Oncostatin M (OSM) is a member of the gp130 cytokine family, including leukemia inhibitory factor (LIF) and interleukin (II)-31, and is involved in T2 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 (LIFR)
- Type 2 receptor: OSM receptor complex (OSMR)

KPL-76 is a fully human monoclonal antibody that targets OSM 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/CCL-2), which has roles in inflammatory responses.
- To assess the ability of KPL-76 in regulating MCP-1/CCL-2 responses in HEK and HDF.

**METHODS**

- To assess the production of the chemokine MCP-1/CCL-2 in HEK cells and the intracellular signaling molecules called STATs (signal transducer and activator of transcription), cells were stimulated with human OSM, LIF, or IL-31; transforming growth factor (TGF)-β, lipoxygenase A (lipoA), 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 0-20 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 antibody control, KPL-76, 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/CCL-2 levels in supernatants were determined using a DuoSet ELISA kit (R&D Systems, Minneapolis, MN).
- MCP-1/CCL-2 and receptor chain mRNA were measured using Nanostar 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/CCL-2 protein levels and mRNA at 24 hours (Figure 1).
- In HEK cells, OSM induced activation of STAT3 and STAT1 as measured by immunoblots for phosphorylated forms (pSTAT) (Figure 2).
- Neither LIF nor IL-31 stimulation (at higher concentrations of 100-1000 ng/mL) induced detectable pSTAT3, pSTAT1, or pSTAT4 in HEK cells.

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, and 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.

**CONCLUSIONS**

- OSM regulates expression of the pro-inflammatory chemokine MCP-1/CCL-2 by HEK and HDF cells.
- OSM synergizes with typical T2 cytokines (IL-4 and IL-13) to induce MCP-1/CCL-2 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/CCL-2 in HEK and HDF cells, suggesting a separate pathway for OSM signaling in these cells.
- KPL-76, at low concentrations, reduced both the OSM induction and the synergistic OSM + IL-4 induction of MCP-1/CCL-2 protein production.
- The potent inhibition of OSM activity by KPL-76 suggests therapeutic potential in T2-mediated disease distinct from KPL-76 inhibition of IL-31 signaling.