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TGF-β Enhances Osteoclast Differentiation in Hematopoietic Cell Cultures Stimulated with RANKL and M-CSF

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TGF-β has been shown to inhibit and stimulate osteoclastogenesis. The purpose of this study was to evaluate the effects of TGF-β in hematopoietic cell cultures stimulated with RANKL and M-CSF. In cocultures of hematopoietic cells and BALC cells (a calvarial-derived cell line), TGF-β inhibited tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cell formation. In contrast, TGF-β enhanced TRAP-positive multinucleated cell formation up to 10-fold in hematopoietic cell cultures containing few osteoblastic/stromal cells. Likewise, TGF-β increased the number of calcitonin receptor (CTR)-positive multinucleated and mononucleated cells in a concentration-dependent manner. An increase in cell size and multinuclearity was also observed in the presence of TGF-β. The stimulatory effects of TGF-β were dependent on the presence of M-CSF and RANKL. When differentiated on bovine cortical bone slices, these cells formed resorption lacunae. These results suggest that TGF-β has a direct stimulatory effect on osteoclastogenesis in hematopoietic cells treated with RANKL and M-CSF.

Osteoclast differentiation requires cell-cell contact between osteoblastic/stromal cells and cells of the hematopoietic lineage. Recently, proteins involved in the cell-cell interaction have been identified. RANKL (receptor activator of nuclear factor NF-κB ligand) [which is identical to TRANCE (TNF-related activation-induced cytokine) and ODF (osteoclast differentiation factor)/OPGL (osteoprotegerin ligand)] was identified as the ligand [for review see Suda *et al.* (1)]. RANKL is a member of the TNF ligand family and is expressed by osteoblastic/stromal cells. RANKL activates its receptor RANK, which is expressed on osteoclasts and osteoclast progenitor cells, to induce osteoclast differentiation and function. A decoy receptor, OPG/OCIF (osteoclastogenesis inhibitory factor), has also been identified [for review see Suda *et al.* (1)]. OPG is a secreted member of the TNF receptor family. OPG binds to RANKL, thus inhibiting osteoclast differentiation or function. Cells of lymphatic system also express RANK, RANKL, and OPG [for review see Kwon *et al.* (2)]. Identification of the proteins that are critical for osteoclast differentiation has greatly enhanced the understanding of the control of osteoclast differentiation and function. In addition, utilization of a soluble form of RANKL allows for stimulation of osteoclast differentiation and function in the absence of osteoblastic or stromal cells.

Transforming growth factor-betas (TGF-βs) represent a family of multifunctional cytokines that stimulate or inhibit cell function and replication in numerous cell types (3). In bone, TGF-β appears to be involved in the regulation of osteoblastic bone formation and osteoclastic bone resorption, as well as, the coupling between these processes [for review see Centrella *et al.* (4)]. Bone is a major storage site for TGF-β and osteoclastic bone resorption releases TGF-β that can modulate bone formation and resorption (4–7). The effects of TGF-β on bone and bone cells vary with the model and the source and isoform of TGF-β examined [for review Mundy (8)]. In osteoclast differentiation cultures, TGF-β has been shown to have biphasic effects (9, 10) or dose dependent inhibitory effects on osteoclast differentiation (11–14). Recent studies have demonstrated that TGF-β upregulates mRNA for OPG (15, 16) in osteoblastic or stromal cells which leads to decreased osteoclast differentiation. Since the previously described effects of TGF-β on osteoclast differentiation were examined in models containing osteoblastic/stromal cells, the inhibition of osteoclast differentiation may be due to an increase in OPG secretion. The purpose of this study was to examine the effects of

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TGF-β in RANKL and M-CSF stimulated bone marrow cultures. TGF-β dramatically increased osteoclast-like cell formation in bone marrow or spleen cell cultures stimulated to differentiation with M-CSF and RANKL. This effect was observed in the absence of supporting osteoblastic/stromal cells and reveals an unsuspected stimulatory affect of TGF-β on osteoclast formation.

METHODS

Cocultures of bone marrow cells and BALC cells. Osteoclast differentiation was studied in cocultures of bone marrow cells and BALC cells (a murine calvarial-derived cell line) as described by John *et al.* (17). Briefly, bone marrow cells from the femurs of male Balb/C mice (aged 10 weeks, Taconic, Germantown, NY) were seeded into 24-well cluster dishes (Costar, Cambridge, MA) at a density of 5×10^4 mononuclear cells/cm^2 in growth media [RPMI media (Life Technologies, Gaithersburg, MD) containing 5% heat-inactivated FBS (Hyclone, Logan, UT) and 1% antibiotic/antimycotic solution (Life Technologies)]. BALC cells (1.5×10^4 cells/cm^2) were cocultured with the bone marrow cells. The cultures were treated with 1,25(OH)$_2$ vitamin D$_3$ (1,25(OH)$_2$D$_3$, 10^{-8} M, Biomol, Plymouth Meeting, PA) in the presence or absence of 0.01–10 ng/ml recombinant human TGF-β1 (TGF-β, R & D Systems, Minneapolis, MN) for 6 days with fresh medium and reagents added on day 3.

