15th Host Pathogen Interaction Forum 2025

November 10 – 13, 2025, Třešť, Czech Republic

Agenda and Abstract book

15th Host Pathogen Interaction Forum 2025

Venue: Třešť, Czech Republic

Date of event: **November 10 – 13, 2025**

Scientific conference with international participation

Military Faculty of Medicine

University Defence

Department of Molecular Pathology and Biology

Hradec Králové

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

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CONFERENCE SPONZORS

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

EDITORIAL

Dear colleagues,

It is our great pleasure to welcome you to the 15th Host Pathogen Interaction Forum, taking place from November 10 – 13, 2025, at the Hotel Třešť Chateau, Czech Republic. Over the past fifteen years, Host Pathogen Interaction Forum has grown into a dynamic and interdisciplinary platform bringing together scientists who share a passion for understanding the intricate relationships between hosts and their pathogens — from molecular mechanisms and immune responses to translational approaches in infection biology.

This year's anniversary meeting offers a diverse and exciting scientific program reflecting the remarkable breadth of the field. We are delighted to host leading international speakers and young researchers alike, presenting cutting-edge findings through keynote lectures or oral presentations. The Forum continues to promote a collegial and open environment that fosters discussion, collaboration, and innovation across disciplines.

The setting of Třešť Chateau provides an inspiring backdrop for both scientific exchange and informal networking. We hope that this meeting will not only stimulate new ideas and collaborations but also strengthen our community dedicated to advancing the understanding of host–pathogen interactions for the benefit of global health.

We warmly thank all contributors, sponsors, and participants whose support and enthusiasm make 15th Host Pathogen Interaction Forum 2025 possible. We wish you an enjoyable and intellectually stimulating conference.

On behalf of the Organizing Committee
15th Host Pathogen Interaction Forum 2025
Třešť, Czech Republic

CONFERENCE VENUE

Nestled in the heart of the Vysočina Region of the Czech Republic, the charming town of **Třešť** offers a perfect blend of history, culture, and natural beauty. Located just 15 kilometers south of Jihlava and within easy reach of both Prague and Brno, Třešť welcomes visitors with its peaceful atmosphere and rich heritage. The town's origins date back to the 14th century, and its story is interwoven with centuries of craftsmanship, trade, and vibrant community life. Třešť has long been known for its cultural diversity — once home to a flourishing Jewish community whose legacy is still reflected in local architecture and traditions.

At the heart of the town stands the beautifully restored Třešť Chateau, a former Renaissance manor transformed into a graceful Baroque residence and today a modern conference and hotel facility managed by the Czech Academy of Sciences. Surrounded by tranquil gardens and forests, the chateau provides a unique and inspiring setting for both scholarly gatherings and moments of relaxation.

Třešť is also famous for its nativity scene (crèche) tradition, dating back more than two centuries. Every Christmas season, the town comes alive with artistic displays crafted by local families — a testament to creativity, community spirit, and dedication to heritage.

Set amidst rolling hills, wooded landscapes, and scenic walking trails, Třešť invites visitors to slow down and enjoy the serene beauty of the Bohemian-Moravian Highlands. This combination makes it an ideal location for reflection, collaboration, and inspiration — qualities that make it a perfect host for the 15th Host Pathogen Interaction Forum 2025.

CONFERENCE CENTRE / Třešť Chateau

The 15th Host Pathogen Interaction Forum 2025 will be held at the beautifully restored **Třešť Chateau**, a historic estate owned by the **Czech Academy of Sciences**, situated in the tranquil town of Třešť in the scenic Vysočina Region. Following an extensive renovation, the chateau now serves as a modern conference and hotel facility, offering elegant meeting rooms, comfortable accommodation, and exceptional hospitality. Surrounded by a picturesque park and peaceful countryside, Třešť Chateau provides an inspiring setting that blends scientific focus with relaxation and informal networking. The venue's combination of historical charm and state-of-the-art facilities makes it an ideal location for the 15th anniversary meeting of Host Pathogen Interaction Forum.

BREAKFAST

Breakfast is included in the accommodation at the Třešť Chateau or participant shall ensure himself.

LUNCHES

Conference lunches are served in the Třešť Chateau from Tuesday to Thursday from approx. 12:00am to 1:30pm (included in the conference fee).

DINNER

Monday, November 10 – Welcome party (included in the conference fee)

Tuesday, November 11 – Dinner will not be organized

Wednesday, November 12 – Conference reception (included in the conference fee)

SOCIAL PROGRAM

Welcome party

Date: Monday, November 10

Time: 6:30pm

Meeting point: Auditorium, Třešť Chateau

Tour to the City of Telč

Date: Wednesday, November 12

Time: 2:30pm

Meeting point: Pick-up will take place in front of Třešť Chateau

This will also serve as the pick-up point for Conference Reception in Telč

Conference Reception

Date: Wednesday, November 12

Time: 7:00pm

Meeting point: restaurant Panský dvůr, Telč

Location: Slavatovská 86, 588 56 Telč

Panský dvůr is part of a renovated historic estate that now serves as a modern leisure and event complex near the UNESCO-listed town centre. The restaurant offers a welcoming atmosphere combining traditional Czech and contemporary cuisine, with seasonal menus and regional specialties. Its spacious interior and terrace make it ideal for both individual visitors and group dining.

SOCIAL PROGRAM – 12 NOVEMBER, 2025

Participants of the conference may register for the activity by writing their names on the sign-up sheet at the conference reception by Tuesday lunchtime.

