13th Host Pathogen Interaction Forum 2018

April 16 – 19, 2018, Slavonice, Czech Republic

Agenda and Abstract book

13th Host Pathogen Interaction Forum 2018

Venue: Slavonice, Czech Republic

Date of event: **April 16th – 19th 2018**

Conference with international participation

Department of Molecular Pathology and Biology

Faculty of Military Health Sciences, University of Defence in Hradec Kralove

&

Czech Immunological Society

The interaction between host and pathogen with enlargement to other aspects of the analysis of biological material

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ORGANIZATORS

University of Defence, Faculty of Military Health Sciences, Department of Molecular Pathology and Biology, Hradec Kralove

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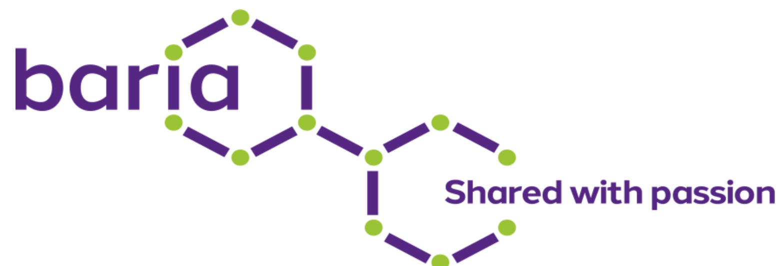

EDITORIAL

Dear colleagues,

,,Host-Pathogen Interaction“ - with that in mind, it is our pleasure to welcome you to the 13th Host Pathogen Interaction Forum 2018 in Slavonice, Czech Republic. The conference is jointly organized by Faculty of Military Health Sciences, University of Defence and Czech Immunological Society. As with the previous conferences, our emphasis on giving members of the audience a range of topics from traditional main area of host-pathogen interaction and enlarged it to comprehend also other aspects of the analysis of biological material. We received a significant number of abstracts from a whole lot of students, doctors, and members of several scientific organizations. We would like to thank the Scientific Committee for their expertise and support.

We have learned from past forums that while many of us may not know each other at first, we immediately recognize that we are among friends who have a common cause. For this reason, you will note that we have long breaks between formal sessions so new friends have plenty of time to get acquainted and trade ideas and innovations. We have also planned several social events, which will give all us the opportunity to relax away from the structure of the meeting place. We have welcoming and farewell receptions and the half day excursions, all designed to provide a relaxing atmosphere with great food and drinks.

We would like to thank Prof. Stulik Jiri (Scientific Secretary) and Mrs. Magdalena Proksova (Organising team) who showed an exemplary example of team work and excellent planning. We are glad you were able to come and hope you have an enjoyable and productive time.

My sincerely thanks to all for making possible this conference!

Klara Kubelkova, Ph.D.
Organizer

,, Make this meeting matter”

CONFERENCE VENUE

The 13th Host Pathogen Interaction Forum 2018 is held in the beautiful and exciting city of Slavonice situated in the southwest of Moravia on the border with South Bohemia, Czech Republic, about a kilometre from the Austrian border with about 2,700 inhabitants. While historically the town belongs to Moravia, it is now part of the South Bohemian Region. The town has a traditional medieval renaissance city centre with Sgraffito covered buildings dating from the 14th to 16th centuries, the oldest dating to 1545. The Sgraffito are the second oldest in the Czech Republic, with older existing only in Prague from 1544. The renaissance character of the town is due to a period of extreme wealth in the 14th to 16th century when Slavonice was an important town on the route from Prague to Vienna. The town and the surrounding countryside were lightly fortified in the period leading up to the Second World War. Some of these small bunker complexes have been repaired and refurbished, with mock battles of Wehrmacht and Czechoslovak forces taking place in summer. The area and defences were never used against the Third Reich as the town and region had to be surrendered to the Third Reich following the Munich Agreement. The original German-speaking population was expelled in June 1945 following the Second World War.

Slavonice is very popular not only for its preserved renaissance town centre but also for the underground tunnel system dating back to the 12th century and surrounding countryside, which is unspoiled by industry.

CONFERENCE CENTRE / Institute Slavonice

Institute Slavonice is an international conference centre, educational facility and event space. Our state-of-the-art renovation of the Town of Slavonice's old school, opened in 1903, has created the finest meeting place between Prague and Vienna for business, professional organizations, clubs, societies and non-profit institutions.Conference Centre also provide the welcome party and the conference reception.

LUNCHES

Conference lunches are served in the hotel ARKADA, Tuesday (from 11am to 2pm), Wednesday and Thursday (from midday to 2pm). The hotel ARKADA is located on the main Slavonice square, 2 min. walk from Institute Slavonice.

DINNER

Monday, April 16 – Welcome party (included in the conference fee)

Tuesday, April 17 – Dinner - will not be organized

Wednesday, April 18 – Conference reception (included in the conference fee)

SOCIAL EVENTS

Welcome party

Date: Monday, April 16

Time: 7pm

Location: Auditorium/Yellow/Blue rooms, Institute Slavonice

Guided tour of Slavonice historical surroundings

Date: Tuesday, April 17

Time: 1pm

Location: pick up in front of Institute Slavonice

Guided tour of of Dukovany Nuclear Power Station

Date: Tuesday, April 17

Time: midday (Note: Bus will not wait for latecomers ☺)

Location: right next to the gate of Institute Slavonice - tourist bus stop

Conference Reception

Date: Wednesday, April 18

Time: 7pm

Location: Cellar and Yellow/Blue rooms, Institute Slavonice

AGENDA

MONDAY (April 16, 2018)

4:00 – 6:45pm	*Arrival of participants, Registation and helpdesk open* (Entrance hall, Institute Slavonice)
7:00 – 7:15pm	**Kubelkova Klara** – Opening (Auditorium**,** Institute Slavonice)
7:15 – 7:50pm	**Sjostedt Anders** – Keynote lecture (Umeå University, Umeå, SE)
7:50 – 12pm	*Welcome party* (Entrance hall/Auditorium/Yellow**/**Blue rooms, Institute Slavonice)

TUESDAY (April 17, 2018)

