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IL-1α and IL-1β Have Different Effects on Formation and Activity of Large Osteoclasts

Diana P. Trebec-Reynolds,[1*] Irina Voronov,[2] Johan N.M. Heersche,[2] and Morris F. Manolson[1,2]

[1] *Department of Biochemistry, University of Toronto, Toronto, ON, Canada*
[2] *Faculty of Dentistry, University of Toronto, Toronto, ON, Canada*

ABSTRACT

Interleukin 1 (IL-1) is a proinflammatory cytokine upregulated in conditions such as rheumatoid arthritis and periodontal disease. Both isoforms, IL-1α and IL-1β, have been shown to activate osteoclasts (OCs), the cells responsible for resorbing bone. Inflammatory conditions are also characterized by increased bone loss and by the presence of large OCs (10+ nuclei). We and others have previously shown that large OCs are more likely to be resorbing compared to small OCs (2–5 nuclei). Moreover, large OCs express higher levels of the IL-1 activating receptor IL-1RI, integrins αv and β3, RANK, and TNFR1, while small OCs have higher levels of the decoy receptor IL-1RII. We hypothesized that IL-1 would have different effects on large and small OCs due to these distinct receptor expression patterns. To test this hypothesis, RAW 264.7 cells were differentiated into populations of small and large OCs and treated with IL-1α or IL-1β (1 and 10 ng/ml). In the presence of sRANKL, both IL-1α and IL-1β increased total OC number and resorptive activity of large OCs. IL-1α stimulated formation of large OCs and increased the number of resorption pits, while IL-1β changed the morphology of large OCs and integrin-β3 phosphorylation. No effects were seen in small OCs in response to either IL-1 isoform. These results demonstrate that IL-1 predominantly affects large OCs. The dissimilarity of responses to IL-1α and IL-1β suggests that these isoforms activate different signaling pathways within the two OC populations. J. Cell. Biochem. 109: 975–982, 2010.

KEY WORDS: OSTEOCLASTS; IL-1; INFLAMMATION; ARTHRITIS

Osteoclasts (OCs) are multinucleated cells that resorb bone. These cells are required for normal bone remodeling; however, increased OC activity leads to bone loss. Rheumatoid arthritis, Paget's disease, multiple myeloma, and periodontal disease are characterized by excessive bone resorption and by the presence of large OCs in the affected areas [Makris and Saffar, 1982; Aota et al., 1996; Singer and Roodman, 1996; Smith et al., 1997], suggesting that an increase in OC size is correlated to pathological resorptive activity.

OC activation, survival, and differentiation are mainly controlled by receptor activator of NF-κB ligand (RANKL) and macrophage colony stimulating factor (M-CSF). Other cytokines, such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and IL-6, are also known to affect OC differentiation and function [Roodman, 1999]. IL-1 is a proinflammatory cytokine present at elevated levels in conditions such as rheumatoid arthritis and periodontal disease [Kanda-Nakamura et al., 1996; Rasmussen et al., 2000; Dinarello, 2002; Strand and Kavanaugh, 2004]. It has two isoforms: IL-1α and IL-1β, which are products of different genes and share 20–30% sequence homology. Both isoforms are synthesized as 31 kDa precursors and are subsequently processed to 17 kDa proteins. IL-1α is proteolytically processed by the protease calpain, while IL-1β is processed by the interleukin converting enzyme (ICE) [Dinarello, 1996]. Due to this difference in processing, IL-1α is mainly active in cell-associated forms as either an intracellular precursor or as a membrane-bound molecule and functions as an autocrine signaling molecule [Dinarello, 1996]. On the contrary, IL-1β is secreted in a processed form and functions as an endocrine signaling molecule [Dinarello, 1996]. Both IL-1α and IL-1β can bind to the two IL-1 receptors: IL-1 receptor I (IL-1RI) and IL-1 receptor II (IL-1RII). IL-1RI heterodimerizes with the IL-1 receptor accessory protein and activates downstream signaling pathways, such as NF-κB and AP-1, and kinases such as ERK1/2 and p38 [Dinarello, 1996]. IL-1RII lacks a cytoplasmic tail and therefore acts as a decoy receptor by

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*Correspondence to: Diana P. Trebec-Reynolds, Faculty of Dentistry, University of Toronto, 124 Edward Street, Room 400, Toronto, ON, Canada M5G 1G6. E-mail: diana.trebec@utoronto.ca
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inhibiting IL-1 signaling [Sims et al., 1994]. IL-1 signaling is also controlled by the naturally occurring antagonist, IL-1 receptor antagonist (IL-1ra), which irreversibly binds to IL-1RI and blocks IL-1 signaling [Dinarello, 1996, 1998].

Increased levels of IL-1β have been reported in cases of osteoporosis and rheumatoid arthritis [Eastgate et al., 1988; Pacifici, 1996], and the effects of IL-1α and IL-1β on OCs have been tested in vitro. Both IL-1α and IL-1β have been found to increase formation as well as the resorptive capacity of OCs in culture [Jimi et al., 1999; Lee et al., 2006; Trebec et al., 2007]. We have previously demonstrated that large OCs (10+ nuclei) were more responsive to IL-1β with respect to resorptive activity than small OCs (2–5 nuclei) [Trebec et al., 2007]. Furthermore, we have reported that the two IL-1 receptors were differentially expressed in OCs: the activating receptor IL-1RI was highly expressed in the more active large OCs, while the expression levels of the decoy receptor IL-1RII was higher in small OCs [Trebec et al., 2007]. These results correlate with in vivo studies by Xu et al. [1996], who demonstrated that in an adjuvant-induced arthritis rat model, IL-1RI is the predominant receptor expressed in the OCs from the areas with more severe bone and cartilage destruction.

As large and small OCs differentially express the IL-1 receptors, we hypothesized that IL-1 would have dissimilar effects on large and small OCs. Moreover, as recent data suggests that IL-1α and IL-1β elicit different responses in other cells [Song et al., 2003; Carmi et al., 2009], we speculated that OC formation and resorptive activity would be differentially affected in response to these two cytokines. Here we show that both isoforms have different effects on the formation and resorptive activity of large OCs while small OCs are not affected.

