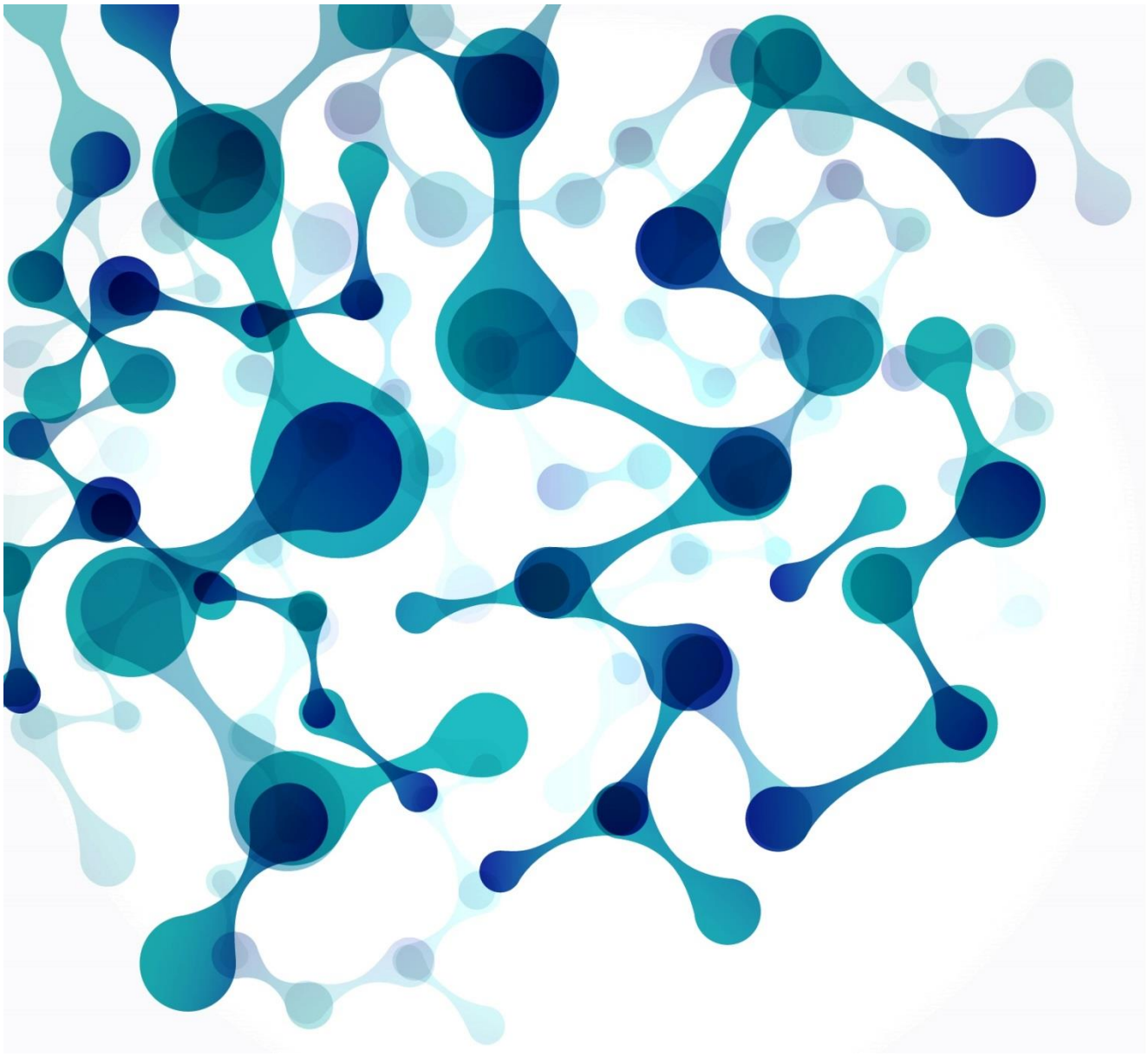

Discussion Forum 2016

Host Pathogen Interaction

May 2 – 5, 2016, Broumov, Czech Republic

Agenda and Abstract book

Discussion Forum 2016

Host Pathogen Interaction

Venue: Broumov, Czech Republic

Date of event: **May 2nd – 5th 2016**

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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&

Czech Immunological Society

CONFERENCE SPONZORS

We gratefully acknowledge the support, involvement and interest of our sponsors and participating organizations:

EDITORIAL

Dear colleagues,

It is our pleasure to welcome you to the Discussion forum 2016 conference in Broumov. The traditional main topic of host-pathogen interaction is enlarged to comprehend also other aspects of the analysis of biological material.

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 excursion, all designed to provide a relaxing atmosphere with great food and drink.

We are glad you were able to come and hope you have an enjoyable and productive time.

Organizers

CONFERENCE VENUE

Discussion Forum 2016 is held in the beautiful and exciting city of Broumov. The city Broumov is a town in the Náchod District near the border with Poland. The municipality is located on the small Stěnava River in the northeast of Bohemia, about halfway between Náchod in the southwest and Polish Wałbrzych in the north. The region of Broumov is geographically and historically a distinctive region with a wild and poetic landscape. For many centuries this region was once a very important cultural center enriched by artistic monuments and edifices, which were built during the administration of the Benedictine monks. It is the centre of the Broumovská vrchovina area of the Central Sudetes range, along with the adjacent Adršpach-Teplice Rocks, a 19 sq. km protected area popular with mountain climbers.

ACCOMODATION

VEBA HOTEL

Participants will be accommodate in First Republic villa, built in 1927 as a wedding gift for the daughter of the owner of a textile factory, will enchant you with its unique atmosphere.

SOLITAIRE

A luxury suite with two spacious rooms, a kitchen, outside grill and three beds is located in a separate stylish building in the middle of the park.

DEPANDANCE

Cosily furnished double rooms with either a double bed or separate beds are located in an adjacent low building belonging to the Hotel and located in the large park.

Hotel Manor House

The Manor House is a newly renovated stylish hotel.

CONGRESS CENTRE

Congress Centre also provide the accomodation.

BREAKFAST

Buffer-styled continental breakfast is served in VEBA hotel and MANOR house, Tuesday to Thursday from 7am to 9am.

LUNCH

Conference lunches are served in VEBA hotel and MANOR house, Tuesday to Thursday from 1pm to 2pm.

DINNER

Monday, May 2 – Welcome party - included in the conference fee

Tuesday, May 3 – Dinner - will not be organized

Wednesday, May 4 – Conference reception - included in the conference fee

SOCIAL EVENTS

Welcome party

Date: Monday, May 2

Time: 7pm

Location: Congress Centre, VEBA hotel resort

Guided tour to Monastery Broumov

Date: Wednesday, May 4

Time: 2pm

Location: pick up in front of VEBA hotel

Conference Reception

Date: Wednesday, May 4

Time: 5:15pm

Location: transport will be ready in front of VEBA hotel

AGENDA

MONDAY (May 2, 2016)

2:30 – 5:30pm	*Arrival of participants, Registation and helpdesk open (VEBA hotel)*
6:00 – 6:10pm	**Kubelkova Klara** – Opening (Congress Centre)
6:10 – 6:35pm	**Stulik Jiri** - *Francisella* immunogenic peptides - searching for Holy Grail, UoD (FoMHS, Hradec Kralove, CZ)
6:35 – 7:00pm	**Macela Ales** – Host-Pathogen Interaction: Innate Immune Recognition: Lesson from *Francisella* models (FoMHS, Hradec Kralove, CZ)
7:00 – 12pm	*Welcome party*

TUESDAY (May 3, 2016)

