

Oncostatin M, leukaemia-inhibitory factor and interleukin 6 trigger different effects on α_1 -proteinase inhibitor synthesis in human lung-derived epithelial cells

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Interleukin 6 (IL-6), oncostatin M (OSM) and leukaemia-inhibitory factor (LIF) share a common signal-transducing subunit in each of their receptors and thus mediate an overlapping spectrum of biological activities. Although all of these cytokines stimulate the production of α_1 -proteinase inhibitor (α_1 -PI) in hepatocyte-derived cells, only OSM is able to up-regulate levels of this inhibitor in epithelial cells originating from the lung. In this study we characterized human lung-derived epithelial-like

HTB58 cells for their ability to synthesize α_1 -PI after treatment with IL-6, OSM and LIF. The results demonstrate that the resistance of HTB58 cells to the effects of IL-6 and LIF was not because of a lack of their individual functional receptors and suggest that OSM utilizes two different receptors, gp130/LIF receptor and gp130/OSM receptor, in lung-derived epithelial cells.

INTRODUCTION

Interleukin 6 (IL-6), oncostatin M (OSM) and leukaemia-inhibitory factor (LIF) have been shown to be functionally related, forming an IL-6 family of cytokines which in addition include interleukin 11, ciliary neurotrophic factor and cardiotrophin-1. Members of this family share a common signal-transducing subunit, gp130, in their receptors [1]. The biological activities of IL-6 result from interaction of the cytokine with a receptor complex consisting of gp130 and a specific ligand-binding protein (α -subunit) (gp80, IL-6R). IL-6R can function in either a membrane-anchored or a soluble form (sIL-6R), each being able to form biologically active complexes with IL-6 [2]. OSM and LIF, on the other hand, induce the assembly of gp130 and another β -subunit (LIFR β). Recently a second OSM-specific receptor has been identified consisting of gp130 heterodimerized with OSMR β [3]. Irrespective of whether a cytokine binds to its α -subunit or directly to the signal-transducing β -subunit, binding of the ligand leads to the formation of gp130 homodimers or gp130 heterodimers with an additional β -subunit, the result being the activation of intracytoplasmic tyrosine kinases (the Janus family of kinases). Induction of the Janus kinases is followed by the phosphorylation and nuclear translocation of the signal transducer and activator of transcription (STAT) family of transcription factors [1]. Although an overlapping spectrum of biological activities of IL-6, OSM and LIF is well explained by the presence of a common gp130 receptor subunit in their receptors, much less is known about differences in the response to these cytokines. Any insensitivity to IL-6 could be due to the lack of an IL-6R [4–6], and, since OSM binding can be found in the absence of LIF binding, the expression of a second OSMR may also be possible [3,7].

α_1 -Proteinase inhibitor (α_1 -PI), a major serine proteinase inhibitor in human plasma, plays an important role in inflammation because of its ability to inactivate neutrophil elastase [8].

Although it is mainly synthesized by hepatocytes, lung-derived epithelial cells have also been shown to be a potential source of this inhibitor [9]. Significantly, in such cells, α_1 -PI synthesis is under the control of OSM but not IL-6, the latter being recognized as the principal regulator of α_1 -PI expression in hepatocytes [10]. In this study we have investigated the mechanisms of IL-6, OSM and LIF action in stimulating α_1 -PI synthesis as a function of their interaction with receptors in a model using lung-derived HTB58 epithelial cells.

EXPERIMENTAL

Stimulating factors

Human recombinant IL-6 (specific activity 10^8 units/mg) and human sIL-6R were prepared as previously described [11,12]. Human recombinant OSM (specific activity 4.7×10^7 units/mg) was kindly provided by Immunex Co. (Seattle, WA, U.S.A.). Human LIF from conditioned medium of Chinese hamster ovary cells, expressing recombinant LIF at 10^5 units/ml, was a gift from Dr. H. Baumann (Roswell Park Cancer Institute, Buffalo, NY, U.S.A.). Dexamethasone (DEX) was purchased from Sigma (St. Louis, MO, U.S.A.).