8:00am – 10:50am	**Session I. - Chairman: Charbit Alain**
8:00 – 8:45am	**Alain Charbit** - Importance of metabolic pathways in *Francisella* pathogenesis (INSERM, Paris, FR)
8:45 – 9:10am	**Kopečková Monika** - Glyceraldehyde-3-phosphate dehydrogenase of *Francisella tularensis* (FoMHS, Hradec Kralove, CZ)
9:10 – 9:35am	**Špidlová Petra** - *Francisella tularensis* D-Ala D-Ala carboxypeptidase DacD is involved in intracellular replication and it is necessary for bacterial cell wall integrity (FoMHS, Hradec Kralove, CZ)
9:35 – 10:00am	*Coffee break – Yellow/Blue rooms*
10:00 – 10:25am	**Mou Sherry** - Characterization of a *Coxiella burnetii* Cvp Mutant Generated by Targeted Gene Deletion (USAMRIID, Maryland, USA)
10:25 – 10:50am	**Stojková Pavla** - HU protein is involved in intracellular growth and full virulence of *Francisella tularensis* (FoMHS, Hradec Kralove, CZ)
11:00am – 2:00pm	*Lunch – ARKADA hotel*
12:00 – 5:00pm	*Social program (Slavonice* surroundings/Dukovany)

WEDNESDAY (April 18, 2018)

8:00 – 12:25am	**Session II. - Chairman: Bevins L. Charles**
8:00 – 8:40am	**Bevins L. Charles** - Paneth Cell Antimicrobials Help Maintain Homeostasis in the Small Intestine (University of California, Davis, USA)
8:40 – 9:05am	**Kubelkova Klara** - A Few Notes on the Spatiotemporal Concept of Innate Immune Recognition (FoMHS, Hradec Kralove, CZ)
9:05 – 9:30am	**Ahmad Jawid** - *Bordetella pertussis* adenylate cyclase toxin modulates functions of host innate immune cells (Institute of Microbiology of the CAS, CZ)
9:30 – 10:00am	**Link Marek** – MHC-II peptidome of *F. tularensis* infected dendritic cells (FoMHS, Hradec Kralove, CZ)
10:00 – 10:30am	*Coffee break* - *Congress foyer*
10:30 – 11.10am	**Rychlik Ivan** - Structure and function of chicken gut microbiota (Veterinary Research Institute, Brno, CZ)
11:10 – 11.35am	**Kubasova Tereza** - Competitive exclusion and colonization of chicken intestinal tract (Veterinary Research Institute, Brno, CZ)
11:35 – 12.00am	**Kollarčíková Miloslava** - Illeal and caecal microbiota of chickens – identification of new opportunistic pathogens of chickens (Veterinary Research Institute, Brno, CZ)
12:00 – 12.25am	**Plzakova Lenka** - Early cellular responses of germ free and specific pathogen free mice to *Francisella tularensis* infection (FoMHS, Hradec Kralove, CZ)
12:30 – 2:00pm	*Lunch – ARKADA hotel*

2:00 – 6:10pm	**Session III. - Chairman: Joshi Lokesh**
2:00 – 2.40pm	**Joshi Lokesh** – Host-Microbial interaction: identification and application of glycan-lectin components as decontamination tools (National University Ireland Galway, Ireland)
2:40 – 3.05pm	**Yang Ruwen** - New insight into HPV related cancer and the role of a potential co-factor *Chlamydia trachomatis* using

	transcriptomic and proteomic methods (German Cancer Research Center, Heidelberg, GE)
3:05 – 3.30pm	**Večerek Branislav** - Dual RNA-seq analysis of human macrophages infected by *Bordetella pertussis* (Institute of Microbiology of the CAS, CZ)
3:30 – 3.55pm	**Hasan Shakir** - *Bordetella pertussis* Adenylate Cyclase Toxin Disrupts Functional Integrity of Bronchial Epithelial Layers (Institute of Microbiology of the CAS, CZ)
3:55 – 4:25pm	*Coffee break* - *Congress foyer*
4:25 – 5.05pm	**Klimentova Jana** - Outer membrane vesicles and nanotubes in *Francisella tularensis* (FoMHS, Hradec Kralove, CZ)
5:05 – 5.25pm	**Volf Jan** - Exosomes in charge? The lesson from B-cell deficient chicken (Veterinary Research Institute, Brno, CZ)
5:25 – 5.40pm	**AMEDIS spol. s.r.o. – Korba Tomáš** – Industrialized Quantitative Proteomics – Thousands of Proteins in Hundreds of Samples
5:40 – 5.55pm	**KRD obchodní společnost s.r.o.** – OpenSPR: New approach to determination ofthe biomolecular interactions with detail information about kinetic data
5:55 – 6.10pm	**MERCK spol. s.r.o. – Kukla Stanislav** – Sensitivity you can count on – a short introduction to new instrument platforms by Merck
7:00pm – 2:00am	*Conference Reception* (Cellar and Yellow**/**Blue rooms, Institute Slavonice)

8:00 – 12:30am	**Session IV. - Chairman: Roesl Frank**
8:00 – 8:40am	**Roesl Frank** - UV irradiation and cutaneous papillomavirus infection: development of non-melanoma skin cancer in a natural animal model (German Cancer Research Center, Heidelberg, GE)
8:40 – 9:05am	**Ďuráčová Miloslava** - Proteomic profiling of virulent phase I and avirulent phase II of *Coxiella burnetii* employing axenic and cell culture-based cultivation (FoMHS, Hradec Kralove, CZ)

9:05 – 9:30am	**Csicsay František** - Proteomic study of antigenicity of *Rickettsia akari* proteins (Biomedical Research Centre, SAS, Bratislava, SK)
9:30 – 9:55am	**Čejková Darina** - Comparative genomics of clinical and environmental isolates of *Campylobacter jejuni* (Veterinary Research Institute, Brno, CZ)
9:55 – 10:30am	*Coffee break* - *Congress foyer*
10:30 – 10:55am	**Prokšová Magdaléna** – Seeking host target of *Francisella* pathogenicity island effectors (FoMHS, Hradec Kralove, CZ)
10:55 – 11:25am	**Stulik Jiri** - Proteome profiling of *Francisella tularensis* dendritic cells invasion (FoMHS, Hradec Kralove, CZ)
11:25 – 11:50am	**Novák Jakub** - Phosphoproteomics of *Bordetella Pertussis* Adenylate Cyclase Toxin Action in Mouse Dendritic Cells Reveales Inhibition of mTOR Pathway and CRTC3-dependent Signaling (Institute of Microbiology of the CAS, CZ)
11:50 – 12:15am	**Kamanová Jana** - *Bordetella* type III secretion system effector BteA: its functional divergence and mechanism of action (Institute of Microbiology of the CAS, CZ)
12:15 – 2:00pm	*Closing the conference, lunch* and *departure of participants*

ORAL PRESENTATION ABSTRACT

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Importance of metabolic pathways in *Francisella* pathogenesis

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Francisella tularensis is a highly infectious Gram-negative bacterium and the causative agent of the zoonotic disease tularemia. Although *Francisella* possesses the capacity to infect numerous mammalian cell types, the macrophage constitutes the main intracellular niche, used for *in vivo* bacterial dissemination. To survive and multiply within infected macrophages, *Francisella* must imperatively escape from the phagosomal compartment. In the cytosol, the bacterium needs to control the host innate immune response and adapt its metabolism to this nutrient-restricted niche.

