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Expression Levels of gp130 in Bone Marrow Stromal Cells Determine the Magnitude of Osteoclastogenic Signals Generated by IL-6-Type Cytokines

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Abstract Interleukin-6 (IL-6)-type cytokines stimulate osteoclast formation by activating the glycoprotein 130 (gp130) receptor subunit on stromal/osteoblastic cells, which in turn leads to signal transducer and activator of transcription 3 (STAT3)-mediated expression of receptor activator of NF-κB ligand (RANKL). Based on evidence that gp130 expression is regulated by a variety of cytokines and hormones, we have determined here whether changes in gp130 levels directly contribute to the magnitude of the osteoclastogenic stimulus delivered by IL-6-type cytokines. To accomplish this, gp130 protein levels were modulated using a tetracycline-regulated expression system in a stromal/osteoblastic cell line, UAMS-32, which supports osteoclast formation. Removal of doxycycline from the culture medium elevated gp130 expression and increased the responsiveness of a STAT-responsive promoter-luciferase construct to IL-6 complexed with its soluble receptor (IL-6+sIL-6R), but diminished the responsiveness to oncostatin M (OSM). IL-6+sIL-6R-stimulated osteoclast formation was greater when osteoclast precursors were cocultured with the cells expressing elevated gp130 levels than when cells expressing low gp130 levels were used. However, increased gp130 levels reduced OSM-stimulated osteoclast formation. These results establish that the level of gp130 in stromal/osteoblastic cells directly modulates the magnitude of the osteoclastogenic response to IL-6-type cytokines such that an increase in gp130 increases the cellular responsiveness to IL-6+sIL-6R but reduces responsiveness to OSM. J. Cell. Biochem. 79:532–541, 2000.

Key words: oncostatin M; osteoblast; RANKL; STAT3; receptor

Glycoprotein 130 (gp130) is the common signal-transducing component of the receptors for the cytokine family that includes interleukin-6 (IL-6), IL-11, oncostatin M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor, and cardiotropin 1 (CT-1) [Kishimoto et al., 1995]. Binding of these cytokines to their specific cell surface receptors results in tyrosine phosphorylation of gp130 by members of the JAK family of tyrosine kinases [Stahl and Yancopoulos, 1993] and subsequent tyrosine phosphorylation of several downstream signaling molecules, including members of the signal transducers and activators of transcription (STAT) family of transcription factors [Boulton et al., 1994; Lutticken et al., 1994]. Phosphorylated STATs then undergo homo- and heterodimerization, translocate to the nucleus, and activate cytokine-responsive gene transcription [Darnell Jr. et al., 1994].

The specific receptor for IL-6, known as gp80, also exists in a soluble form (sIL-6R), but unlike most soluble cytokine receptors, it functions as an agonist by binding to IL-6 and then interacting with membrane-associated gp130 to stimulate JAK/STAT signaling [Kishimoto et al., 1994]. Ligand binding to either membrane-bound or soluble IL-6 receptor activates the JAK/STAT pathway by promoting homodimerization of gp130 molecules [Kishimoto et al., 1994]. In contrast, OSM activates signaling by first binding directly to a single

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gp130 molecule with low affinity [Mosley et al., 1996]. This association then promotes heterodimerization of the OSM–gp130 complex with a gp130-like protein known as OSM receptor β (OSMRβ), resulting in a high-affinity OSM–receptor complex that activates the JAK/STAT pathway [Mosley et al., 1996; Lindberg et al., 1998]. Although activation of gp130 homodimers by IL-6, or gp130-OSMRβ heterodimers by OSM, often has similar effects on a given cell type, notable qualitative differences have been observed, suggesting that the two different receptor complexes are not equivalent in all respects [Tanigawa et al., 1995; Kuropatwinski et al., 1997].