MATERIALS AND METHODS

REAGENTS

Recombinant mouse IL-1α, IL-1β, and IL-1ra were obtained from R&D Systems. The RAW 264.7 cell line (RAW cells) was obtained from American Type Culture Collection (ATCC catalog # TIB-71). Dulbecco's modified Eagle's medium (DMEM), antibiotics (penicillin/streptomycin, Gibco #15140122), and sterile fetal bovine serum (Gibco #12318-028) were obtained from Invitrogen. Fast Red Violet LB Salt (catalog # F-1625) and Naphthol AS-MX (catalog # N-5000) were obtained from Sigma. Antibodies to integrin β3 (catalog # sc-6626) and IL-1RI (catalog # sc-687) were obtained from Santa Cruz. Antibodies to GAPDH (catalog # ab8245) and phosphorylated integrin β3 (catalog # ab38460) were obtained from Abcam. DAPI was obtained from Sigma.

DIFFERENTIATION

Cells were differentiated from RAW cells in the presence of 200 ng/ml sRANKL as previously described in [Manolson et al., 2003; Trebec et al., 2007]. Large and small OCs populations were obtained as previously described either by isolating them on day 3 of culture (small OCs) or day 5 (large OCs), or by separating them after 4–5 days in culture on an FBS gradient [Trebec et al., 2007].

TARTRATE-RESISTANT ACID PHOSPHATASE (TRAP) STAINING

Culture dishes were washed with Ca^{2+} and Mg^{2+}-free PBS and then fixed with formalin for 10 min. Staining for TRAP was carried out as previously described according to the protocol described in BD Biosciences Technical Bulletin #445 with minor modifications [Trebec et al., 2007]. Briefly, TRAP staining solution consisting of 50 mM acetate buffer, 30 mM sodium tartrate, 0.1 mg/ml Naphthol AS-MX phosphate, 0.1% Triton X-100, and 0.3 mg/ml Fast Red Violet LB stain was added to fixed cells for 10–20 min until the desired staining intensity was reached. The TRAP staining solution was then removed and the cells washed three times with dH_2O.

IL-1 EFFECT ON DIFFERENTIATION AND MORPHOLOGY

Cells were plated as described above. RANKL (50 ng/ml) was added to cultures on day 0 (day of plating) in the presence or absence of 1, 10, or 100 ng/ml IL-1β, or in the presence of 1 or 10 ng/ml IL-1α ± 50 ng/ml IL-1ra. Media were changed on day 3. The cultures were stopped on day 5, fixed, TRAP stained, and the number of OCs was counted. To examine the effect of IL-1 isoforms on populations of large and small OCs, the OCs were first differentiated into two populations with sRANKL only. When the majority (≥70%) of cells in culture were small OCs (day 3), or large (days 5–6), the conditions were added for an additional 24 h. At the end of the incubation period the cells were fixed, TRAP stained, and counted. Similar experiments were performed on large and small OCs isolated by an FBS gradient [as previously described, Trebec et al., 2007] after differentiation with 150 ng/ml RANKL for 4–5 days. OCs were replated, treated with conditions for 24 h, fixed, TRAP stained, and counted.

To confirm the change in cellular morphology (polarized or spread) the images of the OCs were obtained using a Leica DM IRE2 microscope (Improvision, OpenLab version 4.0.2). OC area was measured using ImageJ 1.42q (W.S. Rasband, U.S. National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov.ij/, 1997–2009), measuring ~100 total cells/group.

RESORPTION ASSAY

Osteoclastic resorptive activity in the presence or absence of IL-1α (1 and 10 ng/ml) and/or IL-1ra (50 ng/ml) or IL-1β (10 ng/ml) was assessed using Osteologic™ slides. Size separated OCs generated from RAW cells were plated onto slides and incubated for 48 h. In parallel, OCs were plated onto plastic plates, incubated for 48 h, fixed, and TRAP-stained for counting. Osteologic™ slides were stained as previously described [Voronov et al., 2005]. Analysis of resorption pits was carried out using OpenLab™ Software (Improvision).

INTEGRIN β3 PHOSPHORYLATION

RAW cells were differentiated into enriched populations of large and small OCs as described above. Cells were serum starved for 30 min and treated with IL-1α or IL-1β (10 ng/ml) for 0–60 min. Cell lysates were collected every 10 min in octyl-β-D-glucoside containing buffer and protein was collected as previously described [Trebec et al., 2007]. Lysates were then separated on 8% SDS–PAGE gels and transferred on ice for 2 h to nitrocellulose membranes at 50 V, 250 mAmp, or for 1.5 h, at 135 Amp, 75 V. The blots were subjected

to immunoblotting for phosphorylated integrin β3, integrin β3, and for GAPDH to ensure equal loading. Imaging and quantification of results from IL-1β experiments were done using GeneSnap and GeneTools (Syngene) as previously described [Trebec et al., 2007]. Data from IL-1α experiments were obtained using the ChemiDoc XRS HQ2 (Bio-Rad), and analysis was carried out in a similar manner as IL-1β data but using Quantity One® Software with rolling disk background correction.

IMMUNOFLUORESCENCE

RAW cells were differentiated into populations of large and small OCs in the presence of sRANKL on glass chamber slides. At day 5, the cells were serum starved for 30 min, incubated with 10 ng/ml IL-1β for 30 min, and then fixed with cold methanol for 10 min at 4°C. Slides were blocked in 1% normal goat serum in PBS for 1 h, incubated with primary antibodies overnight at 4°C, followed by the addition of FITC- or TRITC-conjugated secondary antibody for 1 h at room temperature. The nuclei were counterstained with DAPI. The slides were visualized at 20× magnification using a Leica DM IRE2 microscope (Improvision, OpenLab version 4.0.2).

