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Normal Human Osteoclasts Formed From Peripheral Blood Monocytes Express PTH Type 1 Receptors and Are Stimulated by PTH in the Absence of Osteoblasts

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Abstract The prevailing view for many years has been that osteoclasts do not express parathyroid hormone (PTH) receptors and that PTH's effects on osteoclasts are mediated indirectly via osteoblasts. However, several recent reports suggest that osteoclasts express PTH receptors. In this study, we tested the hypothesis that human osteoclasts formed in vitro express functional PTH type 1 receptors (PTH1R). Peripheral blood monocytes (PBMC) were cultured on bone slices or plastic culture dishes with human recombinant RANK ligand (RANKL) and recombinant human macrophage colony-stimulating factor (M-CSF) for 16–21 days. This resulted in a mixed population of mono- and multi-nucleated cells, all of which stained positively for the human calcitonin receptor. The cells actively resorbed bone, as assessed by release of C-terminal telopeptide of type I collagen and the formation of abundant resorption pits. We obtained evidence for the presence of PTH1R in these cells by four independent techniques. First, using immunocytochemistry, positive staining for PTH1R was observed in both mono- and multi-nucleated cells intimately associated with resorption cavities. Second, PTH1R protein expression was demonstrated by Western blot analysis. Third, the cells expressed PTH1R mRNA at 21 days and treatment with 10^{-7}M hPTH (1–34) reduced PTH1R mRNA expression by 35%. Finally, bone resorption was reproducibly increased by two to threefold when PTH (1–34) was added to the cultures. These findings provide strong support for a direct stimulatory action of PTH on human osteoclasts mediated by PTH1R. This suggests a dual regulatory mechanism, whereby PTH acts both directly on osteoclasts and also, indirectly, via osteoblasts. J. Cell. Biochem. 95: 139–148, 2005. © 2005 Wiley-Liss, Inc.

Key words: human osteoclasts; PTH receptor; bone resorption

The parathyroid hormone (PTH) type 1 receptor (PTH1R) is a G protein-coupled receptor that is highly expressed in bone and kidney, and mediates the PTH-dependent regulation of mineral homeostasis as well as the actions of PTH-related peptide [Mannstadt et al., 1999]. Since the landmark paper by Rodan and Martin [1981], the prevailing view has been that osteoclasts do not express PTH receptors and that PTH's effects on osteoclasts are mediated indirectly via osteoblasts. However, there have

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been several reports over the years indicating that osteoclasts from several species possess functional PTH receptors. Using immunocytochemistry, Rao et al. [1983] demonstrated intense staining of bovine PTH over osteoclasts in fixed rat bones. The staining was more intense than that observed in both osteocytes and osteoblasts. Teti et al. [1991] and Agarwala and Gay [1992] both demonstrated specific binding of bovine PTH to avian osteoclasts in vitro. In the latter study, living osteoclasts bound PTH in a manner that displayed the properties of receptor-dependent hormone binding, i.e., saturability, time dependence, temperature-dependence, and hormone specificity.

More recently, osteoclast expression of the PTH1R has been demonstrated in deer antler [Faucheux et al., 2002], in rat osteoclasts in culture [Watson et al., 2002], and in sections of rat bone [Gay et al., 2003]. To date, the only evidence for the presence of PTH1R in human osteoclasts has come from the work of Langub et al. [2001], who detected PTH1R mRNA in human osteoclasts in bone biopsies, but PTH1R protein was only detected in patients with secondary hyperparathyroidism. The purpose of the present study was to test the hypothesis that human osteoclasts formed in vitro from peripheral blood monocytes express PTH1R, and that PTH influences osteoclast function in the absence of osteoblasts.

MATERIALS AND METHODS

Human recombinant RANK ligand (RANKL), *E. coli* RANKL, and recombinant human macrophage colony-stimulating factor (M-CSF) were purchased from R&D Systems (Minneapolis, MN). M-CSF was also obtained as a kind gift from the Genetics Institute (Boston, MA). Rat PTH (1–34) was purchased from Sigma (St. Louis, MO). Dulbecco's phosphate-buffered saline (PBS), fetal calf serum, and alpha-minimal essential medium were purchased from Life Technologies (Grand Island, NY), and Ficoll-Paque was from Pharmacia Biotech (Piscataway, NJ). Polyclonal antibodies raised against peptides mapping at the amino terminal (N-20) and carboxyterminal (C-19) of CTR of human origin, and a goat polyclonal antibody raised with an epitope mapping the near amino terminus of the PTH/PTHrP receptor of human origin, and respective blocking peptides were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

