The role of caspase death proteases in the control of intracellular bacterial infections

Safaa Bouheraoua

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Caspases are a family of intracellular proteases that play a critical role in programmed cell death and inflammation. Upon activation, they react in a cascade which results in substrate cleavage and cell death. Programmed cell death is an important tool to remove damaged and infected cells with minimal damage to their surroundings. Therefore, in the context of intracellular bacterial infections, programmed cell death can be used to clear infected cell and remove the replicative niche of the infecting bacteria.

The aim of this project was to identify the role of caspase death proteases in the control of intracellular bacteria. The objectives were to investigate if bacterial infections activate caspases, if caspase activity affects intracellular bacterial growth, and if caspases are able to cleave bacterial effector proteins, which might potentially lead to their inactivation. These questions were examined by establishing infection models using HeLa cells and monocyte derived macrophages (MDM). The cells were infected with Salmonella typhimurium, Listeria monocytogenes wild-type (WT), Listeria monocytogenes ΔLLO, and Mycobacteria bovis BCG.

Infection of HeLa cells with virulent fast-growing bacteria resulted in activation of caspase-3, 7, & 9 as well as cleavage of the caspase-3 substrate parp1. Despite the considerable caspase activity, these infections caused minimal specific cell lysis over the course of the experiment suggesting impaired bacterial dissemination from dead host cells with diminished plasma membrane integrity. Treating infected cells with caspase-3 and -7 inhibitor DEVD or the pan-caspase inhibitor zVAD caused an increase in intracellular bacterial growth while caspase activation using staurosporine caused a decrease in intracellular bacterial growth over time.

Cleavage sites of several bacterial effector proteins by executioner human caspases were predicted using a bioinformatic cleavage website. The predicted cleavage site of listeriolysin O (LLO) by human caspase-7 was identified and tested by western-blot of Listeria monocytogenes WT infected HeLa cells. A possible cleavage product of LLO was observed on an immunoblot suggesting that active caspase-7 directly cleaves and potentially inactivates the major listeria virulence factor LLO. Unfortunately, the MDM infection model did not produce reproducible results.

Hence, it can be concluded that caspase activity does affect intracellular bacterial growth in HeLa cells, potentially by the caspase mediated destruction of major virulence factors.

Director: Professor Michael Walch