Effect of Osteoclast Activating Factor
from Human Leukocytes on Bone Metabolism

LAWRENCE G. RAISZ, RICHARD A. LUBEN, GREGORY R. MUNDY,
JOHN W. DIETRICH, JOHN E. HORTON, and CLARENCE L. TRUMMEL

From the Departments of Pharmacology and Toxicology, Medicine, and
Clinical Dentistry, University of Rochester School of Medicine, Rochester,
New York 14642; Department of Medicine, University of Connecticut Health
Center, School of Medicine, Farmington, Connecticut 06032; and Laboratory
of Microbiology and Immunology, National Institute of Dental Research,
Bethesda, Maryland 20014

Abstract. The effects of osteoclast activating factor (OAF) released by normal human peripheral blood leukocytes cultured with phytohemagglutinin have been examined in organ culture. Like parathyroid hormone (PTH), OAF causes a rapid increase in the release of previously incorporated 45Ca from fetal rat bone after brief or continuous exposure; the bones also lose stable calcium and collagen content. The resorption response to OAF also resembles that of PTH in having a steep dose response curve and being only transiently inhibited by calcitonin and partially inhibited by increasing medium phosphate concentration. OAF-stimulated resorption was inhibited more effectively by cortisol than was PTH stimulation. The response to maximally effective doses of OAF was not enhanced by PTH or prostaglandin E, but submaximal doses gave additive effects. Both OAF and PTH inhibit collagen synthesis in fetal rat calvaria at the concentrations that stimulate bone resorption.

INTRODUCTION

Osteoclast activating factor (OAF) has been found in the supernatant fluid of cultures of normal human peripheral blood leukocytes that have been activated by

Address for reprints is U. S. A. Institute of Dental Research, Walter Reed Army Medical Center, Washington, D. C. 20012. Dr. Raisz's present address is Department of Medicine, University of Connecticut School of Medicine, Farmington, Conn. 06032. Dr. Raisz's present address is U. S. A. Institute of Dental Research, Walter Reed Army Medical Center, Washington, D. C. 20012. Dr. Raisz's present address is the Calcium Research Laboratory, Veterans Administration Hospital, Kansas City, Mo. 64133.

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*Abbreviations used in this paper: BGI, a chemically defined medium developed by Biggers, Gwatkin, and Heyner (1); OAF, osteoclast activating factor; 25-OH D, 25-hydroxy-vitamin D; 1,25-(OH),D, 1,25-dihydroxy-vitamin D; PGE, prostaglandin E; PHA, phytohemagglutinin; PTH, parathyroid hormone; SCT, salmon calcitonin; T/C ratio, treated/control ratio.

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purities were retained on the column and the potency relative to protein content was increased at least twofold. When DEAE cellulose was equilibrated with 0.05 M Tris at pH 7.5, OAF could be eluted by a linear NaCl gradient at approximately 0.1 M and was purified approximately 10-fold relative to Fraction IV OAF.

The PTH used for comparison with OAF was either partially purified on Sephadex G-100 with a potency of approximately 300 U/mg (kindly provided by Dr. William Y. W. Au) or purified on carboxymethyl cellulose with a potency of approximately 2,000 U/mg (kindly provided by Dr. G. D. Dr. G. D. Conn., (Sigma Chemical Co., St. Louis, Mo.), Indomethacin (kindly provided by Dr. Michael Ayvazian, Pfizer Inc., Groton, Conn.), and 1,25-dihydroxy-vitamin D₃ (1,25-(OH)₂D₃, kindly provided by Dr. John DeLuca) were also used. The last five agents were added to the medium in ethanol to a final ethanol concentration less than 1%; a similar concentration of ethanol was added to the control medium.

Bone resorption assay. Bone resorption was measured in organ cultures of the shafts of the radius and ulna of 19-day fetal rats (4). The bones were labeled by injection of the mother with 0.2-0.4 mCi of ⁵⁷Ca 1 day before sacrifice. Bones were cultured in BGJ supplemented with 1 or 4 mg/ml of bovine serum albumin Fraction V (BSA, Armour Pharmaceutical Co.). After 18-24 h of preincubation, bones were transferred to paired treated and control cultures for an additional 48 h, and ⁵⁷Ca was measured in the medium by liquid scintillation counting. In other experiments the bones were not paired and the percentage of ⁵⁷Ca released was measured at the end of the incubation period. In all experiments, stable calcium content of the bone was measured by atomic absorption spectrophotometry and collagen content by analysis of hydroxyproline (5).