Cell culture

HTB58 human lung squamous carcinoma and HepG2 human hepatoma cell lines were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Normal human bronchial epithelial cells were purchased from Clonetics (San Diego, CA, U.S.A.). HTB58 and HepG2 cells were cultured in Eagle's minimal essential medium (MEM) supplemented with 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 100 units/ml penicillin G, 100 μ g/ml streptomycin (all from Gibco,

Abbreviations used: IL-6, interleukin 6; OSM, oncostatin M; LIF, leukaemia-inhibitory factor; IL-6R, interleukin-6-specific receptor; sIL-6R, soluble IL-6R; LIFR β , LIF-specific receptor; OSMR, OSM-specific receptor; α_1 -PI, α_1 -proteinase inhibitor; HTB58, human lung squamous carcinoma; HepG2, human hepatoma; DEX, dexamethasone; MEM, minimal essential medium.

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Grand Island, NY, U.S.A.) and 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA, U.S.A.). Bronchial cells were cultured in serum-free bronchial epithelial cell basal medium containing 0.5 ng/ml human epidermal growth factor, 5 μ g/ml insulin, 0.5 μ g/ml hydrocortisone, 0.5 μ g/ml adrenaline, 10 μ g/ml transferrin, 0.5 ng/ml tri-iodothyronine and 0.4% (v/v) bovine pituitary extract (all from Clonetics). Cells were plated, allowed to grow to confluence before assay, and then treated with various stimulating factors.

Northern-blot analysis

Total RNA was isolated as previously described [13,14]. Northern-blot analysis was carried out by electrophoresis of RNA samples in 1% agarose gels containing 2.2 M formaldehyde, followed by capillary transfer [15] to Hybond-N membranes (Amersham, Arlington Heights, IL, U.S.A.). Filters were hybridized with the following specific probes: 1.4 kb *EcoRI*–*EcoRI* restriction fragment of human α_1 -PI cDNA [16]; 1.0 kb *PstI*–*PstI* restriction fragment of human gp80 cDNA [17]; 1.2 kb *EcoRI*–*HindIII* restriction fragment of human IL-6 cDNA [18] (kindly provided by Dr. T. Kishimoto, Osaka University, Osaka, Japan); plasmid pBluescript containing human β -actin cDNA (American Type Culture Collection). The probes were labelled using the Megaprime Labeling Kit (Amersham). The hybridization was carried out at 65 °C in 0.5 M phosphate buffer, pH 7.0, containing 7% SDS, 1 mM EDTA, 10 mg/ml BSA and 100 μ g/ml herring DNA (in the case of gp80) or in a mixture containing 1 M NaCl, 1% SDS and 10% dextran sulphate (in the case of other probes). Non-specifically bound radioactivity was removed by three washes at 65 °C in 40 mM phosphate buffer, pH 7.0, containing 1% SDS, 1 mM EDTA and 0.5% BSA, followed by three washes at 65 °C in the same buffer without BSA (gp80) or by three washes at 65 °C in a mixture containing 30 mM NaCl, 3 mM sodium citrate and 0.1% SDS (other probes). Autoradiographs were scanned by quantitative densitometry (pdi, sparc station; Sun Microsystems Inc., Mountain View, CA, U.S.A.), and α_1 -PI mRNA was normalized to β -actin mRNA levels.

Determination of α_1 -PI secretion

Culture medium was collected at 24 h after factor(s) addition. The amount of secreted α_1 -PI was determined by rocket immunoelectrophoresis using polyclonal rabbit antibodies against human α_1 -PI (Dako, Carpinteria, CA, U.S.A.). Purified human plasma α_1 -PI (kindly provided by Miles Inc., Berkeley, CA, U.S.A.) was used as a standard. Where indicated, statistical analysis was performed using the Sigma plot software, differences between the various treatment groups being assessed by Student's *t* test.

Determination of sIL-6R and IL-6 production

To quantify IL-6R and IL-6 in culture supernatants, sIL-6R and IL-6 ELISA kits (BioSource International, Camarillo, CA, U.S.A. and Genzyme, Cambridge, MA, U.S.A. respectively) were used. The supernatants were frozen after collection and kept at –20 °C until tested. Assays were performed according to the manufacturers' recommendations.

