), we verified our findings as indicative of the presence of IL-1 by demonstrating inhibition of the conditioned medium effect in the presence of monoclonal antibodies that recognized IL-1 α and IL-1 β (data not shown).

The interassay variability was 29%, 15%, and 18% for IL-1, TNF- α , and GM-CSF, respectively. The interassay variability was 10% for IL-1 and TNF- α and 12% for GM-CSF. Recovery was >90% for all three cytokines. Filtration and refrigeration for up to 1 year resulted in no loss of IL-1, TNF- α , and GM-CSF activity. To rule out the possibility that contamination with levels of lipopolysaccharide (LPS) below the limit of detection of the limulus amoebocyte assay (<10 pg/ml) might account for IL-1 or TNF- α activities detected in the mononuclear cell culture media, mononuclear cells were cultured with polymyxin B (0.5 μ g/ml). Polymyxin B, an antibiotic that blocks LPS-induced cytokine release (41), did not affect the amounts of IL-1 and TNF- α in any sample (data not shown), ruling out a significant LPS contamination.

Biochemical assays. Blood and urinary calcium and creatinine were measured by standard atomic absorptiometric and photometric methods. Total urinary hydroxyproline was measured by the colorimetric method of Prockop and Udenfriend (SmithKline Bioscience Laboratory, Van Nuys, CA) (42). BGP was measured by radioimmunoassay according to the method of Price and Nishimoto (43). 17β -Estradiol was measured by standard radioimmunoassay (Nichols Institute, Los Angeles, CA).

Bone density measurement. BMD of the L₂-L₄ region was measured by dual energy radiography with a QDR-1000 x-ray bone densitometer (Hologic, Waltham, MA) as described (34, 35). The reproducibility of this technique is 0.5% *in vitro* and 1.0% *in vivo* (34).

Statistical analysis. Group mean values were compared by using a two-tailed Student's *t* test, one-way analysis of variance, or multifactor analysis of variance, as appropriate. Subsequent mean comparison tests were performed by using Tukey's honestly significant difference test. Simple linear regression, partial correlation analysis, and stepwise regres-

sion analysis were used to determine the relationship between indices of bone turnover and cytokine secretion.

RESULTS

As shown in Table 1, the women who underwent hysterectomy with oophorectomy and those who underwent simple hysterectomy were comparable for demographic characteristics, bone density, indices of bone turnover, calcium intake, and cytokine secretion. Hysterectomy without oophorectomy did not affect estrogen levels, indices of bone turnover, and cytokine release (data not shown), indicating that surgical stress and/or inflammation does not affect bone turnover and mononuclear cell secretory activity.

In women subjected to hysterectomy and oophorectomy, 17β -estradiol levels decreased from 240 ± 47 to 45 ± 13 pmol/liter ($P < 0.01$) within 1 week after surgery. In the 6 women who did not receive estrogen therapy throughout the study, 17β -estradiol levels remained in the postmenopausal range thereafter. In the 9 women who began estrogen replacement 4 weeks after the operation, 17β -estradiol increased to preoperative levels (228 ± 54 pmol/liter) within 1 week of treatment and plateaued thereafter. Calcium/creatinine and hydroxyproline/creatinine ratios, two markers of bone resorption (36), increased significantly (Fig. 1 A and B) in all 15 women ($P < 0.01$ and $P < 0.05$, respectively) as early as 2 weeks after oophorectomy. In the untreated group, both ratios continued to increase thereafter, attaining peak values 6 weeks after the operation. In the estrogen-treated group, both ratios continued to increase during the first week of treatment (5th week of follow-up) but then decreased significantly ($P < 0.05$) starting after 2 weeks of treatment. These data suggest that changes in bone resorption are detectable 2 weeks after oophorectomy and that there is a latency of at least 1 week between the initiation of estrogen treatment and the manifestation of its effects on bone resorption.

Fig. 1C shows the effects of oophorectomy on circulating levels of BGP, an index of bone formation (44). BGP gradually increased after surgery; however, 4 weeks after the procedure BGP levels were still comparable to preoperative values. In the six women who did not receive estrogen therapy, BGP levels continued to increase during the remainder of the study and became significantly different from baseline ($P < 0.05$) 8 weeks after surgery. In the nine women who were treated with estrogen, BGP levels did not change significantly during the remainder of the study.