Bone collagen synthesis studies. Effects on bone collagen synthesis were assessed using short-term cultures of half calvaria (the central thin portions of the frontal and parietal bone) from 21-day fetal rats. Two to four calvaria were incubated in 5 ml of BGJ with BSA for 24 h at 37°C in a humidified atmosphere of 5% CO₂ in air. The cultures were placed on a rocking platform (Belco Glass Inc., Vineland, N. J.) which tipped them to an angle of 15° at 6 cycle/min. During the last 2 h of incubation 5 μCi/ml of [2,3-³H]proline (New England Nuclear, Boston, Mass., 30 Ci/mole) was added to the medium. At the end of culture the bones were either dialyzed exhaustively against 0.5 M acetic acid or washed in 5% trichloroacetic acid, acetone, and ether, and weighed on a Cahn electrobalance (Cahn Div., Vincent Instruments Corp., Cerritos, Calif.). The weighed bones were homogenized in 0.5 M acetic acid, and aliquots were analyzed for radioactivity in collagenase digestible and noncollagen protein using purified bacterial collagenase (6). The relative proportion of collagen to total protein synthesis (percent collagen synthesized) was calculated using a factor of 5.4 to correct for the greater abundance of proline in collagen than in noncollagen protein.

RESULTS

Supernates of normal human peripheral blood leukocytes cultured with PHA always contained bone-resorbing activity. The amount of crude OAF that produced maximum bone resorption ranged from a twofold con-
OAF for 6–12 h produced as great an increase in 48-h 
$^{46}$Ca release as did continuous exposure; however, when
the concentration was below maximal, the response to
brief exposure was much decreased. Addition of PTH
or PGE$_2$ to maximally effective concentrations of OAF
did not stimulate bone resorption further; however, sub-
maximal concentrations of these agents could produce
additive effects (Table II). In other experiments (data
not shown) addition of OAF to maximally effective

### Table II

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (ng/ml)</th>
<th>4$^{46}$Ca release</th>
</tr>
</thead>
<tbody>
<tr>
<td>OAF</td>
<td>12.5</td>
<td>2.45±0.25‡</td>
</tr>
<tr>
<td>PTH</td>
<td>12.5</td>
<td>1.32±0.07‡</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>10$^{-8}$</td>
<td>1.40±0.14‡</td>
</tr>
<tr>
<td>OAF plus PTH</td>
<td>2× + 12.5 ng/ml</td>
<td>2.82±0.29‡</td>
</tr>
<tr>
<td>OAF plus PGE$_2$</td>
<td>2× + 10$^{-4}$ M</td>
<td>2.44±0.24‡</td>
</tr>
</tbody>
</table>

Bones were treated for 48 h after a 24-h preculture. Values are
means±SE for six to eight cultures. Pure bovine PTH was
used.

* T/C ratio.
† Significantly greater than 1.0 (*P* < 0.05).

doses of PTH or PGE$_2$ did not significantly increase
$^{46}$Ca release further.

As observed previously with PTH (4), the effect of
OAF on percent of total bone $^{46}$Ca release could be
reduced by increasing the phosphate concentration in
the medium from 1 to 3 mM; however, $^{46}$Ca release from
culture controls was also reduced so that T/C ratios
were unchanged. Pretreatment of the bone with 3 mM
phosphate did not alter the subsequent response to OAF
(Table III). Calcitonin blocked the initial effect of
OAF on $^{46}$Ca release; but, as observed previously with
PTH and 25-hydroxy-vitamin D$_3$ (25-OHD$_3$) (7), $^{46}$Ca
release increased during continued administration of su-
pramaximal doses of SCT and OAF (Fig. 3). OAF was
more sensitive to inhibition by cortisol than PTH (Ta-

### Table III

<table>
<thead>
<tr>
<th>PO$_4$ concentration</th>
<th>24-h preculture</th>
<th>48-h $^{46}$Ca release</th>
<th>48-h $^{46}$Ca release</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>OAF</td>
<td>Control</td>
<td>OAF</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>72±5</td>
<td>32±1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>44±4</td>
<td>20±2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>74±7</td>
<td>30±2</td>
</tr>
</tbody>
</table>

Values are means±SE for four cultures or pairs.
* Significantly different from values at 1 mM phosphate,
*P* < 0.05.
ble IV). Sustained inhibition was seen with as little as $10^{-7} \text{M}$ cortisol. Dexamethasone at $10^{-6}$ also blocked OAF when given simultaneously, but neither cortisol nor dexamethasone was effective at this concentration when given after a 24 h pretreatment with OAF (data not shown). The effect of OAF was not blocked by indomethacin at $10^{-4}$ to $10^{-8} \text{M}$ (Table V).