Guided tour of the city of Telč

Telč is a picturesque historic town in the Vysočina Region of the Czech Republic, known for its beautifully preserved Renaissance architecture. Its main square, Zachariáš of Hradec Square, is surrounded by colorful 16th-century houses with arcades and ornate gables, creating one of the most charming urban scenes in Central Europe. At the heart of the town stands the Telč Chateau, a Renaissance masterpiece built on the site of a former Gothic castle. The chateau, together with the old town, was inscribed on the UNESCO World Heritage List in 1992 for its exceptional state of preservation and harmony between architecture and landscape.

Date: **November 12, 2025**

Time: **from 3:00 pm and from 4:30 pm**

Duration: 1,5 hour

Telč Panský Brewery Guided Tour with degustation

The Panský Brewery in Telč was established in the autumn of 2019 in a revitalized complex of a former farmstead in the historic town of Telč. The building itself stands on grounds whose history dates back to the 16th century. The brewmaster, Marcel Hofman, is an experienced professional who has previously helped to launch several other microbreweries. The brewery produces a range of beers under the “Telčský” label, including the light lager Telčský Nádeník 10°, Telčský Holomek 11°, Telčský Hofmistr 12°, and a top-fermented special, Telčský Klíčník 13°. The Panský Brewery is part of the Panský Dvůr Telč complex, which combines traditional craftsmanship with modern hospitality and is located just a short walk from the UNESCO-listed historic center of Telč.

Date: **November 12, 2025**

Time: **from 3:00 pm**

Duration: 2 hours – limited capacity

Telč Panský Wine degustation

Join us for an unforgettable Wine Degustation at Panský dvůr, Telč – an evening dedicated to the art of fine taste. Discover a curated selection of Moravian and international wines in the historic atmosphere of one of Telč’s most beautiful venues. Our expert sommeliers will guide you through the tasting, pairing each wine with local delicacies and sharing the stories behind every bottle.

Date: **November 12, 2025**

Time: **from 3:00 pm**

Duration: 2 hours – limited capacity

AGENDA

MONDAY (November 10, 2025)

4:00 – 5:45pm *Arrival of participants, Registation and helpdesk open* (Entrance hall, *hotel Chateau Třešť*)

6:00 – 6:30pm Keynote lectures

Lokesh Joshi - National Center for Biomedical Engineering Science, University of Galway, IE

06:30 – 12:00pm *Welcome party* (Entrance hall/Auditorium**,** *hotel Chateau Třešť*)

TUESDAY (November 11, 2025)

8:30am – 10:15am Session I.

Chairmans: Gediminas Drabavicius, Ján Matiašovic

8:30 – 9:00am **Gediminas Drabavicius** – Dissecting Host-Pathogen Interactions Using CRISPR Screens: Insights into CDC Toxicity (Vilnius University, Medical Sciences Center, LT)

9:00– 9:15am **Ján Matiašovic** – Current view on the taxonomy of *Streptococcus suis (*Veterinary Research Institute, CZ)

9:15– 9:30am **Monika Zouharová** – Genomic Survey of *Streptococcus parasuis* Isolates from Clinical Cases in Cattle *(*Veterinary Research Institute, CZ)

9:30– 9:45am **Petra Špidlová** – HU protein, inconspicuous player in *Francisella* virulence (Military Faculty of Medicine, UoD, CZ)

9:45– 10:00am **Eva Velecká** – Bacterial HU protein as a regulator of host cell gene expression (Military Faculty of Medicine, UoD, CZ)

10:00– 10:15am **Věra Vozandychová** – Host Macrophage Deubiquitinating Enzymes Exhibit Altered Activity Upon *Francisella tularensis* Infection (Military Faculty of Medicine, UoD, CZ)

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

10:45 – 11:00am **Andreja Zubković** – Long-Term Survival and Encystment Modulation of *Francisella novicida* in *Acanthamoeba castellanii* (University of Rijeka, Medical Faculty, HR)

11:00 – 11:15am **Lucie Balonová** – Role of glycoconjugates in the development of protective immunity against *Francisella tularensis* infection (Military Faculty of Medicine, UoD, CZ)

11:15 – 11:30am **Jana Pavlosková** – In Vitro Characterization of *Francisella tularensis* Infection and Early Immunopeptidomic Insights (Military Faculty of Medicine, UoD, CZ)

11:30 – 11:45am **Pavlína Lásková** – Identification and Validation of CD4+ T Cell Epitopes of the Intracellular Pathogen *Francisella tularensis* (Military Faculty of Medicine, UoD, CZ)

11:45 – 12:00pm **Paulína Mathéová** – Identification of SPI-2 T3SS effectors inhibiting T cell activation (Institute of Microbiology of CAS, CZ)

12:00 – 12:15pm **Alona Dreus** – Role of flagella in translocation of *Salmonella* T3SS effectors (Institute of Microbiology of CAS, CZ)

12:15 – 1:30pm *Lunch – hotel Chateau Třešť*

1:30pm – 3:30pm Session II.

Chairmans: Jana Kamanová, Ivan Rychlík

1:30 – 2:00pm **Milada Pospíšilová** – Bimodal Expression of Type 3 Secretion System 2 and its Effectors Enables Division of Labour (Institute of Microbiology of CAS, CZ)

2:00 – 2:30pm **Jana Kamanová** – Functional specialization of the type III secretion systems (Institute of Microbiology of CAS, CZ)

2:30 – 3:00pm **Ivan Rychlík** – Chickens as model for host-gut microbiota studies (Veterinary Research Institute, CZ)

3:00 – 3:15pm **Company presentation**

3:15 – 3:45pm *Coffee break* **–** *Conference foyer*

3:45 – 4:00pm **Jiří Volf** – Mucus-associated microbiota in chicken caecum (Veterinary Research Institute, CZ)

4:00 – 4:15pm **Lenka Vlasatíková** – Influence of experimental probiotic mixtures on chicken cecal metabolome (Veterinary Research Institute, CZ)

4:15 – 4:30pm **Jana Rájová** – Cecum and Bursa Proteomes in Day-Old Broilers (Veterinary Research Institute, CZ)

4:30 – 5:00pm **Darina Čejková** – Temporal Dynamics of Plasmid-Associated Antibiotic Resistance in the Chicken Gut Microbiome (Brno University of Technology, CZ)

5:00 – 5:15pm **Company presentation**

5:15 – 5:30pm **Company presentation**

WEDNESDAY (November 12, 2025)

8:30 – 12:00pm Session III.

