ORT_32 - Klebsiella pneumoniae VgrG4 protein: structural aspects and functional characterization

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Introduction: *Klebsiella pneumoniae* (KP) is an opportunistic pathogen that is of concern to public health systems around the world, as multi-resistant isolates are frequently identified. One of its virulence factors is the Type VI Secretion System (T6SS), a macromolecular complex that may translocate effector proteins. VgrG proteins are structural components of the tip of T6SS but may also contain a variable C-terminal extension with an effector role. Moreover, VgrGs display antigenic sequences and have successfully immunized mice against bacterial infections. In a previous study, we identified that at least 100 KP isolates present a VgrG containing a conserved C-terminal domain (CTD) of 138 amino acids, although its function is not yet known. Among them, there is the VgrG4 protein from Kp52145 strain. VgrG4- CTD interacts with cytoskeletal proteins and induces the remodeling of actin filaments in macrophages.

Objectives: The aim of this project is to characterize the VgrG4 and VgrG4-CTD proteins.

Methodology: The structure of the VgrG4-CTD was assessed by intrinsic tryptophan fluorescence (ITF) spectroscopy and nano-differential scanning fluorimetry (NanoDSF). Epitopes were predicted using computational methods. VgrG4-CTD and VgrG4 were complexed to a transfection reagent and delivered to macrophages. Infection assays and fluorescence microscopy were performed to analyze the cell cytoskeleton, reactive oxygen species (ROS) production, and expression phagosomal maturation-related proteins. It was also verified whether the proteins were able to modify KP internalization in macrophages by flow cytometry.

Results: ITF experiments revealed a maximum fluorescence of 337 nm to VgrG4-CTD protein and stability study by NanoDSF revealed increase of 350 nm/330 nm fluorescence intensity ratio as a function of temperature, where Tm (inflection temperature of thermal unfolding) was 45.56 ± 4.73°C. Preliminary results suggest that both VgrG4-CTD and VgrG4 proteins were able to induce alterations in the actin cytoskeleton. Moreover, both proteins seem to induce ROS production and to induce RAB7 expression in macrophages. Interestingly, stimulation with both proteins does not appear to play a role in KP internalization in macrophages.

Conclusion: Preliminary data brings interesting insights for the functional characterization of the VgrG4 protein for KP-mediated infection, contributing to the understanding of the molecular mechanisms involved in the host-pathogen interaction.

Keywords: Type VI Secretion System, VgrG, host-pathogen interaction