Sveučilište u Rijeci
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MEDICINSKI FAKULTET - FACULTY OF MEDICINE

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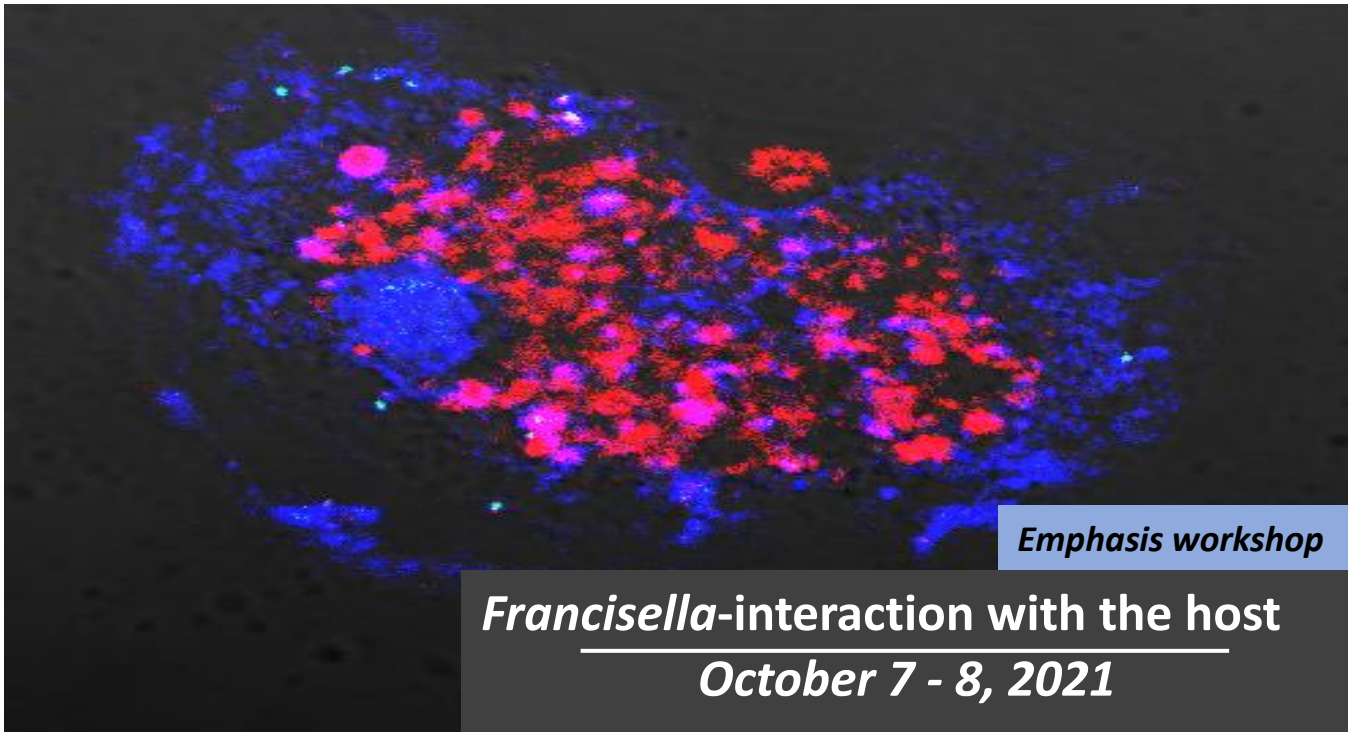

Emphasis workshop

Francisella-interaction with the host

October 7 - 8, 2021

Program Agenda and Abstract Book

Emphasis Workshop

Rijeka, Croatia

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Venue: "Vijećnica" (only for invited speakers), Faculty of Medicine, University of Rijeka, **MS teams**

(**Group -** *Francisella*-interaction with the host)

Organizing Committee:

Marina Šantić

Mateja Ožanič

Valentina Marečić

Mirna Mihelčić

Ina Viduka

Maša Knežević

The aim of the workshop: the research project titled "The role of the intracellular life cycle of *Francisella tularensis* for the pathogenesis of experimental tularemia" financed by the Croatian Science Foundation ending November 5, 2021. We would like to thank all team members and collaborators for a successful collaboration this past 4 years and for greatly improving the project activities and results.