Bone marrow cell cultures. Bone marrow was flushed from the femurs of male Balb/C mice (aged 6–12 weeks) with growth medium as previously described (17). The bone marrow cells were seeded at a density of 2.5×10^5 mononuclear cells/cm^2 in 24-well cluster dishes or in 96-well cluster dishes containing bovine cortical bone slices. The cultures were treated with or without the following factors: recombinant murine M-CSF (M-CSF 1.6–100 ng/ml, R & D Systems), recombinant human soluble RANKL (RANKL 0.1–100 ng/ml, Chemicon, Temecula, CA), TGF-β (0.01–100 ng/ml), 1,25(OH)$_2$D$_3$ (10^{-8} M) and prostaglandin E$_2$ (PGE$_2$, 10^{-7} M, Sigma, St. Louis, MO). For cultures in cluster dishes, the media and factors were replaced on day 3 and cells were fixed on day 6 for analysis of tartrate-resistant acid phosphatase (TRAP)-positive cell formation or calcitonin receptor (CTR) expression. Medium and factors were replaced every 2–3 days in the cultures containing the bone slices and the experiments were terminated on day 10–13.

Spleen cell cultures. Spleens were collected from male Balb/C mice (aged 6–12 weeks) and forced through a 70 μm nylon mesh Cell Strainer (Falcon, Franklin Lakes, NJ) with spleen cell growth medium [α-MEM (Life Technologies) containing 10% heat-inactivated FBS and 1% antibiotic/antimycotic solution]. The cell suspension was centrifuged at 500 $\times$ g for 10 min and resuspended in spleen cell growth medium. The spleen cells were seeded into 24-well cluster dishes at 5×10^5 mononuclear cells/cm^2 and treated with 25 ng/ml of M-CSF, 30 ng/ml of RANKL, and various concentrations of TGF-β for 6 days. Fresh medium and reagents were added on day 3.

TRAP-positive cell quantitation. At the termination of the experiments in cluster dishes, the cultures were fixed with 3.7% formalin for 10 min and stained for TRAP as previously described (17). The number of TRAP-positive multinucleated cells (containing 3 or more nuclei) were determined per well.

CTR immunoperoxidase staining. The methodology for CTR immunoperoxidase staining was adapted from Quinn *et al.* (18) Cultures in 24-well cluster dishes were washed twice with Hanks balanced salt solution (HBSS, Life Technologies) and fixed for 5 min with ice-cold 3% paraformaldehyde (Sigma) in phosphate balanced salt solution (PBS). The fixed cultures were incubated in 0.05% tween in PBS at 56°C for 10 min and then blocked with 3% H_2O_2 in PBS for 5 min at room temperature. The cells were washed with each wash step consisting of three 10-min incubations in PBS. The cells were treated with Dako Protein Block Serum-Free (Dako, Carpinteria, CA) for 5 min and washed. The cells were then incubated for 1 h at 37°C with rabbit anti-mouse CTR antisera described by Perry *et al.* (19) (from Dr. Patrick M. Sexton, Dept. of Pharmacology, University of Melbourne, Parkville, Victoria, Australia) diluted 1:100 in Dako diluent. After washing off the primary antibody, the cultures were incubated for 10 min with biotinylated rat anti-rabbit immunoglobulins (Dako). The cells were incubated with peroxidase (LSAB2, Dako) for 10 min at room temperature, washed, and then incubated for 10 min with DAB (Dako). In some wells preimmune rabbit antisera (from P.M. Sexton) was used in place of the primary CTR antisera to confirm specificity of the immunostaining.

Bone resorption. Bone marrow cells were seeded onto bovine cortical bone slices prepared as described by Sato and Grasser (20). The cells were treated as described in the previous section. On day 10–13, the medium was removed and the bone slices containing the cells were stained for TRAP as described previously (17). A Leitz DMIL (Leica Microscope Systems, Wetzlar, Germany) inverted microscope was used to identify the TRAP-positive cells on the bone slices. The opposite side of the bone slice was notched so that the side containing the resorption lacunae could be mounted upward for resorption lacunae identification by scanning electron microscopy (SEM). The cells were removed by dH_2O lysis (twice for 1 h each), followed by two 15-s bursts of sonication in 0.25 M NH_4OH. The bone slices were rinsed twice with dH_2O and initially preserved in modified Karnovsky's fixative (2.5% gluteraldehyde and 2% formaldehyde in 0.12 M cacodylate buffer). The bones were then rinsed with 0.12 M cacodylate buffer pH 7.2 (Electron Microscopy Sciences, Fort Washington, PA), secondarily fixed in 2% osmium tetroxide (Electron Microscopy Sciences, Fort Washington, PA), rinsed with 0.12 M cacodylate buffer, and dehydrated with serially increasing concentrations of ethanol. Tissue samples were critical point dried, mounted on stubs, sputter coated with gold/palladium (Polaron Equipment Limited, Watford, England), and evaluated with a Philips XL30 (FEI/Philips Electron Optics, Mahwah, NJ) scanning electron microscope.

