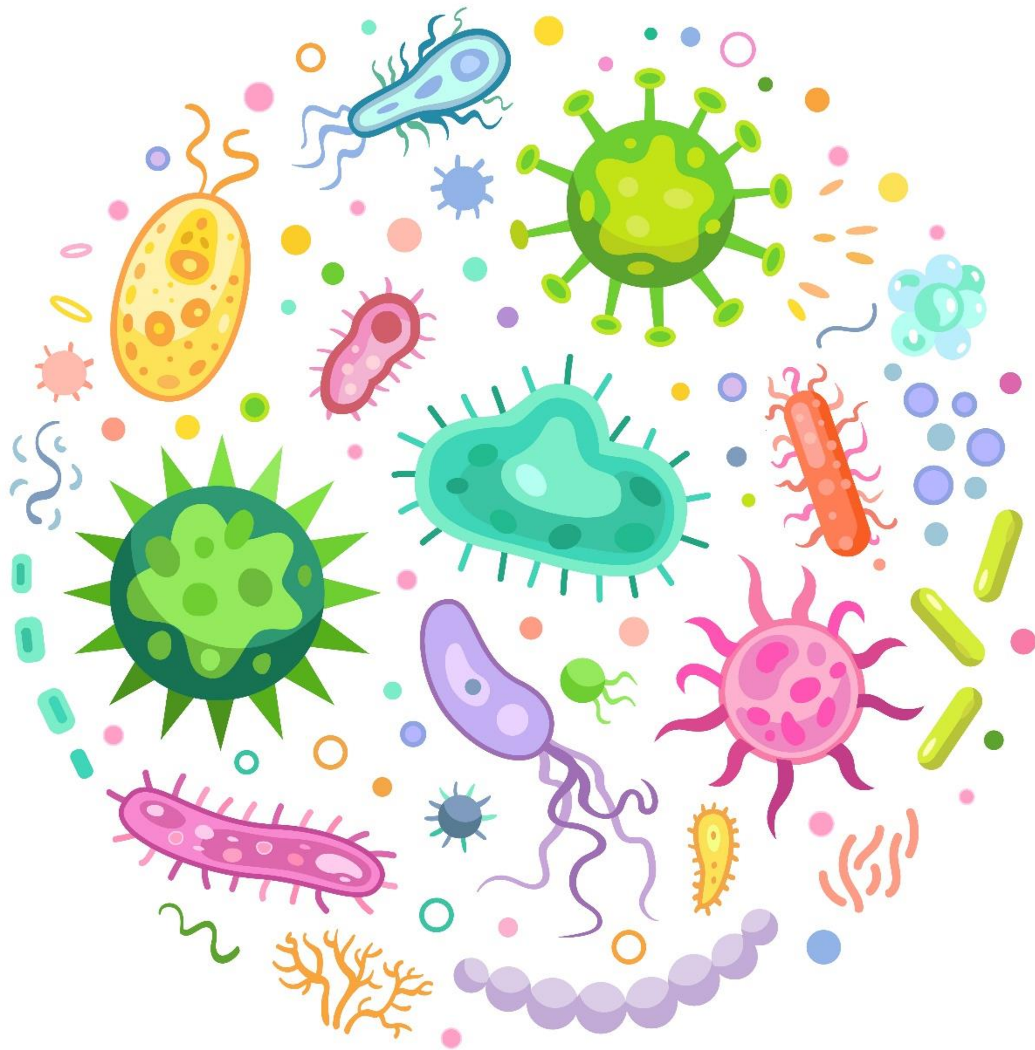

14th Host Pathogen Interaction Forum 2022

November 7– 10, 2022, Kutná Hora, Czech Republic

Agenda and Abstract book

14th Host Pathogen Interaction Forum 2022

Venue: Kutná Hora, Czech Republic

Date of event: **November 07 – 10, 2022**

Scientific 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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CONFERENCE ORGANIZATORS

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

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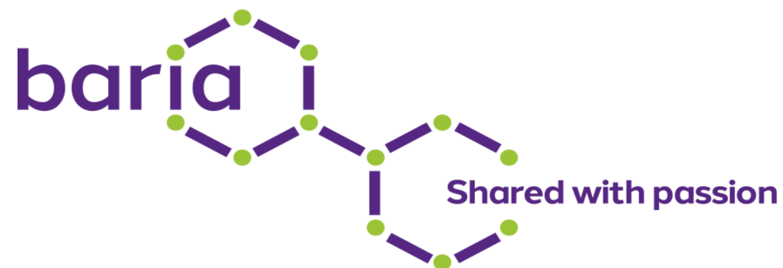

EDITORIAL

Dear colleagues,

We are delighted to have your participation! After a long period during which scientific conferences have been impacted by the coronavirus pandemic, we look forward to welcoming you hopefully in-person once again.

,,Host Pathogen Interaction“ - with that in mind, it is our pleasure to welcome you to the 14th Host Pathogen Interaction Forum 2022 in Kutna Hora, 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.

Despite current pandemic context, the organisation of this meeting is a challenge that we undertake with great enthusiasm, as the predicted dynamics of the local restrictions are likely to allow the organisation of a live event. We strongly believe that an attractive scientific program with excellent speakers and opinion leaders will provide a superb opportunity for broad exchange of knowledge and ideas within our large international community interested in all epidemiological, preventive and therapeutic aspects of host pathogen interactions.

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.

The conference would not have been a success without help of many people, and we would like to acknowledge their contribution. First, we would like to thank all the authors for their excellent submission to HPI Forum 2022. We also express our most sincere appreciation to our website and program committee co-chair, Dr.Pavla Pavlik who showed an exemplary example of team work and excellent planning.

Special thanks to Mr. Baumruk from Allice s.r.o. Meeting and Conference Management for logistic support, invaluable help and support with venue arrangements. Finally, we are especially grateful to the grant MV VJ01030003 obtained from the Czech Ministry of Interior for their financial support.

We are glad you were able to come and hope you have an enjoyable and productive time.We hope that the conference will meet your expectations and your sojourn in Kutna Hora will be pleasant.

My sincerely thanks to all for making possible this conference!

Klara Kubelkova
University of Defence
Organizer

CONFERENCE VENUE

The 14thHost Pathogen Interaction Forum 2022 is held in the beautiful and exciting city of Kutná Horasituated in the Central Bohemian Region of the Czech Republic.

Kutná Hora is one of the most beautiful and historically significant Czech cities. Visitors from both the Czech Republic and abroad come here largely to see a couple of great sights – the Cathedral of St Barbara in the heart of the city and the Ossuary in suburban Sedlec. Many of the sights are associated with medieval silver mining. The town centre, itself a UNESCO heritage site, is very picturesque, with a well-preserved network of cobbled medieval alleys and small squares, various quaint shops, cafés and pubs. Also worth a stroll is the trail along the Vrchlice river beneath the city walls. Enriched by the silver ore that veined the surrounding hills, the medieval city of Kutná Hora became the seat of Wenceslas II's royal mint in 1308, producing silver groschen that were then the hard currency of Central Europe. Boom-time Kutná Hora rivalled Prague in importance, but by the 16th century the mines began to run dry, and its demise was hastened by the Thirty Years' War and a devastating fire in 1770. The town became a Unesco World Heritage Site in 1996, luring visitors with a smorgasbord of historic sights. It looks its flower-bedecked best in May and June but is worth a full day's visit at any time of year.

CONFERENCE CENTRE / Hotel Mědínek

Hotel Mědínek Old Town is located in the historic center of the city and offers conference facilities for organizing events of all types. Our premises are suitable for various purposes, from congresses, conferences, meetings, meetings, corporate events, celebrations to exercises or concerts.

BREAKFAST

Breakfast is included in the accommodation at the hotel Mědínek or participant shall ensure himself.

LUNCHES

Conference lunches are served in the hotel MĚDÍNEK, Tuesday (from 11am to 2pm), Wednesday and Thursday (from midday to 2pm).

DINNER

Monday, November 07 – Welcome party (included in the conference fee)

Tuesday, November 08 – Dinner - will not be organized

Wednesday, November 09 – Conference reception (included in the conference fee)

SOCIAL EVENTS

<u>Welcome party</u>

Date: Monday, November 07

Time: 7:00pm

Location: Auditorium,Hotel Mědínek

<u>Guided tour of the City of silver</u>

Date: Wednesday, November 09

Time: 1:15pm

Location: pick up in front of hotel Mědínek

<u>Conference Reception</u>

Date: Wednesday, November 09

Time: 7:00pm

Location: restaurant Dačický(300 m from the conference centre)

Address: Rybova 8, Kutná Hora

An Old Bohemian restaurant with delicious food, excellent beer and beautiful and stylish environment with a pleasant atmosphere.

AGENDA

MONDAY (November 07, 2022)

3:30 – 5:45pm *Arrival of participants, Registation and helpdesk open* (Entrance hall, hotel Mědínek)

6:00 – 6:30pm **Kubelková Klára**, **Macela Aleš** – Opening (Auditorium, hotel Mědínek)

6:30 – 7:00pm Keynote lecture

Juraj Ivanyi - Potentials of antibody ligands with T cell receptor specifity for the immunotherapy of intracellular infections (Centre for Host-Microbiome Interactions, Guy's Campus of Kings College London, UK)

7:00 – 12:00pm *Welcome party* (Entrance hall/Auditorium, hotel Mědínek)

TUESDAY (November 08, 2022)

8:00am – 10:00am Session I.

Chairmans:Sjostedt Anders, Šantić Marina

8:00 – 8:30am **Sjostedt Anders**– To be defined(Umea University, SE)

8:30– 9:00am **Špidlová Petra** - Arginine 58 is indispensable for proper function of the Francisella tularensis HU protein, and its substitution alters virulence and mediates immunity against wild-type strain (FoMHS, University of Defence, CZ)

9:00– 9:30am **Šantić Marina** – *Francisella* in the environment (University of Rijeka, HR)

9:30– 9:45am **Vozandychová Věra** - Changes of host deubiquitination enzymes during *Francisella tularensis* infection of human macrophages(FoMHS, University of Defence, CZ)

9:45– 10:00am **Mihelčić Mirna**- IL-1β neutralization increased susceptibility to tularemia in autophagy deficient animals during *Francisella tularensis* LVS infection (University of Rijeka, HR)

10:00– 10:30am *Coffee break – Conference foyer*

10:30am – 12:15am Session II.

