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Regulation of the type II oncostatin M receptor expression in lung-derived epithelial cells

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Abstract Oncostatin M (OSM) is a potent modulator of human lung-derived epithelial cell function. This cytokine binds two distinct receptor complexes: type I OSM receptor which is also a functional receptor for leukemia inhibitory factor (LIF), and type II OSM-specific receptor. The role of these two distinct receptors in mediating the response of individual cell types to OSM has not been delineated. In contrast to LIF, OSM induces synthesis of α_1-antichymotrypsin and α_1-antiproteinase inhibitor in lung-derived epithelial cells. The differential responsiveness to LIF and OSM suggested that the response of lung epithelial cells to OSM may be mediated by the OSM-specific receptor. Therefore, we characterized lung-derived epithelial cells for the expression of type II OSM receptor mRNAs, and the regulation of the mRNAs encoding the components of the OSM-specific receptor by cytokines and dexamethasone.

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1. Introduction

Oncostatin M (OSM) is a multifunctional cytokine synthesized by hematopoietic cells as well as activated T lymphocytes and monocytes [1–3]. OSM is functionally related to the interleukin-6 family of cytokines, including IL-6, leukemia inhibitory factor (LIF), IL-11, ciliary neurotrophic factor and cardiotrophin-1 [4,5]. Members of this family mediate their biological effects by inducing homodimerization of gp130 (IL-6, IL-11) or heterodimerization of gp130 and other signaling (β) receptor subunits (other members). Some of these cytokines, including IL-6, also require a ligand-binding subunit that determines receptor specificity but is not directly involved in signaling. Receptor dimerization results in the activation of cytoplasmic tyrosine kinases followed by the phosphorylation and nuclear translocation of the STAT family of transcription factors [4–6].

Biologically active OSM receptor consists of a heterodimer of gp130 and LIFRβ. This gp130·LIFRβ complex, described as type I OSM receptor, is also a functional receptor for LIF. Recently, a second OSM-specific receptor complex has been identified in the human system, consisting of gp130 heterodimerized with an OSM-specific subunit OSMRβ, which forms the type II OSM receptor [7]. Although type I and II OSM receptors are relatively broadly distributed on a variety of cell types [7], the role of these two distinct receptors in mediating the response of individual cell types to OSM has not been delineated.

In addition to bioactivities shared with other members of the IL-6 family of cytokines, OSM exhibits some unique activities [8]. For example, OSM but not LIF or IL-6 upregulates α_1-antichymotrypsin (ACH) and α_1-antiproteinase inhibitor (α_1-PI) levels in lung-derived epithelial cells whereas both LIF and OSM mediate regulation of both these genes in hepatocyte-derived HepG2 cells [9–12]. OSM was found to be one of the most potent 'proinflammatory' inducers of α_1-PI and ACH synthesis in lung-derived epithelial cells [9–12] and cells of epithelial origin, including lung epithelium, have been shown to bind significant amounts of OSM [13]. Thus the role of OSM in the lung is of considerable interest. Although the overlapping spectrum of biological activities of IL-6, OSM and LIF is well explained [5], much less is known about the basis for differences in the response to these cytokines. The specific response to OSM of lung-derived epithelial cells, including normal bronchial epithelial cells (NBEC) and two cell lines, HTB55 and HTB58 (adenocarcinoma and squamous carcinoma respectively), may indicate the utilization of the type II OSM receptor. Lung-derived epithelial cells may thus provide a good model to study potential differences in the levels of expression of the signaling receptor subunits or distinct signal transduction pathways, both of which are likely to account for differences in biological activities between IL-6, OSM and LIF.

In this study we demonstrate that epithelial cells derived from lung express type II OSM receptor mRNAs, with OSMRβ levels most likely being more abundant relative to gp130. Furthermore, we show that the expression of OSMRβ and to a lesser extent gp130 is regulated in these cells by specific inflammatory mediators, including OSM.

2. Materials and methods

2.1. Stimulating factors

Human recombinant OSM and human recombinant transforming growth factor β1 (TGF-β) were purchased from R&D Systems (Minneapolis, MN). Human recombinant IL-1β was obtained from Genzyme (Cambridge, MA). Human LIF from conditioned medium of Chinese hamster ovary cells, containing recombinant LIF at 10^5 U/ml, was a generous gift of Dr. H. Baumann (Buffalo, NY). Dexamethasone (DEX) was purchased from Sigma (St. Louis, MO).