RESULTS

Osteoclast-like cells [TRAP-positive cells containing three or more nuclei] were formed in cocultures of BALC cells and bone marrow cells stimulated with 1,25(OH)$_2$D$_3$ alone or in combination with PGE$_2$ (Table 1). TRAP-positive multinucleated cells are also formed in bone marrow cell cultures treated with RANKL and M-CSF (Table 1). Since bone marrow contains stromal cells which can express RANKL and support osteoclast differentiation, the effects of 1,25(OH)$_2$D$_3$ and PGE$_2$ on osteoclast differentiation were evaluated at the same density as the previously described bone marrow cultures. Ten-fold fewer TRAP-positive multinucleated cells/well were observed when compared to the RANKL and M-CSF stimulated cultures (Table 1).

In 1,25(OH)$_2$D$_3$-stimulated cocultures of bone marrow and BALC cells, TGF-β inhibited TRAP-positive multinucleated cell formation in a concentration-dependent manner (Fig. 1). In contrast, an increase in TRAP-positive multinucleated cell formation was observed in bone marrow cell cultures treated with M-CSF and RANKL (Fig. 2A). A 3–10 fold increase in TRAP-positive multinucleated cell number was routinely seen in these cultures. Likewise, in RANKL and

TABLE 1

Effects of RANKL, M-CSF, $1,25(OH)_2D_3$, and PGE_2 on TRAP-Positive Multinucleated Cell Formation in Bone Marrow/BALC Cell Cocultures and Bone Marrow Cell Cultures

	TRAP-positive multinucleated cells/well	
	Bone marrow/BALC cell cocultures[a]	Bone marrow cell cultures[b]
RANKL + M-CSF	ND	2206 ± 177.0
$1,25(OH)_2D_3$	1070.5 ± 53.3	27.5 ± 5.2
PGE_2	ND	5.7 ± 1.7
$1,25(OH)_2D_3$ + PGE_2	1090.8 ± 89.3	5.2 ± 0.8

[a] Murine bone marrow cells (5×10^4 cells/cm^2) were cocultured with BALC cells (1.5×10^4 cells/cm^2) in the presence of 10^{-8} M $1,25(OH)_2D_3$ and/or 10^{-7} M PGE_2. Medium was changed on day 3 and the cultures were fixed with formalin on day 6. The cells were stained for TRAP and the number of TRAP-positive cells containing 3 or more nuclei per cell was evaluated. Each point represents the mean and standard error of 6 replicates.

[b] Murine bone marrow cells (2.5×10^5 cells/cm^2) were treated with 30 ng RANKL/ml, 25 ng M-CSF/ml, 10^{-8} M $1,25(OH)_2D_3$, and/or 10^{-7} M PGE_2 for 6 days. Medium was changed on day 3 and fresh factors were added. Each point represents the mean and standard error of 6 replicates. ND, not determined.

M-CSF stimulated spleen cell cultures, TGF-β increased the formation of TRAP-positive multinucleated cells in a concentration-dependent manner (Fig. 2B). An increase in calcitonin receptor (CTR)-positive mononuclear (Fig. 2C) and multinucleated (Fig. 2D) cells was observed following treatment with TGF-β. In addition to the increase in the number of CTR-positive multinucleated cells there was an increase in the size of the cells and number of nuclei per cell following TGF-β treatment (Fig. 3). In comparison to the control group (Fig. 3C), fewer mononuclear cells were observed and more and larger multinucleated cells were formed following treatment with 100 ng/ml of TGF-β (Fig. 3A). At 0.01 ng TGF-β/ml (Fig. 3B) more osteoclasts were observed than in the control cultures (Fig. 3C); however, significantly fewer multinucleated cells were present than in the cultures treated with 100 ng TGF-β/ml. Minimal background staining was observed in the cultures immunoperoxidase stained with preimmune serum (Fig. 3D).

A concentration-dependent increase in TRAP-positive multinucleated cell formation was observed with increasing M-CSF concentration in the presence of TGF-β (1 ng/ml) and RANKL (30 ng/ml, Fig. 4A). Likewise, a concentration-dependent increase in TRAP-positive multinucleated cell formation was observed with increasing concentrations of RANKL in the presence of TGF-β (1 ng/ml) and M-CSF (25 ng/ml, Fig. 4B).