STATISTICS

Statistical analysis was performed using SPSS 17.0 for Windows using ANOVA one-way analysis with Games–Howell post hoc tests. Results were considered statistically significant if P-value ≤ 0.05.

RESULTS

IL-1β INCREASES TOTAL OC NUMBER AND IL-1α INCREASES THE PROPORTION OF LARGE OCs

To determine the effects of IL-1α and IL-1β on osteoclastogenesis, RAW cells were cultured in the presence of 50 ng/ml sRANKL and IL-1α or IL-1β for 5 days. The cells were fixed, TRAP stained, and the number of TRAP+ cells with two or more nuclei was counted. Both IL-1α and IL-1β increased the total number of OCs at 10 ng/ml (Fig. 1A,B) while 100 ng/ml of either cytokine was toxic as indicated by cell death (data not shown). The percentage of small OCs formed in culture during differentiation was not affected by either IL-1α or IL-1β (Fig. 2). At the same time, the percentage of large OCs was increased by IL-1α at both 1 and 10 ng/ml concentrations, while IL-1β had no effect. Addition of 50 ng/ml of IL-1ra reversed the IL-1α-induced effects on OCs confirming that these effects were mediated via the IL-1 receptor (Figs. 1B and 2B). These results show that IL-1α and IL-1β have different effects on OC differentiation and that IL-1α is a more potent inducer of OC differentiation than IL-1β.

IL-1β AFFECTS THE MORPHOLOGY OF LARGE OCS

To determine whether IL-1α and IL-1β have an effect on populations of pre-differentiated large and small OCs, the cells were size sorted using FBS gradients and then treated with IL-1α, IL-1β, and IL-1ra for 24 h. After 24 h, IL-1α and IL-1β had no effect on OC number or

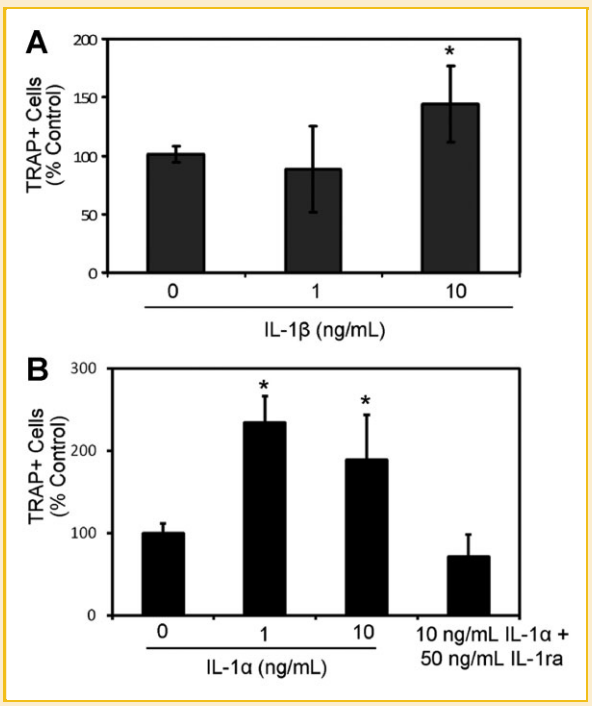

Fig. 1. IL-1α and IL-1β increase OC number. OCs were generated from RAW cells in the presence of 50 ng/ml RANKL and (A) 0, 1, and 10 ng/ml of IL-1β or (B) 0, 1, or 10 ng/ml IL-1α and/or 50 ng/ml of the inhibitor, IL-1ra. Cells were differentiated for 5 days with media changes at day 3. Cells were then fixed, TRAP stained, and counted. Results reflect TRAP+ multinucleated (2+ nuclei) cells expressed as a percentage of control (0 ng/ml IL-1) ± SD from three combined experiments; $^*P < 0.05$ versus control.

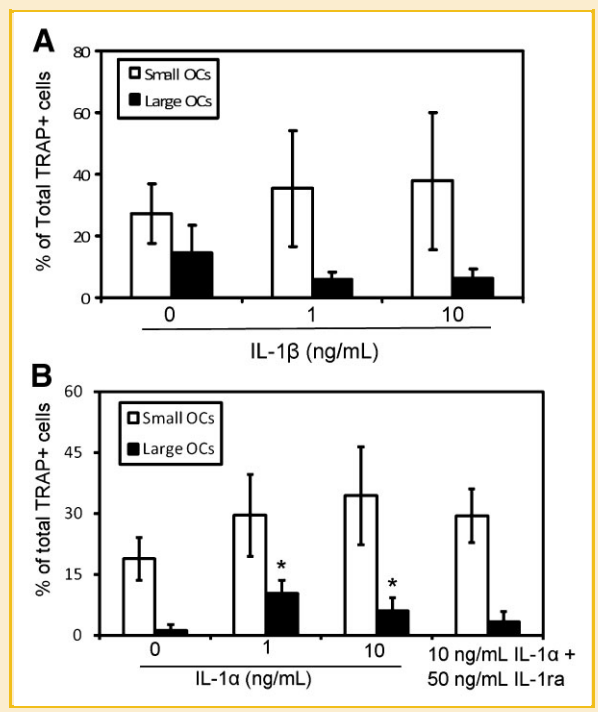

Fig. 2. IL-1α increases the proportion of large OCs formed during differentiation. OCs were generated from RAW cells in the presence of RANKL (50 ng/ml) and 1 and 10 ng/ml of IL-1β (A) or IL-1α (B). Cells were grown for 5 days and then fixed, TRAP stained, and counted. Results reflect the percentage of large and small OCs generated in the presence of IL-1α and IL-1β ± SD from three combined experiments; expressed as a percentage of total TRAP+ (2+ nuclei) cells; $^*P < 0.05$ versus control.