Chairmans: Jana Schwarzerová, Ryan Rego

9:15 – 9:30am **Jana Schwarzerová** – Evaluating Sequence Clustering Tools in PCR Primer Design Pipelines: A Case Study of CD-HIT and MMSeqs2 (University of Technology, CZ)

9:30 – 10:00am **Jiří Zahradník** – The Shape of Adaptation: What SARS-CoV-2 Taught Us About Protein Plasticity and Viral Evolution (Faculty of Medicine, Charles University and BIOCEV, CZ)

10:00 – 10:15am **Katarína Matiašková** – Effectiveness of polyphenol-based formulations in treatment of *Staphylococcus hyicus* skin infection in pigs (Veterinary Research Institute, CZ)

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

10:45 – 11.00am **Helena Langhansová** – *Borrelia burgdorferi* modulates immune tolerance through PD-1/PD-L1 pathway (Faculty of Science, University of South Bohemia, CZ)

11:00 – 11:15am **Ryan Rego** – Grabbing On and Letting Go - Motility of the Lyme Disease Bacterium (Biology Centre of CAS, CZ)

11:15 – 11:30pm **Company presentation**

11:30 – 11:45pm **Company presentation**

12:00 – 1:30pm *Lunch – hotel Chateau Třešť*

2:30pm – 6:00pm *Social program*

6:00pm – 10:30pm *Conference Reception (restaurant Panský dvůr, Telč)*

THURSDAY (November 13, 2025)

9:00 – 12:15pm Session IV.

Chairmans: Ivana Malcová, Ondřej Staněk

9:00 – 9:30am **Jakub Držmíšek** – Flagella-derived Regulation of the Type III Secretion System in *Bordetella pertussis* (Institute of Microbiology of CAS, CZ)

9:30 – 9:45am **Jana Prošková** – The Adhesion Mechanism of *Bordetella pertussis*: A Critical Role of the Mature C-terminal domain of FhaB (Institute of Microbiology of CAS, CZ)

9:45 – 10.00am **Ladislav Bumba** – Extreme C-terminus of the FhaB prodomain is essential for interaction of *Bordetella pertussis* with nasal ciliated epithelial cells (Institute of Microbiology of CAS, CZ)

10:00 – 10.15am **Jana Holubová** – *Bordetella pertussis* Toxin Drive the Emergence of a Unique CD8+ T Cell Subset in the Respiratory Tract (Institute of Microbiology of CAS, CZ)

10:15 – 10.30am **Denisa Petráčková** – Comparative Insights into *Bordetella pertussis* Intracellular Survival in THP-1 Cells and Primary Macrophages (Institute of Microbiology of CAS, CZ)

10:30 – 11:00am *Coffee break – Conference foyer*

11:00 – 11:30am **Ivana Malcová** – Environmental signals shape the T3SS tip filament structure and expression in *Bordetella* (Institute of Microbiology of CAS, CZ)

11:30 – 12:00am	**Michaela Burešová** – Identification of residues involved in fatty acylation of *Bordetella pertussis* adenylate cyclase toxin (Institute of Microbiology of CAS, CZ)
12:00 – 12:15am	**Ondřej Staněk** – The Dermonecrotic Toxin of *Bordetella*: A highly Toxic Protein with an Unknown Role in Pertussis Infection (Institute of Microbiology of CAS, CZ)
12:15 – 2:00pm	*Closing the conference, lunch at hotel Chateau Třešť*
	Departure of participants

ORAL PRESENTATION ABSTRACT

Dissecting Host–Pathogen Interactions Using CRISPR Screens: Insights into CDC Toxicity

Gediminas Drabavicius[1]

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Abstract

Cholesterol-dependent cytolysins (CDCs) are a conserved family of pore-forming toxins secreted by Gram-positive bacteria that disrupt cholesterol-rich membranes, causing cytolysis and triggering strong immune responses. Pneumolysin (PLY) from *Streptococcus pneumoniae* and intermedilysin (ILY) from *S. intermedius* serve as model toxins for dissecting CDC-host interactions. PLY binds directly to cholesterol, driving the pathology of pneumococcal disease, whereas ILY requires human CD59 for pore formation, conferring strict host specificity.

Our previous genome-wide CRISPR knockout (KO) screen with ILY revealed not only canonical cholesterol and CD59 biosynthesis and trafficking pathways but also novel host genes and pathways not previously linked to CDC function, including factors involved in vesicular trafficking, membrane organization, and lipid regulation. These discoveries suggest additional layers of host regulation that influence susceptibility to CDC-mediated damage.

Building on this, we performed complementary CRISPRa and CRISPR-KO screens with ILY and PLY, respectively. These approaches identified overlapping and distinct genetic determinants, highlighting genes that regulate cholesterol synthesis, intracellular transport, and plasma membrane organization, as well as additional pathways whose mechanisms of influence remain unclear. Together, these findings indicate that both cholesterol abundance and its spatial distribution critically shape cellular vulnerability to CDCs, while also pointing toward previously unrecognized host processes that modulate toxin activity.