We have recently addressed the role of two enzymes occupying a central position in glycolysis and gluconeogenesis pathways: GlpX, a fructose 1,6-bisphosphatase; and Fba, a class II fructose-1,6-bisphosphate aldolase. We showed that both enzymes were essential for growth of *Francisella* when gluconeogenic substrates were used as carbon sources, in culture medium as well as in infected macrophages. Remarkably, the enzyme FBA appeared to be also involved in regulatory functions. The direct binding of FBA to several promoter regions and a direct effect of this binding on transcription were experimentally demonstrated.

Other enzymes with regulatory roles might exist and contribute to link metabolism and pathogenic adaptation of this bacterium and/or in other bacterial pathogens.

Glyceraldehyde-3-phosphate dehydrogenase of *Francisella tularensis*

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The GAPDH, an enzyme of glycolytic pathway, is a multifunctional protein, which contributes to adhesion and virulence of many pathogenic bacteria. To investigate the role of GAPDH in virulent strain of *Francisella tularensis* subsp. *holarctica* FSC200 we prepared a viable mutant strain with deleted *gapA* gene and analyzed its phenotype. The results of our in vitro and in vivo experiments revealed that this enzyme is important for full virulence manifestations in *F. tularensis* as well. The *gapA* mutant is characterized by reduced virulence in mice, defective replication inside macrophages, and its ability to induce a protective immune response against systemic challenge with parental wild-type strain. We also demonstrated the multiple localization sites of this protein: In addition to within the cytosol, it was found on the cell surface, outside the cells, and in the culture medium. Recombinant GapA was successfully obtained, and it was shown that it binds host extracellular serum proteins like plasminogen, fibrinogen, and fibronectin. In particular the extracytosolic localization of GapA and its ability to bind to host proteins provide convincing evidence of its additional non-enzymatic functions. Further studies are focused mainly on the elucidation of potential interaction partners both in bacteria and host cells to confirm the further roles of *F. tularensis* GapA.

Francisella tularensis D-Ala D-Ala carboxypeptidase DacD is involved in intracellular replication and it is necessary for bacterial cell wall integrity

Petra Spidlova[1], Pavla Stojkova[1], Vera Dankova[1], Iva Senitkova[1], Marina Santic[2], Dominik Pinkas[3], Vlada Philimonenko[3,4], and Jiri Stulik[1]

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D-alanyl-D-alanine carboxypeptidase, product of *dacD* gene in *Francisella*, belongs to penicillin binding proteins (PBPs) and is involved in remodeling of newly synthetized peptidoglycan. In *E. coli*, PBPs are synthetized in various growth phases and they are able to substitute each other to a certain extent. The DacD protein was found to be accumulated in fraction enriched in membrane proteins from severely attenuated *dsbA* deletion mutant strain. It has been presumed that the DsbA is not a virulence factor by itself but that its substrates, whose correct folding and topology are dependent on the DsbA oxidoreductase and/or isomerase activities, are real virulence factors. Here we demonstrate that *Francisella* DacD is required for intracellular replication and virulence in mice. The *dacD* insertion mutant strain showed higher sensitivity to acidic pH, high temperature and high osmolarity when compared to the wild-type. Eventually, transmission electron microscopy revealed differences in both the size of mutant bacteria and also defects in outer membrane underlying its SDS and serum sensitivity. Taken together these results suggest DacD plays an important role in *Francisella* pathogenicity.

Characterization of a *Coxiella burnetii* Cvp Mutant Generated by Targeted Gene Deletion

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Coxiella burnetii is a ubiquitous zoonotic pathogen and the cause of human Q fever, an acute flu-like illness that can progress to chronic, life-threating endocarditis. *C. burnetii* has a unique intracellular life style that involves acid-activated metabolism within a lysosome-like compartment known as *Coxiella*-containing vacuole (CCV). Biogenesis of CCV requires over 130 bacterial effector proteins delivered into a host cell by the Dot/Icm type IV secretion system. The Dot/Icm system is essential for pathogenesis, however, most functions behind this system and associated effectors remain unknown.

Data obtained from this study illustrates the cloning and characterization of a *C. burnetii* effector mutant *(*∆*cvpC*) involved in the Dot/Icm system, generated by a loop-in/loop-out deletion. A single suicide plasmid was first integrated into 5' or 3' target gene flanking regions. From PCR confirmation, resolution of the plasma cointegrant by a crossover event under sucrose counter selection resulted in a gene deletion. Effector mutant ∆*cvpC* exhibited impaired growth in Raw cells. Assessed virulence by infection of *Galleria mellonella* confirmed attenuation of the mutant. Data suggests *Coxiella* vacuolar protein C playing a critical role in *C. burnetti* replication within a host cell.

HU protein is involved in intracellular growth and full virulence of *Francisella tularensis*

Pavla Stojkova[1], Petra Spidlova[1], Juraj Lenco[2], Helena Rehulkova[1], Lucie Kratka[1] and Jiri Stulik[1]

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The nucleoid-associated HU proteins are small abundant DNA-binding proteins in bacterial cell which play an important role in the initiation of DNA replication, cell division, SOS response, control of gene expression and recombination. HU proteins bind to double stranded DNA non-specifically, but they exhibit high affinity to abnormal DNA structures as four-way junctions, gaps or nicks, which are generated during DNA damage. In many pathogens HU proteins regulate expression of genes involved in metabolism and virulence. Here, we show that the *Francisella tularensis* subsp. *holarctica* gene locus FTS_0886 codes for functional HU protein which is essential for full *Francisella* virulence and its resistance to oxidative stress. Further, our results demonstrate that the recombinant FtHU protein binds to double stranded DNA and protects it against free hydroxyl radicals generated via Fenton´s reaction. Eventually, using an iTRAQ approach we identified proteins levels of which are affected by the deletion of *hupB*, among them for example *Francisella* pathogenicity island (FPI) proteins. The pleiotropic role of HU protein classifies it as a potential target for the development of therapeutics against tularemia.