This workshop will summarize the results of the project, focusing on interaction of *Francisella* with different hosts as well as molecular mechanisms and immune response. Top scientist from Europe, the USA as well as young scientists, will present their recent results and project activities focused on intracellular pathogens *Francisella* and *Legionella*. This two days' workshop should allow scientists, post-docs and students from various fields to gather and share their experiences while enjoying a Croatian / Mediterranean autumn.

Programme:

THURSDAY
October 7th, 2021

14:00 Welcome note - **Goran Hauser, dean**

14:15 ***"The role of the intracellular life cycle of Francisella tularensis for the pathogenesis of experimental tularemia",*** Introduction by **Marina Šantić,** Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Croatia, PI of the project.

14:45 ***"Pathogenic evolution of Legionella through training within amoeba",*** **Yousef Abu Kwaik,** Department of Microbiology and Immunology & Center for Predictive Medicine, University of Louisville Health Sciences Center, Louisville, USA - ***MS teams.***

15:15 - 15:30 ***Coffee break***

15:30 ***"Design of CMV-based vaccine vectors encoding Francisella tularensis epitopes",*** **Tina Ružić,** Center for Proteomics, Faculty of Medicine, University of Rijeka, Croatia.

15:45 ***"Intravacuolar life of Francisella novicida within Dictyostelium discoideum",*** **Valentina Marečić,** Department of Microbiology and Parasitology Faculty of Medicine, University of Rijeka, Croatia.

16:00 ***"Amoeba-grown Francisella species exhibit an alteration in the resistance to disinfectants",*** **Maša Knežević,** Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Croatia.

19:00 ***Dinner***

FRIDAY
October 8th, 2021

9:30 ***"Cell-intrinsic immune defenses against Francisella",*** **Thomas Henry,** CIRI-Centre International de Recherche en Infectiologie, Inserm U1111, CNRS UMR5308, ENS Lyon, University of Lyon, France – ***MS teams.***

10:00 ***"Francisella tularensis induces deacetylation of host mitochondrial proteins",*** **Jiri Stulik,** University of Defense, Hradec Kralove, Czech Republic – ***MS teams.***

10:30 – 11:00 *Coffee break*

11:00 ***"The type IV pili component PilO as a virulence determinant of Francisella novicida",*** **Mateja Ožanič,** Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Croatia.

11:30 ***"Tularemia as a waterborne disease",*** **Max Maurin,** Laboratoire de Bactériologie-Hygiène Hospitalière Institut de Biologie et de Pathologie, CHU Grenoble, France.

12:00 ***"Francisella-the Atg5 depended autophagy in vivo",*** **Ina Viduka,** Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Croatia.

12:15 ***"The role of autophagy protein Atg5 in immune response during Francisella tularensis LVS infection,*** **Mirna Mihelčić,** Department of Microbiology and Parasitology Faculty of Medicine, University of Rijeka, Croatia.

from 12:30-*Lunch, trip, closing remarks and plan for the future*

<u>Important note:</u>

15:00 Thursday, October 14th, 2021 – MS teams

"Characterization of the immune response to tularemia as a prerequisite for an efficacious vaccine", **Anders Sjöstedt,** University of Umea, Sweden.

This workshop has been supported by the Croatian Science Foundation under the project number IP-2016-06-9003 and Uniri-biomed 128 project.

Abstracts:

Mateja Ozanic, Valentina Marecic, Mirna Mihelcic, Ina Viduka, Masa Knezevic, Anders Sjostedt, Jiri Stulik, Marek Link, Maja Abram, **Marina Santic,** ***"The role of the intracellular life cycle of Francisella tularensis for the pathogenesis of experimental tularemia",*** Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Croatia.