Future studies will validate these candidate genes, dissect their mechanistic roles in pore formation, and explore how CDCs exploit host lipid biology. Investigating these novel pathways has the potential to reveal unanticipated aspects of host-pathogen interactions and may ultimately inform strategies to prevent cytolysin-mediated damage or therapeutically modulate cholesterol metabolism.

Current view on the taxonomy of *Streptococcus suis*

Ján Matiašovic[1], Natálie Králová[1,2], Monika Zouharová[1]

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Abstract

Streptococcus suis is one of the most clinically important pig pathogens, but some serotypes also pose a significant threat to human health. Accurate identification of isolates is crucial for assessing their pathogenicity and zoonotic potential. However, the availability of whole-genome sequences of *S. suis* isolates has revealed the broad heterogeneity of this species, indicating the presence of new and candidate species within the *S. suis* complex. *Streptococcus orisratti, S. oriscaviae, S. parasuis, S. ruminantium, S. iners, S. suivaginalis* and *S. hepaticus* have already been excluded from *S. suis* and recognised as distinct species. Another twelve new species have been proposed as separate taxa at the species level. Here, we present the current view on the taxonomy of the *Streptococcus suis* complex. The work was supported from TN02000017 and RO0523 projects.

Genomic Survey of Streptococcus parasuis Isolates from Clinical Cases in Cattle

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Abstract

Streptococcus parasuis, is species closely related to *S. suis* and was formerly classified under *S. suis* serotypes 20, 22, and 26. Recently, based on genomic and phylogenetic analyses, *S. parasuis* is now recognized as a distinct species. It has been implicated in systemic infections in pigs and cattle and is increasingly considered an emerging zoonotic pathogen. Genomic investigations have revealed extensive genetic diversity among *S. parasuis* strains, including multiple lineages and varied capsular polysaccharide gene clusters, potentially affecting virulence and host adaptation. In this study, we performed whole genome sequencing of five bovine isolates originally identified as *S. suis*. The isolates were obtained from four mastitic milk samples and one lung sample from a ten-day-old calf with bronchopneumonia, each from a different farm. Genomic comparisons were conducted between bovine and swine *S. parasuis* isolates, as well as with reference *S. suis* strains representing all known serotypes. Phylogenetic analysis revealed that bovine isolates formed a distinct cluster, closely related to swine *S. parasuis* serotypes 20, 22, and 26. Average Nucleotide Identity values and gene content analysis confirmed high genomic similarity between bovine and swine isolates, supporting their classification within *S. parasuis*, yet suggesting possible emergence of a bovine-adapted lineage. Analysis of the cps locus showed that each bovine isolate harbored a unique cps gene composition. Only two antimicrobial resistance genes were detected across all bovine isolates, and no known virulence genes were identified. These findings highlight the need for further studies to elucidate the pathogenic potential of bovine *S. parasuis* and refine diagnostic tools for species-level discrimination within the *S. suis* complex. Our study expands current knowledge on the genomic diversity of *S. parasuis* and underscores its relevance as a potential bovine pathogen.

This work was supported by grants RO0523 and TN02000017.

HU protein, inconspicuous player in *Francisella* virulence

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Abstract

Francisella tularensis, the causative agent of tularemia, is classified as a highly virulent biological threat due to its potential for aerosolization, which can lead to severe and often fatal pneumonic tularemia. This pathogen exhibits an exceptional ability to persist within phagocytic cells by evading the host immune response. Following internalization, *F. tularensis* escapes from the phagosome into the cytoplasm, where it undergoes extensive replication, ultimately inducing apoptosis in host cells.

HU proteins, members of the nucleoid-associated group of proteins, are an important transcription factors in bacteria. Generally, HU proteins act as DNA sequence-nonspecific binding proteins and are responsible for winding of the DNA chain that leads to the separation of transcription units.

In our study, we characterized the phenotype of the Δ*hupB* deletion mutant and identified the DNA-binding motif of the HU protein within the *Francisella* genome, suggesting both sequence-specific and non-specific modes of interaction. Additionally, we performed an *in silico* search of the mouse genome for the identified motif and analyzed several notable host binding sites. Taken together, our results demonstrate that the HU protein is capable of binding eukaryotic DNA *in vitro*.

This work was supported by the Ministry of Defence of the Czech Republic – DRO of the University of Defence, Military Faculty of Medicine Hradec Kralove, Czech Republic – Medical issues of WMD II, (DZRO-FVZ22-ZHN II).

Bacterial HU protein as a regulator of host cell gene expression

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Abstract

Francisella tularensis is a highly infectious intracellular pathogen, known as a causative agent of zoonotic disease tularemia. Due to its extremely low infectious dose, absence of an effective vaccine, and ease of transmission, it has long been recognized as a potential biological warfare agent. Several virulence factors have been identified, including the *Francisella* pathogenicity island (FPI) gene cluster, as well as the regulatory proteins MglA, SspA, and PigR. In addition, the important role of the HU protein, a histone-like nucleoid-associated protein, has been demonstrated. Deletion of the gene encoding HU results in impaired intracellular replication, decreased synthesis of FPI proteins, and attenuation of pathogenicity both *in vivo* and *in vitro*.

Based on our preliminary evidence, we hypothesize that the HU protein may bind to the host chromatin, thereby functioning as a bacterial effector. Such interaction could alter host gene expression and modulate the host immunity response in favor of bacterial survival during infection.

The aim of this study is to characterize the HU-host DNA interactions using bone-marrow derived macrophages infected with FSC200/HU_HA and FSC200/ΔHU strains. Currently, our work is focused on developing and optimizing a protocol for chromatin immunoprecipitation followed by sequencing (ChIP-Seq) to identify HU associated-DNA motifs in infected bone marrow-derived macrophages.

