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Experimental Oral Pathology

Cytokine and chemokine response of bone cells after dentin challenge *in vitro*

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OBJECTIVE: The aim of this study was to characterize the effects of dentin extracts on cytokine, chemokine and nitric oxide (NO) production by primary rat bone cells.
STUDY DESIGN: Osteoblastic bone marrow cultures were exposed to particulate (D-part), non-particulate (D-n-part) and demineralized dentin extracts and evaluated for proliferative activity, cell morphology, alkaline phosphatase activity and bone-like nodule formation. Cytokine production was assessed by enzyme-linked immunosorbent assay and NO release by the Griess method.
RESULTS: The dentin extracts did not affect osteoblast numbering. Conversely, they up regulated in a dose-dependent manner the production by the osteoblasts of the pro-inflammatory interleukin-1β (IL-1β), tumor necrosis factor-α, IL-6, cytokine-induced neutrophil chemoattractant-1, and of the anti-inflammatory cytokine, IL-10. The NO production was stimulated only by D-n-part.
CONCLUSION: These results demonstrate that dentin induces the production of inflammatory cytokines by osteoblasts and suggest that pro-resorptive pathways might be stimulated when dentin molecules come into contact with bone cells during pathological processes associated with dentin and bone matrix dissolution.
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Keywords: dentin; bone cells; cytokines; chemokines

Introduction

The bulk of the tooth consists of dentin, a mineralized tissue that has a similar biochemical composition to bone. After mineralization the dentin molecules remain trapped in a mineralized phase bound to matrix components or to hydroxyapatite crystals, being exposed or released as a consequence of injuries to the periodontal ligament and dental pulp by, for example, trauma, periodontal disease and orthodontic tooth movement (Ne *et al*, 1999). The exposure of dentin is generally accompanied by resorption of the root surface, which can be promptly repaired, or maintained if the inflammatory stimulus persists. A complex interaction of inflammatory and resorbing cells within the tooth/periodontal ligament microenvironment determines the course of tooth resorption.

Although dentin has been considered to have the potential to alter cell function within the tooth/periodontal microenvironment, including that of osteoblasts (Ogata *et al*, 1997; Takata *et al*, 1998) periodontal ligament cells and human gingival fibroblasts (Ogata *et al*, 1997) and macrophages (Lara *et al*, 2003), the nature of the underlying mechanism is still poorly understood, especially regarding how these cells interact with dental hard tissues and if the release of dentin molecules contributes towards maintaining the resorption or to inducing repair during pathological exposure. Consistent with this, the traumatic root resorption was significantly inhibited in mice immunized with dentin (Wheeler and Stroup, 1993).

Root resorption occurs as a result of osteoclastic activity, which is chiefly governed by osteoblasts (Manolagas, 1995). Cytokines, chemokines and free radicals are of major importance in the process of resorption as local mediators of osteoclast recruitment and activation. In the inflammatory sites, pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and cytokine-induced neutrophil chemoattractant-1 (CINC-1), may coexist with anti-inflammatory cytokines such as IL-10, as well as those with a dual effect such as IL-6 (Manolagas, 1995; Kawashima and Stashenko, 1999).

Interleukin-1 (Fox *et al*, 2000; Tokukoda *et al*, 2001), TNF-α (Azuma *et al*, 2000) and IL-6 (Löwik *et al*, 1989) are potent inducers of bone resorption, regulating osteoclast recruitment and activity either by paracrine or autocrine mechanisms. In opposition to the effects of pro-inflammatory cytokines, IL-10 strongly suppresses periapical bone resorption (Xu *et al*, 1995; Owens *et al*, 1996; Sasaki *et al*, 2000).

The CINC-1, a member of the CXC chemokine family, induces leukocyte chemotaxis at the site of

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inflammation (Nakagawa *et al*, 1994). The effects of CINC-1 on root resorption have not been determined, although human IL-8, a CINC-1 counterpart, has been shown to be expressed in primary osteoblasts (Bilbe *et al*, 1996) and to stimulate the motility of osteoclasts (Fuller *et al*, 1995).

Nitric oxide (NO), a free radical that mediates a variety of pathological phenomena associated with inflammatory processes, also has important effects on bone cell activity (Van't Hof and Ralston, 2001). It has been suggested that NO may be a modulator in the cytokine-induced bone resorption process. Moreover, NO could either stimulate or inhibit the osteoclastic activity in a concentration-dependent pattern (Van't Hof *et al*, 2000).

