Interleukin (IL)-6 Induction of Osteoclast Differentiation Depends on IL-6 Receptors Expressed on Osteoblastic Cells But Not on Osteoclast Progenitors

By Nobuyuki Udagawa,*‡ Naoyuki Takahashi,* Takenobu Katagiri,* Tatsuya Tamura,* Seiki Wada,‡ David M. Findlay,‡ T. John Martin,‡ Hisao Hirota,§‖ Tetsuya Taga,‖ Tadamitsu Kishimoto,§ and Tatsuo Suda*

*From the *Department of Biochemistry, School of Dentistry, Showa University, Tokyo 142, Japan; ‡St. Vincent's Institute of Medical Research, University of Melbourne, Fitzroy, Victoria 3065, Australia; §Department of Medicine III, Osaka University Medical School, Osaka 565, Japan; and ‖Institute for Molecular and Cellular Biology, Osaka University, Osaka 565, Japan*

Summary

We reported that interleukin (IL) 6 alone cannot induce osteoclast formation in cocultures of mouse bone marrow and osteoblastic cells, but soluble IL-6 receptor (IL-6R) strikingly triggered osteoclast formation induced by IL-6. In this study, we examined the mechanism of osteoclast formation by IL-6 and related cytokines through the interaction between osteoblastic cells and osteoclast progenitors. When dexamethasone was added to the cocultures, IL-6 could stimulate osteoclast formation without the help of soluble IL-6R. Osteoblastic cells expressed a very low level of IL-6R mRNA, whereas fresh mouse spleen and bone marrow cells, both of which are considered to be osteoclast progenitors, constitutively expressed relatively high levels of IL-6R mRNA. Treatment of osteoblastic cells with dexamethasone induced a marked increase in the expression of IL-6R mRNA. By immunoblotting with antiphosphotyrosine antibody, IL-6 did not tyrosine-phosphorylate a protein with a molecular mass of 130 kD in osteoblastic cells but did so in dexamethasone-pretreated osteoblastic cells. Osteoblastic cells from transgenic mice constitutively expressing human IL-6R could support osteoclast development in the presence of human IL-6 alone in cocultures with normal spleen cells. In contrast, osteoclast progenitors in spleen cells from transgenic mice overexpressing human IL-6R were not able to differentiate into osteoclasts in response to IL-6 in cocultures with normal osteoblastic cells. These results clearly indicate that the ability of IL-6 to induce osteoclast differentiation depends on signal transduction mediated by IL-6R expressed on osteoblastic cells but not on osteoclast progenitors.

Bone formation and resorption are coupled through the actions of several locally produced growth factors and cytokines. Osteoblastic cells have receptors for bone-resorbing hormones, including parathyroid hormone, PGs, and 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$][1]. However, there is no convincing evidence of direct responses to these bone-resorbing hormones in osteoclast lineage cells (1). Thus the concept that bone-resorbing agents must act first on osteoblastic stromal cells to induce the generation of new osteoclasts has emerged (2–4), although some controversial reports suggest that osteoblastic stromal cells are not required for osteoclast differentiation (5–7).

We developed a coculture system of mouse hematopoietic and primary osteoblastic stromal cells with which to investigate osteoclast development in vitro (8). In these cocultures, osteoclast-like multinucleated cells (OCLs) formed in response to several bone-resorbing factors such as 1α,25-$(OH)_2D_3$, parathyroid hormone, parathyroid hormone–related protein, PGE_2, IL-1, and IL-6 (9–11). OCLs formed in these cocultures satisfied the major criteria of authentic osteoclasts, including tartrate-resistant acid phosphatase (TRAP) activity, calcitonin receptor expression, and formation of resorption pits on dentine slices (8, 12, 13). When osteoclast progenitors and osteoblastic cells

A portion of this work was presented at the XII International Conference on Calcium Regulating Hormones, Melbourne, Australia, 14–19 February 1995, and has been published in abstract form (Udagawa, N., T. Katagiri, N. Takahashi, H. Murakami, I. Nakamura, D. Zhang, T. Tamura, T. J. Martin, and T. Suda. 1995. *Bone* 16:92S).