The identified motifs will form the basis for subsequent analyses, such as *in silico* molecular docking, EMSA, qPCR, and atomic force microscopy. Collectively, these studies will provide a novel insight into *F. tularensis* virulence strategies and determine HU protein as a potential target for therapeutic intervention and vaccine development.

Host Macrophage Deubiquitinating Enzymes Exhibit Altered Activity Upon *Francisella tularensis* Infection

Vera Vozandychova[1], Pavel Rehulka[1], Kamil Hercik[2], Pavla Pavlik[1], Petra Spidlova1, Jiri Stulik[1]

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Abstract

This study focused on investigating changes in the relative abundance and activity of host deubiquitinating enzymes (DUBs) during infection with the Gram-negative bacterium *Francisella tularensis*. Human macrophages differentiated from the THP-1 monocytic cell line were infected with *F. tularensis* subsp. *holarctica* FSC200, and samples were collected at an early infection time point (60 minutes). DUBs were analyzed both in whole cells and in extracellular vesicles released by infected cells.

Multiple methodologies were employed to assess DUB changes: (1) proteomic profiling via LC-MS/MS using enzymatically digested peptides with TMT isobaric labeling and label-free quantification (LFQ), complemented by Western blotting; and (2) gene expression analysis using real-time PCR. Bioinformatic analysis of LC-MS/MS data revealed no significant changes in the relative abundance of cellular DUBs, a result further supported by Western blot and RT-PCR.

To assess enzymatic activity, two activity-based probes — HA-Ub-PA and HA-Ub-VME — were used for enrichment of active DUBs, followed by LC-MS/MS and Western blot analysis. This approach revealed significant activity changes in three DUBs: USP10, UCH-L5, and USP25. Parallel LC-MS/MS analysis of extracellular vesicles from infected cells also detected alterations in these same DUBs, which were again confirmed by Western blotting.

Overall, this study highlights infection-induced changes in the activity, but not abundance, of specific DUBs, offering new insights into the potential roles of USP10, UCH-L5, and USP25 in the host response to *Francisella tularensis* infection.

Long-Term Survival and Encystment Modulation of *Francisella novicida* in *Acanthamoeba castellanii*

Andreja Zubković[1], Linda Jerinić[1], Ina Viduka[1], Maša Antonić[1], Mirna Mihelčić[1], Petra Grbčić[1], Jana Klimentová[2], Jiří Stulík[2], Anders Sjöstedt[3], Marina Šantić[1,4]

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Abstract

Free-living amoebae such as *Acanthamoeba castellanii* are environmental reservoirs for various intracellular pathogens, including *Francisella tularensis*. Our study investigates the survival of *Francisella novicida* within cysts of *A. castellanii*. We also analyze whether *F. novicida* influences the encystment process and aim to elucidate molecular mechanisms underlying amoeba cell death during infection. To assess *F. novicida* survival, *A. castellanii* were infected and incubated in peptone (P), encystation ©, or chemically defined water © media before or after the infection (E-E, P-E, P-C). At 72 h post-infection (hpi), all samples were transferred to chemically defined water for long-term incubation. Samples were collected at specific intervals for up to 1 year. Cysts were differentiated back into trophozoites in glucose-supplemented peptone medium and lysed with 1% saponin to quantify viable bacteria. The confocal microscopy and the transmission electron microscopy were used to visualize intracellular bacteria, while flow cytometry was used to assess cell death. *F. novicida* exhibited the peak of intracellular replication at 72 hpi and remained culturable up to 30 days post-infection. The immunofluorescence confirmed the presence of *F. novicida* in the cysts 365 days post-infection under all tested conditions. These findings suggest that *F. novicida* can survive for a long-term in amoeba cysts in VBNC form. However, after 365 dpi, we were unable to recover it in rich culture media. Our results also confirmed the encystation of the *A. castellanii* infected with *F. novicida*. Ongoing analyses aim to identify the molecular mechanisms induced by *F. novicida* that drive encystment and amoebic cell death.

We found that high MOI infection triggers apoptosis and necrosis in trophozoites, but not cysts, highlighting the complexity of protozoan responses to bacterial intracellular pathogens. In addition, *F. novicida* not only persists long-term within *A. castellanii* cysts but may actively modulate encystment and cell death processes, highlighting its potential role in the environmental survival and transmission.

Role of glycoconjugates in the development of protective immunity against *Francisella tularensis* infection

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Abstract

It is well established that induction of both cellular and humoral immunity is essential for effective protection against tularemia. In terms of humoral immunity, the lipopolysaccharide (LPS) of *Francisella tularensis* plays a key role by stimulating the production of protective anti-LPS antibodies. In its purified form, the LPS is completely inert and lacks endotoxin activity, however, as a part of the bacterial surface, it represents a major virulence determinant. Disruption of LPS biosynthesis therefore results in an attenuated phenotype with impaired ability to induce sufficient protective immunity.

Our previous studies on protein O-linked glycosylation in *F. tularensis* revealed the modification of proteins, particularly pilin, with a single O-antigen subunit of LPS. In the present study, we compared the attenuation and protective potential of two LPS-deficient mutants: the *lpcC* mutant, defective in lipid A core biosynthesis but retaining O-antigen production, and the *wbtDEF* mutant, which completely lacks O-antigen. Both mutant strains were markedly attenuated after subcutaneous infection, however only the *lpcC* strain conferred dose-dependent immune protection against a lethal challenge with the wild-type FSC200 strain. Immunodetection, glycostaining, and glycoproteomic analyses confirmed intact glycosylation, particularly O-antigen modification, of selected glycoproteins in the *lpcC* mutant, whereas such modification was absent in the *wbtDEF* strain. Consistently, sera of *lpcC*-infected mice contained antibodies targeting the O-antigen moiety. These findings underscore the importance of protein glycosylation in *Francisella* and raise the possibility of exploiting glycosylated proteins as subunit vaccine candidates against tularemia.