Cell lysis and Western blotting

Cells were maintained in serum-free MEM for 2 h before stimulation, followed by treatment with the indicated factors for 5 min at 37 °C. Cells were then lysed in 20 mM Tris/HCl, pH 7.5, containing 140 mM NaCl, 1% Triton X-100, 0.5% Nonidet

P40, 1 mM EDTA, 1 mM EGTA, 1 mM sodium orthovanadate and 1 \times inhibitor cocktail (Boehringer-Mannheim, Indianapolis, IN, U.S.A.). Aliquots of lysates were pretreated with pre-immune serum and Pansorbin (Calbiochem-Novabiochem, La Jolla, CA, U.S.A.), as previously described [15], followed by incubation with excess rabbit anti-gp130 or LIFR β antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) or monoclonal anti-gp130 (a gift from Dr. K. Yasukawa, Tosoh, Tokyo, Japan). Immune complexes were precipitated with Protein A-agarose or Protein A/G PLUS-Agarose (Santa Cruz), washed, released by boiling in Laemmli sample buffer and subjected to SDS/PAGE (7.5% gel) [19]. Bands were visualized by enhanced chemiluminescence (Amersham) after electrotransfer to a nitrocellulose membrane and incubation with anti-phosphotyrosine antibodies (Transduction Laboratories, Lexington, KY, U.S.A.).

Biosynthetic labelling and fluorography

Confluent monolayers of normal bronchial epithelial cells were stimulated for 18 h with 50 ng/ml OSM, 50 ng/ml IL-6 and 500 ng/ml sIL-6R. The cells were then rinsed and incubated for 4 h in the presence of methionine-free medium containing stimulating factors and 200 μ Ci/ml [³⁵S]methionine/cysteine (Tran³⁵S-label; ICN Biomedicals, Costa Mesa, CA, U.S.A.). Aliquots of medium were pretreated with pre-immune serum and Pansorbin. The supernatants were then incubated overnight at 4 °C in 20 mM Tris/HCl, pH 7.5, containing 140 mM NaCl, 1% Triton X-100, with excess anti- α_1 -PI antibody. Immune complexes were precipitated with Protein A-agarose and examined by SDS/PAGE (9% gel). Bands were detected by fluorography as described elsewhere [20].

RESULTS

We have previously shown that human normal bronchial epithelial cells as well as lung-derived epithelial cells, such as HTB55, respond to OSM but not IL-6 or LIF in terms of α_1 -PI synthesis [9]. A similar regulation of α_1 -PI was also observed in other lung-derived epithelial HTB58 cells. Figure 1 demonstrates the difference in α_1 -PI levels in hepatocyte-like HepG2 cells and

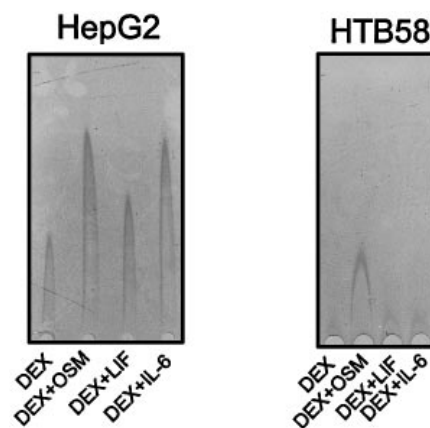


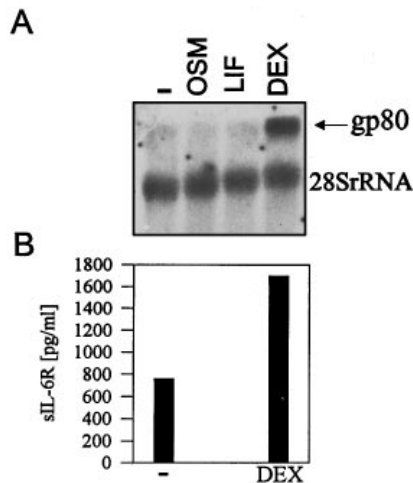
Figure 1 Comparison of the effect of OSM, IL-6 and LIF on α_1 -PI synthesis in HepG2 and HTB58 cells

HepG2 and HTB58 cells were incubated in serum-free MEM containing 10^{-7} M DEX and 90 ng/ml IL-6, 50 ng/ml OSM or 10 units/ml LIF. Samples of medium collected at 24 h were subjected to rocket immunoelectrophoresis using antiserum to α_1 -PI. The gel was stained with Coomassie Blue to demonstrate precipitin lines.

