Determining the Roles of Caspase-4 and Caspase-5 in Inflammasome Activation

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Innate immune system detection of bacterial pathogens within the cytosol leads to the formation of a multiprotein complex termed the inflammasome, which results in proinflammatory cytokine secretion and cell death. An alternative mechanism of inflammasome activation, called the noncanonical inflammasome, initiates inflammatory responses to lipopolysaccharide (LPS), the major lipid component found on the outer membrane of gram-negative bacteria. The human noncanonical inflammasome is composed of the cysteine proteases caspase-4 (CASP4) and caspase-5 (CASP5). Previous work from our lab determined that CASP4 activation leads to cell death and IL-1 family cytokine secretion in human macrophages either infected with *Legionella pneumophila* or transfected with *Escherichia coli* LPS, whereas the role of CASP5 remains unknown in this setting. Additionally, IFN-γ is a cytokine that induces expression of CASP4 and CASP5. In this study, we explore the roles of CASP4 and CASP5 in detection of LPS in the context of IFN-γ priming. We hypothesize that CASP4 and CASP5 work synergistically to induce cell death and IL-1β secretion in response to LPS in the context of IFN-γ priming. We transfect LPS in differentiated THP-1 cells and measure downstream inflammasome responses using cell death and cytokine release assays. Our results show that IFN-γ promotes inflammasome activation in LPS transfection. Next, we will transfect IFN-γ-primed wild-type THP-1 cells and CASP4 or CASP5 CRISPR/Cas-9 knock out cells with LPS. This study will elucidate aspects of human innate immune responses to gram-negative bacterial pathogens.