TRAP-positive multinucleated cells were formed when bone marrow cells were seeded onto bovine cortical bone slices and stimulated with RANKL and M-CSF in the presence or absence of TGF-β (data not shown). SEM analysis of the bone slices following 10 days of culture demonstrated distinct shallow resorption lacunae of various size and shape (Figs. 5A and 5B). Similar results were observed in control cultures (Fig. 5A) and cultures treated with TGF-β (Fig. 5B). No TRAP-positive multinucleated cells or resorption lacunae were observed in cultures stimulated with $1,25(OH)_2D_3$ and PGE_2 (data not shown).

DISCUSSION

The present experiments were undertaken because of our interest in the mechanisms of TGF-β action on osteoclast formation. The results provided the surprising and novel finding that in the absence of a significant number of osteoblastic/stromal cells, TGF-β has a substantial stimulatory effect on osteoclast differentiation in hematopoietic cells treated with RANKL and M-CSF. A 3–10 fold increase in the number of TRAP-positive multinucleated cells was observed following TGF-β treatment of bone marrow cell cultures stimulated with RANKL and M-CSF. This is in contrast to the complete inhibition of differentiation observed in cocultures of bone marrow and BALC cells treated with TGF-β. The inhibitory response confirms our previous findings that TGF-β1 and 3 decrease osteoclast differentiation in bone marrow/BALC cocultures (12, 14). In addition, TGF-β has been shown by others to inhibit

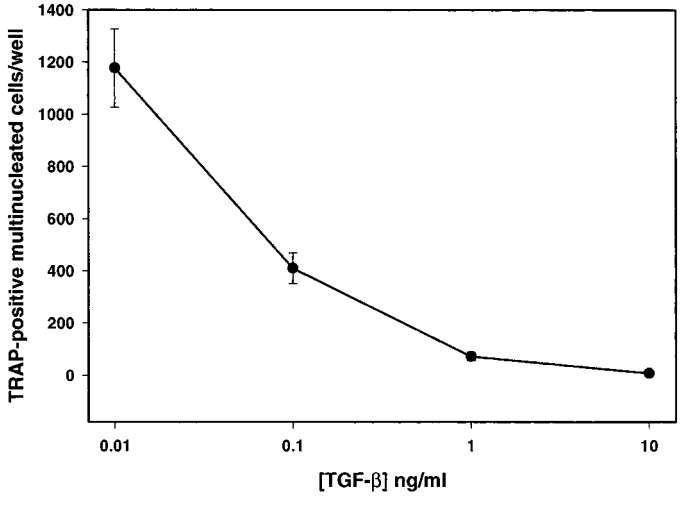

FIG. 1. TGF-β inhibits TRAP-positive multinucleated cell formation in cocultures of bone marrow cells and BALC cells. Murine bone marrow cells were cocultured with BALC cells in the presence of 10^{-8} M $1,25(OH)_2D_3$ and treated with varying concentrations of TGF-β. Medium was changed on day 3 and the cultures were fixed with formalin on day 6. The cells were stained for TRAP and the number of TRAP-positive cells containing 3 or more nuclei per cell were evaluated. The control in the absence of TGF-β was 1912.8 ± 42.9. Data are representative experiment from multiple experiments set up with different cell isolations. Each point represents the mean and standard error of 6 replicates.