Paneth Cell Antimicrobials Help Maintain Homeostasis in the Small Intestine

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The intestinal mucosa interacts with a complex, dense community of microorganisms, including both resident microbiota and many transient microbes entering from food and water borne sources, including enteropathogens. The host relies on multiple complementary functions to maintain homeostasis and avert microbial disease. The epithelium serves as a key arm of the immune system, both by providing a physical barrier and by secreting various antimicrobial factors, most notably antimicrobial peptides and proteins. In the small intestine, specialized epithelial cells called Paneth cells produce abundant quantities of α-defensins and several other antibiotic molecules, including enzymes and lectins. These Paneth cell molecules provide complementary mechanisms of action, and *in vivo* studies support that they promote homeostasis and protection of the intestinal mucosa. Studies in humans suggest that disrupted Paneth cell function may contribute to the pathogenesis of inflammatory bowel disease, and perhaps other chronic diseases. Thus, through synthesis and secretion of antimicrobial factors, Paneth cells help maintain homeostasis at the host-microbe interface in the intestine

A Few Notes on the Spatiotemporal Concept of Innate Immune Recognition

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Innate immune response constitutes the first line of defense against bacterial infection. The dominant role in triggering and streamline of innate immune response plays the innate immune recognition process along with the intrinsic characteristics of the microorganism and host. The epigenetic reprogramming of innate immune cells, creating the hierarchy of immune response functional modules, is critical for inducing and regulating expression of the adaptive immune response. Recent evidence supports the concept that immune response to external stimuli in the form of bacteria is guided by the primary interaction of the bacterium with the host cell. In the case of *Francisella* in vitro models, the literature presents quite different conclusions concerning the outcome of *Francisella* innate immune recognition. Some studies provide evidence that *F. tularensis* LVS represses inflammasome activation, while other studies have demonstrated that *F. tularensis* LVS increases mRNA levels of proinflammatory cytokines followed by increased protein secretion. The in vivo studies demonstrate the relative virulence of *Francisella* depending on the route of infection. These data, together with the data from experiments with host-adapted *Francisella* LVS revealed that the host cells as well as invading *Francisellae* are affected by their "historical memory" and mutually generate, at any given time, an immediate microenvironment affecting all subsequent events in induction of immune responses. Based on data from different *Francisella* models, we present here the basic paradigms of the emerging innate immune recognition concept that respects spatiotemporal character of innate immune recognition. According to this concept, the innate immune recognition is the multistep process that is dependent on the modulation of epigenetic reprograming of innate immune cells by changing microenvironment in time. From this point of view, the cell, with its functional and secretion profile, rather than the host organism in its entirety, seems to be the primary microbe host.

We formulated the spatiotemporal concept of innate immune recognition to understand the innate immune response to infection. To achieve this, we clearly need new multidimensional data sets providing comprehensive, cell-specific, and time-structured information on epigenetic reprogramming of innate immune cells that can provide us with the logic of interplay among immune cells.

Bordetella pertussis adenylate cyclase toxin modulates functions of host innate immune cells

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Bordetella pertussis adenylate cyclase toxin (CyaA) subverts host innate immune responses. CyaA targets cells expressing the complement receptor3 (also known as αMβ2 integrin, CD11b/CD18) and subverts their anti-bacterial functions. The ability of CyaA to dampen innate immune responses depends on its ability to catalyze the conversion of cytosolic ATP into the key signaling molecule, cAMP. Despite extensive research on CyaA toxin action on myeloid phagocytes, it is still not fully understood how *B. pertussis* is able to overcome host innate immune responses to colonize host upper airway. Here, by using flow cytometry, immunoblotting and electron microscopy, we demonstrate that upon translocation into the host cell, CyaA triggers Protein Kinase A activation via cAMP generation to subvert monocyte and macrophage function. Furthermore, CyaA triggers the phenotypic transition of primary human monocytes and renders them inefficient in phagocytic uptake.

MHC-II peptidome of *F. tularensis* infected dendritic cells

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Innate and adaptive immune responses are activated during *Francisella tularensis* infection to protect the infected organism. The role of T-cells has been shown important for the clearance of infection and establishment of protection. As for other bacterial pathogens, knowledge of the *Francisella* molecular targets of T-cells is important for the design of vaccination strategies, monitoring of vaccine efficiency and development of diagnostic tools. We used the mouse bone-marrow derived dendritic cells infected with *F. tularensis* LVS for isolation of peptide-MHC-II complexes and the obtained peptidome were profiled and identified by liquid chromatography high-resolution mass spectrometry. We present the immunoproteomic workflow and point to the challenges when looking for a needle in a haystack.

Structure and function of chicken gut microbiota

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Chickens in a commercial production represent a specific subject for studies on microbiota-host interactions since they are hatched without any contact with adult hens and their colonisation is dependent exclusively on environmental sources. In the youngest chickens, Gram positive *Firmicutes* dominates in the caecum but there is a considerable variation from batch to batch at the level of bacterial species, consistent with random colonisation of chickens from always little bit different environmental sources. However, newly hatched chickens can be colonised by microbiota of nearly any composition and a 24-hour-long contact of newly hatched chickens with an adult hen is long enough to transfer hen microbiota to chickens. Microbiota development in the chickens in commercial production therefore differs from that in the chickens in a contact with a hen. Unfortunately, we observed that experimental inoculation of newly hatched chickens by caecal microbiota of adult hens resulted in an increase of *Campylobacter* in the caeca of newly hatched chickens. Caecal or faecal microbiota from adult hens may therefore protect newly hatched chickens against infection but such inoculation also represents a certain risk associated with pathogen transmission.

An alternative how to avoid the inoculation of chickens with complex microbiota is to obtain individual microbiota members in pure cultures, characterise them by whole genome sequencing, predict their metabolic potential, and carefully select and combine those with likely protective effect but without any adverse effects on chicken's performance. This is a reason why we have already started with the culture of strict anaerobes followed by whole genome sequencing. We have sequence 204 isolates among which we identified 133 different clones belonging to 6 different phyla - *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Elusimicrobia* and *Synergistetes*. Over 70 different clones were already tested in newly hatched chickens and we observed very variable ability of individual cultures to colonise intestinal tract of newly hatched chickens and protect them against infection with *Salmonella* Enteritidis. However, even these initial results indicate that there is a potential for a construction of next generation

of probiotics by careful selection and mixing of pure cultures of particular microbiota members.

