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ARTICLES

Secretion of Tartrate-Resistant Acid Phosphatase by Osteoclasts Correlates With Resorptive Behavior

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Abstract There have been dramatic advances recently in our understanding of the regulation of osteoclastic differentiation. However, much less is known of the mechanisms responsible for the induction and modulation of resorptive behavior. We have developed a strategy whereby osteoclasts can be generated in vitro and released into suspension in a fully-functional state. We now exploit this approach to show that tartrate-resistant acid phosphatase (TRAP) is released by osteoclasts during bone resorption. TRAP release was inhibited by the secretion-inhibitor Brefeldin A, and was not accompanied by LDH release. This suggests that TRAP release is due to secretion, rather than cell death. Consistent with this, TRAP secretion was stimulated by resorbogenic cytokines, was inhibited by the resorption-inhibitor calcitonin, and correlated with excavation of the bone surface. We found that, in contrast to incubation on bone, incubation on plastic, glass, or vitronectin-coated plastic substrates did not induce secretion of TRAP. This suggests that the induction of resorptive behavior in osteoclasts depends upon stimulation by bone matrix of a putative osteoclastic ''mineral receptor.'' Release of TRAP by osteoclasts thus represents not only a productive approach to the analysis of the mechanisms that modulate the rate of resorptive activity, but also a system whereby the mechanism through which bone substrates induce resorptive behavior can be identified. J. Cell. Biochem. 98: 1085–1094, 2006.

Key words: osteoclast; bone resorption; TRAP; bone mineral; secretion

The development and maintenance of the skeleton depends on continual resorption of bone by osteoclasts and its replacement by osteoblasts. There have been considerable advances recently in our understanding of the mechanisms through which osteoclast formation is regulated [Suda et al., 1999; Chambers, 2000; Takayanagi, 2005]. In contrast, much less is known of the mechanisms that modulate their activity once formed, even though this is a major component of the regulation of bone resorption. Thus, after systemic administration of hormones such as CT or PTH in vivo, osteoclasts show morphological evidence of changes in activity, accompanied by changes in plasma calcium concentrations, within 30 min, while a change in osteoclast numbers is not detectable until 24 h later [Miller, 1978].

The resorption of bone by mature osteoclasts depends upon two distinct processes: the induction of resorptive behavior, and the modulation subsequently of its rate. Resorptive behavior involves the formation of a resorptive hemivacuole, bounded peripherally by a ring of F-actin, at the bone-apposed surface, and the extrusion of protons and enzymes into this hemivacuole, onto the bone surface [Vaananen et al., 2000; Wagner and Karsenty, 2001]. It would make good biological sense for this behavior to occur only when the cell is in contact with the appropriate substrate, and there is evidence that this is so: it has been shown that osteoclasts resorb mineralized but not demineralized bone matrix [Chambers et al., 1984]; proton pumping has been reported by osteoclasts incubated on mineralized but not on unmineralized substrates [Zimolo et al., 1995; Lees et al., 2001]; and osteoclasts form actin rings on mineralized but not plastic surfaces [Saltel et al., 2004].

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However, although it seems likely that osteoclasts themselves recognize the bone mineral, this cannot be concluded from the above studies. For example, the resorption and proton pumping experiments mentioned above used osteoclast preparations that also contained osteoblastic cells, so that it might be these contaminating cells that detect bone mineral: osteoblastic cells are known to be central to the regulation of bone resorption [Suda et al., 1999; Chambers, 2000; Takayanagi, 2005]. In the actin ring experiments, osteoclasts were generated on plastic and lifted into suspension for use, and actin rings were not observed on mineralized substrates until 24 h after sedimentation. This suggests that differentiation is necessary, and raises the possibility that actin rings are not seen on plastic because osteoclasts formed on plastic are functionally immature. It might be that bone matrix induces maturation, rather than activation: it is known that bone matrix affects osteoclastic differentiation [Fuller and Chambers, 1989a; Hentunen et al., 1994], and differentiation might be incomplete on plastic [Lader et al., 2001].

The mechanisms that regulate the rate of resorption, once this has been activated by bone mineral, are also poorly understood, largely because the assays used do not distinguish between stimulation of differentiation and stimulation of resorption. Clearly, if, as is usual in such assays, osteoclasts are generated on slices of bone or dentine matrix, then resorption will depend on differentiation. Even those assays in which osteoclasts are generated on plastic and then suspended and sedimented onto mineralized matrix require 48 h before resorption is detected [Akatsu et al., 1992; Wesolowski et al., 1995]. Since osteoclasts isolated from rodents form excavations within 3 h [Fuller and Chambers, 1989b], measurement of resorption in experiments that last 48 h does not distinguish agents that stimulate resorption from those that induce differentiation.