Table 1 Effect of IL-6 family of cytokines, sIL-6R or DEX on α_1 -PI synthesis in HTB58 cells

HTB58 cells were incubated in serum-free MEM containing 50 ng/ml IL-6, 100 ng/ml sIL-6R, 10 units/ml LIF, 50 ng/ml OSM or 10^{-6} M DEX. Aliquots of cell culture medium collected at 24 h were analysed by rocket immunoelectrophoresis to measure secreted α_1 -PI. Results are means \pm S.D. for two determinations of three or four separate experiments. ** $P < 0.01$, * $P < 0.05$, compared with control (Student's t test).

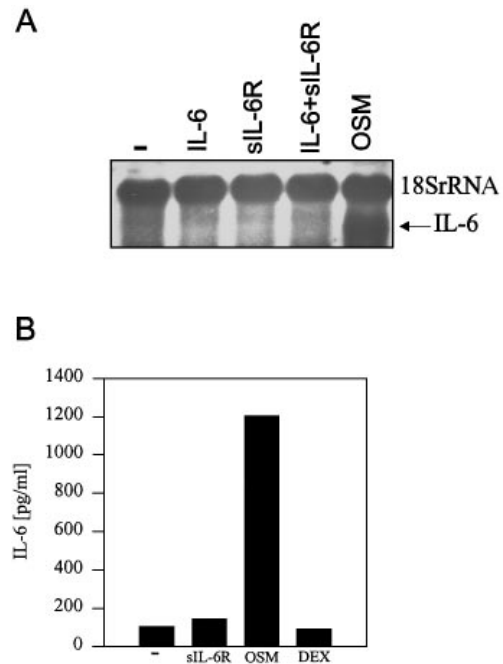
Treatment	α_1 -PI secretion (μ g/ml per 10^6 cells)
Control	0.25 \pm 0.10
IL-6	0.27 \pm 0.14
sIL-6R	0.21 \pm 0.06
IL-6 + sIL-6R	0.21 \pm 0.06
LIF	0.28 \pm 0.20
OSM	0.74 \pm 0.33**
DEX	0.29 \pm 0.06
DEX + IL-6	0.38 \pm 0.20
DEX + LIF	0.42 \pm 0.27
DEX + OSM	1.33 \pm 1.11*

**Figure 2** Expression of gp80 in HTB58 cells

Cells were incubated in serum-free MEM supplemented with 50 ng/ml OSM, 10 units/ml LIF or 10^{-6} M DEX. At 24 h, RNA (A) and (where indicated) medium (B) were collected and subjected to Northern-blot analysis or utilized for soluble receptor measurement respectively. (A) Positions of gp80 and 28 S rRNA are shown. (B) Results are expressed as the mean of two determinations.

HTB58 cells stimulated with cytokines in the presence of the glucocorticoid analogue DEX, a factor known to intensify the effect of cytokines on HepG2 cells [21]. It was found that, in HTB58 cells, the biosynthesis of α_1 -PI was markedly induced by OSM as well as being strongly up-regulated by this cytokine in HepG2 cells. It should be noted that HTB58 cells were far more sensitive to the effect of OSM than the previously examined lung-derived epithelial cell line HTB55 (Table 1 and ref. [9]). In contrast with OSM, neither IL-6 nor LIF caused a statistically significant up-regulation of α_1 -PI release in HTB58 cells ($P > 0.05$) (Table 1), although both cytokines stimulated α_1 -PI synthesis in HepG2 cells. This latter effect was in agreement with previous studies [10].

