Journal of Cellular Biochemistry 89:213–219 (2003)

FAST TRACKS

IL-13 Regulates Vascular Cell Adhesion Molecule-1 Expression in Human Osteoblasts

Leonard Rifas* and Su-Li Cheng

Division of Bone and Mineral Diseases, Department of Internal Medicine, Washington University School of Medicine at Barnes-Jewish Hospital, St. Louis, Missouri 63110

Abstract Activated T cells (Act T) produce multiple cytokines that affect osteoblast function as well as osteoclastogenesis. One of these cytokines, IL-13, is a multifunctional cytokine elaborated by Act T that regulates vascular cellular adhesion molecule (VCAM)-1 expression in endothelial cells. VCAM-1 has also been implicated in osteoclast formation by myeloma cells. We therefore studied whether IL-13 regulates VCAM-1 in human osteoblastic cells since these cells express RANKL, the major osteoclastogenic factor and osteoclast precursors are found adjacent to osteoblasts. Human T cells were activated in the absence or presence of Cyclosporin A (CsA), an inhibitor of the production of most activated T cell cytokines. Conditioned media were assayed for IL-13 by ELISA. Act T produced IL-13 and, unlike other T cell cytokines, this was elevated 3-fold by CsA. Exposure of human osteoblasts (hOB) to doses of recombinant human IL-13 (rhIL-13, 0–10 ng/ml) resulted in an increase of VCAM-1 mRNA (up to 5-fold) within 4 h with a maximum stimulation at 1 ng/ml. CsA had no effect on basal hOB VCAM-1 mRNA expression. Examination of VCAM-1 on the cell surface of hOB, by immunocytochemistry, revealed increasing levels of surface expression of the protein within 16 h after stimulation with doses of rhIL-13 (0.1–10 ng/ml) which were reflective of the mRNAs. IL-6 production was also stimulated in a dose dependent manner with a maximum of 2.5-fold with 1 ng/ml rhIL-13 within 16 h. Since both VCAM-1 and IL-6 showed similar responses to IL-13, IL-6 was examined for its ability to induce VCAM-1. Immunocytochemistry demonstrated no effect of IL-6 on VCAM-1 expression. These data demonstrate that during pathological processes associated with T cell activation, such as rheumatoid arthritis or possibly post-menopausal osteoporosis, T cells may play a pivotal role in osteoclast precursor adhesion to osteoblasts as a first step prior to RANKL signaling. J. Cell. Biochem. 89: 213–219, 2003.

Key words: rheumatoid arthritis; osteoclastogenesis; cell–cell communication; T cells; cytokines

Vascular cellular adhesion molecule (VCAM)-1 is a membrane-bound cellular adhesion molecule, first identified on endothelial cells that had been stimulated by inflammatory cytokines [Osborn et al., 1989]. VCAM-1 mediates adhesive interactions between hematopoietic progenitor cells and stromal cells in the bone marrow and between leukocytes and endothelial cells. T cells bind to a variety of cells upon activation by an immune response. Very late antigen (VLA-4, $\alpha_4\beta_1$), the ligand for VCAM-1 [Elices et al., 1990], is expressed on monocytes, T and B lymphocytes, basophils, and eosinophils [Yusuf-Makagiansar et al., 2002].

Cell–cell communication between osteoclast progenitors and cells of the osteoblast lineage (osteoblasts and stromal cells) is important in the process of osteoclast formation and bone resorption [Yasuda et al., 1998]. Cytokines produced by osteoblasts, such as IL-6, are important in driving osteoclast progenitor cell expansion [Kurihara et al., 1990; Jilka et al., 1992; Roodman, 1992], while physical interaction between osteoclasts and osteoblasts promotes osteoclast differentiation and activation [Lacey et al., 1998; Yasuda et al., 1998] The mechanism leading to osteoclast progenitor-osteoblast adhesion and subsequent osteoclastogenesis may involve VCAM-1 expression [Feuerbach

Grant sponsor: NIAMSD; Grant numbers: AR32087, AR46370.

