Protein kinase R modulates NLRP3 inflammasome in cardiac fibroblasts in sepsis

Xuemei Xu1, Aibin Tao1,2, Raymond Kao1, Claudio Martin1, Tao Rui1,2,3, 1Lawson Health Research Institute, London, ON, Canada. 2The Affiliated People's Hospital of Jiangsu University, Zhenjiang, China, People's Republic of. 3University of Western Ontario, London, ON, Canada

Background: We have reported that sepsis leads to an activation of NLRP3 inflammasome in cardiac fibroblasts (CF) resulting in IL-1β maturation and extracellular release. Increase in IL-1β by the CF induces myocardial dysfunction. The aim of the present study is to identify a modulation pathway that activates NLRP3 inflammasome in the CF. Materials and Methods: CF were isolated and cultured from adult mouse hearts. The CF were primed with LPS (1 μg/ml) for 6 hrs followed by 30 min of ATP (3 mM) treatment to activate NLRP3 inflammasome. PKR activation was assessed by detecting CF caspase-1 p20 (Western) and IL-1β release (ELISA). Results: LPS priming of CF resulted in PKR activation as indicated by increase in PKR phosphorylation at Thr451, and increase in NLRP3 and pro-IL-1β protein expression. Inhibition of PKR (PKR inhibitor and siRNA) prevented the LPS-induced NLRP3 and pro-IL-1β expression in the CF. Inhibition of PKR in CF primed with LPS before ATP showed no effect on NLRP3 and pro-IL-1β expression, but prevented the NLRP3 inflammasome activation as indicated by abolishing the caspase-1 activation and IL-1β releasing. In addition, pretreatment of CF with peroxynitrite (ONOO⁻) decomposition catalyst, FeTPPs, before LPS, prevented the LPS induced PKR phosphorylation, NLRP3 and pro-IL-1β expression in CF. Furthermore, pretreatment of CF with FeTPPs before ATP prevented the NLRP3 inflammasome activation in CF. Conclusion, our results indicate that ONOO⁻/PKR pathway modulates priming and activation of NLRP3 inflammasome in CF in sepsis.

Support or Funding Information
IRF of Lawson Health Research Institute (IRF 2014-25); The Natural Science Foundation of Jiangsu Province, China (BK2015-1332).