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Oncoctatin M-Induced IL-6 Expression in Murine Fibroblasts Requires the Activation of Protein Kinase Cδ¹

David C. Smyth, Christine Kerr, and Carl D. Richards²

Oncostatin M (OSM) is an IL-6/LIF cytokine family member whose role has been identified in a range of biological activities in vitro, including up-regulation of inflammatory gene expression and regulation of connective tissue metabolism. However, the mechanisms through which OSM regulates cellular responses are not completely understood. In this study, we show that activation of the calcium-independent or novel protein kinase C (PKC) isozyme PKCδ is a critical event during OSM-mediated up-regulation of IL-6 expression in murine fibroblasts. The pan-PKC inhibitor GF109203X (bisindolylmaleimide I) reduced secretion of IL-6; however, use of Go6976, an inhibitor of calcium-dependent PKC enzymes, did not. The PKCδ-selective inhibitory compound rottlerin abrogated expression of IL-6 transcript and protein, but only reduced PKC activity for its mediation of inflammatory processes.

Typically, inflammation is potentiated through secretion and elaboration of small soluble factors, including cytokines or by connective tissue cells such as fibroblasts within local inflammatory states (1, 2). Other cytokines associated with inflammatory states include IL-6 and oncostatin M (OSM), three members of the IL-6/LIF cytokine family that are derived from numerous cell types and exhibit pleiotropic functions, including contribution to inflammatory responses (3).

Animal models of inflammatory disease or samples derived from human subjects show elevated levels of IL-6 and OSM at sites of inflammation (4, 5), and studies indicate potential therapeutic benefit may be achieved through inhibitory strategies targeting the bioactivity of these cytokines (6). OSM has been proposed to participate during chronic inflammatory processes (7–9) including rheumatoid arthritis, where its role may be to potentiate pannus formation and cartilage destruction as indicated by studies using adenoviral delivery of murine OSM (mOSM) to murine knee joints (10). OSM may also contribute to chronic inflammatory respiratory diseases (11), though definitive studies proving this role have not been completed.

Our laboratory has been studying the cellular signaling events underlying OSM-mediated regulation of several inflammation-modulating gene products, including IL-6 (8, 12). A functional property OSM shares with other IL-6/LIF cytokines is the capacity to initiate signal transduction by the JAK/STAT and MAPK pathways (13), likely due to shared usage of the common gp130 receptor subunit. In murine systems, OSM acts exclusively through the heterodimer consisting of gp130 and OSMRβ (14), and the use of this signaling platform might allow for OSM-selective activation of factors such as STAT1 and STAT5 and p38 MAPK (15, 16). However, the activation of JAK/STAT and MAPK signaling is not sufficient to mediate maximal OSM effects, prompting the examination of alternate pathways OSM might use in regulation of inflammatory gene expression.

Protein kinase C (PKC) is a family of serine/threonine kinases that are involved in regulation of cellular processes including growth, migration, and inflammatory responses (17), and several PKC isoforms have been shown to be required for regulation of IL-6 expression (18, 19). PKCδ is a calcium-independent, or novel, PKC isozyme whose activity is regulated by stimuli including oxidative stress, UV exposure, and inflammatory cytokines such as IFN-γ and IL-1β (20–22). PKCδ may modulate inflammatory responses, as evidenced by its capability to induce matrix metalloproteinase and chemokine expression in vitro (23, 24), and may play a role during osteoblastic differentiation (25), a process also known to be dependent upon IL-6. PI3K comprises a family of kinases that phosphorylate the 3’ hydroxyl group of phosphatidylinositols. PI3K is known to be activated by

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³ Abbreviations used in this paper: OSM, oncostatin M; mOSM, murine OSM; MLF, murine lung fibroblast; PKC, protein kinase C; PDK-1, phosphoinositide-dependent kinase-1; PDGF, platelet-derived growth factor; siRNA, small interfering RNA; Ct, threshold cycle.

