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

Carl D. Richards,[1] Rohan Gandhi,[2] Fernando Botelho,[1] Lilian Ho,[1] John F. Paolini[2]

[1]McMaster Immunology Research Centre, McMaster University, Hamilton, Ontario, Canada; [2]Kiniksa Pharmaceuticals Corp., Lexington, Massachusetts, USA

BACKGROUND

- 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[1]
- OSM regulates extracellular matrix remodeling by altering the network of matrix metalloproteinases (MMPs), their inhibitors (tissue inhibitors of metalloproteinases [TIMPs]), other enzymes, and chemokines[1]
- Elevated OSM protein levels and mRNA have been documented in various inflammatory diseases, including rheumatoid arthritis, asthma, pulmonary fibrosis, and atopic dermatitis[1-5]
- OSM interacts with 2 receptors in humans[1]:
  - 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[6]

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[7]
- To assess the ability of KPL-716 in regulating MCP-1/CCL-2 responses in HEK and HDF cells

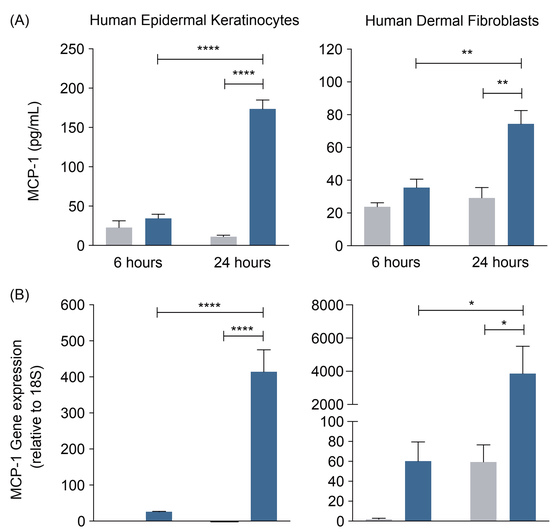
METHODS

- To assess the production of the chemokine MCP-1/CCL-2 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 0–20 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 isotype control, KPL-716, or an anti–IL-31 receptor α (IL-31Rα) 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 DuoSet ELISA kits (R&D Systems, Minneapolis, MN)
- MCP-1/CCL-2 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/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-31stimulation (at higher concentrations of 100 ng/mL) induced detectable pSTAT3, pSTAT1, or pSTAT6 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, 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

Figure 1. OSM Strongly Induced MCP-1/CCL-2 Protein (A) and mRNA (B)



Data are mean ± SEM; two-way ANOVA; *$P<0.05$; **$P<0.01$; ****$P<0.0001$.

Figure 2. OSM Induces STAT Activation in HEK

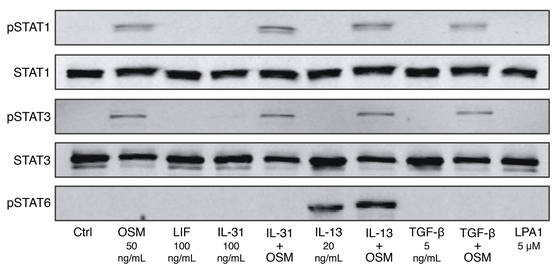
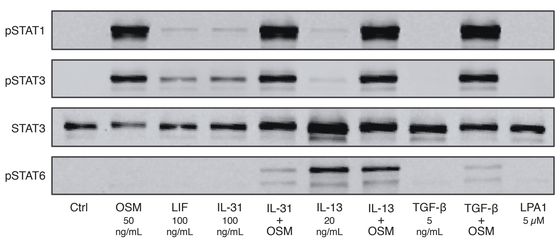
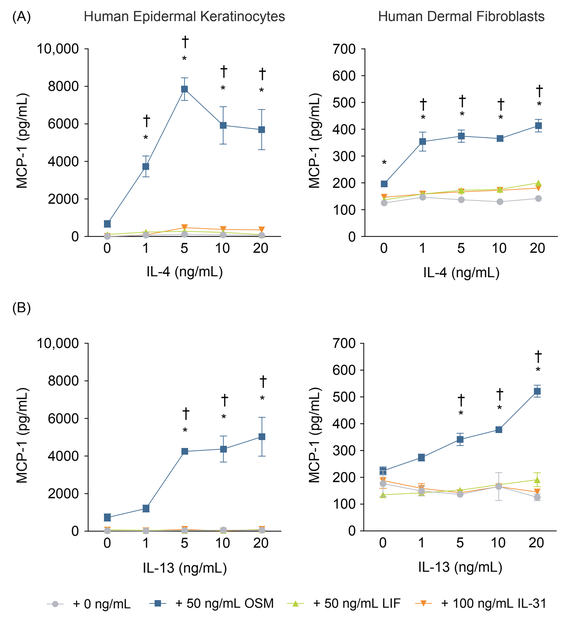