We have developed a novel strategy whereby osteoclasts can be detached from plastic substrates, and, when transferred to bone slices, form excavations in bone within the time-scale achieved by osteoclasts isolated ex vivo. This similarity with the performance of ex-vivo osteoclasts suggests that the cells are fully capable of resorption before they are sedimented. Thus, resorption in this assay represents activation, rather than maturation. This enables an analysis of the mechanisms that regulate the activation and modulation of resorptive behavior.

If contact with mineralized substrates activates resorption, a second question is: how does this occur? To address this question, it would be an advantage if a measure of resorptive activity were available other than bone resorption itself: this would allow us to identify the ligands lacking in other substrates that are responsible for activation of resorptive behavior.

Tartrate-resistant acid phosphatase (TRAP) is highly and specifically expressed in osteoclasts. It is believed to be secreted onto the bone surface, and then endocytosed and transported to the basolateral surface of the osteoclast [Halleen et al., 2003; Vaaraniemi et al., 2004; Ljusberg et al., 2005]. In vivo, serum concentrations correlate with the rate of bone resorption [Stepan et al., 1989; Halleen et al., 2002]. We therefore tested whether release of TRAP into the culture supernatant could be exploited as a surrogate for resorptive behavior in osteoclasts. We found that osteoclasts sedimented onto bone slices and incubated in agents that stimulate bone resorption release significant quantities of TRAP within 3 h. TRAP release correlated with the extent of bone resorption, and was not explicable as release through cell death, but was inhibited by Brefeldin A, an inhibitor of secretion. We also found that, in contrast to sedimentation of osteoclasts onto bone, TRAP release was not observed by osteoclasts sedimented onto plastic or glass substrates, or substrates coated with vitronectin. This suggests a model in which the characteristics of the substrate are crucial in the induction of resorptive behavior in osteoclasts, through a direct action on the osteoclast.

MATERIALS AND METHODS

Media and Reagents

Cells were incubated in MEM with Earle's salts (Invitrogen, Paisley, UK), supplemented with 10% FCS (Perbio Science UK Ltd., Cramlington, Northumberland, UK), 2 mM glutamine, 100 IU/ml benzylpenicillin and 100 μg/ml streptomycin (Sigma, Poole, Dorset, UK), unless stated otherwise. Recombinant human M-CSF was provided by Chiron Corp. (Emeryville, CA). Soluble recombinant murine RANKL was purchased from Insight Biotechnology (Wembley, Middx., UK). Recombinant murine

IL-1α and purified human TGF-β1 were obtained from R and D Systems (Abingdon, Oxon, UK). Salmon calcitonin, brefeldin A and vitronectin were from Sigma. Incubations were performed at 37°C in 5% CO_2 in humidified air, unless stated otherwise. Slices of bovine cortical bone were prepared as previously described [Chambers et al., 1985].

Generation of Osteoclasts on Plastic

Osteoclasts were induced from non-adherent, M-CSF-dependent bone marrow cells as previously described [Wani et al., 1999]. MF1 mice (4–8 weeks old) were killed by cervical dislocation. Femora and tibiae were aseptically removed and dissected free of adherent soft tissue. The bone ends were removed and the marrow cavity flushed out into a petri dish by slowly injecting PBS at one end of the bone using a sterile 21-gauge needle. The bone marrow suspension was passed repeatedly through a 21-gauge needle to obtain a single cell suspension. Bone marrow cells were then washed, resuspended in MEM/FCS and incubated at a density of 3×10^5 cells/ml for 24 h in a 75-cm^2 flask (Greiner Bio-One, Stonehouse, Gloucestershire, UK) with M-CSF (5 ng/ml), to deplete the cell preparations of stromal cells. Non-adherent cells were collected by centrifugation and added to 90 mm diameter cell culture dishes (Greiner) in MEM/FCS, containing M-CSF (50 ng/ml), RANKL (30 ng/ml) and TGF-β (0.1 ng/ml) (7.2×10^6 cells in 25 ml for each dish). Cultures were incubated for 6 days, by which time osteoclast formation was maximal. Cells were fed every 2–3 days by replacing 15 ml of culture medium with an equal volume of fresh medium and cytokines.