IL-6-type cytokines promote osteoclast formation both in vitro and in vivo [Black et al., 1991; Jilka et al., 1992; Girasole et al., 1994; Suda et al., 1996]. Recent studies have shown that these cytokines stimulate osteoclast formation by binding to their receptors on stromal/osteoblastic cells and inducing the expression of receptor activator of NF-κB ligand (RANKL) on these cells [Yasuda et al., 1998; O'Brien et al., 1999]. In addition, IL-6 may increase osteoclast numbers by stimulating proliferation of the hematopoietic precursor of osteoclasts: colony-forming unit granulocyte-macrophage [Suzuki et al., 1989; Kurihara et al., 1990]. Stromal/osteoblastic cell expression of RANKL, together with macrophage colony-stimulating factor, is necessary and sufficient to induce osteoclast differentiation from these hematopoietic precursors [Yoshida et al., 1990; Lacey et al., 1998; Yasuda et al., 1998; Kong et al., 1999]. In vitro osteoclast formation assays suggest that stromal/osteoblastic cells do not normally express membrane-bound IL-6 receptor [Udagawa et al., 1995; Romas et al., 1996]. However, either loss of sex steroids or glucocorticoid treatment may stimulate the expression of IL-6 receptor in these cells [Girasole et al., 1994; Udagawa et al., 1995; Lin et al., 1997]. Nevertheless, stromal/osteoblastic cells robustly support osteoclast formation in response to either IL-6+sIL-6R or OSM, indicating that they possess both gp130 and OSMRβ [Tamura et al., 1993; Romas et al., 1996].

We have shown previously that the levels of gp130 expression in stromal/osteoblastic cells can be modulated by systemic hormones that influence the formation of osteoblasts and osteoclasts [Lin et al., 1997]. Specifically, we showed that whereas estrogen and dihydrotestosterone suppressed gp130 mRNA and protein levels in stromal cell lines and ex vivo bone marrow cultures, 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) and parathyroid hormone (PTH) increased gp130 mRNA [Lin et al., 1997]. Additional studies have demonstrated that gp130 expression is regulated by IL-6-type cytokines, IL-1, tumor necrosis factor-α, retinoic acid, and dexamethasone [Schooltink et al., 1992; Snyers and Content, 1992; Ogata et al., 1994; Romas et al., 1996].

Although these findings clearly demonstrated that gp130 production is regulated by a variety of cytokines and hormones, it is unknown whether changes in gp130 levels lead to corresponding changes in the magnitude of cell responsiveness. In addition, because of the different ways in which ligands such as IL-6 and OSM interact with gp130, it is unclear whether changes in gp130 levels would lead to equivalent changes in the response to these two ligands. Therefore, the significance of gp130 regulation remained speculative. In the studies presented herein, we sought to modulate the levels of gp130 in a stromal/osteoblastic cell line and determine the effects of this change on the magnitude of intracellular signaling and downstream events, such as osteoclast formation, in response to cytokines that activate either gp130 homodimers (IL-6 or IL-6+sIL-6R) or gp130-OSMRβ heterodimers (OSM). We demonstrate that an increase in the level of gp130 has a differential effect on the sensitivity of cells to these ligands. Elevation of gp130 levels increased the sensitivity to IL-6+sIL-6R but had the opposite effect on the sensitivity to OSM.

MATERIAL AND METHODS

Primary Cell Culture and Cell Lines

Primary cultures of murine bone marrow cells were established with marrow aspirates from femur and tibia of 60-day-old Swiss Webster mice as previously described [Jilka et al., 1992]. All procedures were performed in accordance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals. Cells ($0.5 \times 10^6/cm^2$) were cultured in α-minimal essential medium containing 10% fetal bovine serum (FBS) for 5 days before treatment with 10^{-8} M $1,25(OH)_2D_3$. The stromal/osteoblastic cell line UAMS-32, its derivatives, and NIH-3T3 cells were cultured in

phenol red-free α-MEM containing 10% FBS [O'Brien et al., 1999].

Conditional Expression of gp130

Generation of UAMS-32 cells harboring the tTA (TET-OFF) protein was described previously [O'Brien et al., 1999]. To conditionally express gp130 in these cells, a retroviral construct was generated by inserting the murine gp130 cDNA (a gift from T. Kishimoto) with three sequential myc epitopes attached to the carboxyl terminus, into the Hpa I site of the retroviral vector pST [O'Brien et al., 1999]. In this construct, designated pST-gp130, gp130 expression is controlled by the tetracycline-repressible promoter [Gossen and Bujard, 1992]. Retroviral supernatant was prepared and used to infect the UAMS-32-tTA cell line as previously described [O'Brien et al., 1999]. Clonal cell lines were isolated from the pool of transduced cells, and a clone exhibiting elevated expression of tagged-gp130 in the absence of doxycycline was selected for further use and designated UAMS-32-G. Doxycycline was used to repress transgene expression in these cells because it is approximately 100-fold more effective than tetracycline [Gossen et al., 1995]. Conditional expression of gp130 in NIH-3T3 cells was accomplished by first infecting the cells with a retrovirus expressing the tTA protein, as described [O'Brien et al., 1999]. This pool of cells was subsequently infected with the pST-gp130 retrovirus described above, such that culture of these cells in the absence of doxycycline activated expression of the myc-tagged gp130.