Chairmans:Šebo Peter, Kamanová Jana

10:30 – 11:00am **Šebo Peter**- Supress immunity, proliferate and transmit: How the pertusis agent hijacks the immune response of nasal mucosa(Institute of Microbiology of the ASCR, CZ)

11:00 – 11:15am **Bumba Ladislav**- Pertusis toxin suppresses dendritic cell-mediated delivery of *B. pertusis* into lung-draining lymph nodes (Institute of Microbiology of the ASCR, CZ)

11:15 – 11:45am **Kamanová Jana**- *Bordetella* Type II Secretion Injectosome and Effector Proteins (Institute of Microbiology of the ASCR, CZ)

11:45 – 12:00am **Čížková Monika**- Role of BscX and BscY proteins in the *Bordetella* type 3 secretion system (Institute of Microbiology of the ASCR, CZ)

12:00 – 12:15pm **Vysloužil Jan**- News in optical and electron microscopy (Pragolab s.r.o., CZ)

12:15 – 1:15pm *Lunch –hotel Mědínek*

1:15pm – 5:35pm Session III.

Chairmans: Mou Sherry, Šebo Peter

1:15 – 1:45pm **Mou Sherry**- The *Burkholderia pseudomallei* hmqA-G Locus Mediates Competitive Fitness against Environmental Gram-Positive Bacteria (USAMRIID, Maryland, USA)

1:45 – 2:15pm **Malcová Ivana**- Lipid Binding by the N-terminal Motif Mediates Plasma Membrane Localization of *Bordetella* Effector Protein BteA (Institute of Microbiology of the ASCR, CZ)

2:15 – 2:45pm **Romero Allsop Tania , Zmuda Martin, Sedláčková Eliška**- Unraveling the Mechanisms of Action of *Bordetella* Effector Protein BteA (Institute of Microbiology of the ASCR, CZ)

2:45 – 3:00pm	**Grobarčíková Michaela**- Identification of residues involved in posttranslational modification of RTX toxins of Gram-negative pathogens (Institute of Microbiology of the ASCR, CZ)
3:00 – 3:30pm	*Coffee break – Conference foyer*
3:30 – 3:45pm	**Vávrová Pavlína**- Implementation of adavanced methodical strategies to form dual-species biofilm in the research of anti-biofilm acting compounds (Charles University, Faculty of Pharmacy, CZ)
3:45 – 4:15pm	**Černý Ondřej**- CD97 stabilizes the immunological synapse between dendritic cells and T cells and is targeted for degradation by the *Salmonella* effector SteD (Institute of Microbiology of the ASCR, CZ)
4:15 – 4:30pm	**Kambová Milada**- Heterogeneous expression of *Salmonella Typhimurium* SPI-2 T3SS effectors (Institute of Microbiology of the ASCR, CZ)
4:30 – 4:35pm	**Dreus Alona**- *Salmonella* SPI-2 TTSS effectors: Where and When (Institute of Microbiology of the ASCR, CZ)
4:35 – 4:50pm	**Bulvas Ondřej**- Mechanism of mycobacterial inosine-5´-monophospahte gehydrogenase allosteric regulation (Institute of Organic Chemistry and Biochemistry of the ASCR, CZ)
4:50 – 5:05pm	**Dedola Matteo**- Multiprotein organization of the purine metabolism enzymes in mycobacteria(Institute of Organic Chemistry and Biochemistry of the ASCR, CZ)
5:05 – 5:20pm	**Kukla Stanislav**- Proximity Ligation Assay – a powerful tool for study of endogenous protein function(Merck Life Science spol. s r.o., CZ)
5:20 – 5:35pm	**Krist Pavel**- ZEISS - The Most Advanced Light, X-ray and Electron Microscopy(Carl Zeiss spol. s r.o, CZ)

WEDNESDAY (November 09, 2022)

8:00 – 9:45pm Session IV.

Chairmans: Matiašovic Ján, Špidlová Petra

8:00 – 8.15am **Matiašovic Ján**- Development of vaccine against *Streptococcus suis* infection in pig (Veterinary Research Institute, CZ)

8:15 – 8.30am **Zouharová Monika**- Diversity of *Streptococcus suis* strain isolated from sick pigs from farms in the Czech Republic (Veterinary Research Institute, CZ)

8:30 – 8.45am **Matiášková Katarína**- Resistance of *Streptococcus suis* Isolates from the Czech Republic during 2018-2022 (Veterinary Research Institute, CZ)

8:45 – 9.00am **Králová Natálie**- The capsular polysaccharide synthesis locus analysis of *Streptococcus suis* isolates (Veterinary Research Institute, CZ)

9:00 – 9:30am **Kolářová Iva**- Dual role of anti-vector saliva immunity on the outcome of *Leishmania* infection (Department of Parasitology, Faculty of Science, Charles University)

9:30 – 9:45am **Jelínková Kristýna**- Dual role of anti-vector saliva immunity on the outcome of *Leishmania* infection(Veterinary Research Institute, CZ)

9:45 – 10:15am *Coffee break – Conference foyer*

10:15 – 12:00pm Session V.

Chairmans: Rychlík Ivan, Volf Jiří

10:15 – 10.45am **Rychlik Ivan** -How to select novel types of probiotics? (Veterinary Research Institute, CZ)

10:45 – 11.00am **Crhánová Magdalena** - Environmental survival of gut microbiota members and their host species adaptation (Veterinary Research Institute, CZ)

11:00 – 11.15am **Vlasatíková Lenka** - The untargeted metabolomic profiling using LC-MS can distinguish the impacts of different probiotic mixtures on the cecal metabolome of laying chickens in small-scale experiments (Veterinary Research Institute, CZ)

11:15 – 11:30am **Zeman Michal**- Metabolism of selected members of chicken gut microbiota in vivo (Veterinary Research Institute, CZ)

11:30 – 11:45am **Schwarzerová Jana**- Comprehensive analysis of mobile genetic elements in chicken gut microbiome using a novel in-silico approach (Faculty of Electrical Engineering and Communication, Brno Univ, CZ)

11:45 – 12:00am **Čejková Darina**- Horizontal gene transfer network in chicken gut microbiome (Brno University of Technology, CZ)

12:00 – 1:15pm *Lunch – hotel Mědínek*

1:15pm – 6:00pm *Social program (Tour of the City of Silver*)

7:00pm – 12pm *Conference Reception (restaurant Dačický)*

THURSDAY (November 10, 2022)

8:00 – 10:00pm Session VI.

Chairmans: Rösl Frank, Boštík Vanda

8:00 – 8:30am **Rösl Frank** - Cutaneous papillomavirus induced skin carcinogenesis: molecular and immunological aspects (German Cancer Research Center, Heidelberg, GE)

8:30 – 8:45am **Machát Radek** - Innate immune response of common carp (Cyprinus carpio L.) to Koi herpesvirus infection (Veterinary Research Institute, CZ)

8:45 – 9.00am **Pokorná Karolína**- Investigating the Role of UR11 in Hepatitis B Virus Replication ((Institute of Organic Chemistry and Biochemistry of the ASCR, CZ))

9:00 – 9.30am **Boštík Vanda**- Lessons from the COVID-19 Pandemic (FoMHS, University of Defence, CZ)

9:30 – 9.45am **Hrdý Jiří**- Impact of vaccination against COVID-19 on immune responses in patients suffering from autoimmune neurological disorders treated with rituximab or ocrelizumab(Charles University and General University Hospital in Prague, CZ)

9:45 – 10.00am **Vašák Jiří** – The HoloMonitor: holografy in microscopy (KRD s.r.o., CZ)

10:00 – 10:45am *Coffee break – Conference foyer*

10:45 – 12:00am Session VII.

Chairmans: Schabussova Irma, Kozáková Hana

10:45 – 11:15am **Irma Schabussova**- Extracellular vesicles: The missing link between microbiota and the host immunity? (Medical University of Vienna, AT)

11:15 – 11:30am **Bavlovič Jan**- Outer membrane vesicles and nanotubes secretion in *Francisella tularensis* strain with disrupted O-antigen (FoMHS, University of Defence, CZ)

11:30 – 11:45am **Lásková Pavlína** - Validation of *Francisella tularensis* peptides as immunogenic T-cell epitopes (FoMHS, University of Defence, CZ)

11:45 – 12:00am **Hrdý Jiří**- Effect of early postnatal supplementation of newborns by probiotic strain E. coli O83:K24:H31 on allergy incidence, dendritic cells and microbiota (Charles University and General University Hospital in Prague, CZ)

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

ORAL PRESENTATION ABSTRACT

Blank pages for your notes…….

Potentials of antibody ligands with T cell receptor specificity for the immunotherapy of intracellular infections

Juraj Ivanyi

Centre for Host-Microbiome Interactions, Guy's Campus of Kings College London, UK.

Abstract

Immunogenic epitopes of infectious pathogens, expressed in the context MHC molecules on the surface of infected macrophages are recognised by the receptors of host T cells (TcR). Since the associated inflammatory reactions can lead to pathology, potential immunotherapy by single chain antibodies with TcR-like specificity is of interest. The methodology for their selection has been developed and applied mostly toward cancer immunotherapy (Yang et al Theranostics 9:7792, 2019; Duan et al Mol Cancer Ther; 20:1533,2021). Recently, such antibodies have been generated against the CD8 TcR recognised epitopes of infectious pathogens. Selection of antibody ligands against HLA-bound epitopes from phage display libraries was reported in respect of the mycobacterial Acr antigen (Das et al Mol Immunol 101:189, 2018; Int J Biol Macromol 155:305,2020) and the Ag85B antigen (Ortega et al Front. Immunol.11:577815.doi:10.3389/fimmu.2020.577815). The prospects of further development of such antibodies for the immunotherapy of tuberculosis and in respect of other intracellular infections will be discussed.