2.2. Cell culture

HTB58 human lung squamous carcinoma, HTB55 human lung adenocarcinoma and HepG2 human hepatoma cell lines were obtained from the American Type Culture Collection (Rockville, MD). Normal human bronchial epithelial cells were purchased from Clonetics (San

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Abbreviations: OSM, oncostatin M; LIF, leukemia inhibitory factor; IL-6, interleukin-6; IL-1, interleukin-1; FBS, fetal bovine serum; MEM, minimal essential medium

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Diego, CA). Cells were cultured in Eagle's MEM (Biowhittaker, Walkersville, MD) supplemented with 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin G, 100 μg/ml streptomycin (all from Gibco, Grand Island, NY) and 10% heat-inactivated fetal bovine serum (FBS) (Atlanta Biologicals, Norcross, GA). Bronchial cells were cultured in serum-free bronchial epithelial cell basal medium (Clonetics) containing 0.5 ng/ml human epidermal growth factor, 5 μg/ml insulin, 0.5 μg/ml hydrocortisone and epinephrine, 10 μg/ml transferrin, 0.5 ng/ml triiodothyronine and 0.4% v/v bovine pituitary extract (all from Clonetics). Cells were plated, allowed to grow to confluency and then treated with various stimulating factors.

2.3. Northern blot analysis

Total RNA was isolated as previously described [14,15]. Northern blot analysis was carried out by electrophoresis of RNA samples in 1% agarose gels containing 2.2 M formaldehyde, followed by capillary transfer [16] to Hybond-N membranes (Amersham, Arlington Heights, IL). Filters were hybridized with the plasmid containing human OSMRβ cDNA (a generous gift of Dr. B. Mosley, Immunex Corporation, Seattle, WA), human gp130 cDNA (a generous gift of Dr. T. Kishimoto, Osaka University, Osaka, Japan) and *Sma*I-*Bam*HI restriction fragment containing the complete coding sequence of human LIFRβ (a generous gift of Dr. D. Gearing, Immunex Corporation, Seattle, WA). The probes were labeled 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 μg/ml of herring DNA. 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. After probing blots were analyzed using a PhosphorImager (Molecular Dynamics).

3. Results

3.1. Normal bronchial epithelial cells and lung-derived epithelial cell lines express OSMRβ and gp130 mRNA

Northern blot analysis of RNA extracted from lung-derived epithelial cells revealed significant levels of OSMRβ and gp130 mRNAs (Fig. 1). The amounts of both transcripts were compared to that expressed by HepG2 cells. In HepG2 cells which are responsive to IL-6 and to OSM, synthesis of both gp130 and OSMRβ has been reported previously [7]. In agreement with this study HepG2 were found to express high levels of OSMRβ and gp130 mRNA and the signal was greater for OSMRβ than for gp130 (Fig. 1, Table 1). In comparison to HepG2 cells, NBEC appeared to express higher amounts of OSMRβ message but less gp130 message (Fig. 1, Table 1). In HTB55 cells levels of both OSMRβ and gp130 mRNAs were lower than in HepG2 cells and the ratio of OSMRβ to gp130 was even greater than in HepG2 cells (Table 1). A similar ratio of OSMRβ to gp130 to that observed in HepG2 cells was noted in HTB58 cells, although in this case both transcripts were much more abundant (Fig. 1, Table 1).

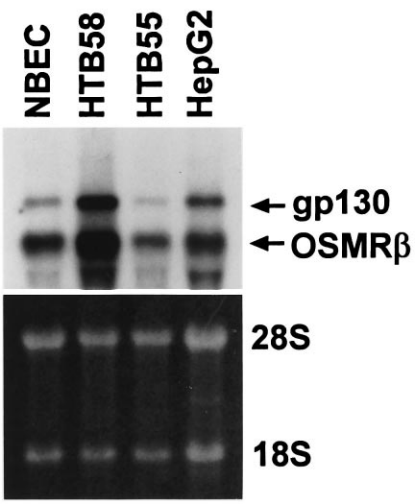

Fig. 1. Comparison of OSMRβ and gp130 mRNA levels in NBEC, HTB55, HTB58 and HepG2 cells. Aliquots of total RNA (5 μg) isolated from the indicated cell types were resolved by gel electrophoresis, transferred to a nylon membrane, and the ethidium bromide-impregnated blot was photographed to demonstrate comparable loading (bands of 28S rRNA and 18S rRNA are indicated). The blot was then hybridized with ^{32}P-labeled gp130 and OSMRβ probes. The blot is representative of three independent experiments.