Despite the recognized role of inflammatory mediators in bone cell activity, the influence on these cells of molecules released from dentin remains undetermined. Thus, the purpose of this study was to examine the effects of dentin extracts on osteoblastic phenotype and cytokine release from dentin-stimulated cells.

Materials and methods

Preparation of dentin extracts

Dentin was obtained from the crowns of impacted teeth recently extracted by the Surgical Service, Department of Oral and Maxillofacial Surgery, Faculty of Dentistry of Ribeirão Preto, University of São Paulo. Informed consent was obtained from all patients. The Institutional Human subjects Committee approved the use of human extracted teeth. Dentin extracts were prepared as previously reported (Lara *et al*, 2003). Adherent soft tissue was removed and the dentin was ground by high-speed diamond rotation under constant irrigation with distilled water. After desiccation at 37 °C, to recover the water-soluble substances potentially eluted during the grinding process, the dentin powder was diluted in phosphate-buffered saline (PBS), and referred to as particulate extract (D-part). Dentin particles were 2–10 μm in diameter, as determined by scanning electron microscopy (SEM). Next, this preparation was centrifuged at $2000 \times g$ for 30 min to obtain the supernatant, referred to as non-particulate extract (D-n-part). Alternatively, dentin powder was demineralized (Smith *et al*, 1990) using 10% (w/v) ethylenediaminetetraacetic acid (EDTA) in the presence of the protease inhibitor phenylmethylsulphonyl fluoride at 5 mM (Gibco, Grand Island, NY, USA). After 14 days, the EDTA-soluble fractions were concentrated by ultrafiltration (Amicon, YM10: WR Grace & Co., Danvers, MA, USA), dialysed against water, diluted in PBS and filtered (0.22 μm), and referred to as demineralized extract (d-Ext). The protein content of fractions was determined by the method of Bradford, using bovine serum albumin as the standard. The protein concentration of the D-part and D-n-part at 5.0 mg ml^{-1} (w/v) was below the detection level of the method (1–2000 μg ml^{-1}). However, each milligram (w/v) of dentin powder resulted in 0.18 μg ml^{-1} of protein after demineralization. Endotoxin contamination was tested by incubation of D-n-part with different concentrations of polymyxin B, as previously described (Morrison and Jacobs, 1976), at 500 ng ml^{-1}, 1 μg ml^{-1} and 2 μg ml^{-1}, for 15 min at 37 °C, and then evaluated for its ability to induce NO production in macrophage cultures. The incubation with polymyxin B did not affect the NO production induced by D-n-part, but significantly reduced the NO levels induced by lipopolysaccharide (control) (*Escherichia coli* 026:B6; Sigma Chemical, St Louis, MO, USA) (500 ng ml^{-1}).

Cell isolation and culture

Cells were obtained from bone marrow of young male Wistar rats using the modified protocol from Maniatopoulos *et al* (1988). The animals were treated in accordance with procedures approved by the Institutional Animal Ethics Committee. Bone marrow cells were seeded in plastic culture flasks and cultured in α-minimal essential medium (α-MEM) supplemented with 15% fetal bovine serum (Gibco), 10^{-7} M dexamethasone, 2.16 g l^{-1} β-glycerophosphate (Sigma), 5 μg l^{-1} ascorbic acid (Gibco), 50 μg ml^{-1} Gentamicin (Gibco) and 0.3 μg ml^{-1} fungizone (Gibco). Cells were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Culture medium was changed three times a week. After 15 days, the cells were removed using 0.25% trypsin (Gibco) and 1 mM EDTA, plated at 4×10^4 cells well^{-1} in 24-well plates and maintained for 4 and 14 days. Cells were stimulated with different concentrations of dentin extracts (0.3–2.0 mg ml^{-1}) for 48 h. Confluent cultures were subjected to serum starvation 48 h prior to treatment. After stimulation, the cells were recovered for cell counting and evaluation of osteoblast parameters. The supernatants were stored at −20 °C until analysis.

Cell proliferation, viability and morphology

Cells were cultured for 2 and 14 days for proliferation evaluation. The culture medium was removed and the wells were washed three times with PBS at 37 °C. Cells were then enzymatically (1 mM EDTA; 0.25% trypsin, Gibco) released and counted using a hemacytometer. The cell viability after dentin treatment was assessed by Trypan Blue (Sigma) (1 mg ml^{-1}) dye exclusion. Cells were evaluated using phase contrast inverted microscopy and by SEM. For SEM analysis, cell cultures were fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.0, postfixed for 2 h in 1% osmium tetroxide (Sigma), dehydrated in graded alcohols, critical point dried, sputter-coated and analyzed in a JEOL JSM 5410 scanning electron microscope (Noran Instruments Inc., Middleton, W1, USA).

