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Death receptors, Fas and TRAIL receptors, are involved in human osteoclast apoptosis ☆,☆☆

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Abstract

Survival and apoptosis are crucial aspects of the osteoclast life cycle. Although osteoclast survival has been extensively studied, little is known about the mechanisms involved in human osteoclast apoptosis. In the present study, cord blood monocytes (CBMs) were used as the source of human osteoclast precursors. When cultured in the presence of M-CSF and RANKL, CBMs formed multinucleated cells that expressed RANK and calcitonin receptor, and were able to resorb bone. These cells expressed TRAIL receptors (R1–R4). Surprisingly, although TRAIL-receptor expression was not detectable in osteoclasts from normal bone, osteoclasts from myeloma specimens did express TRAIL receptors to a variable extent. Significantly, we have shown for the first time that this pathway is indeed functional in human osteoclasts, and that apoptosis occurred and was significantly greater in the presence of TRAIL. In addition, we have shown that a Fas-activating antibody is also able to induce osteoclast apoptosis, as did TGFβ, whereas the survival factor M-CSF decreased apoptosis. Overall, these findings suggest that death receptors, TRAIL receptors and Fas, could be involved in osteoclast apoptosis in humans.

Keywords: Apoptosis; Osteoclast; TGFβ; TRAIL; Fas; Human

Bone remodeling is a continuous physiological process in which bone is resorbed and then new bone is formed. The bone cells responsible for these twin processes include bone-resorbing cells, the osteoclasts, which are multinucleated cells derived from hematopoietic cells of the monocyte-macrophage lineage, and bone-forming cells, the osteoblasts, which are of mesenchymal origin [1]. These events are closely linked and tightly regulated to maintain skeletal homeostasis. Evidence from rodent models suggests that, after producing bone resorption the osteoclasts undergo apoptosis [2]. In addition, bisphosphonates, which are anti-resorption agents, are able to induce osteoclast apoptosis in rodents as well as in humans, both in vitro and in vivo [2,3].

Although we know a lot about the factors that regulate osteoclast formation and osteoclast activity, much less is known about the factors involved in osteoclast apoptosis. Cytokines that enhance osteoclast activity do so in part by increasing osteoclast life span, as has been shown for TNFα, IL1, IL6, and M-CSF. Similarly,

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☆☆ *Abbreviations:* TNF, tumor necrosis factor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TRAIL-R, TRAIL receptor; RANK, receptor activator of NK-κB; RANKL, receptor activator of NK-κB ligand; MNC, multinucleated cell; CTR, calcitonin receptor; CBMs, cord blood monocytes; TGFβ, transforming growth factor β; M-CSF, macrophage-colony-stimulating factor.
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factors that inhibit osteoclast activity block osteoclast formation, and therefore bone resorption, and may therefore also induce osteoclast apoptosis. Such an effect has been observed in murine studies in which TGFβ and estrogens induced osteoclast apoptosis [4], as did osteoprotegerin (OPG) [5].

There is a real need to elucidate this process, because osteoclast life span is the keystone to bone remodeling and could provide a useful target for the treatment of skeletal disorders. However, although considerable progress has been made in our understanding of the mechanisms of osteoclast apoptosis in rodents, little is known about the human mechanism.

The two most common apoptotic pathways involve the activation of one of the membrane death receptors, and that of the mitochondria-activated pathway involving Bcl-2 family members. The induction of apoptosis by extracellular signals involves ligands related to TNF. Conversely, death receptors, cell surface receptors that transmit apoptosis signals, belong to the TNF receptor superfamily. Some death receptors have been described in detail, including CD95 (Fas) that binds CD95 ligand (FasL), and TRAIL receptors that bind TNF-related apoptosis-inducing ligand (TRAIL). To date, five human receptors for TRAIL have been identified and characterized. Of these, TRAIL-R1 and TRAIL-R2 are able to transduce a death signal, however, TRAIL-R3 and TRAIL-R4 do not possess a complete death domain and are not able to mediate apoptosis. Finally, the fifth TRAIL receptor is the soluble decoy receptor, OPG, which inhibits TRAIL-mediated apoptosis [6]. It is important to stress that TRAIL receptors differ in humans and mice, and that they may function by different mechanisms [7].

We know that the osteoclast biology in humans and mice differs in several respects, and it is crucial to evaluate the human counterparts, especially as biotherapies are now being evaluated in human diseases, including inhibitors of RANKL [8,9], TRAIL or anti-TRAIL receptors [10].

Our aim was to study osteoclast apoptosis in a human model using long-term cultures of cord blood monocytes (CBMs) that are able to differentiate into mature osteoclasts under appropriate conditions [11,12]. Interestingly, we show here that this model can be used to study human osteoclast apoptosis, and for the first time, that TRAIL and FasL pathways could be involved in this process.

Materials and methods

Eagle's minimum essential media (MEM), penicillin, streptomycin, fungizone, glutamine, and fetal calf serum (FCS) were purchased from Wisent (Montreal, Que.). Ficoll–Paque was purchased from Amersham Biosciences (Montreal, Que.). Human recombinant (hr) M-CSF, hrGM-CSF, and hrTRAIL were purchased from R&D (R&D Systems, Minneapolis, MN); hrTGFβ1 was purchased from Peprotech (Rocky Hill, NJ), and soluble hrRANKL was kindly provided by Dr. Artur Fernandes (Sherbrooke, Que.). Goat polyclonal antibodies were used to detect TRAIL receptors (R&D Systems) and calcitonin receptor (CTR) (Santa Cruz Biotechnology, Santa Cruz, CA). We used rabbit polyclonal antibodies (Chemicon, Temecula CA) to detect RANK. Mouse monoclonal antibody against Fas (CD95) was purchased from MBL (Watertown, MA).