Harvest of In Vitro-derived Osteoclasts

After formation of osteoclasts on the base of a 90 mm-diameter plastic tissue culture dish, the medium was removed and the cell layer washed three times with PBS without calcium and magnesium. Six milliliters of 0.02% EDTA was added to the dish and cells incubated for 20 min at room temperature. The EDTA was then removed from the dish and replaced with 6 ml of calcium/magnesium-free PBS. A cell scraper (Greiner) was used to scrape the cells into the PBS, and the resulting cell suspension was agitated using a pipette to insure uniform cell dispersal. Seventy five microliters of this cell suspension was added to wells of a 96-well plate (Greiner), each well of which contained a Thermanox coverslip (Invitrogen) or a bone slice in 75 μl MEM/FCS: addition of FCS for sedimentation increased attachment of osteoclasts, while M-CSF and RANKL were without effect (data not shown). In experiments to test the effect of substrate on enzyme release, polystyrene or glass coverslips, or glass coverslips coated with vitronectin (5 μg/ml) were also used. Cells were allowed to sediment for 20 min at 37°C before the coverslips and bone slices were washed and transferred to fresh 96-well plate wells. Cells were incubated in 200 μl MEM/FCS in the presence or absence of cytokines or other agents as described. After incubation, supernatant and lysate enzyme levels and/or bone resorption was assessed as described below.

Tartrate-resistant Acid Phosphatase (TRAP) Cytochemistry

After incubation, some cultures with cells on coverslips or bone slices were fixed in formalin for 10 min, washed, permeabilized in acetone for 10 min, washed and stained for TRAP using the Leucognost-AP cytochemical reagent kit (VWR International Ltd., Lutterworth, Leicester, UK).

Measurement of Release of TRAP and LDH

After incubation of cells for 3–6 h on bone slices or coverslips, supernatants were removed from wells for measurement of enzyme release. Cell lysates, for assessment of enzyme remaining in cells, were then prepared: bone slices and coverslips were washed in PBS, transferred to fresh 96-well plate wells and incubated in 200 μl 0.1% Triton in water (v/v) for 10 min. TRAP enzyme activity was measured by the conversion of *p*-nitrophenyl phosphate to *p*-nitrophenol in the presence of sodium tartrate. Eighty microliters of each supernatant or lysate, diluted appropriately, was added to 96-well plate wells containing 80 μl 0.09 M citrate buffer (Sigma) with 20 mM phosphatase substrate (Sigma) and 80 mM tartaric acid (Sigma) and incubated at room temperature for 40 min. The reaction was stopped by addition of 40 μl of 0.5 M sodium hydroxide. Optical absorbance was measured at 405 nm on an Opsys MR plate reader (Thermo Electron Corporation, Basingstoke, Hampshire, UK) against a standard curve of *p*-nitrophenol (Sigma). LDH enzyme activity was measured using the CytoTox non-radioactive cytotoxicity assay (Promega, Southampton, Hampshire, UK), as per manufacturer's

instructions. For both enzymes, the extent of enzyme activity released into the supernatant as a percentage of total enzyme activity in the supernatant and lysate combined was calculated for each culture assayed.

Assessment of Bone Resorption

Bone slices were immersed in 10% (vol/vol) sodium hypochlorite for 10 min to remove cells, washed, air-dried, mounted onto stubs for scanning electron microscopy and sputter coated with gold. The entire surface of each bone slice was examined in a scanning electron microscope (S90: Cambridge Instruments, Cambridge, UK). The total area resorbed per bone slice was quantified.

Statistical Analysis

The statistical significance of differences between groups was assessed using Student's *t*-test.

RESULTS

Osteoclasts are very difficult to detach from tissue culture substrates [Chambers, 1979], and previous approaches have resulted in cell populations that do not resorb bone until after 48 h of incubation on mineralized substrates. We developed a novel strategy whereby osteoclasts can be detached from plastic, such that they form excavations within a few hours of attachment to bone. To do this, osteoclast-like cells are generated from bone marrow cells by incubation in M-CSF, RANKL and TGF-β for 6 days. The cultures were then incubated in EDTA for 20 min, followed by gentle agitation. Multinuclear cells were then readily detached using a cell scraper, and sedimented onto plastic or bone substrates for further incubation. After 1 h incubation on plastic, the strongly TRAP-positive osteoclast-like cells are well spread (Fig. 1).