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various inflammatory mediators and growth factors, and its principle downstream kinase Akt regulates cell survival and differentiation and promotes synthesis of inflammatory cytokines and chemokines (26–28). Therefore, PI3K may be an important signaling intermediate during the initiation and progression of inflammatory diseases. Studies of Kaposi’s sarcoma indicate human OSM induces activation of PI3K. Recently, we identified PI3K as a pathway activated by OSM in murine fibroblasts, where it had an attenuating role during OSM-induced tissue inhibitor of matrix metalloproteinase (TIMP)-1 expression (29).

In this study, we assess mOSM signaling in murine fibroblasts, examining the potential role of PKCδ and PI3K as novel intracellular components of mOSM signal transduction. Our findings identify PKCδ and PI3K as critical contributors to mOSM regulation of IL-6, a model inflammation-associated gene.

Materials and Methods

Explanted murine lung fibroblast (MLF) cultures

MLFs were derived from explanted cells of finely minced lung tissue taken from C57BL/6 mice (10–12 wk old; Charles River Breeding Laboratories), and were cultured in Earle’s modified MEM (F-15) supplemented with 10% FBS (Invitrogen Life Technologies), 1% penicillin/streptomycin, 0.5% Fungizone, and 0.03% l-glutamine.

Cytokines, Abs, and pharmacologic inhibitors

Reagents were purchased from Sigma-Aldrich unless otherwise indicated. Recombinant mOSM and murine IL-6/ILF were purchased from R&D Systems. Recombinant human platelet-derived growth factor (PDGF)-AA was purchased from Oncogene Research Products. Rabbit polyclonal antisera for STAT3, Akt, phosphotyrosine 705 STAT3, and phosphoserine 473 Akt, and goat polyclonal antisera for PKCδ and goat polyclonal antisera for actin were purchased from Cell Signaling Technologies. Rabbit polyclonal antisera for PKCδ and goat polyclonal antisera for actin were purchased from Santa Cruz Biotechnology. Rabbit polyclonal antisera for PKCδ was purchased from Upstate Biotechnology. Mouse mAbs for PKCδ and phosphoinositide-dependent kinase-1 (PDK-1) were purchased from BD Transduction Laboratories. HRP-conjugated secondary Abs for rabbit and goat antiserum were purchased from Santa Cruz Biotechnology and Sigma-Aldrich, respectively. L294002, roteller, GF109203X, and Go6976 were purchased from Calbiochem.

RNA isolation

Fibroblast cultures grown to near confluence in 75-cm² flasks were stimulated with cytokines as indicated in normal supplemented medium and incubated for 24 h. Where indicated, cultures were subjected to pretreatment with described concentrations of pharmacologic inhibitor reconstituted in DMSO. Occasionally, control cultures were incubated with DMSO alone. Total RNA was extracted from cultures with TRIZol (Invitrogen Life Technologies), according to the manufacturer’s directions. RNA was quantitated by spectrophotometric measurement of absorbance at 260 nm, and RNA quality was assessed by resolving 2–3 μl of sample on 1% agarose gels containing 0.5% agarose.

Quantitative real-time PCR (TaqMan)

One microgram of reverse-transcribed cDNA derived from C57BL/6 lung fibroblast cells cultured as described was analyzed for IL-6 mRNA expression by semiquantitative real-time PCR (TaqMan) as previously described (30), using custom optimized primer-probe pairs (Applied Biosystems). Gene expression was quantitated relative to GAPDH, where relative expression of the target gene was calculated as 2−ddCt, where ddCt is the difference between the threshold cycle (Ct) for the gene of interest and the threshold cycle for GAPDH. In each experiment, the value of the relative expression of the control sample (untreated) was given a value of 1 and the expression of other treatments was plotted relative to the control.