7:00 – 9:00am	*Breakfast –VEBA hotel and MANOR house*
9:00am – 12:20pm	**Session I.: *Chairman:* Worsham P., Stulik J.**
9:00 – 9:20am	**Mou Sherry** - A Putative Carboxypeptidase Is Required for *Francisella tularensis* Virulence (USAMRIID, Maryland, USA)
9:20 - 9:40am	**Spidlova Petra** - D-Ala D-Ala carboxypeptidase is involved in pathogenesis of *Francisella tularensis* (FoMHS, Hradec Kralove, CZ)
9:40 – 10:05am	**Klimentova Jana** - Bacterial membrane vesicles – why do they release them? (FoMHS, Hradec Kralove, CZ)
10:05 – 10:30am	*Coffee break – Congress foyer*
10:30 – 11:05am	**Worsham Patricia** - Updates on Tularemia and Bulkholderia projects (USAMRIID, Maryland, USA)
11:05 – 11:30am	**Link Marek** - Identification of MHC class II bound peptidome of dendritic cells (FoMHS, Hradec Kralove, CZ)
11:30 – 11:55am	**Fabrik Ivo** - Proteomic Mapping of Cell Signaling in Dendritic Cells During Early Interactions with *Francisella tularensis* (FoMHS, Hradec Kralove, CZ)

11:55 – 12:20pm	**Balonova Lucie** - Inactivation of *Francisella tularensis* gene encoding putative flippase has a pleiotropic effect upon production of various glycoconjugates: Evidence for pilin protein modification by O-antigen (FoMHS, Hradec Kralove, CZ)
1:00 – 2:00pm	*Lunch – VEBA hotel and MANOR house*
2:00 – 5:45pm	**Session II. - Chairman: Santic M., Krocova Z.**
2:00 – 2:20pm	**Santic Marina** - The peculiar phenotype of IglG mutant in human macrophages and mice model (University of Rijeka, Medical Faculty, Croatia)
2:20 – 2:35pm	**Putzova Daniela** - Quantitative proteomic analysis of IFN-γ-induced cell surface proteins of mouse macrophages (FoMHS, Hradec Kralove, CZ)
2:35 – 2:55pm	**Sekelova Zuzana** - T-lymphocytes response to Salmonella infection (Veterinary Research Institute, Brno, CZ)
2:55 – 3:15pm	**Maceckova Michaela** - Influence of Salmonella antibodies on *Salmonella enterica* serovars motility (Veterinary Research Institute, Brno, CZ)
3:15 – 3:55pm	*Coffee break - Congress foyer*
3:55 – 4.15pm	**Varmuzova Karolina** - Use of chicken gut microbiota in a protection of newly hatched chickens against *S. Enteritidis* infection (Veterinary Research Institute, Brno, CZ)
4.15 – 4:30pm	**Vasak Jiri** (KRD)
4.30 – 4:45pm	**Senitkova Iva** - Synthetic Biology (HPST)
4:45 – 5:00pm	**Korba Tomas** - OneOmics™ in the Cloud – Connection of Next-Generation Sequencing and Next-Generation Proteomics Utilizing SWATH Acquisition (AMEDIS)
5:00 – 5:15pm	**Pol Jaroslav** (Thermo Fisher Scientific)
5:15 – 5:30pm	**Kukla Stanislav** - Microclass on cell analysis using microcapillary and microfluidics technologies (Merck)
5:30 – 5:45pm	**Prokopova Dana** (BioTech)

WEDNESDAY (May 4, 2016)

7:00 – 9:00am *Breakfast – VEBA hotel and MANOR house*

9:00 am – 12:45 pm Session III. - Chairman: Sebo P., Faldyna M.

9:00 – 9:40am **Sebo Peter** - *Bordetella pertussis* and its adenylate cyclase toxin: How it works and what does it do (Institute of Microbiology of the CAS, CZ)

9:40 – 10:05am **Novak Jakub** - Getting past the guards and teaching them tolerance – lessons from *Bordetella pertussis* (Institute of Microbiology of the CAS, CZ)

10:05 – 10:40am **Hasan Shakir** - Bordetella adenylate cyclase toxin is a unique ligand of the integrin complement receptor 3 (Institute of Microbiology of the CAS, CZ)

10:40 – 11:15am *Coffee break - Congress foyer*

11:15 – 11:50am **Faldyna Martin** - Interferon-alpha downregulates response of porcine monocyte-derived macrophages to in vitro infection with *Mycobacterium avium* subsp. *avium* - the story of CXCL10 (Veterinary Research Institute, Brno, CZ)

11:50 – 12:10pm **Matiaskova Katarina** - Catalase as a potential virulence factor in in vitro interaction between *Haemophilus parasuis* and porcine pulmonar alveolar macrophages (Veterinary Research Institute, Brno, CZ)

12:10 – 12:30pm **Dresler Jiri** - Quantitative and qualitative proteomic analysis of *C. difficile* isolates of clinically relevant PCR-ribotypes (Military Health Institute, Prague, CZ)

12:30 – 12:45pm **McGoldrick Stephan** (BIOMEDICA)

1:00 – 2:00pm Lunch – VEBA hotel and MANOR house

2:00 – 5:00pm Guided tour to Broumov Monastery

5:15 – 5:20pm Transport for Conference Reception (in front of VEBA hotel)

6:00 – 11:00pm *Conference Reception*

6:00 – 6:30pm **Joshi Lokesh** - Glycobiology of pathogenic and commensal bacteria and gut cells (National University Ireland Galway, Ireland)

6:30 – 7:00pm **Utratna Marta** - Investigation of carbohydrate-mediated bacterial adherence and microbial-host interactions for development of biodefence and decontamination strategies (National University Ireland Galway, Ireland)

7:00 – 12:00pm *Farewell dinner with advanture*

THURSDAY (May 5, 2016)

7:00 – 8:30am *Breakfast – VEBA hotel and MANOR house*

8:30am – 12:45pm Session IV. - Chairman: Rösl F., Gekara N.

8:30 – 9:10am **Gekara Nelson** - Recent concepts on the role of the ubiquitin system in innate immune regulation (MIMS, Umea University, Sweden)

9:10 – 9:40am **Volf Jiri** – Transient and prolonged response of chicken caecum mucosa to the colonization with different gut microbiota (Veterinary Research Institute, Brno, CZ)

9:40 – 10:00am **Cejkova Darina** - Chicken gut microbes – from genomics to probiotics (Veterinary Research Institute, Brno, CZ)

10:00 – 10:20am **Hlavova Karolina, Stepanova Hana** - Impact of deoxynivalenol on immune parameters in porcine colostrum (Veterinary Research Institute, Brno, CZ)

10:20 – 10:40am **Oreskovic Zrinka** - In vitro stimulation of porcine monocyte-derived dendritic cells can be influenced by cytokine microenvironment (Veterinary Research Institute, Brno, CZ)

10:40 – 11:15am *Coffee break - Congress foyer*

11:15 – 11:50am **Rösl Frank** - Is there a humoral escape mechanism for papillomaviruses during the development of non-melanoma skin cancer? (German Cancer Research Center, Heidelberg, Germany)

11:50 – 12:10pm **Yang Ruwen** - *Chlamydia trachomatis* and HPV induced cervical cancer: a causal or coincidental interaction? (German Cancer Research Center, Heidelberg, Germany)

12:10 – 12:45pm **Eyer Ludek** - Structure-activity relationships of nucleoside analogues for inhibition of tick-borne encephalitis virus (Veterinary Research Institute, Brno, CZ)

12:45 – 2:00pm *Closing the conference, lunch* and *departure of participants*

ORAL PRESENTATION ABSTRACT

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Francisella immunogenic peptides - searching for Holy Grail

Jiri Stulik, Valerie Sheshko

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Francisella tularensis has been the subject of a surge in the level of research being performed, leading to a substantial increase in knowledge of the pathogenic mechanisms of the organism and the induced immune responses. This information should bring breakthrough in the development of multiple new Francisella vaccine candidates. Historically, live attenuated vaccines have demonstrated the greatest degree of success in protection against tularemia and the greatest promise in recent efforts to develop of a fully protective vaccine. Nevertheless, live vaccines pose a number of risks because they can potentially revert, which means that they can undergo a change in their genetic material that would change them from a safe, harmless version of a pathogen into a virulent and dangerous one. Additionally, people who suffer from chronic illness or are immunocompromised, as well as pregnant women and the elderly can't safely take live vaccinations, and so effective alternatives are needed.

Subunit vaccines circumvent both of these concerns as rather than a whole pathogen, specific fragments of a disease causing agent are used to stimulate the immune system. This is an extremely safe method of immunization, and can be used on virtually everyone in need of vaccination regardless of health status.