[1]*Abbreviations used in this paper:* 1α,25$(OH)_2$ D_3,1α,25-dihydroxyvitamin D_3; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-6R, IL-11R, IL-6 and IL-11 receptor, respectively; sIL-6R, soluble IL-6R; OCL, osteoclast-like multinucleated cells; TRAP, tartrate-resistant acid phosphatase.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/95/11/1461/08 $2.00
Volume 182 November 1995 1461–1468

were cocultured but separated by a membrane filter, no OCLs were formed, even in the presence of bone-resorbing agents. Osteoblastic stromal cells, therefore, play an important role in modulating the development of osteoclast progenitors by mechanisms requiring cell–cell interaction (14–16). At this time, it is not clear whether osteoblastic cells or osteoclast progenitors are the primary target cells for bone-resorbing agents in osteoclast differentiation. In this study, we identified which of the cocultured cells are the target cells for IL-6, which is now recognized as an important osteotropic factor.

IL-6 is a multifunctional cytokine that regulates pleiotropic functions in many types of cells (17). There is much evidence to suggest that IL-6 is an important osteotropic factor. IL-6 is produced by osteoblastic cells in response to bone-resorbing agents (18, 19) and stimulates osteoclastic bone resorption in organ cultures (20). Anti–IL-6 antibody inhibits the bone-resorbing activity of OCLs obtained from giant cell tumors of bone (21). IL-6 causes hypercalcemia in vivo (22, 23) and has been implicated in the bone loss caused by estrogen deficiency. Ovariectomy in the mouse causes a marked increase in ex vivo OCL formation in marrow cultures (24). Jilka et al. (25) have shown that estrogen replenishment inhibits IL-6 production by osteoblastic cells and that anti–IL-6 antibody prevented OCL development in the estrogen-deplete state. Since IL-6–deficient transgenic mice maintained their bone mass after ovariectomy (26), it is likely that IL-6 is involved in the differentiation of osteoclasts and osteoclastic bone resorption in estrogen deficiency. However, the mechanisms by which IL-6 influences osteoclast formation have not been established.

While investigating the action of IL-6 on OCL formation, we found that soluble IL-6 receptors (sIL-6R), which lack both transmembrane and cytoplasmic domains, can also bind IL-6 and mediate IL-6 signaling by interacting with the signal-transducing receptor component, gp130 (27). Whereas mouse IL-6 alone did not stimulate OCL formation in cocultures of mouse bone marrow cells and osteoblastic cells (11), the simultaneous addition of mouse sIL-6R strikingly triggered OCL formation in the cocultures. This suggests that cells in these cultures do not normally express IL-6 receptors but express gp130. IL-11, leukemia inhibitory factor, and oncostatin M, which share gp130 as the common signal transducer (28, 29), similarly stimulated OCL formation in the cocultures. The potency of these cytokines to induce OCLs may reflect the number of respective receptors in bone marrow and/or osteoblastic cells. It is important to emphasize that none of these gp130-stimulatory cytokines could induce OCLs when bone marrow and osteoblastic cells were cocultured without direct contact. Together, these results suggest that the gp130 signal is involved in osteoclast development, although it remains to be clarified which cells in the cocultures primarily require such a signal.

In this study, we first examine the role of IL-6 in OCL formation in the coculture system, and then demonstrate that dexamethasone stimulates expression of membrane-bound IL-6 receptors (IL-6R) on osteoblastic cells, leading to the differentiation of OCLs in response to mouse IL-6 in cocultures without a requirement for added mouse sIL-6R. We also show that osteoblastic cells obtained from transgenic mice constitutively expressing membrane-anchored human IL-6R can support osteoclast differentiation in response to human IL-6 in cocultures with normal spleen cells. This indicates that it is osteoblastic cells rather than osteoclast progenitors that require the gp130 signal during OCL formation in the coculture system.

Materials and Methods

Animals and Drugs. Newborn ddY and 6–9-wk-old male ddY mice were obtained from Shizuoka Laboratories Animal Center (Shizuoka, Japan). $1\alpha,25(OH)_2D_3$ was purchased from Wako Pure Chemical Co. (Osaka, Japan). Dexamethasone was purchased from Sigma Chemical Co. (St. Louis, MO). Mouse rIL-6 and mouse rsIL-6R were prepared as described (11, 30). Human rIL-6 and human rIL-11 were purchased from R&D Systems, Inc. (Minneapolis, MN). Other chemicals and reagents were of analytical grade.

