

# Thymus-derived lymphocytes and their interactions with macrophages are required for the production of osteoclast-activating factor in the mouse

(immune cell interactions/osteoclastic bone resorption)

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**ABSTRACT** A bone-resorbing factor, comparable to the osteoclast-activating factor (OAF) produced from peripheral blood leukocytes, is shown to be produced by murine spleen cells activated with the T-cell mitogen Con A. Murine OAF is demonstrated here as being a product of the interaction between thymus-derived T lymphocytes and macrophages. Activation of T cells in the presence of macrophages with Con A yields culture supernatants with OAF activity. This OAF activity is not dialyzable and is not extracted by lipid solvents. Purified B cells in the presence or absence of macrophages and cocultured with Con A or activated with the B-cell-specific mitogen lipopolysaccharide yield culture supernatants with no detectable OAF activity. Similarly, macrophages cocultured with Con A or activated with lipopolysaccharide fail to yield culture supernatants with bone resorbing activity. These types of immune cell interactions are similar to that required for the production of lymphokines. These data support the hypothesis that one aspect of regulation of bone remodeling is through cells of the immune system.

Human peripheral blood leukocytes have been shown to release *in vitro*, after activation with antigen or the mitogen phytohemagglutinin (PHA), a soluble factor which is able to stimulate osteoclastic bone resorption when added to bone organ cultures (1–3). Consequently, this factor has been called osteoclast-activating factor (OAF) (1). Human OAF has been shown to be a protein by virtue of its sensitivity to trypsin and Pronase and is heat labile. Human OAF does not crossreact with antibodies to parathyroid hormone (PTH) or prostaglandin E (PGE) and is not extracted by lipid solvents, therefore excluding both PGE and metabolites of vitamin D, which are also potent stimulators of bone resorption (4, 5). Human OAF has been reported to have a molecular size range from 9 (6) to 25 kilodaltons (4, 5); another molecular size species of <3.5 kilodaltons has also been reported (7).

OAF production has been linked with increased bone resorption and bone loss in a number of human pathological processes, such as periodontal disease (8), multiple myeloma, lymphosarcoma, and other types of cancer (9, 10). OAF activity has also been demonstrated in supernatants from cultures of tumor cell lines of lymphoid origin (11), activated human tonsil cells (6), and activated mouse spleen cells (12), therefore demonstrating the ability of a number of lymphoid tissues and cell lines to produce such a factor. The production of OAF requires the presence and interaction of lymphocytes and macrophages (3, 13) and this factor has consequently been classified among the lymphokines (14, 15). Further identification of the specific cell types involved in OAF production is still lacking and, more specifically, controversy still exists as to whether, under normal conditions, it is a

T- or a B-lymphocyte product (14) (or both) and whether it might play a role in the local regulation of bone remodeling.

In this report, we have approached the problem of identifying the cell type(s) involved in OAF production by using normal mouse spleen cells cultured *in vitro* because (i) this organ contains the three major immunocompetent cell types—i.e., T lymphocytes (30%), B lymphocytes (50%), and macrophages (10%); (ii) monoclonal antibodies directed against antigens specific for certain cell types are available and well characterized; and (iii) the approach of the role of local OAF production in the regulation of normal bone remodeling required the use of normal, nontransformed cell sources.

The results of our studies suggest that (i) OAF production by normal mouse spleen cells requires the presence and interaction of Thy-1<sup>+</sup> T lymphocytes and Ia<sup>+</sup> macrophages; (ii) B lymphocytes, cultured in the presence of Con A or activated with lipopolysaccharide (LPS), in the presence or absence of macrophages, do not produce detectable amounts of OAF activity; (iii) the presence of B lymphocytes in Con A-activated spleen cell cultures does not alter OAF production by T lymphocytes–macrophage interaction; and (iv) macrophages cocultured with Con A or activated with LPS do not produce detectable amounts of bone-resorbing activity.

