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and murine dendritic cells, respectively (11, 12). Lacey et al. (13) also succeeded in the molecular cloning of a ligand for OPG from an expression library of the murine myelomonocytic cell line 32D. The OPG ligand (OPGL) was identical to ODF (TRANCE/ RANKL). The administration of OPGL (ODF) to mice caused reduced bone volume and extreme hypercalcemia without a significant increase in the number of osteoclasts, suggesting that ODF is involved in the activation of osteoclasts as well (13). Fuller et al. (14) also recently reported that TRANCE (ODF) is involved in the osteoclast activation induced by osteoblastic cells treated with parathyroid hormone. A soluble form of TRANCE induced a striking change in the motility and spreading of isolated rat osteoclasts, and inhibited their apoptosis (14). These results suggest that ODF is necessary for the osteoblast-mediated activation of mature osteoclasts.

The utilization of a soluble form of ODF (sODF) has allowed us to elucidate the role of ODF in osteoclast function in more detail. In the present study, we examined the effects of ODF on the survival, multinucleation, and activation of osteoclasts in comparison with those of M-CSF and IL-1. sODF, M-CSF, and IL-1 promoted the survival and multinucleation of pOCs through their respective receptors, sODF and IL-1, but not M-CSF, stimulated the pit-forming activity of OCLs. sODF, as well as IL-1, activated NF-κB and c-Jun N-terminal protein kinase (JNK) in OCLs. Not only OCIF were removed first from the coculture, using a mixture of collagenase-in 100-mm-diameter dishes for 6 days as described above. The KS4 cells were removed from the dishes by treating with 0.2% collagenase (Wako). To further purify the OCLs, the crude OCL preparation was replated on EDTA according to the method described previously (15). The purity of OCLs in this fraction (crude OCL preparation) was about 5%.

Materials and Methods

Abs and chemicals

A recombinant murine sODF purified by affinity chromatography on an OPG/OCLF-immobilized column and gel filtration chromatography was kindly provided by Snow Brand Milk Products (Tochigi, Japan). The purity of sODF in this preparation was >95% in SDS polyacrylamide gel electrophoresis. Recombinant human IL-1α, recombinant human M-CSF, and murine IL-1 receptor antagonist (IL-1ra) were obtained from R&D Systems (Minneapolis, MN). Echistatin was purchased from Sigma (St. Louis, MO). Anti-human ICαB rabbit polyclonal Abs were purchased from New England BioLabs (Lake Placid, NY). Anti-human Bcl-2, Bcl-xL, and RelA (p65) rabbit polyclonal Abs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-c-Fms mAb was kindly provided by Dr. S-I. Nishikawa (Kyoto University, Kyoto, Japan). Anti-human β-actin mAb was obtained from Boehringer Mannheim Biochemica (Mannheim, Germany).

Coculture system and enrichment of osteoclast-like cells

Osteoblasts obtained from the calvariae of newborn mice and bone marrow cells obtained from the tibiae of male mice were cocultured in αMEM (Life Technologies, Grand Island, NY) containing 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. The purity of osteoblasts/stromal cells as a membrane-associated protein is reduced by sODF. These results indicate that ODF expressed by c-Jun N-terminal protein kinase (JNK) in OCLs. Not only OCIF were removed first from the coculture, using a mixture of collagenase-in 100-mm-diameter dishes for 6 days as described above. The KS4 cells were removed from the dishes by treating with 0.2% collagenase (Wako). To further purify the OCLs, the crude OCL preparation was replated on EDTA according to the method described previously (15). The membranes were hybridized for 15 h at 42°C with radioactive cDNA probes for murine RANK and murine TNF type 1 and type II receptors, which were cloned by RT-PCR and labeled using a multitrand primer oligonucleotide labeling kit (Takara Shuzo, Osaka, Japan). As an internal control, the membrane was rehybridized with a radioactive cDNA probe for mouse GAPDH. Each membrane was then exposed to an x-ray film.