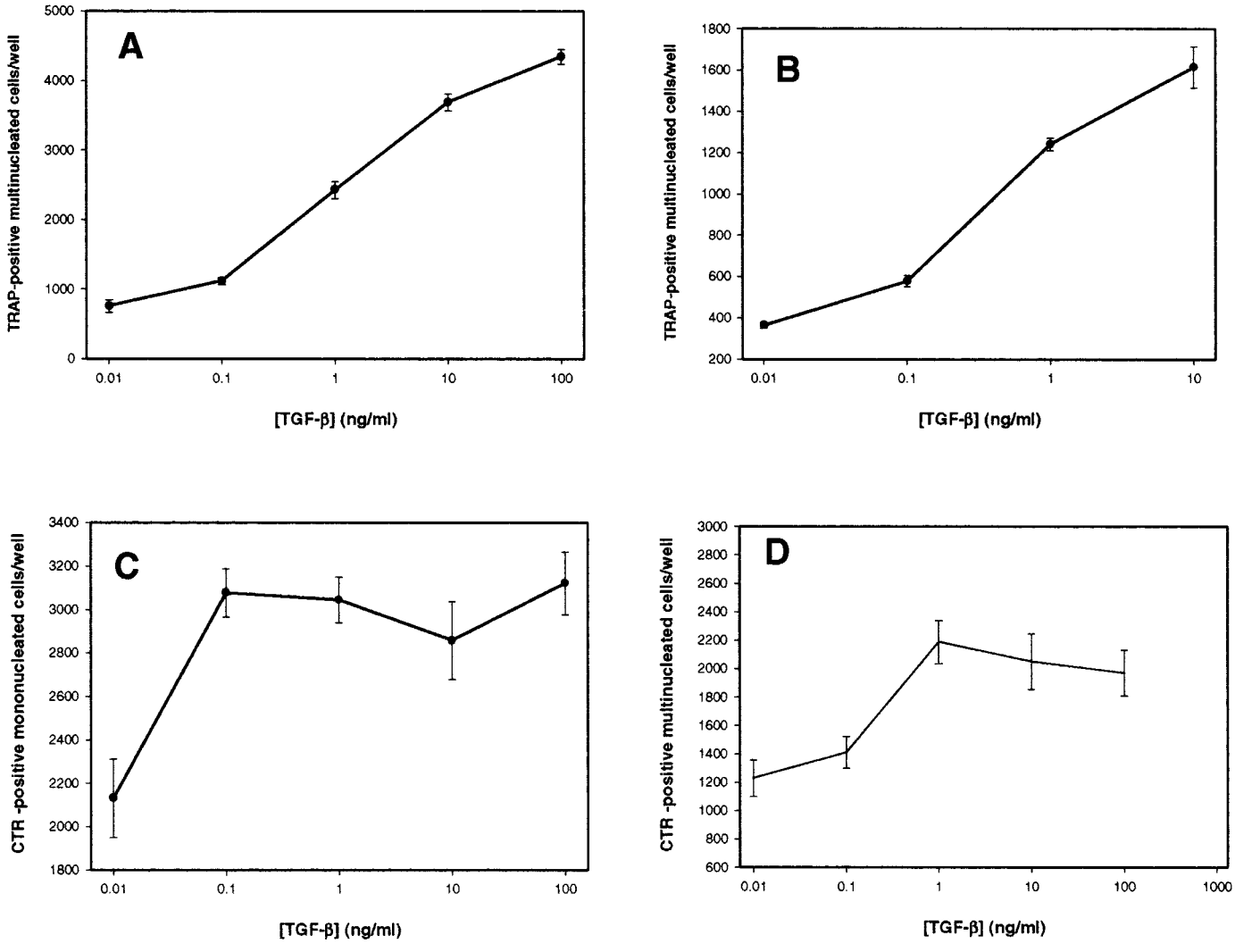

FIG. 2. TGF-β enhances TRAP- and CTR-positive cell formation in bone marrow or spleen cell cultures stimulated with RANKL and M-CSF. Murine bone marrow cells (A, C, D) or spleen cells (B) were treated with 30 ng RANKL/ml, 25 ng M-CSF/ml, and varying concentrations of TGF-β for 6 days. Medium was changed on day 3 and fresh factors were added. In A and B the cultures were fixed with formalin, stained for TRAP, and the number of TRAP-positive cells containing 3 or more nuclei was quantitated. The control in the absence of TGF-β was 333.2 $\pm$ 23.4 for A and 223.7 $\pm$ 14.8 for B. In C and D the cells were fixed with parformaledehyde and CTR immunoperoxidase stained. The number of mononuclear (C) and multinuclear (D, cells containing 3 or more nuclei) cells expressing CTR was evaluated. The controls for C and D were 1969.6 $\pm$ 230.6 and 780.0 $\pm$ 134.7, respectively. Data are a representative experiment from 2–4 experiments set up with different cell isolations. Each point represents the mean and standard error of 5–6 replicates.

osteoclast-like cell formation in human (13) and mouse (11) cocultures of hematopoietic cells and osteoblastic/stromal cells. Shinar and Rodan (10) and Yamaguchi and Kishi (9) observed biphasic effects of TGF-β on TRAP-positive multinucleated cell formation in bone marrow cell cultures seeded at a high density so that stromal cells grow out and support osteoclast differentiation. In the presence of $1,25(OH)_2D_3$, TGF-β increased osteoclast-like cell formation two-fold at 100 pg/ml (10), but inhibited at 1 ng/ml and higher concentrations. The authors suggest that prostaglandin is mediating the effect at low TGF-β concentrations. Yamaguchi and Kishi (9) did demonstrate a slight increase in TRAP-positive cell formation by 10^{-11}–10^{-13} M TGF-β in the absence of $1,25(OH)_2D_3$. In the present experiments, no inhibitory effects of TGF-β were observed in cultures stimulated with RANKL and M-CSF. These findings suggest that TGF-β is a potent stimulator of osteoclast differentiation in RANKL- and M-CSF-treated cultures. In cocultures of hematopoietic and osteoblastic/stromal cells, the predominate effect is inhibition of differentiation at the concentrations examined.