These studies were approved by the Institutional Review Boards of Helen Hayes Hospital and University College London, and informed consent was provided by all subjects. Blood was collected from healthy volunteers by standard venipuncture into heparinized tubes. PBMCs were obtained using Ficoll-Paque as previously described [Breuil et al., 1998]. The $CD14^+$ monocytes ($CD14^+$ Mo), which represent a rich source of osteoclast precursors [Massey and Flanagan, 1999; Nicholson et al., 2000], were isolated by magnetic activated cell sorting (MACS, Miltenyi Biotec, Auburn, CA). Some experiments were done with commercially available $CD14^+$ Mo purchased from Biowhittaker, Inc. (Gaithersburg, MD), stored in liquid N_2, and thawed according to the vendor's instructions. $CD14^+$ Mo were cultured on 16 mm^2 bovine cortical bone slices [Arnett and Dempster, 1986] or in plastic tissue culture wells from Becton Dickinson Labware (Franklin Lakes, NJ) for up to 21 days in the presence of 10 ng/ml RANKL and 25 ng/ml M-CSF. Cos-7 cells were transfected with PTH1R as described previously [Iida-Klein et al., 1997, 2002], and served as a positive control for the presence of PTH1R; untransfected Cos-7 cells served as a negative control. Saos-2 cells also served as a positive control.

Immunocytochemistry

At the end of each experiment, cells were fixed in 100% ice-cold methanol, blocked, and incubated overnight at 4°C with appropriate primary antibodies. Negative controls included cells incubated without primary antibody, and with primary antibody that had been preincubated with a fourfold excess (by weight) of blocking peptide either for 4 h at room temperature or at 4°C overnight. Staining was revealed using the ABC Peroxidase Kit (Vector Laboratories, Burlingame, CA) with the appropriate biotinylated secondary antibody and DAB Chromagen detection method (Polysciences, Warrington, PA). In addition to CTR and PTH1R, immunocytochemical staining was also performed using similar methods with the following antibodies: 23c6 for the vitronectin receptor (Serotec, Oxford, UK), and C-16 for cathepsin K (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

Time Course of Bone Resorption

Culture medium was changed every 3 days and assayed for cross-linked C-telopeptide of

type I collagen (CTx) using the Crosslaps kit (Osteometer, Rodovre, Denmark) as previously described [Breuil et al., 1998].

Effects of PTH on Resorption Pit Formation

The mono-nuclear cell layer was removed from the Ficoll-plasma interface, washed in 10 ml PBS, recentrifuged at 750*g* for 10 min and resuspended in minimum essential medium (MEM) with 10% heat-inactivated fetal calf serum (FCS) (Gibco, Paisley, UK). The mono-nuclear cells (200,000 cells in 100 μl) were sedimented onto bone slices in 96-multiwell plates for 4 h. The bone slices were then rinsed by dipping gently in PBS, transferred to fresh 96-wells containing 200 μl MEM, 10% FCS, 5 ng/ml MCSF, and 5 mEq/l OH^- (as NaOH), and cultured for 4 days. After this 4 day initial culture period, bone slices were transferred to 25 cm^2 flasks containing 1 ml per slice of the same culture medium, plus 1 ng/ml RANKL, and cultured for a further 10 days in a 5% O_2, 5% CO_2, 90% N_2 atmosphere. The hypoxic environment stimulates osteoclast formation strongly [Arnett et al., 2003; Utting et al., 2004]; the operating pH of this medium is ~7.40, which is optimal for osteoclast formation, while allowing partial activation of resorption pit formation by human osteoclasts [Utting et al., 2004]. For the final 48 h, 50 or 100 ng/ml rPTH (1–34), or vehicle were added to the culture medium. Culture medium pH and PCO_2 were monitored by blood gas analyzer (ABL 705, Radiometer, Crawley, Sussex, UK). Cultures were fixed in 2% glutaraldehyde and stained to demonstrate tartrate-resistant acid phosphatase (TRAP) for 40 min. Osteoclasts were defined as TRAP-positive cells with two or more nuclei. The total number of such cells on each bone slice was counted "blind" using transmitted light microscopy. The plan surface area of resorption pits on bone slices was measured "blind" using reflected light microscopy and point-counting morphometry [Hoebertz and Arnett, 2003]. Data were analyzed by analysis of variance.

Western Blots

Western blot analysis was performed following standard protocols using the following equipment: Biorad (Hercules, CA) electrophoresis/western blot system, 7.5% SDS mini gels, broad range molecular weight standards, and Amersham Pharmacia (Little Chalfont, UK) chemiluminescence detection reagents. For detection of PTH1R, Western blot analysis was performed on protein extracted from four different samples; 21 day osteoclast cultures, Cos 7 cells (negative controls), Saos-2 cells (positive controls), Cos 7 cells transfected with the PTH1R (positive controls). Protein/sample of 1 mg was used as starting material. Immunoprecipitation of PTHR1 was performed using a mouse monoclonal antibody to human PTH1R. Samples were loaded onto 7.5% SDS page minigel along with broad range standards. The gel was transferred to PVDF membrane and the Western blot was run at 300 mA for 2 h. Blots were incubated in HRP-conjugated secondary antibody, followed by chemiluminescence substrate, developed, and exposed to X-ray film for 20 s. The breast carcinoma cell line (T47D) obtained from ATCC served as a positive control for the CTR. For negative controls, the antibodies were combined with a fourfold excess by weight of their respective blocking peptides.