Francisella tularensis is a Gram-negative, highly infectious, facultative intracellular bacterium that causes the zoonotic disease tularemia. The genus *Francisella* contains five species: *F. tularensis*, *F. philomiragia*, *F. hispaniensis*, *F. noatunensis* and *F. novicida*. Two subspecies of *F. tularensis*, *tularensis* (Type A) and *holarctica* (Type B) causes most human illness. The *F. tularensis* live vaccine strain (LVS) has been derived from a virulent strain of *F. tularensis* subsp. *holarctica.* Although attenuated for humans, LVS is highly virulent for mice, which makes it an attractive model to study the virulence mechanisms of *F. tularensis.* The only existing vaccine strain, *F. tularensis* LVS, is unlikely to become licensed in the future due to a lack of understanding of the genetic basis for its attenuation. Considering the ease of dissemination and high infectivity, *F. tularensis* subsp. *tularensis* and *F. tularensis* subsp. *holarctica* have been classified by the CDC as a Tier 1 Select Agents. Investigating the molecular and cellular mechanisms of intracellular survival of *F. tularensis* is fundamental to our understanding of its ability to cause tularemia, and is essential for designing strategies for therapeutic interventions and disease prevention by vaccination. The intracellular lifestyle of *F. tularensis* is unique compared to other intracellular pathogens that have been studied. In contrast to phagosomes harboring inert particles which fuse to lysosomes within minutes of internalization, the *Francisella*-containing phagosome (FCP) is arrested at a non-acidified late endosome-like stage, which is followed by gradual bacterial escape into the cytosol. The mechanisms by which *Francisella* manipulate the host cell subcellular organelles remains unclear. It is obvious that *Francisella* cycles through different intracellular compartments; the phagosome (FCP) and the vacuole (FCV). Because of the importance of these subcellular compartments during infection, we established a novel method of isolating the *Francisella*-containing phagosome/vacuole from macrophages. This is an innovative approach in understanding their role in *Francisella* intracellular life cycle. In addition, FCP/FCV were analysed by qualitative proteomics in order to see the changes in protein composition and interaction with the host endocytic pathway during maturation of the vacuoles. Secondly, we assumed that the great part of communication with the host cytosol environment is accomplished by autophagy. It is possible that *Francisella* reseal damaged FCV into the autophagic vacuole later during the infection. Further studies are needed to dissect the molecular mechanisms of autophagy during *Francisella* infection. Many studies have been focused on a genomic region called the *Francisella* pathogenicity island (FPI). Some FPI mutants show a uniform phenotype characterized by lack of phagosomal escape, no intracellular replication, and a loss of virulence in vivo. The *pdpC* mutant of the LVS and the highly virulent *F. tularensis* subsp. *tularensis* strain SCHU S4 strain shows very marginal intracellular replication but other distinct cytopathogenic effects; a phenotype distinct from those of the T6SS core component mutants and all other FPI mutants. We showed that FPI proteins, PdpC, IglC, IglI, PdpE, and IglG, that are effectors of the type VI secretion system are also involved in manipulation of autophagy machinery. Autophagy is also regarded as one of the innate immunity effectors against intracellular bacterial infection. Evidence is emerging that autophagy can be modulated, but also can be, itself, involved in regulation of proinflammatory cytokines. However, the data on the role of autophagy in the regulation of cytokine networks by *Francisella* are still unknown. We propose a new concept using transgenic Atg5 mouse that should contribute to a better understanding of autophagy as host immune response to *Francisella* infections.

Mateja Ozanic[1], Valentina Marecic[1], Masa Knezevic[1], Ina Viduka[1], Pavla Stojková[2], Lena Lindgren[2], Jeanette E. Bröms[2], Anders Sjöstedt[2], Yousef Abu Kwaik[3] and Marina Santic[1], ***"The Type IV pili component PilO is a virulence determinant of Francisella novicida",*** [1]Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2]Department of Clinical Microbiology, Clinical Bacteriology and Laboratory for Molecular Infection Medicine Sweden (MIMS), Umeå University, Umeå, Sweden, [3]Department of Microbiology and Immunology and Center for Predictive Medicine, College of Medicine, University of Louisville, Louisville, KY, USA.