RNA interference

Double-stranded 25-mer RNAs corresponding to target regions of PKCδ were generated as Stealth RNA interference oligonucleotides (Invitrogen Life Technologies). Oligonucleotides were constructed based upon the following sequences isolated from murine PKCδ (GenBank accession number NM_011103): target 1, 5′- (210)-GCCGTTGTATCCACGGTTGCTGATGAT-3′ and target 2, 5′- (1153)-GGACGGTGGTTGAGACAAGCTGATGAT-3′. Duplexed RNA molecules based upon these sequences were constructed and were named PKCδ 210 and PKCδ 1153, respectively. Additionally, scrambled duplexes were generated for both targets (PKCδ 210scr and PKCδ 1153scr). RNA interference duplex oligomers were also constructed for PKCe, PDK-1, and Akt1 as follows: PKCe (NM_011104) 5′-(1582)-CAGCAGGCTGATCACAGGATT-(1606)-3′; PDK-1 (N_M_010162) 5′-(1616)-GAACCTGCGAGAAGGAAAGATG-(1640)-3′; and Akt1 (NM_009652) 5′-(1510)-CAACATCGTGTGGCAGGATGTGTA-(1534)-3′. For RNA interference studies, 1 × 10⁵ C57BL/6 cells/well in 24-well culture dishes were transfected with 20 pmol of the indicated RNA interference or scrambled control using Lipofectamine 2000 (Invitrogen Life Technologies), according to the manufacturer’s protocols. For studies using small interfering RNA (siRNA) targeting PKCδ as described in this study, PKCδ 1153 was the siRNA species used. Following an overnight incubation with the indicated siRNA species, cultures were stimulated with 25 ng/ml mOSM and incubated an additional 24 h. Supernatants from stimulated cultures were collected and used for IL-6 capture ELISA as later described. Culture lysates were also collected and verification of both efficacy and specificity of RNA knockdown was conducted by immunoblot analysis with the indicated Abs.

Immunoblotting

C57BL/6 lung fibroblasts were cultured in 100-mm culture dishes as described and stimulated with cytokines and pharmacologic inhibitors as indicated in Results. Cells were lysed in 50 mM Tris/125 mM NaCl/2 mM EDTA (pH 7.4) containing 1% Triton X-100, 0.03% aprotinin, 50 μg/ml PMSF, and 0.5 mM sodium orthovanadate. Lysates were collected in 1.5-ml Eppendorf tubes, rocked at 4°C for 45 min, centrifuged at 12,000 × g for 10 min, and frozen at −70°C. Protein concentration was determined by Bio-Rad protein assay and 10-μg quantities were loaded onto 8% SDS-PAGE gels. Proteins were transferred onto Immobilon-NC membranes (Millipore), and subsequently blots were blocked with TBS containing 0.2% Tween 20 and 5% low-fat milk powder. Blots were probed with primary Abs as indicated, followed by incubation with HRP-conjugated secondary Abs, and protein expression was visualized by ECL (Amersham Biosciences) on X-Omat film (Kodak).

ELISA

A total of 1 × 10⁵ C57BL/6 lung fibroblasts was cultured in 24-well dishes to subconfluence (~75%) and stimulated with cytokines or pharmacologic inhibitors as indicated for 24 h in normal supplemented medium as previously described. Conditioned medium was collected and stored at −20°C until time of analysis. IL-6 secretion was measured using mouse IL-6 matched capture and biotinylated detection Ab pairs, with recombinant mouse IL-6 as standard (R&D Systems), according to the manufacturer’s protocols.

Immunoprecipitation and PKCδ kinase activity assay

Whole cell lysates generated as described were incubated at 4°C overnight with 2 μg/ml anti-PKCe mAb, followed by 1 h of incubation with 15 μg/ml protein A-agarose. Immune complexes were washed three times in lysis buffer followed by two washes in kinase reaction buffer containing 25 mM Tris (pH 7.5), 0.5 mM EDTA, 5 mM MgCl₂, and 0.5 mM DTT. Beads were resuspended in a 20-μl reaction mix containing kinase buffer and 10 μg of [γ-32P]ATP, and the assay was initiated by addition of 10 μg of histone H1 per reaction. Samples were incubated for 30 min at 37°C with occasional mixing, and the reaction was terminated by addition of 10 μl of 4× Laemmli buffer. Samples were resolved on 12% SDS-PAGE, and gels were dried and exposed to X-Omat film for autoradiography.