*Correspondence to: Leonard Rifas, Division of Bone and Mineral Diseases, Department of Internal Medicine, Washington University School of Medicine, 216 South Kingshighway, St. Louis, MO 63110.
E-mail: lrifas@im.wustl.edu

Received 5 March 2003; Accepted 6 March 2003

DOI 10.1002/jcb.10531

and Feyen, 1997]. T cells produce IL-13, a cytokine which has been shown to induce VCAM-1 expression in endothelial cells [Bochner et al., 1995] and human osteoblasts (hOB) have been reported to bind activated T cells (Act T) via VCAM-1/VLA-4 interaction [Tanaka et al., 1995]. We have previously shown that T cells secrete cytokines which act upon osteoblasts resulting in the expression of IL-6 [Rifas and Avioli, 1999]. We have also recently demonstrated that T cell cytokines up-regulate receptor activator of NF-kappa B ligand (RANKL) in osteoblasts [Rifas et al., 2003], an essential inducer of osteoclastogenesis and osteoclast activity. Furthermore, patients receiving immunosuppressant therapy suffer from osteopenia [Thiebaud et al., 1996] and T cells have also been reported to play a critical role in Cyclosporin A (CsA) induced osteoporosis in the rat [Buchinsky et al., 1996]. Finally, Scopes et al. [2001] have recently shown that IL-13 plays a role in human osteoclast formation in a lymphocyte dependent manner. To explore the relationship between T cell–osteoblast–osteoclast interactions and the possible role of T cells in bone turnover, we examined whether IL-13, a T cell cytokine which is increased by CsA treatment [van der Pouw Kraan et al., 1996], may play a role in the process of osteoclastogenesis by enhancing VCAM-1 expression in hOB.

MATERIALS AND METHODS

All reagents were obtained from Sigma Chemical Co., St. Louis, Missouri, unless otherwise indicated.

Antibodies and Recombinant Cytokine

Sterile, azide free, and low endotoxin preparations of mouse anti-human CD3 monoclonal antibody (clone HIT3a) and mouse anti-human CD28 monoclonal antibody (clone 28.2) as well as mouse anti-human VCAM-1 (CD106) monoclonal antibodies and mouse IgG were obtained from Pharmingen (San Diego, CA). Recombinant human IL-13 and recombinant human IL-6 were obtained from R&D Systems, Minneapolis, MN. Goat anti-mouse IgG-Cy3 conjugate was obtained from the Jackson Laboratory, Bar Harbor, ME.

ELISA Analysis of Cytokine Production

IL-6 and IL-13 were assayed in conditioned media using specific ELISA kits obtained from Amersham Bioscience (Arlington Heights, IL) according to the manufacturer's instructions.

T Cell Isolation and Culture

Peripheral blood mononuclear cells (PBMC) from normal volunteers were obtained from the American Red Cross, St. Louis, MO, as buffy coat byproducts from blood donations, and further purified by separation on Histopaque (1.077 gm/ml) lymphocyte separation medium. Briefly, the buffy coat preparation was diluted 1:1 in PBS, overlayered onto Histopaque and centrifuged at $400\,g$ for 30 min at room temperature. Mononuclear cells were recovered from the interface, washed twice with PBS (Ca^{+2}-, Mg^{+} free) by centrifugation at $300\,g$, 5 min then processed to obtain T cells by negative selection. T cells were enriched from 2×10^8 PBMC using $CD3^{+}$ T cell enrichment columns (R&D Systems) according to the manufacturers instructions as previously described [Rifas and Avioli, 1999]. The cells were suspended in complete medium, counted, pelleted by centrifugation at $300\,g$ then resuspended in complete medium (MEM-α 10% heat inactivated fetal bovine serum, 1% 200 mM L-glutamine and penicillin/streptomycin (100 U and 100 μg/ml, respectively) at 1×10^6 cells/ml.

T Cell Cultures

T cells were cultured in 96 well multiwell plates at 2×10^4 cells in 0.2 ml complete medium. T cells were activated by the addition of anti-human CD3 monoclonal antibody (1 μg/ml) and anti-human CD28 monoclonal antibody (5 μg/ml) in the absence or presence of CsA (100 ng/ml) for a 72 h period. The conditioned media were harvested and frozen at -80°C until assayed for cytokine production.

Preparation of hOB Cultures

hOB cultures were prepared as previously described [Rifas et al., 1994, 1995, 2003; Rifas and Avioli, 1999].