Construction of the Human IL-6R Transgene and Preparation of Transgenic Mice. The IL-6R transgene was prepared by inserting a 1.7-kb fragment of the human IL-6R cDNA (31) spanning the entire coding region into a unique XhoI site of the pCAGGS expression vector carrying the constitutively active chicken β-actin gene promoter (32). This vector was then digested with SacI and HindIII to generate a linear fragment and microinjected into the pronuclei of fertilized BDF1 mouse eggs. Mice were obtained as described (33–35). Newborn transgenic mice were generated by mating animals from human IL-6R–transgenic lines and normal C57BL/6 mice, and their genotypes were determined by PCR analysis of liver DNA from each mouse as described (35).

Coculture System and Determination of Osteoclast Characteristics. Normal osteoblastic cells were prepared from the calvaria of 2-d-old mice by digestion with 0.1% collagenase (Wako Pure Chemical Co.) and 0.2% dispase (Godo Shusei, Tokyo, Japan). Normal bone marrow and spleen cells were obtained from 7-wk-old ddY mice as described (11, 36). To isolate osteoblastic cells from transgenic mice overexpressing human IL-6R, each calvarium from 2-d-old mice was cut into small pieces and embedded into type I collagen gels according to the manufacturer's instructions (Cellmatrix Type I-A; Nitta Gelatin Co., Osaka, Japan). After culture for 3 d, osteoblastic cells grown from the calvaria were collected by treating with PBS containing 0.1% collagenase (36). Spleen cells from transgenic mice were prepared from the splenic tissues of individual littermates (36). Osteoblastic cells were cocultured with bone marrow or spleen cells as described (11, 36). In short, primary osteoblastic cells (10^4 per well) and nucleated spleen cells (5×10^5 per well) or marrow cells (2×10^5 per well) were cocultured in the wells of a 48-well plate (Corning Glass Inc., Corning, NY) with 0.4 ml of α-MEM (GIBCO BRL, Gaithersburg, MD) containing 10% fetal bovine serum (Cytosystems, Castle Hill, NSW, Australia) in the presence of test chemicals. Cultures were incubated in quadruplicate, and cells were replenished on day 4 with fresh medium. OCL formation was evaluated after culturing for 7 d. Adherent cells were fixed and stained for TRAP, and the number of TRAP-positive OCLs was scored as described (11). The expression of calcitonin receptors was also assessed by autoradiography using ^{125}I–salmon calcitonin as described (11).

PCR Amplification of Reverse-transcribed mRNA. Mouse primary osteoblastic cells in flasks (175 cm^2; Nunc Inc., Naperville, IL) were cultured with or without 10^{-7} M dexamethasone for various periods after reaching confluence, and total cellular RNA was extracted using guanidine thiocyanate–phenol chloroform (37). RNA was also prepared from mouse spleen and bone marrow cells.

Single-stranded cDNA was synthesized from 2.5 μg of total RNA by incubating for 1 h at 42°C with avian myeloblastosis virus reverse transcriptase (Promega Corp., Madison, WI) and random hexanucleotides (Promega Corp.). From this reaction mixture, 2.5 μl of 25 μl was amplified by PCR to generate mouse IL-6R mRNA, IL-11R mRNA, and mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. The reaction mixture contained 50 pmol of each primer, 25 mM dATP, dGTP, dCTP, and dTTP (Pharmacia, Uppsala, Sweden), 2 μl of 10× reaction buffer, 1 U of Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN), and sterile distilled water, and was overlayed with 50 μl of paraffin oil. PCR (IL-GR) was performed for 23 cycles at 94°C for 1 min, at 65°C for 1 min, and at 72°C for 1 min in a DNA thermal cycler (model 480; Perkin Elmer Cetus Instruments, Norwalk, CT). Preliminary experiments were performed to ensure that the number of PCR cycles were within the exponential phase of the amplification curve. PCR products were resolved on a 2% wt/vol agarose gel, and the specificity of the reaction was confirmed by Southern transfer onto nylon membranes (Hybond-N; Amersham, Arlington Heights, IL) and hybridization with ^{32}P-labeled internal oligonucleotide probes.