However, the finding of suitable immunogenic components for subunit vaccine construction is major obstacle for subunit vaccine development. This lecture summarizes the current approaches applied for *Francisella* immunogenic antigens identification.

Host-Pathogen Interaction: Innate Immune Recognition Lesson from *Francisella* models

Ales Macela, Klara Kubelkova

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The interaction of hosts with microorganisms has several forms. The two relationships that fundamentally influence the being of the host are commensalism of gut microbiota and parasitism of pathogenic microbes. The critical point for distinguishing one from the other is their original resolution followed by adequate immune response. The second half of 20th century offered several theories on innate immune recognition, from Burnet´s one signal theory, two signal theory of Bretcher and Cohn, defining of antigen-presenting cell (APC) function by Lafferty and Cunningham, up to Janeway´s concept of pathogen recognition receptors (PRRs) and Matzinger´s theory of danger signal.

Francisella tularensis, an intracellular bacterium, has been shown to be sensed on the cell membrane of antigen presenting cell by heterodimer Toll-like receptor 2/1, complement receptor 3, mannose receptor, scavenger receptor class A, and Fc-gamma receptor either alone or in cooperation with others. *Francisella* recruits cholesterol-rich lipid domains with caveolin-1 at the host cell membrane and initiate entry into APC, which generates of first batch of signals. The dominant signal generated by this event is interferon type 1 that can exert their function by autocrine or paracrine manner. The second batch of signals originated from association of *Francisella* with intracellular recognition elements AIM2, pyrin or NLRP3, which finalize twostep process originally controlled by TLR2-mediated ERK phosphorylation (priming) and triggered by recognition of pathogen-associated molecular patterns (PAMPs) by intracellular PRRs. The resulting products, proinflammatory cytokines IL-1-beta and IL-18, are significant results of *Francisella* recognition by APC. On the other hand the activation of AIM2/ASC inflammasome complex induces caspase-1-dependent pyroptosis of host cells or, in some cases, triggers AIM2/ASC-dependent caspase-3-mediated apoptosis. The accumulation of autophagic adaptor protein p62 in macrophage-derived lipid rafts after interaction with *Francisella* and the reentry of *Francisella* into MHC-II positive autophagic vacuole can suggest another source of signals for pathogen recognition.

Which of the signals generated after *Francisella* interaction is critical for innate immune recognition cannot be simply to identify. Rather, it is possible to agree with Rodney E. Langman and Melvin Cohn that no one mechanism can adequately regulate the innate immune recognition.

A Putative Carboxypeptidase Is Required for *Francisella tularensis* Virulence

Sherry Mou, Todd Kijek, Joel Bozue, and Patricia Worsham

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Francisella tularensis is a zoonotic intracellular pathogen and the causative agent of tularemia. After infection of a macrophage, the organism subsequently escapes from the phagosome, replicates to high density in the cytosol and triggers phagocyte death and lysis. Unfortunately, many of the bacterial factors required for these aspects of virulence have not yet been identified. Here we describe the isolation and characterization of a *Francisella tularensis subsp. tularensis* strain Schu S4 mutant that lacks a functional FTT1029 gene. The genome annotation of *F. tularensis* predicts that the FTT1029 locus functions as a D-alanine carboxypeptidase. Our data demonstrated that this mutant was defective for replication in murine J744.A1 cells, A549 cells and also exhibited reduced growth in defined media. When virulence was assessed by intranasal infection of BALB/c mice, the mutant appeared highly attenuated (mutant LD50= > 300 CFU vs the parental strain LD50~1.0 CFU). Complementation studies using the native gene provided in *trans* resulted in 100% restoration of the wild-type phenotypes. Our data suggests that this putative carboxypeptidase plays an important role in the virulence of *F. tularensis* Schu S4.

D-Ala D-Ala carboxypeptidase is involved in pathogenesis of *Francisella tularensis*

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Francisella tularensis is an intracellular pathogen and causative agent of tularemia. After the infection, *Francisella* invades macrophages where it effectively replicates. To enter and to survive in these cells *Francisella* requires a variety of virulence factors, which are still being characterized.
Our results show that the mutant strain with the insertional inactivation of the FTS_1034 gene coding for D-Ala D-Ala carboxypeptidase is highly defective in cellular replication in mouse bone marrow-derived macrophages. The mutant bacteria are also attenuated *in vivo*, however, the higher doses of mutant lead to the development of tularemia and subsequently to the death of BALB/c mice. Further, we demonstrate that the mutant is effectively eliminated from lung and liver tissues when compared to the wild-type strain. But in case of the spleen tissue the mutant bacteria persisted during the whole observed time interval. Complementation *in trans* and *in cis* resulted in the partial or full restoration the wild-type phenotype. Finally, the electron microscopy studies are performed to investigate the fate of mutant bacteria inside the mouse bone marrow-derived macrophages.
All together, these data suggest that D-Ala D-Ala carboxypeptidase contributes to *Francisella* virulence.

Bacterial membrane vesicles – why do they release them?

Jana Klimentová

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Production of outer membrane vesicles (OMV) represent an alternative way of protein secretion that is common to all gram-negative bacteria and is independent of the known secretion systems I-VI. OMV are 20-300nm usually spherical double-layered membranous particles that are released from the gram-negative bacterial outer membranes. They are formed when a portion of outer membrane separates and encapsulates part of the periplasmic space with its contents. As such they contain proteins of the outer membrane and periplasm, phospholipids, lipopolysaccharide and peptidoglycan. Nevertheless, they can harbor also nucleic acids and cytosolic or inner membrane proteins.

OMV play an important role in bacterial physiology as well as in virulence and host-pathogen interaction. They can act both in the defense of bacteria against environmental threats and also in the offense against competitors. Many OMV functions in bacterial physiology and pathology still remain to be elucidated. Nevertheless, the immunomodulatory potential of OMV from a number of bacteria have been studied and employed in the vaccine development.

Updates on *Burkholderia* Animal Modeling and Pathogenesis of *F. tularensis*

Patricia Worsham

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Burkholderia animal modeling. We conducted a direct comparison of BALB/c and C57BL/6 mice challenged by low doses of *B. pseudomallei* by aerosol or IP routes. The two challenge routes produced quite distinct pathologies. Multinucleated giant cells were observed in aerosolized mice, but not those infected by the IP route. Our earlier necropsy, histopathological, and histochemical findings suggested that the African Green Monkey (AGM) is the best model for acute, lethal glanders infection. We have now evaluated the AGM and Rhesus macaque as models for melioidosis and we downselected the AGM as the appropriate model for acute disease.

Francisella tularensis virulence factors. Inactivation of kdsD resulted in a significant attenuation of *F. tularensis* Schu S4. The mutant also demonstrated growth defects in vitro and in macrophage/epithelial cell lines. We were able to restore normal growth in vitro and in macrophages, as well as virulence, with trans complementation. The growth defect in vitro could also be complemented by addition of A5P to the growth medium. As an essential virulence factor, this gene product represents a potential new target for development of medical countermeasures. The mutants themselves may have promise as live vaccine candidates.

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army. Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.

Identification of MHC class II bound peptidome of dendritic cells

Marek Link, Stanislava Porkertová, Lucie Balonová, Jiří Stulík

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Antigen presenting cells play an important role in initiating and shaping the host adaptive immune response during pathogen infection. These cells present pathogen-derived antigens in a complex with their MHC molecules on the cell surface for recognition by specific T-cells. The recognition by T-cells governs the specificity of evolving T-cell mediated immunity.

The identification of antigenic fragments bound to MHC molecules became an important part of immunological studies. Immunoproteomics is one of the approaches that can be used for identification of MHC-bound peptides. We present this approach for global identification of self-peptidome bound on MHC class II molecules of primary bone-marrow derived dendritic cells. The procedure is based on immunoaffinity purification of MHCII-peptide complexes, isolation of obtained peptides, and subsequent analysis by liquid chromatography high-resolution mass spectrometry. The general properties of obtained self-peptidome are described. Additionally, the peptidome of dendritic cells treated with a model antigen is reported as a proof of principle that will be further exploited for identification of presented peptides from *Francisella tularensis* antigens.