RT-PCR

These experiments were conducted following our previously published procedures [Iida-Klein et al., 1997, 2002]. Total RNA was extracted and 5 μg of RNA was reverse-transcribed at 42°C for 50 min using SuperScript II (Invitrogen, Carlesbad, CA) in the presence of Oligo dT (0.5 μg), dNTPs (10 μM each), and dithiothreitol (1 mM). The reaction was terminated by heating the mixture at 70°C for 15 min. 100 ng of RT products were then amplified by PCR, using Taq polymerase (Clontech, La Jolla, CA). The primer pairs of human, PTH1R (forward primer, 5′-ACTCGTGAACGGGAGG TGTTTG-3′, backward primer, 5′-GAGGATGAAGTTGAGCACA ATGG-3′, size 600, 560–1,159 bp, NCBI access no. NM_00316.1) were specifically designed as previously described [Iida-Klein et al., 2002]. Primers for GAPDH (forward primer, 5′-TGA-AGGTCGGTGTGAACGGATTTGGC-3′, backward primer, 5′-CATGTAGGCCAT GAGG TCCACCAC-3′, size 983 bp, Clontech, La Jolla, CA) were used for standardization of the band intensity. The PCR was conducted by 5-min initial denaturing at 95°C, followed by repeated cycles of denaturing at 94°C for 45 s, annealing at the specific temperature for each primer pair for 45 s , and extension at 72°C for 60 s. The annealing temperature and cycle number for the PTH1R and GAPDH were 57°C and 60°C and 35 and 30 cycles, respectively. The last extension was 7 min. The PCR products were

electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and subjected to quantitative scanning densitometry using SigmaGel software (SPSS, Inc., Chicago, IL).

Scanning Electron Microscopy

After immunocytochemical staining, the cells were stripped from the bone slices by ultrasonication for 3 min in 0.25M NH_4OH and slices were dehydrated and air-dried from acetone. Specimens were viewed in a JEOL JSM 840A scanning electron microscope after sputter-coating with a 50 nm layer of gold [Breuil et al., 1998].

RESULTS

Characterization of Osteoclast Cultures

Incubation of $CD14^+$ Mo on bone slices for 21 days resulted in a mixed population of multi- and mono-nuclear cells. All the cells showed specific staining for the CTR (Figs. 1 and 4). No staining was observed when a fourfold excess of blocking peptide was added or when primary antibodies were omitted. In Western blots, the CTR antibody recognized a single band at approximately 65 kDa, which corresponds to the CT receptor (Fig. 2). To verify that there were no cells present that did not stain for the CTR, we counted the total number of CTR-positive cells on bone slices. We then stained the slices with toluidine blue to reveal any cells that had not stained for the CTR and recounted the same slices. The cell number was the same before and after toluidine blue staining (Fig. 3). Similar results were obtained using $CD14^+$ Mo from three individual donors. All cells present also stained positively for the vitronectin receptor and cathepsin K, markers that are highly expressed in osteoclasts (data not shown).

There was extensive resorption of the bone slice underlying the CTR-positive cells (Fig. 4). Frequently the outline of a resorbed area corresponded exactly with the shape of the accompanying CTR-positive cell (Fig. 4A,B). Furthermore, mono-nuclear, CTR-positive cells were frequently associated with small resorption cavities, suggesting that they were capable of resorption (Fig. 4). Further evidence for this was obtained by scanning electron microscopy (Fig. 5). The high level of bone resorption in these cultures was confirmed by measurement of medium CTx concentration (Fig. 6). Resorption commenced after 7 days and increased rapidly thereafter.