Immunohistochemistry

Osteoblasts were seeded onto 10 mm coverglasses in 24 well multiwell tissue culture plates in MEM-α, 10% HIFBS at 2×10^4 cells/cm^2. After a 24 h incubation period, the cells were stimulated for 16 h with either rhIL-13 or rhIL-6 as described in the text. The cells were then washed twice with ice cold (4°C) PBS/BSA (0.1% BSA:PBS, w:v) then incubated with either

mouse anti-human VCAM-1 monoclonal antibody (2 μg/ml in PBS/BSA) or mouse IgG for 1 h at 4°C. The cells were then washed three times with ice cold PBS/BSA and incubated with goat-anti mouse IgG-CY3 conjugate at 1:200 dilution in PBS/BSA for an additional 1 h at 4°C. The cell layer was washed twice more with PBS/BSA then fixed with 4% paraformaldehyde in PBS for 30 min at room temperature followed by three washed with PBS at room temperature and one quick rinse in deionized water. Coverslips were mounted onto slides with Aqua Polymount (Polysciences, Inc., Warrington, PA.) mounting medium and pictures taken with a Zeiss inverted microscope equipped with epi-fluorescence and a digital camera.

RNA Isolation and Northern Blot Analysis

Primary hOB were passed into 100 mm diameter tissue culture plates at 1×10^4 cells/cm^2 and allowed to grow for 7 days to reach confluence. The medium was changed and rhIL-13 at either 0.1, 1.0, or 10 ng/ml was added to each dish. Cells were allowed to incubate for 4 h then cell layers were washed 3× with PBS. To assess the effect of CsA on basal VCAM-1 gene expression, cells were incubated with CsA (100 ng/ml) for 24 h. In all cases, total RNA was extracted from the cell monolayers with an RNeasy kit (Qiagen, Chatsworth, CA) according to the manufacturers instructions. The quantity and quality of RNA were routinely tested spectrophotometrically using A_{260}/A_{280}. Twenty μg of total RNA from each preparation were electrophoresed on formaldehyde-containing 1% agarose gels. Prior to transfer, the gels were stained with ethidium bromide and rRNA bands photographed. RNA was then transferred to Nytran nylon membranes using the Turboblotter system (Schleicher & Schuell, Keene, New Hampshire, UK). The membranes were prehybridized with Hybrisol I (Oncor, Gaithersburg, MD) at 42°C for 3 h followed by hybridization in the same solution but with cDNA probe overnight at 42°C. The membranes were probed with [^{32}P]deoxy-CTP-labeled cDNA for human VCAM-1 (ATCC 116412) then in some instances, stripped and reprobed with [^{32}P]deoxy-CTP-labeled cDNA for human β-actin. Both probes were generated using the multiprime random primer labeling kit obtained from Amersham Biosciences according to the procedures provided. The membranes were washed twice with 2× SSC and 0.5% SDS for 15 min at room temperature and a single wash with 0.2 × SSC and 0.5% SDS at 52°C. Visualization of the extent of hybridization was performed by autoradiography at −80°C using Hyperfilm-MP (Amersham Biosciences). Relative loading efficiency was determined either by the 28S ribosomal RNA staining pattern or β-actin.

RESULTS

CsA Superinduces IL-13 in Act T

To investigate the nature of activated T cell response in the presence of CsA, T cells were cultured alone or in the presence of a combination of antibodies against human CD3 and CD28 for 72 h then assayed for IL-13 (Fig. 1). The results showed that stimulation with antibodies to either CD3 or CD28 alone induced very low levels of IL-13. A combination of anti-CD3 and anti-CD28 antibodies proved to be a potent stimulator of IL-13. Treatment of Act T with CsA resulted in a 3-fold induction of IL-13 production. These data demonstrate that although CsA inhibits the production of most T cell cytokines [Rifas and Avioli, 1999], it augments the production of IL-13 [van der Pouw Kraan et al., 1996].