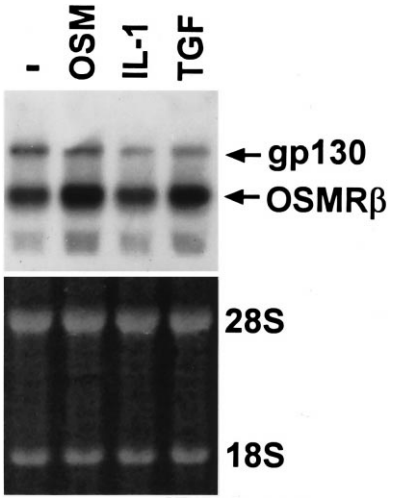

Fig. 2. Regulation of expression of the type II OSM receptor components in NBEC. Cells were incubated for 24 h with 50 ng/ml OSM, 5 U/ml IL-1 or 10 ng/ml TGFβ. Total RNA was then isolated and subjected to Northern blot analysis. Similar amounts of ethidium bromide-stained 28S and 18S rRNA are visualized in the bottom panel. In the upper panel the positions of the gp130- and OSMRβ-specific bands are indicated. The blot is representative of three independent experiments.

3.2. Expression of OSMRβ and gp130 in lung-derived epithelial cells is regulated by cytokines and dexamethasone

Factors which modulate function of lung epithelial cells were then studied as potential regulators of type II OSM receptor components in NBEC including, OSM, IL-1 [9–12] and TGF-β [17]. OSM and TGFβ caused significant increases in OSMRβ mRNA levels; IL-1, however, had no effect (Fig. 2). OSM was also effective in upregulating gp130 mRNA

Table 1
Relative quantitation of OSMRβ and gp130 mRNA levels in lung-derived epithelial cells

	OSMRβ	gp130	OSMRβ/gp130
NBEC	28.5	10.1	2.8
HTB58	53.9	36.6	1.5
HTB55	19.8	4.3	4.6
HepG2	26.5	16.5	1.6

The Northern blot presented in Fig. 1 was exposed to a PhosphorImager screen, and the resulting image was scanned. Arbitrary numbers indicate the relative amounts of OSMRβ- and gp130-specific mRNAs in NBEC, HTB58, HTB55 and HepG2 cells.