Oligonucleotides were synthesized on a DNA synthesizer (model 381A; Applied Biosystems, Inc., Foster City, CA). The oligonucleotides for mouse IL-6R were 5′-CCTGTGTGG-GGTTCCAGAGGAT-3′ (3′ primer complementary to nucleotides 980–1001) and 5′-CTGCCAGTATTCTCAGCAGCTG-3′ (5′ primer complementary to nucleotides 488–519). The products were verified by hybridization with the internal oligonucleotide, 5′-CACAACGAAGCGTTTCACAGCTT-3′, by Southern hybridization. The oligonucleotides for mouse IL-11R (38) were 5′-GGAGGCCTCCAGAGGGT-3′ (3′ primer complementary to nucleotides 661–677) and 5′-GGGTCCTCCAGGGGTCCAG-TATGG-3′ (5′ primer complementary to nucleotides 133–156). The products were verified by Southern hybridization with the internal oligonucleotide, 5′-CTCCTGTACTTGGAGTCCAGG-3′. To ensure equal starting quantities of DNA for the experiments, and to allow semiquantitation of the PCR products representing IL-6R or IL-11R, reverse-transcribed RNA samples were also amplified using oligonucleotide primers specific for GAPDH (39). Oligonucleotides for GAPDH were 5′-AACGGATACAT-TGGGGGTAG-3′ (complementary to nucleotides 701–720) and 5′-CATGGAGAAGGCTGGGGCTC-3′ (complementary to nucleotides 306–325). The products were verified by hybridization with the internal oligonucleotide, 5′-GCTGTGGGCAAGGT-CATCCC-3′.

Immunoblotting. After incubating osteoblastic cells with or without dexamethasone (10^{-7} M) for 48 h in flasks (25 cm^2; Corning Glass Inc.), they were stimulated with or without IL-11 (500 ng/ml) or IL-6 (1 μg/ml) for 7 min. The cells were washed in situ with ice-cold PBS, lysed in 100 μl of lysis buffer (0.5% NP-40/10 mM Tris-HCl, pH 7.6/150 mM NaCl/5 mM EDTA/2 mM Na_3Vo_4/1 mM PMSF/5 μg of aprotinin/ml), then detergent-insoluble materials were pelleted by centrifugation (15,000 *g* for 20 min at 4°C) as described (40). The solubilized proteins were separated on a 7.5% SDS–polyacrylamide gel, then transferred to a membrane (Immobilon-P; Millipore Corp., Bedford, MA). The proteins were immunostained with antiphosphotyrosine mAb (PY20) (Seikagaku Kogyo, Tokyo, Japan) using an enhanced chemiluminescence Western blotting detection system (Amersham) according to the manufacturer's recommendations.

Results

Effects of Dexamethasone on OCL Formation Induced by IL-6. In the first series of experiments, we examined TRAP-positive OCL formation in cocultures of bone marrow and osteoblastic cells from ddY mice. IL-11 (10 ng/ml) was used as a positive control to stimulate OCL formation in the cocultures. In the absence of dexamethasone, mouse IL-6 (100 ng/ml) failed to induce OCL formation in the cocultures. The simultaneous addition of mouse sIL-6R (200 ng/ml) and mouse IL-6 (100 ng/ml) to the cocultures strikingly triggered OCL formation (Fig. 1 *A*). In marked contrast, when 10^{-7} M dexamethasone was added to the cocultures, mouse IL-6 (100 ng/ml) alone also stimulated OCL formation. In the presence of dexamethasone, mouse IL-6 (10–400 ng/ml) dose dependently stimulated OCL formation without the help of mouse sIL-6R (data not shown). Dexamethasone alone had no effect on OCL formation. An autoradiographic study using labeled calcitonin revealed that TRAP-positive multinucleated and mononuclear cells formed in these cocultures possessed calcitonin receptors (data not shown).

Expression of IL-6R and IL-11R mRNA on Osteoblastic Cells and Osteoclast Progenitors. We investigated the expression of IL-6R on osteoblastic, spleen, and bone marrow cells by

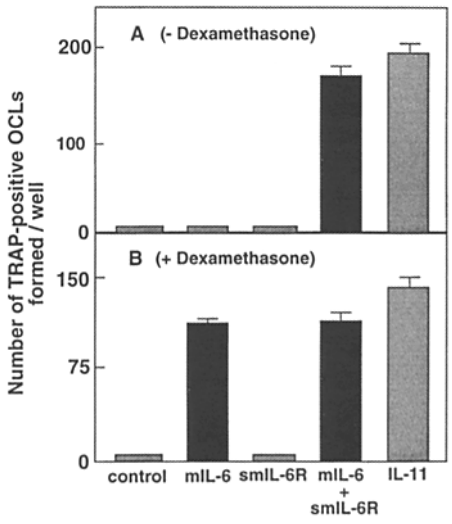

Figure 1. Effects of mouse IL-6 (*mIL-6*) and soluble mouse IL-6R (*smIL-6R*) on TRAP-positive OCL formation in cocultured mouse bone marrow and osteoblastic cells in the absence (*A*) or presence (*B*) of dexamethasone. Mouse bone marrow and primary osteoblastic cells were cocultured in the presence or absence of mIL-6 (100 ng/ml), smIL-6R (200 ng/ml), and 10^{-7} M dexamethasone, separately or in combination. IL-11 (10 ng/ml) was a positive control to stimulate TRAP-positive OCL formation. After culture for 7 d, TRAP-positive OCLs were counted. Data are expressed as the means ± SEM of quadruplicate cultures.