IL-13 Induces IL-6 in hOB

Since IL-13 induces IL-6 in human endothelial cells [Sironi et al., 1994], and we demonstrated an elevated secretion of IL-13 by activated human T cells, we examined whether IL-13 might induce IL-6 secretion from hOB. hOB were grown to confluence then exposed to

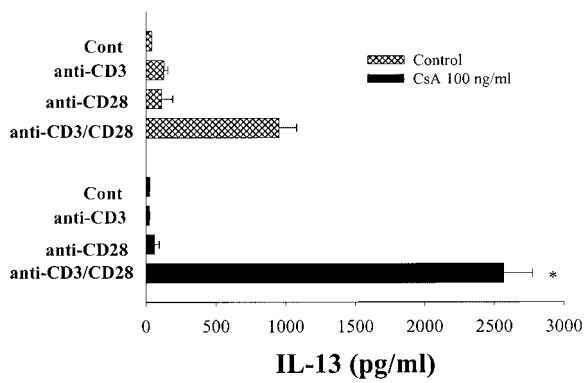

Fig. 1. Effect of activation and Cyclosporin A (CsA) on IL-13 production in human T cells. T cells were activated with anti-CD3 and/or anti-CD28 monoclonal antibodies and cultured in the presence or absence of CsA (100 ng/ml) for 72 h. Supernatants were assayed for IL-13 by ELISA. *$P < 0.02$ vs. control anti-CD3/CD28.

rhIL-13 (0–10 ng/ml) for 72 h. This dose encompassed the maximum amount of IL-13 (~3 ng/ml) produced by Act T in the presence of CsA. The conditioned media were collected and assayed by a specific ELISA for IL-6 (Fig. 2). rhIL-13 induced IL-6 secretion in a dose dependent fashion. A low dose (0.1 ng/ml) of rhIL-13 induced a 35% increase in IL-6 ($P < 0.001$), whereas 1 and 10 ng/ml rhIL-13 induced an approximately 2.5-fold increase ($P < 0.001$ and $P < 0.008$, respectively).

IL-13 Induces VCAM-1 Gene Expression in hOB

Since CsA enhanced, rather than inhibited IL-13 induction in T cells, we examined the effect of IL-13 on VCAM-1 expression in hOB (Fig. 3A). Cells were treated with a dose curve of rhIL-13 (0–10 ng/ml) and VCAM-1 mRNA steady state levels were examined by Northern blot analysis. Untreated hOB constitutively expressed low levels of VCAM-1 mRNA. Stimulation with a low dose (0.1 ng/ml) of rhIL-13 for 4 h induced a 2-fold increase, while 1 ng/ml induced a 5-fold increase, in VCAM-1 expression. This level of expression was not enhanced any further with 10 ng/ml rhIL-13. To determine whether CsA may have an effect on VCAM-1 expression, hOB were incubated in the absence or presence of CsA then mRNA steady state levels examined by Northern blot analysis (Fig. 3B). The results demonstrated that CsA has no effect on VCAM-1 gene expression.

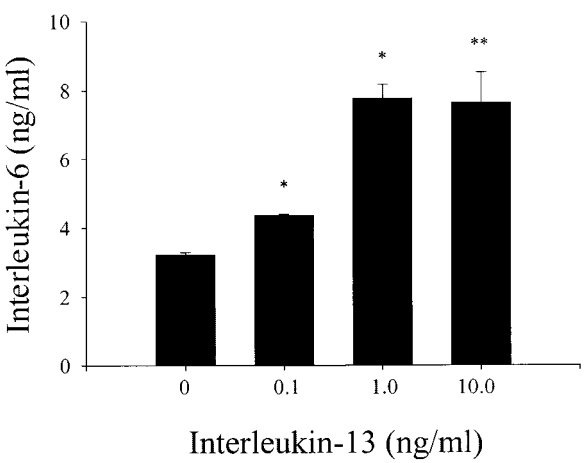

Fig. 2. IL-13 induces IL-6 production in human osteoblastic cells. Cells were treated with rhIL-13 (10 ng/ml) for 72 h. Supernatants were analyzed for IL-6 by ELISA. Data represent the mean ± SEM. of four independent cultures. *$P < 0.001$; **$P < 0.008$ vs. control.

