TIME-DEPENDENT EFFECTS OF ONCOSTATIN M IN THE PRESENCE OF IL-1β ON CCL2/MCP-1 mRNA EXPRESSION IN HUMAN PROXIMAL TUBULAR CELLS

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Introduction: In response to tubular injury, production and secretion of cytokines, chemokines or extracellular matrix components by human proximal tubular cells (PTC) directly contributes to the development of tubulointerstitial inflammation and fibrosis. Oncostatin M (OSM) represents a cytokine involved in a variety of biological activities such as inflammation, remodeling of extracellular matrix, hematopoiesis, and modulation of cell growth and differentiation. Besides its possible role in tubulointerstitial fibrogenesis recent studies provided evidence for a function of this IL-6 family cytokine in the renal inflammatory response.
Methods: Cell culture, siRNA-mediated gene silencing, real-time PCR.
Results: In the present study we report time-dependent dual effects of OSM on the expression of the proinflammatory chemokine CCL2/MCP-1 mRNA in human PTC. Utilizing proximal tubular HK-2 cells we found that OSM (10 ng/ml) inhibits IL-1β- (10 ng/ml) and TNF-α-mediated (10 ng/ml) mRNA expression of the profibrotic matricellular proteins TSP-1 and TNC after 24 h while it acts synergistically with these two proinflammatory cytokines to induce CCL2 mRNA expression for up to 24 h. Interestingly, incubation of the cells with OSM in the presence of IL-1β for 48 h led to an inhibition of CCL2 mRNA levels. Time course experiments in two independent human PTC lines (HK-2 and RPTEC/TERT1) with OSM alone revealed a rapid induction of this chemokine within the first hour of ligand administration. In HK-2 cells, this stimulation subsequently returned towards basal levels in between 3 h and 24 h of incubation and finally switched into a significant OSM-mediated 70% inhibition of basal CCL2 mRNA expression after 48 h. In contrast to OSM, which stimulated both STAT1/3 and ERK1/2 signaling, IL-1β led to a strong phosphorylation of p65 NFκB/RelA, SMAD2/3 and p38 MAPK in HK-2 cells. Selective silencing of these signaling molecules revealed that p65 NFκB/RelA is involved in IL-1β-mediated stimulation of CCL2 mRNA, and that superinduction of CCL2 mRNA expression in the presence of both OSM and IL-1β at least partially depends on STAT3 signaling.

Fig. 1A

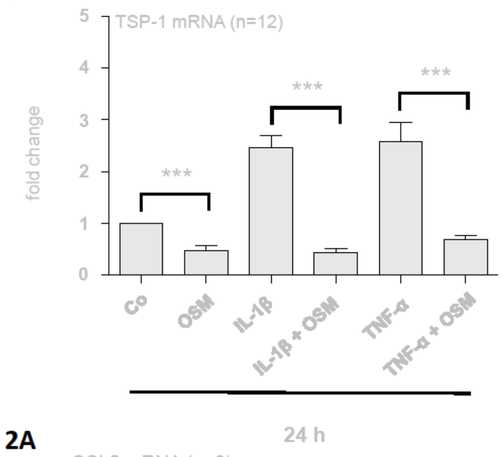

Fig. 1B

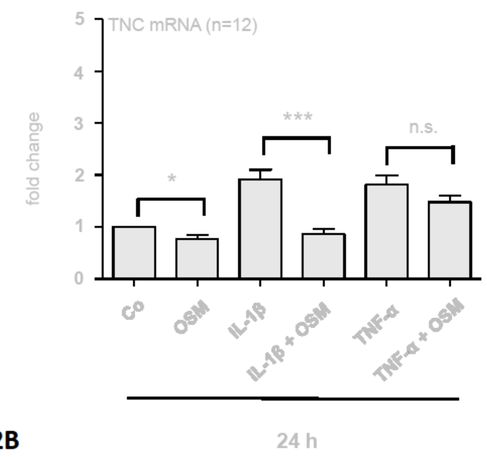

Fig. 1. Effects of OSM on TSP-1 (A) and TNC (B) mRNA expression in the absence and in the presence of IL-1β or TNF-α. HK-2 cells were serum- and supplement-starved for 48 h, and were then stimulated for 24 h with OSM alone, IL-1β alone, TNF-α alone (at a concentration of 10 ng/ml each) or with OSM in the presence of either IL-1β (IL-1β + OSM) or TNF-α (TNF-α + OSM). Data are presented as fold induction above TSP-1 (*A*) or TNC (*B*) mRNA control levels after normalizing to GAPDH mRNA expression. Each data point indicates the average of 12 independent experiments with error bars corresponding to SEM (*$P < 0.05$; ***$P < 0.001$).