The spontaneous release of IL-1 and TNF- α (Fig. 2 A and B) as well as the PHA-induced release of GM-CSF (Fig. 2C) from peripheral blood mononuclear cells were also affected

Table 1. Clinical data (mean \pm SE) and preoperative values for indices of bone turnover, bone density, and mononuclear cell cytokine production

	Hyster- ectomized	Oophor- ectomized
Age, years	39.8 \pm 2.3	41.9 \pm 2.4
Weight, kg	66.4 \pm 5.1	67.1 \pm 4.0
Ca intake, mmol/day	17.7 \pm 3.9	17.6 \pm 3.8
17β -Estradiol, pmol/liter	263 \pm 55	240 \pm 47
BMD, g/cm ²	1.1 \pm 0.1	1.1 \pm 0.3
Hydroxyproline/creatinine, mmol/mol	20.6 \pm 3.4	18.5 \pm 1.8
Calcium/creatinine, mmol/mol	0.14 \pm 0.03	0.15 \pm 0.03
BGP, ng/dl	8.6 \pm 1.4	7.9 \pm 1.9
IL-1, units per 10 ⁶ cells	39.2 \pm 12.7	32.0 \pm 14.3
TNF- α , pg per 10 ⁶ cells	1459 \pm 386	1300 \pm 281
GM-CSF, pg per 10 ⁶ cells	209.1 \pm 74.7	233.9 \pm 71.0

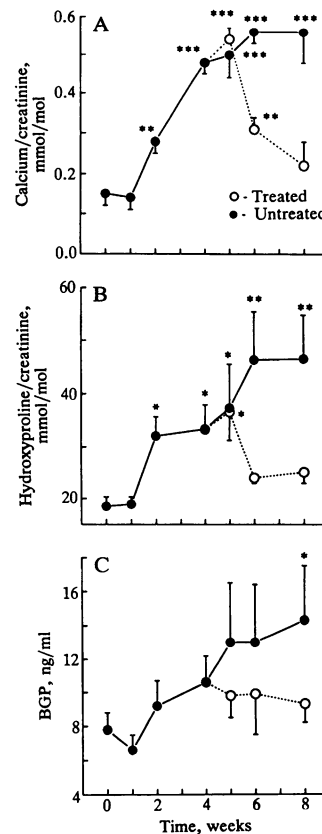


FIG. 1. Effect of oophorectomy and subsequent estrogen therapy on the fasting excretion of calcium/creatinine (A) and hydroxyproline/creatinine (B) and circulating BGP (C). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with baseline. Multifactor analysis of variance revealed that treatment, time, and their interaction were significant sources of variation for hydroxyproline/creatinine (time, $P < 0.005$; treatment, $P < 0.05$) and calcium/creatinine (time, $P < 0.0001$; treatment, $P < 0.05$). Treatment was the only significant source of variation of BGP ($P < 0.05$).

by surgically induced menopause. All three cytokine levels increased significantly in the first month after oophorectomy: GM-CSF levels became significantly ($P < 0.05$) higher than baseline as early as 1 week after surgery, and IL-1 and TNF- α levels increased significantly ($P < 0.05$) 2 weeks after surgery. In untreated subjects, IL-1, TNF- α , and GM-CSF continued to increase during the 2nd month of follow-up and reached the highest levels at 8 weeks. In the estrogen-treated group, IL-1 continued to increase during the 1st week of treatment. The highest concentration of this cytokine was, in fact, noted at the end of that week (5 weeks after surgery). Conversely, GM-CSF and TNF- α levels were lower, although not significantly, at 5 than at 4 weeks after surgery. However, all three cytokines decreased significantly ($P < 0.05$) after 2 weeks of estrogen treatment, reaching preoperative levels by the end of the 4th week of estrogen treatment.

When the data were further analyzed by multifactor analysis of variance, both estrogen treatment and time (as well as the interaction between those two factors) were significant sources of variation for 17β -estradiol levels, indices of bone resorption, and cytokine secretion. Estrogen treatment but not time contributed to the variance of BGP. Moreover, multiple range tests confirmed that the increase in GM-CSF reached significance 1 week after surgery and preceded similar increases in IL-1 and TNF- α and indices of bone resorption.