Immunoblotting

Immunoblots of extracts from wild-type or retrovirally transduced UAMS-32 cells or primary bone marrow cultures were performed as previously described [Lin et al., 1997]. The following dilutions of antibodies were used: anti-myc (Santa Cruz Biotechnology, Santa Cruz, CA), 1:6700 ; anti-gp130 (Upstate Biotechnology, Lake Placid, NY), 1:4,000; and anti-β-actin (Santa Cruz), 1:12,500. The intensity of the immunoreactive bands (scanned into a digital format) was quantified using image analysis software (Molecular Dynamics, Sunnyvale, CA).

Transient Transfections

Transient transfection of retrovirally transduced UAMS-32 or NIH-3T3 cells, plated at 2×10^4 cells/well in 24-well plates, was performed using LipofectAMINE (Gibco, Grand Island, NY) as previously described [O'Brien and Manolagas, 1997]. The promoter-reporter construct used in this assay, p4xAPRE (a gift from I. Matsumura), contained a firefly luciferase gene controlled by a composite promoter consisting of four STAT-binding elements from the α2-macroglobulin promoter inserted upstream from a minimal Jun B promoter [Nakajima et al., 1996]. Luciferase values were normalized to beta-galactosidase activity resulting from co-transfection of the plasmid pSVβ-gal (Promega, Madison, WI).

Osteoclast Formation Assay

Nonadherent bone marrow cells were prepared by removing femurs from 30- to 90-day-old C57BL/6J mice and flushing the marrow cavity with α-MEM (Gibco) containing 15% FBS (Hyclone Laboratories, Logan, UT). Marrow cells were seeded at a density of 2.5×10^5 cells/cm^2 in the same medium and cultured for 48 h, after which nonadherent cells were collected. UAMS-32-G cells were seeded at 2.5×10^3 cells/cm^2 in α-MEM containing 10% FBS and cultured in the presence or absence of 100 ng/ml doxycycline for 2 days before addition of nonadherent bone marrow cells at a density of 2×10^4 cells/cm^2. Osteoclast-inducing cytokines were added at the indicated concentrations and the cocultures were maintained in 100 ng/ml doxycycline at 37°C in 5% CO_2 for 6 days. Doxycycline at this concentration had no effect on osteoclast formation in cocultures using wild-type UAMS-32 cells in response to either IL-6+sIL-6R or OSM (data not shown). On day 3, one half of the medium was replaced with fresh medium and cytokines. After 6 days, cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) [Girasole et al., 1992].

RESULTS

Conditional Expression of gp130 in UAMS-32 Cells

To demonstrate the extent of regulation of gp130 expression in bone marrow cells, we treated primary murine bone marrow cultures with vehicle or $1,25(OH)_2D_3$ for 24 h and measured changes in gp130 protein levels. $1,25(OH)_2D_3$ stimulated gp130 levels by 10-fold relative to vehicle-treated cells (Fig. 1A). To

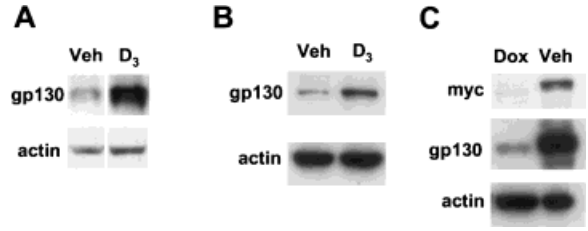

Fig. 1. Modulation of gp130 expression in primary bone marrow and UAMS-32 cells. **A:** Lysates from primary murine bone marrow cells, treated with vehicle or $1,25(OH)_2D_3$ (10^{-8} M) for 24 h, were analyzed by immunoblot with anti-gp130 or anti-actin antibodies. **B:** UAMS-32 cells were treated with vehicle or $1,25(OH)_2D_3$ (10^{-8} M) for 24 h and cell lysates were prepared and analyzed by immunoblot with consecutive incubation with anti-gp130 and anti-actin antibodies. **C:** UAMS-32-G cells were treated with vehicle or 100 ng/ml doxycycline for 48 h and lysates were analyzed by immunoblot with anti-myc, anti-gp130, and anti-actin antibodies.