The *Francisella tularensis* ClpB - a unique chaperone regulating T6SS

Athar Alam[1], Igor Golovliov[2], Eram Javed[3], Rajender Kumar[4], Jörgen Ådén[5], **Anders Sjöstedt**[6]

[1]*Department of Clinical Microbiology, Umeå University, Sweden*

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Abstract

Francisella tularensis possesses an atypical type VI secretion system (T6SS), which is essential for its virulence. The chaperone ClpB, a member of the Hsp100/Clp family and critically required during heat-shock, is esential for type VI secretion (T6S). We investigated whether the role of ClpB related to T6S was dependent on its disaggregation activity, which is dependent on its interaction with the DnaK/Hsp70 chaperone system. Key residues of the ClpB-DnaK interaction were identified by molecular dynamic simulation and verified by targeted mutagenesis. Using such targeted mutants, it was found that the *F. tularensis* ClpB-DnaK interaction was dispensable for T6S, intracellular replication, and virulence in a mouse model, but, as expected, essential for handling of heat shock. Moreover, by mutagenesis of key amino acids of the Walker A, Walker B, and Arginine finger motifs of each of the two Nucleotide-Binding Domains, their critical roles for heat shock, T6S, intracellular replication, and virulence were identified. In contrast, the N-terminus was dispensable for heat shock, but required for T6S, intracellular replication, and virulence. Complementation of the Δ*clpB* mutant with a chimeric *F. tularensis* ClpB expressing the N-terminal of *Escherichia coli*, led to reconstitution of the wild-type phenotype. In prototypical T6SS of enterobacteria, ClpV interacts via conserved domains with the T6SS-component IglB and provides the energy needed for T6S via its ATPase activity. Our further work is aimed to identify how ClpB interacts with the T6SS of *F. tularensis* and data will be presented in this regard. Collectively, the data demonstrate that the ClpB-DnaK interaction, critical for the disaggregation activity of ClpB-DnaK, does not contribute to T6S, whereas the N-terminal displayed critical roles for T6S and virulence.

Arginine 58 is indispensable for proper function of the *Francisella tularensis* HU protein, and its substitution alters virulence and mediates immunity against wild-type strain

Pavla Pavlik, **Petra Spidlova**

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Abstract

HU protein, a member of the nucleoid-associated group of proteins, is an important transcription factor in bacteria. Generally, HU protein acts as a DNA sequence nonspecific binding protein and it is responsible for winding of the DNA chain that leads to the separation of transcription units. Here, we identified potential HU protein binding sites using the ChIP-seq method and two possible binding motifs in *F. tularensis* FSC200 depending upon growth conditions. We also confirmed that FSC200 HU protein is able to introduce negative supercoiling of DNA in the presence of topoisomerase I. Next, we showed interaction of the HU protein with a DNA region upstream of the *pigR* gene and inside the *clpB* gene, suggesting possible regulation of PigR and ClpB expression. Moreover, we showed that arginine 58 and partially arginine 61 are important for HU protein's DNA binding capacity, negative supercoiling induction by HU, and the length and winding of FSC200 chromosomal DNA. Finally, in order to verify biological function of the HU protein, we demonstrated that mutations in arginine 58, arginine 61, and serine 74 of the HU protein decrease virulence of FSC200 both *in vitro* and *in vivo* and that immunization using these mutant strains is able to protect as many as 100% of mice against wild-type challenge. Taken together, our findings deepen knowledge about the role of the HU protein in tularemia pathogenesis and suggest that HU protein should be addressed in the context of tularemia vaccine development.

This work was supported by the Ministry of Defence of the Czech Republic - Long-term organization development plan Medical Aspects of Weapons of Mass Destruction of the Faculty of Military Health Sciences, University of Defence (DZRO-ZHN-2017 and DZRO-FVZ-ZHN-II).

Francisella in the environment

Maša Knežević[1], Valentina Marečić[1], Ina Viduka[1], Mateja Ožanič[1], Mirna Mihelčić[1],

Marina Šantić[1,2]

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Abstract

Francisella tularensis is a gram-negative bacterium, the causative agent of the zoonotic disease tularemia. Tularemia has been reported primarily in areas near water sources. Our and other *in vitro* studies have shown that *F. tularensis* subsp. *tularensis*, *F. holarctica*, and *F. novicida* can replicate in *Acanthamoeba castellanii* and *Hartmannella vermiformis*, making amoebae an important environmental reservoir for bacteria. Surprisingly, in our study, *F. novicida* grown in amoebae were more sensitive to decontamination with benzalkonium chloride, didecyldimethylammonium chloride (DDAC) and formic acid, and polyhexamethylene biguanide (PHMB). It is really fascinating why *Francisella* behaves differently from other intracellular pathogens, and the goal of this project is to explore these mechanisms. Our results show that autophagy plays a critical role in the maintenance of *Francisella* infection not only in human and mouse macrophages, but also in the long-term survival of the bacteria in amoebae. Moreover, our results show that *Francisella* species are responsible for suppressing apoptosis of *A. castellanii* during the first 6 hours of infection. One of the possible reasons for this is that the bacteria delay host cell death by the time it takes for them to complete their intracellular life cycle. Overall, control of amoeba cell death mechanisms is critical for *Franciella* survival in the aquatic environment.

Changes of host deubiquitination enzymes during *Francisella tularensis* infection of human macrophages

Vera Vozandychova[1], Pavel Rehulka[1], Kamil Hercik[2], Jiri Stulik[1]

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Abstract

This work was focused on identification of relative quantitation changes in deubiquitination enzymes of the host cell during infection with Gram-negative bacteria *Francisella tularensis*.

Francisella tularensis subsp. FSC200 infection was performed on human macrophages differentiated from the monocytic cell line THP-1. Two early infection time intervals – 10 min and 60 min – were chosen for the experiment. Relative changes of deubiquitination enzymes present both in cell and in released vesicles of infected cells were measured at different levels using several methodologies: 1) proteomic level (LC-MS analysis of enzymatically digested peptides - untargeted and targeted; Western blotting); 2) expression level (real-time PCR).

Bioinformatics processing of the data obtained from LC-MS/MS experiment identified several cellular DUBs with relative quantitation changes. These DUBs (UCH-L5, USP10, USP25, OTUB1, OTUD6B) were selected for verification by Western blotting and real-time PCR. Western blot analysis performed in cell lysates showed minimal changes for selected DUBs. Real-time PCR analysis of infected cells lysates at the mRNA level of individually analyzed DUBs did not confirm most of the changes identified in the LC-MS/MS experiments. The simultaneous LC-MS/MS analysis of isolated extracellular vesicles of infected cells also identified changes of several DUBs (UCH-L5, USP10, and USP25), but further experiments are to be done for confirmation of these results.

Changes in cellular DUBs identified by mass spectrometry based proteomic analysis were only partially verified by Western blot analysis and were not confirmed by corresponding real-time PCR analysis. Therefore, a targeted proteomic analysis with selected specific peptides for individual DUBs will be employed to solve the

question about possible DUBs regulation during *Francisella tularensis* infection process.

IL-1β neutralization increased susceptibility to tularemia in autophagy deficient animals during *Francisella tularensis* LVS infection

Mirna Mihelčić[1], Ina Viduka[1], Maša Knežević[1], Valentina Marečić[1], Mateja Ožanič[1], and Marina Šantić[1].

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Abstract

Autophagy is a conserved cellular degradation pathway, which involves the delivery of cytoplasmic substrates to lysosomes for degradation. Among 41 identified ATG proteins, ATG5 protein seems to have indispensable role in formation of autophagosome. Autophagy also plays an indisputable role in the regulation of immune response, contributing to the host defense against pathogen. *Francisella tularensis*, an intracellular pathogen, survive autophagy process, and require autophagy for its intracellular growth. ATG5-dependent autophagy is critical in the regulation of host's innate and adaptive immune response, impacting subsequent inflammatory response as well. IL-1β is an important pro-inflammatory cytokine and its secretion is regulated through the inflammasome induced caspase-1 activation upon infection. The link between autophagy and IL-1β secretion has been interpreted differently. The aim of the study was to investigate an interplay between ATG5-induced autophagy and IL-1β, and their role in pathogenesis of tularemia. We used a model of transgenic mice, lacking ATG5 in cells of the myeloid lineage, bacterium *F. tularensis* LVS (live vaccine strain) and IL-1β neutralizing antibody. ATG5-deficient and control mice were infected intradermally and IL-1β neutralizing antibody or isotype control antibody were inoculated intraperitoneally. Cytokine and chemokine levels in sera were determined by Luminex assay. Levels of selected pro-inflammatory and anti-inflammatory cytokines in spleen, liver and lungs were analyzed by RT-PCR, and the inflammatory cell infiltration in the lungs and liver were analyzed by immunohistochemistry. Our findings reveal that IL-1β neutralization, together with autophagy deficiency in myeloid cells, increased susceptibility to tularemia. In contrast, treatment of control group of mice with IL-1β antibody, have a beneficial effect on course of tularemia, suppressing bacterial

Suppress immunity, proliferate and transmit: How the pertussis agent hijacks the immune response of nasal mucosa

Jana Holubová, Nela Klímová, Ondřej Staněk, Ladislav Bumba a **Peter Šebo**

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Abstract

Mouse models of pertussis pneumonia served in characterization of *B. pertussis* virulence mechanisms. However, the biologically most relevant catarrhal disease stage and *B. pertussis* transmission has not been adequately reproduced in adult mice due to limited proliferation of the human-adapted pathogen on murine nasopharyngeal mucosa. We used immunodeficient C57BL/6J MyD88 KO mice to achieve *B. pertussis* proliferation to human-like high counts of 10^8 viable bacteria per nasal cavity to elicit rhinosinusitis accompanied by robust shedding and transmission of *B. pertussis* bacteria to adult co-housed MyD88 KO mice. Experiments with a comprehensive set of *B. pertussis* mutants revealed that pertussis toxin, adenylate cyclase toxin-hemolysin, the T3SS effector BteA/BopC and several other known virulence factors were dispensable for nasal cavity infection and *B. pertussis* transmission in the immunocompromised MyD88 KO mice. In contrast, mutants lacking the filamentous hemagglutinin (FhaB) or fimbriae (Fim) adhesins infected the nasal cavity poorly, shed at low levels and failed to productively infect co-housed MyD88 KO or C57BL/6J mice. FhaB and fimbriae thus appear to play a critical role in *B. pertussis* transmission.

