Cytokine Expression In Heart Tissue of Caspase 1 Knock Out, Community-Acquired MRSA Sepsis infected Mice

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Abstract

Intro: Community-acquired MRSA sepsis is an important clinical problem leading to multiple organ failures, including cardiac dysfunction. Studies show caspase 1 inhibition reduced myocardial dysfunction in other cases such as heart failure and ischemia-reperfusion injury. In this study, we seek to identify the role of caspase 1 in sepsis-associated myocardial cytokine expression.
Materials and Methods: Caspase 1 KO mice and control NOD1 mice were infected intravenously with CA-MRSA strain TCH1516. RNA was isolated from heart tissue and quantitative PCR was performed to measure cytokine expression levels.
Results: Caspase 1 KO mice showed no significant change in mortality. However, TNF-α, IL-1β, and IL-6 expression in the heart were reduced significantly, especially in later stages of infection.
Discussion: Caspase 1 did not lead to reduced mortality, but it reduced expression of inflammation makers. This suggests other cytokines not affected by caspase 1 or alternative mechanisms may contribute to cardiac dysfunction. Reduced inflammation due to lack of caspase 1 may be contributing to mortality as well.

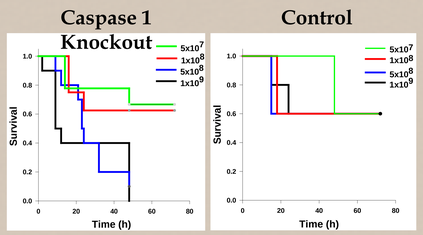
Introduction

- *Staphylococcus aureus* is a gram-positive bacterium that naturally colonizes many areas of the human body.[1]
- It is a pathogen that causes many infections, including soft tissue infections, pneumonia, and sepsis.[2]
- Methicillin resistant *S. aureus* (MRSA) originally evolved in hospital settings.[3]
- In the 1990s, new community-acquired MRSA strains (CA-MRSA) appeared in individuals with no hospital exposure and are responsible for a large and increasing portion of MRSA infections.[3]
- Sepsis is associated with bacterial infections where an excessive inflammatory response can lead to multiple organ dysfunction syndrome which includes myocardial dysfunction.[4]
- Previous studies observed an increase of inflammation markers TNF-α and IL-1βin cardiomyocytes of gram negative bacterial sepsis animal models.[5]
- The pathophysiology and mechanism of myocardial dysfunction in CA-MRSA sepsis have not been studied.
- Caspase 1 is associated with cardiac dysfunction and up regulation of of TNF-αand IL-1βin heart failure and ischemia-reperfusion injury. Therefore, we hypothesized that capase1 knock-out mice would display lower rates of cardiac dysfunction-associated mortality and less cardiac up regulation of pro inflammatory mediators.[6,7]
- In this experiment, cytokine expression levels of TNF-α, IL-1β, and IL-6 in CA-MRSA infected mice was measured to identify their role in the mechanism underlying CA-MRSA sepsis.

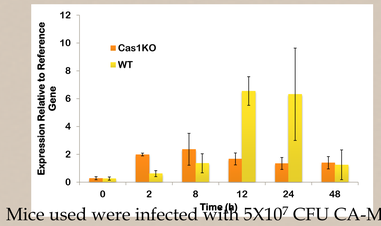
Materials and Methods

- **Infection:** CA-MRSA strain TCH1516, a clinical isolate from a lethal case of sepsis, was obtained from ATCC.[8] Cas1 KO mice and control NOD1 mice were infected intravenously via tail vain.
- **Echocardiography:** The fractional shortening of the mice hearts was calculated following echocardiography data obtained using an Acuson Sequoia 512 echocardiogram machine with a 15L8 8-14 mHz probe.
- **Heart Tissue:** At indicated time points after infection, CO_2 inhalation was used to kill the mice, and the hearts were removed and flash frozen in liquid nitrogen.
- **RNA Isolation:** Heart tissue was ground in glass dounces with PureZol (Bio-Rad), and RNA was isolated from heart tissue using the Aurum™ Total RNA Fatty and Fibrous Tissue Kit (Bio-Rad).
- **Quantitative PCR**: RNA was reverse transcribed to produce cDNA, and Q-PCR was performed using the SybrGreen mix (Bio-Rad). Primers were optimized to concentrations and annealing efficiencies of 90-105%. Constitutively expressed genes, HPRT1 and Rpl32 , were used as reference genes.

Mouse Survival at different Infection doses of CA-MRSA

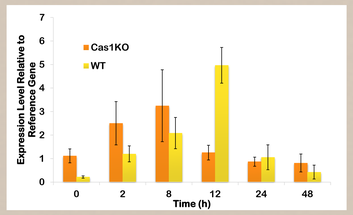


Expression Levels of IL-1β In hearts of CAS1 KO and control Mice infected with CA-MRSA



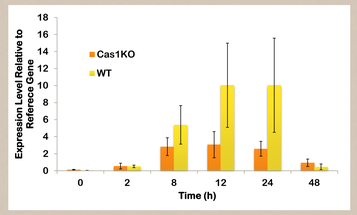
- Mice used were infected with 5X10^7 CFU CA-MRSA

Expression Levels of TNF-α in hearts of CAS1 KO and control Mice infected with CA-MRSA



- Mice used were infected with 5X10^7 CFU CA-MRSA

Expression Levels of IL-6 in hearts of CAS1 KO and control Mice infected with CA-MRSA



- Mice used were infected with 5X10^7 CFU CA-MRSA

Results

- Mice infected with fewer than 5 x 10^7 CFU showed lower and delayed mortality relative to higher CFU infections but displayed measurable illness phenotypes.
- Echocardiography, in our lab, showed substantially reduced shortening fractions in Cas1 KO mice at 8 hours, and a non-significant trend towards a reduced shortening fraction in WT mice at 12 hours (Data not shown).[9]
- Caspase 1 KO mice displayed a trend towards greater mortality at higher inocula, but this was not statistically significant.
- IL-1βand IL-6 showed greatest up-regulation at 12 and 24 hour marks of disease. These up regulation were reduced substantially in caspase 1 KO mice however ($p<0.05$).
- TNF-α also showed a substantial down regulation at the 12 hour mark of infection in caspase 1 KO mice compared to WT mice ($p<0.05$).

Discussion

- The mechanism of septic cardiac dysfunction is still not fully understood, and most previous studies investigated gram-negative bacterial sepsis models only.
- Previous studies linked caspase 1 to myocardial dysfunction in heart failure and ischemia repercussion injury. Inhibition of all caspases has been shown to reduce cardiac dysfunction in gram negative septic models.[6,10]
- Our results showed mortality rates were equivalent or greater in CA-MRSA infected Caspase 1 KO mice than in WT mice, despite lower myocardial expression of the major inflammatory mediators TNF-α, IL-1β, and IL-6, previously associated with myocardial dysfunction ..
- Possible mechanisms that may contribute to these results:
  - More cytokines are contributing to myocardial dysfunction and death than those associates with caspase 1 in this study.
  - Eliminating caspase 1 activity is detrimental resulting in insufficient inflammation needed to fight infection.
  - Absence of caspase 1 may trigger an alternative, acute apoptotic pathway.[11,12]
- **Future Directions**:
  - Perform RNA microarray assay on heart tissue to identify other cytokines/inflammation mediators up regulated in septic shock.
  - Use caspase 1 inhibitors instead of Knocking out gene.

References

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