We found that incubation in RANKL or IL-1 for 6 h induced these cells to release significant quantities of TRAP into the supernatant (Fig. 1). There was an accompanying reduction in TRAP found in the lysate. While RANKL alone induced a significant increase in the percentage of TRAP released into the supernatant, in combination with IL-1, there was a six-fold increase in TRAP release, compared to control cultures. For subsequent experiments RANKL was combined with IL-1 as a potent resorbogenic stimulus.

We noted that the quantity of TRAP released did not match the reduction in lysate TRAP, suggesting that TRAP is partly consumed during the resorptive process, or is unstable in culture medium. We have found (data not shown) that the latter process contributes significantly to this deficit.

Significant release of TRAP was detectable after 3 h of incubation, and release continued for the ensuing 3 h (Fig. 2). Cultures incubated in M-CSF alone showed substantially less TRAP release throughout the incubation period. Release of TRAP showed a similar pattern to that of bone resorption: there was no excavation of the bone surface by osteoclasts incubated in M-CSF alone, while in cultures incubated in RANKL and IL-1 excavations were already present after 3 h, and the proportion of bone surface that was excavated increased progressively for the remainder of the incubation period.

RANKL and IL-1 are both survival factors for osteoclasts [Fuller et al., 1998; Jimi et al., 1998]. This makes it unlikely that the increased release of TRAP induced by RANKL and IL-1 is due to apoptosis, especially since this release was associated with a considerable increase in bone resorption. We also found that TRAP release substantially exceeded release of LDH (Fig. 3).

TRAP release by osteoclasts incubated on bone was potently inhibited by CT, which also strongly suppressed bone resorption (Fig. 4). This further correlates TRAP release with resorptive behavior. CT was able to completely inhibit even the release of TRAP induced by the potent resorbogenic combination of RANKL and IL-1.

We found that release of TRAP was also strongly inhibited by Brefeldin A, an inhibitor of vesicular secretion [Misumi et al., 1986] (Fig. 5). Brefeldin A inhibited TRAP release, even inhibiting those small quantities released by cells incubated in M-CSF alone. The ability of Brefeldin A to suppress both bone resorption and TRAP release by osteoclasts suggests that secretion is required for both processes.

These results strongly suggest that release of TRAP by osteoclasts occurs through a secretory process, and reflects bone-resorptive activity by these cells. To investigate this notion further, we tested whether incubation of osteoclasts on