Alkaline phosphatase (ALP) activity

The ALP activity was measured colorimetrically (ALP kit 104; Sigma) in cell lysates obtained by treatment of cultures for 30 min with 0.1% lauryl sulfate (Sigma), and assayed by the hydrolysis of ρ-nitrophenol phosphate in alkaline buffer solution at 37 °C for 30 min. Absorbance of product, ρ-nitrophenol, was determined at 410 nm and the ALP concentration was quantified using a standard curve. Results were expressed as μmol ρ-nitrophenol produced per hour (Units ml^{-1}) per cell number.

Mineralized nodule formation
After 14 days of culture and 48 h of stimulation, the cells were washed three times with PBS and attached cells were fixed in 10% formalin for 2 h at room temperature. The specimens were then dehydrated in a graded series of alcohol and stained with 2% Alizarin red S (Sigma) pH 4.3. The percentage of mineralized nodules was calculated as a percentage of total well area using software for image analysis (Image Tool, University of Texas, Health Science Center, San Antonio, TX, USA).

Cytokine production
The concentrations of IL-1β, TNF-α, IL-6, CINC-1 and IL-10 in conditioned medium were determined using a sandwich enzyme-linked immunosorbent assay as previously described (Taktak *et al*, 1991). Briefly, microtiter plates were coated overnight at 4 °C with immunoaffinity-purified polyclonal sheep antibodies against each cytokine (2 μg ml^{-1}). After blocking, samples and standards were incubated at room temperature for 2 h. Rabbit biotinylated immunoaffinity-purified polyclonal antibodies to each cytokine at 1:1000 dilution were added, followed by incubation at room temperature for 1 h. Finally, 50 μl of avidin-HRP (1:5000) was added to each well; after 30 min the plates were washed and the color reagent o-phenylenediamine (OPD) (40 μg well^{-1}) was added. After 15 min, the reaction was stopped by adding 75 μl of 1 M sulfuric acid and absorbance was measured at 490 nm using a microplate reader (Spectra Max 250: Molecular Devices Corporation, Sunnyvale, CA, USA). The concentration of each cytokine was calculated from a standard curve (4–4000 pg ml^{-1}) prepared for this purpose. Antibodies were kindly supplied by Dr Steve Poole (National Institute for Biological Standards and Control, NIBSC, London, UK).

Nitrite measurement
The secretion of NO was evaluated by measuring NO_2^- accumulation in culture supernatants by the Griess reaction (Green *et al*, 1982). Briefly, 50 μl of sample was mixed with an equal amount of Griess reagent containing 1% sulphanilamide (Sigma) and 0.1% naphthylethylenediamine dihydrochloride (Sigma) in 2% phosphoric acid. Absorbance was measured at 540 nm and nitrite concentration was determined using a standard curve of sodium nitrite (Merck & Co. Inc., Whitehouse Station, NJ, USA).

Statistical analysis
Numerical values are means ± standard error of mean (s.e.m.). The data were analyzed by one-way ANOVA with Bonferroni's post-test. Statistical significance was considered to be achieved at $P < 0.05$.

Results

Effects of dentin extracts on cell viability and proliferation
The particulate, non-particulate and demineralized dentin extracts had no cytotoxic effect on primary osteoblasts which retained a viability >95%. In the same way, osteoblast proliferation was not affected by either D-part or D-n-part treatment at concentrations of 0.3–2.0 mg ml^{-1} (Figure 1a).