EMSA and JNK assay

For the EMSA, nuclear extracts were prepared according to the method described by Dignam et al. (18). The sequence of the NF-κB-binding oligonucleotide used as a radioactive DNA probe was 5'-AGCCGGG GACTTCCCGAG-3'. The DNA binding reaction was performed at room temperature in a volume of 20 μl, which contained the binding buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 4% glycerol, 100 mM NaCl, 5 mM DTT, 2 μg/ml EDTA (mg/ml) BSA, 3 μg of poly(dI-dC), 1 x 10^5 cpm of a 32P-labeled probe, and 8 μg of nuclear proteins. After incubation for 15 min, the samples were electrophoresed on native 5% acrylamide/0.25% TBE gels. The gels were dried and exposed to an x-ray film. For the determination of JNK activity, purified OCLs treated with sODF or IL-1 were washed twice with ice-cold PBS, then lysed in a lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM DTNB, 1 mM Trition X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na2VO3, 1 μM leupeptin, 1 mM PMSF). The JNK activity of the cell lysates was determined using a stress-activated protein kinase (SAPK)/JNK kinase assay kit (New England Bio Labs).

Survival and cell fusion assays

The survival rate of OCLs was measured as reported previously (5, 19). After OCLs were purified, some of the cultures were subjected to TRAP staining. TRAP-positive MNCs containing more than 3 nuclei were counted as living OCLs. Other cultures were further incubated in the presence or absence of sODF. After incubation for indicated periods, the remaining OCLs were counted. To examine the effect of sODF on the fusion of osteoclasts, we repleted pOCs (15,000 cells/well) on 96-well culture plates with or without various increasing concentrations of sODF. After culture for 18 h, the cells were fixed and stained for TRAP. Some cultures were also treated with IL-1ra (1 μg/ml), anti-c-Fms Ab (10 μg/ml), or OCIF (100 ng/ml). The number of TRAP-positive MNCs with more than 10 nuclei was counted as pOC-derived OCLs. Actin rings in pOC-derived OCLs were also visualized by rhodamine-conjugated phalloidin staining, as previously described (15). Results are expressed as the means ± SEM of three cultures.

Immunoblotting analysis and immunofluorescence microscopy

After purified OCL preparations were cultured for various periods in the presence of ODF, the cells were washed twice with ice-cold PBS and then lysed in a lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 10 μg/ml aprotinin, 20 μg/ml leupeptin, and 1 mM PMSF). The cell lysates (20 μg of protein) were resolved by 10% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA). After blocking with 2% BSA in TBST, the ICαB, Bcl-2, and Bcl-xL Abs (1/1000 dilution) were added in TBST containing 2% BSA and visualized by an enhanced chemiluminescence assay using reagents from Amersham and exposed to an x-ray film. The immunofluorescence analysis was done as described previously (6). Anti-RelA (p65) Abs (1 μg/ml) was used for the immunostaining.

Pit formation assay

pOC preparations (15,000 cells/0.1 ml/well) were seeded on dentine slices (4-mm diameter) which had been placed in 96-well plates. After incubation for 2 h, dentine slices were transferred to 48-well plates (one slice/well) in the presence or absence of ODF. Pit formation by pOCs was determined after culture for 24 h. For the pit formation assay, cells were removed from dentine slices, and the resorbed area was stained with Mayer’s hematoxylin after culture for 24 h. For the pit formation assay, cells were removed from dentine slices, and the resorbed area was stained with Mayer’s hematoxylin after culture for 24 h. For the pit formation assay, cells were removed from dentine slices, and the resorbed area was stained with Mayer’s hematoxylin after culture for 24 h.
into the EcoRI site of an expression vector that carries the promoter region of human EF1α gene (pEF-BOS) (20). COS 7 cells were transfected with the expression vectors (16.6 μg/100-mm-diameter dish) by cationic liposomes (DMRIE-C, Life Technologies) according to the manufacturer’s recommendation. The supernatant was harvested 48 h later, passed through a 0.45-μm filter, incubated with anti-FLAG M2 affinity gel (Kodak, New Haven, CT), and eluted with FLAG peptide (250 μg/ml, Kodak) as outlined in the manufacturer’s protocol. The eluant was dialyzed against PBS, and the protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL).