Cord blood monocyte cultures. Human umbilical cord blood was obtained from healthy women, under a protocol approved by the local Ethics Committee. Mononuclear leukocyte suspensions were isolated from heparinized umbilical cord blood by density-gradient centrifugation, washed, and suspended in MEM with antibiotics, glutamine, and 10% FCS. They were plated at a density of 3×10^6/ml on 8-well chamber/slides (Lab-Tek, Biosciences, Bedford, MA). After being incubated overnight, the cells were washed to remove non-adherent cells. The CBMs were cultured for a further 3 weeks in the same medium supplemented with GM-CSF (100 pg/ml) for 3 days, and then with M-CSF (25 ng/ml) and RANKL (75 ng/ml). The medium was changed every 2–3 days.

In some experiments, 72 h after removing M-CSF and RANKL, factors that could have an effect on osteoclast apoptosis were added at the end of the culture, 24 h before the time scheduled for the analysis of apoptosis. Different concentrations of TRAIL (10–200 ng/ml), a Fas-activating antibody (50–400 ng/ml), TGFβ1 (0.1–2 ng/ml), and M-CSF (1–25 ng/ml) were added where appropriate.

Bone resorption evaluation in cultures performed on cortical bone slices. CBMs were settled on devitalized bone slices and then cultured for 3 weeks under the same conditions as above. The bone slices were then removed, washed with sodium hydroxide and distilled water, sonicated to remove cell debris, and stained with 1% toluidine blue containing 1% sodium borate. Optical light microscopy with epi-illumination was used to determine bone resorption.

Bone-marrow biopsies. Bone-marrow biopsies were obtained at diagnosis from three patients with multiple myeloma and from three subjects with normal bone. This protocol had been approved by the local Ethics Committee. Each subject gave his/her informed consent. These biopsies were fixed in formol and embedded in paraffin wax for histological study.

Immunohistochemistry. Immunohistochemistry was performed using a biotin–streptavidin–peroxidase kit (LSAB+ kit, Dako, Carpinteria, CA) to stain paraffin-embedded bone biopsy sections and cultured cells in three experiments after a 3-week culture period. Tissue sections were deparaffinized in 100% xylene and then rehydrated by serial incubations in ethanol, followed by PBS. Tissue sections were pre-treated by microwave heating in citrate buffer, pH 7.3, for 10 min. The specimens were incubated with the primary antibody or with the same concentration of normal goat or mouse serum (Dako) overnight at 4 °C in a humid chamber. Other controls were performed omitting the primary antibody. The dilutions used for the primary antibodies were 1/250 for anti-CTR, 1/400 for anti-RANK, 1/100 for anti-TRAIL-R1, -R3, and R-4, and 1/250 for anti-TRAIL-R2 antibodies. Specimens were treated with 3% H_2O_2 in PBS for 5 min to inactivate endogenous peroxidase activity. Sequential incubations were then performed with biotinylated secondary antibody and peroxidase-labeled streptavidin (Dako). Staining was revealed with AEC chromogen (Dako). The slides were counterstained and mounted in Glycergel (Dako). Only multinucleated cells (MNCs), cells with three or more nuclei, were taken into account, and the results were expressed as a percentage of the labeled cells (means ± SEM). To evaluate the co-expression of CTR and TRAIL receptors, a double immunofluorescence method was used on cultured cells. We used mouse anti-goat antibodies labeled with the fluorescent marker Alexa 488 or 546 (Molecular Probes, Eugene, OR) as secondary antibodies.

Study of osteoclast apoptosis. Apoptosis was visualized using Hoechst fluorescent dye (bisbenzimide H33342, Sigma, Oakville, Ont.). The TACS Blue Label kit (R&D Systems) was used to detect and

quantify apoptosis, and to investigate the kinetics of apoptosis in this model. Osteoclast cells are adherent and so this TUNEL-derived method permits in situ visualization of DNA fragmentation at the single cell level by immunochemistry. At the end of the cultures, cells are fixed in 3.7% formaldehyde solution, washed, and permeabilized using cytonin. Endogenous peroxidase activity is quenched using hydrogen peroxide. Biotinylated nucleotides are then incorporated by terminal deoxynucleotidyl transferase (TdT). Streptavidin–horseradish peroxidase conjugate is then added, followed by the substrate, TACS Blue Label (R&D Systems). The enzyme reaction generates an insoluble, colored precipitate where DNA fragmentation has occurred. As positive controls, and after incubation in cytonin and washing, some samples were treated with nuclease to generate DNA breaks in every cell. Cells stained with TACS Blue Label were then counterstained with Nuclear Fast Red that allows all cells to be visualized. The stained samples are examined using a light microscope. Only MNCs were taken into account, and the results were expressed as a percentage of the labeled cells (means ± SEM). As RANKL and M-CSF are survival factors, osteoclast apoptosis was determined after the withdrawal of M-CSF and RANKL.

Immunocytochemical double-labeling was performed using CTR expression, and apoptosis was determined in order to identify the multinucleated cells of the osteoclast lineage.

Statistical analysis. Results were expressed as means ± standard error of the mean (SEM), and the significance was determined using a Student's paired t test. Statistical significance was defined as $P < 0.05$.