Competitive exclusion and colonization of chicken intestinal tract

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Colonization of intestinal tract of chickens begins immediately after hatching and hen is the first and most important source of microbiota. However, chickens in commercial poultry production system are hatched in near sterile environments and contact between hens and chickens is eliminated. The initial colonization of the intestinal tract in chickens is therefore dependent on the microbiota present in the hatchery or the housing environment, and if a pathogen appears in the environment, the poorly populated gut of young chickens may represent an ideal ecological niche for its multiplication.

In this study we therefore focused on gut microbiota of chickens which were in contact with adult hen and composition of chicken microbiota after application of microbiota cultured from faecal material of adult. Contact of newly hatched chickens with an adult hen enabled the colonization of chicken intestinal tract with nearly complete gut microbiota of hen. Furthermore, we tested to what extent the hen can be replaced by administration competitive exclusion products derived from adult hen. We tested Aviguard, a commercial product based on competitive exclusion of pathogens from the chicken caecum, and two bacterial mixtures cultured from fecal material of adult. Administration of Aviguard or bacterial mixtures increased chicken resistance to Salmonella challenge 10-100 fold. All products contained high number of different gut anaerobes but only a few of them were capable of efficient colonization of chicken caecum. This shows that only a few bacterial species out of those present in the competitive exclusion products efficiently colonize chicken intestinal tract. Moreover, if these bacterial species were obtained in pure cultures, it should be possible to prepare similar products but of clearly defined composition.

Illeal and caecal microbiota of chickens – identification of new opportunistic pathogens of chickens

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In this study we addressed the issue of slightly different development of gut microbiota in 4 successive batches of commercially hatched broiler parent chickens. Microbiota composition was determined in chickens 5 to 18 weeks of age, in 100 and 160 samples originating from the ileum or caecum, respectively. When planning this study, we expected that we will come across a batch with a compromised performance what should allow for identification of microbiota of suboptimal composition. This happened and in one of the batches with suboptimal performance we identified increased abundance of *Helicobacter* forming over 80 % of ileal microbiota in individual chickens. Moreover, the same owner provided us also with samples of adult hens 53 weeks of age and with decreasing egg production. In this case, caecal microbiota was enriched for *Fusobacterium mortiferum* forming over 30 % of total microbiota is 3 different hens. Though none of the identified unusual microbiota members represented a well recognised pathogen, their overgrowth can be associated with decreased performance. These bacterial species may therefore represent new opportunistic pathogens of chickens in which zoonotic potential cannot be excluded.

Early cellular responses of germ free and specific pathogen free mice to *Francisella tularensis* infection

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Francisella tularensis is a small, facultative intracellular Gram-negative coccobacillus, which caused tularemia in humans and in animals. Both *in vitro* and *in vivo*, *Francisella* spp. infect and proliferate inside phagocytic cell types, non-phagocytic hepatocytes, epithelial cells, and B cell lines. Information available about immune response to *Francisella* spp. comes from animal model studies. Our experiments have shown the data, which were documented the different pattern of cellular behavior in an environment unaffected by microbiota using the model of germ free (GF) animals associated with *F. tularensis* subsp. *holarctica* strain LVS FSC155. The mammalian intestine is colonized by a large and diverse community of microbes, referred as the gut microbiota. These microbes have the capacity to influence the body's physiology. The microbiota can also regulate different types of inflammatory processes during microbial infections. GF mice, having a sterile intestine, thus represent a suitable model for the study of the early stages of host-pathogen interactions at the naïve immunological background.

In our experiments we monitored the response of several cell subtypes during the first two days post infection at the site of infection (in peritoneum), and in one of the central lymphoid organs (in spleen). The relative frequency of cell subpopulations tested the basic difference between the response of GF mice and SPF mice to *F. tularensis* LVS infection. The Gr1+ and F4/80+ cells of GF mice in peritoneum demonstrated the mutual frequency changes in the two distinguishable waves. The data also demonstrate surprising behavior of cell subpopulations tested in the spleen. In both murine models the dominating frequency of CD19+ cells were changed to dominating frequency of CD3+ cells during first 12 h post infection. Our data collected from GF as well as SPF animal models clearly demonstrate the decisive role of primary interaction between invading bacterium and the host cell that the microbe first encounters. Our experiments have also shown the data of cellular responses are more important in peritoneum till 12 hour post infection.

This work was supported by Long-term Organization Development Plan 1011 from the Ministry of Defense of Czech Republic and Specific research project no: SV/FVZ201707 from the Czech Ministry of Education, Youth and Sport.

Host-Microbial interactions: identification and application of glycan-lectin components as decontamination tools

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Many microbial interactions with host cells require the participation of lectins/adhesins and glycans on both microbial and host surfaces. These interactions lead to biological signaling responses which determine the host-microbial relationship and further alters the cell surface glycosylation of both organisms. Thus, dynamic glycosylation-mediated cross-talk contributes to the pathogenic or mutualistic response. Although an important area to elucidate, progress is impeded by a lack of high throughput tools.

To facilitate our investigations in to the role and response of glycosylation in host-microbial interactions, we have established cell-based assays, a suite of glycomics microarrays including novel mucin microarrays, and glycogenomic methods. We have also constructed a number of specific carbohydrate recognition molecules to facilitate rapid detection and anti-infective strategies.

Leveraging these methods, we have developed an innovative non-toxic and environmentally friendly method to capture pathogens and decontaminate physical surfaces. This technology has been demonstrated in laboratory conditions and is being developed for further human and animal applications against microbial contaminations.

New insight into HPV related cancer and the role of a potential co-factor *Chlamydia trachomatis* using transcriptomic and proteomic methods

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Certain types of Human papilloma viruses (HPV) are the etiological agents for cervical cancer. However, not all infections of high-risk HPVs will finally lead to cancer since most HPV infections are cleared without any consequences. *Chlamydia trachomatis* (*Ctr*) is the most prevalent sexual transmitted bacteria and is an obligatory intracellular pathogen exhibiting tropism in endocervical epithelial cells. Over the past decades, *Ctr* is thought to be a potential co-factor for cervical cancer formation, but there are also studies that did not show such a correlation.

To address this question in molecular terms, we stably expressed HPV16 E6 and E7 in spontaneously immortalized NOKs (normal oral keratinocytes) and performed SILAC (stable isotope labeling by amino acids in cell culture) as well as RNAseq analysis with or without *Ctr* infection.