## MATERIALS AND METHODS

**Mice.** C57BL/6J, C3H/DiSn, C3H/HeJ, and C3H/HeSn mice were obtained from The Jackson Laboratory. For all experiments mice were matched for sex and age.

**OAF Production.** Spleen cell suspensions were washed and adjusted to 10<sup>7</sup> cells per ml in RPMI 1640 containing 1% heat-inactivated fetal calf serum, 100 mM L-glutamine, 25 mM Hepes, 50 μM 2-mercaptoethanol, and antibiotics. Spleen cells were cultured at 1 ml in Costar 24-well cluster tissue culture dishes containing 2.5 μg of Con A (Sigma) per ml. The same conditions were used for purified T cells. B-cell activation was accomplished by culturing the purified B cells with 40 μg of LPS 055:B5 (Difco) per ml. Cultures were incubated for 48 hr at 37°C with 5% CO<sub>2</sub> in air. At the end of the culture period the cultures were spun to remove the cells and the culture supernatants were filtered through 0.45-μm Millipore filters. In all experiments OAF-containing supernatants were used within hours of termination of culture.

**Purification of T Cells and B Cells.** *Panning.* Fisher 100 × 15 mm Petri dishes were coated with 50 μg of affinity-purified goat anti-mouse Ig. To each plate 30 × 10<sup>6</sup> spleen cells were added and incubated for 1 hr at 4°C. The T cells and macrophages were removed by swirling the plates (16). It

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Abbreviations: OAF, osteoclast-activating factor; PHA, phytohemagglutinin; LPS, lipopolysaccharide; PTH, parathyroid hormone; PGE, prostaglandin E.

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has previously been shown that these cells are >95% Ig negative by fluorescence (16). B cells were collected by incubating the plates at 37°C for 60 min, followed by vigorously pipetting the dishes with phosphate-buffered saline/2% fetal calf serum. This fraction is >95% Ig positive by fluorescence. B-cell fractions were then treated with anti-Thy-1 antiserum and complement to remove residual T cells. The remaining cells were <1%  $\theta$  positive by fluorescence.

**Column Separation.** Column separation of cells into T and B cells was accomplished by the technique of Wigzell (17).

**Treatment of Cells with Antiserum.** Spleen cell suspensions or purified T cells and macrophages were incubated with monoclonal anti-Thy-1.2 antiserum (J1J, kindly provided by John Sprent) at a final concentration of 1:1000 in RPMI 1640/5% fetal calf serum for 30 min at room temperature. Cells were washed in RPMI 1640 and resuspended in selected batches of low-background rabbit complement, diluted 1:7. The cells were incubated for 30–40 min at 37°C, washed, and adjusted to the appropriate concentration. An identical treatment was used to remove Ia<sup>+</sup> cells: monoclonal anti I-A (Y3P) and anti I-E (Y-17) were used at a final concentration of 1:100.

**Preparation of Spleen-Adherent Cells.** Spleen-adherent cells were prepared by placing  $50 \times 10^6$  spleen cells in 10 ml of RPMI 1640/5% fetal calf serum in 100-mm Costar tissue culture dishes and incubated at 37°C for 2 hr. After the incubation the nonadherent cells were removed by swirling the dish and decanting the cell suspension. This procedure was repeated twice. To remove the bound spleen-adherent cells, 4 mg of lidocaine (Invenex, Chagrin Falls, OH) per ml was added to cover the surface of the dish and the plates were incubated for 30 min at 4°C. Adherent cells were collected by vigorous pipetting of the dishes with RPMI 1640. Adherent cells were then treated with monoclonal anti-Thy-1 antibody plus complement to remove residual T cells.

**Treatment with Indomethacin.** Indomethacin (Sigma), an irreversible inhibitor of prostaglandin synthesis, was dissolved in 95% ethanol to 1 mM and diluted to a working concentration in RPMI 1640. Treatment of cells was by one of two methods: (i) indomethacin was added directly to the cell cultures or (ii) cells were incubated with indomethacin for 1 hr at 37°C and subsequently washed three times. The concentration of indomethacin used was either 1 or 10  $\mu$ M. Although equivalent amounts of ethanol were added to control cultures, no effect was ever noted at the concentration used.