Francisella tularensis is a highly pathogenic intracellular bacterium that causes the disease tularemia. While its ability to replicate within cells has been studied in much detail, bacterium also encodes a less characterised type 4 pili (T4P) system. T4Ps are dynamic adhesive organelles identified as major virulence determinants in many human pathogens. In *F. tularensis*, the T4P is required for adherence to the host cell, as well as for protein secretion. Several components, including pilins, a pili peptidase, a secretin pore and two ATPases, are required to assemble a functional T4P, and these are encoded within distinct clusters on the *Francisella* chromosome. While some of these components have been functionally characterised, the role of PilO, if any, still is unknown. Here, we examined the role of PilO in the pathogenesis of *F. novicida*. Our results show that the PilO is essential for pilus assembly on the bacterial surface. In addition, PilO is important for adherence of *F. novicida* to human monocyte-derived macrophages, secretion of effector proteins and intracellular replication. Importantly, the *pilO* mutant is attenuated for virulence in BALB/c mice regardless of the route of infection. Following intratracheal and intradermal infection, the mutant caused no histopathology changes, and demonstrated impaired phagosomal escape and replication within lung liver as well as spleen. Thus, PilO is an essential virulence determinant of *F. novicida*.

Tina Ružić[1], Mateja Ožanič[2], Berislav Lisnić[1,3], Astrid Krmpotić[3], Marina Šantić[2], Stipan Jonjić[1,3], ***"Design of CMV-based vaccine vectors encoding Francisella tularensis epitopes",*** [1] Center for Proteomics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2] Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [3] Department of Histology and Embryology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia.

Conventional strategies of vaccination which induce protective humoral immune response have not successfully dealt with the issue of different infectious agents against which T cells play an important role in adaptive immunity. A promising approach against these microbial pathogens are the vaccines which can generate potent and long-lasting cellular immunity against infectious agents based on the robust CD8+ T cell response. Cytomegaloviruses (CMVs) are excellent inducers of antigen specific CD8+ T cells, which accumulate over time. Therefore, CMVs genetically engineered to express foreign antigens are attractive live replicating viral vaccine vectors due to their large genomes and numerous immunomodulatory genes which can be manipulated to modulate their vaccine properties. We have previously shown that murine CMV (MCMV) vectors to foreign antigens conferred excellent protection against bacterial and tumor challenges. We have now constructed MCMV vectors encoding *Francisella tularensis* epitopes IAFTKYPSL or ISLNNFVSL, in place of the MCMV immunodominant CD8+ T-cell epitope and tested the capacity of IFNγ production by *F. tularensis* epitope-specific CD8+ T cells upon immunization with these vectors and infection with *F. tularensis* (LVS).

Valentina Marecic[1], Olga Shevchuk[2], Mateja Ozanic[1], Marek Link[3], Rok Kostanjsek[4], Jiri Stulik[3], Masa Knezevic[1], Ina Viduka[1] and Marina Santic[1], ***"Intravacuolar life of Francisella novicida in Dictyostelium discoideum",***[1]Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2]Leibniz Institute for Analytical Sciences, Dortmund, Germany, [3]Department of Molecular Pathology and Biology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic, [4]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.

Francisella is a highly infectious gram-negative bacterium that causes disease tularemia in humans and animals. It can survive and replicate in a wide variety of cells, including macrophages, dendritic cells, amoeba, and arthropod-derived cells. However, the intracellular lifecycle of a bacterium is different depending on the cell type. After infection of mammalian cells, the bacterium is shortly localized within early phagosome, followed by an escape to the cytosol where it replicates. In contrast, in amoeba *Acanthamoeba castellanii* and *Hartmanella vermiformis*, bacterium is replicating within the membrane-bound vacuole. Over the last years, amoeba *Dictyostelium discoideum* emerged as a powerful model to study the intracellular cycle and virulence of many pathogenic bacteria. We used *D. discoideum* as a model for infection and isolation of *Francisella*-containing vacuole (FCV), formed after the entry of bacteria in amoeba. Our results showed that once inside the *D. discoideum*, *F. novicida* is localized in a vacuole. Thereby, we established a method for the isolation of FCV. The proteome of isolated FCVs was determined by mass spectrometry. The proteome analyses revealed 689 host proteins, including 9 Rab family small GTPases. This approach will contribute to our understanding of the host-pathogen interactions and process of pathogen vacuole formation since vacuoles containing bacteria represent direct contact between pathogens and their hosts.