Fig. 2A

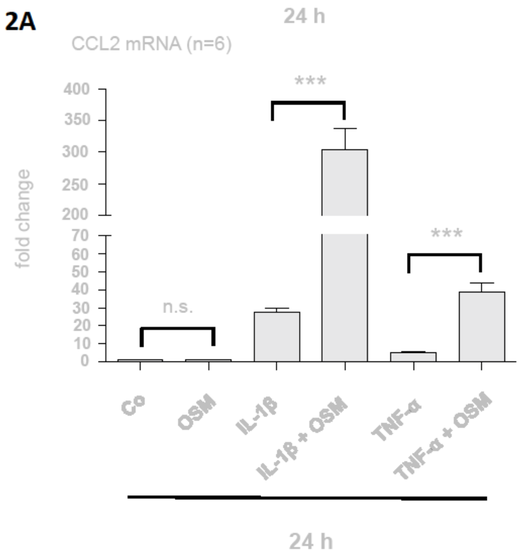

Fig. 2B

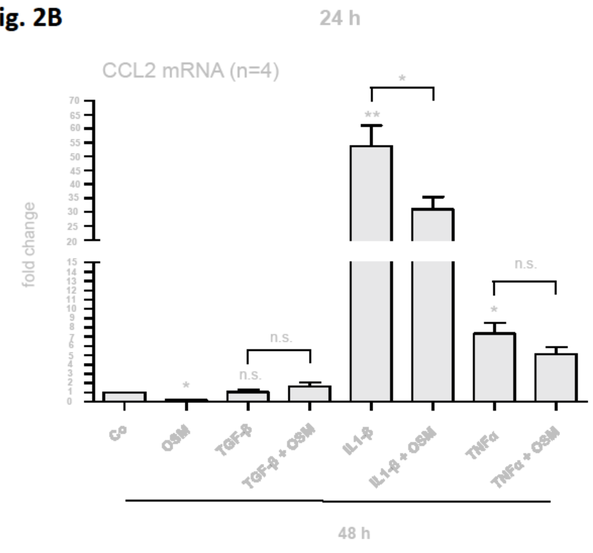

Fig. 2. Effects of OSM on CCL2 mRNA expression in the absence and in the presence of IL-1β, TNF-α or TGF-β1. HK-2 cells were serum- and supplement-starved for 48 h, and were then stimulated for 24 h (A) or for 48 h (B) with OSM alone, IL-1β alone, TNF-α alone, TGF-β1 alone (at a concentration of 10 ng/ml each) or with OSM in the presence of either IL-1β (IL-1β + OSM), TNF-α (TNF-α + OSM) or TGF-β1 (TGF-β1 + OSM). Data are presented as fold induction above CCL2 mRNA control levels after normalizing to GAPDH mRNA expression. Each data point indicates the average of 6 (A) or 4 (B) independent experiments with error bars corresponding to SEM (*$P < 0.05$; **P < 0.01 ***P < 0.001).