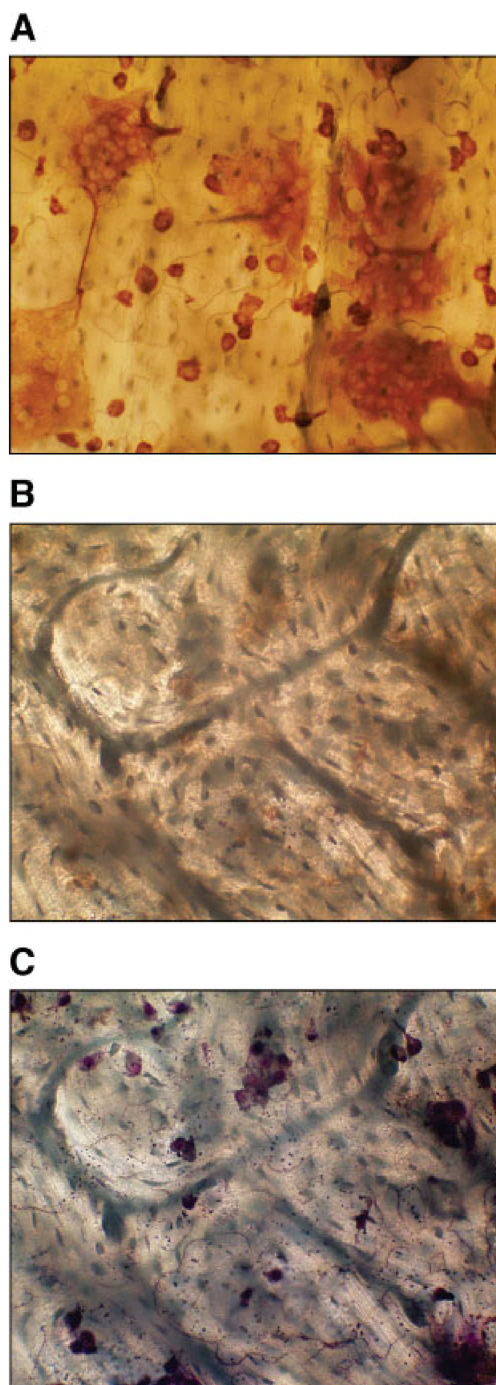

Fig. 1. Mono- and multi-nuclear cells stained for CTR using the N-20 antibody (**A**). Note the lack of staining in control samples incubated with blocking peptide (**B**). Panel (**C**) shows the same slice as in (B) counterstained with toluidine blue to reveal that cells were present in the control sample.

Demonstration of PTH1R

$CD14^+$ Mo cultured in the presence of M-CSF and RANKL for 21 days were stained for PTH1R. Controls included omission of the prim-

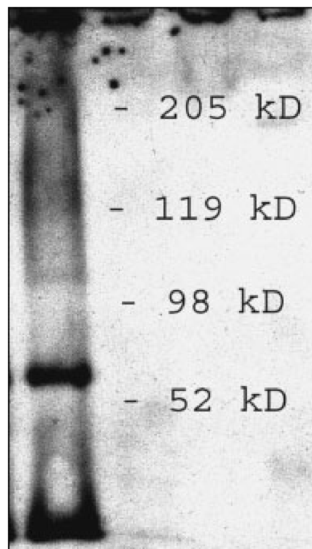

Fig. 2. Western blot from 14-days osteoclast-forming human Peripheral blood monocytes (PBMC) culture indicating the presence of a single band at approximately 60 kD corresponding to the human CTR. The presence of CTR was revealed by the CTR-N20 antibody.

ary antibody and preabsorption of the primary antibody with a fourfold excess of blocking peptide. Positive staining was observed in mono- and multi-nucleated cells intimately associated with resorption cavities (Fig. 7A), whereas staining was markedly reduced in samples in which the primary antibody had been preincubated with blocking peptide (Fig. 7B).

Western blot analysis was performed on protein extracted from four different samples; 21-days osteoclast cultures, Cos-7 cells (negative controls), Saos-2 cells (positive controls), and Cos-7 cells transfected with the PTH1R (positive controls). Figure 8 shows the presence of PTH1R in 21-day osteoclast cultures as

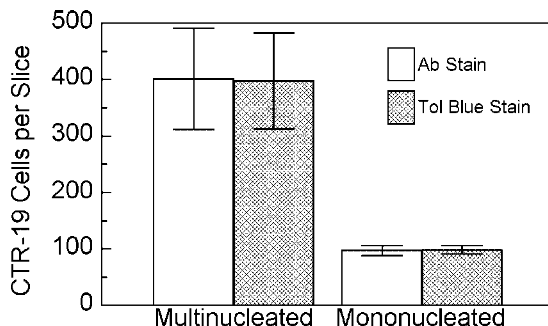

Fig. 3. Multi- and mono-nuclear cell numbers as determined on slices stained with the CTR-C19 antibody and then after counter-staining the same slices with toluidine blue.

well as in positive controls (Cos-7-PTH1R and Saos-2), but not in the negative control (untransfected Cos-7).

$CD14^+$ monocytes were treated with M-CSF (25 ng/ml) and RANKL (10 ng/ml) for 21 days in the presence of either 10^{-7}M hPTH (1–34) or vehicle. As shown in Figure 9, treatment with PTH (1–34) reduced PTH1R mRNA expression by 35%. This result was reproduced in two independent experiments using different RT products.