address the significance of altered gp130 levels on cell responsiveness, we sought to specifically modulate gp130 expression in a cell line that supports osteoclast formation in response to IL-6-type cytokines. For these studies, we used a murine bone marrow stromal/osteoblastic cell line, designated UAMS-32, that supports osteoclast formation from hematopoietic precursors in response to IL-6+sIL-6R or OSM [O'Brien et al., 1999]. These cells do not respond to IL-6, unless sIL-6R is provided, nor do they respond to LIF or IL-11, because of lack of specific receptors for these cytokines (data not shown). The multinuclear cells formed in cocultures with UAMS-32 cells possess the phenotypic characteristics of authentic osteoclasts, including the expression of calcitonin receptors and TRAP, as well as the ability to form pits on devitalized bone slices [O'Brien et al., 1999]. UAMS-32 cells express gp130 and, as with primary bone marrow cells and other cell lines, this expression is stimulated by $1,25(OH)_2D_3$ (Fig. 1B). However, because $1,25(OH)_2D_3$ and other gp130 modulators also regulate the expression of many other genes, we used the tetracycline-regulated expression system [Gossen and Bujard, 1992] to specifically and conditionally increase the expression of gp130 in UAMS-32 cells.

Conditional expression of gp130 in UAMS-32 cells was accomplished by first transducing the cells with a retrovirus expressing the tetracycline-regulated transcription factor tTA. This pool of cells was then transduced with a second virus containing a myc-tagged murine gp130 cDNA inserted downstream from a promoter responsive to tTA. From this pool of cells, a clonal line, designated UAMS-32-G, was isolated that expressed the myc-tagged gp130 after removal of doxycycline from the culture medium (Fig. 1C). Quantitation of the gp130 band intensity, normalized to actin, indicated that the level of gp130 was approximately eightfold higher in the absence of doxycycline.

Increased gp130 Levels Alter the Magnitude of Intracellular Signaling

To determine the effect of increased gp130 levels on the magnitude of intracellular signaling, UAMS-32-G cells were transiently transfected with a promoter-luciferase construct that is responsive to the activated STAT3 transcription factor (Fig. 2A). The cells were then treated with increasing concentrations of IL-6+sIL-6R in the presence or absence of doxycycline. In cells with low gp130 (doxycycline present), IL-6+sIL-6R dose-dependently stimulated STAT3-dependent promoter activity (Fig. 2B). However, in cells with higher gp130 (doxycycline absent), the effect of IL-6+sIL-6R was 1.3- to 2.0-fold greater at each concentration at which there was a statistically significant difference in luciferase activity (Fig. 2B). Surprisingly, and in contrast to the results with IL-6+sIL-6R, OSM-stimulated promoter activity was decreased 2.1- to 2.7-fold in the high-gp130 condition, compared to the low-gp130 condition (Fig. 2C). These results demonstrate that elevated gp130 levels increase signaling intensity in response to IL-6+sIL-6R but reduce it in response to OSM. The finding that signaling responsiveness to IL-6+sIL-6R did not increase by the same magnitude as gp130 levels (eightfold) may be because of limiting amounts of a factor or factors downstream of gp130 in the signaling pathway, such as STAT3.

Increased gp130 Levels Alter the Magnitude of Cytokine-Stimulated Osteoclast Formation

To determine whether the changes in gp130 levels were biologically significant, we compared the ability of UAMS-32-G cells, expressing low or high gp130, to support osteoclast formation when cocultured with nonadherent bone marrow cells and increasing concentrations of IL-6+sIL-6R or OSM. As expected, cells expressing higher levels of gp130 were more sensitive to the osteoclastogenic actions of IL-6+sIL-6R, in that they were able to sup-