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In Vitro Characterization of *Francisella tularensis* Infection and Early Immunopeptidomic Insights

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Abstract

Francisella tularensis (*Ft*) is a Gram-negative, facultative intracellular pathogen that causes the zoonotic disease tularemia. In addition to its natural occurrence, it is classified as a potential biothreat due to aerosol transmission and low infectious dose. Current vaccine candidates, such as the live vaccine strain (LVS), offer limited protection, particularly against the highly virulent *Ft* subsp. *tularensis*. A promising alternative under investigation is SchuS4ΔclpB, an attenuated strain derived from subsp. *tularensis*, generated by deletion of the *clpB* gene. This mutant shows reduced virulence and improved protective efficacy in animal models.

In this work, we set up MS-based immunopeptidomics workflow to identify bacterial antigens potentially involved in the vaccine-induced T cell response. Focusing on $CD4^+$ T cells, we aimed to identify *Francisella* peptides presented on MHC class II molecules following infection of bone marrow-derived dendritic cells (BMDCs) with SchuS4ΔclpB. To characterize the infection model, we determined the multiplicity of infection (MOI) and evaluated cell viability, bacterial distribution, intracellular replication, and dendritic cell activation. Cell viability showed a time- and dose-dependent decline, decreasing from 84% at MOI 5 to 67% at MOI 20 and 52% at MOI 80 by 27 hours post-infection. At 1 hour post-infection, bacterial distribution increased with MOI, with 5% of BMDCs infected at MOI 20 and 11% at MOI 80. Intracellular bacterial proliferation showed a statistically significant increase over time. Flow cytometry analysis showed upregulation of MHC II and CD86 on BMDCs compared to uninfected controls, indicating cellular activation.

Further, we performed immunopeptidome analysis to obtain an initial profile of bacterial antigens presented during infection and to determine the optimal bacterial load for infection. BMDCs were infected at MOIs of 5, 20, and 80, followed by isolation of MHC II peptide complexes and LC-MS/MS analysis of immunopeptides. We have identified thousands of endogenous MHC II peptides across the tested conditions, and importantly, several *Francisella* peptides were hidden in the self-peptidome. Based on these results, we selected the optimal MOI for subsequent experiments, which will be performed in biological replicates to ensure robustness, reproducibility, and maximal coverage in $CD4^+$ T cell epitope identification.

Identification and Validation of CD4⁺ T Cell Epitopes of the Intracellular Pathogen *Francisella tularensis*

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Abstract

Francisella tularensis is a Gram-negative, facultative intracellular, and highly virulent bacterium that causes the serious zoonotic disease tularemia. In laboratory settings, the attenuated strain *F. tularensis* LVS is commonly used as a model organism due to its ability to induce a strong and protective immune response in mice. At sublethal doses, both innate and adaptive immune components are activated and cooperate to promote long-term survival and bacterial clearance. Among these, T cell-mediated mechanisms play a critical role. However, the specific antigens that serve as T cell epitopes during *F. tularensis* infection remain poorly characterized.

This study builds on previous immunopeptidomic experiments that identified bacterial peptides presented on MHC-II molecules during *in vitro* infection of murine bone marrow-derived dendritic cells (BMDCs) with *F. tularensis* LVS. To evaluate the biological relevance of these candidate epitopes, their immunogenicity was subsequently assessed and validated in an *in vivo* model of infection. The findings could contribute to a deeper understanding of host immune responses and may support the development of tools for immune monitoring and the identification of protective antigens.

Epitope identification was performed by stimulating whole-cell suspensions or isolated T cells obtained from the spleens, lymph nodes, or peripheral blood of *F. tularensis* LVS-immunized mice. Cytokine responses, including IFN-γ, TNF-α, and IL-2 production, were measured using the ELISpot assay following stimulation with candidate peptides. Based on these analyses, we report the identification and *in vivo* validation of ten novel *F. tularensis* epitopes, including both major and minor antigens capable of eliciting antigen-specific T cell responses.

Identification of SPI-2 T3SS effectors inhibiting T cell activation

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Abstract

Salmonella enterica remains a major global health threat, responsible for millions of cases of infection each year. CD4+ T cells play a crucial role in host defense against *Salmonella*. After infection activated CD4+ T cells produce INF-γ which enhance bactericidal capacity of macrophages. The intracellular pathogen *Salmonella enterica* relies on the *Salmonella* Pathogenicity Island-2 (SPI-2) type III secretion system (T3SS) and its effectors for survival and replication within host cells and systemic dissemination. The SPI-2 T3SS effectors SteD, SteE, and SseI are known to influence processes leading to activation of host adaptive immunity. SteD inhibits antigen presentation by MHC II, SteE drives polarization of macrophages to an anti-inflammatory state, and SseI alters migration of infected phagocytic cells. In this study, we focus on combined role of SteD, SteE and SseI in the inhibition of adaptive immunity. Infection with this strain led to increased activation of T cells both *in vitro* and *in vivo*. Understanding how *Salmonella* dampens immune response can lead to new strategies to enhance host immunity and potentially to the development of the next generation of attenuated vaccines.