The increase in osteoclast differentiation observed in the absence of BALC cells suggests that TGF-β acts on osteoclast progenitor cells or other cells in the bone

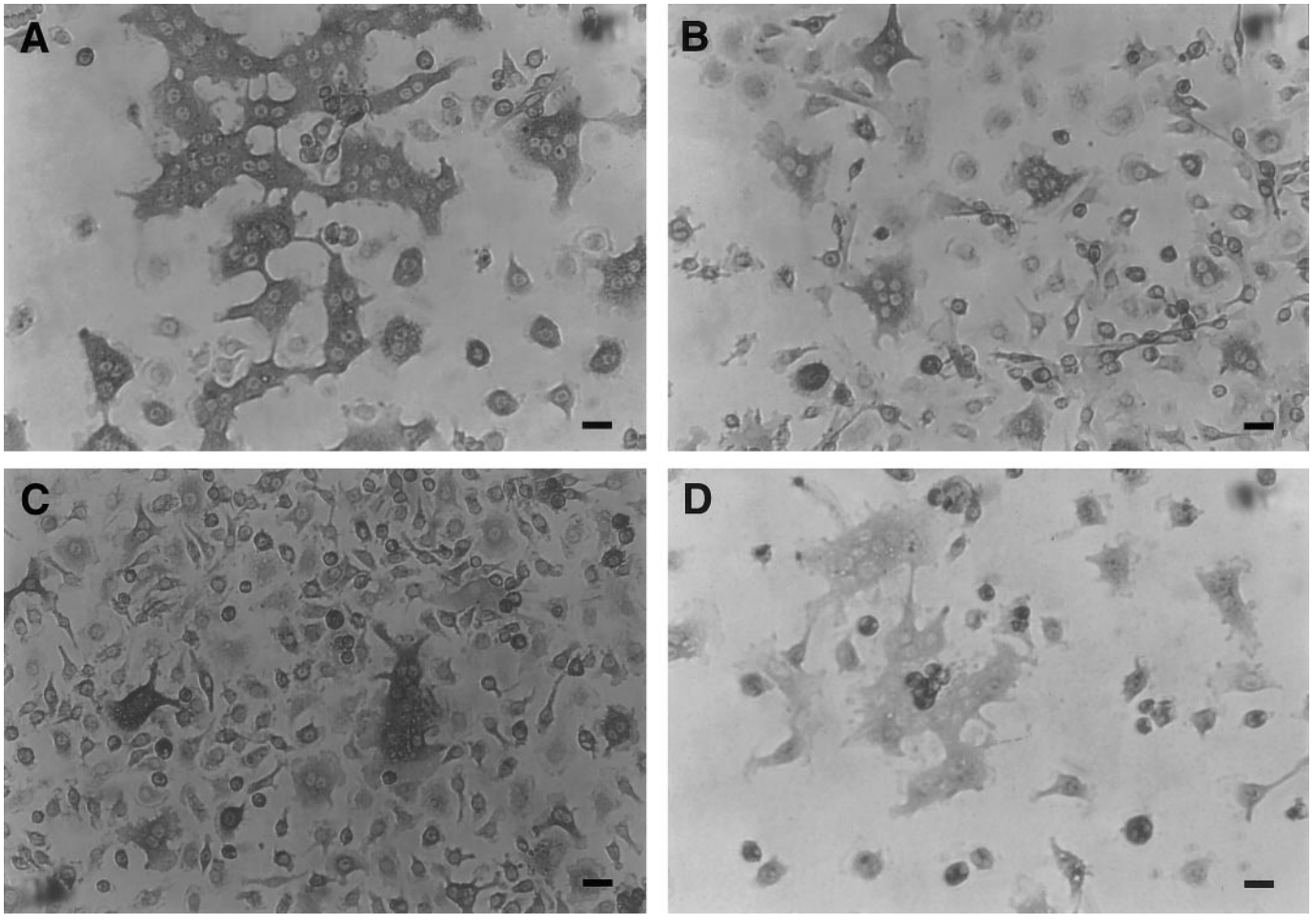

FIG. 3. CTR expression in osteoclast-like cells differentiated in RANKL, M-CSF, and TGF-β-stimulated bone marrow cultures. Murine bone marrow cells were treated with 30 ng RANKL/ml, 25 ng M-CSF/ml, and varying concentrations of TGF-β for 6 days. Medium was changed on day 3 and fresh factors were added. The cells were fixed with paraformaldehyde and immunoperoxidase stained with a CTR-specific antibody or preimmune rabbit serum. (A) CTR immunoperoxidase-stained cultures differentiated in the presence of 100 ng TGF-β/ml. (B) CTR immunoperoxidase-stained cultures differentiated in the presence of 0.01 ng TGF-β/ml. (C) CTR immunoperoxidase-stained control cultures differentiated in the absence TGF-β. (D) Preimmune rabbit serum immunoperoxidase stained cultures differentiated in the presence of 100 ng TGF-β/ml. Data are representative experiment from 4 experiments set up with different cell isolations. Bar, 20 μm.

marrow or spleen cell cultures besides stromal cells. Recent studies have demonstrated that TGF-β increases OPG mRNA expression (15, 16) in osteoblastic cells, which can lead to a decrease in osteoclast differentiation. Thus, in cultures containing osteoblastic/stromal cells TGF-β may increase OPG secretion, which inhibits osteoclast differentiation. Bone marrow contains stromal cells, which can support osteoclast differentiation. However, at the density used in these studies, the numbers of TRAP-positive multinucleated cells induced by $1,25(OH)_2D_3$ and PGE_2 are low (27 or less per well). Since the effects of $1,25(OH)_2D_3$ and PGE_2 are mediated through the osteoblastic/stromal cells (25), this observation suggests that few stromal cells are present in these cultures. Thus, the predominant effect of TGF-β in the RANKL and M-CSF stimulated cultures is probably mediated through another cell type.