To determine whether the lack of an IL-6R was responsible for the resistance of the HTB58 cells to IL-6, we first examined the

**Figure 3** Expression of IL-6 in HTB58 cells

Cells were incubated in serum-free MEM supplemented with 50 ng/ml IL-6, 100 ng/ml sIL-6R, 50 ng/ml OSM or 10^{-6} M DEX. At 24 h, RNA (A) and medium (B) were collected and subjected to Northern-blot analysis or utilized for IL-6 measurement respectively. (A) Positions of IL-6 and 18 S rRNA are shown. (B) Results are expressed as the mean of two determinations.

ability of these cells to express IL-6R (gp80). Figure 2(A) shows that unstimulated, OSM- and LIF-treated HTB58 cells expressed very low levels of gp80 mRNA. However, treatment with DEX, a factor known to up-regulate gp80 mRNA levels [22], markedly increased gp80 expression in these cells, which suggested that the latter gene was not silenced. Since cells expressing membrane-bound IL-6R can release soluble receptor into the culture medium [2], we next demonstrated the increased ability of HTB58 cells to release sIL-6R after treatment with DEX (Figure 2B). Since these results indicate that HTB58 cells are able to express IL-6R, we also investigated the desensitization of the cells by endogenously produced IL-6, as a possible mechanism for the lack of responsiveness to this cytokine. In HepG2 or melanoma cell lines transfected or transduced with IL-6 cDNA respectively [5,23], a lack of responsiveness to IL-6 was observed, most probably because of the occupation of all available receptors by this cytokine. As illustrated in Figure 3, HTB58 cells were quite capable of synthesizing IL-6, and, more significantly, this was markedly up-regulated by OSM, but not by sIL-6R, a combination of sIL-6R with IL-6, or by DEX.

If the IL-6R was occupied by endogenously produced IL-6, addition of sIL-6R should have restored IL-6 sensitivity [6,23]. Thus we next examined the effect of sIL-6R on α_1 -PI expression. Although sIL-6R added in combination with IL-6 slightly up-regulated α_1 -PI expression (Figure 4), the level of stimulation never reached that caused by OSM, even though sIL-6R was used in 10 times higher concentration. Moreover, as shown in Table 1, the effect of sIL-6R added in combination with IL-6 on α_1 -PI synthesis in HTB58 cells was not statistically significant ($P > 0.05$). Furthermore, normal bronchial epithelial cells, like HTB58 cells, were not significantly sensitized to the effect of

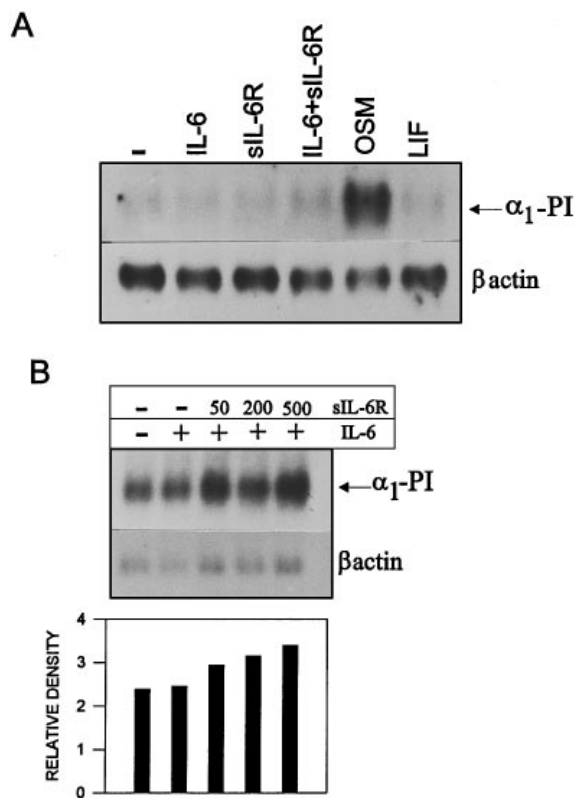


Figure 4 Effect of sIL-6R on α_1 -PI expression in HTB58 cells

Cells were incubated in serum-free MEM supplemented with 50 ng/ml IL-6, 100 ng/ml sIL-6R, 50 ng/ml OSM and 10 units/ml LIF (A), or 50 ng/ml IL-6 and the indicated amounts of sIL-6R (B). Total cellular RNA collected at 24 h was subjected to Northern-blot analysis. The blot was hybridized to an α_1 -PI probe followed by rehybridization to a β -actin probe. Autoradiographs were scanned by densitometry, and α_1 -PI mRNA was normalized to the amount of β -actin (B, lower panel).