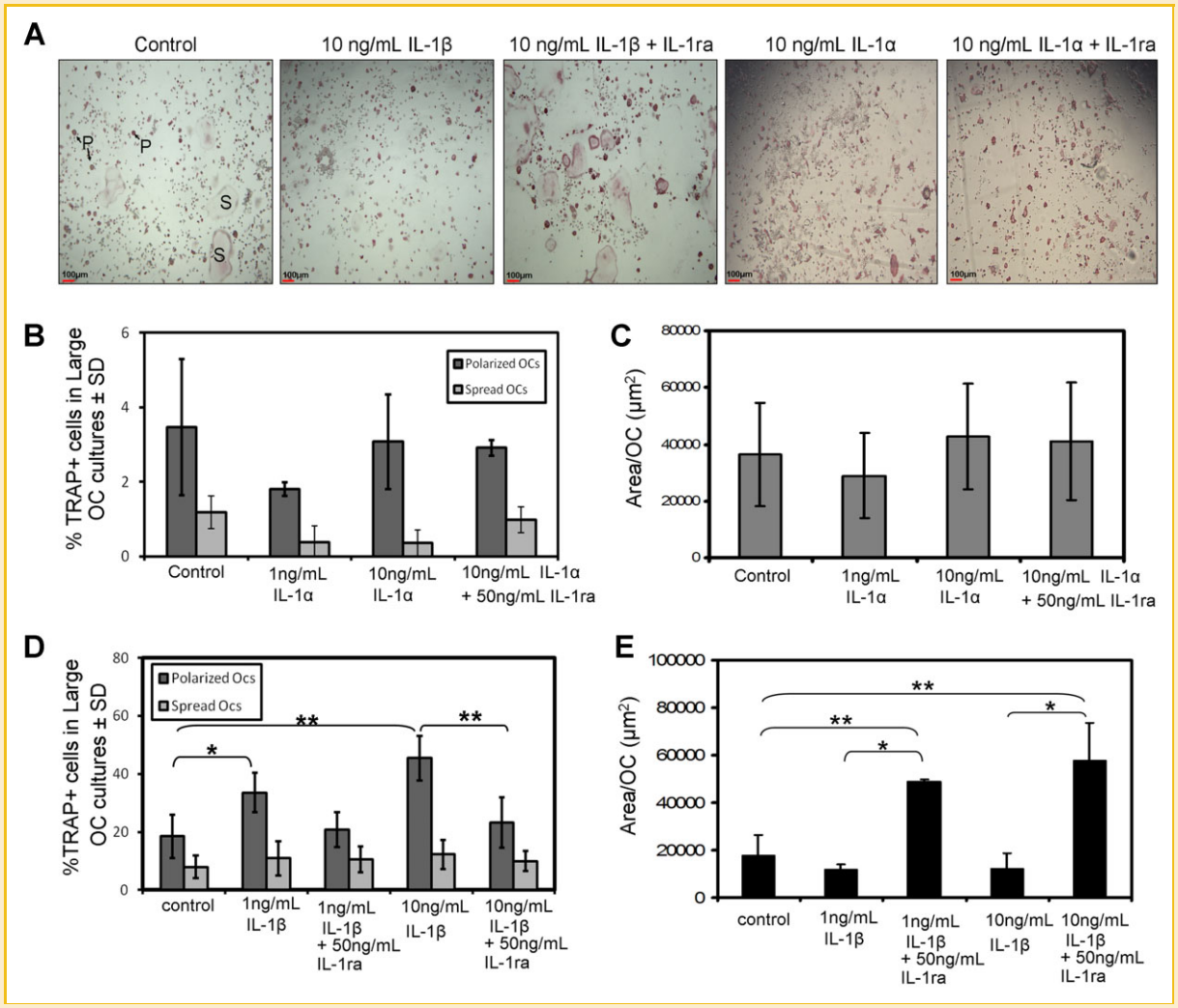

Fig. 3. IL-1β affects morphology of large OCs. Large and small OCs were generated from RAW cells in the presence of RANKL, size separated on an FBS gradient, replated, and incubated in the presence of IL-1α or IL-1β for 24 h. Cells were fixed, TRAP-stained, and analyzed for cell morphology. A: Depicts typical morphologies of large OCs in culture. P, polarized cells; S, spread cell. B,D: Cells were counted and expressed as a percentage of total cells in culture ± SD of results represent three combined experiments; $^{**}P \leq 0.01$; $^{*}P \leq 0.05$. C,E: Areas of ~100 large OCs from each group were measured, calculated as area per OC, and presented as mean ± SD of three combined experiments; $^{**}P \leq 0.01$; $^{*}P \leq 0.05$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

multinucleation of pre-differentiated cells (data not shown); however, cell morphology was affected (Fig. 3A). In OCs, polarized morphology is associated with actively resorbing or migrating cells, while spread morphology is associated with cells being in a resting state [Lakkakorpi and Vaananen, 1996]. To quantify changes in morphology, the number of large OCs (10+ nuclei) with polarized or spread morphologies was counted. IL-1α had no effect on cell morphology in large OC cultures (Fig. 3B), while IL-1β significantly affected the number of large OCs adopting a polarized cell morphology (Fig. 3D). This effect could be reversed by IL-1ra (Fig. 3D) confirming that this effect is IL-1 mediated. Both isoforms of IL-1 had no effects on the morphology of small OCs (data not shown).

To verify differences in morphology, the planar area of OCs was measured. Since a polarized cell has a smaller planar area than a cell with a spread morphology (Fig. 3A) [Saltel et al., 2004], treatment with the cytokines should have an effect on area per OC. The average area of large OCs was significantly lower in IL-1β-treated cultures compared to those cells in the presence of inhibitor (Fig. 3E). Surprisingly, no difference was seen between the IL-1β-treated cultures and the controls. This discrepancy could be due to differences in the experimental parameters: Figure 3D only shows the number of polarized or spread cells, without reflecting the degree of change in cell shape, while Figure 3E depicts a more accurate assessment of the degree of change in cell morphology. Interestingly, presence of the antagonist had a significant effect on cell area suggesting that IL-1ra drastically reverses OC morphology in IL-1β-treated cultures. No significant differences were seen in the areas of large OCs treated with IL-1α (Fig. 3C) or in small OC cultures treated with either cytokine (data not shown).