Statistics

Statistical analyses were conducted using Sigma Stat (SPSS) applying the Student’s t test except where use of one-way ANOVA is indicated.

Results

OSM-mediated PI3K activation in murine fibroblasts

It has been shown that human OSM induces activation of PI3K in Kaposi’s sarcoma cell lines; therefore, we examined C57BL/6 lung fibroblasts to determine whether PI3K was activated by OSM in murine systems. Immunoblots of whole cell lysates demonstrated that mOSM treatment induced phosphorylation of Akt (Fig. 1A), although not as potently as PDGF. By contrast, IL-6 stimulation of cells induced little, if any, detectable Akt phosphorylation. Time course analysis of mOSM-stimulated fibroblasts showed maximal...
Akt phosphorylation at 20 min poststimulation and remained detectable at 45 min, whereas cultures treated with murine IL-6 demonstrated markedly lower phosphorylation of Akt (Fig. 1B). Akt phosphorylation was not detectable at any time point post mOSM or murine IL-6 stimulation in the presence of LY294002, a widely used inhibitor of PI3K activity. Immunoblot analysis of STAT3 showed that mOSM as well as murine IL-6 and murine LIF induced STAT3 tyrosine phosphorylation, and mOSM induction of STAT3 phosphorylation was not affected by LY294002 (Fig. 1C). Subsequently, we examined the role of PI3K activity on regulation of IL-6.

Effects of PI3K inhibition on IL-6 expression

Our laboratory has shown that mOSM is a more potent inducer of gene products including IL-6 and eotaxin-1 relative to other gp130 cytokines in murine fibroblast cell lines. As we had determined that PI3K inhibition affected OSM signaling in C57BL/6 fibroblasts, we assayed IL-6 gene expression induced by OSM following LY294002 treatment. mOSM potently up-regulated IL-6 mRNA levels in C57BL/6 lung fibroblasts as determined by quantitative real-time PCR (TaqMan) (Fig. 2A), consistent with our previous findings. LY294002 treatment reduced levels of IL-6 mRNA transcription, and protein secretion was reduced by PI3K inhibition in a dose-responsive manner, which was statistically significant at inhibitor concentrations of 5 μM (Fig. 2B).

Pharmacologic inhibition of PKCδ results in decreased expression of IL-6

Studies using human gastric cell lines indicated a potential requirement for activation of PKC family enzymes in the regulation of IL-6 by OSM (31). Furthermore, several isoforms of PKC have been shown to be dependent upon PI3K activation. Therefore, we examined the effects of PKC inhibition on IL-6 expression in murine fibroblasts. Use of the pan-specific PKC inhibitor Go6976 and the PKCδ-selective inhibitor rottlerin. Go6976 did not inhibit mOSM-stimulated IL-6 secretion when used at 20 nM (Fig. 3B). However, rottlerin pretreatment inhibited mOSM-induced IL-6 secretion in a dose-responsive manner, which was statistically significant at inhibitor concentrations of 5 μM.

PI3K-dependent Akt phosphorylation in murine fibroblasts is induced by OSM. A, C57BL/6 lung fibroblasts (MLF) were cultured in 100-mm dishes to near confluence in serum-containing medium, followed by 3 h of incubation in serum-free conditions before stimulation. For stimulation, cells were either left untreated or treated with 25 ng/ml mOSM, 25 ng/ml murine IL-6, or 25 ng/ml PDGF-AA. Following a 15-min stimulation, whole cell lysates were collected and 10 μg per sample was denatured in 4× Laemmli sample buffer, resolved on 8% SDS-PAGE, and subjected to immunoblotting for phosphoserine 473 Akt (p-ser473Akt) or total Akt (Akt).