Results

CBMs are able to differentiate in bone-resorbing cells under appropriate conditions

When cultured for 3 weeks in MEM supplemented with FCS 10% in the presence of soluble RANKL (75 ng/ml) and MCSF (25 ng/ml), CBMs formed multinucleated cells that expressed phenotype markers for osteoclasts. The expression of CTR and RANK, osteoclast markers, by cultured CBMs was investigated by immunohistochemistry (Figs. 1A–C); the proportion of labeled MNCs formed in long-term cultures of CBMs was estimated (results of five experiments, expressed as the mean proportion of positive cells ± SEM). The proportion of CTR-positive cells was 72% ± 2 and the proportion of RANK-positive cells was 75% ± 4 of the multinucleated cells. In addition, CBMs cultured on the surface of cortical bone resulted in the formation of bone resorption pits (Fig. 1D). Thus, these findings make it clear that we have a good and reliable model for generating cells with all the phenotypic markers of mature human osteoclasts.

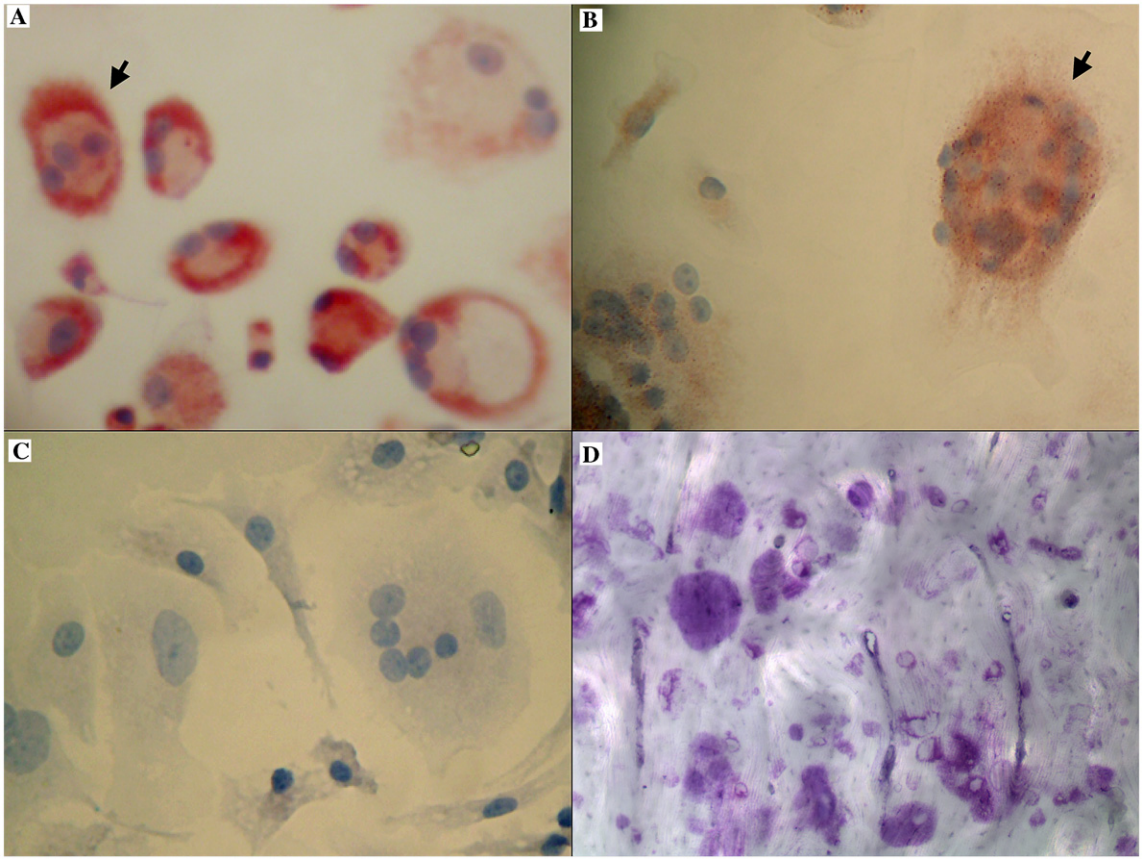

Fig. 1. Cultured CBMs expressed osteoclast phenotype. When cultured for 3 weeks in the presence of M-CSF and RANKL, CBMs formed multinucleated cells that expressed CTR (A) and RANK (B). No cells were stained in experiments in which rabbit or goat polyclonal Ig was used instead of the primary antibody (400×) (C). When CBMs were cultured for 3 weeks on bone slices, they formed resorption pits (200×) (D).

Apoptosis may occur in osteoclasts after survival factors have been withdrawn

At the end of the culture, after withdrawing RANKL and M-CSF, and lowering the concentration of FCS to 5% for 24 h, apoptosis was evaluated. Hoechst staining with a DNA-binding fluorochrome was used to detect apoptosis in MNCs, and DNA fragmentation was observed in MNCs (Figs. 2A and B). TACS Blue labeling, a TUNEL-derived method that can be used to investigate the apoptosis of adherent cells, was used to quantify the number of apoptotic MNCs (Fig. 2C). Under the conditions described above, apoptosis occurred in $58.6 \pm 2.34\%$ of the MNCs ($n = 10$).

In addition, to characterize the apoptotic cells, the expression of CTR, an osteoclast marker, was determined in conjunction with apoptosis in some experiments, and we found that $72.77 \pm 6.12\%$ of the multinucleated apoptotic cells were indeed osteoclasts ($n = 6$).