BOTH IL-1α AND IL-1β INCREASE RESORPTION BY LARGE BUT NOT BY SMALL OCs

To assess OC resorptive capacity in response to IL-1α and IL-1β, populations of large and small OCs were plated on Osteologic™ slides. Both isoforms increased resorption by large OCs and this effect was partially reversed by IL-1ra (Fig. 4A). Moreover, large OCs stimulated with IL-1α formed greater numbers of pits/OC than those treated with IL-1β (Fig. 4B), while large OCs stimulated with IL-1β generated larger pits than those treated with IL-1α (Fig. 4C). These results demonstrate that IL-1α is a more potent inducer of bone resorption. The resorption area, number, and size of pits per OC of small OCs were not affected by both cytokines (Fig. 4A–C).

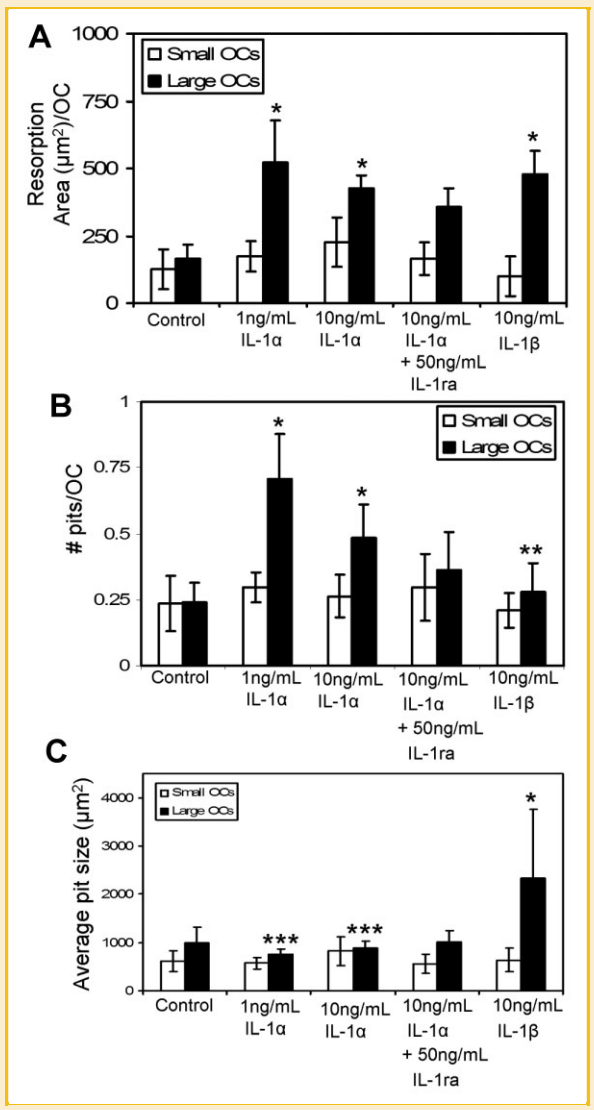

Fig. 4. IL-1α affects resorption and pit number in large OC cultures. RAW 264.7 cells were differentiated with RANKL for 4 days. Cells were then size separated on an FBS gradient into populations of large and small OCs and replated onto Osteologic™ slides in the presence or absence of IL-1α, IL-1β, and/or IL-1ra and incubated for 48 h. The slides were stained using Von Kossa staining and resorption area and pit number were evaluated. Cells with the same conditions were also plated in parallel for TRAP staining and counting. The results are expressed as resorption/OC (A), number of resorption pits, pits/OC (B), and the average size of the resorption pits (C). The data are presented as the means of combined experiments ± SD; $^{*}P < 0.05$ versus control; $^{**}P < 0.05$ versus 10 ng/ml IL-1α; $^{***}P < 0.05$ versus 10 ng/ml IL-1β.

IL-1β TRIGGERS INTEGRIN β3 PHOSPHORYLATION

The results shown in Figure 3 indicate that IL-1β, but not IL-α, affects cell morphology and by implication, cytoskeletal rearrangement. It is known that OC cytoskeletal rearrangements are mediated by integrins [Nakamura et al., 1999; Duong et al., 2000; Huang et al., 2001], which are activated by phosphorylation [Hynes, 2002; Nakamura et al., 2003]. Thus, we investigated whether IL-1α and/or IL-1β affect integrin signaling, by examining the phosphorylation status of β3, the predominate OC integrin. Large variations were seen in β3 phosphorylation with IL-1α treatment and we were unable to draw any conclusions for this cytokine (data not shown). In contrast, IL-1β increased integrin β3 phosphorylation in large OCs at 20 and 30 min (Fig. 5) but had no effect on β3 phosphorylation in small OCs (data not shown). These results show that the morphological changes induced by IL-1β are likely mediated through integrin activation.

INTEGRIN β3 AND IL-1RI LOCALIZE TO PODOSOMES AND PODOSOME BELTS OF LARGE OCs

Activation of integrin β3 can be induced by cytokines like IL-1 by activating serine/threonine kinases [Singh et al., 1997, 1999]. It has