B, MLF cultured as described in A were either left untreated or treated with 25 ng/ml recombinant mOSM or murine IL-6 (mIL-6) over the indicated times, following a 15-min pretreatment with DMSO vehicle or 20 μM PI3K inhibitor LY294002 (LY). Cell lysates were collected and resolved by SDS-PAGE followed by immunoblotting for phosphoserine 473 Akt (p-ser473Akt) or total Akt (Akt).

C, MLF were cultured as described and left untreated or stimulated with mOSM, murine IL-6, murine LIF, or mOSM in the presence of 15-min 20 mM LY294002 pretreatment. Cell lysates were collected and resolved by SDS-PAGE followed by immunoblotting for phosphotyrosine 705 STAT3 (p-tyr705STAT3) or total STAT3 (STAT3).

FIGURE 1. PI3K-dependent Akt phosphorylation in murine fibroblasts

FIGURE 2. Effects of PI3K inhibition by LY294002 on OSM-induced IL-6 mRNA and protein expression in murine fibroblasts. A, C57BL/6 lung fibroblasts (MLF) were either left untreated or treated with 25 ng/ml mOSM for 24 h, in the presence or absence of LY294002 pretreatment, or with 25 ng/ml mIL-6. One microgram of reverse-transcribed CDNA was subjected to TaqMan for quantitation of IL-6 with normalization to GAPDH. Data presented are the fold increase in mRNA expression relative to unstimulated control, with error bars corresponding to SD. *, p < 0.01 compared with mOSM-stimulated controls. B, MLF were either left untreated or treated with 25 ng/ml mOSM for 24 h, in presence or absence of LY294002 pretreatment at the indicated concentrations. Each assay condition was performed in quadruplicate. Conditioned medium was collected and subjected to capture ELISA for IL-6. Error bars represent SD. *, p < 0.01 compared with mOSM-stimulated controls.

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mOSM-stimulated controls. B PKC fibroblasts (Fig. 3).

Expression of PKC expression, we conducted kinase activity assays using C57BL/6 lung fibroblasts. Expression of PKC expression in murine fibroblasts. Expression of PKC expression in C57BL/6 lung fibroblasts. Expression of PKC expression in C57BL/6 lung fibroblasts.

significant at a concentration of 3 μM, the IC_{50} concentration for rottlerin, and at concentrations as low as 1 μM in C57BL/6 lung fibroblasts (Fig. 3C).

PKCδ is activated in C57BL/6 lung fibroblasts stimulated with mOSM

To verify the involvement of PKCδ during mOSM-induced IL-6 expression, we conducted kinase activity assays using C57BL/6 lung fibroblasts. Expression of PKCδ was confirmed by immunoblotting of immunoprecipitated protein from whole cell lysates (Fig. 4A), and activation of PKCδ was assessed by kinase activity assay using histone H1 as a substrate. mOSM stimulation resulted in a marked increase of PKCδ-mediated histone H1 phosphorylation (Fig. 4A). IL-6 also induced PKCδ activation. mOSM-stimulated PKCδ activity was inhibited by LY294002 pretreatment as indicated by reduced histone H1 phosphorylation compared with mOSM stimulation alone. Rottlerin pretreatment did not affect PKCδ activity at 5 μM concentrations; however, higher concentrations (10 and 15 μM) inhibited PKCδ in a dose-dependent manner. The observed effects of LY294002 were not due to a lack of PKCδ enzyme, as evidenced by immunoblotting of the membrane used for kinase activity (Fig. 4A). Immunoblotting from input lysate used for kinase activity reactions demonstrated LY294002 reduction of Akt phosphorylation (Fig. 4B). Whole cell lysates from cultures stimulated with mOSM and pretreated with rottlerin showed partial inhibition of Akt phosphorylation, indicating the possibility that rottlerin affects the activity of kinase enzymes other than PKCδ. We did not observe coimmunoprecipitation of Akt or MAPK enzymes ERK1/2 and p38 with PKCδ following mOSM stimulation (data not shown).