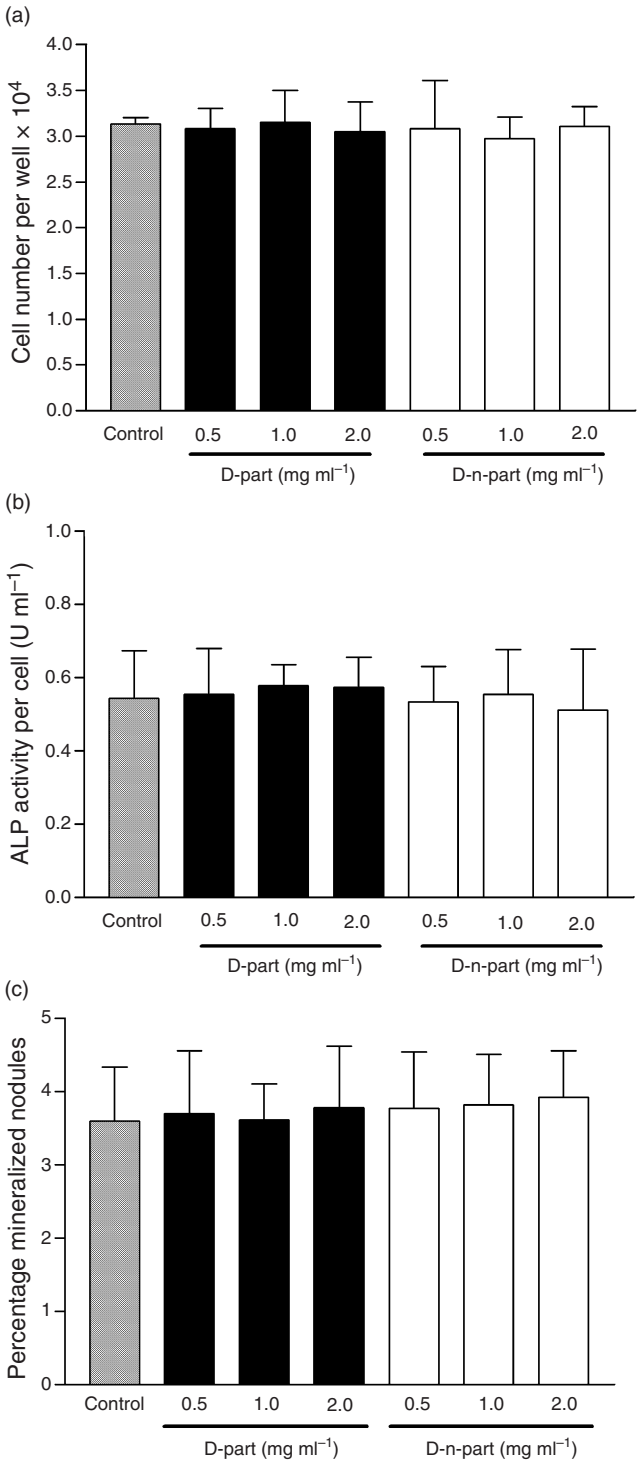

Figure 1 Effect of dentin extracts on bone cell growth and differentiation. Confluent cultures at 12 days were incubated for 48 h with D-part and D-n-part at different concentrations. Number of cells (**a**) and ALP activity (**b**) were measured as described in Material and methods. Formation of mineralized deposits (**c**) was estimated by histochemical assay. These results represent the mean of five independent experiments ± s.e.m.

Effects of dentin extracts on osteoblast markers
In order to investigate whether dentin could affect osteoblast differentiation, we evaluated the effect of extracts on osteoblastic markers. We observed that neither D-part nor D-n-part at 0.3–2.0 mg ml^{-1} affected the ALP activity or formation of calcium deposits (Figure 1b,c). Observation of cultures by phase contrast microscopy and SEM showed that osteoblast morphology changed from spindle-shaped, in the first days of culture, to a polygonal appearance. No morphologic changes were observed after dentin treatment for 48 h at early and later stages of cell differentiation. The treatment with d-Ext did not affect the osteoblast markers of differentiation (data not shown).

Cytokine release by dentin extracts-stimulated bone cells
An increase in IL-1β, TNF-α, IL-6, CINC-1 and IL-10 secretion was observed in fully-differentiated (at 14 days) osteoblasts (Figure 2a–e). Larger amounts of TNF-α were produced in response to dentin extracts, followed by CINC-1, IL-10, IL1-β and IL-6. Non-particulate extracts showed a more potent stimulatory effect for IL-1β, TNF-α and CINC-1 production than D-part. On the contrary, the IL-6 production was greater for the D-part-treated group, at a concentration of 1.0 mg ml^{-1}, than for the D-n-part-group (Figure 2c). Similar levels of IL-10 were detected for both groups (Figure 2e). A significant production of IL-1β (control: 4.32 ± 2.31; d-Ext: $214.21^* \pm 35.89$ pg ml^{-1}; $n = 3$, $^*P < 0.01$) and TNF-α (control: 211 ± 32.15; d-Ext: $1141.0^* \pm 116.12$ pg ml^{-1}; $n = 3$, $^*P < 0.01$) was also stimulated by demineralized extract treatment (d-Ext; 9 μg ml^{-1}). IL-6, CINC-1 and IL-10, but not IL-1β and TNF-α secretion were also detected in dentin-treated early-differentiated (at 4 days) osteoblasts (data not shown).