Evaluation of TRAIL-receptor expression in CBMs

Immunocytochemistry analysis was performed in long-term cultured CBMs, using antibodies directed against TRAIL receptors (R1–R4). This evaluation was performed at the end of the CBM cultures. We found that multinucleated cells expressed TRAIL receptors, TRAIL-R1 was expressed in $67 \pm 2\%$, TRAIL-R2 in $55 \pm 4\%$, TRAIL-R3 in $49 \pm 6\%$, and TRAIL-R4 in $36 \pm 8\%$ of the MNCs ($n = 3$). In addition, immunocytochemical double-labeling was performed using both anti-death receptor antibodies against TRAIL receptors and anti-CTR antibodies in order to identify the multinucleated cells of the osteoclast lineage. Some multinucleated cells did indeed express both of these markers. Results for TRAIL-R1 expression are shown in Figs. 3A–E.

TRAIL-receptor expression in osteoclasts in normal bone and in myeloma lesions

TRAIL-receptor expression was investigated in vivo in myeloma, a condition characterized by a high level of osteoclastogenesis, and in normal bone. Immunohistochemistry was performed on normal bone-marrow biopsies ($n = 3$), and on myeloma specimens ($n = 3$). In all normal bone-marrow biopsies, the TRAIL receptors, TRAIL-R1 and TRAIL-R3, were found to be expressed by mononuclear cells in the bone marrow. These cells were identified as myeloid cells, especially eosinophils and neutrophils (Fig. 4B). In contrast, TRAIL-R2 and TRAIL-R4 expression was not detected. In these normal specimens, the bone cells, which

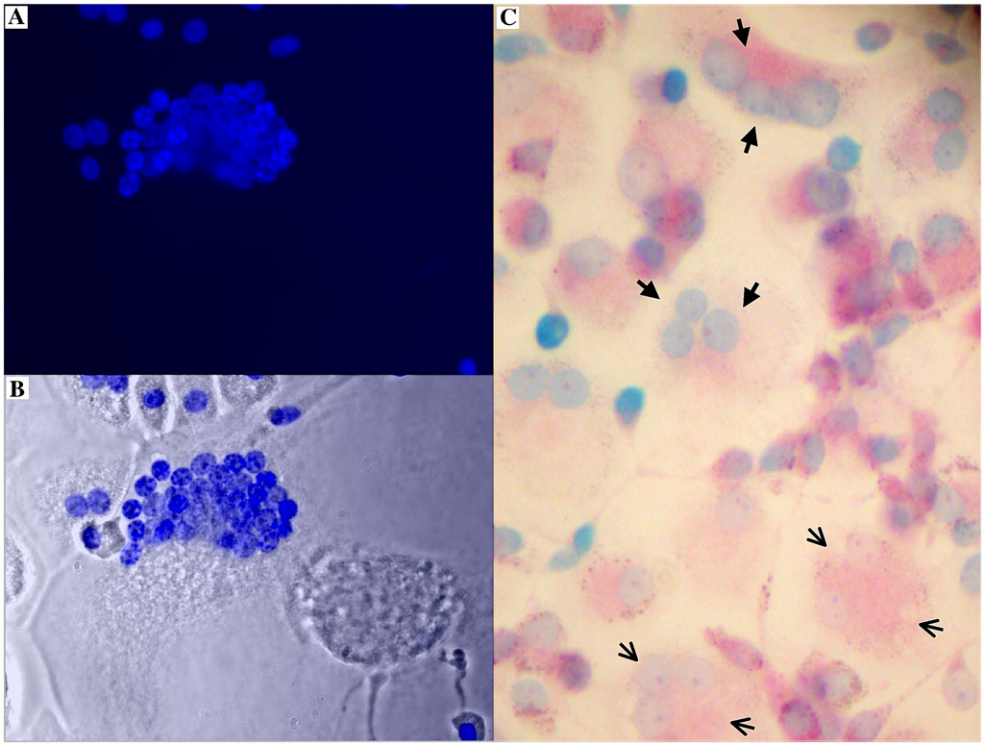

Fig. 2. Apoptosis in cultured CBMs. Nuclear fragmentation can be observed in osteoclast cells using a DNA-binding fluorochrome (Hoechst) added at the end of the culture (A,B). To quantify the number of apoptotic cells, TACS Blue labeling was used, resulting in blue staining of the nuclei of the apoptotic cells (positive multinucleated cells are delineated by bold arrows, and negative cells by thin arrows) (C).