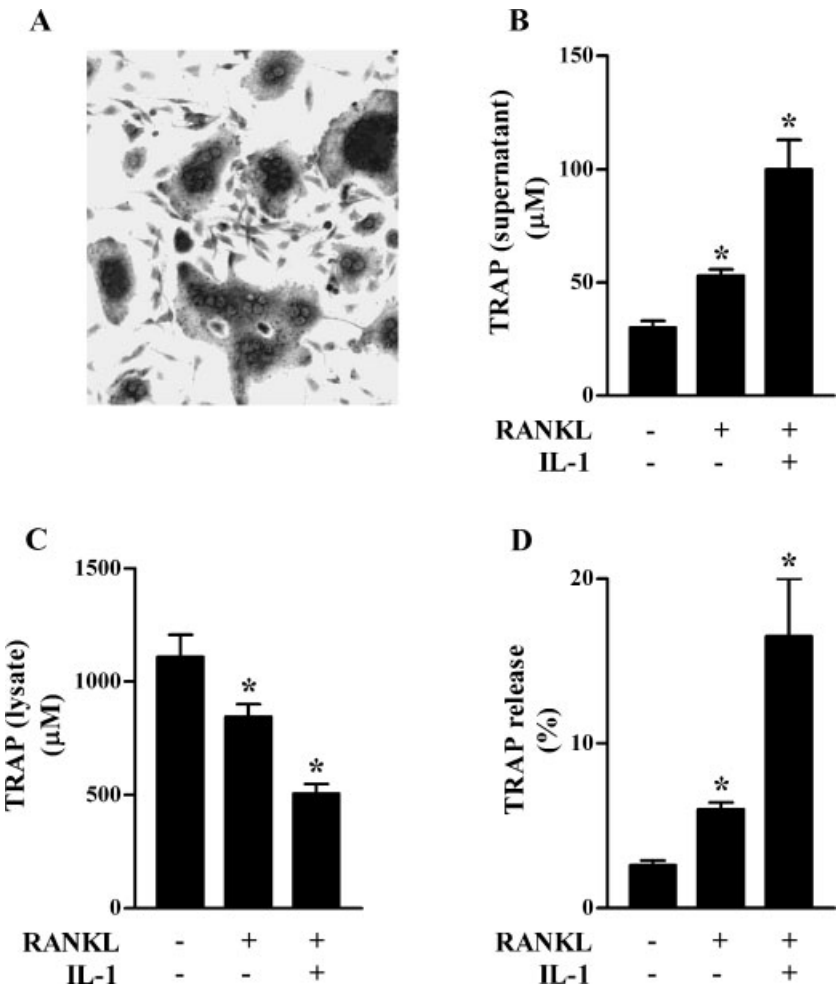

Fig. 1. Osteoclasts incubated on bone release TRAP into the supernatant. After incubation of M-CSF-dependent bone marrow cells in M-CSF, RANKL and TGF-β for 6 days, cells were harvested by incubation in EDTA for 20 min, followed by detachment from the plastic with a cell scraper. **A**: cells harvested as described above and sedimented onto a plastic coverslip, and stained for TRAP after 1 h of incubation in M-CSF, showing many TRAP-positive multinuclear cells. **B**–**D**: cells harvested as above and sedimented onto bone slices for 20 min, and then placed in new wells for incubation for 6 h in M-CSF (50 ng/ml) with or without RANKL (30 ng/ml) and IL-1 (10 ng/ml). Supernatants and lysates were then taken for assessment of TRAP activity. TRAP release (%) was calculated as TRAP activity in the supernatant, as a percentage of the total activity in the supernatant and lysate combined. n = 6 cultures per variable. $*P < 0.05$ versus control.

substrates other than bone matrix were associated with release of TRAP. We found (Fig. 6) that, while osteoclasts incubated on bone released a considerable proportion of TRAP in response to cytokines, TRAP release was not increased by these cytokines in osteoclasts incubated on artificial substrates, including those coated with vitronectin.

DISCUSSION

TRAP is strongly expressed in osteoclasts [Ek-Rylander et al., 1991; Angel et al., 2000; Hayman et al., 2001]. Deletion of the gene that codes for TRAP leads to an increase in the quantity of bone, suggesting that the enzyme assists the resorptive process [Hayman et al., 1996]. One proposal is that the enzyme generates free radicals that help to dissolve collagen fragments released from bone by cathepsin K [Halleen et al., 1999]. Alternative roles include actions as an osteopontin phosphatase [Andersson et al., 2003], in iron transport [Saunders et al., 1985], and as a growth factor [Bazer et al., 1991; Sheu et al., 2003]. Whatever its function, it has long been known that TRAP levels are increased in the serum of individuals who have a high rate of bone resorption [Stepan et al., 1989; Halleen et al., 2002]. We reasoned that measurement of TRAP release in culture might