Figure 3. OSM Induces STAT Activation in HDF



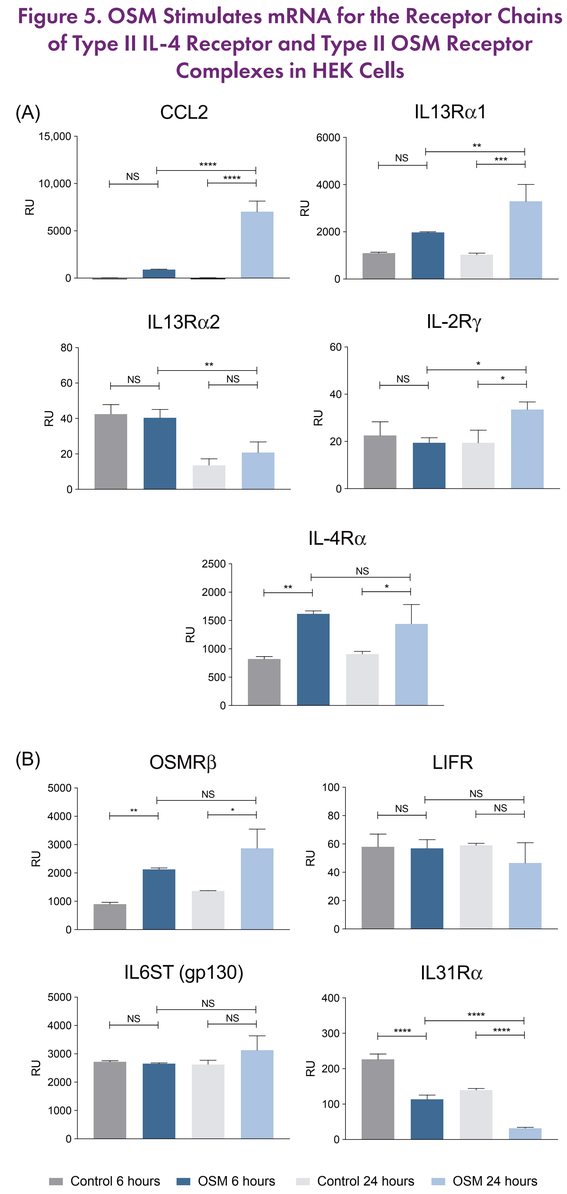
- A dose-dependent increase in MCP-1/CCL-2 production was observed for IL-4 or IL-13 in combination with OSM in both HEK and HDF cells (**Figure 4**)
  - IL-4 or IL-13 alone did not induce MCP-1/CCL-2 levels at any concentration assessed
  - Co-stimulation of IL-4 (or IL-13) with LIF or IL-31 did not result in any changes in MCP-1/CCL-2 levels
- In HEK cells, OSM significantly induced mRNA for the receptor chains of type II IL-4 receptor (IL-4Rα/IL-13Rα-1) to 1000–2000 Relative Units (RU) and OSMRβ/gp130 to 1000–3000 RU (**Figure 5**)
  - Levels of IL-13Rα-2 or IL-2Rγ (type I IL-4 receptor) were very low (<40 RU, where 10 RU is considered non-detectable or at background levels)
  - Levels of LIFRα were very low and IL-31Rα was reduced (≤200 RU) compared with OSMRβ and gp130
- Analysis of mRNA from HDF cells showed the exact same trends as HEK cells (data not shown)

Figure 4. OSM Synergizes With IL-4 or IL-13 in Induction of MCP-1/CCL-2 in HEK and HDF



Data are mean ± SEM; two-way ANOVA; *$P<0.05$ vs control; †$P<0.05$ vs OSM alone.