Maša Knežević[1], Valentina Marečić[1], Mateja Ožanič[1], Nikolina Špoljarić[1], Ina Viduka[1], Marija Ćurlin[2], Yousef Abu Kwaik[3], Mirna Mihelčić[1] and Marina Šantić[1], ***"Amoeba-grown Francisella species exhibit an alteration in the resistance to disinfectants",*** [1]Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2]Department of Histology, Faculty of Medicine, University of Zagreb, Croatia, [3]Department of Microbiology and Immunology and Center for Predictive Medicine, College of Medicine, University of Louisville, Louisville, KY, USA.

Francisella tularensis is a highly infectious, gram-negative intracellular bacterium and the causative agent of tularemia. Since *Francisella* can enter and multiply in amoeba cells, one of the primary goals of this study was to determine whether *Acanthamoeba castellanii*-grown *Francisella* strains exhibits alteration in the resistance to disinfection, when compared to *in vitro*-grown bacteria. The disinfectant efficacy studies were performed with different disinfectants on three bacterial strains: *F. novicida*, *F. philomiragia* and *F. tularensis* subsp. *holarctica* LVS, grown on an agar or within amoeba. After treatment with disinfectants, the bacterial viability was determined by a colony- forming unit, transmission electron microscopy and the leakage of intracellular fluid. Our results have shown that didecyldimethylammonium chloride combined with isopropyl alcohol (D1 disinfectant) was the most effective in bacterial killing, all bacteria strains were destroyed after only 10s of treatment. Surprisingly, in comparison to *in vitro*-grown strains, *A. castellanii*-grown *Francisella* strains were more sensitive to decontamination by the benzalkonium chloride combined with didecyldimethylammonium chloride and formic acid (D2), and the polyhexamethylene biguanide (D3). The results of this study clearly demonstrate that *Francisella* grown in *A. castellanii* become more susceptible to disinfectant treatment, which has been proven on three investigated bacterial strains.

Thomas Henry, ***"Cell autonomous immune defences against Francisella",*** CIRI-Centre International de Recherche en Infectiologie, Inserm U1111, CNRS UMR5308, ENS Lyon, University of Lyon, France.

Francisella tularensis is an intracellular pathogen replicating in the macrophage cytosol. While highly virulent strains of *F. tularensis* subspecies tularensis are stealth pathogens largely escaping immune recognition, the less virulent strain *Francisella novicida* is detected by the immune system. Macrophages have developed cell autononous mechanisms to detect *F. novicida* in their cytosol and trigger IFN-γ-induced antimicrobial responses. Particularly, I will present how inflammasome activation and the ensuing inflammatory cell death, termed pyroptosis restricts *F. novicida* replication. Furthermore, the role of Guanylate Binding Proteins (GBPs) in orchestrating these immune responses will be presented as well as the difference in GBPs recruitment between *F. novicida* and another cytosol-dwelling pathogen, *Shigella flexneri*.

Jiri Stulik, ***"Francisella tularensis induces deacetylation of host mitochondrial proteins",*** University of Defense, Hradec Kralove, Czech Republic.

Francisella tularensis is an intracellular bacterial pathogen, which bypasses stimulation of adaptive immunity by evading activation of infected dendritic cells (DCs). While the underlying mechanisms of DC suppression remain elusive, it was shown previously that *Francisella* transiently reprograms host mitochondria to fuel noninflammatory oxidative metabolism supporting bacterial replication. Our data show that in DCs, virulent *Francisella* directly associates with mitochondria after its phagosomal escape to cytosol and that the early interaction does not compromise mitochondrial integrity. Instead, *Francisella*-infected DCs enhance metabolic flux via TCA cycle and increase expression and activity of mitochondrial SIRT3 deacetylase, which coincides with the mitochondria-specific downregulation of protein acetylation. Rather than affecting particular proteins, deacetylation in mitochondria occurs globally implying that *Francisella* tunes DC noninflammatory mitochondrial metabolism on systemic level. Collectively, the presented data identify host mitochondrial protein acetylation as a potential target of pathogens hijacking host metabolic pathways.