Effect of dentin on nitric oxide production
Only D-n-part at 0.7 and 1.0 mg ml^{-1} induced significant levels of NO_2^- at 14 days (Figure 2f). In these same groups, we observed that most of the cells (70%) showed positive expression for the inducible nitric oxide synthase (NOS II) enzyme (data not shown).

Discussion

The primary approach of studies that evaluated the interaction of dentin and bone cells has been to understand the mechanisms of the periodontal regeneration and osteoinduction (Ogata *et al*, 1997; Takata *et al*, 1998). However, the signaling mechanisms that affect the cell function in these sites were not fully elucidated. Dentin molecules become available in tooth-surrounding milieu when dentin matrix is broken down by osteoclasts during the resorption process (Nesbitt and Horton, 1997). We previously reported that dentin extracts triggered an intense cell migration in a time- and dose-dependent manner, as well as macrophage expression of IL-1β, TNF-α, NO and hydrogen peroxide (Lara *et al*, 2003). In the present work, we observed that dentin extracts were able to dose-dependently stimulate bone cells to release the inflammatory mediators IL-1β, TNF-α, IL-6, CINC-1, IL-10, and NO, which are implicated in the regulation of resorption (Azuma *et al*, 2000; Fox *et al*, 2000; Sasaki *et al*, 2000; Tokukoda *et al*, 2001). Of these, IL-1β, TNF-α, IL-6 and IL-10 have been shown to be produced in inflamed pulp and periapical tissues and may be involved in the pathogenesis of bone and root resorption associated with these diseases (Kawashima and Stashenko, 1999). Consistently, significant inhibition of root resorption and a decrease in the number of resorbing cells have been observed after immunoneutralization of IL-1 and TNF-α in rats (Zhang *et al*, 2003). In opposition to pro-inflammatory actions, IL-10 suppresses the recruitment and differentiation of osteoclasts (Xu *et al*, 1995; Owens *et al*, 1996) and also inhibits the infection-stimulated periapical bone resorption (Sasaki *et al*, 2000). Furthermore, IL-6 and NO have dual actions on the resorption processes (Löwik *et al*, 1989; Al-Humidan *et al*, 1991; Van't Hof *et al*, 2000; Van't Hof and Ralston, 2001).

Additionally, a significant production was also observed of CINC-1, a member of the CXC chemokine and IL-8 family, which is a potent chemotactic factor for neutrophils *in vitro* and *in vivo* (Nakagawa *et al*, 1994). Considering that leukocyte chemotaxis is a key event in inflammation, this result suggests that released dentin may play a role in the initial events of the inflammatory response, which is in accord with our previous demonstration that neutrophil migration is induced by dentin sialoprotein (DSP) and dentin phosphoprotein (DPP) (Silva *et al*, 2004). Furthermore, IL-1β, TNF-α, macrophage inflammatory protein-2 and cytokine-induced neutrophil chemoattractant (KC) have key roles in neutrophil recruitment induced by DSP and DPP (Silva *et al*, 2004). Consistent with the hypothesis that dentin may have a role in its own resorption through pro-inflammatory properties, the dentin immunoneutralization procedure protects mice from traumatic root resorption (Wheeler and Stroup, 1993).

Despite the effect of dentin on cytokine production, dentin extracts did not affect the bone cell behavior as determined by cell proliferation and morphology, alkaline phosphatase activity and bone-like nodule formation in primary bone marrow cultures, a well-characterized model to study the functions and regulatory mechanisms of primary osteoblasts. On the contrary, the exposure of an osteoprogenitor cell line to guanidine/EDTA dentin extracts suppressed the cell proliferation and differentiation (Takata *et al*, 1998), a result which could be due to the different cell types and experimental conditions.