Moreover, the adenylate cyclase (ACT) and the pertussis (PT) toxins of *Bordetella pertussis* are known to exert potent immunomodulatory activities that synergize to suppress host defense. We thus compared also the mouse lung infection capacities of *B. pertussis* (Bp) mutants (Bp AC- or Bp PT-) producing enzymatically inactive toxoids. Despite accelerated and near complete clearance from the lungs of immunocompetent Balb/c mice by day 14 of infection, the PT- bacteria accumulated within the lymphoid tissue of lung-draining mediastinal lymph nodes (mLNs). In contrast, the wild type or AC- bacteria colonized the lungs but did not enter into mLNs. Lung infection by the PT- mutant triggered an early arrival of migratory conventional dendritic cells with associated bacteria into mLNs, where the PT- bacteria entered the T cell-rich paracortex of mLNs by day 5 and proliferated in clusters within the B-cell zone (cortex) of mLNs by day 14, being eventually phagocytosed by infiltrating neutrophils. Finally, only infection by the PT- bacteria triggered an early production of anti-B. pertussis serum IgG antibodies already within 14 days of infection, indicating that action of the pertussis toxin blocks DC-mediated delivery of *B. pertussis* bacteria into mLNs. Thereby PT action prevents

bacterial colonization of mLNs and early adaptive immune response to *B. pertussis* infection, thus enabling high level colonization of the nasopharyngeal mucosa and efficient transmission to new hosts.

Pertussis toxin suppresses dendritic cell-mediated delivery of *B. pertussis* into lung-draining lymph nodes

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Abstract

The adenylate cyclase (ACT) and the pertussis (PT) toxins of *Bordetella pertussis* exert potent immunomodulatory activities that synergize to suppress host defense in the course of whooping cough pathogenesis. We compared the mouse lung infection capacities of *B. pertussis* mutants (AC^- or PT^-) producing enzymatically inactive toxoids and confirm that ACT action is required for maximal bacterial proliferation in the first days of infection, whereas PT action is crucial for persistence of *B. pertussis* in mouse lungs. Despite accelerated and near complete clearance from the lungs by day 14 of infection, the PT^- bacteria accumulated within the lymphoid tissue of lung-draining mediastinal lymph nodes (mLNs). In contrast, the wild type or AC^- bacteria colonized the lungs but did not enter into mLNs. Lung infection by the PT^- mutant triggered an early arrival of migratory conventional dendritic cells with associated bacteria into mLNs, where the PT^- bacteria entered the T cell-rich paracortex of mLNs by day 5 and proliferated in clusters within the B-cell zone (cortex) of mLNs by day 14, being eventually phagocytosed by infiltrating neutrophils. Finally, only infection by the PT^- bacteria triggered an early production of anti-*B. pertussis* serum IgG antibodies already within 14 days of infection. These results reveal that action of the pertussis toxin blocks DC-mediated delivery of B. pertussis bacteria into mLNs and prevents bacterial colonization of mLNs, thus hampering early adaptive immune response to *B. pertussis* infection.

Bordetella Type III Secretion Injectosome and Effector Proteins

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Abstract

Pertussis, or whooping cough is an infectious disease of the respiratory tract caused mainly by the bacterium Bordetella pertussis. The incidence of pertussis is steadily increasing in most industrialized countries despite massive vaccination programs. This calls for a better understanding of the pathogenesis of B. pertussis. 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 components of the type III secretion system (T3SS) that translocates the so-called effectors BteA and BopN into host cells. Here, we solved the crystal structure of BopN and found that it is a T3SS gatekeeper that promotes the efficient and polarized transfer of BteA into host cells. We also show that the cytotoxic and plasma membrane-localizing BteA effector has undergone divergent evolution within classical Bordetella species. A single amino acid insertion into the BteA protein of B. pertussis, the insertion of alanine at position 503, attenuated its cytotoxic efficacy. Interestingly, the production of Bp BteAΔA503 increases virulence of B. pertussis B1917 in a mouse model of intranasal infection (reduced LD50), but results in less inflammatory pathology in infected mouse lungs at sublethal doses. This suggests that A503 insertion in the T3SS effector Bp BteA may represent an evolutionary adaptation that fine-tunes B. pertussis virulence and host immune response.

Role of BscX and BscY proteins in the *Bordetella* type 3 secretion system

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Abstract

The type 3 secretion system (T3SS), also known as an injectosome, is a widespread macromolecular nanomachine that enables the delivery of bacterial effector proteins directly from the bacterial cytosol into the cytosol of host cells. Despite many T3SS subunits are genetically and structurally conserved among different Gram-negative bacteria, there are some additional components that are distinct and species specific. In the *Bordetella* genus, these include BscX and BscY, small subunits of the T3SS protein, which are homologous to the *Yersinia* YscX and YscY proteins. These proteins appear to be part of the export gate, orchestrating the secretion of early substrates, but their structure and function in the *Bordetella* T3SS apparatus remain unknown. Here, we determined solution structure of BscX and BscY proteins by nuclear magnetic resonance (NMR) spectroscopy. BscX and BscY form a tight heterodimeric complex, where six helical folds of BscY are wrapped by BscX made up of the N-terminal unstructured region and three C-terminal helices. The single-gene *bscX* or *bscY* deletion mutants of *B. bronchiseptica* cells did not exert any cytotoxic activity against HeLa cells, indicating that the presence of the BscX-BscY heterodimer is critical for proper function of the secretion apparatus. Futhermore, the removal of the first 22 amino acids from BscX rendered the bacterial mutant non-cytotoxic on HeLa cells, indicating that the N-terminal unstructured region of BscX is structurally essential. These results pave the way for understanding the structure-function relationship of the BscX and BscY proteins in the Bordetella T3SS.

The *Burkholderia pseudomallei hmq*A-G Locus Mediates Competitive Fitness against Environmental Gram-Positive Bacteria

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Abstract

Burkholderia pseudomallei, a saprophytic soil bacterium is responsible for melioidosis in human and animals. *Burkholderia pseudomallei* naturally resides in water, soil, and the rhizosphere and its success as an opportunistic pathogen is dependent on the ability to persist in these harsh habitats long enough to come into contact with a susceptible host. In addition to adapting to limiting nutrients and diverse chemical and physical challenge, *B. pseudomallei* also has to interact with a variety of microbial competitors.

There have been few reports investigating the molecular mechanism(s) utilized by *B. pseudomallei* to survive and persist in ecological niches harboring microbial competitors. Here, we report the isolation of Gram-positive bacteria from multiple environmental source and show that ~45% of these isolates are inhibited by *B. pseud*omallei in head-to-head competition assay. Two competition-deficient *B.pseudomallei* transposon mutants were identified that contained insertion mutations in the *hmq*A-G operon. This large biosynthetic gene cluster encodes the enzymes that produce a family of secondary metabolites call 4-hydroxy-3-methyl-2-alkylquinolines (HMAQs). Liquid chromatography and mass spectrometry conducted on extracted filter-sterilized culture supernatants revealed five HMAQs and N-oxide derivatives that were produced by the parental strain but were absent in an isogenic *hmq*D deletion mutant. This result demonstrate that *B. pseudomallei* inhibit the growth of environmental Gram-positive bacteria in a contact-independent manner via the production of HMAQs by the *hmq*A-G operon.

Lipid Binding by the N-terminal Motif Mediates Plasma Membrane Localization of Bordetella Effector Protein BteA

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Abstract

The respiratory pathogens Bordetella pertussis and Bordetella bronchiseptica employ a type III secretion system (T3SS) to inject a 69-kDa BteA effector protein into host cells. BteA is known to be targeted to lipid rafts in the plasma membrane (PM) of host cells. However, the exact molecular mechanisms underlying the interaction of BteA with the plasma membrane and its cytotoxic activity in the course of Bordetella infections are poorly understood. The BteA protein consists of two functional domains, an N-terminal lipid raft-targeting (LRT) domain and a C-terminal domain required for its cytotoxicity. We have shown that the recombinant LRT domain binds negatively charged membrane phospholipids such as phosphatidylinositol-4,5-bisphosphate (PIP2), phosphatidic acid (PA), and phosphatidylserine (PS). The association of BteA with PM is influenced by electrostatic and hydrophobic interactions of its LRT domain. Charge-reversal substitutions in the L1 region of the LRT disrupted PM localization of BteA without affecting its cytotoxic activity during B. bronchiseptica infection of HeLa cells. Therefore, LRT-mediated targeting of BteA to the cytosolic leaflet of the plasma membrane of host cells is not required for its cytotoxicity. This finding suggests the existence of an intracellular target of BteA action.

Unraveling the Mechanisms of Action of Bordetella Effector Protein BteA

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Abstract

Classical Bordetella species cause respiratory infections in mammals, such as pertussis in humans caused by Bordetella pertussis and B. parapertussis or chronic respiratory infections in a variety of animals caused by B. bronchiseptica. These pathogenic bacteria encode a multicomponent type III secretion system (T3SS) that contributes to the evasion of host protective responses against B. bronchiseptica and translocates the effector protein BteA into the host cells. In the host cells, BteA localises to lipid rafts and triggers non-apoptotic and caspase-1-independent cell death. However, the molecular mechanism of BteA action and its molecular targets are unknown.

To elucidate the mechanisms underlying the action of BteA, we apply a range of genetic, cell-biology and biochemical approaches. First, we perform a genome-wide CRISPR/Cas9-mediated screen in which a pool of human epithelial knock-out cells is generated using a gRNA library and tested for resistance to BteA-induced cell death. Second, we analyse cellular signalling pathways activated by BteA. Finally, we aim to produce a soluble BteA protein and gain structural insights into its mechanism of action. The status of our research will be presented.

Identification of residues involved in posttranslational modification of RTX toxins of Gram-negative pathogens

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Abstract

Bordetella pertussis adenylate cyclase toxin (CyaA) and *Escherichia coli* α-hemolysin (HlyA) belong to the Repeats in ToXin (RTX) family and play key roles in virulence of numerous Gram-negative pathogens. CyaA translocates unique N-terminal AC domain into the cytosol of phagocytes and undermines their bactericidal functions by unregulated conversion of ATP to cAMP. Both CyaA and HlyA then permeabilize the membrane of eukaryotic cells by forming cation-selective pores. The toxins bind preferentially to cells expressing β_2 integrins but can also penetrate the membrane of cells not expressing β_2 integrins. CyaA and HlyA are synthesized as protoxins and are activated by covalent posttranslational acylation that is catalyzed by the dedicated acyltransferases CyaC and HlyC, respectively. The acyls are linked at ε-amino groups of two lysine residues located within conserved acylation sites, namely Lys860 and Lys983 in CyaA and Lys564 and Lys690 in HlyA. Using chimeric CyaA/HlyA molecules, we identified the sequences essential for acyltransferase-mediated acylation of CyaA and HlyA and site-directed mutagenesis defined the residues involved in recognition of the toxin acylation sites by the acyltransferases. We show that substitution of Tyr990 and Arg991 in CyaA and of Tyr697 and Arg698 in HlyA, reduces the extent of acylation of Lys983 in CyaA and of Lys690 in HlyA, respectively. We further showthat these substitutions reduce the cytotoxic and cytolytic capacity of both toxins towards model sheep erythrocytes and human macrophage THP-1 cells. Using AlphaFold prediction and homologous modeling of CyaC on the known structure of ApxIC, we aim to identify residues directly engaged in the interaction between the acyltransferase and the RTX toxin.