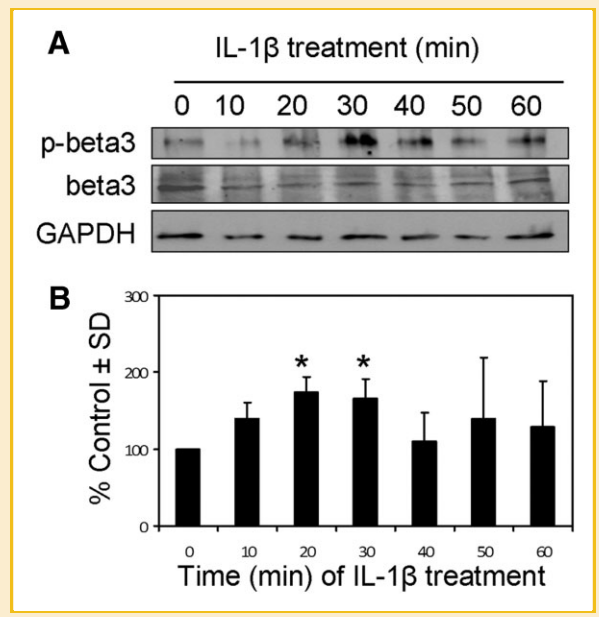

Fig. 5. IL-1β increases phosphorylation of integrin β3 in large OCs. RAW 264.7 cells were differentiated with sRANKL into populations of large or small OCs. Cells were serum starved for 30 min and then stimulated with IL-1β for the indicated time periods. Cell lysates were run on 8% SDS–PAGE gels and immunoblotted for phosphorylated integrin β3 (p-beta3), integrin β3 (beta3), and GAPDH. A: Representative blot for large OC experiments. B: Quantification of Western blotting, combined results from three independent experiments in large OCs normalized to GAPDH. Data are presented as a percentage of control (time 0). $^{*}P < 0.05$ versus control.

been reported in fibroblasts that IL-1 receptors cluster at the sites of focal adhesions [Qwarnstrom et al., 1988]. Integrins, the major adhesion receptors, are also present in focal adhesions [Geiger et al., 2009]. To investigate in OCs whether IL-1RI is localized to podosomes (the OC focal adhesions) to facilitate integrin phosphorylation, cells were analyzed using immunofluoresence (Fig. 6). The results demonstrated that unstimulated cells display diffuse cytosolic staining of IL-1RI and β3. In cells treated with IL-1β, IL-1RI and β3 were predominantly located in the cell periphery and seemed to colocalize at podosomes and podosome belts, the regions of OC attachment. This same phenomenon was observed to a lesser extent in mid-sized OCs and not observed in small OCs. This suggests that the signaling cascade induced by IL-1β and leading to cytoskeletal rearrangements, might be initiated by the co-localization of IL-1RI and αvβ3.

DISCUSSION

Differences in resorptive activity of large and small OCs have been previously demonstrated [Lees et al., 2001]. Here we show for the first time that large and small OCs respond differently to IL-1α and IL-1β. Our data demonstrate that IL-1α and IL-1β have a differential effect on the proportion of large and small OCs generated during differentiation, the number of resorption pits per OC, and the phosphorylation levels of β3 integrin. Furthermore, these cytokines predominantly affected populations of large OCs and appeared to have little effect on small OCs.

The difference in responses between large and small OCs could be due to differential expression of activating receptors. We have shown previously that large OCs express higher levels of the signaling receptor, IL-1RI, while small OCs expressed higher levels of the decoy receptor IL-1RII [Trebec et al., 2007]. Compatible with this, large OCs predominantly expressing the signaling receptor were responding to IL-1, while small OCs expressing higher levels of the decoy receptor were not affected.

It has been demonstrated that IL-1α and IL-1β have different affinities for their receptors: IL-1α has a higher affinity for IL-1RI, while IL-1β has a higher affinity for IL-1RII. Furthermore, IL-1ra binds with the highest binding affinity and irreversibly to IL-1RI [Arend et al., 1994]. This could explain our observations that IL-1α had a greater effect on OC number, proportion of large OCs, and bone resorption at lower concentrations when compared to the effects of IL-1β (1 ng/ml for IL-1α vs. 10 ng/ml for IL-1β). Differences in