Effect of PTH on Bone Resorption

Human peripheral blood mono-nuclear cells were cultured with RANKL and M-CSF for 14 days and then exposed to 50 or 100 ng/ml rat PTH (1–34) or vehicle for a further 2 days. Bone resorption was reproducibly increased by three to fourfold in the presence of 50 or 100 ng/ml rat PTH (1–34) (Fig. 10). There was a non-significant trend towards an increase in osteoclast number in the presence of PTH.

DISCUSSION

This paper adds to the growing body of literature [Rao et al., 1983; Agarwala and Gay, 1992; Langub et al., 2001; Faucheux et al., 2002; Watson et al., 2002; Gay et al., 2003] supporting the view that osteoclasts express functional PTH receptors and shows for the first time that the resorptive function of human osteoclasts is regulated by PTH in the absence of osteoblasts. The presence of the receptor was demonstrated by four independent techniques. First, using immunocytochemistry, positive staining for PTH1R was observed in both mono- and multi-nucleated cells intimately associated with resorption cavities. Second, PTH1R protein expression was demonstrated by Western blot analysis. Third, the cells expressed PTH1R mRNA at 21 days and treatment with 10^{-7}M hPTH (1–34) reduced PTH1R mRNA expression by 35%; such homologous downregulation of PTH1R has previously been reported in a number of cell types, including embryonic chicken bone cells [Teitelbaum et al., 1986], rat fetal osteoblasts [Jongen et al., 1996], Saos-2 osteoblast-like cells [Fukayama et al., 1992], and opossum and human kidney cells [Abou-Samra et al., 1994; Chauvin et al., 2002]. Finally, bone resorption was reproducibly increased by two to threefold when PTH (1–34) was added to the cultures. We cannot comple-

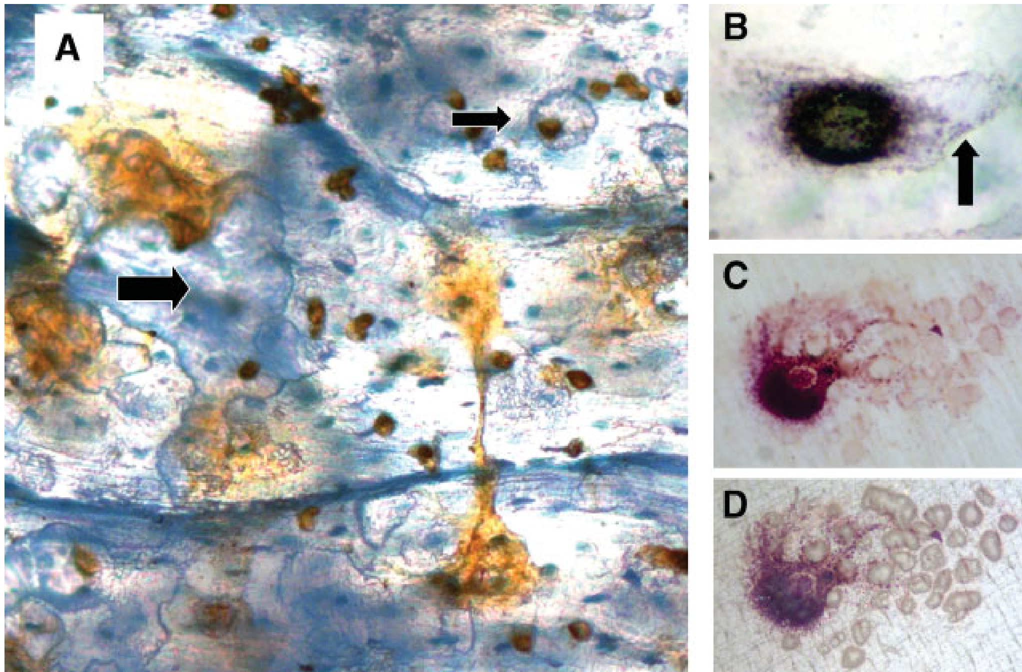

Fig. 4. **A**: Multi- and mono-nuclear cells stained with the CTR-C19 antibody. Note large resorption cavities (large arrow, left) associated with multi-nuclear cells and a small cavity (small arrow, right) associated with a mono-nuclear cell; (**B**) mono-nuclear cell stained with the CTR-C19 antibody and underlying resorption pit (arrow); (**C**,**D**) mono-nuclear cell stained for tartrate-resistant acid phosphatase (TRAP) and associated small pits viewed under transmitted light (C) and reflected light (D).