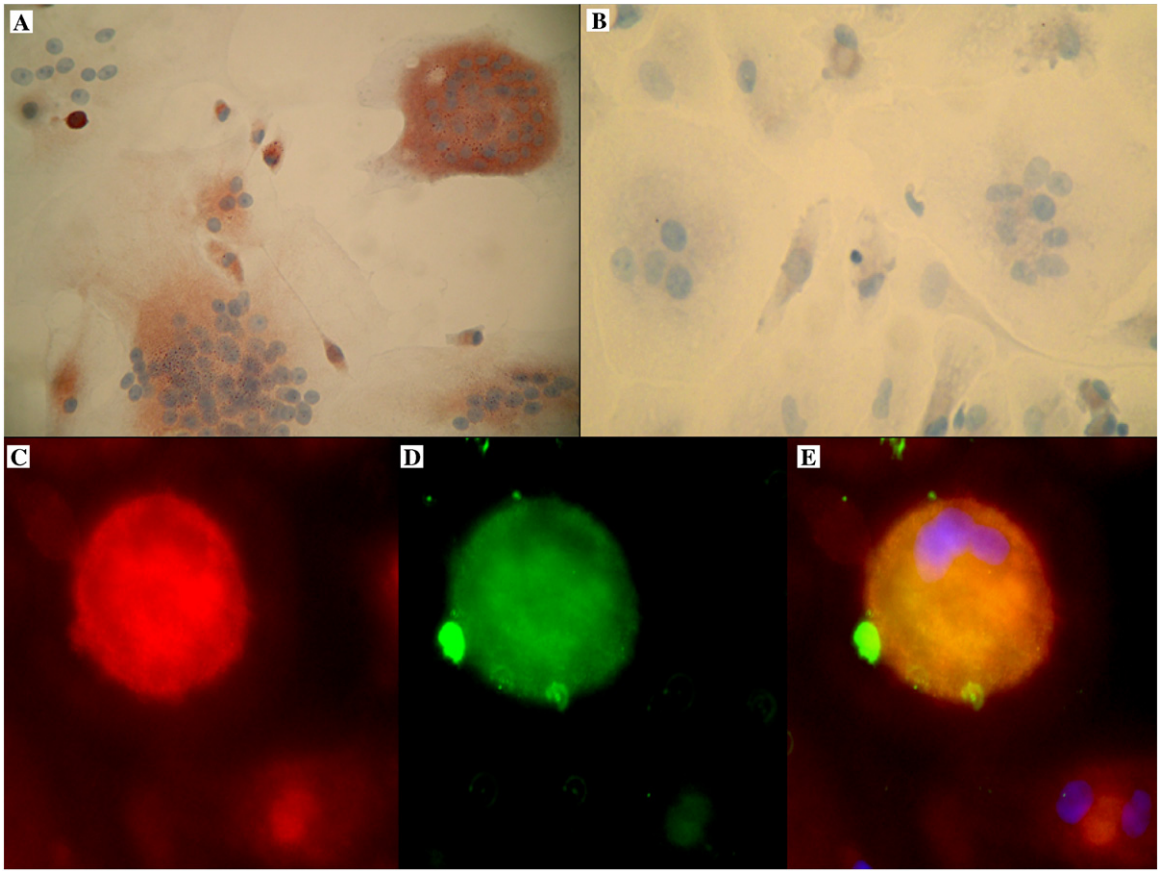

Fig. 3. Expression of death receptors by cultured CBMs evaluated by immunofluorescence using double labeling. In long-term cultures of CBMs, some osteoclasts expressed TRAIL-R1 (A), and no cells were stained in experiments using goat polyclonal Ig (B). In addition, in double-labeling experiments using fluorescent antibodies, some multinucleated cells expressed both a TRAIL receptor (TRAIL-R1) stained in red (C) and the CTR, stained in green (D). This is clear from the co-localization of these markers (E), where in addition to immunochemistry, Hoechst staining was visualized, showing the multinucleation.

included very few osteoclasts, were not stained by any of the antibodies (Fig. 4A). In myeloma lesions, which are characterized by having a high bone turnover and numerous osteoclasts close to areas of resorbed bone, the pattern of expression of TRAIL receptors differed. TRAIL-R3 and TRAIL-R4 were strongly expressed in osteoclasts (Fig. 4C), whereas the expression of TRAIL-R1 was weaker (Fig. 4D), and some osteoclasts were negative. We were not able to find any expression of TRAIL-R2 in osteoclasts from these biopsies.

Evaluation of osteoclast apoptosis

As the correlation between the pattern of expression of the death receptors and the sensitivity to the pro-apoptotic molecules may be difficult to establish [13], functional determinations were required to confirm the potential role of the TRAIL apoptotic pathway in osteoclast apoptosis. In addition, we evaluated Fas-induced apoptosis in order to explore the second main pathway involving membrane death receptors. At the end of the CBM cultures, after the M-CSF and RANKL had been removed, different concentrations of a Fas-activating antibody (50–400 ng/ml) and recombinant TRAIL (10–200 ng/ml) were added 24 h before the apoptosis was determined. In addition, TGFβ, a factor known to induce osteoclast apoptosis in murine osteoclasts, was also evaluated in these experiments at concentrations of 0.1–2 ng/ml. Finally, as M-CSF is a survival factor for osteoclasts, increasing concentrations of M-CSF (1–25 ng/ml) were also used in these experiments.

Results from 10 experiments revealed that the percentage of apoptotic MNCs was $58 \pm 2.3\%$ in control cultures, $69.4 \pm 3.8\%$ in the presence of TRAIL at a concentration of 10 ng/ml ($p = 0.013$ vs. controls), $74.5 \pm 3.8\%$ at 50 ng/ml ($p = 0.017$ vs. controls), $75.6 \pm 3.2\%$ at 100 ng/ml ($p = 0.003$ vs. controls), and $77.6 \pm 3.3\%$ at 200 ng/ml ($p = 0.0007$ vs. controls) (Fig. 5A); the percentage of apoptotic MNCs was $62.8 \pm 2.4\%$ in the presence of a Fas-activating antibody at a concentration of 50 ng/ml (ns vs. controls), $67.5 \pm 3.4\%$ at 100 ng/ml ($p = 0.003$ vs. controls), $69.3 \pm 1.6\%$ at 200 ng/ml ($p = 0.001$ vs. controls), and $75.2 \pm 3.6\%$ at 400 ng/ml ($p = 0.001$ vs. controls) (Fig. 5B); the per-