Implementation of advanced methodical strategies to form dual-species biofilm in the research of anti-biofilm acting compounds

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Abstract

Currently, it is known that microorganisms, including multidrug-resistant ones, prefer a lifestyle in complex communities called biofilms which are in nature mostly polymicrobial. These microbial consortia provide microorganisms more protection and it has been demonstrated that mutual interactions among biofilm-forming microbes also contribute to the enhancement of antimicrobial resistance.

One of the pitfalls in anti-infective drug discovery research also lies in the low correlation between results of the candidate compounds' activity *in vitro* compared to results from the clinical trials. The primary goal of our research is to reflect this potential gap and introduce some conditions mimicking the host environment and leading to robust dual-species biofilm biomass formation *in vitro*, with the key biofilm attributes corresponding to biofilm resistance.

Microorganisms colonizing wounds or venous catheters and forming poly-species biofilms *in vivo* were chosen, namely methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Candida albicans*. Four different cultivation media (nutrient-poor/rich), supplemented with human plasma, eventually erythrocyte cell lysate, were chosen for this study. For the determination of the formation of the most robust biofilm, the exposure to 50-fold higher MIC of ciprofloxacin and anidulafungin was evaluated, using Alamar blue method. Furthermore, for the establishment of the optimal conditions for biofilm formation *in vitro*, parameters such as viability, equal representation of individual species, presence of the biofilm matrix, *etc.*, were evaluated, as well.

Under the influence of various nutrient conditions of cultivation media robust dual-species biofilms with different cell phenotypes and rates of their typical attributes have been formed.

In conclusion, the aim of our study is the implementation of relevant and valid conditions for the formation of robust dual-species (*S. aureus*/*S. epidermidis* and *C. albicans*) biofilm *in vitro* with the key attributes linked to the adaptive resistance of these microbial consortia. In addition, the subsequent insight and comparative analysis especially the composition of the extracellular biofilm matrix can offer possible explanations for different resistance profiles revealed in dual-species microbial consortia formed *in vitro* under different nutrient conditions.

The study was supported by the Czech Science Foundation project No. 20-19638Y, and the Ministry of Health of the Czech Republic, grant nr. NU21-05-00482.

CD97 stabilises the immunological synapse between dendritic cells and T cells and is targeted for degradation by the Salmonella effector SteD

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Abstract

The Salmonella enterica effector SteD depletes mature MHC class II (mMHCII) molecules from the surface of infected antigen-presenting cells through ubiquitination of the cytoplasmic tail of the mMHCII β chain. This requires the Nedd4 family HECT E3 ubiquitin ligase Wwp2 and a tumorsuppressing transmembrane protein adaptor Tmem127. Here, through a proteomic screen of dendritic cells, we found that SteD targets the plasma membrane protein CD97 for degradation by a similar mechanism. SteD enhanced ubiquitination of CD97 on K555 and mutation of this residue eliminated the effect of SteD on CD97 surface levels. We showed that CD97 localises to and stabilises the immunological synapse between dendritic cells and T cells. Removal of CD97 by SteD inhibited dendritic cell-T cell interactions and reduced T cell activation, independently of its effect on MHCII. Therefore, SteD suppresses T cell immunity by two distinct processes.

Heterogeneous expression of Salmonella Typhimurium SPI-2 T3SS effectors

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Abstract

Infection by Salmonella can lead to its systemic spread to various organs. This spread is mediated by intracellular colonization of macrophages, where the bacterium survives in the Salmonella-containing vacuole (SCV). Salmonella survival in SCV is enabled by translocation of effectors of the Salmonella pathogenicity island 2 (SPI-2)-encoded Type III secretion system (T3SS) into the host cells. Using a fluorescent reporter system, we show that the expression of the SPI-2 T3SS effectors is heterogeneous. Therefore, we aim to i) unravel how does the Salmonella population in one host cell benefit from heterogeneous expression of the SPI-2 T3SS effectors, ii) identify bacterial genetic factors influencing expression heterogeneity of the SPI-2 T3SS effectors, and iii) identify the factors in SCV that drive heterogeneous expression of the SPI-2 T3SS effectors. This could lead to a better understanding of the heterogeneous behaviour of other intracellular pathogens and consequently to a removal of the beneficial heterogenous behaviour in order to cure the bacterial infection.

Salmonella SPI-2 TTSS effectors: Where and When

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Abstract

Many bacteria, including the human food-born pathogen Salmonella Typhimurium, use effectors translocated by type III secretion systems (TTSS) to create optimal niche for their survival and replication in the host, and to suppress the host immune response. However, expression of the large secretion machinery and of all its effectors is metabolically costly. Therefore, we hypothesize that the timing of production and translocation of particular effectors is finetuned to maximize the benefits from their functions during the specific phase of the infection. Using fluorescence reporters and split luciferase systems, we correlated expression and translocation of selected Salmonella effectors with their known roles in the infection. Further research will help us to detect cooperative relationships between effectors and could uncover new functions of less understood effectors.

Mechanism of mycobacterial inosine-5′-monophosphate dehydrogenase allosteric regulation

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Abstract

Inosine-5′-monophosphate dehydrogenase (IMPDH) is a crucial purine metabolism enzyme that is well established as a promising drug target against mycobacterial infections. However, because most previous biochemical and structural studies were conducted with IMPDH lacking its regulatory CBS domain, little is known about the allosteric regulation of- mycobacterial IMPDH. In this work, we describe the novel allosteric regulation of full-length IMPDH and its underlying molecular mechanism. First, isolated recombinant IMPDH from *Mycobacterium smegmatis* was used for *in vitro* biochemical characterization. Testing the impact of selected purine nucleotide compounds on IMPDH activity showed the dramatic inhibitory effect of their combination at biologically relevant concentrations. This regulation is only present in the full-length enzyme, not in the CBS-domain-deleted variant. Next, cryo-electron microscopy was used to determine a series of molecular structures of full-length IMPDH with the nucleotide ligands bound to the CBS domain. Structural changes in the active/inhibited forms of IMPDH enabled us to propose the interdomain crosstalk that leads to changes in the catalytic core of the enzyme. The described mechanism represents a novel example of the regulation of IMPDH activity, with potential for use in the design of antimycobacterial IMPDH-targeting drugs.

Multiprotein organization of the purine metabolism enzymes in mycobacteria

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Abstract

Mycobacterium tuberculosis (*Mtb*), a causative agent of tuberculosis, is the first bacteria per deaths worldwide. The increasing number of multidrug-resistant *Mtb* strains is currently a big threat for the public health and new therapeutic targets are urgently needed. The *de novo* purine biosynthesis pathway regulation in mycobacteria is far from being understood and a deeper view can unveil important details on their biology. The *de novo* pathway, involving tandem 11 enzymes reactions, produces essential molecules for the viability of the bacteria and it is believed it can be used as a drug target to block the bacterial replication. Our aim is to investigate the possible presence of the purinosome, a putative protein complex, that may prevent the dispersion and/or the degradation of highly reactive/instable metabolites, fastening the entire process. There are evidences of its presence in mammalians cells, suggesting that it is usually assembled when the cells undergo stress conditions as hypoxia, lack of nutrients, low pH, etc. We examined the possible *in vivo* protein interactome in the model species *Mycobacterium smegmatis*. Immuno-precipitation followed by mass spectrometry analysis revealed no interacting partners for 1st and 3rd enzyme of the pathway purF and purL, indicating the absence of strong non-covalent bonds between proteins. To examine the possibility of weak non-covalent bonds, formaldehyde was used as crosslinker, but since its properties don't allow the analysis of the data by mass spectrometry, a de-crosslinking protocol is being developed.

Developmen vaccine against *Streptococcus suis* infection in pig

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Abstract

Development of vaccines against streptococcal infection is challenging due to nature of the interaction between the pathogen and the host immune system. In last decades, vaccines based on conjugates of capsular polysaccharide and carrier protein were proved to be protective against various bacterial pathogens, including streptococcal infections in humans. However, the manufacturing cost is limiting for their use in animals. Although *Streptococcus suis* infection in pig is currently considered to be one of most important bacterial disease, an effective commercial vaccine is still not available. We thus tested an experimental vaccine against *Streptococcus suis* serotype 2 based on capsular polysaccharide conjugated to chicken ovalbumin and compared its immunogenicity and protectivity with a vaccine based on CRM197 conjugate. Ovalbumin was selected as a cheap alternative to recombinant carrier proteins widely used in vaccines for human use. We found that the ovalbumin-based experimental vaccine successfully induced immune response in pigs, and the IgG antibody response was even higher than after immunization with capsular polysaccharide-CRM197 conjugate. Protectivity of vaccination against infection was evaluated in the challenge experiment and was found promising for both conjugates.

This work was supported by Ministry of Agriculture of the Czech Republic (Institutional Support No.MZE-RO0518) and by the National Agency for Agricultural Research (Project No. QK1810193).

Diversity of *Streptococcus suis* strains isolated from sick pigs from farms in the Czech Republic

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Abstract

Streptococcus suis is a significant cause of mortality in piglets and growing pigs worldwide. The pathogenic strains causes meningitis, arthritis, endocarditis, polyserositis, and septicemia and are also considered an emerging zoonotic pathogen. Serotyping and multilocus sequence typing are primary methods to differentiate strains. The objective of this study was to characterize the diversity of 552 *S. suis* isolates collected between 2018 and 2022 originating from sick pigs from farms in the Czech Republic. Using molecular methods, serotype 7 (15.2%) was determined to be predominant among *S. suis* isolates, followed by serotypes 2, 1/2, 9, 8, 4, 3, 1, 29, 31, 16, 12, and 15. Other serotypes were identified either rarely (up to 10 cases) or not at all, 18.5% of isolates were non-typeable. Multilocus sequence typing revealed 56 sequence types, of which 29 was identified as novel. The predominant sequence types were ST29 and ST28. No direct relationship between serotypes and sequence types was detected. Eleven of the 23 serotypes identified contained multiple sequence types, with the number of different sequence types within a single serotype ranging from 2 to 6. The distribution of serotypes and sequence types showed the large diversity of the *S. suis* strains in pig farms.