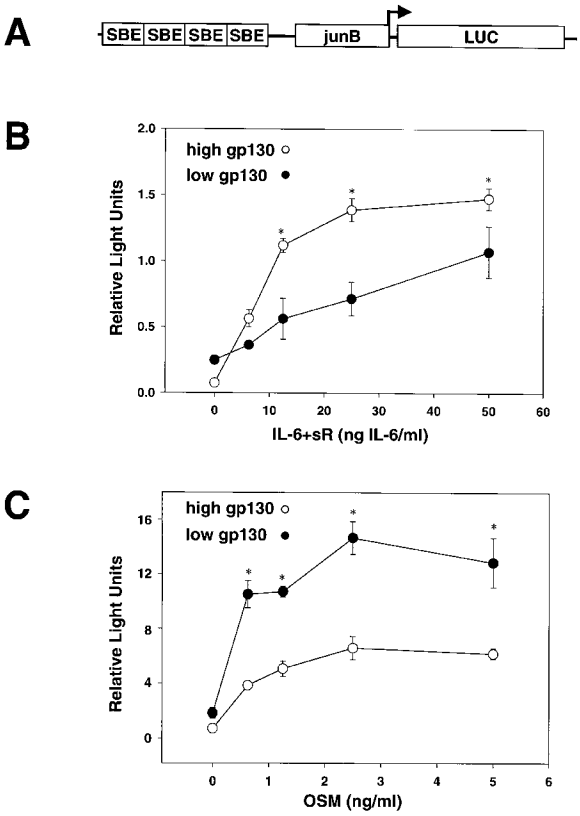

Fig. 2. Elevated gp130 increases transcriptional responsiveness of cells to IL-6+sIL-6R but decreases responsiveness to oncostatin M (OSM). UAMS-32-G cells were transiently transfected with a promoter-luciferase construct containing several signal transducers and activators of transcription (STAT)-binding elements (SBE) inserted upstream from the junB promoter **(A)**. After transfection, the cells were maintained in the presence (black circles) or absence (white circles) of doxycycline and were treated with increasing concentrations of interleukin-6 (IL-6) complexed with its soluble receptor (IL-6+sR) **(B)** or OSM **(C)** for 5 h and luciferase activity was determined. In (B), only the value for IL-6 is shown, but for each dose, the concentration of sIL-6R was twice that of IL-6. The values represent the mean normalized luciferase activity of three independent transfections ± SD. $*P < 0.003$ vs. same concentration of IL-6+sIL-6R or OSM by one-way analysis of variance.

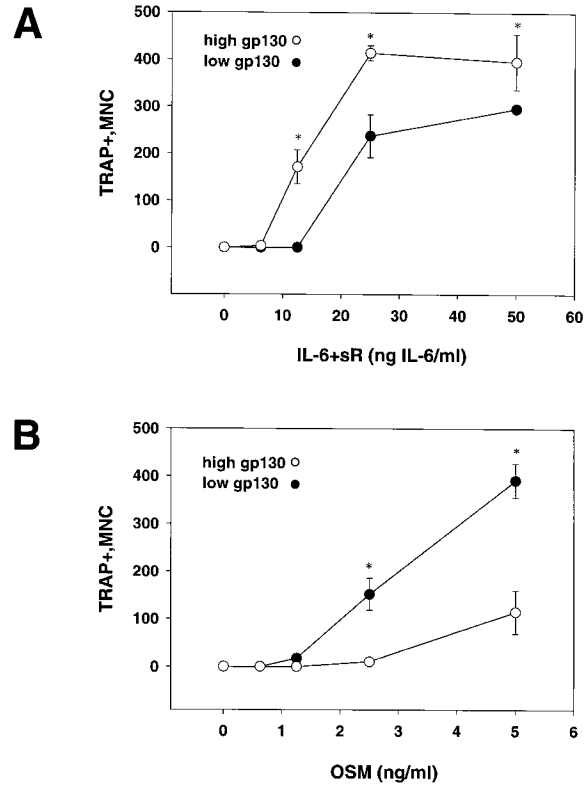

Fig. 3. Stromal/osteoblastic cells with elevated gp130 levels exhibit a greater osteoclastogenic response to interleukin-6 (IL-6) complexed with its soluble receptor (IL-6+sR) but a lower response to oncostatin M (OSM). UAMS-32-G cells were cocultured with nonadherent bone marrow cells and the indicated amount of IL-6+sIL-6R **(A)** or OSM **(B)**, in the presence or absence of doxycycline for 6 days. In A, only the value for IL-6 is shown, but for each dose, the concentration of sIL-6R was twice that of IL-6. After the culture period, multinucleated cells that stained positive for tartrate-resistant acid phosphatase (TRAP+,MNC) were enumerated (black circles, low gp130; white circles, high gp130). The values shown are the mean of triplicate cultures ± SD. $*P < 0.006$ vs. same concentration of IL-6+sIL-6R or OSM using one-way analysis of variance.