Fig. 3A

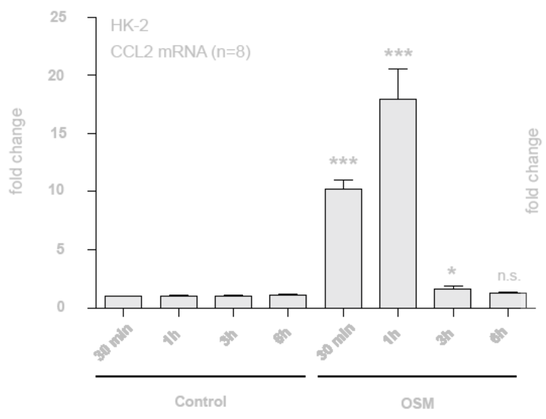

Fig. 3B

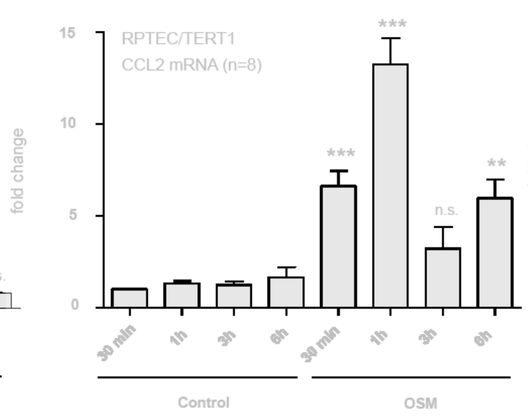

Fig. 3C

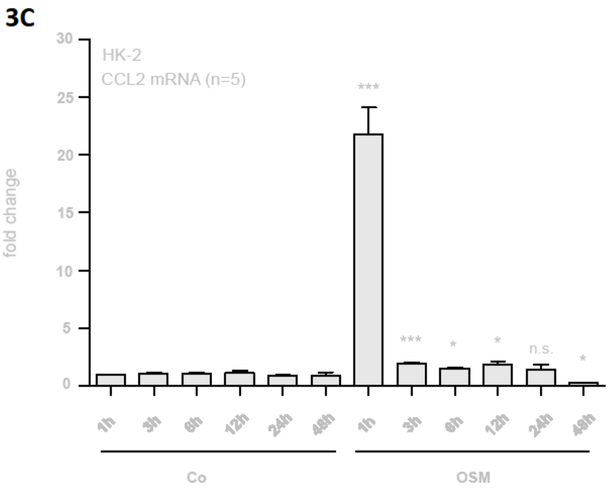

Fig. 3. Time-dependent effects of OSM on CCL2 mRNA expression in human PTC. *A and C*: HK-2 cells were serum- and supplement-starved for 48 h, and subsequently stimulated with 10 ng/ml OSM for the indicated periods of time. *B:* RPTEC/TERT1 cells were grown to a confluent state, serum- and supplement-starved for 48 h, and subsequently stimulated with 10 ng/ml OSM for the indicated periods of time. Data are presented as fold induction above CCL2 mRNA control levels after normalizing to GAPDH mRNA expression. Each data point indicates the average of 8 (*A, C*) or 5 (*B*) independent experiments with error bars corresponding to SEM (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Fig. 4

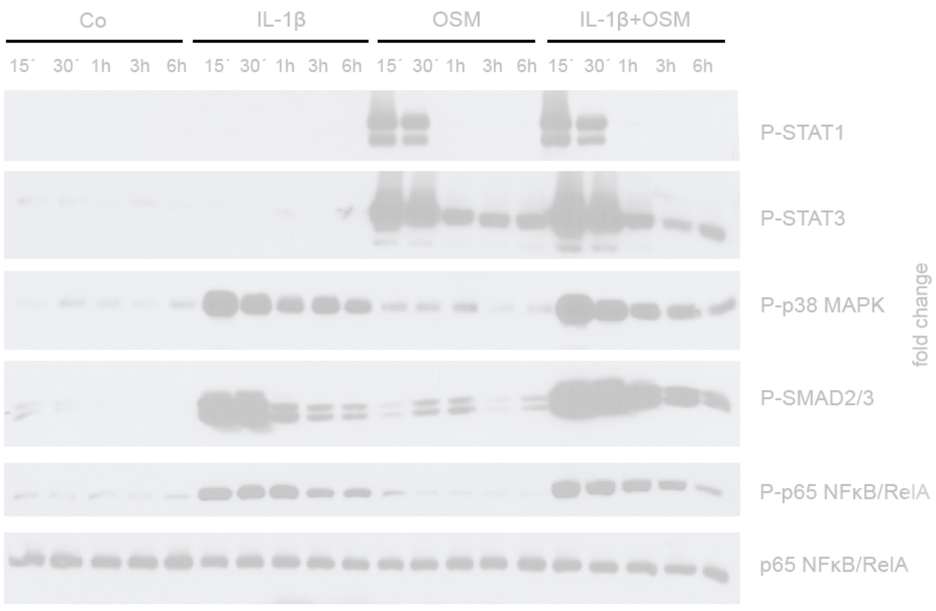

Fig. 4. Time-dependent effects of IL-1β and OSM on phosphorylation of STAT1, STAT3, p65 NFκB/RelA, p38 MAPK and SMAD2/3 in human PTC. Serum starved HK-2 cells were stimulated with IL-1β, OSM (10 ng/ml each) or a combination of the two cytokines (IL-1β + OSM) for the indicated periods of time. Protein expression of p65 NF-κB/RelA indicates equal protein loading. One representative Western blot of 3 separate experiments is depicted.