Our initial results show that, as the major factor of cervical cancer, expression of HPV16 E6 and E7 in NOKs cells results in alterations of multiple cellular pathways including significant downregulation of interferon signaling (e.g. IFI6, ISG15, OAS2, OAS3), downregulation of cell adhesion and migration (e.g. KLK7, FN1, THBS1, THBD) and upregulation of cell cycle progression (CDKN2B, PGF, HTRA1, respectively). Acute infection with *Ctr* leads to significant changes in numerous host genes (e.g. LAMB3, MAP2K2, LAMC1, MTOR, NFKB2, RAC2, ITGAV) and pathway analysis shows that these genes are tightly related to cancer signaling.

In addition, we identified common targets of HPV16 oncogenes and *Ctr* (FN1, G0S2, C3, respectively) that are regulated in a synergistic manner. Further interpretation of the data by integrative analysis of transcriptome and proteome data is currently ongoing to reveal the connections of the changes observed.

This study provides new insights into HPV related cancer by identifying novel targets of HPV16 oncogenes and offers distinct evidence for a contribution of *Ctr* infection as a potential co-factor in HPV induced carcinogenesis.

Dual RNA-seq analysis of human macrophages infected by *Bordetella pertussis*

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Bordetella pertussis is the Gram-negative causative agent of human whooping cough (pertussis), a highly contagious respiratory disease that remains one of the ten most common causes of death from infectious diseases worldwide. Therefore, there is an urgent need for a better understanding of molecular mechanisms underlying the pathogenesis of *B. pertussis* infection. Macrophages are one of the first cells of the innate immune system to face *B. pertussis* cells and clear the primary infection. Nevertheless, the activities of wide spectrum of virulence factors help *B. pertussis* cells to manipulate cell signaling, evade the killing and survive and thrive in human macrophages. Macrophages thus may provide a hideout to extend infection and potentially to spread to new hosts. To identify phagocyte-specific adaptation responses during the infection as well as pathogen-specific remodeling of the gene expression in the course of adaptation to intramacrophage environment we have decided to perform dual RNA-seq analysis of human THP-1 macrophages infected with *B. pertussis* cells. At first we had to standardize the protocol for differentiation of THP-1 monocytic cell line into macrophages and optimize the infection protocol to obtain significant number of *Bordetella*-specific reads for transcriptomic analysis. We will present optimized infection protocol as well as our preliminary data showing that both pathogen and host substantially modulate the gene expression during the infection.

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Bordetella pertussis Adenylate Cyclase Toxin Disrupts Functional Integrity of Bronchial Epithelial Layers

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The airway epithelium restricts the penetration of inhaled pathogens into the underlying tissue and plays a crucial role in the innate immune defense against respiratory infections. The whooping cough agent, *Bordetella pertussis*, adheres to ciliated cells of the human airway epithelium and subverts its defense functions through the action of secreted toxins and other virulence factors. We examined the impact of *B. pertussis* infection and of adenylate cyclase toxin-hemolysin (CyaA) action on the functional integrity of human bronchial epithelial cells cultured at the air-liquid interface (ALI). *B. pertussis* adhesion to the apical surface of polarized pseudostratified VA10 cell layers provoked a disruption of tight junctions and caused a drop in transepithelial electrical resistance (TEER). The reduction of TEER depended on the capacity of the secreted CyaA toxin to elicit cAMP signaling in epithelial cells through its adenylyl cyclase enzyme activity. Both purified CyaA and cAMP-signaling drugs triggered a decrease in the TEER of VA10 cell layers. Toxin-produced cAMP signaling caused actin cytoskeleton rearrangement and induced mucin 5AC production and interleukin-6 (IL-6) secretion, while it inhibited the IL-17A-induced secretion of the IL-8 chemokine and of the antimicrobial peptide beta-defensin 2. These results indicate that CyaA toxin activity compromises the barrier and innate immune functions of *Bordetella*-infected airway epithelia.

Outer membrane vesicles and nanotubes in *Francisella tularensis*

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Francisella tularensis is a gram-negative, facultative intracellular bacterium, causing a severe disease known as tularemia. Being one of the most infectious pathogenic bacterium it is classified as a potential biological warfare agent by the Working Group on Civilian Biodefence. Outer membrane vesicles (OMV) secreted by gram-negative bacteria play an important role in bacterial physiology, virulence and host-pathogen interaction. Immunomodulatory potential in some bacteria has also been reported. It has been described previously that *Francisella* spp. apart from the classical spherical OMV produces also less usually shaped outer membrane nanotubes.

In this study OMV were isolated from culture medium of *F. tularensis* subsp. *holarctica* FSC200 by medium concentration followed by high-speed centrifugation and density gradient purification. Transmission electron microscopy of the low density fraction revealed a rich mixture of vesicles (spherical and tubular all together). Proteomic analysis of the purified OMV fraction revealed ca 300 proteins. This fraction was rich in outer membrane proteins, lipoproteins, immunoreactive proteins as well as previously described virulence factors. The interaction of purified OMV with murine primary bone marrow-derived macrophages (the predominant host cell type for *F. tularensis*) was visualized by fluorescence microscopy. Rapid entry of OMV into the cells was observed as well as prolonged viability of the macrophages which suggests their role in host-pathogen interaction.

We have also isolated OMV from bacteria cultivated *in vitro* under several cultivation conditions that simulated the diverse conditions of the *F. tularensis* life cycle. These included conditions mimicking the environment inside the mammalian host cell (oxidative stress, low pH, elevated temperature) and low temperature that mimicked the external milieu.

Low pH and high temperature lead to the most prominent increase of OMV production. Moreover, OMV isolated in high temperature contained extremely long nanotubes in comparison with other cultivation conditions. Semi-quantitative

proteomic comparison of OMV derived from different cultivation conditions resulted in distinct groups of proteins characteristic for each stress.

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Exosomes in charge? The lesson from B-cell deficient chicken

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The onset of immunoglobulin expression and secretion was identified as the most prominent caecum response to the colonization of digestive tract with non-pathogenic commensal microbiota during our recent experiments. On the other hand, gastrointestinal manifestations in Ig deficiencies are rather rare. As this is a bit controversial, we decided to uncover the role of antibodies in maintaining of gut homeostasis and therefore the chicken model deficient in B-cell development was constructed and involved in host - microbiota response study. The unbiased mass spectrometry of caecum wall protein was performed at the age of 4 and 8 weeks to identify the differentially expressed proteins. As the secreted antibodies were reported also as the host instrument to influence the microbiota composition, bacterial 16S rRNA parallel sequencing was performed and also the chicken proteins that may directly interact with the microbiota were identified in luminal content. Concerning the microbiota composition, only minor differences were identified between Ig-deficient and wild-type group. Although the pro-inflammatory IL8 and IL17 were slightly up-regulated at mRNA level (fold 6.2 and 2.6 respectively) at 4 weeks of age, these differences disappeared until week 8. The further analysis of differentially expressed proteins in the caecal wall samples led us to identification of microbiota responding genes/proteins and indicated the role of extracellular exosomes as the mediator of homeostasis of the gut.