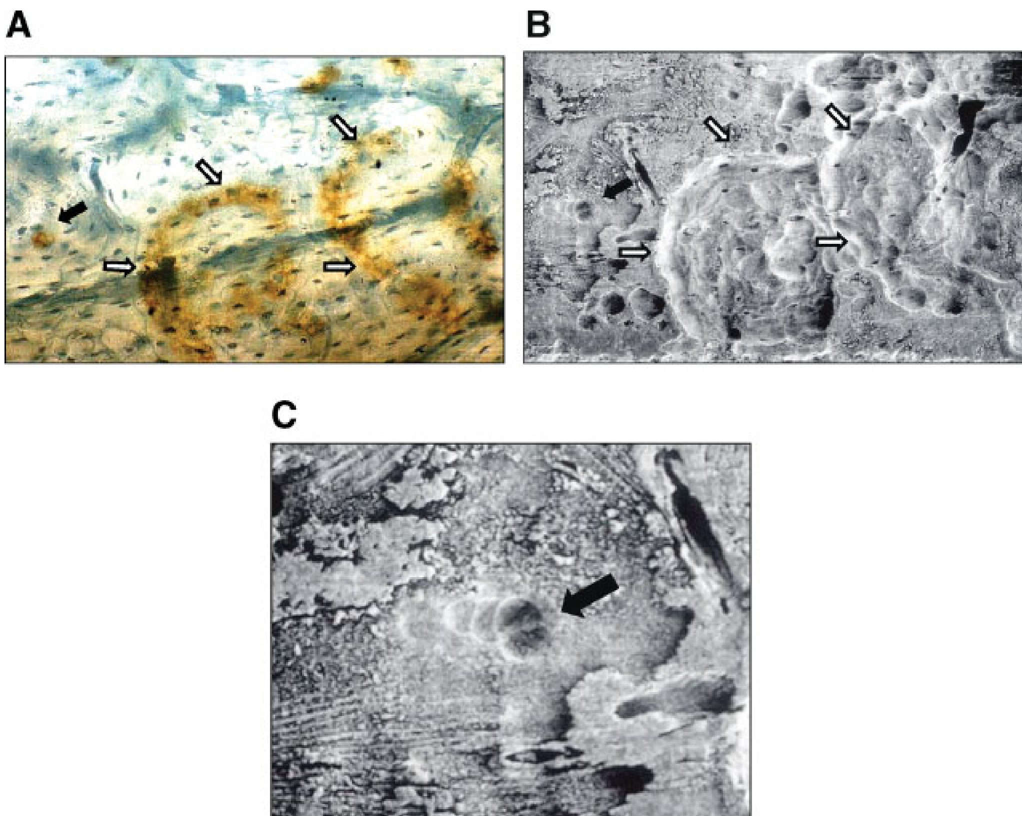

Fig. 5. **A**: Multi-nuclear cells (open arrows) and a mono-nuclear cell (closed arrow) stained with the CTR-C19 antibody; (**B**) scanning electron micrograph of the resorption pits created by the cells shown in (A). The open arrows indicate the edges of the two pits created by the multi-nuclear cells; the closed arrow indicates the pit created by the mono-nuclear cell. This is shown at higher power in (**C**).

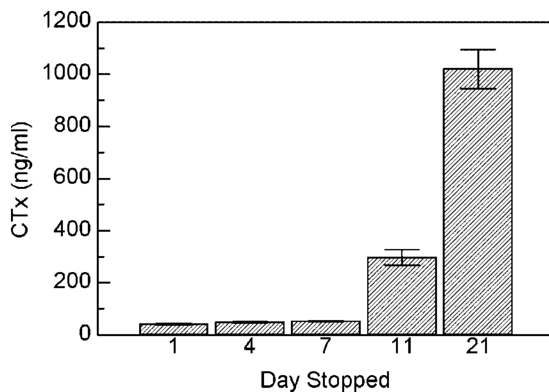

Fig. 6. Time course of bone resorption in osteoclast-forming hPBMC cultures as assessed by the type I collagen telopeptide (CTx) concentration in the medium collected at the indicated time points.

tely rule out the possibility that PTH1R expression may have been influenced by the culture conditions in which the osteoclasts are formed, which is a very different microenvironment to that present in vivo. However, we would note that the presence of PTH1R in chick and rat osteoclasts has been demonstrated under a wide variety of cell culture conditions [Teti et al., 1991; Agarwala and Gay, 1992; Watson et al., 2002] and has also been detected in situ in sections of animal and human bone [Rao et al., 1983; Langub et al., 2001; Faucheux et al., 2002; Gay et al., 2003].