Christopher Price[1], Snake Jones[1], Marina Santic[3], and **Yousef Abu Kwaik[1,2],** **"An accidental pro-inflammatory response of macrophages to an amoeba-adapted effector of *Legionella*",** [1]Department of Microbiology and Immunology, [2]Center for Predictive Medicine, College of Medicine, University of Louisville, KY, [3] Faculty of Medicine, University of Rijeka, Croatia.

Legionella pneumophila has evolved in aquatic environments to proliferate within free-living amoebae. Upon accidental transmission to humans through environmental aerosols, *L. pneumophila* replicates within alveolar macrophages. Excessive glucose ex vivo or hyperglycemia in diabetic patients, shifts macrophages towards aerobic glycolysis and differentiate into a M1 pro-inflammatory phenotype. Here we show *L. pneumophila* injects into human monocyte-derived macrophages (hMDMs) an amylase (LamA) which rapidly degrades glycogen causing cytosolic hyper-glucose and a shift to aerobic glycolysis. A rapid pro-inflammatory response is triggered that partially restricts pathogen proliferation ex vivo and in vivo through IDO1-mediated depletion of host tryptophan. In amoebae, the LamA-mediated degradation of amoebal glycogen deprives the host from the essential resources for synthesis of the cellulose cyst wall, subverting amoeba encystation. Therefore, LamA of *L. pneumophila* has evolved to interfere with

encystation of amoebae but results in an accidental pro-inflammatory response in macrophages that partially restricts pathogen proliferation.

Max Maurin, ***"Tularemia as a waterborne disease",*** Laboratoire de Bactériologie-Hygiène Hospitalière Institut de Biologie et de Pathologie, CHU Grenoble, France.

Francisella tularensis is the etiological agent of tularemia. This zoonosis usually manifests by a localized infection at the *F. tularensis* inoculation site (e.g., a skin eschar, pharyngitis, and conjunctivitis) associated with regional lymphadenopathy. Rarely, more severe diseases such as pneumonia and sepsis are observed. *F. tularensis* can infect or colonize a broad range of vertebrate animal species. Humans are most frequently infected by these animals, particularly lagomorphs (hares are wild rabbits) and small semi-aquatic rodents. Tularemia can also occur through the bite of arthropods (mainly Ixodidae ticks and mosquitoes in specific areas). Mosquitoes are supposedly contaminated during their aquatic larval stage. In tularemia endemic areas, infections also occur from *F. tularensis* contaminated hydro-telluric environments. Waterborne tularemia can occur via consuming contaminated water (e.g., spring water and water from water wells) or contact with contaminated water (e.g., while swimming or fishing). Both mosquito-borne and waterborne tularemia cases suggest that *F. tularensis* has an aquatic reservoir. Hence, this bacterium has been detected in many water environments, including in fresh or brackish water. However, the modes and duration of *F. tularensis* survival in the water environment remain unknown. Current hypotheses include: 1/ interactions of this bacterium with protozoa, especially amoebae such as *Legionella pneumophila*; 2/ slowing down of bacterial metabolism and replication under specific conditions (low temperature, salinity, etc.); 3/ biofilm formation; and 4/evolution towards a viable but non-cultivable state (VBNC) as described for many different bacterial species. I aim to summarize the current medical and scientific aspects of waterborne tularemia and our own experience in this area.

Ina Viduka[1], Mirna Mihelčić[1], Mateja Ožanič[1], Valentina Marečić[1], Maša Knežević[1], Marija Ćurlin[2], Sanja Štifter[1], Anders Sjöstedt[3], Marina Šantić[1], ***"Francisella - the Atg5 depended autophagy in vivo",*** [1]Department of Microbiology and Parasitology, Faculty of Medicine, University of Rijeka, Rijeka, Croatia, [2]Department of Histology, Faculty of Medicine, University of Zagreb, Croatia, [3]Department of Clinical Microbiology, Umeå University, Umeå, Sweden.