Fig. 5

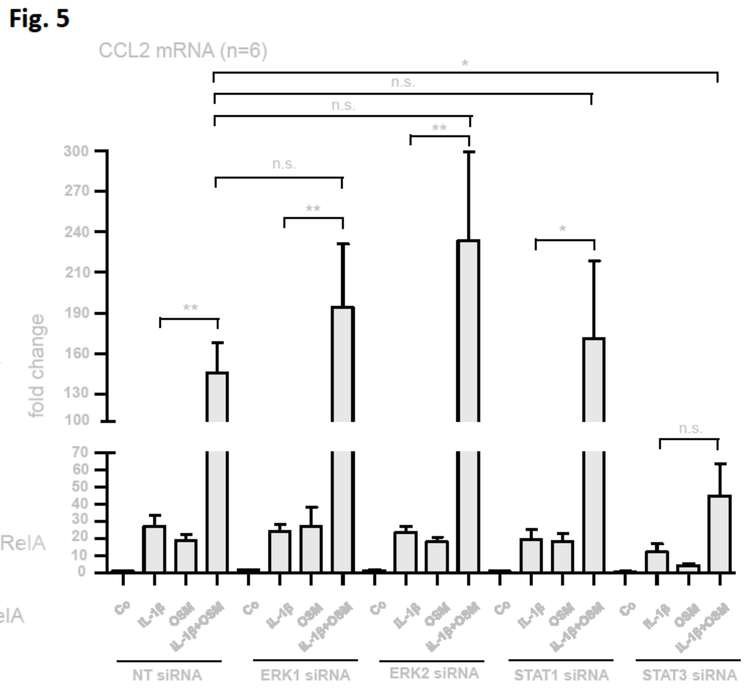

Fig. 5. Silencing of STAT3 attenuates the additive stimulatory effect of OSM on CCL2 mRNA expression in the presence of IL-1β. HK-2 cells were transfected with non-targeting siRNA (NT siRNA), ERK1 siRNA, ERK2 siRNA, STAT1 siRNA or STAT3 siRNA. 72 h post-transfection cells were treated with 10 ng/ml IL-1β alone, 10 ng/ml OSM alone or with a combination of the two cytokines (IL-1β + OSM) for 1 h. The efficiency and selectivity of siRNA treatments and their effect on CCL2 mRNA expression was assessed by real-time PCR analysis. Each data point indicates the average of 6 independent experiments with error bars corresponding to SEM (*$P < 0.05$; **$P < 0.01$).

Fig. 6

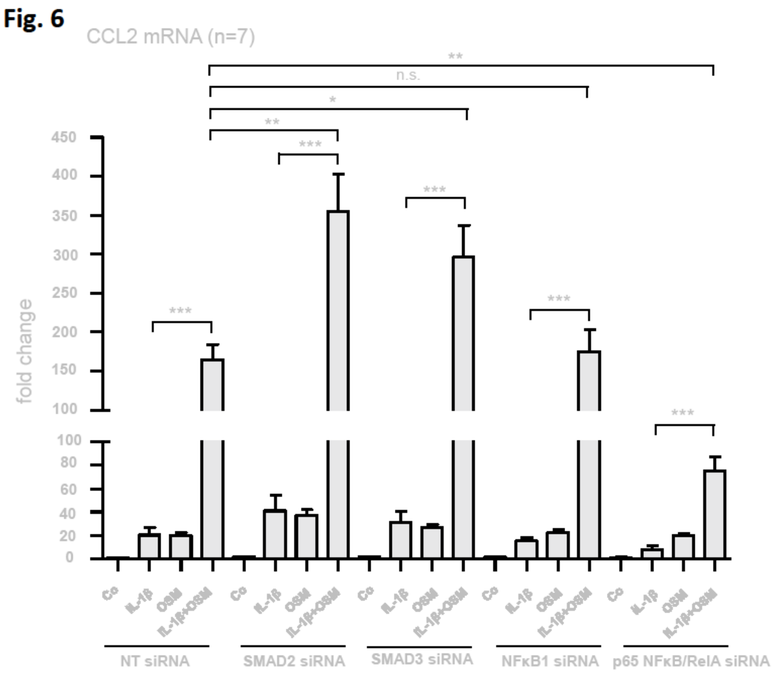

Fig. 6. Differential effects of SMAD2, SMAD3, NFκB1 and p65 NFκB/RelA silencing on CCL2 mRNA expression in HK-2 cells. HK-2 cells were transfected with non-targeting siRNA (NT siRNA), SMAD2 siRNA, SMAD3 siRNA, NFκB1 siRNA or p65 NFκB/RelA siRNA. 72 h post-transfection cells were treated with 10 ng/ml IL-1β alone, 10 ng/ml OSM alone or with a combination of the two cytokines (IL-1β + OSM) for 1 h. The efficiency and selectivity of siRNA treatments and their effect on CCL2 mRNA expression was assessed by real-time PCR analysis. Each data point indicates the average of 7 independent experiments with error bars corresponding to SEM (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Conclusion: In human PTC, OSM in the presence of IL-1β exerts early synergistic effects on the expression of proinflammatory CCL2 via STAT3 and p65 NFκB/RelA, respectively, while inhibiting this gene at later time points, e.g. after 48 h. It is tempting to speculate that, with respect to the expression of the proinflammatory chemokine CCL2, OSM may stimulate acute inflammation via its synergistic effect with other proinflammatory cytokines early after injury, but may attenuate chronic inflammation and fibrogenesis at later time points.

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