The particulate extract used in this work can be phagocytosed by osteoblasts and there is evidence that this phenomenon activates the cells (Vermes *et al*, 2001). However, it seems that it was not the case in this study, as non-particulate extract was more effective in inducing the production of the majority of inflammatory mediators analyzed. The fact that demineralized extract also induced significant production of IL-1β and TNF-α reinforces the notion that the observed effects were

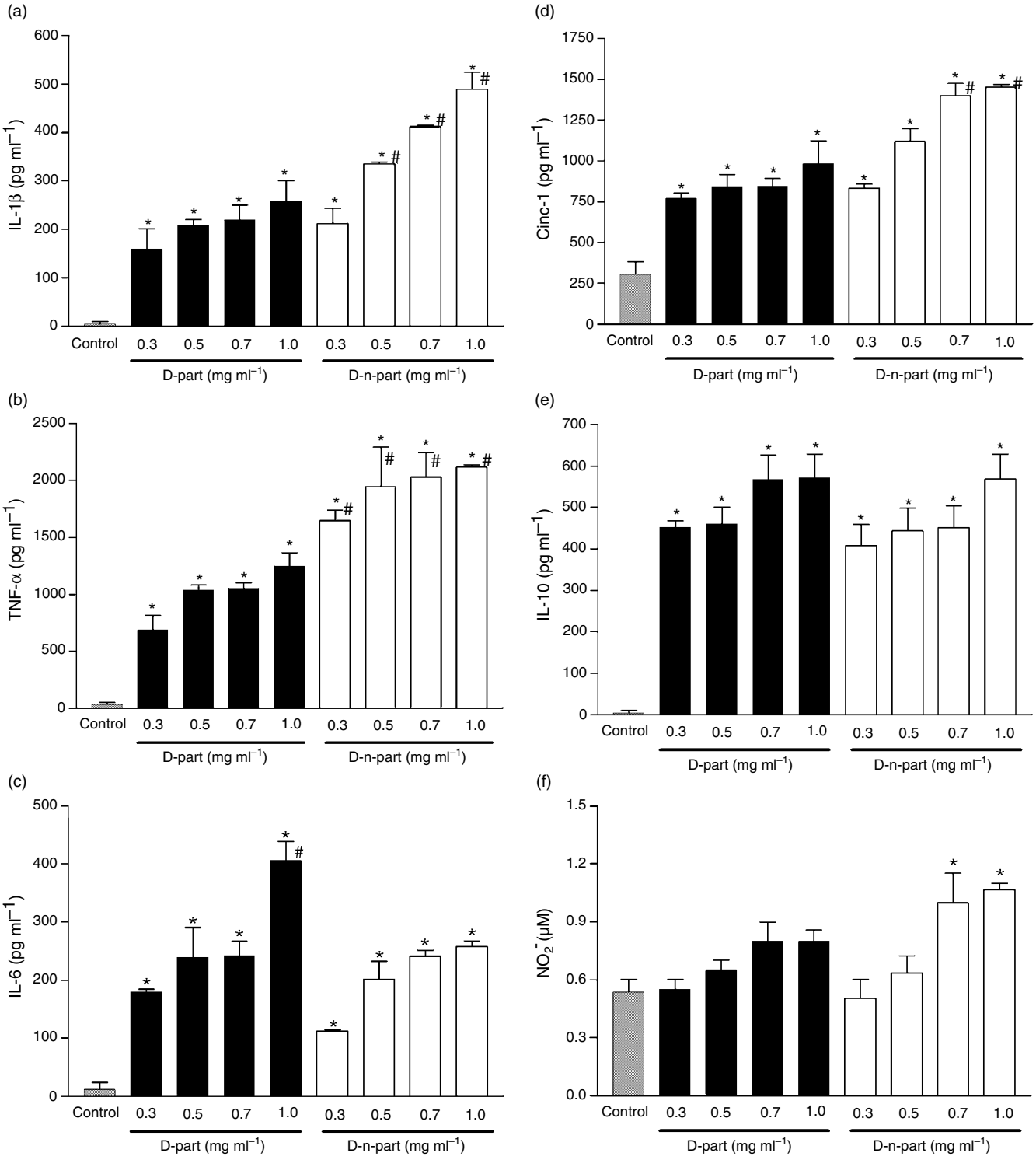

Figure 2 Dose-dependent effects of dentin extracts on production of interleukin (IL)-1β (**a**), tumor necrosis factor (TNF)-α (**b**), IL-6 (**c**), cytokine-induced neutrophil chemoattractant-1 (CINC-1) (**d**), IL-10 (**e**) and nitric oxide (**f**) by bone cells. Confluent cultures at 12 days were stimulated with the indicated concentrations of D-part and D-n-part extracts for 48 h and compared with cells incubated with media alone (control). Conditioned media were collected and IL-1β, TNF-α, IL-6, CINC-1 and IL-10 levels were measured by enzyme-linked immunosorbent assay. Nitrite secretion was determined by the Griess method. Each column represents the mean $\pm$ s.e.m. $^*P < 0.05$ *vs* control, $\#P < 0.05$ when comparing two dentin extracts