This work was supported by Ministry of Agriculture of the Czech Republic (Institutional Support No.MZE-RO0518) and by the National Agency for Agricultural Research (Project No. QK1810193).

Resistance of *Streptococcus suis* Isolates from the Czech Republic during 2018–2022

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Abstract

A determination of minimal inhibition concentration for the monitoring of antimicrobial resistance (AMR) was carried out in 506 field isolates of *Streptococcus suis*, originating from sick pig on farms in the Czech Republic in the period 2018–2022. Moreover, the correlation between AMR and the serotype of the isolates was determined. A very high level of susceptibility of *S. suis* isolates was found to amoxicillin, in combination with clavulanic acid and sulfamethoxazole potentiated with trimethoprim. None of the tested isolates were resistant to these antimicrobial substances. Only two isolates were found to be intermediately resistant to enrofloxacin in 2020. With regard to ceftiofur, one isolate was intermediately resistant in 2020 and 2022, and two isolates were intermediately resistant in 2018 and 2021. A low level of resistance was detected to ampicillin and to florfenicol. With regard to penicillin, a medium level of resistance was detected in 2018, but a low level of resistance was found in the following years. On the contrary, a high or very high level of resistance was found to tetracycline. Using molecular and serological methods, serotype 7 (16.4%) was determined to be predominant among *S. suis* isolates, followed by serotypes 1/2, 2, 9, 4, 3, 1, 29, 16, and 31. Other serotypes were identified among the investigated strains either rarely (up to 10 cases) or not at all. A relatively high percentage of isolates (15.6 %) were detected as non-typeable. Dependence of resistance upon serotype assignment could not be proven in all but serotype 31, wherein all isolates (n = 17) were resistant or intermediately resistant to clindamycin, tilmycosin, tulathromycin, and tetracycline. The resistance to clindamycin and tetracycline may be related to the high consumption of these antibiotics on pig farms at present or in previous years. Macrolides (tilmicosin and tulathromycin) and tiamulin are not suitable for the treatment of streptococcal infections, but are used on pig farms to treat respiratory infections caused by gram-negative bacteria, so they were included in the study.

The study was supported by the Ministry of Agriculture of the Czech Republic (MZE-RO0518) and by the National Agency for Agricultural Research (QK1810193).

The capsular polysaccharide synthesis locus analysis of *Streptococcus suis* isolates

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Abstract

Streptococcus suis is an important pig pathogen that occurs worldwide and causes great economic losses. One of the important virulent factors of this zoonotic agent is its capsular polysaccharide (CPS). Based on the structure and composition of the capsular polysaccharide, we distinguish *S. suis* serotypes. Due to its phenotypic and genotypic diversity, more than 33 serotypes are currently known. 123 field samples of *S. suis* from Czech farms were sequenced and their cps synthesis loci were compared with reference serotypes. After comparing the cps loci, it was found that there is a group in our collection whose isolates can be classified into previously reported serotypes. The second group of isolates were similar to the reference serotypes, but had either mutations or gene insertions/deletions. Other isolates have been grouped as having cps loci identical to recently described novel cps loci (NCLs) and other strains have NCLs not yet described. This indicates the great diversity of *S. suis* in the Czech republic.

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Host-*Leishmania* interactions – an essential role of the vector

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Abstract

Leishmania (Kinetoplastida) are unicellular digenetic parasites alternating two different hosts in their life cycle - the invertebrate vector and the vertebrate host. The host species cover wild, domestic, and accompanying animals as well as humans. There are about 20 *Leishmania* species that are medically important, causing leishmaniasis, a group of diseases with various clinical symptoms. Leishmaniases are traditionally divided into 3 basic clinical forms - cutaneous, mucocutaneous, and visceral. The latter one being fatal if left untreated. Leishmaniases are endemic in 98 countries in tropical and subtropical regions (incl. favourite holiday destinations in the Mediterranean region such as France, Italy, Croatia, Egypt, and Tunisia), about 350 million people are living at risk of infection, the prevalence is about 12 million people, and about 50,000 die each year from leishmaniases. In the Czech Republic, human leishmaniasis is not autochthonous, however, several imported cases are reported each year. There is no vaccination available for humans.

The clinical symptoms depend on several factors, including *Leishmania* species, the virulence of the parasite, the status of the host´s immune system, and – last but not least – on the history of host-vector interactions. *Leishmania* parasites are transmitted by phlebotominae sand flies during blood feeding. In 1988, Titus and Ribeiro published a ground-breaking study demonstrating that the vector is more than a flying syringe. They showed that the vector molecules, delivered alongside *Leishmania* into the host skin, substantially influence the effectiveness of pathogen transmission. The presentation will reveal the background of this essential role of the vector on pathogen transmission, the nature of molecules behind it, and how they can be utilised in human benefit.

Dual role of anti-vector saliva immunity on the outcome of *Leishmania* infection

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Abstract

Leishmaniases are diseases caused by intracellular protozoan parasites of the genus *Leishmania*. The parasite multiply in monocytes and macrophages and is transmitted by female sand flies during blood feeding. Exposure to vector saliva prior to *Leishmania* infection has been shown to protect against severe leishmaniasis. This effect is believed to be related to anti-sand fly saliva delayed type hypersensitivity reaction in sensitised host, leading to a more effective immune defense against the *Leishmania* parasites. The aim of this study was to determine whether the disease outcome might be affected by the timing between the last exposure to vector saliva and *Leishmania* infection, a factor neglected in so far published studies. Experiments were performed on BALB/c mice sensitised by *Phlebotomus duboscqi* bites and subsequently co-injected by *Leishmania major* and *P. duboscqi* salivary gland lysate, 48 hours or one week after the last exposure to *P. duboscqi* bites. In agreement with published studies, sensitised mice infected 1 week after the last *P. duboscqi* exposure were protected against severe leishmaniasis as demonstrated by smaller dermal lesions and lower parasite load in the affected tissue, when compared to non-sensitised controls. Such effect was, however, diminished in mice with shorter timespan (48 hours) between the last exposure to *P. duboscqi* saliva and the *L. major* inoculation. Moreover, several immunological parameters were altered by this timing, such as levels of anti-*P. duboscqi* and anti-*L. major* specific antibodies as well as the presence of the effector cells controlling *Leishmania* infection - neutrophils and M1 macrophages. In summary, our results suggest that the disease outcome is strongly influenced by the ongoing anti-vector saliva immune response at the site of *Leishmania* transmission. The timespan between the last exposure to vector saliva of the sensitised host and the *Leishmania* transmission is an important, yet neglected, factor affecting the leishmaniasis outcome by changing the host skin immune microenvironment. The

understanding of this phenomenon might affect the implementation of control programs, including leishmaniasis vaccine development.

How to select novel types of probiotics?

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Abstract

Probiotics are viable microorganisms with positive effect on its host. The most frequently used probiotics belong to lactic acid bacteria, mostly Lactobacilli. While Lactobacilli are indeed of key importance for food or feed fermentation, their efficacy when used for gut colonisation is sometimes controversial. Why this is so and what characteristics should be considered when designing novel types of probiotics for gut colonisation? Using a chicken model, we have found, that only these bacterial species from the gut which do not express any form aerobic survival, colonise intestinal tract after a single dose administration. These include Bacteroidetes, Selenomonadales and strictly anaerobic Proteobacteria. On the other hand, aerotolerant Lactobacilli and spore-forming Clostridia do not colonise chicken intestinal tract after a single dose administration. If these bacteria are used as probiotics, these have to be supplied continuously and their effect will likely disappear shortly after their withdrawal. However, there are additional alternatives for effective probiotics. Spores of Bacillus sp. are sometimes used as probiotics and these may mimic permanent exposure of chickens to spores of Clostridiales. Another possibility is to use mucus-associated microbiota members. Conditions at the mucosal layer differ from those in gut lumen and mucosal microbiota are in more intimate contact with host than microbiota in lumen. Bacteroidetes and Firmicutes colonise gut lumen and not mucosal surfaces in the chickens. Other bacteria such as Mucispirillum or Helicobacter dominate in the mucus although their selection as probiotics will have to be performed more carefully as these may be at the border of probiotics and pathogens.

Bacteria with positive influence on chicken performance can be sough in other compartments such as respiratory tract or skin. In addition to chicken colonisation, probiotics can be selected to colonise external environment to suppress multiplication of pathogens outside chicken host. All these concepts we gradually develop to improve chicken health and to reduce need for antibiotic therapy. However, the same way of thinking can be used for use of probiotics in other animal species like pigs or even humans.

Environmental survival of gut microbiota members and their host species adaptation

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Abstract

Gut microbiota of warm-blooded omnivorous vertebrates consists of approximately 1000 different bacterial species, which taxonomically mostly belong to two major phyla, Firmicutes and Bacteroidetes. Majority of gut microbiota members are obligate anaerobes, which are inactivated upon exposure to air, but the others are capable to survive in aerobic conditions – e.g. aerotolerant bacteria, facultative anaerobes or spore-forming bacteria. Interestingly, there exists an inverse relationship between aerobic survival and ability to colonize intestinal tract. In this study we have compared gut microbiota composition of chickens, pigs and humans to identify broadly distributed bacterial species and determined if there are any host-specific differences.

Microbiota composition in 140 samples was compared. 37 originated from ceca of adult hens, 50 from pig rectal swabs and 44 from adult human fecal samples. Total DNA was purified from all the samples and PCR products covering V3/V4 variable regions of 16S rRNA gene were sequenced using MiSeq platform. Obtained data were analyzed using QIIME and Clustal Omega software.

In the gut microbiota of humans, pigs and chickens were recorded particularly phyla Firmicutes and Bacteroidetes. The microbiota of chickens was enriched for Bacteroidetes, while Firmicutes were more abundant in both mammalian species. Host adaptation was further evaluated at the level of OTUs. From a total, 561 OTUs, 168 of them were detected only in chickens, 159 in pigs and 118 in humans. 33 OTUs were recorded at a similar abundance in the microbiota of all 3 hosts. When host specificity was further investigated for each host separately, host adapted

OTUs belonged mostly to Bacteroidetes while OTUs distributed in more than one host belonged mainly to Firmicutes.