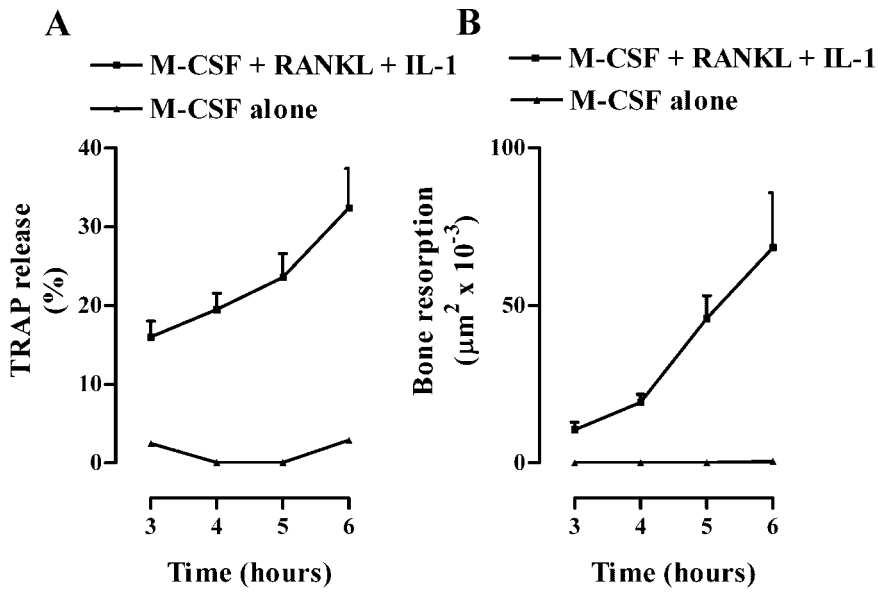

Fig. 2. Release of TRAP by osteoclasts correlates with bone resorption. After incubation of M-CSF-dependent bone marrow cells in M-CSF, RANKL and TGF-β for 6 days, cells were harvested by incubation in EDTA for 20 min, followed by detachment from the plastic with a cell scraper. The cells were then sedimented onto bone slices for 20 min, and then placed in new wells for incubation for 3–6 h in M-CSF (50 ng/ml) with/without RANKL (30 ng/ml) and IL-1 (10 ng/ml). Supernatants and lysates were then taken for assessment of TRAP activity. TRAP release (%) was calculated as TRAP activity in the supernatant, as a percentage of the total activity in the supernatant and lysate combined. After cell lysis, the surface area of bone that showed excavation was measured in a scanning electron microscope. n = 6 cultures per variable.

therefore represent a surrogate for bone resorptive behavior of osteoclasts in vitro.

We found that release of TRAP by osteoclasts incubated on bone slices correlated with bone-resorptive activity. Moreover, agents that stimulate bone resorption also stimulated TRAP release, and release was strongly suppressed by CT, which inhibits bone resorption. Release was inhibited by Brefeldin A, an inhibitor of secretion, and could not be attributed to cell death, since the proportion of TRAP released by resorbing osteoclasts was substantially greater that the proportion of LDH released. Furthermore, the cytokines that we found to induce the release of TRAP are known to suppress, rather than cause, apoptosis in osteoclasts; and the correlation between TRAP release and bone resorption makes it unlikely that release can be attributed to apoptosis. Thus, TRAP appears to be released from resorbing osteoclasts in our cultures by a secretory process.

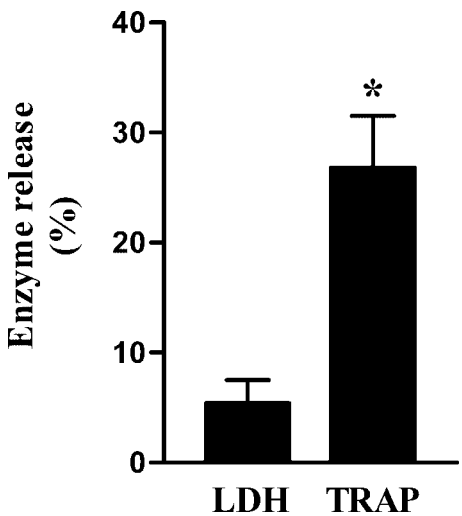

Fig. 3. Release of TRAP by osteoclasts in response to resorbogenic cytokines does not reflect release of LDH. Osteoclasts were suspended and sedimented onto bone slices. TRAP and LDH activity were measured in supernatant and lysate after 6 h of incubation in M-CSF (50 ng/ml), RANKL (30 ng/ml) and IL-1 (10 ng/ml). n = 6 cultures per variable. *$P < 0.05$ versus LDH.

Osteclasts isolated from rodents and sedimented onto slices of bone form excavations in the bone surface within 3 h of the initiation of incubation [Fuller and Chambers, 1989b]. We have found that osteoclasts released from plastic similarly undertake resorptive activity very soon after sedimentation onto bone slices, and have already formed excavations within 3 h. TRAP release showed a similar tempo. This suggests that TRAP release by osteoclasts reflects the induction of resorptive behavior, and that osteoclasts formed on plastic and