RNA interference targeting PKCδ inhibits IL-6 secretion

Because PKCδ-independent effects mediated by rottlerin have also been suggested in prior studies, we used RNA interference strategies to selectively abrogate PKCδ expression in C57BL/6 lung fibroblasts and subsequently examined IL-6 production in response to mOSM stimulation. Forty-eight hour incubation

![Image](http://www.jimmunol.org/Downloaded from http://www.jimmunol.org/)

**FIGURE 3.** Effects of PKC inhibition upon OSM-induced IL-6 protein expression in murine fibroblasts. A, C57BL/6 lung fibroblasts (MLF) were cultured in 24-well dishes and either left untreated or treated with 25 ng/ml mOSM for 24 h following a 30-min pretreatment with DMSO (Control) or 7.5 μM GF19203X (GF). Each assay condition was conducted in quadruplicate. Conditioned medium was collected and subjected to capture ELISA for IL-6. Error bars represent SD. *, p < 0.01 compared with mOSM-stimulated controls. B, MLF were cultured as described in A and either left untreated or treated with 25 ng/ml mOSM for 24 h following pretreatment with DMSO (Control), 5 μM rottlerin (rott) or 20 nM Go6976 (Go). Conditioned medium was collected from quadruplicate assays and IL-6 was measured by capture ELISA. Error bars represent SD. *, p < 0.01 compared with mOSM-stimulated controls. C, MLF were cultured as described and either left untreated or treated with 25 ng/ml mOSM in the presence or absence of a 15-min pretreatment with rottlerin at the indicated concentrations. Conditioned medium was collected from quadruplicate assays and IL-6 was measured by capture ELISA. Error bars represent SD. *, p < 0.01 compared with mOSM-stimulated controls.

**FIGURE 4.** PKCδ is activated by OSM in C57BL/6 lung fibroblasts through a mechanism involving PI3K activation. A, C57BL/6 lung fibroblasts (MLF) were cultured as described and either left untreated or treated with 200 nM PMA, 25 ng/ml mIL-6, 25 ng/ml mOSM (left). Cultures were preincubated with DMSO vehicle, and additional mOSM-stimulated cultures were pretreated for 15 min with 20 μM LY294002 or 5 μM rottlerin. MLF were pretreated for 15 min with the indicated concentrations of rottlerin and subsequently treated with 25 ng/ml mOSM (right). Following a 5-min stimulation, cells were harvested and ~750 μg of whole cell lysates were immunoprecipitated at 4°C overnight with monoclonal anti-PKCδ Ab, followed by a 1-h incubation with protein A-agarose. Immune complexes were subjected to kinase activity assay using histone H1 as a substrate in presence of 10 μCi per reaction of [γ-32P]ATP. Reactions were stopped with 4× Laemmli buffer, resolved on 12% SDS-PAGE and proteins were transferred to nitrocellulose. Determination of PKCδ loading was performed by immunoblotting using anti-PKCδ Ab (top) while PKCδ kinase activity was assessed by autoradiograph detection of γ-32P-labeled histone (bottom). B, A portion of MLF whole cell lysates from experiments described were 8% SDS-PAGE and subjected to immunoblotting for phospho-erine 473 Akt (p-ser473Akt) or total Akt (Akt).
PKCδ, but not PKCe or PI3K-dependent kinases PDK-1 or Akt1, is required for IL-6 expression

