ImmunoTools *special* Award 2018

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Antimicrobial proteins (AMPs) and myocardial cytokine release

Bacterial infections are one of the major threats for human health, still claiming millions of lives each year. In particular, a pathology known as sepsis that arises subsequent to microbial infections is a prominent challenge to modern medicine. Despite intense effort in basic and clinical research the morbidity and mortality of sepsis and septic shock have remained high. Sepsis encompasses a spectrum of illness that ranges from minor symptoms to systemic inflammatory response and multiple organ dysfunction, with the heart as one of the most frequently affected organs . Mortality due to septic cardiac dysfunction has been encountered especially in critically ill patients , where an increased heart rate may act as an independent risk factor.

The majority of cases of septic cardiomyopathy is caused by a disproportional immune response to e.g. LPS from Gram-negative bacteria. Conventional antibiotics may kill bacteria, but simultaneously release bacteria-derived wall-fragments, such as LPS, which causes systemic and cardiac inflammation and Ca^{2+}-dysregulation during septic cardiomyopathy. The released microbial stimuli are recognized by cell surface receptors of the innate immune system, such as CD14 and Toll-like receptors (TLR). Receptor binding activates intracellular signaling cascades leading to the release of potent inflammatory mediators as interleukins, tumor necrosis factor alpha (TNF-α), gamma interferon (IFN-γ), macrophage inflammatory protein-2 (MIP-2), prostaglandin E2, and reactive oxygen species (ROS). These factors cause immune cell activation and migration into tissues, endothelial leakage, coagulation, and other profound physiological alterations that, when combined, lead to tissue hypoperfusion and organ failure.

Although the main sources of cytokines in the heart are infiltrating/resident immune cells and cardiac fibroblasts, cardiomyocytes contribute to the inflammatory reaction, particularly via secretion of IL-6 and TNF-α.

Besides initiating the inflammatory response, LPS directly affects cardiac function via myocardial TLR-4 signaling or by direct interaction with HCN channels conducting the pacemaker current I_f .

Antimicrobial peptides (AMPs) are small biological molecules (<10 kDa) and were identified as important parts of the innate immunity in species ranging from bacteria and insects to mammals. Unlike conventional antibiotics, some AMPs are known to kill bacteria without releasing pro-inflammatory factors, thus preventing the devastating consequences of the pro-inflammatory cascades in severe sepsis and septic shock. In addition to their broad-spectrum activity against bacteria, fungi, and viruses they have the ability to bypass the common resistance mechanisms of standard antibiotics. The newly designed synthetic antimicrobial peptide 19-2.5 (Pep2.5) belongs to the class of synthetic anti-lipopolysaccharide peptides. It was demonstrated that Pep19-2.5 neutralizes the inflammatory responses triggered by both Gram-negative and Gram-positive bacteria and reduces levels of inflammatory cytokines in human tissue and a murine sepsis model.

Recent reports strongly suggest that the interaction between leukocytes, non-myocytes (mainly cardiac fibroblasts) and cardiomyocytes, possibly mediated by cytokine signaling, plays an important role in controlling the inflammatory reaction. Therefore, controlling cytokine secretion from resident cardiomyocytes is one plausible strategy for preventing tissue damage.

The aim of this study is to investigate the effect of Pep19-2.5 on the release of inflammatory mediators by isolated murine and human cardiomyocytes in response to LPS treatment. Especially, we want to study the release of the main inflammatory mediators that contribute to myocardial depression in sepsis including interleukins (IL-1β, IL-2, IL-6), IFN-γ and TNF-α using qPCR and Elisa. Further, we want to evaluate whether Pep19-2.5 affects the expression of TLR-4 and CD14 in LPS treated cardiomyocytes via qPCR and FACS analysis. This study will contribute to a project investigating the molecular mechanism of sinoatrial beat-to-beat variability under septic conditions.

Cohen J. The immunopathogenesis of sepsis. Nature. 2002; 420(6917):885-891.
2. Fernandes CJ, Jr. and de Assuncao MS. Myocardial dysfunction in sepsis: a large, unsolved puzzle. Critical care research and practice. 2012; 2012:896430.
3. Hoke RS, Muller-Werdan U, Lautenschlager C, Werdan K and Ebelt H. Heart rate as an independent risk factor in patients with multiple organ dysfunction: a prospective, observational study. Clinical research in cardiology : official journal of the German Cardiac Society. 2012; 101(2):139-147.
4. Flierl MA, Rittirsch D, Huber-Lang MS, Sarma JV and Ward PA. Molecular events in the cardiomyopathy of sepsis. Molecular medicine. 2008; 14(5-6):327-336.
5. Schuerholz T, Brandenburg K and Marx G. Antimicrobial peptides and their potential application in inflammation and sepsis. Crit Care. 2012; 16(2):207.
6. Alexander C and Rietschel ET. Bacterial lipopolysaccharides and innate immunity. Journal of endotoxin research. 2001; 7(3):167-202.
7. Annane D, Bellissant E and Cavaillon JM. Septic shock. Lancet. 2005; 365(9453):63-78.
8. Porter KE and Turner NA. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmacol Ther. 2009; 123(2):255-278.

9. Aoyagi T and Matsui T. The Cardiomyocyte as a Source of Cytokines in Cardiac Injury. Journal of cell science & therapy. 2011; 2012(S5).
10. Fallach R, Shainberg A, Avlas O, Fainblut M, Chepurko Y, Porat E and Hochhauser E. Cardiomyocyte Toll-like receptor 4 is involved in heart dysfunction following septic shock or myocardial ischemia. Journal of molecular and cellular cardiology. 2010; 48(6):1236-1244.
11. Scheruebel S, Koyani CN, Hallstrom S, Lang P, Platzer D, Machler H, Lohner K, Malle E, Zorn-Pauly K and Pelzmann B. I(f) blocking potency of ivabradine is preserved under elevated endotoxin levels in human atrial myocytes. Journal of molecular and cellular cardiology. 2014; 72:64-73.
12. Heinbockel L, Sanchez-Gomez S, Martinez de Tejada G, Domming S, Brandenburg J, Kaconis Y, Hornef M, Dupont A, Marwitz S, Goldmann T, Ernst M, Gutsmann T, Schurholz T and Brandenburg K. Preclinical investigations reveal the broad-spectrum neutralizing activity of peptide Pep19-2.5 on bacterial pathogenicity factors. Antimicrob Agents Chemother. 2013; 57(3):1480-1487.
13. Schuerholz T, Doemming S, Hornef M, Martin L, Simon TP, Heinbockel L, Brandenburg K and Marx G. The anti-inflammatory effect of the synthetic antimicrobial peptide 19-2.5 in a murine sepsis model: a prospective randomized study. Crit Care. 2013; 17(1):R3.