TGF-β may act directly on the osteoclast progenitor cell population to stimulate differentiation. Previous studies have provided evidence that osteoclast-like cells do express TGF-β receptors and respond to TGF-β. Zheng *et al.* (21) showed expression of mRNA for type I and II TGF-β receptor in osteoclast-like cells from human giant cell tumor of bone. In mouse organ culture, TGF-β stimulates osteoclastic bone resorption through a prostaglandin-mediated mechanism (22, 23), but inhibits in rat long bone culture (22). Transgenic mice overexpressing TGF-β2 exhibit increased osteoclast formation and resorption and develop an osteopenic phenotype consistent with the possibility that locally produced TGF-β enhances osteoclast differenti-

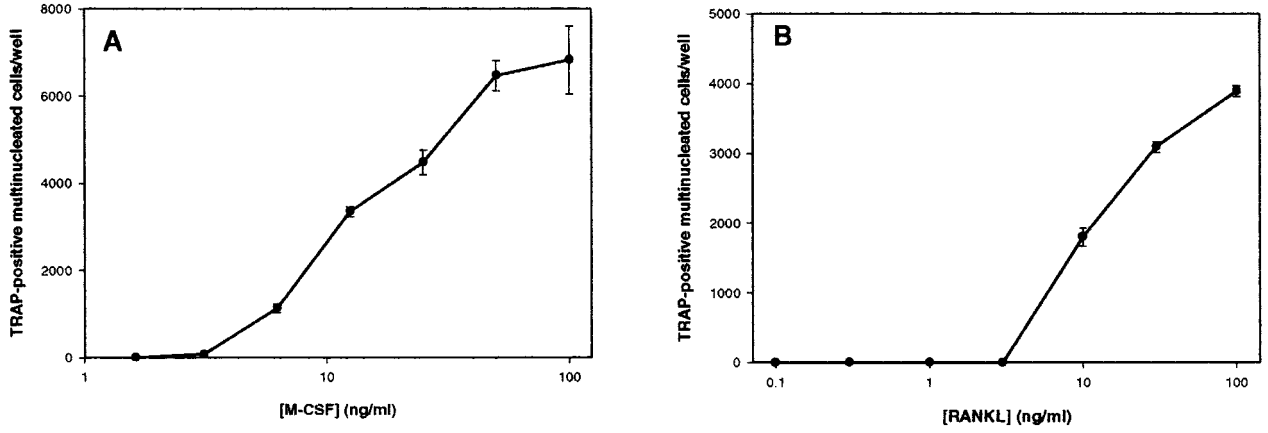

FIG. 4. TGF-β-enhanced osteoclast differentiation is dependent on the RANKL and M-CSF concentration in the culture. Murine bone marrow cells were treated with 1 ng TGF-β/ml in the presence of varying concentrations of RANKL or M-CSF for 6 days. Medium was changed on day 3 and fresh factors and media were added. On day 6, cultures were fixed with formalin, stained for TRAP, and the number of TRAP-positive cells containing 3 or more nuclei was quantitated. In A, RANKL was added at 30 ng/ml and the M-CSF concentration was varied. In B, M-CSF was added at 25 ng/ml and the RANKL concentration was varied. Data are representative experiment from 2 experiments set up with different cell isolations. Each point represents the mean and standard error of 6 replicates.

ation and resorption (24). However, these studies do not demonstrate a direct effect of TGF-β on osteoclasts or progenitor cells. It is also possible that the effects of TGF-β are mediated through other cells in the bone marrow or spleen cell population since these populations contain many cell types.

Activated T-lymphocytes express RANKL [for a review see Kwon *et al.* (2)] and secrete factors that are known to modulate osteoclast differentiation and function. It is possible that the T-lymphocytes in the bone marrow or spleen cell population are responding to TGF-β and in turn regulating osteoclast differentiation. Previous studies have shown that T-lymphocytes regulate osteoclast differentiation (17, 26, 27) through soluble factors. Although the cells that mediate the effects of TGF-β in the RANKL and M-CSF-stimulated cultures are not known at this time, it is clear that TGF-β significantly enhances osteoclast differentiation.