**Bone collagen synthesis assays.** After 24-h culture of fetal rat calvaria with high concentration of either PTH or OAF, the incorporation of a 2-h pulse of [{}^{3}H]-proline into collagenase-digestible protein was inhibited by more than 50% (Table VI). The incorporation of proline into noncollagen protein was less affected, so that collagen synthesis represented a smaller percentage of newly synthesized protein. This occurred with both crude and purified OAF preparations. In this system PTH and OAF were more effective both as stimulators of $^{40} \text{Ca}$ release and inhibitors of collagen synthesis than $1,25-(\text{OH})_{2}\text{D}_{3}$ (Table VI, experiment B). The results were similar with purified DEAE-OAF; only inhib-

**TABLE IV**

*Effect of Cortisol on Stimulation of $^{40} \text{Ca}$ Release by PTH, 1.6 $\mu$g/ml, or Crude OAF, 1X Concentrate, from Cultured Fetal Rat Bones*  

<table>
<thead>
<tr>
<th>Dilution</th>
<th>$^{40} \text{Ca}$ release</th>
<th>T/C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH/control</td>
<td>80±9 28±1</td>
<td>2.87±0.31*</td>
</tr>
<tr>
<td>PTH + cortisol $10^{-7}$/cortisol $10^{-7}$</td>
<td>75±4 20±1</td>
<td>3.67±0.15*</td>
</tr>
<tr>
<td>PTH + cortisol $10^{-5}$/cortisol $10^{-5}$</td>
<td>59±8 18±1</td>
<td>3.31±0.07*</td>
</tr>
<tr>
<td>PTH + cortisol $10^{-2}$/cortisol $10^{-2}$</td>
<td>26±5 17±1</td>
<td>3.11±0.17</td>
</tr>
<tr>
<td>OAF/control</td>
<td>82±3 28±1</td>
<td>2.94±0.10*</td>
</tr>
<tr>
<td>OAF + cortisol $10^{-7}$/cortisol $10^{-7}$</td>
<td>24±2 20±1</td>
<td>1.19±0.08</td>
</tr>
<tr>
<td>OAF + cortisol $10^{-5}$/cortisol $10^{-5}$</td>
<td>20±1 18±1</td>
<td>1.15±0.04</td>
</tr>
<tr>
<td>OAF + cortisol $10^{-2}$/cortisol $10^{-2}$</td>
<td>23±2 17±1</td>
<td>1.36±0.08*</td>
</tr>
</tbody>
</table>

Values are means±SE for four to eight cultures.  
* Significantly greater than 1.0, $P < 0.01$.

**TABLE V**

*Effect of Indomethacin on Stimulation of $^{40} \text{Ca}$ Release from Cultured Fetal Bone by Crude OAF, One-half Dilution*

<table>
<thead>
<tr>
<th>Indomethacin</th>
<th>48-h $^{40} \text{Ca}$ release, OAF</th>
<th>T/C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.60±0.19†</td>
<td></td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>1.67±0.11†</td>
<td></td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>1.96±0.18†</td>
<td></td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>1.96±0.17†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SE for four cultures.  
* T/C ratio.  
† Significantly greater than 1.0, $P < 0.01$.

**DISCUSSION**

The present studies indicate that the bone resorbing factor secreted by human leukocytes, which we have named OAF, acts much like PTH in organ cultures of fetal rat bone. Crude and partially purified preparations of OAF gave similar effects except that the cruder preparations more often showed loss of effect at supramaximal concentrations. This could have been due to toxic contaminants since at high concentrations certain OAF preparations inhibited $^{40} \text{Ca}$ release below controls. The ascending portions of the dose-response curves were steep and had similar slopes at all steps of purification. The similarity of slope, together with the chromatographic results (2), suggest that the stimulation of bone resorption by OAF could be ascribed to the effects of a single material. The slope of the dose-response curve for PTH was not significantly different from that for OAF while that for vitamin D metabolites is less steep and for PGEs much less steep (8). The first three agents all act rapidly and have similar effects on removal of mineral and matrix.

The interactions of OAF, PTH, and PGEs were examined largely because all three agents are potential mediators of neoplastic and inflammatory bone loss. Effects were additive at low concentrations, but PTH and PGEs did not increase resorption further in the presence of high concentrations of OAF. This probably reflects the limited capacity of the tissue to respond and need not indicate a common receptor or mechanism of action. In preliminary studies treatment with OAF, unlike PTH and PGEs, has not increased cyclic 3’,5’-adenosine monophosphate concentrations in cultured bone (L. G. Raisz, and W. A. Peck, unpublished observations).