IL-6 in the presence of sIL-6R, although they were strongly responsive to OSM (Figure 5).

Ligand-induced dimerization of signal-transducing subunits induces tyrosine phosphorylation of several proteins, including the β components of the receptor complex [1]. To determine whether sIL-6R forms a functional receptor with gp130, we determined whether gp130 is phosphorylated on addition of IL-6 in combination with sIL-6R. As illustrated in Figure 6(A), the induction of gp130 tyrosine phosphorylation was observed in HTB58 cells treated with IL-6 in combination with sIL-6R but not with IL-6 alone. Significantly, both OSM and LIF were effective in the stimulation of tyrosine phosphorylation of gp130 and LIFR β , which clearly indicates the expression of functional LIF receptors in HTB58 cells (Figures 6A and 6B). Finally, by using a monoclonal antibody to gp130, we detected a protein predominantly phosphorylated in response to OSM which migrated slightly more slowly than classical gp130 (Figure 6C). A tyrosine-phosphorylated protein exhibiting a similar migration pattern to that of gp130 was detected in unstimulated as well as stimulated cells (Figure 6C). However, since we did not observe tyrosine phosphorylation of gp130 in control as well as IL-6 stimulated cells when polyclonal anti-gp130 antibodies were used (Figure 6A), we conclude that this protein most probably did not represent gp130.

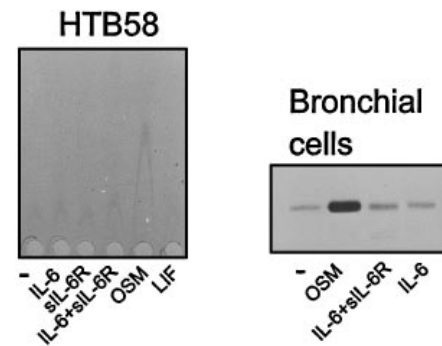


Figure 5 Effect of sIL-6R on α_1 -PI synthesis in HTB58 and normal bronchial epithelial cells

HTB58 cells were incubated in serum-free MEM containing 90 ng/ml IL-6, 100 ng/ml sIL-6R, 50 ng/ml OSM or 10 units/ml LIF. Samples of medium collected at 24 h were subjected to rocket immunoelectrophoresis using antiserum to α_1 -PI. The gel was stained with Coomassie Blue to demonstrate precipitin lines (left). Bronchial epithelial cells were incubated for 18 h in bronchial epithelial cell basal medium supplemented with 50 ng/ml OSM, 50 ng/ml IL-6 and 500 ng/ml sIL-6R. Cells were subjected to radiolabelling for 4 h with [35 S]methionine/cysteine. Aliquots of cell medium were then immunoprecipitated with human anti- α_1 -PI. Immunoprecipitates were subjected to SDS/PAGE followed by fluorography (right).

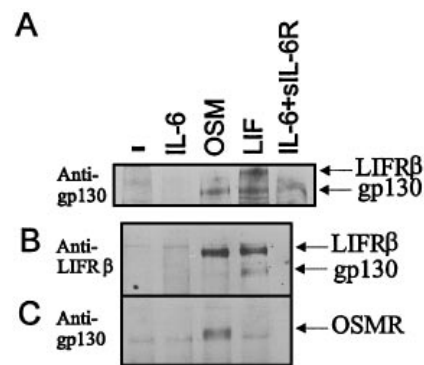


Figure 6 Tyrosine phosphorylation of gp130 and LIFR β in HTB58 cells stimulated with IL-6, sIL-6R, OSM and LIF

Confluent cultures of HTB58 cells were maintained in serum-free MEM for 2 h and then incubated for 5 min in the absence (control) or presence of 50 ng/ml IL-6, 200 ng/ml sIL-6R, 50 ng/ml OSM or 10 units/ml LIF. Cells were lysed as described in the Experimental section and immunoprecipitated with a rabbit anti-gp130 antibody (A), a rabbit anti-LIFR β antibody (B) or a monoclonal anti-gp130 antibody (C). Immunoprecipitates were subjected to Western-blot analysis with an anti-phosphotyrosine antibody. Bands were visualized by enhanced chemiluminescence. The positions of gp130, LIFR β and presumable OSMR β (OSMR) are indicated.