Proteomic Mapping of Cell Signaling in Dendritic Cells During Early Interactions with *Francisella tularensis*

Ivo Fabrik, Marek Link, Lenka Plzakova, Daniela Putzova, Ivona Pavkova, Pavel Rehulka, Zuzana Krocova and Jiri Stulik

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Francisella tularensis is known for its capability to escape from the phagosome and to replicate in the cytosol of invaded host cells. Finely-tuned interaction between the bacterium and the host cell is best described for infected macrophages – however these are not the only phagocytes affected by *Francisella* infection. While lesser in numbers compared to macrophages, dendritic cells represent interesting targets for *Francisella* because of their complex role in the induction of adaptive immunity needed for the control of the infection. Our goal was to monitor *Francisella*-dendritic cell interaction during the first crucial steps of the invasion process by the means of mapping of host cell signaling. Employing stable isotope labeling of primary bone-marrow dendritic cells for LC-MS-based relative quantitation of phosphoproteome changes, we were able to construct protein-protein interaction network consisting of proteins involved in *Francisella* entry into the host cell. Time-dependent changes in protein phosphorylation also revealed potentially activated kinases. In the future work, we would like to focus on differences in the host signaling between dendritic cells infected by virulent and attenuated strains of *Francisella*, respectively.

Inactivation of *Francisella tularensis* gene encoding putative flippase has a pleiotropic effect upon production of various glycoconjugates: Evidence for pilin protein modification by O-antigen

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Francisella tularensis utilizes surface glycoconjugates such as lipopolysaccharide, capsule, and capsule-like complex for its protection against inhospitable conditions of the environment. *Francisella* species also possesses a functional glycosylation apparatus by which specific proteins are O-glycosidically modified. Through the present study, we seek to extend the knowledge of protein glycosylation machinery in *F. tularensis* and examine the consequences of disrupting the *FTS_1402* gene of the putative *protein glycosylation locus (pgl)*. Based on the homology to *Campylobacter* PglK flippase, FTS_1402 is predicted to play a role as a transporter of the nascent glycan across the inner membrane before its addition to target protein. Surprisingly, inactivation of the *FTS_1402* gene had a pleiotropic effect upon production of multiple surface glycoconjugates (e.g. glycoproteins, lipopolysaccharide, capsule/capsule-like complex), which all had preserved structures but were synthesized in smaller amounts when compared to the highly virulent parental strain. It resulted in unexpectedly marked attenuation of *FTS_1402* mutant with enhanced sensitivity to serum complement, which however, provided protection against subsequent systemic challenge. This observation is of particular interest since a complete lack of surface glycoconjugates usually results in a failure to elicit protective immune response. Moreover, we provide evidence that a pilin glycoprotein PilA is modified with a single O-antigen unit of LPS in the *F. tularensis* FSC200 strain and its derived mutants.

The Peliculiar phenotype of ΔiglG mutant inhuman macrophages and mice model

Ozanic M[1], Marecic V[1], LindgrenM[2], Sjöstedt A[2], **Santic M[1]**

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Several bacterial pathogens interact with their host through protein secretion involving type VI secretionsystem (T6SS). *Francisella tularensis* is a highly pathogenic intracellular bacterium that causes the disease tularemia. Proteins encoded by the *Francisella* pathogenicity island (FPI), which constitute a type VI secretion system, are essential for the escape of the bacterium from the phagosome followed by productive intracellular replication. I has been shown that T6SS in *Francisella* is distinct since all substrates of *F. tularensis* T6SS, except for VgrG, are unique to the species. Many of the FPI proteins were secreted into the macrophage cytosol, dependent on the functional components of DotU, VgrG, IglC and IglG. In addition, PdpC seems to have a regulatory role in expression of iglABCD. Since previous results showed peculiar phenotype of ΔpdpC and ΔiglC mutants in mouse macrophages their unique behavior was futher studies in this study. Our results show that both ΔpdpC and ΔiglC mutants of the live vaccine strain (LVS) of *F. tularensis* did not replicate within human monocytes derived macrophages (hMDM). The ΔpdpC mutants did not escape from the FCV, neither caused cytopythogenicity in primary macrophages and was attenuated in a mice model. Interestingly, ΔiglC mutant escaped from the FCV within hMDMs and caused the pathological changes in the spleen and liver tissues of intradermally infected C57BL/6 mice. The ΔiglC mutant with its unique phenotype could be used as a potential vaccine candidate.

Quantitative proteomic analysis of IFN-γ-induced cell surface proteins of mouse macrophages

Putzova D.[1], Härtlova A.[2], Bock, T. [3], Wollscheid B. [3], Stulik J.[1]

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Francisella tularensis is a highly virulent intracellular pathogen that causes severe disease upon inhalation only of 10 organisms. The ability to infect and survive inside macrophages is crucial to its virulence. Interferon-γ (IFN-γ) limits the intracellular replication of *Francisella* in macrophages and is therefore considered to be the key host innate immune factor against *Francisella.* We employed the MS-based Cell Surface Capturing method in combination with SILAC approach to investigate qualitative and quantitative changes in the glycoprotein composition of the macrophage cell surfaceome upon IFN-γ stimulation. We identified 312 N-glycopeptides corresponding to 154 cell surface N-glycoproteins. Of these 154 glycoproteins, 64 were annotated in the CD nomenclature. Upon stimulation, five glycoproteins were found to be differentially expressed. These proteins include MHC I molecules, high-affinity Fc receptor (CD64), ITIM-containing killer cell lectin receptor (Ly49b), bone marrow stromal antigen 2 (Bst-2, CD317) and sphingosine-1-phosphate receptor (S1pr1, CD363). Bst-2 acts as an innate immune factor against viral infection by blocking virion release from host cell surface. S1pr1 is involved in cell migration. We therefore investigated the Bst-2 and S1pr1 glycoproteins expression levels upon the effect of ongoing *Francisella tularensis* infection. We found decreased expression of Bst-2 on the cellular surface upon bacterial infection followed by IFN-γ stimulation. On the contrary, *Francisella tularensis* increased the expression level of the S1pr1 protein, pointing towards the crucial regulatory role of both proteins during infection. In conclusion, our study suggests a role of Bst-2 and S1pr1 in *Francisella in vivo* dissemination.

T-lymphocytes response to *Salmonella* infection

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T-lymphocytes play an important role in a coordination of chicken immune response to *Salmonella* infection. This is why we determined protein expression in CD4, CD8 and γδ T-lymphocytes from infected, vaccinated and infected, and naïve chickens.
T-lymphocytes were isolated from chicken spleen by fluorescence-activated cell sorting four days after intravenous infection with *S*. Enteritidis. Proteome of T-lymphocytes was characterized by LC-MS/MS. For protein identification, the LC-MS/MS was performed separately for each sample while for the protein quantification, T-lymphocytes proteins from different chicken groups were labelled by dimethylation and processed in a single LC-MS/MS analysis.

Based on the protein identification, 5067 different proteins were detected in T-lymphocytes from infected, 5680 from vaccinated and infected, and 5893 proteins in T-lymphocytes from naïve chickens. An alternative expression of these proteins was determined by dimethyl labelling quantification method. Out of all quantified proteins, 595 proteins passed the criteria for significance and were further analysed.

Influence of *Salmonella* antibodies on *Salmonella enterica* serovars motility

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Salmonella, the member of the *Enterobacteriaceae* family, is a Gram-negative bacteria that invades digestive system of its host as a result of digestion of bacteria from contaminated food, water or hands. Once *Salmonella* crosses intestinal cell wall, it can cause gastroenteritidis or typhoid fever. Among all serovars of *Salmonella enterica*, we focused on *Salmonella* Typhimurium, *Salmonella* Derby and *Salmonella* Infantis as the most prevalent serovars in pig. We previously observed that suckling piglets of sows vaccinated with an *S.* Typhimurium-based inactivated vaccine are protected against homologous strain challenge. Because of similar antigenic structures between *Salmonella* Typhimurium and Derby, we assumed crossprotectivity of anti-*S.* Typhimurium vaccine to *S.* Derby challenge. Unfortunately, only only limited crossprotectivity was found in subsequent *in vivo* experiment. This is in accordance with our *in vitro* motility tests that explained limited protectivity of *S*. Typhimurium serum antibodies against *S*. Derby and *S*. Infantis and limited protectivity of *S*. Infantis serum antibodies against *S*. Typhimurium and *S*. Derby. In next experiment, we tested impact of antibodies induced by trivalent vaccine (vaccine against *S*. Typhimurium, *S*. Derby and *S*. Infantis) in all three serovars and we found that motility of these strains was effectively inhibited. Altogether we can conclude that motility test may be potentially beneficial in assumption of vaccine efficiency against challenge strain.