**[<sup>3</sup>H]Thymidine Incorporation.** Varying numbers of spleen cells were placed in flat-bottom 96-well microculture trays (Falcon) with 2.5  $\mu$ g of Con A per ml or 40  $\mu$ g of LPS per ml in a total volume of 200  $\mu$ l of complete medium. After 24 hr, 1  $\mu$ Ci (1 Ci = 37 GBq) of [<sup>3</sup>H]dThd (New England Nuclear) was added and at 48 hr the cultures were harvested on glass fiber strips with a MASH II (Microbiological Associates). The dried filters were assayed for radioactivity in Betafluor (National Diagnostics, Somerville, NJ) in a Beckman scintillation counter. Results were expressed as the average ( $\pm$ SEM) cpm of triplicate cultures. Controls contained no mitogen.

**Bone Resorption Assay.** The bone resorption assay was performed as described by Raisz and Niemann (18). Eighteen-day pregnant rats were injected with 200  $\mu$ Ci of <sup>45</sup>CaCl<sub>2</sub> (Amersham) and sacrificed 24 hr later. The fetuses were removed and their ulna and radii were dissected free of soft tissue. The cartilaginous ends were removed and the calcified shafts were precultured for 24 hr in BGJb medium (GIBCO) supplemented with bovine serum albumin (Pentex, bovine serum albumin fraction V, Miles), 2 mM L-glutamine, and penicillin/streptomycin. The paired bones were then transferred to fresh medium mixed 1:1 or 2:1 with either the supernatant to be tested or the correspondent control medium and were cultured for 2 days. The ratio of <sup>45</sup>Ca released

into the medium from treated vs. control bone cultures is used as a measure of bone resorption: a ratio significantly >1 indicates a stimulation of bone resorption. PTH-(1–34) (Beckman) was used in all experiments as a positive control for the ability of the bone cultures to respond to a stimulation of bone resorption and gave an average ratio of  $1.39 \pm 0.13$  at 1 unit/ml. Precultures were performed for 24 hr to allow any unbound <sup>45</sup>Ca to leak from the bone into the medium.

## RESULTS

**Con A-Activated Spleen Cell Supernatants Induce Bone-Resorbing Activity.** In a first series of experiments, spleen cell suspensions were activated with the T-cell-specific mitogen Con A. The supernatants from these cultures were tested after 48 hr for their ability to mobilize <sup>45</sup>Ca from prelabeled fetal rat long bones *in vitro*. This lectin induced both a mitogenic response, as monitored by [<sup>3</sup>H]dThd incorporation (Table 1, experiment a), and a significant, dose-dependent release of <sup>45</sup>Ca from bone (Table 1, experiment b) to an extent comparable to PGE at 10  $\mu$ M and PTH at 1 unit/ml (Table 1, experiment b). Con A, added directly to the organ cultures, did not stimulate bone resorption.

**Comparison of the OAF Production by Spleen Cell Fractions Stimulated with Con A.** To determine which cell type(s) was required for OAF production, spleen cells were separated into T and B cells. B cells were positively selected by adherence to plastic plates (panning) coated with affinity-purified goat anti-mouse Ig antibody. The plate-adherent B cells were subsequently treated with monoclonal anti-Thy-1 antiserum and complement to remove residual T cells. The use of these two techniques leaves a population of B cells with no detectable T cells as measured by (i) direct staining with fluorescent anti-Thy-1 antiserum, (ii) proliferation response after Con A activation, and (iii) the ability to secrete antibody in the presence of interleukin 2 (formerly T-cell growth factor). T cells (and macrophages) were isolated by one of two techniques. The first technique was to pass spleen cells through Wigzell columns that have complexes of mouse Ig–anti-mouse Ig bound to glass beads; the column nonadherent fraction contains T cells and macrophages. The second technique was panning, in which the nonadherent fraction was used as a source of T cells and macrophages. By using both techniques, B-cell contamination of the T-cell fraction was <1% by fluorescence. These two highly purified cell fractions (B cells and T cells with macrophages) were then adjusted to a concentration of  $10^7$  cells per ml and activated with Con A (2.5  $\mu$ g/ml) in culture. Only the supernatants from the T cell/macrophage fraction were able to induce bone resorption and did so in a manner similar to the spleen cell suspensions, therefore suggesting that the B cells did not affect the production of OAF (Table 1, experiment c). To test whether B cells could produce OAF in the presence of macrophages, 10% spleen-adherent cells were added back to the B-cell fraction. Neither the B-cell fraction nor the B-cell fraction with added macrophages was able to induce bone resorption in our system when cultured with Con A (Table 1, experiment c).