port osteoclast formation at a lower concentration of IL-6+sIL-6R and stimulated the formation of 1.8- and 1.3-fold more osteoclasts at the two highest doses of IL-6+sIL-6R, respectively (Fig. 3A). In contrast, cells with elevated gp130 were less sensitive to the osteoclastogenic actions of OSM (Fig. 3B). Specifically, higher concentrations of OSM were required to stimulate osteoclastogenesis using cells with elevated gp130, and at the highest concentration of OSM, osteoclast numbers were approximately 3.4-fold lower relative to cells expressing low levels of gp130.

Elevated gp130 Levels Alter the Responsiveness of NIH-3T3 Cells to IL-6+sIL-6R and OSM, but Not to IL-6 in the Absence of Its Soluble Receptor

To determine whether the differential effect of elevated gp130 levels on signaling initiated by IL-6+sIL-6R and OSM was cell specific, we generated NIH-3T3 cells conditionally expressing gp130. These cells demonstrated levels of gp130 elevation similar to that shown for the UAMS-32-G cells (data not shown). Analysis of

STAT3 activation by increasing concentrations of IL-6+sIL-6R or OSM yielded results that were very similar to those obtained with the UAMS-32-G cells (Fig. 4A,B; compare with Fig. 2). Because NIH-3T3 cells possess the specific α-receptor for IL-6 (gp80), whereas UAMS-32 cells do not, we determined whether altered gp130 levels had any effect on the sensitivity of the cells to this cytokine in the absence of its soluble receptor. In contrast to the results with the IL-6+sIL-6R complex or OSM, elevated gp130 levels did not have a significant effect on the magnitude of STAT3 activation in response to increasing concentrations of IL-6 alone (Fig. 4C).

DISCUSSION

The expression of gp130 is regulated by a variety of cytokines and hormones [Schooltink et al., 1992; Snyers and Content, 1992; Ogata et al., 1994; Romas et al., 1996; Lin et al., 1997]. Based on this, it has been previously hypothesized that changes in gp130 levels lead to changes in cell responsiveness to IL-6-type cytokines [Lin et al., 1997]. To directly address this issue, we generated cell lines that conditionally expressed either relatively low or high levels of gp130, depending on the presence of the antibiotic doxycycline. Stromal/osteoblastic UAMS-32 cells with elevated gp130 levels were more sensitive, i.e., exhibited a response of greater magnitude, to a given concentration of IL-6+sIL-6R, as measured by STAT3 transcriptional activity and support of osteoclast formation. In sharp contrast, UAMS-32 cells with elevated gp130 were less sensitive to OSM, as assessed by the same criteria. A similar result was obtained in NIH-3T3 cells with elevated gp130, i.e., they were more responsive to IL-6+sIL-6R and less responsive to OSM, as measured by STAT3 transcriptional activity. These results represent the first direct demonstration that changes in gp130 expression levels can have a significant impact on the magnitude of cellular responsiveness, depending on the ligand and the types of receptor present on the cell.

The increased sensitivity to IL-6+sIL-6R resulting from elevated gp130 levels is most likely caused by an increase in the number of functional receptors. The IL-6+sIL-6R complex binds directly to gp130 and promotes its homodimerization and activation by JAKs [Murakami et al., 1993]. Our results suggest that a given amount of ligand, in this case consisting of the IL-6+sIL-6R complex, can activate more receptors on a cell that has a greater receptor density. Consistent with this idea, T-cell clones expressing higher levels of the T-cell-receptor α chain were more sensitive to subsaturating