OAF resembles PTH in being less effective at high medium phosphate concentrations but still stimulates re-
TABLE VI
Effect of PTH, 1 μg/ml, 1,25-(OH)2D3, or OAF on [3H]Proline Incorporation into Collagenase Digestible (CDP) and Noncollagen Protein (NCP) in Fetal Rat Calvaria

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specific activity</th>
<th>Collagen synthesized</th>
<th>4Ca release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDP</td>
<td>NCP</td>
<td>%</td>
</tr>
<tr>
<td>Experiment A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>270±20*</td>
<td>280±10</td>
<td>15.0±8</td>
</tr>
<tr>
<td>PTH, 1 μg/ml</td>
<td>130±10*</td>
<td>290±20</td>
<td>7.7±0.3*</td>
</tr>
<tr>
<td>Crude OAF, 1× concentration</td>
<td>70±10*</td>
<td>240±10*</td>
<td>5.2±0.5*</td>
</tr>
<tr>
<td>Experiment B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>182±25</td>
<td>496±74</td>
<td>6.4±0.1</td>
</tr>
<tr>
<td>1,25-(OH)2D3, 10 ng/ml</td>
<td>120±23</td>
<td>386±78</td>
<td>5.6±0.3*</td>
</tr>
<tr>
<td>PTH, 2 μg/ml</td>
<td>62±7*</td>
<td>260±24*</td>
<td>4.1±0.1*</td>
</tr>
<tr>
<td>Fraction IV OAF, 4× concentration</td>
<td>60±6*</td>
<td>243±13*</td>
<td>4.4±0.2*</td>
</tr>
</tbody>
</table>

Values are means±SE for six to eight half calvaria. Bones were cultured for 24 h and pulsed for last 2 h with 5 μCi/ml [3H]proline.

* Significantly different from control, P < 0.05.

Absorption in high phosphate medium. In fact, OAF activity was first detected using medium RPMI 1640 which has a phosphate concentration over 5 mM (2). OAF showed the characteristic initial inhibition by calcitonin followed by escape (7). Failure to inhibit OAF or PTH with indomethacin suggests that prostaglandin synthesis does not mediate the resorptive effects of these two agents. The concentration of indomethacin used has been shown to inhibit complement dependent stimulation of prostaglandin synthesis and bone resorption (9) in our culture system. The only difference we detected between OAF and PTH effects on bone resorption was in their susceptibility to inhibition by cortisol. The reason for this difference is not known; however, it might explain the clinical effectiveness of glucocorticoids in some forms of hypercalcemia and their ineffectiveness in hyperparathyroidism.

In short-term cultures of fetal rat calvaria, PTH and OAF both inhibited the incorporation of [3H]proline into collagenase-digestible protein. Noncollagen protein labeling was less inhibited. This could represent a specific inhibition of osteoblastic activity which has been postulated for PTH on the basis of autoradiographic studies (10). PTH and OAF appeared to be more potent inhibitors than 1,25-(OH)2D3 even when the vitamin D metabolite was added in a dose 10 times that required to produce maximal stimulation of bone resorption. We have found that prostaglandins also inhibit collagen synthesis in calvaria, but only at concentrations (10-4 to 10-5 M) which are high relative to those that stimulate resorption (10-4 to 10-5 M) (11). The rough reciprocal relation between inhibition of osteoblastic collagen synthesis and stimulation of osteoclastic bone resorption in culture need not indicate any connection between the two phenomena at the cellular level. Moreover, in the case of OAF, it is not certain that these two effects are mediated by the same substance, although the persistence of both effects through two stages of purification supports this possibility. DEAE-OAF is not pure and may still contain toxic contaminants. This seems unlikely since OAF inhibited collagen labeling at concentrations which did not affect noncollagen protein labeling. These results do not bear on the specificity of OAF for bone. We are currently testing whether these preparations influence metabolism of other tissues.

Despite its powerful effects on bone in vitro, we have been unable to increase serum calcium concentrations by injection of large quantities of OAF in intact or thyroparathyroidectomized rats (Raisz, unpublished observations). A similar dissociation between in vitro and in vivo effects has been observed with prostaglandins (12). This could have been due to rapid inactivation of OAF in the circulation which prevented its reaching bone in sufficient concentrations. Direct measurements of OAF in blood will be required to assess this possibility. There is now some evidence that OAF production may be clinically important. OAF-like factors have been found in the supernates of cultured lymphoid cell lines as well as short-term cultures of bone marrow cells aspirated from patients with myeloma (8, 13). OAF could also be released in chronic inflammation by activated lymphocytes (14). The effects of OAF we observed in vitro are consistent with the nature of the bone lesions in periodontal disease and in myeloma. In both disorders, bone destruction is osteoclastic, local, and not regularly associated with an increase in bone formation. It is even more difficult to
ascribe a physiologic function to OAF. If bone marrow lymphocytes produced OAF during their proliferation, this might enhance endosteal bone resorption and facilitate the rapid development of the medullary cavity. If so, some forms of osteopetrosis in animals and man in which bone marrow formation and bone resorption are both decreased and medullary bone formation appears to be excessive could be related to deficient OAF production.

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REFERENCES