The functional significance of PTH1R expression in osteoclasts is still to be defined, but clearly, it provides for the possibility of dual regulation of osteoclast function by direct action on osteoclasts as well as indirect effects mediated by osteoblasts. Early studies suggested that PTH activated carbonic anhydrase and regulated acid production in avian and rat osteoclasts, respectively [Anderson et al., 1985; Hunter et al., 1988]. Datta et al. [1996] reported that PTH treatment of rat osteoclasts caused a rapid and dramatic increase in superoxide anion production, whereas treatment of osteoblast-like cells with PTH had no such effect. Faucheux et al. [2002] showed that deer antler osteoclast formation was stimulated by PTHrP via a mechanism that only partially involved the RANKL pathway. Langub et al. [2001] found that in patients with secondary hyperparathyroidism there was a positive correlation between the percentage of osteoclasts containing PTH1R protein and resorption depth, an index of osteoclast activity. All these previous observations are consistent with the present finding of a direct stimulatory action of PTH on human osteoclasts. Recently, Gay et al. [2003] have proposed the intriguing hypothesis that PTHrP, which is produced by both osteoblasts and osteoclasts, may play a role in the communication between osteoblasts and osteoclasts, i.e., PTHrP produced by osteoblasts may regulate osteoclast activity via PTH1R in osteoclasts, and vice versa.

The model employed in our studies was human osteoclasts formed in vitro from PBMC. This model was first developed in 1996 [Fujikawa et al., 1996; Matayoshi et al., 1996; Purton et al., 1996]. Later studies demonstrated that osteoclasts were derived largely from $CD14^+$ positive monocytes [Massey and Flanagan, 1999; Nicholson et al., 2000] and that the presence of RANKL and M-CSF provided a sufficient condition for substantial osteoclast

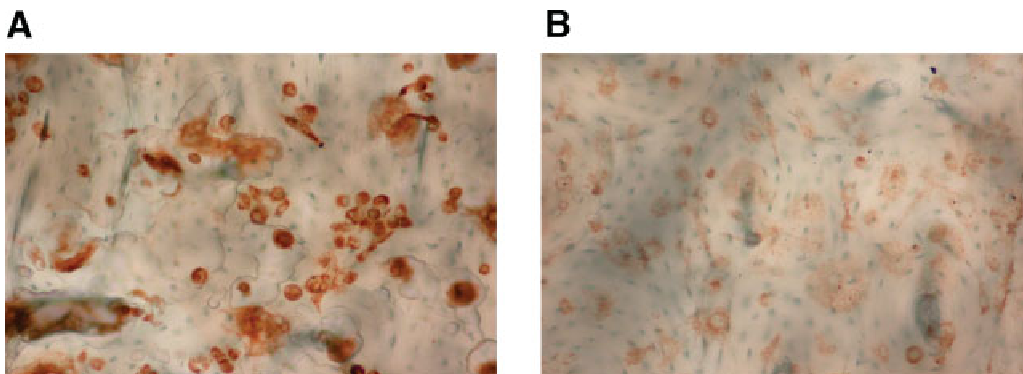

Fig. 7. Mono- and multi-nuclear cells in osteoclast-forming hPBMC cultures on bone slices stained with anti-PTH1R (PTH1R, PTH type 1 receptors) antibody (**A**); abundant resorption pits are evident. Note that markedly reduced staining in cells treated with antibody that had been incubated with blocking peptide (**B**).

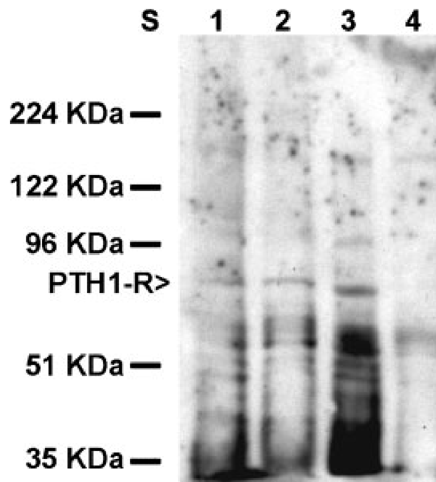

Fig. 8. Western blot detection of PTH1R. Lanes: S, molecular weight standards; 1 Cos-7 cells transfected with PTH1R (positive control); 2, Saos-2 cells (positive control); 3, osteoclasts from 21-day hPBMC culture; 4, Cos-7 cells (negative control).

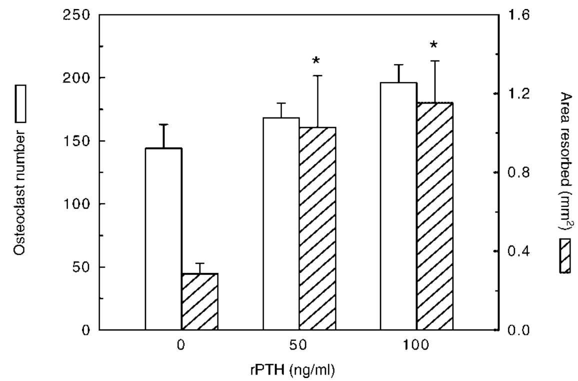

Fig. 10. Parathyroid hormone (PTH) stimulation of resorption pit formation in hPBMC cultures on bone slices (hatched bars). Rat PTH (1–34) or vehicle was added for the final 2 days of culture (14–16 days). Significantly different from vehicle: $*P < 0.05$; Values are mean ± SEM (n = 6). PTH also caused smaller, non-significant increases in numbers of osteoclasts (open bars).