To further assess the requirement for PKCδ during mOSM-induced IL-6 expression, we examined the effects of RNA interference knockdown of PKCe, a closely related PKC isoform. Additionally, as our results indicated a dependence of PI3K for both IL-6 expression and PKCδ activity, we also examined whether PDK-1, known to phosphorylate various PKC enzymes, or Akt1, a ubiquitous downstream target of PI3K-PDK-1 activity, contributed to OSM-induced effects mediated through PKCδ. mOSM-induced IL-6 secretion by C57BL/6 MLF was also not affected by PKCe siRNA pretreatment (Fig. 6A) and ablation of PKCe expression by siRNA did not influence PKCδ kinase activity as demonstrated in Fig. 6B.
PDK-1-targeted RNA interference did not affect mOSM-induction of IL-6 (Fig. 6A), and we observed an apparent increase in PKCε/H9254 activity in cells depleted of PDK-1 (Fig. 6B). Akt1 knockdown using RNA interference effectively reduced expression and activity assessed by Thr308 phosphorylation. However, it did not influence mOSM-induced IL-6 secretion (Fig. 6C). The siRNA treatment targeting PKCε, PDK-1, or Akt1 did not affect PKCδ protein levels. These results indicate a requirement for PI3K–PKCδ in mediating mOSM stimulation of IL-6 gene expression in murine fibroblast cell lines that is independent of PDK-1 and Akt1.

Discussion

Our results indicate that OSM induces both PKCδ and PI3K/Akt activation in murine fibroblasts, two pathways used in addition to the JAK/STAT and MAPK signaling mechanisms previously established in other studies (29, 30). We show that mOSM-mediated maximal up-regulation of IL-6 expression in murine fibroblasts is dependent...
upon a pathway involving the activity of PKCδ. Additionally, we indicate that PI3K activity is selectively induced by OSM, and in explant cultures of MLFs PI3K is an important pathway mediating IL-6 up-regulation. These findings reveal a novel and mOSM-specific signal transduction pathway in murine fibroblasts, which is involved in IL-6 gene regulation in vitro.

Previously, we had demonstrated that expression of a variety of gene products, including IL-6 and proinflammatory mediators, such as MCP-1 and eotaxin-1, are more potently induced by mOSM relative to other gp130 cytokine family members (30, 32). Several studies have also shown that OSM is uniquely involved in the activation of a variety of gene products associated with inflammatory responses, including plasminogen activator (33), cyclooxygenase-2 (34), and several matrix metalloproteinases (35). Additionally, when transiently overexpressed in vivo, OSM is capable of inducing substantial inflammation that includes immune cell infiltration, fibroblast hyperplasia and alterations of the extracellular matrix network within connective tissue in mouse models (10, 36). The broad spectrum of activities mediated by OSM likely reflects its stimulation of multiple signaling pathways, some of which may regulate discrete patterns of gene expression. Our studies presented here suggest that activation of PI3K and PKCδ contribute to OSM-induced effects, and further investigation is required to assess the activity this putative pathway in other cell types that respond to OSM.

PKCδ belongs to the novel, or diacylglycerol-dependent and calcium-independent family of PKC isoforms. Evidence indicating PKCδ contributes to OSM regulation of osteoblastic factor expression from human osteosarcoma cell lines (25) along with results presented in this study suggest further examination of the role of PKCδ in OSM-mediated gene regulatory events is merited, particularly due to our indication that PKCδ is an important molecule mediating mOSM-dependent up-regulation of inflammation-associated factors such as IL-6. The potency of inhibition of IL-6 gene expression by rotterlin suggested that PKCδ may be an essential intracellular factor for mOSM-mediated up-regulation of IL-6. Several studies have called into question the specificity of rottlerin and rottlerin activity has been shown to be indirectly associated factors such as IL-6. The potency of inhibition of IL-6 gene expression by rottlerin suggested that PKCδ may be an essential intracellular factor for mOSM-mediated up-regulation of IL-6. Several studies have called into question the specificity of rottlerin and rottlerin activity has been shown to be indirectly associated with molecules such as 3'-phosphatidylinositol. Additionally, our findings using siRNA to deplete Akt1 indicate that Akt1 does not influence mOSM-induced IL-6 expression.

Disclosures

The authors have no financial conflict of interest.

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