**Effect of Anti-Thy-1 Antiserum Pretreatment on the Ability of Spleen Cells or Purified T Cells and Macrophages to Produce OAF.** More definitive evidence for the role of T lymphocytes in OAF production has been obtained by specifically killing T cells by pretreatment with monoclonal anti-Thy-1 antibody and complement (Table 1, experiment d). As a control, spleen cells were treated with complement alone; this treatment did not alter the ability of the supernatants to induce bone resorption after stimulation with Con A. However, treatment of spleen cells or purified T cells and macrophages with anti-Thy-1 antiserum completely blocked their ability to produce active supernatants. We concluded from this series of experiments that T cells are necessary for the

Table 1. Identification of the cell types required for OAF production by Con A-stimulated spleen cell suspensions

Experiment	Cells per well, no.	Mitogen	Mitogenic response, cpm
a	$2 \times 10^5$	+	$150,011 \pm 1693$
	+	-	$6,648 \pm 467$
	$1 \times 10^5$	+	$74,061 \pm 2607$
	+	-	$2,899 \pm 85$
	$5 \times 10^4$	+	$17,153 \pm 1144$
	+	-	$1,125 \pm 89$
Condition		$^{45}\text{Ca}$ release (T/C ratio)	Number of pairs
b	PGE <sub>2</sub> , 10 $\mu\text{M}$	$1.45 \pm 0.23^*$	6
	PTH, 1 unit/ml	$1.39 \pm 0.13^*$	12
	Spleen cells cultured without Con A	$0.89 \pm 0.07$	6
	OAF (spleen cells cultured with Con A)		
	10%	$1.07 \pm 0.07$	6
	30%	$1.05 \pm 0.08$	6
	50%	$1.36 \pm 0.08^*$	29
	60%	$1.60 \pm 0.09^*$	36
	80%	$1.35 \pm 0.07^*$	6
	100%	$1.37 \pm 0.11^*$	6
c	Spleen cells	$1.36 \pm 0.08^*$	29
	T cells + M $\phi$	$1.34 \pm 0.08^*$	24
	B cells	$0.99 \pm 0.06$	6
	B cells + M $\phi$	$0.99 \pm 0.09$	6
d	Spleen cells + C	$1.65 \pm 0.07^*$	24
	Spleen cells + anti-Thy-1 + C	$1.09 \pm 0.03^\dagger$	23
	T cells + M $\phi$ + C	$1.38 \pm 0.27^*$	6
	T cells + M $\phi$ + anti-Thy-1 + C	$0.88 \pm 0.07^\dagger$	6
e	Spleen cells + C	$1.81 \pm 0.24^*$	6
	Spleen cells + anti-I-A + C	$1.29 \pm 0.19^\dagger$	6
	Spleen cells + anti-I-E + C	$1.26 \pm 0.08^{*\dagger}$	6
f	Spleen cells	$1.36 \pm 0.08^*$	29
	Spleen cells + indomethacin, 10 $\mu\text{M}$	$1.37 \pm 0.15^*$	12