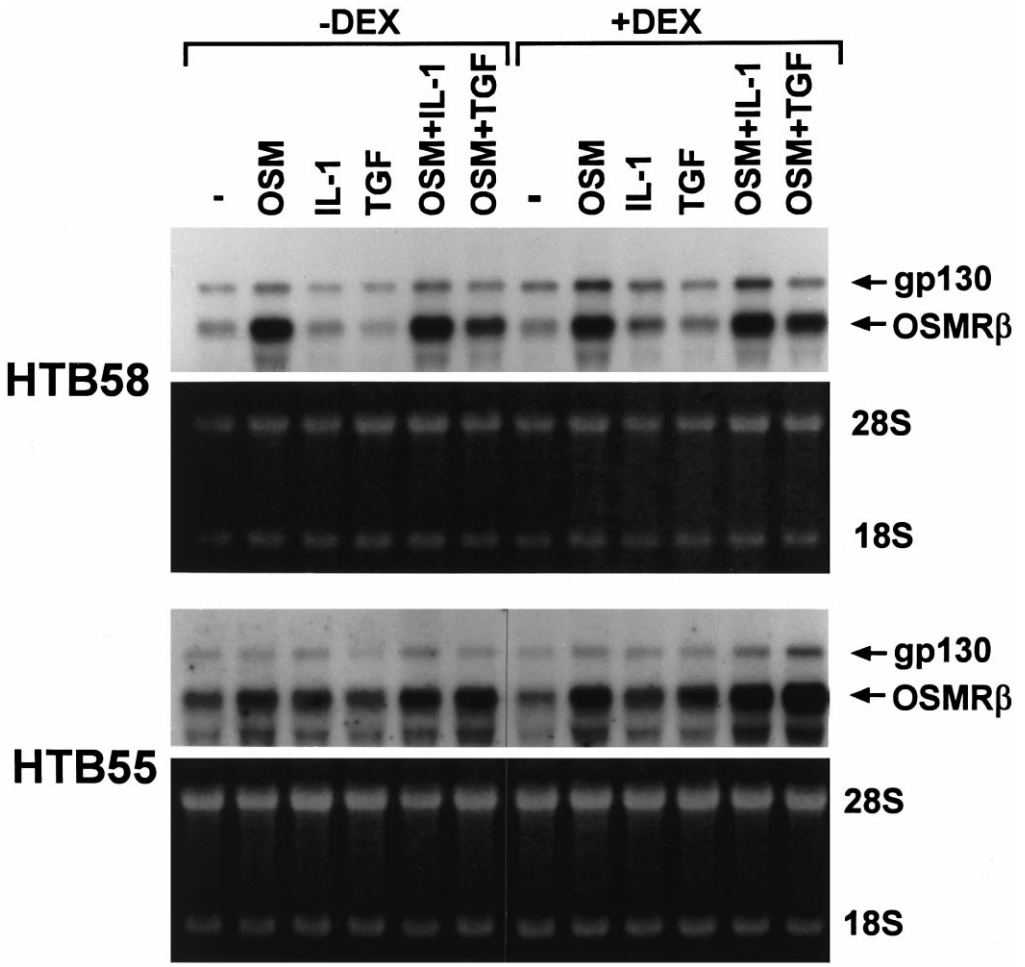

Fig. 3. Regulation of gp130 and OSMRβ mRNA levels in HTB58 and HTB55 cells. Cells were incubated for 24 h with 50 ng/ml OSM, 5 U/ml IL-1, 10 ng/ml TGFβ and/or 10^{-6} M DEX. The levels of gp130 and OSMRβ were then determined by Northern blot analysis. The blot was photographed to demonstrate equal loading (bands of 28S RNA and 18S rRNA are shown) and then hybridized to ^{32}P-labeled gp130 and OSMRβ probes. The blots are representative of three independent experiments.

levels, whereas both TGFβ and IL-1 reduced the amount of gp130 transcripts.

In order to compare the regulation of OSMR in NBEC to other lung-derived epithelial cells which expressed different ratios of OSMRβ to gp130 mRNA, we extended our study to HTB58 and HTB55 cells. Treatment of both cell types with OSM led to stimulation of OSMRβ and gp130 expression, although in the case of HTB55 cells the effect of OSM was more evident in the presence of the glucocorticoid analog dexamethasone (DEX), a factor capable of intensifying the effect of cytokines on these cells [9–12]. DEX given alone significantly upregulated gp130 levels in HTB58 cells. When given in combination with cytokines, DEX generally increased the levels of gp130 mRNA and, to a lesser extent, OSMRβ mRNA in both cell types. IL-1 alone appeared not to affect the expression of OSMR but given in combination with OSM, or more markedly OSM and DEX, IL-1 further enhanced the upregulation of the mRNA levels for both components of the receptor in HTB58 and HTB55 cells. In contrast, the effect of TGFβ was found to be cell-specific. TGFβ generally acted as a negative regulator of OSMR expression in HTB58 cells and as a stimulator in HTB55 cells. Interestingly, in the HTB58 cells, the most substantial increase in OSMRβ and gp130 mRNA levels was noted when the cells were treated with a combination of TGFβ, OSM and DEX.

3.3. *OSM and LIF exert different effects on expression of type I and II receptors*

Since OSM significantly increased type II OSM receptor levels it was interesting to determine if it had any effect on LIFRβ and ultimately type I receptor. We found that treatment with OSM caused a reduction in the levels of LIFRβ transcripts in HTB58 cells (Fig. 3). We previously demonstrated that HTB58 cells have the potential to utilize type I receptor since LIF treatment induced tyrosine phosphorylation of gp130 and LIFRβ [12].

We therefore examined the effect of LIF on type I and II receptors in these cells. In the presence of DEX, LIF similarly to OSM, stimulated OSMRβ and to a lesser extent gp130 mRNA levels, but, in contrast to OSM, did not reduce LIFRβ mRNA levels (Fig. 4). The increase in OSMRβ and gp130 levels by LIF was also observed in NBEC (data not shown).

