CICMD Abstract

Activation of cardiomyocyte NLRP3 inflammasome via calcium mobilization

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Background: Myocarditis is inflammation of the myocardium, typically caused by infection. Myocarditis occurs in over 1.5 million patients annually and can lead to adverse cardiac events, such as arrhythmia, dilated cardiomyopathy, and sudden death. The NOD-like receptor pyrin-containing protein 3 (NLRP3) inflammasome has been shown to correlate with myocarditis severity. Calcium can activate the NLRP3 inflammasome, but the current literature does not address whether this occurs in cardiomyocytes during myocarditis. I hypothesize that, in myocarditis, cardiomyocytes undergo NLRP3 inflammasome activation in response to calcium mobilization following viral infection. The membrane attack complex (MAC) is a pore that forms on the cell surface following complement cascade activation. MAC is a proposed source of external calcium that floods the cell. Sarcoplasmic reticulum calcium ATPase 2a (SERCA2a) is a pump on the sarcoplasmic reticulum that clears calcium from the cytoplasm to storage. SERCA2a is the proposed mechanism of internal calcium dysregulation for this study.

Methods: I investigated the external source of calcium *in vitro* by observing calcium kinetics in cardiomyocytes with MAC formation using live-cell imaging. Using fluorescently fixed cell imaging, I observed NLRP3 inflammasome activation in cardiomyocytes, accompanied by MAC formation. To elucidate the impact of internal calcium dysregulation *in vitro*, I conducted a Caspase-1Glo Assay on coxsackievirus B3-infected cardiomyocytes transfected with a SERCA2a modulator. In addition to *in vitro* investigations, I examined the efficacy of an adeno-associated virus that modulates SERCA2a and apoptosis activity in our viral myocarditis mouse model by RT-PCR and histology.

Results: Calcium dynamics differed between MAC-positive and MAC-negative groups and are being further assessed based on rise and decay rates. The NLRP3 inflammasome showed higher activation levels in MAC-positive groups compared to MAC-negative groups. The Caspase-1Glo Assay revealed that infected cells with SERCA2a modulation had significantly decreased caspase-1 activity (NLRP3 inflammasome activity; $p=0.0134$). *In vivo,* mice that received the adeno-associated virus with a bicistronic vector modulating SERCA2a and apoptosis had significantly less inflammation, as determined by histology ($p=0.0117$), as well as lower caspase 1 and Nlrp3 inflammasome gene expression, as determined by RT-PCR ($p=0.0040$; $p=0.0045$, respectively).

Conclusions: MAC and SERCA2a both can influence calcium dynamics and NLRP3 inflammasome activation in cardiomyocytes *in vitro*. The literature suggests that an influx of extracellular calcium can trigger SERCA2a dysfunction *in vivo.* Therefore, the interplay between the two mechanisms needs to be investigated. Furthermore, I will determine whether MAC inhibition significantly reduces inflammasome activation in vivo and whether the therapeutic rescues cardiac function during myocarditis, as assessed by echocardiogram and electrocardiogram.