UV irradiation and cutaneous papillomavirus infection:

development of non-melanoma skin cancer in a natural animal model

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Cutaneous human papillomaviruses (HPVs) are considered as cofactors for non-melanoma skin cancer (NMSC) development, especially in conjunction with UVB exposure. Using the natural infection model *Mastomys coucha,* which reflects the human situation in many aspects, we provide evidence that only skin infected with *Mastomys natalensis* papillomavirus (MnPV) progressed to NMSC after UV irradiation. Animals developed either well-differentiated keratinizing SCCs still with supporting productive infections with high viral loads and transcription or poorly differentiated non-keratinizing SCCs, almost lacking viral DNA and in turn transcriptional activity. Notably, animals with the latter, however, still showed strong seropositivity, clearly verifying a preceding MnPV infection. Moreover, the mere presence of MnPV could induce γH2AX foci, indicating that viral infection without prior UVB exposure can already perturb genome stability of the host cell. Like in humans, the majority of SCCs harbored *Trp53* mutations especially at two hot-spots in the DNA-binding domain, resulting in a loss of function that favored tumor dedifferentiation, counter-selective for viral maintenance. Such a constellation provides a reasonable explanation for making continuous viral presence dispensable during carcinogenesis as observed in patients with NMSC.

Proteomic profiling of virulent phase I and avirulent phase II of *Coxiella burnetii* employing axenic and cell culture-based cultivation

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Coxiella burnetii is an obligate intracellular bacterial pathogen, causative agent of multiple outbreaks of zoonotic disease Q fever which is worldwide with an increasing tendency. The bacterium is also considered as important biothreat agent and is characterized as category B pathogens in the bacterial list of biological warfare agents. To date, the only defined and characterized Coxiella virulence determinant is the lipopolysaccharide, which is used to distinguish between virulent and avirulent organisms and appears after frequent passaging. The deeper study of novel determinant of *Coxiella burnetii* is complicated and demands advanced techniques for axenic as well as culture-based cultivation together with new approaches in the high-resolution mass spectrometry. This project is the first proteomic study comparing virulent and avirulent *C. burnetii* obtained from different axenic and culture-based cultures. Virulent *Coxiella burnetii* RSA 493 phase I and avirulent *Coxiella burnetii* RSA 439 phase II were cultivated and L929 mouse fibroblasts were infected by both *C. burnetii* strains. Biological replicates were cultured and amount of the bacteria was analyzed using qPCR of genome equivalents. Samples for the analysis by liquid chromatography on-line coupled with mass spectrometer - Q-Exactive (Thermo Scientific) - were prepared based on the Filter Aided Sample Prep protocol followed by tryptic digestion. The MS/MS data were processed and label free quantification was then performed to compare all groups. This study compares the axenic media and *in vivo* cultures at the proteomic level and these data were matched with the genome sequences of the cultured strains. The most prominent changes were represented by proteins connected with lipopolysaccharide biosynthesis outside the known deleted region

from the avirulent Phase II and the proteins connected with the Type IV secretion system. Unique comparative proteomic analysis of *in vivo* and *in vitro* cultured *C. burnetii* proposed new virulence determinants

Proteomic study of antigenicity of *Rickettsia akari* proteins

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Rickettsia akari is a small obligate intracellular bacterial pathogen and the etiological agent of Rickettsialpox. This underestimated rickettsial disease is transmitted to humans by an arthropod vector (*Liponyssoides sanguineus*). Occurrences of Rickettsialpox have been reported from diverse parts of Europe and American continents. Accessible data on immunogenicity of *R. akari* is limited and can lead to misdiagnosis of the disease, therefore identification of immunogenic proteins can improve the recognition of illness and accurate treatment. The goal of this study is to investigate the protein profile of *R. akari* and study the antigenicity of its proteins using two dimensional gel electrophoresis (2D), immunoblotting techniques and identification by Mass spectrometry. Our results showed positive spots in 2D protein gel staining by Coomasie blue, confirming the presence of proteins ranging between 130 and 10 kDa and p interval from 3 to 10. The corresponding 2D immunoblot revealed the known ones rickettsial immunogenic proteins: OmpB (Uniprot: A8GPL7), Chaperone protein DnaK (Uniprot: A8GMF9) and 60 KDa chaperonin (Uniprot: A8GPB6). In addition, we were able to detect five more specific immunogenic proteins for *R. akari*, i.e. Peptidoglycan-associated lipoprotein (Uniprot: A8GPW0), Cell surface antigen (Uniprot: A8GNM2), Membrane protease subunits (Uniprot: A8GN16), Superoxide dismutase (Uniprot: A8GNP0) and a 44 kDa Uncharacterized protein (Uniprot: A8GP63). Interestingly, a pattern of rickettsial lipopolysacharide was identified by patient sera. These results can contribute to the improvement of Rickettsialpox diagnosis.

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Comparative genomics of clinical and environmental isolates of *Campylobacter jejuni*

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Campylobacteriosis is major bacterial food-borne disease in industrialized countries in recent years with a large economic burden. The major causative agent of human campylobacteriosis is *Campylobacter jejuni* which accounts for 90 % of human cases. *C. jejuni* is considered as a member of commensal microbiota in birds, thus the raw or undercooked poultry products are the major sources of human cases. However, *C. jejuni* strains can also survive in water and waterborne outbreaks of campylobacteriosis are also widely and frequently reported. Epidemiologic and genomic studies revealed that genetically distinct *C. jejuni* isolates circulate throughout the globe and even within the same host. In order to identify genotypes associated with clinical and environmental sources, we determined and compared genome sequences of 15 *C. jejuni* isolates originating from water sources (5 isolates) and human beings (10 isolates).

The total pan-genome of sequenced isolates comprises 2345 proteins, whereas core-genome consists of 1371 proteins. Comparing genomes of human and water isolates, thirty-six proteins were only associated with human isolates. These human-associated proteins were mainly involved in sugar, amino and fatty acid metabolism, transport, iron uptake and cell wall biosynthesis. On contrary, water-associated proteins included eight hypothetical proteins. In addition, human isolates showed intra-strain variation in homopolymeric tract length in genes coding for flagellar and other motility proteins. These data indicate that human isolates of *C. jejuni* are more virulent and showed increased adaptation to a novel host.