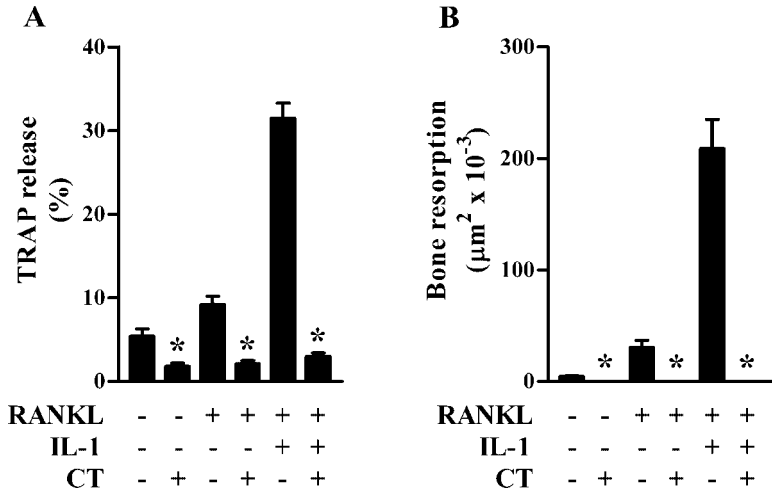

Fig. 4. CT suppresses TRAP release and bone resorption by osteoclasts incubated on bone. Osteoclasts were sedimented onto bone slices, and incubated for 6 h in M-CSF (50 ng/ml) with or without RANKL (30 ng/ml), IL-1 (10 ng/ml) and CT (1 ng/ml). TRAP activity was measured in the supernatant and lysate, and expressed as the activity released into the supernatant as a percentage of the total TRAP activity in the culture. Bone resorption was measured, after removal of lysate, in a scanning electron microscope. n = 6 cultures per variable. *$P < 0.05$ versus CT-free control.

detached into suspension are functionally mature.

We noted that there was an inverse relationship between release of TRAP into the supernatant, and the quantity of TRAP remaining in cells after incubation: the cells from cultures stimulated to resorb bone contained smaller quantities of TRAP. This is unlikely to be due to cell death, as discussed above, and therefore suggests that osteoclasts resorbing in vitro secrete TRAP more rapidly than it is replaced. This decline might not occur in vivo if the bone micro-environment enables receptor-mediated reuptake of TRAP to occur more efficiently than is possible in the more diffusible conditions of tissue culture.

In contrast to osteoclasts sedimented onto bone slices, we found that sedimentation onto

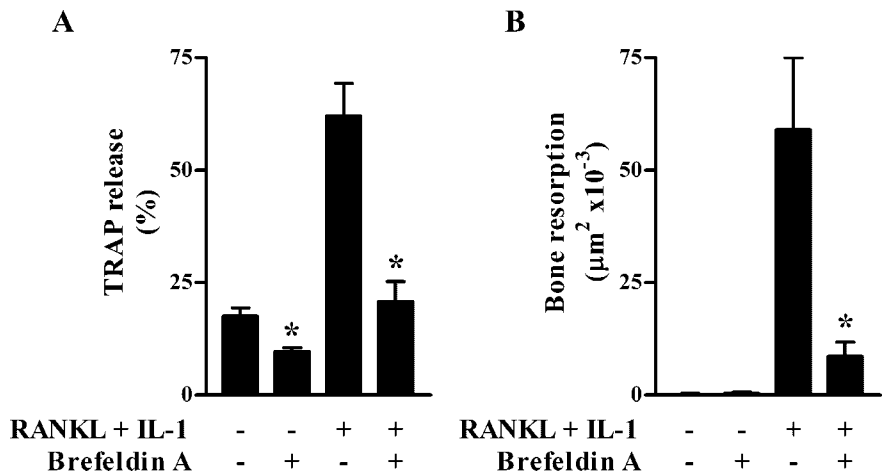

Fig. 5. Brefeldin A suppresses osteoclastic release of TRAP. Osteoclasts were sedimented onto bone slices, and incubated with M-CSF (50 ng/ml) with or without RANKL (30 ng/ml), IL-1 (10 ng/ml) and brefeldin A (10 μg/ml) for 6 h. TRAP activity was measured in the supernatant and lysate, and expressed as the activity released into the supernatant as a percentage of the total TRAP activity in the culture. Bone resorption was measured, after lysate-extraction, in a scanning electron microscope. n = 6 cultures per variable. *$P < 0.05$ versus brefeldin A-free control.