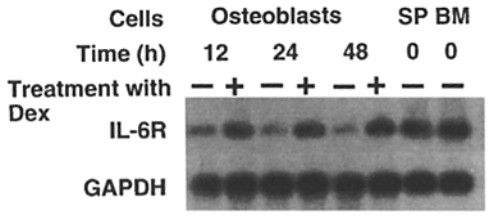

Figure 2. Expression of IL-6R mRNA on mouse osteoblastic cells and the osteoclast progenitors, mouse spleen (*SP*) and bone marrow (*BM*) cells. Osteoblastic cells were incubated with (+) or without (−) 10^{-7} M dexamethasone (*Dex*) for 12, 24, and 48 h. The spleen and bone marrow cells were isolated immediately before use. Total RNA was reverse transcribed and amplified by 23 cycles of PCR for mouse IL-6R mRNA and 20 cycles for mouse GAPDH mRNA using the specific primers described in Materials and Methods. PCR products were transferred to a nylon membrane and hybridized with ^{32}P-labeled internal sense oligonucleotides specific to the mouse IL-6R or the GAPDH sequences, respectively.

reverse transcription PCR with mouse IL-6R–specific oligonucleotides. Mouse primary osteoblastic stromal cells expressed only a low level of IL-6R mRNA. Treatment of osteoblastic cells with 10^{-7} M dexamethasone for 12–48 h markedly increased IL-6R mRNA expression (Fig. 2). Fresh mouse spleen and bone marrow cells, however, constitutively expressed relatively high levels of IL-6R mRNA (Fig. 2). In contrast, IL-11R mRNA was constitutively expressed on osteoblastic cells irrespective of the presence or absense of dexamethasone (Fig. 3). Fresh mouse spleen and bone marrow cells also expressed IL-11R mRNA (Fig. 3). The results suggest that OCL formation in the cocultures in response to gp130-stimulatory cytokines may be determined by the expression of a ligand-binding chain of the receptor complex in osteoblastic cells. A sandwich ELISA did not detect mouse sIL-6R (30) in the conditioned medium of cocultures of bone marrow and osteoblastic cells, either in the presence or absense of dexamethasone (detection limit, 1.0 ng/ml; data not shown).

Analysis of Protein Tyrosine Phosphorylation in Osteoblasts. The signals induced by cytokines that use gp130 as a signal transducer trigger the activation of some tyrosine kinases at an early step of the signal transduction pathway (29, 40). To evaluate whether the IL-6 and IL-11 signaling pathways

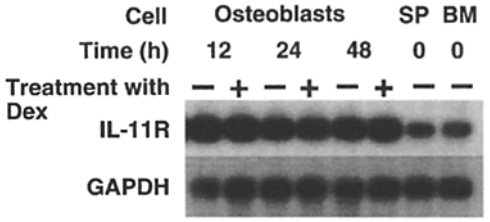

Figure 3. Expression of IL-11R mRNA on mouse osteoblastic cells and the osteoclast progenitors, mouse spleen (*SP*) and bone marrow (*BM*) cells. Osteoblastic cells were incubated with (+) or without (−) 10^{-7} M dexamethasone (*Dex*) for 12, 24, and 48 h. The spleen and bone marrow cells were isolated immediately before use. Total RNA was reverse transcribed and amplified by 30 cycles of PCR for mouse IL-11R mRNA and 20 cycles for mouse GAPDH mRNA using the specific primers described in Materials and Methods. PCR products were transferred to nylon membranes and hybridized with ^{32}P-labeled internal sense oligonucleotides specific to the mouse IL-11R or GAPDH sequences, respectively.