4. Discussion

Many of the overlapping biological effects of IL-6 related

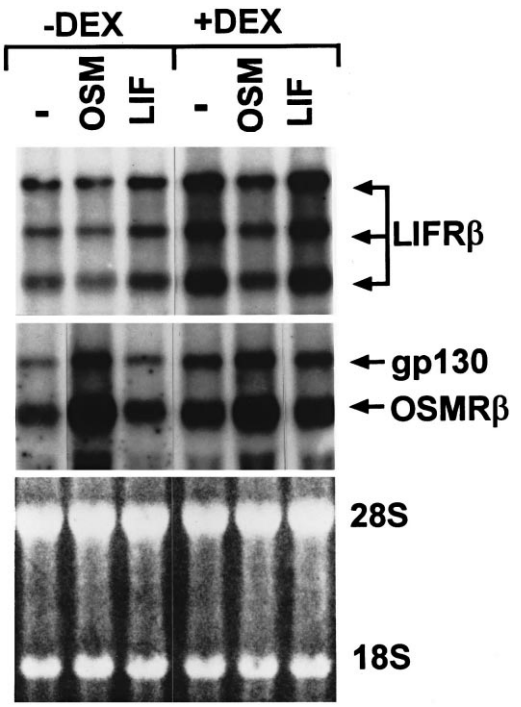

Fig. 4. Effect of OSM and LIF on expression of type I and II OSM receptors in HTB58 cells. Cells were incubated for 24 h with 50 ng/ml OSM, 10 U/ml LIF and/or 10^{-6} M DEX. The levels of LIFRβ, gp130 and OSMRβ were then determined by Northern blot analysis. The blot was photographed to demonstrate equal loading (bands of 28S RNA and 18S rRNA are shown) and then hybridized to ^{32}P-labeled LIFRβ probe. The same samples of RNA were used for parallel hybridization with gp130 and OSMRβ probes.

cytokines can be attributed to the presence of a common receptor subunit, gp130, or in the case of OSM and LIF, to binding of the same receptor consisting of two components, gp130 and LIFRβ [5]. However, significant functional differences between LIF and OSM have also been described and, some cell types are incapable of binding LIF in spite of expressing high affinity functional OSM receptors [8]. Taken together, these findings suggested the existence of an OSM-specific receptor. Recently, an alternative subunit, OSMRβ, of an OSM receptor complex was cloned [7]. This subunit associates with the low affinity OSM·gp130 complex to form a high affinity heterodimeric receptor that is capable of transducing OSM-specific signaling events. Although the signal transduction pathways that mediate OSM-specific responses have not yet been fully delineated, OSM can activate pathways linked to MAP kinase [8], *src*-related kinases [18] and more notably a JAK-STAT pathway that includes STAT3, STAT1 and STAT5 [5,19,20]. Interestingly, activation of STAT5 has been reported to be mediated by the type II OSM receptor [19]. The biological activities of OSM, some of which are OSM-specific and some of which are shared by LIF, can be explained by utilization of a dual receptor system. However, although a dual receptor system is evident in the human system, in mice OSM apparently utilizes only its specific receptor complex [21].

We previously demonstrated in the human system that several genes are specifically regulated by OSM but not other members of the IL-6 family of cytokines [9–12]. Our understanding of the specificity of the response to OSM and LIF will depend on identifying the role of the two potential receptors in mediating these responses. There are several major findings in this study: (i) lung-derived epithelial cells express both subunits of type II OSM receptor, i.e. gp130 and OSMRβ, (ii) OSMRβ mRNA levels appeared to be higher than gp130 in these cells, suggesting that the levels of gp130 may be the limiting factor in the formation of the type I and type II receptors, (iii) there is no consistent difference in the ratio of OSMRβ to gp130 between HepG2 (responsive to OSM, LIF and IL-6) and lung-derived epithelial cells (responsive only to OSM), (iv) OSMRβ and gp130 mRNA levels are regulated by specific mediators, (v) type II receptor is upregulated by either OSM or LIF whereas type I receptor is downregulated by OSM in HTB58 cells. The expression of OSMRβ and gp130 in all lung-derived epithelial cell types examined indicates that the type II OSM-specific receptor may mediate the effects of OSM on lung-derived epithelial cells. Furthermore, based on the high ratio of OSMRβ mRNA relative to gp130 mRNA levels, it is likely that there is an excess of OSMRβ produced that can successfully compete to form dimers with the gp130 subunit even if the gp130 is expressed in limited amounts and in the presence of LIFRβ.

The expression of OSM type II receptor in lung-derived epithelial cells was found to be regulated by cytokines, of which OSM was the most potent stimulator. The cytokines that regulated the steady state mRNA levels of the components of the OSM type II receptor are produced by activated monocytes and T cells and can be produced by infiltrating cells at sites of inflammation. Importantly, lung-derived epithelial cells are stimulated by OSM and IL-1 for the production of serine proteinase inhibitors, proteins which have been postulated to prevent tissue damage associated with inflammation [9–12]. The additive effects of OSM and IL-1 on synthesis of these inhibitors can be explained by the present study showing the role of both cytokines in upregulation of OSMR expression. Moreover, since TGFβ was also found to regulate OSMR levels it may also act as a modulator of OSM effects in lung-derived epithelial cells. However, such a role of TGFβ remains to be determined.

The finding that LIF was capable of modulating type II receptor levels in HTB58 and NBEC further supports the concept that the inability of LIF to induce α_1-PI and ACH synthesis in these cells is not due to the lack of functional type I receptors. Different effects of OSM and LIF resulting in downregulation of LIFRβ by OSM and upregulation of OSMRβ by both cytokines may suggest that in the presence of both OSM and LIF preference will be given to OSM due to the preferential increase in type II OSM receptors.

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