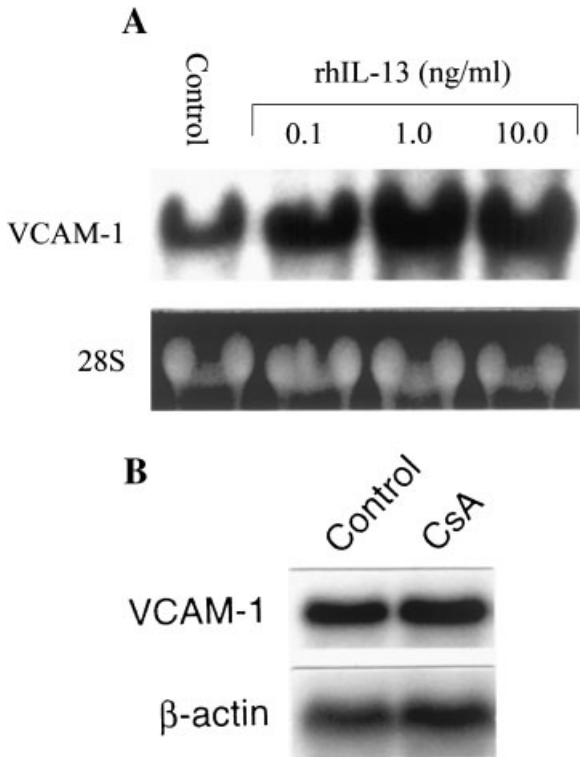

Fig. 3. Northern blot analysis of IL-13 induction of VCAM-1 in hOB. Confluent cultures were treated with (**A**) various doses of rhIL-13 for 4 h prior to isolation of total RNA and Northern blot analysis for VCAM-1 as described in Materials and Methods. **B**: RNA was isolated from hOB after a 24 h treatment with vehicle or 1 μg/ml CsA and analyzed for VCAM-1 expression by Northern blot analysis. The membrane was stripped and reprobed to detect β-actin.

IL-13 Increases VCAM-1 Cell Surface Expression

To further explore the effect of IL-13 on VCAM-1 expression, osteoblasts were incubated for 16 h with a dose range of rhIL-13 (0–10 ng/ml) then subjected to immunocytochemistry for VCAM-1. Figure 4 shows that control cells expressed basal levels of surface VCAM-1. Expression increased substantially after treatment with 0.1 ng/ml IL-13, while a maximum affect was observed with concentrations of 1–10 ng/ml rhIL-13, similar to that found with the mRNA. Since IL-13 induces IL-6, cells were also incubated for 16 h with rhIL-6 (1 or 10 ng/ml) then subjected to immunocytochemistry for VCAM-1. IL-6 did not increase basal expression of VCAM-1 on the surface of hOB.

DISCUSSION

We have demonstrated for the first time in the studies presented herein that IL-13 is a potent regulator of VCAM-1 in hOB. Act T may play a