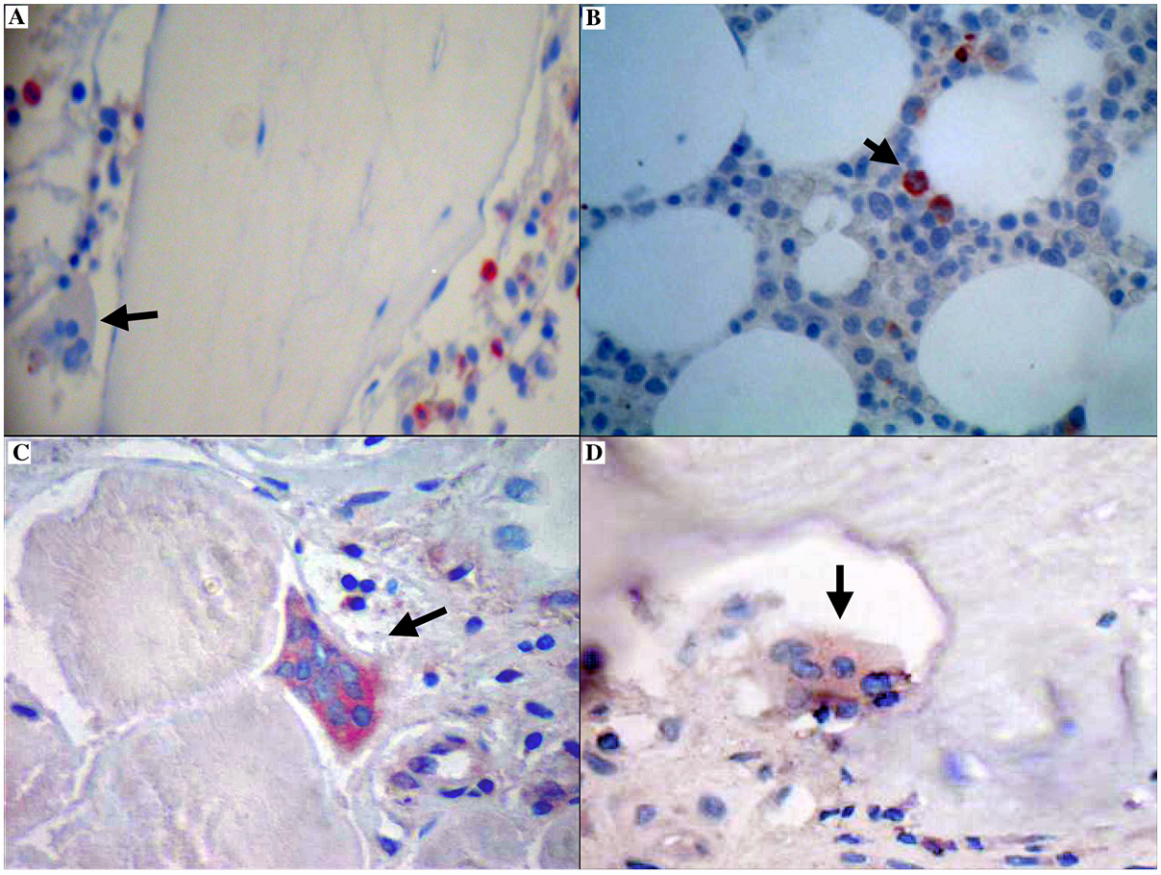

Fig. 4. Expression of TRAIL receptors in human bone-marrow biopsies. In all the normal bone-marrow biopsies, the bone cells, which included very few osteoclasts, were not stained with any antibody (A). In these normal specimens, TRAIL-R1 (and -R3, data not shown) were found to be expressed by mononuclear cells in the bone marrow. These cells were identified as myeloid cells, mainly eosinophils and neutrophils (B). In myeloma lesions, TRAIL-R3 (and TRAIL-R4, data not shown) (C) were strongly expressed in osteoclasts, the expression of TRAIL-R1 was weaker (D), and no expression of TRAIL-R2 was detected.

centage of apoptotic MNCs was $70.6 \pm 4.5\%$ in the presence of TGFβ1 at a concentration of 0.1 ng/ml ($p = 0.04$ vs. controls), $74.6 \pm 3.4\%$ at 0.5 ng/ml ($p = 0.03$ vs. controls), $75.3 \pm 3\%$ at 1 ng/ml ($p = 0.002$ vs. controls), and $78.4 \pm 3.6\%$ at 2 ng/ml ($p = 0.001$ vs. controls) (Fig. 5C). Finally, in eight experiments we found that the percentage of apoptotic MNCs was $51.2 \pm 5.3\%$ in control cultures, $58.8 \pm 4.3\%$ in the presence of M-CSF at a concentration of 1 ng/ml, $49.1 \pm 5.7\%$ at 5 ng/ml, $38.7 \pm 9.3\%$ at 10 ng/ml, and $25.1 \pm 5.6\%$ at 25 ng/ml ($p < 0.001$ vs. controls) (Fig. 5D).