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Use of chicken gut microbiota in a protection of newly hatched chickens against *S. Enteritidis* infection

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Non-typhoid *Salmonella enterica* serovars are among the most common causative agents of food-borne diseases worldwide. Since poultry is the most frequent reservoir of *Salmonella* for humans, different interventions in chickens aimed at the decrease of *Salmonella* prevalence are understood as an effective measure how to decrease *S*. *enterica* incidence in humans. On of such interventions is the use of different probiotic or competitive exclusion products. However, since microbiota compositions changes in chickens of different age, in this study we tested whether microbiota from donor hens of different age will equally protect chickens against *Salmonella* infection. To address this aim, newly hatched chickens were orally inoculated with ceacal extracts from 1-, 3-, 16-, 28- and 42-week-old donors and 7 days later, the chickens were infected with *Salmonella* Enteritidis. The experiment was terminated 4 days later. In the second experiment, we shortened the period between inoculation with gut microbiota and *S*. Enteritidis challenge including the therapeutic use of microbiota inoculation. Groups of newly hatched chickens were therefore inoculated with caecal extract of 35-week-old donors either on day 1 of life followed by *S*. Enteritidis infection on day 2, or were inoculated on day 1 of life by a mixture of caecal extract and *S*. Enteritidis, or were infected with *S*. Enteritidis infection on day 1 followed by therapeutic administration of caecal extract from 35-week-old donors on day 2. This experiment was terminated when the chickens were 5 days old. Both *Salmonella* culture and chicken gene expression confirmed that inoculation of newly hatched chickens with microbiota from 3-week-old or older chickens protected them against *S*. Enteritidis challenge. On the other hand, microbiota from 1-week-old donors failed to protect chickens against *S*. Enteritidis challenge. Microbiota from 35-week-old hens protected chickens even within 24 hours after administration. However, simultaneous or therapeutic microbiota administration failed to protect chickens against *S*. Enteritidis infection. Gut microbiota can be used as a preventive measure against *S*. Enteritidis infection but its composition and early administration is critical for their efficacy.

Bordetella pertussis and its adenylate cyclase toxin: How it works and what does it do"

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Bordetella adenylate cyclase toxin (ACT) is secreted from bacteria by a 'push-pull' mechanism through a T1SS 'channel-tunnel' assembly. This is promoted by the C-terminal folding nucleus that emerges with the C-terminal secretion signal from the T1SS conduit on bacterial surface and facilitates Ca^{2+}-driven stacking of adjacent RTX repeats blocks. These form β-roll structures, serving as Brownian ratchets that promote vectorial folding of the translocating ACT polypeptide as it emerges from the T1SS duct. The secreted toxin then targets myeloid phagocytes bearing the complement receptor 3 (CR3, $\alpha_M\beta_2$ integrin CD11b/CD18 or Mac-1), such as neutrophil, macrophage or dendritic cells (DC, $CD11b^{high}$). ACT recognizes a positively charged loop of the CD11b subunit of CR3 near the hinge region outside of the I-domain of CD11b and inserts directly across phagocyte membrane. ACT-mediated Ca^{2+} influx then induces calpain-mediated cleavage of talin, enabling ACT to hijack the receptor and mobilize it into membrane lipid rafts. There, translocation of the AC domain across cell membrane is completed across a tightly sealed protein-lipid interface. The AC binds cytosolic calmodulin and catalyzes conversion of ATP to cAMP, generating supraphysiologic cAMP levels that subvert phagocyte functions, causing phagocyte impotence due to inactivation of the Syk kinase and block of signaling of leukocyte receptors. Activation of PKA through cAMP next provokes transient inactivation of the small GTP-ase RhoA, causing rapid and unproductive cell ruffling. In parallel, transient activation of the tyrosine phosphatase SHP-1 occurs by an as yet unknown PKA-dependent mechanism and causes inhibition of oxidative burst and block of expression of iNOS and of bactericidal NO production in phagocytes. Simultaneously, activated SHP-1 causes stabilization of BimEL and activation of Bax, provoking induction of apoptosis. Influx of calcium ions and relocation into membrane rafts also allows ACT to escape rapid endocytic removal from cell surface, thus enabling a subpopulation of ACT molecules to oligomerize into small cation-selective pores that permeabilize cells for potassium efflux. This contributes to induction of maturation of dendritic cells that is, however, hijacked by cAMP signaling that compromises the capacity of DCs to stimulate antigen-specific T cell immune responses. Migration of the incompletely mature DCs into lymph nodes then likely contributes to suppression of adaptive host immune responses to the pathogen

and support bacterial colonization of the host in early stages of infection. Later in infection, ACT action provokes NALP3 inflammasome activation in dendritic cells, which likely contributes to late inflammatory response and eventual development of Th1/Th17 polarized immune responses that support eventual clearance of the bacterial infection.

Getting past the guards and teaching them tolerance – lessons from *Bordetella pertussis*

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Bordetella pertussis, the agent of whooping cough, subverts phagocyte functions through powerful immunomodulatory action of its toxins. Among them, a prominent role 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, cyclic adenosine monophosphate (cAMP). To fill the gap in understanding of the immunosuppressive signaling of CyaA, we have undertaken here an unbiased quantitative phosphoproteomic approach towards deciphering of mechanisms by which CyaA hijacks the dendritic cells that play a central role in induction of adaptive immune responses. Mouse bone marrow-derived dendritic cells were exposed to action of CyaA or of its non-enzymatic toxoid variant (dCyaA) and global phosphoproteome changes were analyzed using the SILAC-based mass spectrometry. Bioinformatic identification of cAMP-regulated phosphoproteins revealed numerous pathways that are manipulated by CyaA-provoked signaling. These are in particular linked to actin cytoskeleton homeostasis regulation by small GTPase signaling, nuclear export and metabolism of mRNA. The performed pathways analysis provides a detailed picture of changes leading to the paralysis of host cell defense mechanisms, revealing interesting hints on the so far overlooked involvement of chromatin remodeling mechanisms in infection by the pertussis agent.

Bordetella adenylate cyclase toxin is a unique ligand of the integrin complement receptor 3

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Bordetella adenylate cyclase toxin (CyaA) penetrates phagocytes expressing complement receptor 3 (CR3) and paralyzes their bactericidal functions by unregulated conversion of ATP to the key signaling molecule cAMP. CR3 belongs to a group of cell surface adhesion and signaling integrin receptors that are essential for metazoan existence. CR3 and some other integrins contain an I-domain that is a major ligand-binding site. The ligands preferentially engage the active forms of the integrins and trigger signaling cascades that alter numerous cell functions. Here we found that CyaA preferentially binds an inactive form of CR3 outside of its I-domain, using a positively charged segment located at the interface between the beta-propeller and the thigh domain of the integrin. CyaA binding did not trigger downstream signaling of CR3 in human monocytes and CyaA-catalyzed elevation of cAMP effectively blocked CR3 signaling initiated by a natural ligand. This unprecedented type of integrin-ligand interaction distinguishes CyaA from all other known ligands of the I-domain-containing integrins and provides a mechanistic insight into the previously observed central role of CyaA in the pathogenesis of *B. pertussis*.