Results

RANK, a novel TNF receptor family member, has been shown to be a signal-transducing receptor of ODF (12, 21). Total RNA was obtained from spleen cells, bone marrow cells, purified OCLs, primary osteoblasts, myoblastic C2C12 cells, and bone marrow-derived stromal ST2 cells, and the expression of RANK mRNA was examined in the RNA preparations (Fig. 1). Few contaminating osteoblasts (alkaline phosphatase-positive cells) or monocyte-macrophages (F4/80 Ag-positive cells) were detected in the purified OCL preparations in this study (data not shown). The expression of other TNF receptor family members, such as TNFRI and TNFRII, was also examined in the same membrane. TNFRI mRNA was similarly expressed in most of the cells examined in this study. TNFRII mRNA was highly expressed by OCLs, and the expression level by the other cells was much lower (Fig. 1). OCLs also highly expressed RANK mRNA (Fig. 1).

The overexpression of RANK was sufficient to activate NF-κB (12). The ligand-dependent NF-κB activation was also demonstrated by the cotransfection of RANKL with RANK in human 293 cells and T cells (12). We then examined whether sODF activates NF-κB in OCLs. sODF activated NF-κB in OCLs in a dose-dependent manner (Fig. 2A). Fig. 2B shows the time course of change in the activation of NF-κB and the levels of IκBα in OCLs after stimulation with sODF. sODF transiently activated NF-κB in

**FIGURE 1.** Expression of RANK mRNA by OCLs. Total RNA (10 mg) extracted from spleen cells (lane 1), bone marrow cells (lane 2), purified OCLs (lane 3), C2C12 cells (lane 4), ST2 cells (lane 5), and primary osteoblasts (lane 6) was separated in a 1.0% formaldehyde-agarose gel and hybridized with the cDNA probes for RANK, TNF type I receptor, TNF type II receptor, and GAPDH as described under Materials and Methods.

**FIGURE 2.** sODF activates NF-κB in purified OCLs. A, Purified OCLs were treated with increasing concentrations of sODF for 30 min. B, Time course of changes in the activation of NF-κB and levels of IκBα in OCLs after stimulation with sODF. Purified OCLs were treated with sODF (100 ng/ml) for the indicated periods. C, Effects of OCIF on the activation of NF-κB in OCLs treated with sODF. sODF (100 ng/ml) (lanes 2 and 3) or IL-1 (10 ng/ml) (lanes 4 and 5) was incubated with OCIF (100 ng/ml) for 1 h at 37°C. Purified OCLs were treated with sODF or IL-1 in the absence (lanes 2 and 4) or presence (lanes 3 and 5) of OCIF for 30 min. NF-κB activity in the nuclear extracts was determined by an EMSA, and the amount of IκBα was determined by immunoblotting, as described under Materials and Methods. D, Translocation of RelA (p65) into the nuclei of OCLs in response to sODF. Purified OCLs were treated with sODF (100 ng/ml) for 30 min. Cells were then fixed and incubated with Abs against RelA (p65), followed by FITC-conjugated anti-rabbit immunoglobulins. The subcellular localization of FITC-labeled RelA (P65) was observed by fluorescence microscopy.
OCLs, and the maximal activation occurred at 30 min. The degradation of IκBa coincided with the activation of NF-κB (Fig. 2B). OCIF, a decoy receptor of ODF, prevented the sODF-induced NF-κB activation, but it did not affect at all the IL-1-induced NF-κB activation in the OCLs (Fig. 2C). The immunocytochemical analysis revealed that RelA (p65) was translocated from the cytoplasm into almost all the nuclei of the OCLs within 30 min after the sODF stimulation (Fig. 2D).

JNK is a signal transducer commonly activated by TNF-related ligands (22). TRANCE (ODF) has been shown to activate JNK in T cells and in bone marrow-derived dendritic cells (11). We examined whether sODF activates JNK in mature OCLs. JNK was rapidly activated in OCLs after the stimulation with sODF, in a dose-dependent manner (Fig. 3A and B). The activation of JNK was also induced in OCLs treated with 10 ng/ml of IL-1 (Fig. 3C). OCIF blocked the JNK activation induced by sODF but not that induced by IL-1 (Fig. 3B and C).