BGP correlated weakly with IL-1 ($r = 0.26$; $P < 0.05$) and not at all with TNF- α or GM-CSF. Moreover, significant correlations were found between indices of bone resorption and all three cytokines. However, when the contribution of each cytokine to the variance of indices of bone resorption was examined by stepwise regression analysis and partial correlation analysis, TNF- α was found to account for a larger fraction of the variance of calcium/creatinine ($R^2 = 0.42$; $P < 0.001$) than either IL-1 ($R^2 = 0.06$; $P < 0.05$) or GM-CSF ($R^2 = 0.01$; P value not significant) and to be the only significant predictor of hydroxyproline/creatinine ($R^2 = 0.24$; $P < 0.005$). These data suggest that the changes in

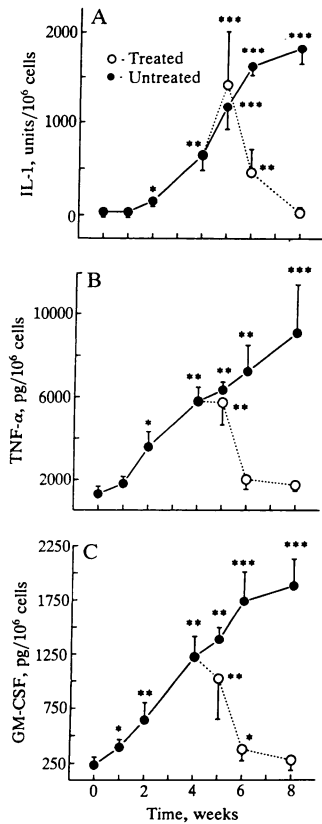


FIG. 2. Effect of oophorectomy and subsequent estrogen therapy on mononuclear cell spontaneous secretion of IL-1 (A), and TNF- α (B) and on the PHA-induced excretion of GM-CSF (C). *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$ compared with baseline. Time, treatment, and their interaction were significant sources of variation by multifactor analysis of variance for IL-1 (time, $P < 0.0001$; treatment, $P < 0.01$), TNF- α (time, $P < 0.0001$; treatment, $P < 0.001$), and GM-CSF (time, $P < 0.0001$; treatment, $P < 0.0001$).

cytokine secretion associated with oophorectomy reflect changes in bone resorption and that TNF- α is, among the three cytokines, the one that best predicts bone resorption.

DISCUSSION

In this study, surgically induced menopause was associated with an early increase in the monocytic secretion of GM-CSF, a cytokine that stimulates osteoclast recruitment and differentiation (30), and with subsequent, simultaneous increments in biochemical indices of bone resorption and IL-1 and TNF- α activities. The lack of significant changes in cytokine levels after simple hysterectomy indicates that it is the change in ovarian hormone status, rather than surgical stress or inflammation and healing, that accounts for the postovariectomy increases.

In this study, cytokines were measured in the media of mononuclear cells, which contain <5% lymphocytes and negligible amounts of other cells. Under these conditions, both monocytes and lymphocytes constitutively produce IL-1, TNF- α , and GM-CSF (45, 46). Lymphocytes secrete large amounts of GM-CSF when stimulated with PHA, although the induction of an optimal response requires the presence of monocytes (47). Thus, it is likely that lymphocytes may have contributed to the production of GM-CSF, and perhaps the IL-1 and the TNF- α , activities measured in our samples. Whether the changes in cytokine secretion observed in this study are the result of a regulatory effect of estrogen on monocytes, lymphocytes, or both remains to be established.

Although the significance of the relationship between estrogen levels and monokine secretion is uncertain, there are reasons to believe that it may be relevant to the skeletal impact of menopause. Menopause is followed by a period of rapid bone loss (5, 6), which is thought to result from an increase in bone resorption and in the overall rate of bone turnover (9, 10). Several lines of evidence suggest a role for mononuclear cell immune products, and in particular for