mediated by dentin molecules, not being due to the micro-particles present in the D-part extract. We should consider that, despite the analogous effects induced by D-part and D-n-part extracts, the mechanism involved in the secretion of cytokines may differ. The fact that D-part is insoluble could suggest that it may act as

matrix for osteoblasts. However, the observation that D-part did not affect the osteoblast differentiation parameters suggests that this is not the case.

The observation that regulatory factors are incorporated into the dentin matrix (Finkelman *et al*, 1991; Roberts-Clark and Smith, 2000) points to the likely importance of these factors as regulators of hard tissue resorption acting on the recruitment and activation of tooth-surrounding cells including bone and periodontal ligament cells (Ogata *et al*, 1997; Takata *et al*, 1998) and inflammatory cells (Lara *et al*, 2003). In fact, similar to bone, dentin represents a significant storage site for growth factors such as epidermal growth factor, fibroblast growth factor and transforming growth factor (TGF)-β super family members such as TGF-β, bone morphogenetic proteins and others (Finkelman *et al*, 1991; Roberts-Clark and Smith, 2000) which can act on dental and periodontal cells through interaction with their cognate cell surface receptors (Parkar *et al*, 2001). Besides, it has been shown that osteopontin, a protein found in bone and dentin, is involved in the inflammatory cell recruitment during the inflammatory response, bone remodeling, and cell-mediated immunity (O'Regan and Berman, 2000). Further studies should focus on which specific constituents in dentin have the effects on bone cells.

Our results support the idea that there is a balance between cytokines released from osteoblasts to regulate the possible involvement of dentin proteins in inflammatory events coupled with their release at root resorption sites. A better understanding of factors that lead to the production of inflammatory mediators and their *in vivo* effects on bone cells might facilitate the development of new therapies for inflammatory hard tissue resorption.

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References

Al-Humidan A, Ralstron SH, Hughes DE *et al* (1991). Interleukin-6 does not stimulate bone resorption in neonatal mouse calvariae. *J Bone Miner Res* **6:** 3–8.

Azuma Y, Kaji K, Katogi R *et al* (2000). Tumor necrosis factor-alpha induces differentiation of and bone resorption by osteoclasts. *J Biol Chem* **275:** 4858–4864.

Bilbe G, Roberts E, Birch M *et al* (1996). PCR phenotyping of cytokines, growth factors and their receptors and bone matrix proteins in human osteoblast-like cell lines. *Bone* **19:** 437–445.

Finkelman RD, Mohan S, Jennings JC *et al* (1991). Quantification of growth factors IGF-I, SGF/IGFII and TGF-beta in human dentin. *J Bone Miner Res* **5:** 717–723.

Fox SW, Fuller K, Chambers TJ (2000). Activation of osteoclasts by interleukin-1: divergent responsiveness in osteoclasts formed *in vivo* and *in vitro*. *J Cell Physiol* **184:** 334–340.

Fuller K, Owens JM, Chambers TJ (1995). Macrophage inflammatory protein-1 alpha and IL-8 stimulate the motility but suppress the resorption of isolated rat osteoclasts. *J Immunol* **154:** 6065–6072.

Green LC, Wagner DA, Glogowski J *et al* (1982). Analysis of nitrate, nitrite, and [^{15}N] nitrate in biological fluids. *Anal Biochem* **126:** 131–138.

Kawashima N, Stashenko P (1999). Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. *Arch Oral Biol* **44:** 55–66.

Lara VS, Figueiredo F, Silva TA *et al* (2003). Dentin induced *in vivo* inflammatory response and *in vitro* activation of murine macrophages. *J Dent Res* **82:** 460–465.

Löwik CWGM, van der Pluijm G, Bloys H *et al* (1989). Parathyroid hormone (PTH) and PTH-like protein (PLP) stimulate interleukin-6 production by osteogenic cells: a possible role of interleukin-6 in osteoclastogenesis. *Biochem Biophys Res Commun* **162:** 1546–1552.

Maniatopoulos C, Sodek J, Melcher AH (1988). Bone formation *in vitro* by stromal cells obtained from bone marrow of young adult rats. *Cell Tiss Res* **254:** 317–330.