cpm and T/C ratio values are given as mean  $\pm$  SEM. T/C ratio, treated/control ratio; C, complement; M $\phi$ , macrophages. Experiments a-f are as follows: experiment a, response of C57BL/6J spleen cells to the mitogen Con A; experiment b, comparative effects of PTH, PGE<sub>2</sub>, and OAF on  $^{45}\text{Ca}$  release from fetal rat long bones; experiment c, comparison of OAF production by spleen cell fractions stimulated with Con A; experiment d, effect of anti-Thy-1 antiserum pretreatment on the ability of spleen cells or purified T cells and macrophages to produce OAF; experiment e, effect of anti-Ia antiserum pretreatment on the ability of spleen cells to produce OAF; experiment f, effect of indomethacin treatment on OAF production by Con A-activated spleen cell suspensions.

\*Value significantly different from 1 (a  $^{45}\text{Ca}$  release T/C ratio significantly higher than 1 indicates bone resorption,  $P < 0.05$ ).

$^\dagger$ Value significantly different from cultures not pretreated with antiserum.

production of OAF.

**Effect of Anti-Ia Antiserum Pretreatment on the Ability of Spleen Cells to Produce OAF.** Because the first series of experiments indicated that B cells, which bear Ia antigens on

their surface (class II antigens), did not participate in OAF production, another series of experiments were done to assess the contribution of the other Ia-bearing subset, the macrophages, in OAF production. Pretreatment of spleen cells with monoclonal antisera directed at either the A $_{\alpha}$ :A $_{\beta}$  or E $_{\alpha}$ :E $_{\beta}$ <sup>†</sup> molecules markedly decreased the ability of the remaining cells to produce bone-resorbing activity after Con A stimulation (Table 1, experiment e). These data indicate that Ia<sup>+</sup> macrophages as well as Thy-1<sup>+</sup> T cells are required for OAF production.

**The Bone-Resorbing Activity Is Not Due to Prostaglandins.** The possibility that this bone-resorbing activity was due to prostaglandins was then examined. The following observations ruled out PGE as being responsible for the induction of resorption. (i) Radioimmunoassay of PGE<sub>2</sub> (New England Nuclear) showed concentrations between 0.01 and 1 nM in our active supernatants, values that are lower than the first active concentration required by others in this bone resorption assay (19) and four orders of magnitude lower than the concentration of PGE<sub>2</sub> required in our assay to reach similar ratios as our OAF preparation (see Table 1, experiment b). (ii) No difference in PGE<sub>2</sub> concentration could be found between Con A-activated and nonactivated spleen cell cultures, despite a marked difference in the abilities of these supernatants to induce resorption (Table 1, experiment b). (iii) Indomethacin treatment (10  $\mu\text{M}$ ) lowered the PGE<sub>2</sub> concentration by a factor of 10 (data not shown) without affecting the bone-resorbing activity (Table 1, experiment f). (iv) Extensive dialysis of our supernatants through a Spectropor 2 membrane (12,000–14,000 molecules weight cutoff) against buffer or medium did not alter their resorbing activity, whereas PGE<sub>2</sub> (350 daltons) should be readily dialyzable under these conditions. However, in all culture supernatants, whether stimulated with mitogen or not, detectable levels of PGE<sub>2</sub> were present. We therefore proceeded to extensive lipid extraction by ethyl acetate treatment of our supernatants to remove prostaglandins: this lipid extraction failed also to alter the bone-resorbing activity of our supernatant.

**Effect of LPS Activation on OAF Production by B Cells and Macrophages.** In a second series of experiments, LPS, a specific B-cell mitogen, was used to test whether highly purified B lymphocytes were able to produce a bone-resorbing factor after appropriate stimulation. LPS, having previously been shown to directly stimulate bone resorption *in vitro* (20), was first tested for its ability to increase  $^{45}\text{Ca}$  release from bone organ cultures. As expected, LPS significantly increased  $^{45}\text{Ca}$  release in the culture medium (Table 2, experiment b) but only moderately. To have an appropriate control group for this LPS background activity, substrains of the C3H strain were selected: C3H/HeJ is a substrain in which B cells bind LPS but undergo a low mitogenic response (21). This substrain was therefore used as a control for LPS carry-over in the bone culture medium. B cells from two other substrains (C3H/DiSn and C3H/HeSn) that also bind LPS but undergo a high mitogenic response after binding constituted the experimental groups (Table 2, experiment a). Supernatants from B-lymphocyte cultures of these three substrains after stimulation with LPS did not show any OAF activity, whether there was a mitogenic response or not (Table 2, experiment c). The results show that B cells and macrophages that are capable of responding to LPS by proliferation (B cells) and subsequently go on to secrete their biologically active product (antibody and monokines, respectively) fail to secrete any detectable OAF activity. On the other hand, the T cells/macrophage fraction of these same substrains

