Oncostatin M (OSM) is a member of the gp130 cytokine family, including leukemia inhibitory factor (LIF) and interleukin (IL)-31, and is involved in Th2 inflammation, epidermal integrity, and fibrosis. OSM regulates extracellular matrix remodeling by altering the network of matrix metalloproteinases (MMPs), their inhibitors (tissue inhibitors of metalloproteinases [TIMPs]), other enzymes, and chemokines. Elevated OSM protein levels and mRNA have been documented in various inflammatory diseases, including rheumatoid arthritis, asthma, pulmonary fibrosis, and atopic dermatitis. OSM interacts with 2 receptors in humans: Type 1 receptor: LIF receptor complex (LIFRα/gp130) Type 2 receptor: OSM receptor complex (OSMRβ/gp130) KPL-716 is a fully human monoclonal antibody that targets OSMRβ and simultaneously inhibits both IL-31 and OSM signaling.

**OBJECTIVES**

- To characterize the in vitro responses of human epidermal keratinocytes (HEK) and human dermal fibroblasts (HDF) to OSM in comparison to LIF and IL-31, using the chemokine monocyte chemoattractant protein 1 (MCP-1/CCL2), which has roles in inflammatory responses.
- To assess the ability of KPL-716 in regulating MCP-1/CCL2 responses in HEK and HDF cells.

**METHODS**

- To assess the production of the chemokine MCP-1/CCL2 and the intracellular signaling molecules called STATs (signal transducer and activators of transcription), cells were stimulated with human OSM, LIF, IL-31, transforming growth factor (TGF)-β, lipoprotein A (LPA), or combinations of IL-31 + OSM, IL-13 + OSM, and TGF-β + OSM for 30 minutes or 24 hours.
- To characterize synergistic responses of OSM with human IL-4 or IL-13, cells were stimulated with 0-200 ng/mL of the cytokines alone or in combination with OSM, LIF, or IL-31 for 24 hours.
- To determine antibody-mediated neutralization, cells were stimulated with 2x concentrated isotype control, KPL-716, or an anti-IL-31 receptor α (IL31Ra) antibody (final concentrations of 0.1, 0.01, 0.001, and 0.0001 µg/mL) after 1-hour pre-incubation with antibody or media alone. OSM or OSM + IL-4 were added to cells and incubated for an additional 24 hours.
- MCP-1/CCL2 levels in supernatants were determined using DuoSet EIA kits (R&D Systems, Minneapolis, MN).
- MCP-1/CCL2 and receptor chain mRNAs were measured using Nanostring technology (Seattle, WA) or quantitative real-time polymerase chain reaction (qRT-PCR).
- Experiments shown are representative of ≥3 separate experiments.
- Data are presented as mean ± standard error of the mean (SEM).
- One-way analysis of variance was used to determine statistical significance (P<0.05).

**RESULTS**

- OSM (50 ng/mL) significantly induced MCP-1/CCL2 protein levels and mRNA at 24 hours (Figure 1).
- In HEK cells, OSM induced activation of STAT3 and STAT1 as measured by immunoassays for phosphorylated forms (pSTAT) (Figure 2).
- Similarly, in HDF cells, OSM induced phosphorylation of STAT3 and STAT1 (Figure 3) — LIF or IL-31 minimally activated pSTAT3 and pSTAT1 but with lower signals compared with OSM — In both cell lines, OSM + IL-13 induced pSTAT1, 3, and 6 signals comparable to each cytokine alone, and TGF-β + OSM did not result in detectable differences from levels induced by OSM alone.
- Analysis of mRNA from HDF cells showed the exact same trends as HEK cells (data not shown).
- To determine antibody-mediated neutralization, cells were stimulated with 2x concentrated isotype control, KPL-716, or an anti-IL-31 receptor α (IL31Ra) antibody (final concentrations of 0.1, 0.01, 0.001, and 0.0001 µg/mL) after 1-hour pre-incubation with antibody or media alone. OSM or OSM + IL-4 were added to cells and incubated for an additional 24 hours.
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- Experiments shown are representative of ≥3 separate experiments.
- Data are presented as mean ± standard error of the mean (SEM).
- One-way analysis of variance was used to determine statistical significance (P<0.05).

**CONCLUSIONS**

- OSM regulates expression of the pro-inflammatory chemokine MCP-1/CCL2 by HEK and HDF cells.
- OSM synergizes with typical Th2 cytokines (IL-4 and IL-13) to induce MCP-1/CCL2 in these cells.
- OSM induces mRNA expression of the Type II IL-4 receptor chains.
- LIF and IL-31 did not synergize with IL-4 or with IL-13 to induce MCP-1/CCL2 in HEK and HDF cells, suggesting a separate pathway for OSM signaling in these cells.
- KPL-716, at low concentrations, reduced both the OSM induction and the synergistic OSM + IL-4 induction of MCP-1/CCL2 protein production.
- The potent inhibition of OSM activity by KPL-716 suggests therapeutic potential in Th2-mediated disease distinct from KPL-716 inhibition of IL-31 signaling.

**REFERENCES**


**DISCLOSURES**

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637 Osmotic M induction of Monocyte Chemoattractant Protein 1 (MCP-1) in Human Epidermal Keratinocytes Is Inhibited by Anti-Oncostatin M Receptor β Monoclonal Antibody KPL-716

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