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Knock Out of Cell Death Pathway Components Results in Differential Caspase Expression in Response to HCV Infection †

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Abstract: Introduction: Pyroptosis (inflammatory programmed cell death) is induced after the activation of an inflammasome, ultimately resulting in pore formation and cell lysis. One factor in the pathology associated with chronic hepatitis C virus (HCV) infection is non-inflammatory caspase-3-mediated apoptosis. Our lab has found both apoptosis and pyroptosis occurring in HCV-infected Huh-7.5 cells. In the context of some viral infections, pyroptosis is beneficial to the virus; for others, pyroptosis is believed to represent an innate antiviral response. This study aimed to test the effects of knocking out components of the inflammasome pathway on caspase activation in HCV-infected cells. Methods: FAM-FLICA (Carboxyfluorescein - Fluorochrome Inhibitor of Caspases) probes or antibodies were used to visualize active caspase-1 and active caspase-3 in vitro. Huh-7.5 cells with components of the pyroptotic or apoptotic pathways knocked out (NLRP3, GSDM-D or caspase-3) were used to determine the effects of their absence on the virus and caspase activation using confocal microscopy and flow cytometry. Results: Increased levels of caspase-1 were consistently observed in HCV-infected cells compared to those in uninfected cells, and these levels increased with subsequent days post-infection. The inhibition of inflammasome activation using knock out cell lines induced the differential activation of caspase-1 and caspase-3, with the inhibition of pyroptosis, resulting in a trend towards greater expression of caspase-3, indicative of apoptosis. The inhibition of NLRP3 did not fully stop caspase-1 activation, but it was decreased. The flow cytometry results revealed a small sub-set of cells positive for both caspase-1 and caspase-3. Conclusions: These data confirm the occurrence of pyroptosis in HCV-infected cells and demonstrate the involvement of the NLRP3 inflammasome, although other inflammasome sensors might be involved. Since the inhibition of one cell death pathway resulted in the increased activation of the other, along with the presence of double-positive cells, there may be cross-talk between apoptotic and pyroptotic pathways; the role of this cross-talk during infection remains to be elucidated.

Keywords: Hepatitis C Virus; Pyroptosis; Apoptosis; Programmed Cell Death; Inflammasome