DISCUSSION

Data have been accumulating that indicate the local production of α_1 -PI in the lung [9]. Since α_1 -PI deficiency is often correlated with disturbances in lung function [24], it is important to determine the mechanisms controlling expression of this inhibitor in this organ, as they might be useful in developing therapeutic strategies. We have previously demonstrated the ability of epithelial-like lung-derived HTB55 cells as well as normal bronchial epithelial cells to synthesize α_1 -PI [9]. Here, we report that other lung-derived epithelial HTB58 cells express α_1 -PI. Signifi-

cantly, the strong stimulatory effects of OSM, but not IL-6 or LIF, observed in each cell type examined raise the possibility that OSM plays a unique role in controlling α_1 -PI synthesis in epithelial cells originating from lung.

OSM, IL-6 and LIF share some functional similarity, including similar regulation of α_1 -PI synthesis in hepatocyte-derived cells. Differences in the response to these cytokines observed in lung-derived epithelial cells could arise from differential expression of individual ligand-specific receptors. Significantly, IL-6-mediated induction of osteoclast differentiation has been reported to depend on IL-6R expressed on osteoblastic cells [4]. Furthermore down-regulation of IL-6R has been suggested as one of the mechanisms responsible for IL-6 resistance in melanoma cells [5]. In this study, the sensitivity of lung-derived epithelial cells to the effect of specific cytokines was investigated using HTB58 cells as a model. Our results demonstrate that HTB58 cells were capable of IL-6R expression and that this level increased significantly after DEX treatment. It is important to note that gp80 mRNA levels were also up-regulated in DEX-treated HTB58 cells [25]. However, treatment of the cells with DEX in combination with recombinant IL-6 did not significantly change the level of α_1 -PI synthesis, indicating that a lack of effect of IL-6 is not due to either an absence or low levels of IL-6R.

Stimulation of HTB58 cells with IL-6 did not cause the phosphorylation of gp130, which suggests that this receptor was inactive, possibly because of desensitization. This may be explained by trapping of IL-6R by endogenously produced IL-6, making these cells unresponsive to exogenous IL-6. In any event, regardless of whether IL-6R is either not expressed or non-functional, addition of soluble IL-6R could be expected to restore the sensitivity of the cell to IL-6 [6,23]. The data presented in this paper demonstrate that sIL-6R in combination with IL-6 induced phosphorylation of gp130. This, however, did not lead to strong stimulation of α_1 -PI synthesis, compared with that triggered by OSM, suggesting that the unavailability of IL-6R is not a major mechanism responsible for the lack of an IL-6 effect. A similar finding has been reported for IL-6-resistant melanoma cell lines, which, although being responsive to OSM, were not only resistant to IL-6 but could not have their sensitivity restored by addition of sIL-6R [5].

In this study we have also presented evidence that lung-derived epithelial cells expressed functional receptors for LIF, as demonstrated by their ability to respond to this cytokine through phosphorylation of the gp130/LIFR β heterodimer. However, as in the case of IL-6, the effect of LIF was not manifested by an up-regulation in the production of α_1 -PI in these cells. Significantly, OSM also triggered the assembly of gp130/LIFR β receptor. However, HTB58 cells responded to OSM through tyrosine phosphorylation of another protein co-precipitating with antibodies to gp130. This protein most probably represents OSMR. OSMR has been reported to consist of a dimer of gp130 with another protein of molecular mass 160–180 kDa [7,26].

The strong effect of OSM on lung-derived epithelial cells is manifested not only in the regulation of α_1 -PI synthesis but also that of anti-chymotrypsin [25]. Our data suggest that this effect results in the utilization of either OSMR or a combination

of two different receptors consisting of gp130/LIFR β and gp130/OSMR. Although the amounts of OSMR in lung-derived epithelial cells remain to be established, it is important to note that epithelial-like cells show relatively high levels of expression of this receptor [3]. OSM has previously been found to be a much more potent inducer of certain biological responses compared with LIF or IL-6 [3–7]. Thus lung-derived epithelial cells may provide a good model for studying either different functional levels of β receptor subunits or different signal-transduction pathways, both of which are likely to be responsible for differences in biological activities between OSM, LIF and IL-6.