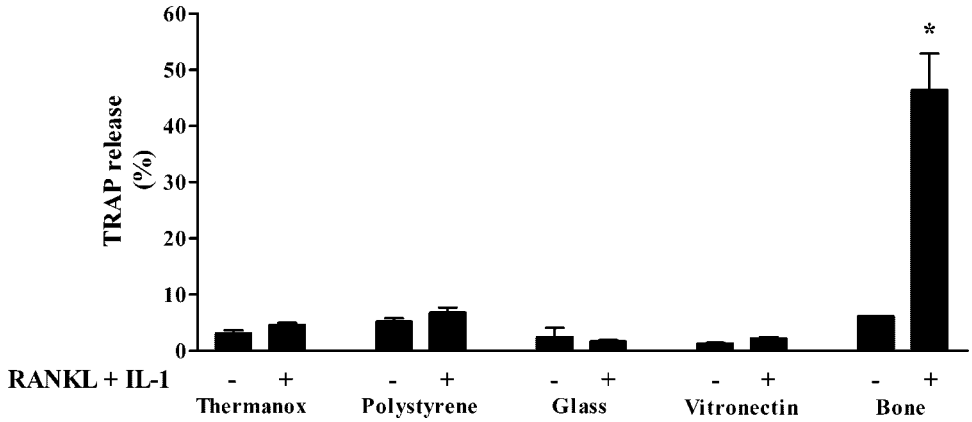

Fig. 6. Artificial substrates do not induce TRAP secretion by osteoclasts. Osteoclasts were sedimented onto Thermanox, polystyrene, or glass coverslips, or glass coverslips pre-coated with vitronectin, or onto bone slices. Cultures were incubated for 6 h in M-CSF (50 ng/ml), RANKL (30 ng/ml) and IL-1 (10 ng/ml). TRAP activity was then measured in the supernatant and lysate, and expressed as the activity released into the supernatant as a percentage of the total TRAP activity in the culture. n = 6 cultures per variable. $^*P < 0.05$ versus all other cultures.

artificial substrates did not lead to TRAP release. This represents further evidence that the release of TRAP by osteoclasts on bone reflects resorptive behavior. The observation also supports our previous suggestion, using ex-vivo cells, that osteoclasts recognize some mineral-dependent characteristic of bone that leads, via a putative "mineral receptor" to the induction of resorptive behavior [Chambers et al., 1984]. Our results are also consistent with the observation that osteoclasts form actin ring structures on mineralized substrates that differ distinctively from those formed on non-mineralized substrates [Saltel et al., 2004]. The results are also consistent with data showing that mineralized but not plastic substrates induce proton pumping in osteoclasts [Zimolo et al., 1995; Lees et al., 2001]. Our present results show that the activation response is due to an interaction between specifically osteoclasts and the mineralized substrate. The above data support a model in which osteoclastic recognition of mineralized substrates induces resorptive behavior.

The identity of the receptor that mediates osteoclastic recognition of mineralized bone is of substantial importance: the putative mineral receptor is the mechanism through which resorptive behavior in osteoclasts is induced. Thus, it would be a prime target for inhibition, for the prevention of bone resorption that causes osteoporosis and other common diseases of bone. Therefore, its identity is a question of considerable interest.

Recently, two receptor systems that mediate contact-dependent interactions between the osteoclast and its environment have been identified, that might be candidate partners for this receptor. These are two mutually-redundant receptors for unknown ligands, that act through proteins that possess immunoreceptor tyrosine-based activation motifs (ITAMs) [Koga et al., 2004]. Current evidence makes it unlikely that they represent the putative mineral receptor. Mice deleted for the ITAM that is the partner for OSCAR, the receptor for the unknown osteoblastic ligand, have virtually normal bones. The other, the unknown receptor that acts through the ITAM DAP-12, is required for osteoclast formation on plastic, yet plastic does not, unlike bone, induce release of TRAP. Furthermore, we would expect normal or increased numbers of osteoclasts in the bone of the ITAM knockouts if their osteoclasts were incompetent for resorption—as occurs, for example in mice deleted for Src [Lowe et al., 1993]—but osteoclasts are reduced or almost absent in the single and double ITAM knockouts. Thus, in osteoclasts these ITAMs appear to be essential primarily for the induction of differentiation.

Our finding that vitronectin-coated substrates did not induce TRAP release by osteoclasts suggests that the vitronectin receptor is also not the receptor responsible for the recognition of mineralized matrix. This conclusion is consistent with the observation that the vitronectin receptor mediates adhesion of osteoclasts

not only to bone but to plastic [Helfrich et al., 1992], which does not cause TRAP release; and that mice deleted for the vitronectin receptor do not develop osteopetrosis [Feng et al., 2001]. The surprising ability of vitronectin-receptor-deficient mice to develop a normal marrow cavity suggests that some other receptor system is responsible for the activation of resorptive function in osteoclasts.

The assay we have described should facilitate analysis of the mechanisms through which bone matrix activates resorptive behavior in osteoclasts. In addition, the assay will be of value in measuring osteoclastic performance. At present this depends upon morphology-based measurement of the rate of excavation of mineralized substrates, or measurement of the concentration of collagen fragments released from bone into the supernatant during incubation. Application of these assays is limited by productivity and cost. The assay described herein thus represents not only a means to identify the putative mineral receptor, but also a productive approach to the analysis of the cellular and humoral mechanisms through which the rate of bone resorption is modulated.

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