Interferon-alpha downregulates response of porcine monocyte-derived macrophages to in vitro infection with *Mycobacterium avium* subsp. *avium* - the story of CXCL10

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Macrophages are crucial cells involved in a setup of antimycobacterial immunity. Formation of tubercles is dependent on the Th1-lymphocytes produced interferon gamma (IFNγ) which can stimulate macrophages. Macrophages, vice versa, produced many chemokines including CXCL10 which is responsible for Th1-lymphocyte recruitment. Viral infections can be considered as on of the reasons of break through antimycobacterial immunity. Once viral particles / nucleic acids are recognized by infected cells via TLR3/7, interferons of type I are produced. Aim of the study was to test whether interferon-alpha (IFNα) can influence response of porcine monocyte-derived macrophages (MDMF) to *in vitro* infection with *Mycobacterium avium* subsp. *avium*. MDMF were exposed *in vitro* to the influence of IFNα and/or IFNγ and then infected with mycobacteria. Response of the cells was detected on mRNA level as a relative expression of mRNA for pro- and antiinflammatory cytokines and CXCL10. As expected, the infection led to strong and weak pro- and antiinflammatory response, respectively. Treatment with IFNγ led to generally weaker response, except CXCL10 which were upregulated 3times more when compared to the infection alone. Expression of antiinflammatory TGFβ and IL10 was practically ceased. Contrary to that, treatment with IFNα did not suppress antinflammatory response so much. However, proinflammatory response was suppressed and it was true particularly for CXCL10 which was downregulated at least 10times. As a conclusion, our results show that IFNα produced as a response to viral infection can inhibit Th1-associated response by downregulation of Th1-associated chemokine CXCL10. The study was supported by Ministry of Education, Youth and Sport of the Czech Republic (LO1218) and Ministry of Agriculture of the Czech Republic (RO0516, QJ1310258).

Catalase as a potential virulence factor in *in vitro* interaction between *Haemophilus parasuis* and porcine pulmonar alveolar macrophages

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H. parasuis is a part of the normal flora of the respiratory tract of pigs, but it can also induce severe diseases. To date, 15 serovars of *H. parasuis*, differing in virulence, have been descibed. The aim of this study was to compare the immune responses of porcine alveolar macrophages (PAMs) to *in vitro* infection with various *H. parasuis* serovars of different virulence. Using qRT-PCR, the expression of IL-1β, TNFα, IL-8, IL-10 and IL-1Ra mRNA was detected in macrophages at 4 and 24 hours after *H. parasuis* infection. Stimulation of PAMs with *H. parasuis* up-regulated the expression of all selected genes and it was higher after 24 hours post-infection except of TNF-alpha. But there was no significant difference between serovars of different virulence. On the other hand, virulent strains were able to suppress production of reactive oxygen species (ROS) in PAMs more than avirulent strains, indicating the presence of defence mechanism against respiratory burst. The capsular polysaccharide (CPS) as the surface cell structure and as described virulence factor of this bacterium was used to investigate its ability in anti-ROS defence mechanism. CPS isolated from a field strain 132 originated from a death case of *H. parasuis* infection was able to reduce production of ROS in PAMs. Based on isolation protocol of CPS, one can expect protein contamination. Therefore, protein identification was performed by the mass spectrometry. Several proteins with the anti-ROS potential were identified. One of them – catalase of molecular weight approx. 50 kDa – was the most abundant. This is in accordance with the fact, that protein fraction about 50kDa separated from an ultrasound lysate of the same strain 132 was efficient to inhibit the oxidative burst. It is proposed that catalase serves as a virulence factor for *H. parasuis* during the colonization and progression of infection circumventing the effects of the oxidative stressors. Yet, more research is stil needed to determine the anti-oxidant enzymes and their role in this respiratory pathogen. The study was supported by Ministry of Agriculture of the Czech Republic (QJ1210120) and Ministry of Education, Youth and Sport of the Czech Republic (LO1218).

Quantitative and qualitative proteomic analysis of *C. difficile* isolates of clinically relevant PCR-ribotypes

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Clostridium difficile is a causative agent of *C. difficile* infection (CDI) with significant mortality and economic burden. The aim of this proteomic study was primarily focused on potential virulence factors; among them the *Clostridium difficile* toxin A and B (TcdA and TcdB) and binary *C. difficile* transferase (CDT) have the prominent role. In addition, the label-free analyses of culture supernatants of selected representative PCR-ribotypes of *C. difficille* were performed in order to reveal new potential connections.

The panel of *C. difficile* strains in the experiment comprised of ribotypes occurring in Europe: ribotype 027, referred as hypervirulent, 176, genetically related to 027 lineage and ribotypes 001,014,005, 012, 078. Non-toxigenic PCR-ribotype 010 was involved as negative control. The supernatants of cultures were precipitated and subsequently processed based on the Filter Aided Sample Prep (FASP) protocol, analysed using liquid chromatography on-line coupled with mass spectrometer Q-Exactive (Thermo Scientific) and the MS/MS data were label free quantified. For the TcdA and TcdB quantitative analysis, the synthetic peptide standards of TcdA and TcdB were employed.

In the first part of the study, TcdA and B were proved as a significantly produced only in the PCR-ribotypes 027 and 176, genetically related cluster linked with severe outcome of CDI and outbreaks. In addition, the presence of both CDT /A and B type/ was proved in the secreted fraction with a high level. Interestingly, neither TcdA/TcdB nor CDT were detected in the PCR-ribotype 078, so called as hypervirulent, apparently representing different cluster proved by PCA analysis and via hierarchical clustering. Finally, TcdA production was proved in the PCR-ribotypes 005 and 012 while in the ribotypes PCR-001, 078 and 001 it remained undetected.

The employment of heavy labelled peptides enabled precise relative quantitative analysis of TcdA and TcdB and proved that only highly clinically relevant PCR-ribotypes are able to produce highest levels of both of these toxins under the defined conditions. Moreover, this preliminary study also showed the feasibility of detection known and possible new virulence factors via LFQ approach.

Glycobiology of pathogenic and commensal bacteria and gut cells

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All bacterial interactions with host cells require the participation of lectins/adhesins and glycans on both bacterial and host surfaces. These interactions lead to the biological signaling response which determines 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 in the context of the human gastrointestinal environment, we have established cell-based assays, a suite of glycomics microarrays including novel mucin microarrays, and glycogenomic methods to investigate. We have used these tools to explore *Campylobacter jejuni* and *Helicobacter pylori* interactions as well as elucidate the carbohydrate ligands of commensal species. We have also constructed a number of specific carbohydrate recognition molecules to facilitate rapid detection and anti-infective strategies.

The combination of existing techniques and novel high throughput methods has yielded insights in to global glycomic changes in pathogenic and commensal strains.

Investigation of carbohydrate-mediated bacterial adherence and microbial-host interactions for development of biodefence and decontamination strategies

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Glycosylation is a key modification of proteins and lectin-carbohydrate interactions are essential in many host-microbial processes including adherence, colonization and infection. To study important carbohydrate-mediated microbial interactions, high-throughput lectin and glycan microarrays are increasingly utilised. These platforms can be custom made to cover a wide range of specificities, carbohydrates presented on glycoproteins and neoglycoconjugates (NGCs) and allow screening of multiple interactions with low sample and probe usage, providing high data yield at the same time. After an initial microarray screening the most significant binding partners can be further explored using other glycobiology techniques.

A number of specific interactions in microbial pathogenesis and commensalism were investigated using these glycomics microarrays to elucidate the role of glycosylation and its potential role in development of biodefence and decontamination strategies. The total surface glycome of three nonpathogenic surrogates of *Bacillus antracis* was compared using lectin microarrays and changes in glycosylation were profiled for vegetative cells and spores. Interactions of commensal and pathogenic strains of *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa* with lectins and host specific carbohydrates were analysed for rapid identification of pathogenic species in public water systems. Overall, we demonstrate a novel approach for studying lectin-carbohydrate interactions and the potential applications of glycomics microarrays in microbial research.

Recent concepts on the role of the ubiquitin system in innate immune regulation

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Key to the activation of the innate immune system are the pattern-recognition receptors (PRRs) including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and cytoplasmic DNA receptors (CDRs). While essential for protection against infections, activation of PRRs require tight control to avert inflammatory diseases. The mechanisms underlying this strict regulation are unclear. The reversible attachment to and removal of ubiquitins from proteins by ubiquitin ligases and deubiquitinases respectively is a versatile system that regulates diverse aspects of biology including the immune system. Recently we have identified the H2A deubiquitinase MYSM1 (H2A-DUB), as a master negative regulator of PRR pathways. MYSM1 is a nuclear metalloprotease previously described as a key component of epigenetic signaling machinery. However, I will present new data showing that in response to infections or inflammation, MYSM1 is rapidly enriched in the cytoplasm where it interacts with and disrupts key PRR signaling complexes. I will also discuss our *in vivo* data demonstrating the physiological role of MYSM1 during infection and inflammation. Overall, during this seminar, the central importance of MYSM1 as a negative regulator of the innate immune system that protects against an overzealous self-destructive immune response will be highlighted.