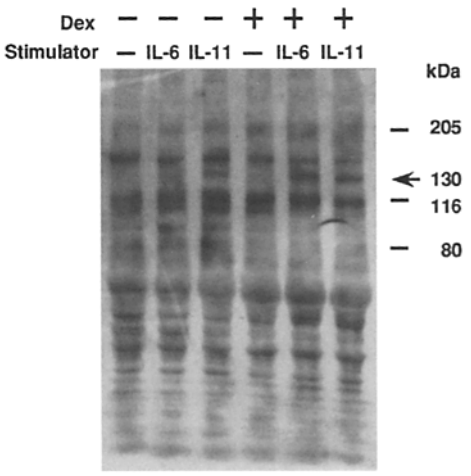

Figure 4. Protein tyrosine phosphorylation in osteoblastic cells. Osteoblastic cells were incubated with (+) or without (−) 10^{-7} M dexamethasone (*Dex*) for 48 h, then stimulated with or without mouse IL-6 (1 μg/ml) or IL-11 (500 ng/ml) for 7 min. Antiphosphotyrosine immunoblots of NP-40 detergent-soluble protein (30 μg/sample) were performed as described in Materials and Methods.

are initiated by the activation of tyrosine kinases in osteoblastic cells, we examined protein tyrosine phosphorylation in osteoblastic cells incubated with dexamethasone followed by IL-6 or IL-11. Fig. 4 shows Western blots against an antiphosphotyrosine antibody. Without dexamethasone pretreatment, IL-6 stimulation did not tyrosine-phosphorylate the 130-kD protein in osteoblastic cells, whereas IL-11 stimulation did. When osteoblastic cells were first incubated with 10^{-7} M dexamethasone for 48 h, both IL-6 and IL-11 tyrosine-phosphorylated the 130-kD protein (Fig. 4).

OCL Formation in Cocultures of Spleen and Osteoblastic Cells from Transgenic Mice Overexpressing Human IL-6R. To examine whether OCL formation in our coculture system is

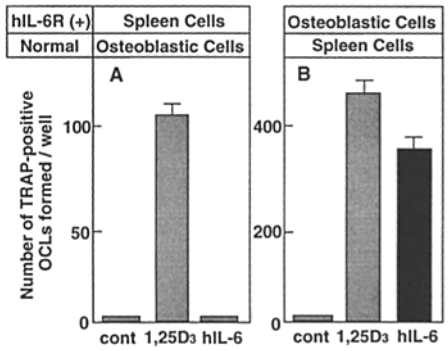

Figure 5. TRAP-positive OCL formation in cocultures of spleen or osteoblastic cells obtained from transgenic mice overexpressing human IL-6R [*hIL-6R(+)*] and their counterparts from normal ddY mice (*Normal*). Cells were cocultured in the presence or absence of human IL-6 (50 ng/ml). $1\alpha,25(OH)_2D_3$ (*1,25D3*) (10^{-8} M) was used as a positive control to stimulate TRAP-positive OCL formation. (*A*) Cocultured spleen cells from transgenic mice overexpressing human IL-6R and normal ddY osteoblastic cells. (*B*) Cocultured normal ddY spleen and osteoblastic cells from transgenic mice overexpressing human IL-6R. After culture for 7 d, TRAP-positive OCLs were counted. Data are expressed as the means ± SEM of quadruplicate cultures.

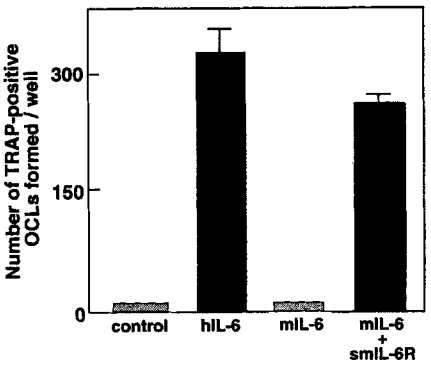

Figure 6. TRAP-positive OCL formation in cocultures of normal ddY mouse–derived spleen cells and osteoblastic cells obtained from transgenic mice overexpressing human IL-6R. Cocultures were incubated in the presence or absence of mouse IL-6 (*mIL-6*) (100 ng/ml), soluble mouse IL-6R (*smIL-6R*) (200 ng/ml), and human IL-6 (*hIL-6*) (50 ng/ml), separately or in combination. After 7 d, TRAP-positive OCLs were counted. Data are expressed as the means ± SEM of quadruplicate cultures.

