Title of the PhD thesis:

The molecular mechanisms of the interaction of *Francisella tularensis* and antigen presenting cells

Abstract:

Over the past few decades, reductionist approaches dominated in the study of biological systems and helped to understand many basic principles that underlie host-pathogen interaction. Nevertheless, recent advances in genomics, transcriptomics and proteomics have enabled to design new type of experiments and get thus novel information of all the components of biological systems, as well as to characterize interaction among them. Profiling of proteins, as main carriers of biological activity, is especially of great interest in order to elucidate the molecular mechanisms of infection in space and time.

Nowadays, there is a need to develop new antibiotic and anti-viral drugs, extensively investigate the respective infectious agents in regard to pathogen-specific signaling processes or enzymes that do not occur in human cells. However, pathogens have evolved different strategies to avoid the host defense mechanisms. The fact that pathogens exploit many factors of the host cell signaling machinery for finding safe niche for their replication provides the basis for an alternative, host-directed strategy to prevent establishing of infection in a host.

This PhD thesis applies quantitative proteomics approach to research topics in relation to the interaction of *Francisella tularensis* with macrophages from the host point of view. Two major biology topics are studied in this thesis.

The first part of the PhD thesis was focused on the key signaling pathways controlling the entry of *Francisella tularensis* LVS into murine J774.2 macrophages. Firstly, we have shown that plasma membrane organization of membrane rafts is critical to *Francisella tularensis* internalization into macrophages. Disruption of host membrane raft organization by cholesterol-depleting agent, methyl-β-cyclodextrin, and cholesterol-binding reagent, filipin, resulted in the inhibition of bacterial uptake. Moreover, we employed quantitative stable isotope labeling by amino acids in cell culture-proteomic approach in order to elucidate dynamic changes in the protein composition of membrane raft region upon *F. tularensis* internalization. Upon *Francisella* infection, a signaling adaptor protein p62 was specifically recruited into the plasma membrane domain region. We determined the association of p62 with ubiquitinated proteins around bacteria by immunofluorescence microscopy. Moreover, our data demonstrated that *Francisella tularensis* interacts with autophagic pathway via p62 interaction very early after internalization (10min).

In the second part, we investigated the dynamic protein profile of macrophage surfaceome upon either IFN-γ stimulation or *Francisella* infection. We employed the MS-based recently developed Cell Surface Capturing in combination with SILAC approach to investigate qualitatively and quantitatively changes in the protein composition of cell surfaceome of macrophages upon IFN-γ stimulation. We were able to identify a total of 152 cell surface proteins of J774.2 macrophages. Of these, seven proteins were found to be differentially expressed upon IFN-γ perturbation. These proteins include MHC I molecules, high-affinity Fc receptor (CD64), intercellular adhesion molekule (CD54), sphingosine-1-phosphate receptor and bone marrow stromal antigen 2 (BST-2, CD317). Furthermore, we investigated the effect of ongoing bacterial infection on IFN-γ - induced macrophage phenotype. We found out the decrease of BST-2 expression upon bacterial infection followed by IFN-γ stimulation.

Keywords:

Infectomics, intracellular bacterium, *Francisella tularensis,* macrophage, interferon gamma, quantitative proteomics, membrane protein