Figure 5. OSM Stimulates mRNA for the Receptor Chains of Type II IL-4 Receptor and Type II OSM Receptor Complexes in HEK Cells

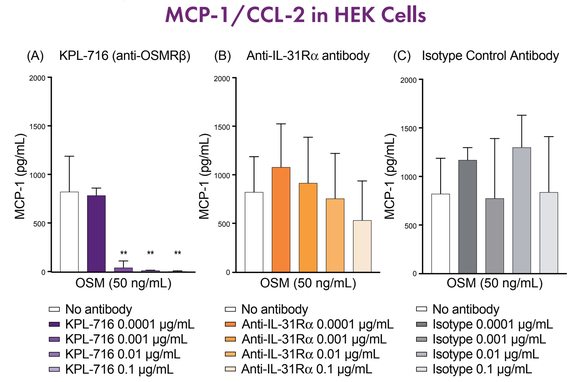


Data are shown as Nanostring counts corrected to 3 housekeeping genes (ACTB, PPIB, UBC).
Data are mean ± SEM; one-way ANOVA; *$P<0.05$; **$P<0.01$; ***$P<0.001$; ****$P<0.0001$.

Effect of KPL-716 (anti-OSMRβ antibody) on HEK cells

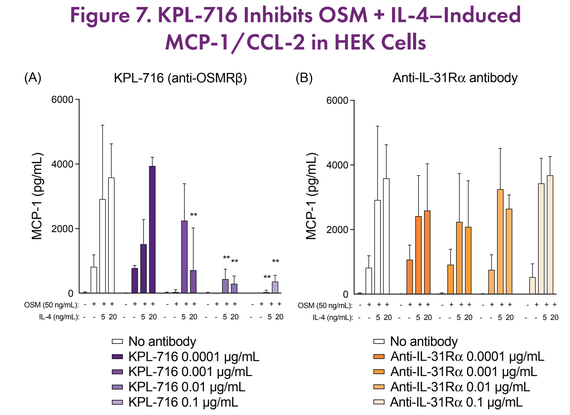
- KPL-716 significantly attenuated the cellular MCP-1/CCL-2 response to OSM (**Figure 6**)
  - At concentrations of KPL-716 of 0.001 µg/mL and higher, MCP-1/CCL-2 levels were markedly reduced
- KPL-716 significantly reduced MCP-1/CCL-2 levels associated with the synergistic response to OSM and IL-4 at both concentrations of IL-4 (5 and 20 ng/mL; **Figure 7**)
- Anti-IL-31Rα or isotype control antibody had no significant effect on the OSM-induced or OSM + IL-4–induced responses at any concentration tested

Figure 6. KPL-716 Inhibits OSM-Induced MCP-1/CCL-2 in HEK Cells



Data are mean ± SEM; two-way ANOVA; **$P<0.01$, no antibody vs KPL-716 at concentration of 0.001–0.1 µg/mL.

Figure 7. KPL-716 Inhibits OSM + IL-4–Induced MCP-1/CCL-2 in HEK Cells



Data are mean ± SEM; two-way ANOVA; **$P<0.01$, no antibody vs KPL-716.

CONCLUSIONS

- OSM regulates expression of the pro-inflammatory chemokine MCP-1/CCL-2 by HEK and HDF cells
- OSM synergizes with typical TH2 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-716, 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-716 suggests therapeutic potential in TH2-mediated disease distinct from KPL-716 inhibition of IL-31 signaling

REFERENCES

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DISCLOSURES

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