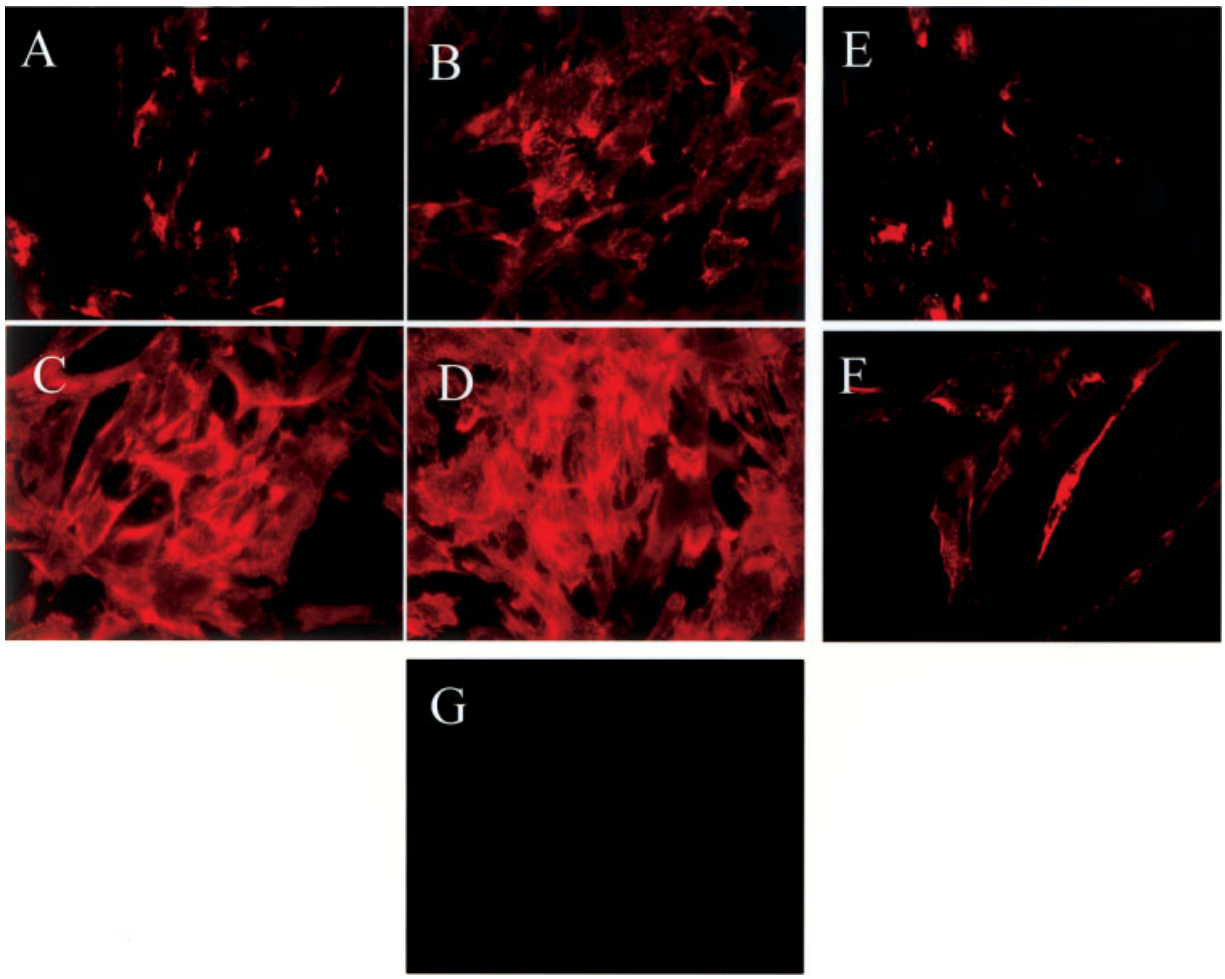

Fig. 4. IL-13, but not IL-6, induces VCAM-1 surface expression in human osteoblastic cells. hOB were subcultured and seeded onto 10 mm glass coverslips at 1×10^4 cells/cm^2. After a 24 h incubation period, in order to allow the cells to attach, the cells were washed two times with medium then incubated with either (**A**) medium alone or rhIL-13 at (**B**) 0.1 ng/ml; (**C**) 1.0 ng/ml; (**D**) 10 ng/ml; or IL-6 at (**E**) 1 ng/ml; (**F**) 10 ng/ml for 16 h. The cells were then stained for VCAM-1 as described in Materials and Methods. The antibody control (**G**) was incubated with mouse IgG. Original magnification ×40.

vital role in the process of bone turnover since both cells of the osteoblast lineage, as well as cells of the immune system, reside in the bone marrow compartment and cytokines released by Act T have modulating effects on osteoblast [Rifas et al., 2003], as well as on osteoclast [Weitzmann et al., 2001] development and activity. One such activity may be the up-regulation of VCAM-1 in osteoblasts, leading to an enhanced accumulation of osteoclast precursors at the site of bone turnover. In this regard, Feuerbach and Feyen [1997] have recently demonstrated that VCAM-1 is involved in the interaction between bone marrow stromal cells and osteoclast precursor cells during the early stages of osteoclastogenesis. Bone marrow stromal cells represent the earliest precursors in the osteoblast lineage [Cheng et al., 1994]. Furthermore, the interaction of murine myeloma cell $\alpha_4\beta_1$-integrin with bone marrow stromal cell VCAM-1 increases osteoclast formation [Michigami et al., 2000]. Thus it is not surprising to find that osteoblasts express VCAM-1 as well. These data are supported by the findings of others [Tanaka et al., 1995] who have shown that antibodies to VCAM-1 inhibited T cell binding to osteoblasts. The local up-regulation of VCAM-1 in human osteoblastic cells by IL-13 may further enhance the interaction between T cells and osteoblasts, resulting in a sustained increased production of IL-6 at the local level. Since IL-6 has been implicated to be an important stimulator of osteoclast recruitment and activation [Kurihara et al., 1990; Jilka et al., 1992; Roodman, 1992], the up-regulation of VCAM-1 in osteoblasts by T

cells via IL-13, and the direct stimulation of IL-6 production by IL-13 in osteoblasts, may eventually lead to increased osteoclast activity at sites of inflammation, resulting in increased bone resorption.