We reported that the activation of NF-κB is involved in the survival of OCLs promoted by IL-1 (6). Like IL-1, sODF prolonged the survival of OCLs in a dose-dependent manner (Fig. 4A). Addition of OCIF with sODF prevented the sODF-induced survival of OCLs. To the contrary, OCIF could not prevent the IL-1- or M-CSF-induced survival of OCLs. OCIF did not accelerate spontaneous cell death of OCLs (Fig. 4A). TRANCE (ODF) has been shown to induce the survival of dendritic cells, which was mediated by up-regulation of Bcl-xL (23). To determine whether sODF induces Bcl-2 or Bcl-xL, we measured their expression in OCLs treated with sODF. A Western blot analysis showed that the expression of Bcl-2 and Bcl-xL in OCLs was not affected by sODF (Fig. 4B).

Wesolowski et al. (16) developed a method for obtaining highly purified (>90%) TRAP-positive mononuclear or binuclear pOCs released by the “disintegrin” echistatin from cocultures of murine osteoblastic cells (MB1.8 cells) with murine bone marrow cells. We reported that IL-1 and M-CSF both prolonged the survival and induced the multinucleation of pOCs in the absence of osteoblast/stromal cells (7). A small number of TRAP-positive MNCs (pOC-derived OCLs) were formed within 2–5 h in the control cultures (7), but they disappeared by 18 h of the incubation period (Fig. 5A). To determine whether the effects of SDF, IL-1, and M-CSF on the survival and multinucleation of pOCs are mediated through their respective receptors, OCIF, IL-1ra, and Ab against c-Fms (M-CSF receptor) were added to pOC cultures (Fig. 5B). IL-1ra inhibited only the IL-1-induced multinucleation of pOCs, and anti-c-Fms Ab inhibited only M-CSF-induced multinucleation of pOCs (Fig. 5B). The survival and multinucleation of pOCs induced by sODF were inhibited by adding OCIF but not by adding IL-1ra or anti-c-Fms Ab (Fig. 5B). The formation of ringed structures of OCLs, and the maximal activation occurred at 30 min. The degradation of IκBa coincided with the activation of NF-κB (Fig. 2B). OCIF, a decoy receptor of ODF, prevented the sODF-induced NF-κB activation, but it did not affect at all the IL-1-induced NF-κB activation in the OCLs (Fig. 2C). The immunocytochemical analysis revealed that RelA (p65) was translocated from the cytoplasm into almost all the nuclei of the OCLs within 30 min after the sODF stimulation (Fig. 2D).

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F-actin dots (actin rings) in osteoclasts is closely related to the osteoclast function (24). Actin rings were distributed at the periphery of the sODF-induced OCLs with smooth contours (Fig. 5C). This suggests that the smooth contour of pOC-derived OCLs is somehow related to the sODF-induced pit-forming activity.

pOCs were then cultured for 24 h on dentine slices in the presence or absence of sODF (Fig. 6). The number of pOC-derived OCLs remaining on the slices in the cultures treated with sODF was much greater than that in the control cultures (data not shown). Resorption pits on dentine slices were observed in the culture treated with sODF (Fig. 6A). The pit-forming activity of pOCs induced by sODF was inhibited by adding OCIF (Fig. 6B). M-CSF also prolonged the survival and induced the multinucleation of pOCs, but no resorption pits were formed in the cultures treated with M-CSF (7).

We then examined the effect of interactions between ODF and RANK on the activation of NF-κB and JNK by adding sRANK to purified OCLs. Addition of sRANK significantly reduced the number of OCLs in a dose-dependent manner (Fig. 7). Proliferation of stromal cells in bone marrow cultures appeared to not be affected by the addition of sRANK or OCIF. Addition of sRANK as well as OCIF reduced the activity of NF-κB and JNK (Fig. 7B). Moreover, the sODF-mediated stimulation of the survival, multinucleation, and pit-forming activity of pOCs was also abrogated by the addition of sRANK (Table I).

**Discussion**

We previously reported that both IL-1 and M-CSF induced the survival and multinucleation of OCLs/pOCs, but only IL-1 stimulated their pit-forming activity (7). The present study clearly showed that, like IL-1, sODF stimulated not only the survival and multinucleation of OCLs/pOCs but also their pit-forming activity. Our results are consistent with the findings by Fuller et al. (14), who demonstrated that TRANCE (ODF) is involved in the osteoclast activation induced by osteoblastic UMR 106 cells treated with parathyroid hormone. It was also shown that OPGL (ODF) administered into mice induced osteoclastic bone resorption without a significant increase in the number of osteoclasts (13). These results suggest that ODF is a factor essentially involved in not only osteoclast differentiation but also osteoclast activation.