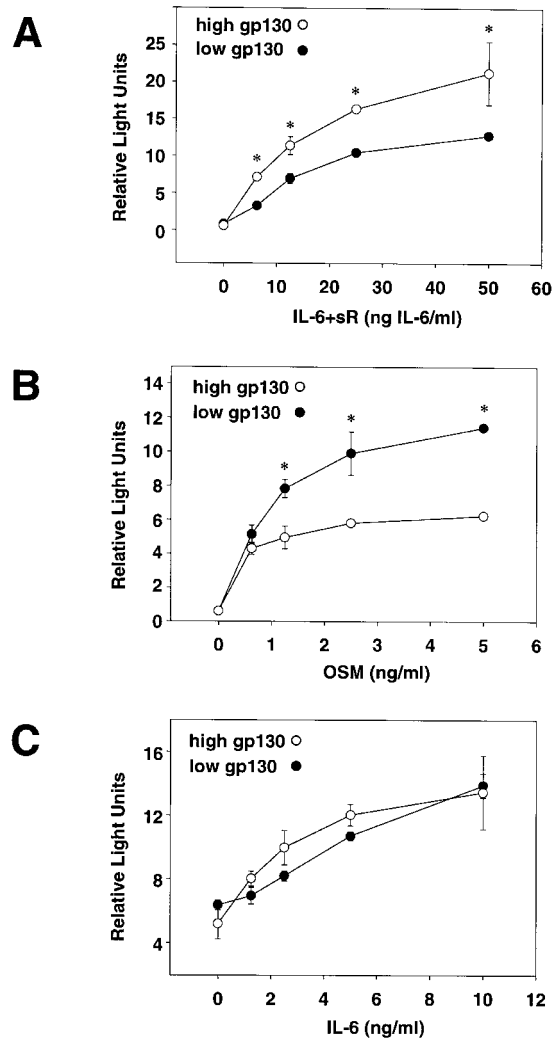

Fig. 4. Elevated gp130 expression in NIH-3T3 cells results in increased sensitivity to interleukin-6 (IL-6) complexed with its soluble receptor (IL-6+sIL-6R), decreased sensitivity to oncostatin M (OSM), and unaltered sensitivity to IL-6 alone. NIH-3T3 cells conditionally expressing elevated gp130 were transiently transfected with a promoter-luciferase construct containing several signal transducers and activators of transcription–binding elements in the presence (black circles) or absence (white circles) of doxycycline. After transfection, the cells were treated with increasing concentrations of IL-6+sIL-6R **(A)**, OSM **(B)**, or IL-6 **(C)** for 5 h and luciferase activity was determined. In (A), only the value for IL-6 is shown, but for each dose, the concentration of sIL-6R was twice that of IL-6. The values represent the mean normalized luciferase activity of three independent transfections ± SD. $^*P < 0.02$ vs. the same concentration of IL-6+sIL-6R or OSM by one-way analysis of variance.

amounts of stimulating peptide [Blichfeldt et al., 1996]. Higher receptor density probably increases receptor activation by increasing the likelihood of receptor–ligand interaction. Another possible explanation for our results is that increased gp130 density may facilitate homodimer formation once the IL-6+sIL-6R complex binds to a gp130 monomer. However, this does not appear to be the case, because the sensitivity to IL-6, in the absence of sIL-6R, was not altered by elevated gp130 in cells expressing membrane-bound IL-6R. The latter finding indicates that the level of membrane-bound IL-6R, but not gp130, is a limiting factor for IL-6 responsiveness, at least in the absence of sIL-6R. However, it is possible that elevation of gp130 expression may increase sensitivity to IL-6 in some cell types that express very low levels of gp130.

In view of the increased sensitivity to the IL-6+sIL-6R complex, and the lack of effect on the sensitivity to IL-6, the reduced sensitivity to OSM resulting from elevated gp130 levels was unexpected. Murine OSM first binds to gp130 with low affinity, and this complex, in turn, associates with OSMRβ to form a high-affinity signaling receptor [Mosley et al., 1996; Lindberg et al., 1998]. OSM binding to gp130 alone, without heterodimerization with OSMRβ, does not result in signal transduction [Mosley et al., 1996]. Therefore, the most likely explanation of our findings is that increased gp130 levels promoted formation of low-affinity, nonsignaling complexes, thereby inhibiting formation of high-affinity receptors, thus leading to reduced responsiveness to OSM. The increased responsiveness observed with IL-6+sIL-6R, and the lack of effect with IL-6 alone, demonstrate the specificity of the effect and argue against it being artifactual. Thus, these results suggest that the ratio of gp130 to OSMRβ may play a role in determining the sensitivity of cells to OSM.