<sup>†</sup>A $_{\alpha}$ :A $_{\beta}$  = E $_{\alpha}$ :E $_{\beta}$  represents class II glycoprotein antigens detected on the surface of both mature B cells and macrophages. The genes that code for these determinants are found on chromosome 17 within the major histocompatibility locus.

Table 2. Inability of the B-cell mitogen LPS to induce OAF production by spleen cell suspensions

Experiment	Condition	Mitogen	Mitogenic response, cpm
a	C3H/DiSn	+	122,283 ± 3125
		-	1,880 ± 223
	C3H/Hen	+	114,167 ± 1165
		-	2,036 ± 136
	C3H/HeJ	+	24,647 ± 533
		-	820 ± 132
b	Bones + LPS	<sup>45</sup> Ca release (T/C ratio)	Number of pairs
	Spleen cells + LPS	1.14 ± 0.06*	12
c	C3H/DiSn	1.29 ± 0.13*	12
	C3H/HeSn	0.98 ± 0.06	12
	C3H/HeJ	1.04 ± 0.13	12
d	Spleen cells + C	1.00 ± 0.05	6
	Spleen cells + anti-Thy-1 + C	1.12 ± 0.05	6
		1.15 ± 0.05*	6

cpm and T/C ratio values are given as mean ± SEM. T/C ratio, treated/control ratio; C, complement. Experiments a-d are as follows: experiment a, response of C3H substrain B cells to the mitogen LPS ( $2 \times 5$  cells were used for all determinations); experiment b, effect of LPS on direct stimulation of bone resorption by fetal rat long bones in culture and on OAF production by spleen cell suspensions; experiment c, effect of LPS on OAF production by B lymphocytes from C3H substrains; experiment d, effect of anti-Thy-1 antiserum pretreatment on the ability of LPS-activated spleen cell suspensions to induce bone resorption.

\*See footnote to Table 1.

showed a normal ability to produce OAF when stimulated with Con A. The slight resorbing activity observed with spleen cell suspensions stimulated with LPS was not above LPS background and was not affected by treatment with anti-Thy-1 antiserum and complement (Table 2, experiment d), therefore further demonstrating that (i) this background is not due to T-cell-dependent OAF production but rather to a direct stimulation of bone resorption by LPS or the release of other soluble products by LPS-activated macrophages and (ii) LPS-activated B cells or macrophages (or both) cannot produce an amount of OAF comparable to T cells and macrophages activated with Con A.

## DISCUSSION

We conclude from these studies that, in the mouse, OAF production requires an interaction between activated Thy-1<sup>+</sup> T lymphocytes and Ia<sup>+</sup> macrophages (or their soluble products). In addition, this study shows that B cells or macrophages (or both), activated with Con A or LPS, cannot produce any detectable bone-resorbing factor.