Discussion

Survival and apoptosis are of major importance in the osteoclast life cycle, and can lead to crucial changes in bone resorption in diseases such as osteoporosis or malignant osteolysis. Our aim was to investigate the apoptotic pathways involved in the osteoclast apoptosis that occurs at the end of the bone resorption process, and that could be regulated to control the number and activity of osteoclasts. The originality of this work lies in the fact that it investigated this process in human osteoclasts. In in vitro murine or human osteoclast differentiation models, soluble RANKL enables osteoclast precursors to differentiate into mature osteoclasts in the presence of M-CSF [14]. We used CBMs as the source of human osteoclast precursors for in vitro experiments. When cultured in the presence of M-CSF and RANKL, CBMs are transformed into fully differentiated osteoclasts, multinucleated cells that express RANK, CTR, the vitronectin receptor ($\alpha_v\beta_3$), and tartrate-resistant acid phosphatase, and are able to resorb bone [11,12].

In rodents, osteoclast apoptosis has been studied both in mature osteoclasts in vivo, and in in vitro models of osteoclast differentiation [2], however, it is very important to stress that osteoclast apoptosis has rarely been investigated in humans. It has been demonstrated in in vitro cultures using murine bone marrow as a source of osteoclast progenitors that osteoclast apoptosis can occur in the absence of both RANKL and M-CSF, and that caspases, including caspase-8, -9, and -3, are involved in this process [15,16]. These find-

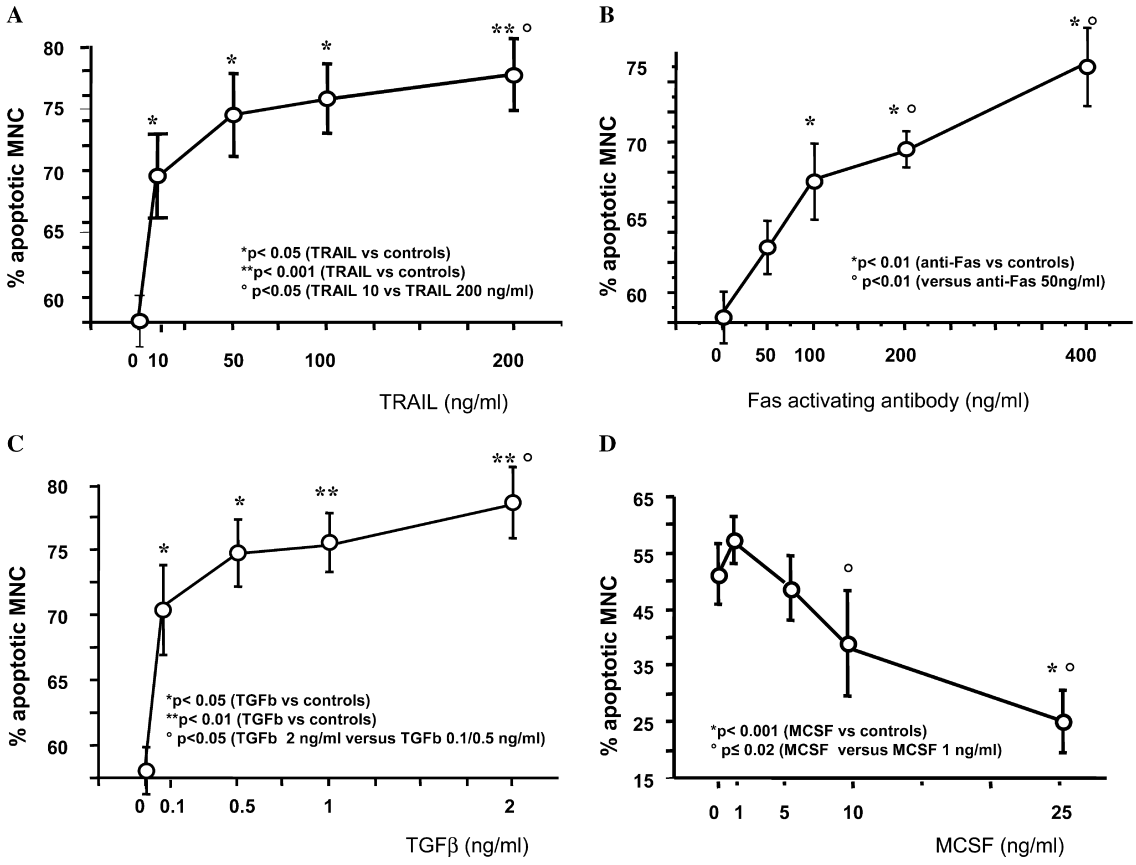

Fig. 5. Induction of apoptosis in long-term cultures of CBMs. At the end of the CBM cultures, after the M-CSF and RANKL had been removed, various concentrations of hrTRAIL (10–200 ng/ml) (A), of a Fas-activating antibody (50–400 ng/ml) (B), of hrTGFβ (0.1–2 ng/ml) (C), and of M-CSF (1–25 ng/ml) (D) were added 24 h before the apoptosis was assessed using TACS Blue labeling. Results are expressed as the percentage of apoptotic MNCs.

ings suggest that both the extracellular and the mitochondrial pathways are involved in osteoclast apoptosis.

In long-term cultures of CBMs, we showed that apoptosis occurred after the survival factors (M-CSF, RANKL) had been removed, and that the rate of apoptosis increased significantly in the presence of TGFβ1, a pro-apoptotic agent, and decreased in the presence of M-CSF, a well-known osteoclast survival factor. A limitation of our study arises from the use of a TUNEL assay to evaluate apoptosis, because blue staining of the cytoplasm and nucleus may occur in instances of necrosis, and is not completely specific for apoptosis. However, we observed significant and dose-dependent changes in the rate of cell death, that increased from 55% to 75% in the presence of pro-apoptotic agents, and decreased to 25% in the presence of M-CSF, an anti-apoptotic agent. We therefore believe that our findings are relevant to apoptosis. We removed M-CSF and RANKL for 72 h before studying apoptosis on the basis of previous studies showing that in the presence of FCS, about 50% of mature osteoclasts survived after a 72-h culture, and that adding M-CSF or caspase inhibitors prevented the cell death of these cells [17].