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REFERENCES

- 1 Kishimoto, T., Taga, T. and Akira, S. (1994) *Cell* **76**, 253–262
- 2 Rose-John, S. and Heinrich, P. C. (1994) *Biochem. J.* **300**, 281–290
- 3 Mosley, B., De Imus, C., Friend, D., Boiani, N., Thoma, B., Park, L. S. and Cosman, D. (1996) *J. Biol. Chem.* **271**, 32635–32643
- 4 Udagawa, N., Takahashi, N., Katagiri, T., Tamura, T., Wada, S., Findlay, D. M., Martin, T. J., Hirota, H., Taga, T., Kishimoto, T. and Suda, T. (1995) *J. Exp. Med.* **182**, 1461–1468
- 5 Silvani, A., Ferrari, G., Paonessa, G., Toniatti, C., Parmiani, G. and Colombo, M. P. (1995) *Cancer Res* **55**, 2200–2205
- 6 Cichy, J., Rose-John, S., Pryjma, J. and Travis, J. (1996) *Biochem. Biophys. Res. Commun.* **227**, 318–321
- 7 Thoma, B., Bird, T. A., Friend, D. J., Gearing, D. P. and Dower, S. K. (1994) *J. Biol. Chem.* **269**, 6215–6222
- 8 Potempa, J., Korzus, E. and Travis, J. (1994) *J. Biol. Chem.* **269**, 15957–15960
- 9 Cichy, J., Potempa, J. and Travis, J. (1997) *J. Biol. Chem.* **272**, 8250–8255
- 10 Baumann, H. and Gauldie, J. (1994) *Immunol. Today* **15**, 74–80
- 11 Van Dam, M., Mullberg, J., Schooltink, H., Stoyan, T., Brakenhoff, J., Heinrich, P. C. and Rose-John, S. (1993) *J. Biol. Chem.* **268**, 15285–15290
- 12 Vollmer, P., Peters, M., Ehlers, M., Yagame, H., Matsuba, T., Kondo, M., Yasukawa, K., Meyer zum Buschenfelde, K. and Rose-John, S. (1996) *J. Immunol. Methods* **199**, 47–54
- 13 Scherrer, K. and Darnell, J. E. (1962) *Biochem. Biophys. Res. Commun.* **7**, 486–490
- 14 Rose-John, S., Dietrich, A. and Marks, F. (1988) *Gene* **74**, 465–471
- 15 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- 16 Long, G. L., Chandra, T., Woo, S. L. C., Davie, E. W. and Kurachi, K. (1984) *Biochemistry* **23**, 4828–4837
- 17 Schooltink, H., Stoyan, T., Lenz, D., Schmitz, H., Hirano, T., Kishimoto, T., Heinrich, P. C. and Rose-John, S. (1991) *Biochem. J.* **277**, 659–664
- 18 Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatsu, A., Tsunasawa, S., Sakijama, F., Matsui, H., Takahara, Y., Taniguchi, T. and Kishimoto, T. (1986) *Nature (London)* **324**, 73–76
- 19 Laemmli, U. K. (1970) *Nature (London)* **227**, 680–684
- 20 Bonner, W. M. and Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88
- 21 Baumann, H., Richards, C. and Gauldie, J. (1987) *J. Immunol.* **139**, 4122–4128
- 22 Rose-John, S., Schooltink, H., Lenz, D., Hipp, E., Dufhues, G., Schmitz, H., Schiel, X., Hirano, T., Kishimoto, T. and Heinrich, P. C. (1990) *Eur. J. Biochem.* **190**, 79–83
- 23 Mackiewicz, A., Schooltink, H., Heinrich, P. C. and Rose-John, S. (1992) *J. Immunol.* **149**, 2021–2027
- 24 Crystal, R. G. (1990) *J. Clin. Invest.* **85**, 1343–1350
- 25 Cichy, J., Potempa, J. and Travis, J. (1995) *J. Clin. Invest.* **95**, 2729–2733
- 26 Bellido, T., Stahl, N., Farruggella, T. J., Borba, V., Yancopoulos, G. D. and Manolagas, S. C. (1996) *J. Clin. Invest.* **97**, 431–437