Seeking host targets of *Francisella* pathogenicity island effectors

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Francisella tularensis is an intracellular, Gram-negative bacteria able to enter, survive and replicate within the host phagocytic cells. Because of its high infectivity, ability to cause disease via inhalation route, and absence of a vaccine approved for human use, the pathogen is classified as a potent biothreat agent. Despite increasing knowledge about its virulence, crucial molecular interactions during the infection have not been characterized. The aim of our study is to identify eukaryotic proteins targeted by *F. tularensis* effectors which were reported to be secreted via type VI secretion system. To this end, we employed affinity purification of ectopically expressed *F. tularensis* proteins combined with stable isotope labeling with amino acids in cell culture (SILAC). Proteins enriched from the host cells were identified via mass spectrometry. We identified several eukaryotic proteins with different abundance in enrichments from a mock culture and the culture with ectopically expressed *F. tularensis* proteins. Nevertheless, their factual involvement in *F. tularensis* virulence has to be further confirmed.

This work was supported by the Ministry of Defense (A long-term organization development plan no. 1011) and by Ministry of Education, Youth and Sport (Specific research project no: SV/ FVZ201509), Czech Republic.

Proteome profiling of *Francisella tularensis* dendritic cells Invasion

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Francisella tularensis is a highly infective Gram-negative bacterium, also known as the causative agent of tularemia. As an intracellular pathogen, *Francisella* is able to invade and to proliferate inside dendritic cells while avoiding their effective activation and maturation. Our goal was therefore to map detergent resistant membrane (DRM) fractions and phosphoproteome changes in dendritic cells infected by *Francisella* in order to identify host signaling pathways involved in *Francisella*-dendritic cell interaction.

Bone marrow-derived dendritic cells were labeled by SILAC and infected by fully virulent *F. tularensis* FSC200 strain or its attenuated mutant counterpart Δ*dsbA*. Infected cells were lysed at 10 min for DRM analysis and at three time points p.i. (10, 30 and 60 min) for phosphoproteomics. Quantified DRM proteins and phosphosites from WT- and Δ*dsbA*-infected BMDC lysates were compared by Student t-test and those with $p<0.05$ were considered differentially regulated. Western blot analysis was employed for the verification of proteomic data and for detection of phosphosites not identified by mass spectrometry. The quantitative real-time PCR was used for quantification of cytokine mRNA.

In total, we were able to identify 1 215 proteins from DRM fractions and more than 17,000 phosphosites from *Francisella*-infected dendritic cells. The entry of *Francisella* was associated with strong activation of GTPase family proteins resulting in cytoskeleton reorganization and there were no quantitative differences between dendritic cells response to virulent and attenuated mutant strain. In contrast only virulent *Francisella* induced the second wave of protein phosphorylation in infected cells at 60 min postinfection. The most prominent feature of the latter signaling phase was the stimulation of ERKs and p38 kinases and the activation of NF-κB, Jun, and CREB transcription factors. Using p38/SAPK2 inhibitor the role of the p38 kinase in the CREB phosphorylation and the early expression of *Il12b* and *Il10* was uncovered.

We conclude that this second signaling wave represents the initial event of dendritic cells activation where virulent and attenuated *Francisella* strains differ.

Phosphoproteomics of *Bordetella Pertussis* Adenylate Cyclase Toxin Action in Mouse Dendritic Cells Reveales Inhibition of mTOR Pathway and CRTC3-dependent Signaling

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Gram negative aerobic coccobacillus *Bordetella pertussis*, the agent of whooping cough (or pertussis), subverts host protective functions through powerful immunomodulatory actions of its toxins and other virulence factors. The prominent role here is played by the adenylate cyclase toxin (CyaA) that delivers into phagocytic cells its adenylyl cyclase enzyme domain and paralyzes bactericidal functions of the cells through unregulated conversion of cytosolic ATP into the key signaling molecule, cAMP.

To fill the gap in our understanding of its immunosuppressive signaling, we have used here SILAC-based phosphoproteomics of CyaA-intoxicated primary murine bone marrow-derived dendritic cells.

Bioinformatic identification of cAMP-regulated phosphoproteins revealed inhibition of mTOR signaling pathway through the activation of mTORC1 inhibitors TSC2 and PRAS40. Besides that, CyaA toxin action promoted inhibitory phosphorylation of SIK family kinases, which are important immunomodulators, resulting in dephosphorylation of the transcriptional coactivator CRTC3. This finding provides possible explanation for CyaA-induced IL-10 production. Observed alterations of phosphorylation status of multiple chromatin remodelers, including HDAC5, then provided interesting hints on the so far overlooked involvement of chromatin remodeling mechanisms during infection by the pertussis agent.

Bordetella type III secretion system effector BteA: its functional divergence and mechanism of action

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Pertussis, or whooping cough, is a respiratory infectious disease, primarily caused by the bacterium *Bordetella pertussis*. The occurrence of pertussis in the most developed countries is steadily increasing despite massive vaccination programs. This calls for better understanding of *Bordetella pertussis* pathogenesis. *B. pertussis* and the closely related respiratory pathogens *B. parapertussis* and *B. bronchiseptica* share a nearly identical virulence control system BvgAS and numerous virulence factors, including the components of the type III secretion system (T3SS) machinery. The T3SS effector protein BteA is encoded in the genome of *Bordetellae* and the molecular basis of its activity and its role in the *B. pertussis* virulence remain elusive.

Here, we confirmed the presence of active T3SS in a recent clinical isolate *B. pertussis* 1917 and further suggested divergent evolution of BteA effector in *Bordetellae*. Indeed, *B. pertussis* 1917 cytotoxicity to infected cells is negligible as compared to cytotoxicity caused by *B. bronchiseptica* D445 infection. A single amino acid insertion in BteA protein sequence of *B. pertussis* 1917, insertion of alanine at position 503, is responsible for the observed lack of cytotoxicity. Importantly, this amino acid insertion is conserved in the genus *B. pertussis* as compared to *B. bronchiseptica* and deletion of A503-encoding codon in *B. pertussis* 1917 chromosome leads to *B. pertussis* cytotoxic levels that are similar to the ones induced by *B. bronchiseptica*. The biological role of this amino acid insertion and conservation in the *B. pertussis* genome is under investigation, the status of which will be as well presented.

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