mediated by the gp130 signal, which is initiated in osteoblastic cells, cocultures were made using different combinations of osteoblastic cells and osteoclast progenitors either from transgenic mice overexpressing human IL-6R or from normal mice and were stimulated with human IL-6. In cocultured spleen cells containing osteoclast progenitors obtained from transgenic mice overexpressing human IL-6R and osteoblastic cells from normal ddY mice, $1\alpha,25(OH)_2D_3$ (10^{-8} M) stimulated OCL formation, whereas human IL-6 (50 ng/ml) did not (Fig. 5 *A*). In contrast, osteoblastic cells obtained from transgenic mice overexpressing human IL-6R supported OCL formation in response to human IL-6 (50 ng/ml) when cocultured with normal spleen cells (Fig. 5 *B*). Osteoblastic cells obtained from normal littermates did not exhibit such a capability (data not shown). Mouse IL-6 (100 ng/ml) did not stimulate OCL formation, even in the cocultures of spleen cells from normal ddY mice and osteoblastic cells from transgenic mice overexpressing human IL-6R (Fig. 6). This is because mouse IL-6 is specific to mouse IL-6R, whereas human IL-6 binds to both mouse IL-6R and human IL-6R (30). Incubating these cocultures with mouse IL-6 (100 ng/ml) together with mouse sIL-6R (200 ng/ml) resulted in OCL formation (Fig. 6).

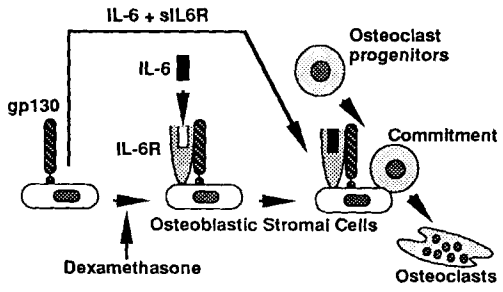

Figure 7. The mechanism of IL-6–induced osteoclast formation and the role of dexamethasone.

Discussion

We reported that mouse IL-6 alone does not stimulate OCL formation in cocultures of mouse bone marrow and osteoblastic cells (11). Mouse sIL-6R strikingly triggered OCL formation in response to mouse IL-6 in the cocultures. IL-11, leukemia inhibitory factor, and oncostatin M, all of which use gp130 as a signal transducer, similarly stimulated OCL formation in the cocultures without the help of their respective soluble receptors (11). Our findings indicate that the stimulation of gp130 signaling in the cells in cocultures (osteoclast progenitors and/or osteoblastic stromal cells) plays an important role in OCL formation and that expression of cytokine-specific receptor chains determines responsiveness to gp130-stimulatory cytokines, for example, IL-11 and IL-6.

Both IL-11 and IL-6 stimulate mouse myeloid and lymphoid cells (41). Not only IL-6, but also IL-11 induced acute phase proteins in hepatoma cells and isolated hepatocytes (17, 42). A series of studies have shown that the biological actions of IL-11 in hematopoietic cells overlap those of other gp130-mediated cytokines. IL-11 inhibits lipoprotein lipase activity and preadipocyte differentiation in mouse stromal cells (43–45). In contrast, the activity of IL-6 to inhibit adipogenesis is very low compared with that of IL-11, leukemia inhibitory factor, and oncostatin M in mouse preadipocyte cell lines (44, 46).

IL-6 as such has no direct effect on mouse osteoblastic stromal cells (47, 48). However, simultaneous addition of sIL-6R and IL-6 to osteoblastic cell cultures caused a marked change in cell growth and alkaline phosphatase activity (48). These findings suggest that mouse osteoblastic stromal cells do not express functional IL-6R. Consistent with these results, our study clearly shows that the expression of IL-6R mRNA on mouse primary osteoblastic cells was not sufficient to mediate an IL-6 effect. Dexamethasone strikingly upregulated IL-6R mRNA expression and tyrosine phosphorylation in response to IL-6 in osteoblastic cells. In contrast, IL-11R mRNA was constitutively expressed in mouse primary osteoblastic cells, which was consistent with the finding that a transcript homologous with IL-11R mRNA is expressed in skeletogenic cells (chondroblastic and osteoblastic progenitor cells) (49). IL-11 induced OCL differentiation in cocultures of bone marrow and osteoblastic cells, whereas IL-6 failed to do so. However, in the presence of dexamethasone, IL-6 stimulated OCL formation without the help of mouse sIL-6R. These results indicate that dexamethasone induces functional IL-6R expression in osteoblastic stromal cells, which confers upon them the ability to trigger the differentiation of postmitotic osteoclast precursors into osteoclasts (Fig. 7).