Although gp130 is not required for osteoclast differentiation in developing mice [Kawasaki et al., 1997], IL-6, IL-11, OSM, and LIF stimulate osteoclastogenesis in vitro [Tamura et al., 1993]. More important, IL-6 is required for the increase in osteoclast differentiation and bone loss associated with loss of sex steroids and hyperparathyroidism [Jilka et al., 1992; Poli et al., 1994; Bellido et al., 1995; Grey et al., 1999]. Consistent with this evidence, loss of estrogen increases the sensitivity of the osteoclastogenic response to IL-6 [Girasole et al., 1994]. In line with this, we have shown previously that sex steroids suppress the expression of both components of the IL-6 receptor, gp80 and gp130, in stromal/osteoblastic cells in vitro [Lin et al., 1997]. We have also demonstrated that ovariectomy in mice increases the expression of these two genes in ex vivo bone marrow cultures, as determined by reverse transcriptase–polymerase chain reaction (RT-PCR) and in situ RT-PCR [Lin et al., 1997]. These findings have suggested that increased expression of gp80 and/or gp130, after loss of estrogens, may augment IL-6 action by making cells more responsive to a given level of IL-6. The percentage of bone surface covered by osteoclasts can increase two- to threefold after loss of sex steroids or decrease by the same amount after long-term immobilization or glucocorticoid treatment [Jilka et al., 1996; Weinstein et al., 1997, 1998; Zerwekh et al., 1998]. Therefore, the changes in osteoclast formation we observed in the present study are consistent with those that occur in conditions that lead to bone loss, suggesting that modulation of gp130 levels may be biologically relevant.

Our results demonstrate that changes in gp130 levels alone would not be sufficient to alter the responsiveness of cells to IL-6 in the absence of its soluble receptor. However, elevated levels of sIL-6R, as well as IL-6, were found in serum from patients with primary and secondary hyperparathyroidism and correlated with markers of bone resorption [Rusinko et al., 1995; Grey et al., 1999]. The observations reported herein, together with previous studies demonstrating that PTH stimulates gp130 expression in osteoblastic cells [Romas et al., 1996; Lin et al., 1997], suggest that PTH excess may lead to increased gp130, which would result in increased sensitivity to circulating IL-6+sIL-6R, possibly contributing to increased osteoclast differentiation in this condition. In addition, the observation that IL-6-type cytokines themselves can stimulate the expression of gp130 [Lin et al., 1997; O'Brien and Manolagas, 1997] suggests that, once activated, the gp130 signaling pathway might be self-amplifying and result in even greater sensitivity of the cells to IL-6+sIL-6R.

Changes in gp130 expression might also modulate other stromal/osteoblastic cell responses to IL-6-type cytokines, such as differentiation and survival [Bellido et al., 1997, 1998; Jilka et al., 1998]. In addition, it has recently been demonstrated that gp130 activa-

tion is required for the survival of cardiac myocytes undergoing biomechanical stress [Hirota et al., 1999]. Depending on the particular IL-6-type cytokine involved in this process, changes in gp130, mediated by systemic hormones like estrogen and $1,25(OH)_2D_3$, or the IL-6-type cytokines themselves, might increase or decrease the survival of cardiac myocytes in patients with congestive heart failure. CT-1 is thought to play a role in cardiac myocyte survival [Pennica et al., 1995a] and binds directly to the LIFRβ but does not bind to gp130 alone [Pennica et al., 1995b]. Therefore, considering the results of the present study, changes in gp130 levels would not be expected to affect the sensitivity to CT-1. However, because circulating levels of IL-6+sIL-6R may also be increased in some patients with cardiac failure, altered gp130 levels may play a role in the decision between cardiac survival or apoptosis [Testa et al., 1996].

In conclusion, we have provided direct evidence that altered levels of gp130 change the magnitude of the cellular response to IL-6-type cytokines. This evidence is consistent with the hypothesis that increased osteoclast formation seen in estrogen deficiency and hyperparathyroidism may in part be caused by altered gp130 expression, which in turn alters the sensitivity of stromal cells to the IL-6+sIL-6R complex. Whether osteoclast differentiation increases or decreases in response to changes in gp130 levels in other conditions may depend on the particular IL-6-type cytokines involved. Finally, our results suggest that alterations of gp130 levels may have a significant impact on a number of biological responses because of the broad range of functions of IL-6-type cytokines.

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