The untargeted metabolomic profiling using LC-MS can distinguish the impacts of different probiotic mixtures on the cecal metabolome of laying chickens in small-scale experiments

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Abstract

Extensive use of antibiotics in food animals is generally accepted as a public health concern. The ability of probiotics to replace their benefits as protection against pathogens, growth promotion, and others have already been described. Finding the most efficient mixture of probiotic strains, however, remains an issue due to the extreme complexity of parameters needed to be taken into account. Empirical testing of newly designed mixtures is necessary. An effect of probiotics, similar to antibiotics, is often apparent only in large-scale experiments which makes the testing of new probiotic mixtures very expensive. Chickens treated with commercial probiotic mixture AVIGUARD® were compared with chickens treated with a mixture of 31 anaerobic bacterial strains (known to be able to colonize chicken cecum) and with untreated chickens, 5 chickens per group. The untargeted mass spectrometry metabolomic analysis of methanol extracts from cecal contents was able to distinguish between all experimental groups. This approach was found effective for distinguishing treatments with different probiotic mixtures in small-scale experiments. Improved bioavailability of the feed-derived substances such as soy isoflavones, feruloyl putrescine, and L-carnitine was found as one possible mechanism of better performance of commercial probiotic mixture.

Metabolism of selected members of chicken gut microbiota *in vivo*

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Abstract

The commercial production of chickens dramatically differs from chickens raised in the backyards. This difference in their lifestyle influences also their gut microbiome. In commercial production, chickens are held without any contact with parent birds and the outdoor environment for their whole life. The administration of probiotics can compensate for the lack of parents' microbiota, however, the development of optimal probiotic composition is still in progress.

Commercial chicks (ISA-Brown egg-lying hybrid) were inoculated by individual strains from the set of 19 anaerobic bacteria isolated previously. Protein sequences originated from the whole-genome shotgun-sequencing of tested bacteria and were annotated by Prokka using the Swiss-Prot and the HAMAP databases. Purified caecal proteins isolated from 8-day-old chicks were digested and their mass spectra were analysed by LTQ-Orbitrap VelosPro hybrid mass spectrometer. Protein data were processed in ProteomeDiscoverer and R statistical software. Metabolic pathways of detected proteins were searched in iPATH and MetaCyc.

Bacterial strains representing phyla Actinomycetota, Bacillota, Bacteroidota and Pseudomonadota were able to successfully colonize chicken caecum, however, they differed in overall abundance. The most effective colonisers belonged to the phylum Bacteroidota. Of tested bacteria, *Bifidobacterium* (Actinomycetota) strain expressed mostly proteins of fructose 6-phosphate pathway thus degrading saccharides to lactate and acetate. Dominant was also the capture of vitamin B12 from the environment in exchange for the release of vitamin B1 into the environment. Protein expression in Pseudomonadota differed for different strains. Sugar metabolism was suppressed and aminoacid degradation served as a source of carbon. Byproducts entered the Krebs cycle as fumarate. Bacillota expressed mainly oxidoreduction flavoproteins and proteins responsible for fatty acid production. Bacillota also imported sugars from the environment. High production of transport proteins for sugars and aminosugars were observed for Bacteroidota which were coupled with degradation machinery for saccharides. Proteins for fatty acid modifications also belonged among the most abundant.

A combination of next-generation sequencing and proteome analysis can be applied to the analysis of potential probiotic strains as well as particular strains found in complex samples.

Comprehensive analysis of mobile genetic elements in chicken gut microbiome using a novel *in-silico* approach

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Abstract

Antibiotic use in farming for decades led also to the selection pressure on chicken commensal bacteria which adapted to those changes by the acquisition of antibiotic resistance genes via horizontal gene transfer. Extensive gene transfer between gut bacteria is mediated by mobile genetic elements such as plasmids, integrative conjugative elements and phages.

We have established a bacterial culture collection originated from healthy chicken ceca with respect to gather novel commensal bacteria. Extracted gDNA from each isolate was sequenced on Illumina platform and assembled by SPAdes. Draft genome sequences were annotated by the RAST tool and de-replicated by dRep tool. To identify traits of horizontal gene transfer, almost identical genes (> 99 % id over 100 % length) present in different bacterial species were extracted by BLAST; extracted protein-coding sequences were functionally annotated by the eggNOG-mapper, CD-Batch tool and CCD search.

Genomes of 279 bacterial isolates from healthy chicken ceca were analyzed in this study; the isolates belonged to 7 different phyla. Within the analysis pipeline we identified 1,418 different genes co-shared by at least two different bacterial families. In total, 634 genes are prevalent among Gram positive bacteria, 785 genes are prevalent among Gram negative bacteria and 8 genes were found in both. The further analysis of identified genes with assigned function and associated with mobilome. Interestingly, we identified novel putative aminoglycoside resistance proteins, uridine phosphorylase (protein may be involved in drug metabolism) and protein with ATPase domain.

On summary, we identified 1,418 genes envisaged to drive horizontal gene transfer between individual chicken gut microbiota members, not only across

different genera and families but also between more distant bacterial taxa. Based on our comprehensive analysis we suppose many hypothetical genes represent important yet an unknown section of resistome or mobilome.

Horizontal gene transfer network in chicken gut microbiome

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Abstract

Antibiotics have been widely administered as preventive treatment in first days of chicken life in commercial husbandries since newly-hatched chickens are prone to invasive pathogens, especially chicken gut is an outstanding niche for infection. Antibiotic use in farming for decades led also to the selection pressure on chicken commensal bacteria which adapted to those changes by acquisition of antibiotic resistant genes via horizontal gene transfer. Extensive communication between bacteria is characteristic for gut microbiota, thus they are considered reservoirs of antibiotic resistant genes. Therefore, we have identified and characterized key mobile genetic elements such as antibiotic resistance genes and plasmids in chicken gut microbiota using the combination of genomic and cutting-edge metagenomic approaches. In addition, we also have determined which bacterial taxa are predominantly associated with dissemination of mobile genetic elements.

Cutaneous papillomavirus induced skin carcinogenesis: molecular and immunological aspects

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Abstract

In my talk I will further describe our unique animal system for studying cutaneous papillomavirus (cPV) infection in a natural host. This model completely reflects the situation in humans in terms of the appearance of skin lesions induced by cPVs. Moreover as shown previously, cPVs act in combination with UV *via* a "hit-and-run" mechanism in the development of non-melanoma skin cancer (NMSC).

In a follow-up, seroconversion during the complete course of infection was monitored. Here, we identified a novel mechanism how these viruses escape from humoral immune surveillance. Using different isoforms of the major capsid protein L1, the virus first produces longer versions of L1 that could not form a mature capsid, but evades the immune system by producing non-neutralizing antibodies against this viral decoy. Consequently, viral amplification and spread can continue. Only after a delay of four months the animals finally started making neutralizing antibodies, now directed against the shorter form of L1 that actually makes up the mature viral particles, encapsulating progeny viral DNA.

Finally, I will also discuss the implication of these findings, particularly in the light of our recent results where we showed that – in contrast to L1 short –the only 34 amino acid longer L1 isoform vaccine completely failed to prevent skin tumor formation after experimental infection.

Innate immune response of common carp (*Cyprinus carpio L.*) to Koi herpesvirus infection

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Abstract

Common carp is the most economically important fish in Czech Republic. However, a profit, earned by breeding of common carp, can be reduced by many diseases, among which viral diseases are the most serious matter. Koi herpesvirus (KHV) disease is probably the most serious viral disease affecting common carp. KHV disease affects only common carp, its strains and some hybrids of common carp and other cyprinids. KHV disease morbidity and mortality can reach one hundred percent, especially in juvenile individuals. Mortality rates of KHV disease could be significantly different among different strains of common carp. Further, KHV is able to kill diseased carp within one week after infection. Importance of KHV disease is even higher, because neither effective KHV treatment nor vaccine are available on the market in EU.

The aim of presented study is to show current discoveries in innate immune response of common carp to KHV. Immune responses to KHV of two common carp breeds were compared: amur wild carp, which is highly resistant to KHV and Koi carp, which is susceptible to KHV. Analysis was focused on known antiviral immune response mechanisms, in particular class I interferon signalling, mechanism of complement cascade and cell-mediated cytotoxicity.

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Investigating the Role of URI1 in Hepatitis B Virus Replication

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Abstract

Despite the availability of a vaccine, the hepatitis B virus (HBV) still presents a global health problem. Chronic HBV infection has a long asymptomatic phase that can ultimately result in liver cirrhosis and cancer. The currently available treatments rarely lead to a functional cure. Today, HBV infects more than 250 million people worldwide and causes more than 800 000 deaths every year.

HBV protein HBx plays a crucial role in hepatitis B virus replication and is necessary for the initiation of HBV genome transcription. By LC-MS/MS analysis, we have identified URI1 (Unconventional Prefoldin RPB5 interactor) as an HA-HBx interacting partner. The aim of our study is to characterize the role of URI1 in HBV replication.

URI1 is a component of the prefoldin-like complex. This complex contributes to the assembly of several protein complexes and plays a role in the stabilization of cellular signaling complexes such as ATM, ATR, or mTOR. URI1 also interacts with cellular transcription factors and plays a role in transcription regulation.

To investigate the role of URI1 in HBV replication, we have analyzed the effect of URI1 silencing on HBV infection in HepG2-NTCP cell line. Our data show that URI1 level affects the replication of HBV. We have also tested the effect of silencing of other components of the prefoldin-like complex. We are analyzing the possible effect of URI1 on HBV entry and on the transcription of viral cccDNA.

Lessons from the COVID-19 Pandemic

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Abstract

The risks that we face today are many and varied. They include tipping points in the environmental system due to climate change or mass biodiversity loss, a natural global pandemic, also malicious or accidentally harmful, use of artificial intelligence or malicious use of, or unintended consequences from, advanced biotechnologies.

Each of these global catastrophic risks could cause unprecedented harm. A pandemic could speed around our hyper-connected world, threatening hundreds of millions, potentially billions of people. It is an epidemic extending over a wide geographic area, affecting whole continents. It involves the high incidence of a disease over a large territory over a specific period of time. Unfortunately, a number of epidemic and pandemic outbreaks in recent time including SARS (China, 2002-2003), H1N1 (global, 2009-2010), MERS (Saudi Arabia, 2012 and South Korea, 2013), Ebola (West Africa, 2014-2015), and the Zika virus (Americas, 2015-2016) have illustrated painful shortcomings in the global capacity to predict and respond to outbreaks of unfamiliar or emerging diseases (several of which are also considered climate sensitive).