The survival and multinucleation of pOCs induced by M-CSF, IL-1, and sODF were inhibited by anti-c-Fms (M-CSF receptor) Ab, IL-1ra (antagonist of IL-1 type 1 receptor), and OPG/OCIF (a decoy receptor of ODF), respectively (Fig. 5). OPG/OCIF specifically inhibited activation of NF-κB and JNK in OCLs induced by sODF but not that induced by IL-1 (Figs. 2 and 3). These results suggest that M-CSF, IL-1, and ODF have similar effects on the survival and multinucleation of osteoclasts via respective receptors. A soluble form of TNFR has been shown to inhibit the activity of TNF-α and TNF-β (25–28). Here, sRANK inhibited the...
OCL formation in bone marrow cultures treated with sODF together with M-CSF and blocked the sODF-induced activation of NF-κB and JNK in OCLs (Fig. 7). Nakagawa et al. (21) recently reported that polyclonal Abs against the extracellular domains of RANK induced OCL formation in spleen cell cultures in the presence of M-CSF. This indicates that the clustering of RANK is required for the RANK-mediated signal transduction for osteoclastogenesis. In contrast, the Fab fragment of anti-RANK Abs completely inhibited ODF-mediated osteoclastogenesis (21). These results suggest that RANK is the sole receptor of ODF responsible for inducing differentiation and activation of osteoclasts.

We have reported that M-CSF is indispensable for both the proliferative phase and the differentiation phase of osteoclast development (29). ODF and M-CSF cannot be replaced by other cytokines in inducing osteoclast differentiation. The present study shows that M-CSF and IL-1, as well as ODF, prolonged the survival of OCLs and induced their fusion. ODF and IL-1 but not M-CSF induced the pit-forming activity of purified OCLs/pOCs in culture. These results suggest that ODF is the sole factor for inducing differentiation and activation of osteoclasts.

Fig. 8 summarizes the role of cytokines examined in this study in the regulation of osteoclast differentiation and function.

sODF activated NF-κB in OCLs, which coincided with the degradation of IκBα (Fig. 2B). Our immunocytochemical analysis revealed that RelA (p65) was translocated from the cytoplasm into almost all of the nuclei of OCLs within 30 min after sODF stimulation (Fig. 2D). This action of ODF was quite similar to that of IL-1 previously reported (6, 7). Wong et al. (11) reported that a soluble form of TRANCE (ODF) induced JNK activation in T cells but not in splenic B cells and bone marrow-derived dendritic cells. sODF as well as IL-1-activated JNK in OCLs (Fig. 3). In contrast, M-CSF activated JNK but not NF-κB in OCLs, though it supported the survival and fusion of OCLs/pOCs (our unpublished observation). These results suggest that the survival and fusion of osteoclasts are not sufficient for inducing osteoclast function.

TRANCE (ODF) has been shown to induce the survival of dendritic cells through the up-regulation of Bcl-xL (23). It was also reported that targeting of both Bcl-xL and SV40 large T Ag to cells of the osteoclast lineage immobilized osteoclast precursors (30). These results suggest that antiapoptotic proteins such as Bcl-xL and Bcl-2 are involved in the survival of osteoclasts. However, neither Bcl-2 nor Bcl-xL in OCLs was up-regulated by sODF in our culture condition. IL-1 failed to induce the expression of Bcl-2 and Bcl-xL in OCLs (31). This suggests that ODF supports the survival of osteoclasts through a mechanism different from the up-regulation of Bcl-2 and Bcl-xL.