Role of flagella in translocation of *Salmonella* T3SS effectors

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Abstract

Salmonella enterica, a prominent bacterial pathogen, relies on flagella for motility and chemotaxis. Interestingly, the flagellar membrane complex shares structural similarities with the type III secretion systems (T3SS) injectosome encoded in the *Salmonella* pathogenicity islands SPI-1 and SPI-2. While the SPI-1 and SPI-2 injectosomes are well-known for translocation of virulence factors into host cells, the potential role of flagella as a secretion apparatus in this process remains poorly understood. Here, we used a split-luciferase assay to investigate the translocation of T3SS effectors in various *Salmonella* mutant strains defective in flagellar assembly and translocation. Surprisingly, *Salmonella* strains lacking flagella exhibited a significant reduction in the translocation of several effector proteins and bacterial invasion of host cells, highlighting a previously underrated role of flagella in facilitating effector delivery. These findings suggest that flagella may play a critical, yet overlooked, role in the early stages of infection, potentially enhancing bacterial invasion or contributing to host-pathogen interactions in ways not previously described.

Bimodal Expression of Type 3 Secretion System 2 and its Effectors Enables Division of Labour

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Abstract

Intracellular *Salmonella* residing in a *Salmonella*-containing vacuole within host cells translocate effectors through the Type 3 Secretion System encoded on *Salmonella* pathogenicity island 2 (SPI-2 T3SS) enabling them to survive and proliferate within host cells and eventually employ a systemic lifestyle. It has been suggested but not extensively studied that not all intracellular *Salmonella* express the SPI-2. Here we show that the expression of the SPI-2 T3SS as well as its effectors is bimodal. Also, the ON and OFF states are not hereditary and can be changed. The bimodality was dependent on SsrB, the transcriptional master regulator of SPI-2 T3SS and its effectors. SPI-2^{OFF} *Salmonella* benefited from the presence of *Salmonella* expressing SPI-2 (SPI-2^{ON}) residing in the same host cell and providing the necessary SPI-2 T3SS effectors. In the presence of the SPI-2^{ON} bacteria, the SPI-2^{OFF} bacteria proliferated faster than the SPI-2^{ON} bacteria, conceivably as due to a decreased energetic burden. The SPI-2^{OFF} *Salmonella* escaped more from the infected host cell thus potentially serving as a pool for dissemination. In summary, our findings propose a model of the SPI-2 bimodality among intracellular *Salmonella* resulting in a division of labour potentially allowing more efficient bacterial spreading.

Functional specialization of the type III secretion systems

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Abstract

Mesophilic *Aeromonas* species are Gram-negative bacteria that are ubiquitous in aquatic ecosystems. They are frequently detected in the intestinal microbiota of fish, but can also cause fish mortality, resulting in significant economic losses. In humans, mesophilic *Aeromonas* are increasingly recognized as opportunistic pathogens associated with gastroenteritis, wound infections and septicemia. Their pathogenicity is linked to several virulence factors, including the type III secretion system (T3SS), which remains poorly understood.

The reference strain *A. schubertii* ATCC 43700 encodes two distinct T3SSs. The first, API-1, is related to the *Yersinia* Ysc family and the second, API-2, belongs to the *Salmonella* Ssa-Esc family. To investigate their contribution to virulence, we generated ΔAPI1 and ΔAPI2 mutants and examined their interactions with epithelial HeLa cells and predatory amoebae. Our data showed that API-1, but not API-2, was required for cytotoxicity in HeLa cells, where API-1 activity induced both apoptotic and necrotic cell death. In contrast, API-2 enhanced bacterial survival in predatory amoebae, suggesting a role in environmental persistence and fitness.

A comparative proteomic analysis of bacterial secretomes identified seven effectors of API-1. These included the previously described AopH and AopO as well as five newly identified proteins named AopI, AopJ, AopL, AopT and AopU. The translocation of these effectors into host cells was verified using a split-luciferase reporter assay. Functional assays showed different activities. AopL, which is homologous to *Vibrio* VopQ, induced caspase-3-independent necrosis, while AopI, a homolog to *Pseudomonas* ExoY, inhibited caspase-3 activation, suggesting a pro-survival function. For API-2, two effector candidates, PteC and PteD, were detected that likely support bacterial survival in protozoan hosts.

In summary, our results show a functional divergence between the two T3SS of *A. schubertii*.

Chickens as model for host-gut microbiota studies

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Abstract

Around 240 million chickens are hatched annually in the Czech Republic what corresponds to approx. 1 million chickens produced every working day. This makes this animal simply available and high production also reduces cost of a single chick. Moreover, since chicks are hatched in hatcheries without any contact with parents, millions of chicks are available every week and these might be colonised with microbiota of various composition. Although ignoring some facts which are influenced by raising of chicks in the absence of adult birds may lead to confounding conclusions on development of chicken gut microbiota, once correctly understood, there is no longer better animal model than chicken. Newly hatched chicks can be oral inoculated with faecal material from adult hens and microbiota increasing chicken resistance can be selected. If the selection is modified towards body weight increase, microbiota positively affecting growth can be identified. Following administration of faecal material, microbiota colonising gut lumen and gut mucosal surfaces can be defined. It is also possible to have an access to chicks 1, 2, 3 or 4 days of age and define fine development of gut microbiota before and after beginning of feed income. Finally, not only bacteria but also phages specific against particular bacterial species can be easily tested in chickens.

Mucus-associated microbiota in chicken caecum

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Abstract

Gut of warm-blooded animals, including chicken, is colonized by complex mostly anaerobic microbial community, which influence the host health and performance. Although the gut content is mixed by peristaltic movements, the microniches which enable attachment were shown to be inhabited by specific local microbial communities. In this study, the differences in microbiota composition among the mucus scrapings and luminal content were investigated in week old chickens and adult hens.