Manolagas SC (1995). Role of cytokines in bone resorption. *Bone* **17:** 63–67.

Morrison DC, Jacobs DM (1976). Binding of polymyxin B to the lipid A portion of bacterial lipopolysaccharides. *Immunochem* **13:** 813–818.

Nakagawa H, Komorita N, Shibata F *et al* (1994). Identification of cytokine-induced neutrophil chemoattractant (CINC), rat GRO/CINC-2α and CINC-2β, produced by granulation tissue in culture: purification, complete amino acid sequences and characterization. *Biochem J* **301:** 545–550.

Ne RF, Whitherspoon DE, Gutmann JL (1999). Tooth resorption. *Quintessence Int* **30:** 9–25.

Nesbitt SA, Horton MA (1997). Trafficking of matrix collagens through bone-resorbing osteoclasts. *Science* **276:** 266–269.

O'Regan A, Berman JS (2000). Osteopontin: a key cytokine in cell-mediated and granulomatous inflammation. *Int J Exp Pathol* **81:** 373–390.

Ogata Y, Niisato N, Moriwaki K *et al* (1997). Cementum, root dentin and bone extracts stimulate chemotactic behavior in cells from periodontal tissue. *Comp Biochem Physiol* **116:** 359–365.

Owens JM, Gallagher AC, Chambers TJ (1996). IL-10 modulates formation of osteoclasts in murine hemopoietic cultures. *J Immunol* **157:** 936–940.

Parkar MH, Kuru L, Giouzeli M *et al* (2001). Expression of growth-factor receptors in normal and regenerating human periodontal cells. *Arch Oral Biol* **46:** 275–284.

Roberts-Clark DJ, Smith AJ (2000). Angiogenic growth factors in human dentine matrix. *Arch Oral Biol* **45:** 1013–1016.

Sasaki H, Hou L, Belani A *et al* (2000). IL-10, but not IL-4, suppresses infection-stimulated bone resorption *in vivo*. *J Immunol* **165:** 3626–3630.

Silva TA, Lara VS, Silva JS *et al* (2004). Dentin sialoprotein and phosphoprotein induce neutrophil recruitment: a mechanism dependent of IL-1β, TNF-α and CXC chemokines. *Calcif Tissue Int* (in press).

Smith AJ, Tobias RS, Plant CG *et al* (1990). *In vivo* morphogenetic activity of dentine matrix proteins. *J Biol Buccale* **18:** 123–129.

Takata T, D'Errico JA, Atkins KB *et al* (1998). Protein extracts of dentin affect proliferation and differentiation of osteoprogenitor cells *in vitro*. *J Periodontol* **69:** 1247–1255.

Taktak YS, Selkirk S, Bristow AF *et al* (1991). Assays of pyrogens by interleukin-6 from monocytic cell lines. *J Pharm Pharmacol* **43:** 578–582.

Tokukoda Y, Takata S, Kaji H *et al* (2001). Interleukin-1beta stimulates transendothelial mobilization of human peripheral blood mononuclear cells with a potential to differentiate into osteoclasts in the presence of osteoblasts. *Endocr J* **48:** 443–452.

Van't Hof RJ, Ralston SH (2001). Nitric oxide and bone. *Immunol* **103:** 255–261.

Van't Hof RJ, Armour KJ, Smith LM *et al* (2000). Requirement of the inducible nitric oxide synthase pathway for IL-1-induced osteoclastic bone resorption. *PNAS* **97:** 7993–7998.

Vermes C, Chandrasekaran R, Jacobs JJ *et al* (2001). The effects of particulate wear debris, cytokines, and growth factors on the functions of MG-63 osteoblasts. *J Bone Joint Surg Am* **83:** 201–211.

Wheeler TT, Stroup SE (1993). Traumatic root resorption in dentine-immunized mice. *Am J Orthod Dentofac Orthop* **103:** 352–357.

Xu LX, Kukita T, Kukita A *et al* (1995). Interleukin-10 selectively inhibits osteoclastogenesis by inhibiting differentiation of osteoclast progenitors into preosteoclast-like cells in rat bone marrow culture system. *J Cell Physiol* **165:** 624–629.

Zhang D, Goetz W, Braumann B *et al* (2003). Effect of soluble receptors to interleukin-1 and tumor necrosis factor alpha on experimentally induced root resorption in rats. *J Periodontal Res* **38:** 324–332.