We established transgenic mice overexpressing human IL-6 (34). Although there was massive plasmacytosis in these mice overexpressing human IL-6, no abnormality was apparent in bone. Likewise, normal bone and bone marrow were formed in mice overexpressing human IL-6R (Hirota, H., unpublished observation). In cocultures of spleen cells from transgenic mice overexpressing human IL-6R and normal osteoblastic cells, osteoclasts were not formed in response

to human IL-6. In contrast, osteoblastic cells obtained from transgenic mice overexpressing human IL-6R supported osteoclast differentiation in response to human IL-6 in cocultures with normal spleen cells. These results strongly indicate that IL-6–induced osteoclast differentiation depends on signal transduction mediated by IL-6R expressed on osteoblastic cells but not on osteoclast progenitors (Fig. 7).

Cytokines that share gp130 as the common signal transducer trigger activation of unknown tyrosine kinases at an early step in their signal transduction pathways (28, 29). The JAK family of protein kinases (JAK1, JAK2, and TYK2) and the latent cytoplasmic transcription factor APRF/STAT3 participate in IL-6 signaling (40, 50, 51). Yin et al. (52) have identified a 130-kD tyrosine-phosphorylated protein induced by IL-11 as JAK2 tyrosine kinase in a preadipocyte cell line. Levy et al. (53) have reported that the JAK family of tyrosine kinases (JAK1, JAK2, and TYK2) is tyrosine-phosphorylated by oncostatin M in mouse primary osteoblastic cells. Lowe et al. (54) have also reported that IL-11 causes a modest elevation in JAK2 phosphorylation in mouse primary osteoblastic cells. Our results show that, like IL-11, IL-6 tyrosine-phosphorylates a protein with a molecular mass of 130 kD in osteoblastic cells exposed to dexamethasone, although further studies are necessary to determine whether this protein is a JAK-related kinase.

Glucocorticoids regulate some hormone and cytokine actions in a variety of cells and tissues. Insulin-like growth factor I receptors in rat bone cells (55), β_2-adrenergic receptors in smooth muscle cells (56), and calcitonin receptors in osteoclasts (57) are upregulated by glucocorticoids. Snyers et al. (58) have reported that dexamethasone upregulated the number of IL-6R on human epithelial cells and hepatoma cells. Like the occurrence of osteoporotic disorders in Cushing's syndrome, prolonged treatment with pharmacological doses of glucocorticoids causes secondary osteoporosis (59). This clinical phenomenon is well characterized by increased osteoclastic bone resorption and suppression of osteoblastic bone formation. Although there are several reports discussing the effect of glucocorticoids on osteoclastic bone resorption (60–62), the mechanism of the glucocorticoid action in stimulating osteoclastogenesis and bone resorption has not been elucidated. This study indicates that dexamethasone acts on osteoblastic cells to induce osteoclast differentiation in response to IL-6 (Fig. 7). It is therefore possible that the early increase in bone resorption during glucocorticoid therapy is mediated at least in part by the increase in IL-6 sensitivity of osteoblastic cells.

Serum levels of sIL-6R progressively increase after ovariectomy in women, and estrogen prevents this increase (63). Such a pathological role of IL-6 has also been recognized in patients with rheumatoid arthritis. Morever, we reported that synovial fluids from patients with rheumatoid arthritis contained both IL-6 and sIL-6R at sufficiently elevated levels to induce OCL formation in mouse cocultures (64). These findings suggest that the elevated levels of both IL-6 and sIL-6R form a complex that causes bone destruction in these patients.

In conclusion, IL-6–induced osteoclast differentiation depends on signal transduction mediated by IL-6R that is expressed on osteoblastic cells but not on osteoclast progenitors. This study will further our understanding of signal transduction during osteoclast development induced by gp130-mediated cytokines.

We thank Dr. H. Murakami (Pharmaceutical Research Center, Meiji Seika Kaisha, Kanagawa, Japan) and Dr. I. Nakamura (Department of Orthopedic Surgery, School of Medicine, University of Tokyo, Tokyo, Japan) for valuable discussions.

This work was supported by grants-in-aid 06404067 and 06771640 from the Ministry of Science, Education, and Culture of Japan, and a Program Grant from the National Health and Medical Research Council of Australia. N. Udagawa is C. R. Roper Research Fellow, University of Melbourne.

Address correspondence to Dr. Tatsuo Suda, Department of Biochemistry, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan.

Received for publication 14 April 1995 and in revised form 5 July 1995.

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