Focusing our work on death receptors, we have shown that osteoclast-like cells from CBM cultures expressed TRAIL receptors, both TRAIL-R1 and -R2, which are able to transduce apoptosis, and TRAIL-R3 and -R4, which are decoy receptors. These data are in accordance with previous studies showing that human osteoclast precursors express TRAIL receptors [18,19]. In addition, Fas has been shown to be expressed by human osteoclasts obtained from peripheral blood monocytes [18,20]. Significantly, we have shown here for the first time that these pathways are indeed functional as apoptosis occurred in human osteoclasts obtained from long-term cultures of CBMs, and was significantly greater in the presence of TRAIL, or a Fas-activating antibody. Although these data were obtained in an in vitro model of osteoclast differentiation, the observation of TRAIL-receptor expression in human bone specimens is further evidence suggesting that the TRAIL pathway may be involved in vivo. The relevance of the TRAIL-receptor expression in bone lesions from myeloma is difficult to interpret, because of the small number of specimens investigated. Nevertheless, it is of interest to note that none of these receptors were expressed in

normal bone, suggesting that the TRAIL pathway could be induced in osteoclasts in vivo under pathological conditions. In addition, the over-expression of the decoy receptors (TRAIL-R3 and TRAIL-R4) may contribute to the defect of osteoclast apoptosis that could be involved in diseases characterized by a high rate of osteoclast formation and bone resorption, such as myeloma. These results are in agreement with recent data showing that osteoclasts from PBMC generated in the presence of myeloma T cells over-expressed TRAIL-R4 and had a reduced expression of TRAIL-R1 [18]. Overall, the combination of these findings suggests for the first time in a human model that the TRAIL and FasL pathways could be involved in osteoclast apoptosis in humans.

Previous findings have suggested that the Fas–FasL pathway may be involved in murine osteoclast apoptosis [20,21]. Apoptosis pathways have been studied using cultures from mouse bone marrow, as well as RAW 264.7 cells. These cells expressed Fas, and a Fas-activating antibody induced apoptosis in mature osteoclasts [20]. RANKL could modulate Fas expression, by up-regulating osteoclast progenitors, and down-regulating mature osteoclasts [22]. In addition, when Fas blocking antibodies are added to mouse bone-marrow cultures that have been treated with M-CSF, TNFα, and IL12, apoptotic changes are inhibited [21]. However, the effects of defects in the Fas–FasL system in murine models in vivo are controversial [20,23]. The potential role of Fas in human osteoclast physiology has been highlighted by recent data showing that DcR3, a secreted member of the TNF receptor superfamily, induces osteoclast formation by promoting TNFα production [24]. It is interesting to note that DcR3 has been shown to be the decoy receptor for several members of the TNF family, including the Fas ligand. It is not clear, however, whether DcR3 acts by neutralizing FasL. Nevertheless, our findings show clearly that Fas stimulation induced osteoclast apoptosis in human osteoclasts.

Unlike the expression of FasL, which is mainly restricted to hematopoietic progenitors, activated T cells, NK cells, and lymphoid tissues [25], TRAIL mRNA expression occurs in many tissues [26]. TRAIL is produced by monocytes and NK cells, and appears to be an important effector molecule used mainly in the host defense against transformed cells [27–29]. In addition, TRAIL may play a role in the apoptosis of antigen-bearing dendritic cells induced by TRAIL-expressing activated T cells [30]. This suggests that TRAIL pathway could be involved in normal cells. TRAIL −/− deficient mice did not display any bone abnormalities, and in vitro osteoclast differentiation was normal in these mice, suggesting that this factor cannot play an essential part in bone physiology under normal conditions [31]. However, these studies do not conclusively rule out the possibility that the TRAIL–TRAIL-R pathway may be involved in osteoclast apoptosis. There may be some redundancy in the pathways involved in osteoclast apoptosis. Furthermore, human and murine apoptotic pathways may be different. Indeed, the TRAIL pathway could be involved in human osteoclast physiology. It has been shown in a recent study by Zauli et al. [19] that human peripheral blood mononuclear cells, which were used as osteoclast precursors, expressed TRAIL receptors, and that TRAIL inhibited osteoclast differentiation and blocked osteoclast bone resorption. Our findings support these data, as we found that osteoclast-like cells did indeed express TRAIL receptors. In addition, and contrary to the findings of Zauli et al., we found that in our model TRAIL induced osteoclast apoptosis. This discrepancy may have been due to differences in the experimental procedures, as we removed RANKL and M-CSF 72 h before adding TRAIL.

Although osteoclast apoptosis and its regulation are still not fully understood in animal models, considerable progress has been made during the past decade, and this is of great importance for our understanding of the behavior of osteoclast cells in bone resorption and bone remodeling. Indeed, bone resorption, as the first stage of bone renewal, is the keystone to the bone remodeling process, and its regulation is of major importance in controlling bone homeostasis. Our data, showing that the two main apoptotic pathways involving TRAIL receptors and Fas, could be implicated in osteoclast apoptosis in humans, are therefore of great interest, as they suggest potential targets that could be used to treat skeletal disorders.

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