The most resonant topic this year is undoubtedly SARS-CoV-2 and the crisis associated with it. That problem must be confronted from a multidimensional perspective

Impact of vaccination against COVID-19 on immune responses in patients suffering from autoimmune neurological disorders treated with rituximab or ocrelizumab

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Abstract

B cell-depleting therapy targeting CD20 molecule with rituximab (RTX) or ocrelizumab (OCR) affects humoral immune response after vaccination. It remains unclear whether these therapies influence T-cell-mediated immune response against SARS-CoV-2 after immunization. We aimed to evaluate the humoral and cellular immune response to the COVID-19 vaccine in a cohort of patients with multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOSD), and myasthenia gravis (MG). Patients with MS (83), NMOSD (19), MG (7) under RTX (n=47) or OCR treatment (n=62) were vaccinated twice with mRNA BNT162b2 vaccine. Antibodies were quantified using the SARS-CoV-2- IgG chemiluminescence immunoassay targeting the spike protein. SARS-CoV-2-specific T-cell responses were quantified by interferon γ release assays (IGRA). Immunocompetent vaccinated individuals (n=41) were included as controls. Almost all immunocompetent controls developed antibodies against the SARS-CoV-2 trimeric spike protein, but only 42.05% of the patients under anti-CD20 (RTX or OCR) treatment seroconverted. There was no correlation between circulating B cells and the levels of antibodies. Even patients with a low proportion of circulating CD19 B cells (<1%, 71 patients) had detectable SARS-CoV-2 specific antibody responses. This response was even higher in patients with longer than 3 weeks intervals of

vaccination. SARS-CoV-2 specific T cell response measured by released interferon γ was detected in 94.39% of the patients, independently of a humoral immune response. The majority of MS and NMOSD patients developed SARS-CoV-2-specific T cell response. The data suggest that vaccination can induce SARS-CoV-2-specific antibodies in a part of anti-CD20 treated patients. The response represented by levels of antibodies was better in individuals, who completed vaccination within more than 3 weeks. This work was supported by AZV NU22-A-150.

Extracellular vesicles: The missing link between microbiota and the host immunity?

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Abstract

Probiotic bacteria have been shown to reduce allergic sensitization in mice and humans. Escherichia coli A0 34/86 (EcO83; serotype O83:K24:H31) is a Gram-negative probiotic bacterium. Oral application of EcO83 to newborns reduced the development of sensitization later in life but had no beneficial effects on allergic lung disease. Recently, we have shown that the intranasal application of EcO83 reduced several hallmarks of allergic airway inflammation in mice. Importantly, signalling through the TLR4 was required for the allergy-preventive effects. Extracellular vesicles derived from bacteria have been recognized as efficient carriers for the delivery of biomolecules to recipient cells, and to efficiently regulate host pathophysiology. The potential of probiotic bacteria-derived outer membrane vesicles (OMVs) on the prevention or treatment of allergy is unclear. Herein, we isolated OMVs from EcO83 (EcO83-OMVs) and studied their effect in a model of experimental allergic airway inflammation.

EcO83-OMVs were isolated by ultracentrifugation. HEK293 cells expressing NOD1, NOD2, TLR2, and TLR4 and bone marrow-derived derived dendritic cells (BMDCs) from wild type (WT) and TLR4KO BALB/c mice were stimulated with EcO83-OMVs. The production of cytokines was measured by ELISA. Mouse model of ovalbumin-induced airway inflammation was used to study immunotherapeutic effects of EcO83-OMVs.

Stimulation of HEK293 NOD1, NOD2, TLR2, and TLR4 cells with EcO83-OMVs increased the production of IL-8 suggesting the involvement of these receptors in the signalling by EcO83-OMVs. Stimulation of WT BMDCs with EcO83-OMVs increased the production of IL-23, IL-12, TNFα, IL-1β, and IL-6, while BMDCs from TLR4KO mice exhibited reduced production of these cytokines. Intranasal application of EcO83-OMVs reduced allergic airway hyperresponsiveness and the level of eosinophils in lungs in comparison to sham-treated controls, and increased the numbers of pulmonary neutrophils, indicating that EcO83-OMVs might cause a shift from Th2 towards Th1 response.

Here we have shown that i) EcO83-OMVs are recognized by NOD1, NOD2, TLR2, and TLR4, ii) EcO83-OMVs induce production of pro-and anti-inflammatory cytokines in a TLR4-dependent manner, and iii) intranasal application of ECO83-OMVs reduce the development of experimental allergy. Our research suggests that probiotic-derived OMVs might be a novel treatment option for allergic diseases in humans.

Outer membrane vesicles and nanotubes secretion in *Francisella tularensis* strains with disrupted O-antigen

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Abstract

Francisella tularensis is highly infectious gram-negative coccobacillus causing disease known as tularemia. This bacterium, as well as other gram-negative bacteria, produces outer membrane vesicles (OMVs), which are usually spherical particles with size of 20-250 nm. Moreover, *Francisella* produces an unusual tubular shape of OMVs. OMVs of *Francisella* contain enormous number of virulence factors and immunomodulatory proteins and are one of the major secretory pathways. We have previously described that they take part in the entry of *Francisella* into macrophages and the possibility of their potential use as a subunit vaccine is also offered.

The aim of the current study was to find some hypervesiculating strains which would serve as source of OMVs for further studies of their protective potential. Because OMVs emerge from the outermost surface of the bacterial cell, we focused on the secretion of OMVs in several mutant *Francisella* strains with disrupted surface structures (namely the O-antigen and type IV pili). O-antigen in *Francisella* is not only the structural component of LPS but also forms another important virulence factor – the O-antigen polysaccharide capsule. Some hypervesiculating strains were present in group of mutants with disrupted O-antigen structure. The phenotype of the mutant strains was monitored by the growth curves, vesiculation rates, their sensitivity to the serum complement, and proliferation inside murine bone marrow macrophages. The morphology of OMVs as well as the bacteria was visualized by electron microscopy. Regarding producing of OMVs, all strains showed lower ability to form the tubular OMVs in comparison with wild type. Some strains formed tubular protrusions from their outer membrane, but their stability was low. All the mutant strains exhibited higher sensitivity to the bactericidal effect of normal human serum and lower ability to proliferate in macrophages in comparison to the wild type. According to our results, the presence of LPS and the O-antigen capsule on the surface of *Francisella* seems to be critical not only for its virulence but interestingly also for the exceptional tubular shape of its OMVs. The detected

hypervesiculating strains also present an interesting source of better yields of vesicular material for future studies of their protective effect.

Validation of *Francisella tularensis* peptides as immunogenic T-cell epitopes

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Abstract

F. tularensis is a gram-negative, facultative intracellular and highly virulent bacterium. It is the causative agent of tularemia, a zoonotic disease of humans and other mammals. In the mouse model of infection, the survival of sublethal *F. tularensis* LVS infection is dependent on the correct functioning and cooperation of innate and adaptive immune system, and the immunity mediated by T-cells is critically required for long-term survival and clearance of *Francisella*. However, the particular T-cell antigens/epitopes are not well documented yet and we established experiments for identification of *Francisella* $CD4^+$ T-cell targets.

In previous immunopeptidomics experiments, bacteria peptides presented on MHC class II molecules were identified after *in vitro* infection of murine BMDCs by *Francisella*. We present here the design and first results of experiments aiming to verify if these peptides also operate as $CD4^+$ T-cell epitopes and enhance the immune response in mice.

Cell suspensions or isolated $CD4^+$ T-cells were obtained from spleens and lymph nodes of mice immunized with sublethal dose of *F. tularensis* LVS. These cells were stimulated with synthetic *Francisella* peptides arranged into three peptides pools and IFN-γ or TNF-α effectors and cell proliferation was measured. We found the following order of reactivity, peptide pool 3 >> peptide pool 2 > peptide pool 1, based on a few biological replicates performed. The peptide pool 3 was consistently reactive in most replicates, stimulated most IFN-γ producing T-cells as measured by ELISpot and stimulated production of IFN-γ and TNF-α into culture supernatants. The reactivity of peptide pools 2 and 3 was either not so consistent across replicates or the production of measured effectors was lower.

Based on these results and established procedures, we plan to perform the study with individual peptides to identify bacterial $CD4^+$ T-cell targets. If successful,

we hope this knowledge could bring new possibilities into immune monitoring of infection and identification of immune protective antigens.

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Effect of early postnatal supplementation of newborns by probiotic strain *E. coli* O83:K24:H31 on allergy incidence, dendritic cells and microbiota

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Abstract

Mutual interactions of microbiota with host immune system is critical for the setting and maintenance of the homeostasis preventing development of inappropriate immune responses leading to development of immune mediated disorders such as autoimmunity and allergy. Adequate stimulation of neonatal immune system with microbes is promoting maturation of neonatal immune system. Probiotic administration seems to be a rational approach to support development of the neonatal immune system. On the other hand, our knowledge of the modes of actions of probiotics is still scarce. Probiotic strain *Escherichia coli* O83:K24:H31 (EcO83) was administered to neonates of allergic mothers (i.e. neonates with higher risk for allergy development) within 48 hours after delivery and the impact of this early postnatal supplementation on allergy incidence and selected immune markers has been analyzed 10 years after the primary EcO83 administration. We have observed decreased allergy incidence in ten-year-old children supplemented by EcO83 (13 of 52 children were allergic) in comparison with non-supplemented children of allergic mothers (16 of 42 children were allergic). Dendritic cells in peripheral blood of EcO83 supplemented children have lower cell surface presence of CD83 as determined by flow cytometry. Immunomodulatory capacity of EcO83 on dendritic cells was tested *in vitro*. Both myeloid and plasmacytoid dendritic cells derived from cord blood progenitor cells increased CD83 expression together with IL-10 secretion after EcO83 stimulation. The impact of early postnatal EcO83 supplementation on gut microbiota composition has been analyzed at the age of ten years. No significant difference in diversity and composition has been observed in children supplemented early postnatally by EcO83 in comparison with non-supplemented children of healthy and allergic mothers. Early postnatal EcO83 supplementation lowered allergy incidence in children of allergic mothers. It seems that the beneficial effect of EcO83 is mediated via modulation of immunoregulatory capacities including promotion of functional capacities of regulatory T cells and

dendritic cells. This work was supported by Charles University research program Cooperatio IMMU207032.

Následuje firemní reklama - jednostránkově

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