Although the requirement for a lymphocyte-macrophage interaction for the production of OAF by human peripheral blood leukocytes has previously been demonstrated (3, 22), the question of whether it is a T or B lymphocyte (or both) remained unresolved. Chen *et al.* (23) concluded that, after enrichment by rosette formation, both T-cell- and B-cell-enriched fractions could release OAF. The B-cell fraction released OAF even when unstimulated. These studies, however, remain questionable given the lack of highly specific purification techniques. Contamination of the B-cell-enriched fraction by T cells could account for the observed OAF production. In our hands, B cells purified by positive selection and subsequent treatment with anti-Thy-1 antiserum and

complement to eliminate all detectable contaminating T cells, cultured with Con A or activated with LPS in the presence or absence of macrophages, were not able to induce bone resorption. Previous work by other investigators has shown that numerous B-cell tumor lines, including multiple myeloma cells as well as T-cell tumor lines, release bone-resorbing activity (9, 10). This would suggest that B cells are able, under these circumstances, to make OAF. However, as discussed by Mundy *et al.* (11), this ability might be due to the tumor transformed state of the cells that could allow B cells to express a normally repressed gene coding for OAF. Alternatively, this resorbing activity could be related to one of the bone-resorbing factors of malignancy (24). Our results indicate that in nontumor-bearing, normal murine spleen cells, only the T lymphocyte is able to express this gene.

It is not possible to determine from these experiments whether OAF is a product of the T lymphocytes or of the macrophages, following the coculture of these two cell types. It is noteworthy that macrophages cultured in the presence of Con A or activated with LPS did not yield culture supernatants containing detectable amounts of bone-resorbing activity. Notwithstanding the fact that Con A is not thought to directly stimulate macrophages, LPS activation of these cells is both potent and specific and is a well-established way to induce secretion of interleukin 1, a monokine recently reported to induce bone resorption (25). Our observations therefore suggest that, in our system, the bone-resorbing activity is not due to interleukin 1 production by macrophages but due to OAF production, most likely by T lymphocytes. Further work will be necessary to dissociate these two molecules and establish the final cell source of OAF.

The question of whether these cellular interactions between T lymphocytes and macrophages leading to OAF production require prostaglandin synthesis, as suggested by Yoneda and Mundy for human OAF production (13), has not been specifically addressed in this study. The observation that the presence of indomethacin in Con A-activated murine spleen cell cultures did not prevent OAF production does not exclude a role for prostaglandins because very low concentrations of this compound might be sufficient to mediate the cellular interactions. These experiments solely demonstrate that our bone-resorbing activity cannot be attributed to prostaglandins.

These results are of importance in the understanding of both pathological resorption and the normal bone remodeling process because T cells, which are known to act synergistically with macrophages in numerous immunological reactions (15), are shown here to interact with macrophages for the release of a factor able to activate osteoclasts, themselves considered to be of the mononuclear phagocyte lineage (26-28). This could therefore be viewed as another instance in which T cells activate macrophages to mediate a specific function—i.e., bone resorption. In addition, monocytes have been shown to be able to mobilize <sup>45</sup>Ca from bone *in vitro* (29, 30) and to be chemotactically attracted by some components of this tissue (31-33). Furthermore, at least one instance in which T cells or a thymic factor (or both) control bone resorption *in vivo* has been reported in the literature (34, 35): osteopetrotic (*op/op*) rats, in which osteoclasts are present but not active, have been shown to resume normal osteoclastic bone resorption when injected with normal thymic cell suspensions, therefore also raising the possibility of a role for the T lymphocytes in osteoclast activation, both *in vivo* and *in vitro*. Finally, T lymphocytes have been shown to have receptors for PTH (36, 37), calcitonin (38), and 1,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub> (39), the three major calcium-regulating hormones with specific effects on bone cells and bone remodeling; vitamin D metabolites have also been shown to have marked effects on the differentiation of cells of the

mononuclear phagocyte system (40) in addition to their effects on bone remodeling. Finally, it has recently been suggested (41) that diphosphonates, which inhibit bone resorption, also impair T-cell, but not B-cell, functions. These data provide further evidence for an increasing body of information that links the immune system with bone remodeling and suggest the possibility of a role for lymphocytes and macrophages in the regulation of the process. Further experiments to delineate the role of the macrophage [and/or dendritic cell, which is also Ia positive (42)] and the particular subset of T cells responsible for OAF production as well as purification of this lymphokine will be necessary to better establish its role in normal and pathological bone remodeling.

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