formation [Matsuzaki et al., 1998; Quinn et al., 1998; Shalhoub et al., 1999]. An important methodological consideration in the present study was the production and verification of a purified population of human osteoclasts. This was particularly necessary for the experiments in which the endpoints were the identification of PTH1R mRNA and protein. The characterization of the cells as osteoclasts hinged on two important attributes: expression of the calcitonin receptor and the ability to resorb bone. The calcitonin receptor is widely recognized as a specific marker of the osteoclast phenotype in the bone/marrow environment [Lee et al., 1995; Quinn et al., 1999]. Earlier in vitro studies used the technically challenging approach of autoradiography to demonstrate the presence of CTR in human osteoclasts formed in vitro as antibodies to the human CTR had not been developed [e.g., Breuil et al., 1998; Faust et al., 1999; Massey and Flanagan, 1999]. We have shown here that commercially available antibodies to the human CTR recognize human osteoclasts formed in vitro. Furthermore, all the cells formed from $CD14^+$ Mo in the presence of RANKL and M-CSF expressed the CTR. Such a homogeneous population of human cells expressing the major phenotypic markers of osteoclasts represents a useful model for molecular biology and biochemical studies.

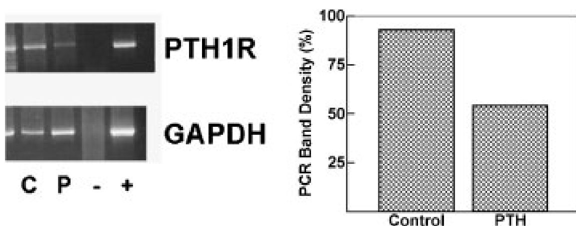

Fig. 9. PCR analysis of PTH1R and GAPDH mRNA expression in 21-day osteoclast cultures. Lanes were loaded with PCR product from: C, 21-day control culture; P, 21-day culture treated with 10^{-7}M PTH throughout; (–), no PCR product (negative control); (+), plasmid DNA (positive control).

James et al. [1999] described a method for purification of functional osteoclast precursors from osteoclastoma-derived mono-nuclear cells. The present method offers a number of advantages over that method in that it is not dependent on the availability of osteoclastomas and the cells studied are not tumor cells. Furthermore, it is possible using our method to study osteoclasts formed in vitro from patients with known bone diseases and, thereby, gain insight into the underlying pathogenetic mechanisms. As an example of this type of potential application, Neale et al. [2000] reported that circulating monocytes obtained from patients with Paget's disease show enhanced sensitivity to RANKL and 1,25 dihydroxyvitamin D and differentiate into more active osteoclasts in vitro than do those from control subjects. In a similar vein, osteoclasts formed in vitro from PBMC of patients with malignant osteopetrosis were found to be non-functional [Flanagan et al., 2000; Helfrich and Gerritsen, 2001]. Menaa et al. [2000] have also described a technique for

obtaining large numbers of purified human osteoclasts but this method uses bone marrow cells, which are less accessible than the peripheral monocytes used in the present method.

CTR-positive, mono-nuclear cells were found to be capable of bone resorption in this study. Resorption by mono-nuclear cells in vitro has previously been reported both in animal and human studies [Prallet et al., 1992; Sarma and Flanagan, 1996; Nicholson et al., 2000]. Based on a detailed analysis of resorption cavities in human iliac crest biopsies, Eriksen [1986] has calculated that as much as two thirds of the cavity may be excavated by mono- rather than multi-nucleated osteoclasts. Using similar culture conditions, Nicholson et al. [2000] demonstrated that $CD14^+$ Mo give rise to a mixed population of mono- and multi-nucleated cells that exhibited marked resorptive capacity. These authors concluded that the large numbers of mono-nucleated cells present at 21 days indicated heterogeneity in the maturity of the $CD14^+$ osteoclast precursor phenotype and in the potential of these cells to become osteoclasts. They suggested that with longer incubation times these cells might indeed form multi-nucleated osteoclasts. Our finding that all the mono-nuclear cells express the calcitonin receptor and that they are capable of bone resorption indicates a much more homogenous population in terms of expression of the osteoclast phenotype.

In conclusion, using a purified population of human osteoclasts generated in vitro, we have obtained evidence that human osteoclasts express PTH1R at the mRNA and protein level. Furthermore, human osteoclast activity was stimulated by PTH in the absence of osteoblasts. These findings suggest that there is a dual regulatory mechanism, whereby PTH acts directly on human osteoclasts and also, indirectly, via osteoblasts. Appreciation of this dual regulatory mechanism may ultimately advance our understanding of the complex effects of PTH on the human skeleton.

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