To survive and replicate within the host cells, intracellular pathogens use various strategies. *Francisella tularensis* is a highly virulent intracellular pathogen that causes tularemia in humans and animals. Early after infection, *Francisella* escapes the phagosome and reaches the cytosol, where it becomes a target of the autophagy pathway. The autophagy mechanism detects and eliminates cytosolic pathogens by producing double-membrane vacuoles called autophagosomes. In the canonical autophagy pathway, the Atg5 protein is essential for the formation of the autophagosome. Previous *in vitro* studies showed that *Francisella* avoids elimination and provides nutrients via autophagy. The role of Atg5 dependent autophagy in the pathogenesis of tularemia is still unknown. We used mice deficient for Atg5 protein in the myeloid lineage in our experiments. We determined that Atg5-dependent autophagy increases mice mortality after intradermal infection. Our study showed for the first time that Atg5 protein is responsible for the efficient intracellular replication of *Francisella in vivo*.

Mirna Mihelčić[1], Ina Viduka[1], Maša Knežević[1], Valentina Marečić[1], Mateja Ožanič[1], Kjell Eneslätt[2], Maja Abram[1], Anders Sjöstedt[2], and Marina Šantić[1], ***"The role of autophagy protein Atg5 in immune response during F. tularensis LVS infection",*** [1]Department of Microbiology and Parasitology, Faculty of Medicine University of Rijeka, Rijeka, Croatia, [2]Department of Clinical Microbiology, Umeå University, Umeå, Sweden.

Autophagy, as an evolutionary conserved cellular mechanism, is a crucial in the host response to intracellular bacterial pathogens. During the autophagy-mediated response, microorganisms may undergo direct degradation in autophagolysosomes. Autophagy also plays an indisputable role in the regulation of the host's innate and adaptive immune response. *Francisella tularensis*, as a highly virulent intracellular pathogen has developed survival strategies to evade degradation within the double-membrane vacuole during autophagy process. To investigate the role of autophagy in the immune response to *F. tularensis*, live vaccine strain (LVS), transgenic mice deficient in Atg5 of cells of the myeloid lineage were used. Upon intradermal infection, at indicated time point, immunological responses in Atg5 deficient and control mice were explored. Cytokine and chemokine levels in sera were analyzed by Luminex method, mRNA cytokine levels in the lungs were determined by and RT-PCR, and the inflammatory cell infiltration in the lungs was analyzed by immunohistochemistry. Obtained results indicate that that Atg5 deficiency in myeloid cells attenuated pro-inflammatory response during *Francisella* LVS infection. Atg5 deficient littermates revealed decreased infiltration of T cells and macrophages in the lungs as well as diminished pro-inflammatory cytokine response.

Anders Sjostedt, ***"Characterization of the immune response to tularemia as a prerequisite for an efficacious vaccine",*** University of Umea, Sweden.

F. tularensis (Ft) is a highly virulent facultative intracellular bacterium that causes the severe disease tularemia in humans and many mammalian species. Natural outbreaks of tularemia are widespread over the Northern hemisphere and a significant health problem, in particular in parts of Sweden and Finland and incidences are very high during years of outbreaks; up to 1,000/100,000; comparable to influenza epidemics. Thus, tularemia is a significant local public health problem and protecting the population through vaccination would lead to considerable health gains.

We hypothesized that an efficacious vaccine would be possible to develop and we have a promising candidate vaccine, ΔclpB, which is highly attenuated and demonstrates excellent protective efficacy. To define how the vaccine effectuates protection, we have utilized a human in vitro model in conjunction with a unique resource of a vast collection of samples collected from immune individuals following vaccination or infection with Ft. In addition, we have utilized a mouse and rat co-culture model. The data demonstrate that the co-culture models closely mimic the in vivo situation. Using these models, protective efficacy was analyzed as well cytokine patterns and flow cytometry analyses. Thereby protective correlates were identified. Altogether, our current work has revealed mechanisms whereby the efficacious new vaccine exerts its protective effects.