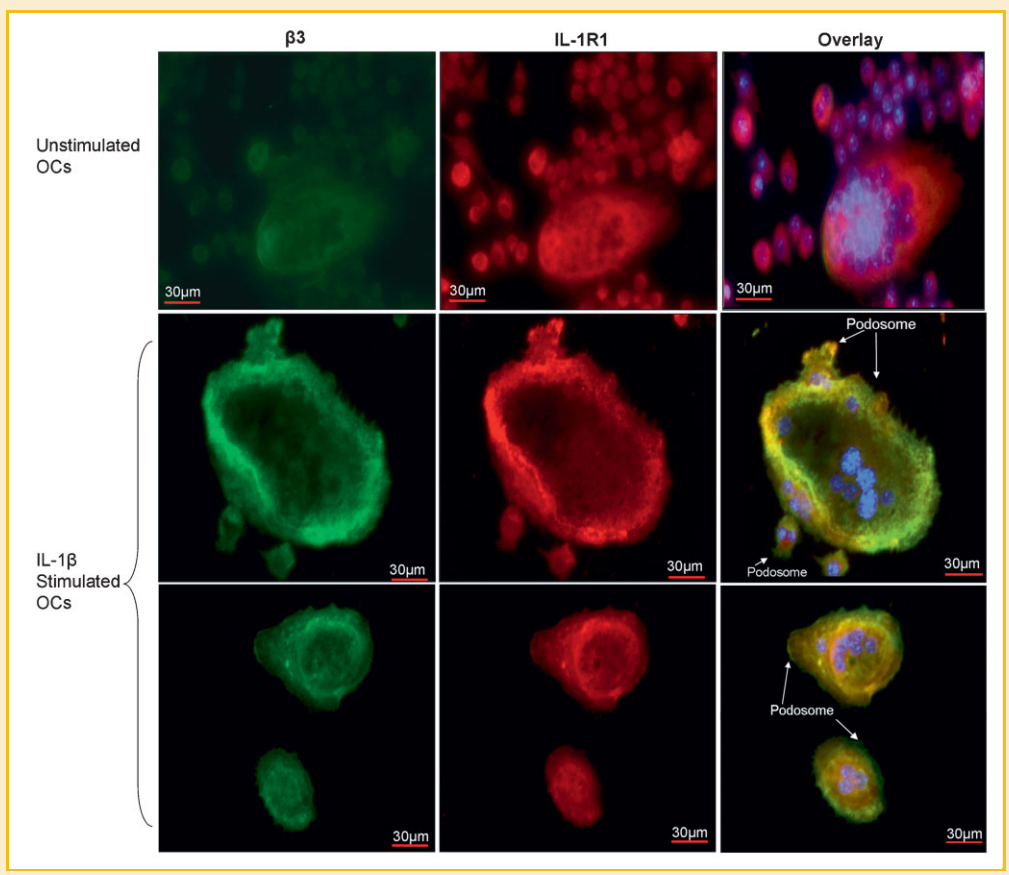

Fig. 6. Integrin β3 and IL-IRI localize to podosomes and podosome belts of large OCs. RAW 264.7 derived small and large OCs were grown on glass chamber slides. They were then serum starved for 30 min, then stimulated with 10 ng/ml of IL-1β for 30 min. Slides were then fixed and immunostained for IL-IRI (red) and β3 (green) and nuclei were stained with DAPI (blue). Images are representative of three independent experiments displaying OCs of various sizes: large OC (10+ nuclei, middle horizontal row), mid-size OCs (7 nuclei), and small OCs (2–5 nuclei) (bottom horizontal row).

affinities, however, cannot explain the differences in cell morphology and integrin phosphorylation since IL-1β significantly affected these parameters, while IL-1α (Figs. 3 and 5) had no effect. Nor can affinities explain why IL-1ra significantly reversed the effects on cell morphology induced by IL-1β and not IL-1α-treated cultures.

In OCs, changes in cell morphology indicate the status of the cell: polarized cells are believed to represent actively resorbing or migrating cells, while cells with a spread morphology are believed to be resting [Lakkakorpi and Vaananen, 1996]. It is of note that IL-1β, but not IL-1α, significantly increased the number of polarized cells (Fig. 3). However, this change in cell morphology did not affect bone resorption as the cells treated with either cytokine resorbed the same area per OC (Fig. 4A). Surprisingly, IL-1α significantly increased the number of resorption pits per OC compared to the cells treated with IL-1β (Fig. 4B), even though the number of polarized cells was not different from the controls (Fig. 3B). A possible explanation is that IL-1α, but not IL-1β, affects cell migration and an increase in migration could explain the higher number of resorption pits. Alternatively, IL-1 could affect pit depth and pit size. While the depth of the pits was not measured, our results indicate that pits formed by OCs treated with IL-1β had a larger surface area compared to IL-1α-treated cells (Fig. 4C). It is possible that IL-1α promotes bone resorption by increasing cell migration and the formation of numerous but smaller pits while IL-1β induces OCs to form fewer but larger pits.

To resorb, OCs attach to the bone surface and form a sealing zone, and this process is mediated by integrins [Nakamura et al., 1999]. We have previously shown that large OCs express five-fold higher levels of integrin β3 compared to small OCs [Trebec et al., 2007]. Our data, presented in Figure 5, demonstrate that IL-1β increased β3 integrin phosphorylation levels in large OCs. The molecular mechanism of how IL-1β treatment increased integrin phosphorylation is not clear. Integrins are the adhesion receptors present in focal adhesions and podosomes [Hynes, 2002; Geiger et al., 2009] and treatment of fibroblasts with IL-1 results in IL-1 receptors clustering at focal adhesions [Qwarnstrom et al., 1988]. Furthermore, several protein kinases directly associate with the cytosolic domain of IL-1RI [Singh et al., 1997, 1999]. Therefore, it is possible that IL-1β binding to its receptor, IL-1RI, activates the kinases bound to the cytosolic tail of the receptor, which in turn phosphorylate the integrin and initiate cytoskeletal rearrangements. In support of this hypothesis, our results show IL-1RI and αvβ3 co-localizing upon cytokine stimulation (Fig. 6).

Here we show that IL-1α and IL-1β, although considered to have similar biological functions [Dinarello, 1996], display differential effects in OCs. Differential and non-redundant effects of IL-1α and IL-1β were similarly seen in tumor cells [Song et al., 2003]. Levels of IL-1β are increased in plasma and synovial fluid of rheumatoid arthritis patients, while levels of IL-1α are the same as healthy controls [Eastgate et al., 1988, 1991; Lettesjo et al., 1998]. Determining specific differences affecting OC differentiation and activation induced by IL-1α and IL-1β signaling under inflammatory conditions could lead to better understanding of the resulting pathological bone loss and identify better therapeutic targets in rheumatoid arthritis and other inflammatory diseases.

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REFERENCES

Aota S, Nakamura T, Suzuki K, Tanaka Y, Okazaki Y, Segawa Y, Miura M, Kikuchi S. 1996. Effects of indomethacin administration on bone turnover and bone mass in adjuvant-induced arthritis in rats. Calcif Tissue Int 59:385–391.

Arend WP, Malyak M, Smith MF, Jr., Whisenand TD, Slack JL, Sims JE, Giri JG, Dower SK. 1994. Binding of IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist by soluble IL-1 receptors and levels of soluble IL-1 receptors in synovial fluids. J Immunol 153:4766–4774.

Carmi Y, Voronov E, Dotan S, Lahat N, Rahat MA, Fogel M, Huszar M, White MR, Dinarello CA, Apte RN. 2009. The role of macrophage-derived IL-1 in induction and maintenance of angiogenesis. J Immunol 183:4705–4714.

Dinarello CA. 1996. Biologic basis for interleukin-1 in disease. Blood 87: 2095–2147.

Dinarello CA. 1998. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. Int Rev Immunol 16:457–499.

Dinarello CA. 2002. The IL-1 family and inflammatory diseases. Clin Exp Rheumatol 20:S1–S13.

Duong LT, Lakkakorpi P, Nakamura I, Rodan GA. 2000. Integrins and signaling in osteoclast function. Matrix Biol 19:97–105.

Eastgate JA, Symons JA, Wood NC, Grinlinton FM, di Giovine FS, Duff GW. 1988. Correlation of plasma interleukin 1 levels with disease activity in rheumatoid arthritis. Lancet 2:706–709.

Eastgate JA, Symons JA, Wood NC, Capper SJ, Duff GW. 1991. Plasma levels of interleukin-1-alpha in rheumatoid arthritis. Br J Rheumatol 30:295–297.