Both TRAP-positive multinucleated cell formation and CTR-positive multinucleated cell formation were increased by TGF-β in M-CSF and RANKL stimulated bone marrow cultures. In osteoclast differentiation cultures, CTR expression has been shown to correlate

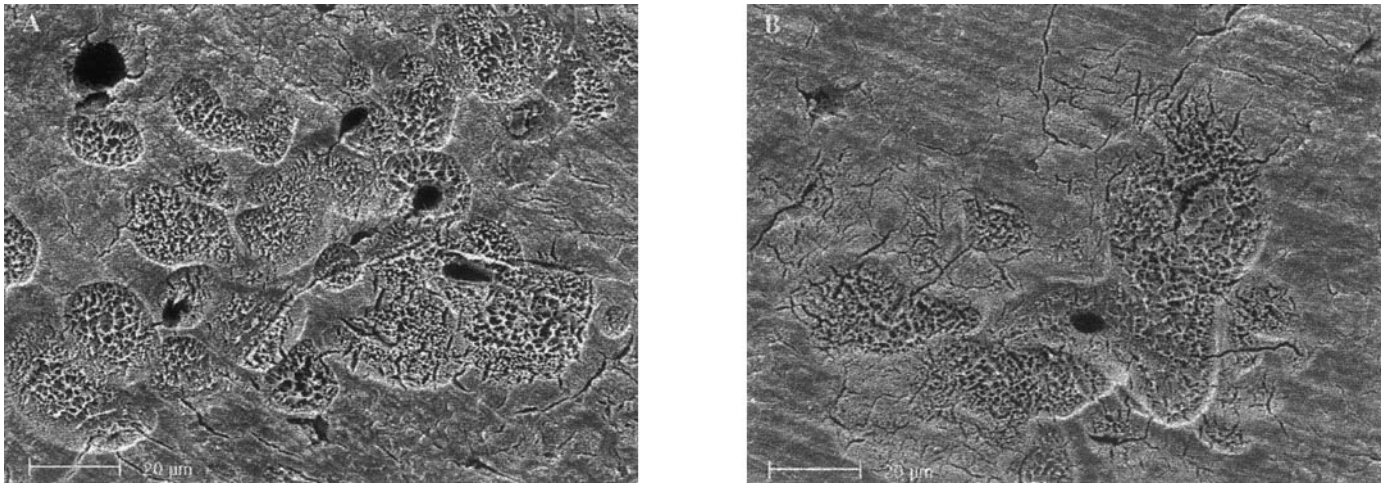

FIG. 5. Resorption lacunae formation by osteoclast-like cells differentiated in RANKL and M-CSF-stimulated bone marrow cultures treated with or without TGF-β. Murine bone marrow cells were seeded onto bovine cortical bone slices and treated with 30 ng RANKL/ml, 25 ng M-CSF/ml, and varying concentrations of TGF-β for 10 days. Medium was changed every 2–3 days and fresh factors and media were added. The cells were lysed by NH_4OH-treatment and the bones were fixed with Karnovsky's fixative and processed for SEM. (A) Resorption lacunae formed in control cultures. (B) Resorption lacunae formed in cultures treated with 100 ng TGF-β/ml. Data are representative experiment from 2 experiments set up with different cell isolations. Bar, 20 μm.

with bone resorption (18, 28, 29); whereas, multinucleated cell number does not correlate with bone resorption. Thus, the TRAP-positive cell numbers may not be reflective of osteoclast number. Quantitation of CTR-positive cells in the cultures provides a better indication of osteoclast differentiation (18). The number of CTR-positive mononuclear cells, as well as multinuclear cells, was increased in these cultures suggesting that TGF-β stimulates the differentiation of mononuclear cells and not just fusion. It is clear that the size of the multinucleated cells is increased by TGF-β. It appears that either mononuclear or multinucleated cells continue to fuse leading to larger cells. This may just be due to the enhanced number of osteoclasts or progenitor cells in close proximity to each other, thus leading to larger cells.

The multinucleated cells generated in these cultures express TRAP and CTR and form resorption lacunae on cortical bone slices as demonstrated by SEM analysis. These results indicate that the cells formed in the presence or absence of TGF-β are osteoclasts. These observations confirm the findings of others that soluble RANKL and M-CSF can promote osteoclast formation *in vitro* (1, 30, 31). The results of this study demonstrate that TGF-β stimulates the formation of osteoclasts and that the stimulatory effects of TGF-β were only observed in RANKL and M-CSF stimulated bone marrow cultures, and not cocultures. These data suggest that the overall effect of TGF-β on osteoclast differentiation is dependent on the cell population. It appears that inhibitory effects are observed when large numbers of osteoblastic/stromal cells are present. In the presence of minimal numbers of stromal cells, the predominating effect of TGF-β is a substantial increase in osteoclast differentiation, a finding that may be significant in some states of pathologic bone resorption.

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