Transient and prolonged response of chicken caecum mucosa to the colonization with different gut microbiota

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Although it is well established that there are continuous interactions between a host and microbiota of the intestinal tract, little is known how these develop immediately after birth. In this study we determined protein and gene expression in the caeca of newly hatched chickens inoculated with cecal contents sourced from hens of different ages. Over 250 proteins exhibited modified expression levels in response to microbiota inoculation. The most significant inductions were observed for ISG12-2 (IFI6), OASL, ES1, LYG2, DMBT1-L, CDD, ANGPTL6, B2M, CUZD1, IgM and Ig lambda chain. Of these, ISG12-2, ES1 and both immunoglobulins were expressed at lower levels in germ-free chickens compared to conventional chickens. In contrast, CELA2A, BRT-2, ALDH1A1, ADH1C, AKR1B1L, HEXB, ALDH2, ALDOB, CALB1 and TTR were expressed at lower levels following inoculation of microbiota. When chicks were given microbiota preparations from different age donors, the recipients mounted differential responses to the inoculation which also differed from the response profile in naturally colonized birds. For example, B2M, CUZD1 and CELA2A responded differently to the inoculation with microbiota of 4- or 40-week-old hens. The increased or decreased gene expression could be recorded 6 weeks after the inoculation of newly hatched chickens. To characterize the proteins that may directly interact with the microbiota we characterized chicken proteins that co-purified with the microbiota and identified a range of host proteins including CDD, ANGPTL6 and DMBT1-L. In conclusion, a single microbiota inoculation on a day of hatch triggered long lasting changes in the gene expression in the cecum of treated individuals dependent on microbiota composition. These results show that different microbiota communities can induce very different responses in newly hatched chickens offering the potential to manipulate microbiota with probiotic strategies to improve overall host performance and health.

Chicken gut microbes – from genomics to probiotics

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The gut represents a microbial ecosystem with a complex population of microorganisms that survive by maintaining a symbiotic relationship with the host. The interactions between gut microbiota and host provide stimuli for the host's immune system, keeping it activated and ready for response to pathogens. The microbiota and its metabolic pathways also provide the host with nutrients that otherwise the host would not receive, for instance, short-chain fatty acids are produced by Bacteroides and Clostridium species. One of the product of fiber fermentation, butyrate, serves as energy source for epithelial cells and induces the production of mucin and antimicrobial peptides in these cells and simultaneously suppress the expression of virulence factors of pathogens. Thus, the gut microbiota is a key player participating in immune response, nutrient uptake and production of metabolites essential for the host, and consists of many health-promoting bacteria, known as probiotics.

In order to identify probiotic bacteria and their function in the chicken gut, we isolated anaerobes from ceca, a reservoir of probiotic strains. So far, 54 strains have been isolated and characterized. Extracted DNA from each isolate was subjected to DNA sequencing on the NextSeq platform, followed by *de novo* read assembly via IDBA-UD software and *ab initio* gene prediction using the Prokka pipeline. Using this approach, metabolic pathways for every isolated strain were proposed.

In parallel, newly-hatched male chickens were inoculated with isolated strains, challenged by *Salmonella* Enteritidis at the age of 7 days and sacrificed 4 days later. The evaluated parameters for every chicken at the age of sacrifice were: body weight; *Salmonella* enumeration in liver; and a relative quantity immune response to infection.

Based on our analysis, candidates for probiotic strains were of *Pseudoflavonifractor*, *Ruminococcus* or *Cloacibacillus* clades. Chickens inoculated by strains of these clades showed a very good protection against the salmonella infection considering the immune response values and salmonella counts. Moreover, strains of *Pseudoflavonifractor* clade represent spore-forming butyrate producers, which are ideal probiotic candidates. However, the analysis is still ongoing, and it is too early to draw conclusions.

Impact of deoxynivalenol on immune parameters in porcine colostrum

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Piglets are born agammaglobulinemic and their postnatal immunity is fully dependent on colostrum intake. It is well known that the colostrum contains large amounts of antibodies and also lots of immune cells. However, colostrum can also contain non-beneficial components, including mycotoxins. Currently, we focused on mycotoxin deoxynivalenol (DON) produced by *Aspergillus*, *Penicillium* and *Fusarium* ssp. The source of DON is contaminated animal feed which caused serious problem in many Czech farms last year (2015) and simultaneously increased piglets mortality occurred. Mycotoxin excretion to colostrum is probably connected with this problem. If piglets survive, mycotoxin could have negative impact on wide range of biological functions, including immune system. We focused especially on immune cells. The cellular, mainly lymphocytic components of colostrum awoke an interest after findings indicating that the immune cells may be transferred from mother to offspring organs via colostrum and that these cells are able to respond to specific antigen stimulation in piglet organs. We found that the most predominant lymphocyte subpopulation in colostrum were cytotoxic T cells followed by CD4+CD8+ double positive T cells, CD2+CD8+ γδ T cells, and NK cells. Helper T cells and other γδ T cell subpopulations were present in colostrum in very low percentages.

The impact of DON on suckling piglet health have been clearly documented, however its real amount hasn´t been tested yet. Future this fact, one of the aims of our study is to define real DON concentrations in colostrum and its correlation with concentration in feed. We succeeded in the development of a highly sensitive and specific method for the qualitative and quantitative determination of deoxynivalenol (DON) in pig colostrum using liquid chromatography combined to high resolution Orbitrap-based mass spectrometer (UHPLC-(HR)MS), type QExactive (Thermo Fisher Scientific). The method was validated for DON in animal colostrum according to EU regulations 657/2002/EC (linearity, precision, accuracy, CC∝, CCβ, specificity). The detected DON concentration in colostrum allowed us to define the impact of this mycotoxin on colostral leukocytes.

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In vitro stimulation of porcine monocyte-derived dendritic cells can be influenced by cytokine microenvironment

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Dendritic cells (DC) are considered to be primary antigen presenting cells driving differentiation of naive T cells into distinct T cell types with ability to induce primary immune response. Since microenvironment in which DC are present is important for their activation, effect of interleukin-4 and interferon-gamma on porcine monocyte-derived DC (MDDC). MDDC were generated from CD14+ peripheral blood monocytes isolated by magnetic sorting and cultivated for 4 days in the presence of IL4 and GM-CSF. Differentiated MDDC were cultivated under influence of IL4 or IFN-γ or left without an additional cytokine treatment and stimulated with protein KLH for 48h. Total RNA was isolated and activation of cytokines was measured by using quantitative RT-PCR. Also, cellular supernatants were analyzed by ELISA test. As expected, presence of IFN-γ drives MDDC to produce more proinflammatory cytokines including IL1-β, IL12 and IL23. On the other hand, cytokines produced under influence of IL4 were more anti-inflammatory, including IL10 and TGF-β. This experiment clearly showed that expression of certain cytokines was dependent on cytokine environment DC were exposed to. Also, it suggests that in vitro stimulation of DC can be used as a model for further experiments in vaccine studies.
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Is there a humoral escape mechanism for papillomaviruses during the development of non-melanoma skin cancer?

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There is increasing evidence that certain types of cutaneous human papillomaviruses (HPVs) are involved in the development of non-melanoma skin cancer (NMSC). *Mastomys coucha*, an African rodent, is a useful preclinical model of papillomavirus infection. It is persistently infected with MnPV, which is the causing agent of both premalignant and malignant skin tumors. To understand how these animals develop skin tumors even they were not immunological compromised, we performed a detailed serological analysis also in the context of their ability to induce neutralizing antibodies. An important and still unanswered question is why the antibody response during natural infection is not sufficient to prevent tumor formation.