Geiger B, Spatz JP, Bershadsky AD. 2009. Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol 10:21–33.

Huang WY, Valles S, Qwarnstrom EE. 2001. Translocation of the IL-1 receptor to focal adhesions is regulated through the C-terminal end of the cytoplasmic domain. Cell Biol Int 25:309–317.

Hynes RO. 2002. Integrins: Bidirectional, allosteric signaling machines. Cell 110:673–687.

Jimi E, Nakamura I, Duong LT, Ikebe T, Takahashi N, Rodan GA, Suda T. 1999. Interleukin 1 induces multinucleation and bone-resorbing activity of osteoclasts in the absence of osteoblasts/stromal cells. Exp Cell Res 247:84–93.

Kanda-Nakamura C, Izumi Y, Sueda T. 1996. Increased expression of interleukin-1 receptors on fibroblasts derived from inflamed gingiva. J Periodontol 67:1267–1273.

Lakkakorpi PT, Vaananen HK. 1996. Cytoskeletal changes in osteoclasts during the resorption cycle. Microsc Res Tech 33:171–181.

Lee SK, Gardner AE, Kalinowski JF, Jastrzebski SL, Lorenzo JA. 2006. RANKL-stimulated osteoclast-like cell formation in vitro is partially dependent on endogenous interleukin-1 production. Bone 38:678–685.

Lees RL, Sabharwal VK, Heersche JN. 2001. Resorptive state and cell size influence intracellular pH regulation in rabbit osteoclasts cultured on collagen-hydroxyapatite films. Bone 28:187–194.

Lettesjo H, Nordstrom E, Strom H, Nilsson B, Glinghammar B, Dahlstedt L, Moller E. 1998. Synovial fluid cytokines in patients with rheumatoid arthritis or other arthritic lesions. Scand J Immunol 48:286–292.

Makris GP, Saffar JL. 1982. Quantitative relationship between osteoclasts, osteoclast nuclei and the extent of the resorbing surface in hamster periodontal disease. Arch Oral Biol 27:965–969.

Manolson MF, Yu H, Chen W, Yao Y, Li K, Lees RL, Heersche JN. 2003. The a3 isoform of the 100-kDa V-ATPase subunit is highly but differentially expressed in large (≥10 nuclei) and small (≤5 nuclei) osteoclasts. J Biol Chem 278:49271–49278.

Nakamura I, Pilkington MF, Lakkakorpi PT, Lipfert L, Sims SM, Dixon SJ, Rodan GA, Duong LT. 1999. Role of alpha(v)beta(3) integrin in osteoclast migration and formation of the sealing zone. J Cell Sci 112(Pt 22):3985–3993.

Nakamura I, Rodan GA, Duong le T. 2003. Regulatory mechanism of osteoclast activation. J Electron Microsc (Tokyo) 52:527–533.

Pacifici R. 1996. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. J Bone Miner Res 11:1043–1051.

Qwarnstrom EE, Page RC, Gillis S, Dower SK. 1988. Binding, internalization, and intracellular localization of interleukin-1 beta in human diploid fibroblasts. J Biol Chem 263:8261–8269.

Rasmussen L, Hanstrom L, Lerner UH. 2000. Characterization of bone resorbing activity in gingival crevicular fluid from patients with periodontitis. J Clin Periodontol 27:41–52.

Roodman GD. 1999. Cell biology of the osteoclast. Exp Hematol 27:1229–1241.

Saltel F, Destaing O, Bard F, Eichert D, Jurdic P. 2004. Apatite-mediated actin dynamics in resorbing osteoclasts. Mol Biol Cell 15:5231–5241.

Sims JE, Giri JG, Dower SK. 1994. The two interleukin-1 receptors play different roles in IL-1 actions. Clin Immunol Immunopathol 72:9–14.

Singer F, Roodman GD. 1996. Paget's disease of bone. In: Bilezikian J, Raisz LG, Rodan GA, editors. Principles of bone biology. San Diego: Academic Press. pp 969–977.

Singh R, Huang S, Guth T, Konieczkowski M, Sedor JR. 1997. Cytosolic domain of the type I interleukin-1 receptor spontaneously recruits signaling molecules to activate a proinflammatory gene. J Clin Invest 100:419–428.

Singh R, Wang B, Shirvaikar A, Khan S, Kamat S, Schelling JR, Konieczkowski M, Sedor JR. 1999. The IL-1 receptor and Rho directly associate to drive cell activation in inflammation. J Clin Invest 103:1561–1570.

Smith MD, Triantafillou S, Parker A, Youssef PP, Coleman M. 1997. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. J Rheumatol 24:365–371.

Song X, Voronov E, Dvorkin T, Fima E, Cagnano E, Benharroch D, Shendler Y, Bjorkdahl O, Segal S, Dinarello CA, Apte RN. 2003. Differential effects of IL-1 alpha and IL-1 beta on tumorigenicity patterns and invasiveness. J Immunol 171:6448–6456.

Strand V, Kavanaugh AF. 2004. The role of interleukin-1 in bone resorption in rheumatoid arthritis. Rheumatology (Oxford) 43(Suppl 3):iii10–iii16.

Trebec DP, Chandra D, Gramoun A, Li K, Heersche JN, Manolson MF. 2007. Increased expression of activating factors in large osteoclasts could explain their excessive activity in osteolytic diseases. J Cell Biochem 101:205–220.

Voronov I, Heersche JN, Casper RF, Tenenbaum HC, Manolson MF. 2005. Inhibition of osteoclast differentiation by polycyclic aryl hydrocarbons is dependent on cell density and RANKL concentration. Biochem Pharmacol 70:300–307.

Xu LX, Kukita T, Nakano Y, Yu H, Hotokebuchi T, Kuratani T, Iijima T, Koga T. 1996. Osteoclasts in normal and adjuvant arthritis bone tissues express the mRNA for both type I and II interleukin-1 receptors. Lab Invest 75:677–687.