IL-1, in this process. Although there is no published evidence for a role of IL-1 in physiologic bone turnover, increased monocytic IL-1 activity is typical of medical conditions characterized by rapid bone remodeling and decreased bone mass, including high-turnover osteoporosis (32), fasting (resorptive) idiopathic hypercalciuria (48), and endometriosis (49). Similarly, localized and generalized osteoporosis are frequent complications of conditions in which IL-1 is thought to play a pathogenetic role, such as rheumatoid arthritis (50). Studies in rodents reveal that IL-1 also causes the appearance of new, discrete areas of resorption, resulting in bone loss and hypercalcemia (29), and that TNF- α causes hypercalcemia and increased bone resorption *in vivo* while it depresses bone formation (28). Although less is known about the role of GM-CSF in bone remodeling, this cytokine may condition osteoclast recruitment and differentiation (31). Furthermore, since parathyroid hormone has been found to stimulate the secretion of GM-CSF from bone cells (51) and GM-CSF increases IL-1 and TNF- α release from mononuclear cells (52), GM-CSF would appear to link systemic and local control of bone resorption. It is also possible, however, that monokine secretion from blood monocytes may be irrelevant with respect to postmenopausal bone loss. Our observations could be, in fact, relevant only to the extent that they suggest a regulatory effect of estrogen on marrow resident mononuclear cells, a hypothesis that remains to be proven.

In previous studies, we have shown that IL-1 activity increases after natural menopause and that treatment of postmenopausal women with ovarian steroids restores premenopausal IL-1 levels (33). Those data and the results of the present study suggest that manipulation of the estrogen status *in vivo* is associated with changes in blood mononuclear cell secretory activity *in vitro*. This association is apparently not restricted to IL-1 but affects other major mononuclear cell secretory products, such as TNF- α and GM-CSF. Estrogen receptors have recently been found in macrophages (53). Moreover, treatment *in vitro* of human monocytes with either estrogen or progesterone has been shown to regulate both IL-1 and TNF- α (54-56). These data support the hypothesis of a direct regulatory interaction between mononuclear cells and ovarian steroids and suggest that the bone-sparing effect of these hormones may be related, at least in part, to their ability to modulate monokine secretion. The finding that changes in cytokine secretion precede and/or coincide with corresponding changes in bone resorption after both oophorectomy and initiation of estrogen treatment, although not conclusive, is consistent with this hypothesis. It is also possible that monokine-independent mechanisms initiate the postmenopausal acceleration of bone turnover. In this model, the increased secretion of mononuclear cell immune products could be visualized as a response to activated bone remodeling. However, should this be the case, increased levels of mononuclear cell secretory products should be found only after an increase in bone resorption. Moreover, IL-1 and TNF- α are known to induce bone resorption *in vitro* and *in vivo* (24, 29). Thus, it is likely that the substantial release of mononuclear cell immune products associated with oophorectomy would have an influence on bone remodeling.

Although the present study did not reveal how estrogen regulates cytokine release, we speculate that a mononuclear cell-bone matrix interaction may account for this phenomenon. The lack of fever or other signs of "acute phase reaction" (19), despite elevated cytokine levels, suggests that cytokine secretion occurs locally, rather than systemically. In other studies, we have observed that collagen fragments and other bone matrix constituents released in the local microenvironment with bone resorption stimulate monocytic IL-1 (57) and GM-CSF secretion (unpublished observations) in a manner similar to PHA and LPS. In preliminary studies, we have also observed that estrogen decreases the monocytic

secretory response to bone matrix fragments (unpublished observations). Therefore, our working hypothesis is that with estrogen deficiency bone resorption-regulating cytokines are released in specific sites, upon activation of remodeling units, as a result of a local interaction between mononuclear cells and bone matrix fragments. Because of the ability of all three cytokines to promote osteoclast recruitment and differentiation (30, 31) and because of the autocrine effect (58) and the powerful bone-resorbing activity of both TNF- α and IL-1 (24, 25), a cascade of events leading to an amplification of bone resorption and further cytokine production could be initiated. Estrogen replacement could in turn decrease the monocytic secretory response to the bone matrix fragments, resulting in a lower number of mature osteoclasts available for the activation of new remodeling units.

Among the potential confounding factors that should be considered in interpreting our findings are the persistence of significant levels of circulating estrogen during the first week of follow-up and a prolonged lag time between changes in bone resorption and changes in biochemical indices of bone resorption. However, these factors are unlikely to have affected our data. Estradiol, the major estrogenic substance secreted by the human body, reaches postmenopausal blood levels within 2 days after oophorectomy (59). Likewise, studies in postmenopausal women subjected to rapid changes in hormonal status have revealed that significant changes in hydroxyproline/creatinine ratio, calcium/creatinine ratio, and BGP (60) can occur within 7 days. Thus, although a cause-effect relationship remains to be established, our results indicate that an increased production of mononuclear cell immune products may contribute to the postmenopausal increase in bone turnover and the bone-sparing effect of estrogen replacement.

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