We have confirmed, by immunofluorescence, the presence of VCAM-1 on the surface of osteoblasts and that this expression is increased by IL-13. The method used for the immunocytochemisty, i.e., staining unfixed cells at 4°C, insured that only cell surface ligands interacted with the primary antibody. Subsequent fixation after staining allowed for the cross-linking of the primary/secondary antibody complex to the surface VCAM-1. The results provided very specific surface expression without the complications of either internal antibody-ligand formation or fixation artifacts.

The IL-13 production studies were performed with $CD3^+$ T cells that include both $CD4^+$ and $CD8^+$ T cell subsets. Of interest is that both $CD4^+$ and $CD8^+$ T cells produce IL-13 and that such production is enhanced in both cell types by CsA [van der Pouw Kraan et al., 1996]. Moreover, the ability of CsA to further enhance the production of IL-13 by Act T may result in osteopenia as a consequence of CsA treatment. Although CsA inhibits most cytokines expressed by Act T [Quesniaux, 1993], including TNF-α [Rifas and Avioli, 1999], another T cell cytokine that can induce VCAM-1 [Carter and Wicks, 2001], it does not suppress the expression of VCAM-1 in hOB. Thus, our accumulated data may help explain the recent studies by Epstein and co-workers who reported that the effect of CsA on bone turnover is mediated by T cells [Buchinsky et al., 1995, 1996]. Furthermore, the data presented herein support our hypothesis that as a consequence of T cell activation and production of IL-13 in the local bone marrow environment, VCAM-1 is upregulated in hOB. Although we have found that IL-13 induces low levels of IL-6 in hOB, as has been previously reported [Frost et al., 1998, 2001], other cytokines elaborated by T cells are more potent in inducing IL-6 [Rifas and Avioli, 1999]. Nonetheless, IL-6 induction by T cells may lead to the expansion of osteoclast precursors. Once osteoclast precursors are expanded, they may bind to osteoblasts via VCAM-1 allowing for close contact to RANKL. RANKL induction of the precursors would then lead to osteoclast maturation, activation, and subsequent bone resorption.

ACKNOWLEDGMENTS

The authors thank Mrs. Aurora Fausto for preparation of the human osteoblast cultures.

REFERENCES

Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. 1995. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. J Immunol 154:799–803.

Buchinsky FJ, Ma Y, Mann GN, Rucinski B, Bryer HP, Paynton BV, Jee WS, Hendy GN, Epstein S. 1995. Bone mineral metabolism in T lymphocyte-deficient and -replete strains of rat. J Bone Miner Res 10:1556–1565.

Buchinsky FJ, Ma Y, Mann GN, Rucinski B, Bryer HP, Romero DF, Jee WS, Epstein S. 1996. T lymphocytes play a critical role in the development of cyclosporin A-induced osteopenia. Endocrinology 137:2278–2285.

Carter RA, Wicks IP. 2001. Vascular cell adhesion molecule 1 (CD106): A multifaceted regulator of joint inflammation. Arthritis Rheum 44:985–994.

Cheng SL, Yang JW, Rifas L, Zhang SF, Avioli LV. 1994. Differentiation of human bone marrow osteogenic stromal cells in vitro: Induction of the osteoblast phenotype by dexamethasone. Endocrinology 134:277–286.

Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, Lobb RR. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell 60:577–584.

Feuerbach D, Feyen JH. 1997. Expression of the cell-adhesion molecule VCAM-1 by stromal cells is necessary for osteoclastogenesis. FEBS Lett 402:21–24.

Frost A, Jonsson KB, Brandstrom H, Ohlsson C, Ljunghall S, Ljunggren O. 1998. Interleukin-13 inhibits cell proliferation and stimulates interleukin-6 formation in isolated human osteoblasts. J Clin Endocrinol Metab 83:3285–3289.

Frost A, Jonsson KB, Brandstrom H, Ljunghall S, Nilsson O, Ljunggren O. 2001. Interleukin (IL)-13 and IL-4 inhibit proliferation and stimulate IL-6 formation in human osteoblasts: Evidence for involvement of receptor subunits IL-13R, IL-13Ralpha, and IL-4Ralpha. Bone 28:268–274.

Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. 1992. Increased osteoclast development after estrogen loss: Mediation by interleukin-6. Science 257:88–91.

Kurihara N, Bertolini D, Suda T, Akiyama Y, Roodman GD. 1990. IL-6 stimulates osteoclast-like multinucleated cell formation in long term human marrow cultures by inducing IL-1 release. J Immunol 144:4226–4230.

Lacey DL, Timms E, Tan H-L, Kelley MJ, Dunstan CR, Burgess T, Elliot R, Colombero A, Elliot G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian Y-X, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176.

Michigami T, Shimizu N, Williams PJ, Niewolna M, Dallas SL, Mundy GR, Yoneda T. 2000. Cell-cell contact between marrow stromal cells and myeloma cells via VCAM-1 and

alpha(4)beta(1)-integrin enhances production of osteoclast-stimulating activity. Blood 96:1953–1960.

Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, Lobb R. 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell 59: 1203–1211.

Quesniaux VF. 1993. Immunosuppressants: Tools to investigate the physiological role of cytokines. Bioessays 15:731–739.

Rifas L, Avioli LV. 1999. A novel T cell cytokine stimulates interleukin-6 in human osteoblastic cells. J Bone Miner Res 14:1096–1103.

Rifas L, Fausto A, Scott MJ, Avioli LV, Welgus HG. 1994. Expression of metalloproteinases and tissue inhibitors of metalloproteinases in human osteoblast-like cells: Differentiation is associated with repression of metalloproteinase biosynthesis. Endocrinology 134:213–221.

Rifas L, Kenney JS, Marcelli M, Pacifici R, Cheng SL, Dawson LL, Avioli LV. 1995. Production of interleukin-6 in human osteoblasts and human bone marrow stromal cells: Evidence that induction by interleukin-1 and tumor necrosis factor-alpha is not regulated by ovarian steroids. Endocrinology 136:4056–4067.

Rifas L, Arackal S, Weitzmann MN. 2003. Inflammatory T cells rapidly induce differentiation of human bone marrow stromal cells into mature osteoblasts. J Cell Biochem 88:650–659.

Roodman GD. 1992. Interleukin-6: An osteotropic factor? J Bone Miner Res 7:475–478.

Scopes J, Massey HM, Ebrahim H, Horton MA, Flanagan AM. 2001. Interleukin-4 and interleukin-13: Bidirectional effects on human osteoclast formation. Bone 29: 203–208.

Sironi M, Sciacca FL, Matteucci C, Conni M, Vecchi A, Bernasconi S, Minty A, Caput D, Ferrara P, Colotta F. 1994. Regulation of endothelial and mesothelial cell function by interleukin-13: Selective induction of vascular cell adhesion molecule-1 and amplification of interleukin-6 production. Blood 84:1913–1921.

Tanaka Y, Morimoto I, Nakano Y, Okada Y, Hirota S, Nomura S, Nakamura T, Eto S. 1995. Osteoblasts are regulated by the cellular adhesion through ICAM-1 and VCAM-1. J Bone Miner Res 10:1462–1469.

Thiebaud D, Krieg MA, Gillard-Berguer D, Jacquet AF, Goy JJ, Burckhardt P. 1996. Cyclosporine induces high bone turnover and may contribute to bone loss after heart transplantation. Eur J Clin Invest 26:549–555.

van der Pouw Kraan TC, Boeije LC, Troon JT, Rutschmann SK, Wijdenes J, Aarden LA. 1996. Human IL-13 production is negatively influenced by CD3 engagement. Enhancement of IL-13 production by cyclosporin A. J Immunol 156:1818–1823.

Weitzmann MN, Cenci S, Rifas L, Haug J, Dipersio J, Pacifici R. 2001. T cell activation induces human osteoclast formation via receptor activator of nuclear factor kappaB ligand-dependent and -independent mechanisms. J Bone Miner Res 16:328–337.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. 1998. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597–3602.

Yusuf-Makagiansar H, Anderson ME, Yakovleva TV, Murray JS, Siahaan TJ. 2002. Inhibition of LFA-1/ICAM-1 and VLA-4/VCAM-1 as a therapeutic approach to inflammation and autoimmune diseases. Med Res Rev 22:146–167.