THE ROLE OF ONCOSTATIN M SIGNALLING IN SEPSIS AND ORGAN INJURY

Authors: Pang Y. Young MD, Barbara Pedrycz, Valerie A. Luyckx MBBCH, Catherine A. Compston MSc, Thomas F. Mueller MD PhD, Rachel G. Khadaroo MD PhD

Division of General Surgery, Departments of Surgery and Division of Nephrology and Immunology, Department of Medicine, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB

Background

Sepsis is a major clinical problem in critical care that contributes considerably to inpatient morbidity and mortality worldwide. Sepsis is a significant complication in many areas of clinical care, including trauma, burns, and transplantation. Despite significant advances in goal-directed therapy for sepsis and septic shock, mortality remains high (17-42%). The primary contributor to adverse outcomes in sepsis is the development of multiple organ dysfunction syndrome (MODS). MODS can manifest in injury to a wide variety of end-organs, leading to a cascade of effects ranging from acute kidney injury (AKI) to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

Understanding the pathophysiology of the development of MODS in sepsis is critical to the development of new diagnostic and therapeutic measures. The focus of research into the mechanisms of sepsis and MODS has historically been on the activation and dysregulation of the immune and inflammatory response. The current model for the pathogenesis of sepsis describes an initial surge in pro-inflammatory cytokines, described as the systemic inflammatory response syndrome (SIRS). This phase is followed by a rise in anti-inflammatory cytokines, termed the compensatory anti-inflammatory response syndrome (CARS).

Oncostatin M (OSM) is a 28 kDa glycoprotein in the Interleukin-6 (IL-6) family of cytokines. OSM binds either a Type I or Type II OSM receptor (OSMR) leading to activation of intracellular signalling cascades, including JAK-STAT and MAPK pathways. OSM has wide-ranging effects on hematopoiesis, cell growth and differentiation, and remodelling of the extracellular matrix. The utility of OSM in the study of sepsis is that OSM is a significant immunomodulator with both pro-inflammatory and anti-inflammatory activities.

There is limited literature on the role of OSM in sepsis, but studies on patients with sepsis do show that OSM levels increase significantly, up to several hundred-fold. Due to the nature of OSM as an important immunomodulator of the IL-6 family of cytokines, we hypothesize that signalling via the OSM/OSMR axis plays a major role in mediating local and systemic injury in an animal model of sepsis.
Methods

Male wild-type (WT) C57BL/6 and OSMR knockout (OSMR−/−) mice between the ages of 15 - 20 weeks were obtained. A standardized protocol of cecal ligation and puncture (CLP), involving 50% ligation of the cecum with puncture using a 16 gauge needle, was performed on both WT and OSMR−/− animals. Controls consisted of sham laparotomy with full exposure of the cecum, with no ligation and puncture. Animals were closely monitored for a period of 24 hours, and subsequently euthanized.

Serum cytokine levels were measured using a multiplex fluorescent detection system. Tissue homogenates were obtained from lung and kidney tissue. Tissue was analyzed using myeloperoxidase activity assays, quantitative RT-PCR, ELISA, and Western blots.

Statistical analysis was performed using Student's t-test or one-way ANOVA.

Results

A total of 12 WT and 7 OSMR−/− mice underwent the CLP procedure. In addition, 3 controls were performed of each group. Overall mortality in the WT group was 41.7% compared to 0% in the OSMR−/− group, demonstrating that OSMR deficiency may have a protective benefit in this model of abdominal sepsis.

Serum cytokine analysis was carried out on levels of IL-1β, IL-6, IL-10, IL-17, IFN-γ, and TNF-α. There were no significant differences in the levels of IL-1β, IL-17, IFN-γ, and TNF-α between WT and OSMR−/− groups. There was a significant difference between IL-6 levels in WT CLP animals and OSMR−/− CLP animals (41355 pg/mL vs 790 pg/mL, p < 0.05), and with IL-10 levels (4791 pg/mL vs 1293 pg/mL, p < 0.05).

Tissue analysis of both the lung and kidney was performed to determine the effects of OSMR deficiency on end organ injury. Myeloperoxidase (MPO) activity assays were performed on lung tissues as a surrogate measure of neutrophil infiltration, as a marker of lung injury. There was no statistical difference in lung injury between WT and OSMR−/−. There was evidence of kidney injury measured by two methods. Serum blood urea nitrogen (BUN) was measured demonstrated significantly higher levels of BUN in WT animals compared to OSMR−/− mice (121 mg/dL vs 36 mg/dL, p<0.01). Quantitative RT-PCR showed a trend towards higher renal tissue levels of the pro-inflammatory cytokine, IL-6 (36.89 pg/mL vs. 0.15 pg/mL, p=0.19), and significantly higher levels of the tissue injury marker, Ngal (3036 pg/mL vs. 712 pg/mL, p=0.049), in WT animals compared to OSMR−/− animals.

Discussion

Elucidating the activation of inflammatory signalling pathways is critical to a clear understanding of the pathogenesis of sepsis and MODS. This study focused on OSM, a member
of the IL-6 family of cytokines, which acts as an immunomodulatory cytokine. These findings suggest that OSM plays an important role in mediating inflammatory signalling.

Functional deficiency of OSM through knockout of the OSMR gene appears to provide a beneficial effect on survival in sepsis, with a trend towards decreased mortality in OSMR\(^{-}\) animals. The effects on the systemic cytokine profile and end organ injury was investigated to determine the role of a functional deficiency of the OSM/OSMR axis. The systemic cytokine profile demonstrated notably attenuated of IL-6 and IL-10 levels in OSMR\(^{-}\) animals. The effects on lung and kidney were investigated due to the prominent role of lung and kidney injury in MODS. There was no significant difference in lung injury, but significantly reduced kidney injury with OSMR deficiency.

The findings of this study align with the current model for the pathogenesis of sepsis, in which there is dysregulation of the pro-inflammatory and anti-inflammatory response, highlighted by SIRS and CARS. Blunting of both responses in the OSMR\(^{-}\) mice reduced kidney injury and provides a trend towards improved survival. OSM/OSMR signalling may be one of the early upstream signals in acute inflammatory signalling in sepsis.

Additionally, the role of OSM appears to be much more specific to stimulating the renal acute phase response. Further investigation is required to outline the degree of organ specificity of the OSM response, and to determine the role of OSM in mediating injury in other organs, such as in the heart or liver.

There are limitations with this study. Long term effects of OSM/OSMR deficiency are unknown. Based on mice studies using IL-6 knockouts, there are deleterious effects on hepatic acute phase response and hepatocyte regeneration following injury. Blunting of the IL-6 response through inhibition of the OSM axis may result in similar effects on healing and repair. Further studies are underway to examine the longer term effects of the OSMR deficiency in the septic model. Additional studies to examine the effect of direct inhibition of OSM through targeted antibodies at the time of sepsis are also merited.

**Conclusion**

The OSM/OSMR axis plays a significant role in immune activation in a CLP model of sepsis. Deficiency of the OSM/OSMR axis leads to suppression of the release of pro-inflammatory and anti-inflammatory cytokines, leading to significant alteration of the systemic cytokine profile. There is evidence of reduced inflammation and injury in the kidney, suggesting a role in organ-specific activation of an injury response during sepsis. Elucidation of this unique pathway may suggest a novel strategy for the prevention and treatment of sepsis and associated organ injury.