Inspection of the DNA sequences encoding the viral capsid protein L1 of several *high-risk* HPV types revealed that two potential open reading frames exist for L1, which could lead to a long or a short form of the L1 protein. Transcription of L1 genes starting from the first ORF encodes a long form of the L1 protein ($L1_{LONG}$), which lacks the ability to produce proper assembled VLPs. Notably, the same situation can be detected in MnPV.

Based on a recent study, our results suggest that anti-MnPV L1 antibodies raised in vaccinated animals are different from those of naturally infected animals, which seem to react with a longer L1 variant with additional residues at its N-terminus ($L1_{LONG}$) instead of $L1_{SHORT}$, indicating a possible switch from the expression of long to short L1 protein during the course of infection. The implications and consequences will be discussed.

Chlamydia trachomatis and HPV induced cervical cancer: a causal or coincidental interaction?

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Chlamydia trachomatis is the most prevalent sexual transmitted bacteria and is an obligatory intracellular pathogen which exhibits tropism in endocervical epithelial cells. Over the past decades, Chlamydia trachomatis has been widely studied as a potential co-factor in high-risk HPV induced cervical cancer development due to the fact that Chlamydia infection itself can induce series of changes in host cells such as DNA damage, down regulation of P53 and up regulation of hTERT which will then favour cell cycle progression and apoptosis inhibition and possibly support cellular transformation. Meanwhile, emerging epidemiological researches indicate a strong association of chlamydia infection and HPV induced cervical while others suggest no correlation between these two events at all. The controversial conclusions from epidemiological studies and the absence of concrete evidence from basic researches lead us to ask the question: what's the role of chlamydia infection in HPV induced cervical cancer.

In our preliminary research, we employed a pseudovirion-based Gaussia luciferase assay to measure HPV entry with or without Chlamydia infection. Our results show that in HeLa cells, a pre-existed chlamydia infection can significantly increase the infectivity of HPV pseudovirus. Another aspect of interest in this preliminary research is to identify the impact of Chlamydia infection on HPV E6 and E7 oncogenes. Our results in both acute infection and persistent Chlamydia infection models suggest no significant changes in E6 and E7 transcription after chlamydia challenge. However, at a protein level, our preliminary results showed a band shifting in western blot detection of HPV18 E7 which suggests chlamydia could get involved in the post-translational modifications of HPV oncoproteins. More experiments are running at the moment to further identify the type of the modifications and to determine the biological functions of these modifications. Considering the limited information getting from western-blot, proteomic approaches such as SILAC will be the plan for the next step to get more information about a global change in host cell proteome after chlamydia infection in cells with or without HPV oncogenes. For this purpose we generated immortalized human keratinocytes retrovirally transduced with HPV16/18 E6 and E7 respectively.

Structure-activity relationships of nucleoside analogues for inhibition of tick-borne encephalitis virus

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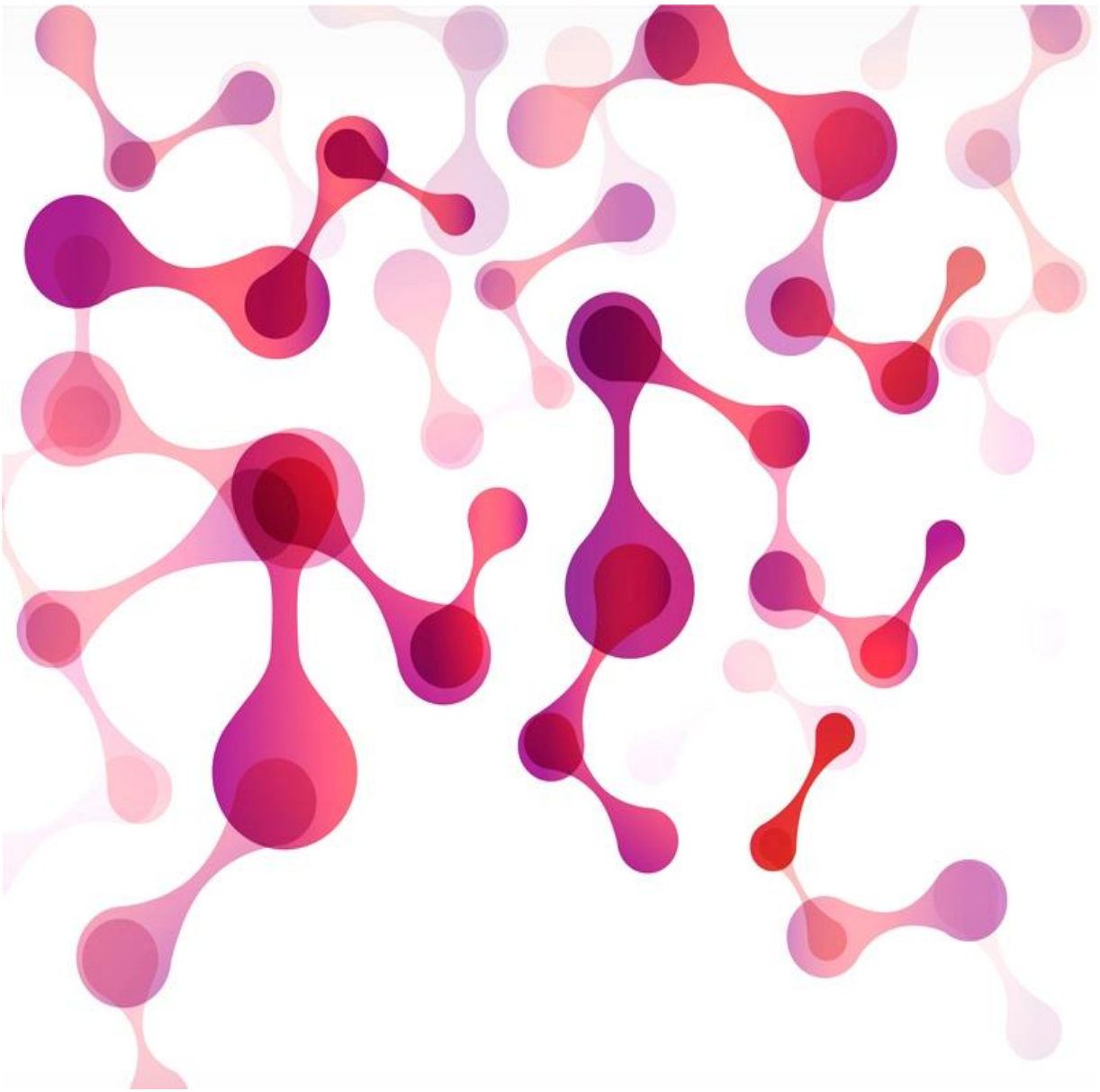
TBE represents one of the most serious arboviral neuro-infection in Europe and northern Asia. As no specific antiviral therapy is available at present, there is an urgent need for efficient drugs to treat patients with TBEV infection. Using two standardised *in vitro* assay systems, we evaluated a series of 29 nucleoside derivatives for their ability to inhibit TBEV replication in cell lines of neuronal as well as extraneural origin. The series of tested compounds included 2´-*C*- or 2´-*O*-methyl substituted nucleosides, 2´-*C*-fluoro-2´-*C*-methyl substituted nucleosides, 3´-*O*-methyl substituted nucleosides, 3´-deoxynucleosides, derivatives with 4´-*C*-azido substitution, heterobase modified nucleosides and neplanocins. Our data demonstrate a relatively stringent structure-activity relationship for modifications at the 2´, 3´, and 4´ nucleoside positions. Whereas nucleoside derivatives with the methylation at the C2´ position or azido modification at the C4´position exerted a strong TBEV inhibition activity (EC_{50} from 1.5 to 32.9 μM) and low cytotoxicity *in vitro*, substitutions of the O2´ and O3´ positions led to a complete loss of anti-TBEV activity (EC_{50} > 50 μM). Moreover, some structural modifications of the heterobase moiety resulted in a high increase of cytotoxicty *in vitro*. High antiviral activity and low cytotoxicity of C2´ methylated or C4´ azido substituted pharmacophores suggest that such compounds might represent promising candidates for further development of potential therapeutic agents in treating TBEV infection.



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