ABSTRACT

Project Code: RSA5580047

Project Title: STRUCTURAL DETERMINANTS OF MEMBRANE CHANNEL FORMATION AND RECEPTOR RECOGNITION WITHIN THE VACUOLATING CYTOTOXIN VacA – A MODEL VIRULENCE FACTOR FROM HUMAN PATHOGENIC *HELICOBACTER PYLORI*

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The vacuolating cytotoxin (VacA) from human-pathogenic *Helicobacter pylori* represents one of the most intensively characterized virulence factors of this bacterium. It is involved in the colonization of the gastric epithelium, the establishment of a prolonged inflammation and induces a multitude of cytopathic symptoms in the affected host. Within this project, it was proposed to establish molecular biological and biochemical work on VacA with a view to structure-mechanism relations. As practically no technical experience with VacA was available when the project started, the work was initiated by evaluating basic procedures for cloning, expression, purification and biochemical assays for VacA. In the first phase of this project, we have accomplished isolation, purification to homogeneity and an *in vitro* assay of apoptotic activity of a VacA toxin obtained from a Thai patient with gastrointestinal lymphoma. Sequencing revealed that the Thai isolate VacA is structurally similar to *H. pylori* s1/m2 type strains, whereas homology to the 60190 model strain was found to be lower. The purified VacA toxin exhibited significant apoptotic activity on two epithelial cell lines, T84 and MDCK, as revealed by DAPI staining, whereby the observed activity was significantly higher on MDCK cells. Preliminary experimental data have shown, however, that yield and purity of the recombinant VacA protein were insufficient for further biochemical and biophysical experiments, in particular for an intended x-ray crystallographic analysis of the pore-forming p33 domain. Therefore, further investigations on expression and purification of VacA led us to an intensive characterization of product toxicity within the recombinant *E. coli* host. We were able to demonstrate that biosynthesis of subdomains of VacA containing the p33 moiety was conducive to stalled growth of the host and low product yields. Currently we explore an improved expression system which uses a VacA-DHFR fusion protein that appears to be non-toxic for *E. coli* and offers substantial advantages for purification of recombinant VacA.

Keywords: *Helicobacter pylori*; VacA cytotoxin; sequence; apoptosis; assay; fusion protein.