The activation of NF-κB has been shown to increase cellular resistance to apoptosis (32–36). Using antisense oligodeoxynucleotides to NF-κB (RelA/p65 and p50) and proteasome inhibitors that inhibit the degradation of IκB, we have shown that the activation of NF-κB is involved in the survival of OCLs promoted by IL-1 (6). The activation of NF-κB appears to be also involved in the ODF-induced survival of OCLs/pOCs. The precise role of JNK in apoptosis is controversial. Strong activation of JNK was induced by apoptosis-inducing stresses such as UV and hydrogen peroxide (37–39). Using the knockout mice of the SEK1 gene, which encodes a direct upstream kinase of JNK, SEK1-induced signals were shown to play a protective role against various cytotoxic stimuli (40). TNF receptor-associated factor (TRAF) 2 is a signal-transducing protein of the TNF receptor family (39). The activation of JNK was impaired, but the activation of NF-κB was induced in thymocytes obtained from dominant negative (DN)
TRAF2-transgenic mice and in embryonic fibroblasts obtained from TRAF2-deficient mice (41, 42). These thymocytes and fibroblasts were rather apoptotic in the presence of TNF-α. Furthermore, thymocytes from IκBαDN and TRAF2 DN double-transgenic mice were more sensitive to TNF-induced apoptosis than those from normal mice and IκBαDN- or TRAF2 DN-transgenic mice (43). Therefore, JNK-mediated signals appear to collaborate with NF-κB in inducing the antiapoptotic action induced by sODF.

We previously showed that OCLs expressed IL-1 type 1 receptors (6). Xu et al. (44) reported that intense signals for IL-1 type I receptor mRNA were detected in active osteoclasts in an adjuvant arthritis model in rats, whereas mRNA of IL-1 type II receptor, which serves as a decoy receptor, was expressed preferentially in resting osteoclasts. Bone histological studies of OPG/OCIF knock-out mice revealed that physiological bone resorption was regulated mainly by ODF and OPG/OCIF (45). IL-1 appears to be involved in pathological bone resorption, such as that observed in rheumatoid arthritis and periodontal bone diseases.

Recent studies indicate that the cytoplasmic tail of RANK interacts with TRAF1, TRAF2, TRAF3, TRAF5, and TRAF6 (46–49). Mapping of the structural requirements for TRAF/RANK interaction revealed that selective TRAF-binding sites clustered in two distinct domains of the RANK cytoplasmic tail. In particular, TRAF6 interacted with the membrane-proximal domain of the cytoplasmic tail distinct from binding sites for TRAF1, -2, -3, and -5. When the TRAF6 interaction domain was deleted, RANK-mediated NF-κB activation was completely inhibited, and JNK activation was partially inhibited (48). N-terminal truncation of TRAF6 (TRAF6 DN) also inhibited RANKL-induced NF-κB activation (48, 49). These results suggest that TRAF6 transduces a signal involved in RANK-mediated activation of osteoclast function.

Double knockout mice of p50 (NF-κB1) and p52 (NF-κB2), subunits of NF-κB, showed severe osteopetrosis because of the

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**Table I. Effects of sRANK on the survival, multinucleation, and pit-forming activity of osteoclasts promoted by sODF**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (%)</th>
<th>Multinucleation (no. of pOC-derived OCLs/well)</th>
<th>Pit formation (no. of pits/slice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.0 ± 2.8</td>
<td>30.3 ± 5.7</td>
<td>0</td>
</tr>
<tr>
<td>ODF</td>
<td>71.5 ± 6.4</td>
<td>123.0 ± 2.7</td>
<td>87.5 ± 12.0</td>
</tr>
<tr>
<td>sODF + OCIF</td>
<td>29.5 ± 3.5*</td>
<td>41.0 ± 1.4*</td>
<td>26.0 ± 12.7**</td>
</tr>
<tr>
<td>sODF + sRANK</td>
<td>35.0 ± 7.1**</td>
<td>62.5 ± 6.4**</td>
<td>**</td>
</tr>
</tbody>
</table>

* Purified OCLs or pOcs were incubated with sODF (100 ng/ml) in the presence or absence of OCIF (100 ng/ml) or sRANK (1 μg/ml) for 24 h. A survival assay (OCLs), cell fusion assay (pOcs), and pit formation assay (OCLs) were performed as described under Materials and Methods. Similar results were obtained from two independent experiments. Significantly different from the cultures treated with sODF: *, p < 0.01; **, p < 0.05.
impaired osteoclast differentiation (50, 51). The osteopetrotic disorder was cured by normal bone marrow transplantation. These results indicate that osteoclast progenitors are impaired in the deficient mice. sODF activated NF-κB in the target cells including osteoclasts. This suggests that the ODF-induced activation of NF-κB in osteoclast progenitors also plays a crucial role in their differentiation into osteoclasts. Further studies are necessary to elucidate the molecular mechanism of the action of ODF in osteoclast differentiation and function.

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References