Our results clearly showed that in adult hens the microbiota is significantly more complex and more differentiated compared to week old chickens. Mucus layer of adult hens was enriched with species adapted for this specific niche. Mucus-associated species belonging to genuses *Mucispirillum*, *Brachyspira*, *Treponema*, *Helicobacter* and *Desulfovibrio* were highly prevalent (73–100 %) and comprised high percentage of all local microbiota (together up to 63 %). In contrast, those bacteria were missing in week-old chickens and their mucus layer was dominated by *Ruminococcus torques*, the spore forming mucus degrading bacteria of human origin, instead. However, our experiments with co-housing of adult hen together with just hatched chickens clearly show that those bacteria can be transmitted to chickens and are able to colonize them in the very beginning of life. Such colonization is abrogated in commercial chicken production.

The high proportion of mucus-associated bacteria in mucus scrapings suggest that those specialists could represent the exclusive colonizers of deeper mucus adjacent to host mucosa and play therefore a key role in host-microbiota interaction. At the same time, our results demonstrate that in the vast majority of studies based on fecal microbiota sequencing, the mucus-associated species are deeply underestimated. In mice, it was demonstrated that the gut microbiota is fundamental for the formation of a proper protective mucus layer. They stimulate the MUC2 protein expression, mucus secretion and its penetrability and change the glycosylation profile of mucins. Moreover, the occupation of the mucin binding sites by beneficial mucus-associated bacteria protects the host from pathogen adhesion. In conclusion, the composition and interactions of mucus-associated microbiota with

the host deserves increased attention of scientific community and their role has been underestimated so far.

Influence of experimental probiotic mixtures on chicken cecal metabolome

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Abstract

The number of studies concerning changes in the chicken cecal metabolome as a result of different treatments has been steadily increasing since 2019, when the first such study was published. It has been shown that the cecal metabolome is influenced by many factors, including the addition of dietary supplements, farming methods, stress factors, food composition and quantity, antibiotic treatment, or probiotic inoculation. However, generalizing the causes of observed changes is very difficult since the studies were conducted under different conditions, with various breeds, ages, feeds, etc. To address this issue, we inoculated chickens with the same bacterial mixtures in two independent biological experiments and compared them with chickens without any treatment. One group was inoculated with a mixture Q9 of nine strains previously proven effective against *Salmonella* Enteritidis colonization. Three more mixtures were tested with different additional strains with potential beneficial effects added to the mixture Q9. We focused only on metabolites that were consistently influenced in both experiments. In the group inoculated with mixture Q9, we observed an increase in B vitamins, purine molecules such as adenosine, cytidine, guanine, and guanosine, and metabolites important for proper nervous system function, including biopterin, dihydropterin, and urocanate. We also found increased levels of tryptophan-derived metabolites with potential anti-inflammatory effects. Sinapine, a feed-derived metabolite with potential antinutritional effects, was one of the most notably reduced molecules. Further experiments are needed to clarify the impact of these metabolic changes on chicken health.

Cecum and Bursa Proteomes in Day-Old Broilers

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Abstract

Chickens are the most widespread farm animals in the world. In commercial hatcheries, chickens are hatched without any contact with their parents. However, contact with adult birds is known to facilitate the transfer of chicken-adapted gut microbiota, which increases the chick's resistance to enteric diseases. Since the immune system in chickens matures after hatching, the hatching window represents a critical period in their life. Most of mortalities in poultry flocks occur within the first week of life and are often associated with bacterial infections. The largest gut-associated lymphoid organ in chickens is the bursa of Fabricius. Shortly after hatching, bursal B cells are exposed to gut-derived antigens, which may include bacterial antigens, as the bursal lumen is directly connected to the gut lumen. Using 16S rRNA sequencing, we identified bacterial species colonizing cecum immediately after hatching, including *Escherichia coli, Enterococcus faecalis, Clostridium disporicum, Zhenhengia yiwuensis, Clostridium paraputrificum, Clostridium tertium,* and *Terrisporobacter petrolearius*. Subsequently, total protein purified from the chicken caecum, cecal content, and the bursa of Fabricius was analysed by mass spectrometry, and the obtained spectra were searched against strain-specific protein databases generated from known genomic sequences. This approach allowed us to characterize both the chicken proteome and the microbiota metaproteome. The identification of proteins revealed some metabolic pathways and processes occurring during the hatching period. Such knowledge may contribute to preventing pathological events in commercial hatcheries and farms and to the rational design of poultry-specific probiotic supplements.

Temporal Dynamics of Plasmid-Associated Antibiotic Resistance in the Chicken Gut Microbiome

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Abstract

The rapid expansion of intensive poultry farming has driven extensive antibiotic use, accelerating the accumulation of antibiotic resistance genes (ARGs). The chicken gut acts as a reservoir for these genes and provides favorable conditions for their horizontal transfer through mobile genetic elements such as plasmids. This enables commensal bacteria to pass ARGs to pathogens, facilitating their spread and raising the risk of transmission to humans. In this study, long-read sequencing was applied to characterize the plasmidome and resistome of fecal samples collected from three barns of a commercial broiler farm. All chickens received enrofloxacin in their first days of life, with one house also treated with sulfamethoxazole/trimethoprim. For comparison, metagenomic profiling using short-read sequencing was performed on the same samples. The analysis revealed diverse ARGs conferring resistance to 26 antibiotic classes. A strong genetic link was identified between MOBP-type plasmids and fluoroquinolone resistance within the farm. Temporal dynamics showed progressive mobilization of these ARGs, indicating an increasing potential for horizontal transfer. While fluoroquinolone resistance expanded over time, diaminopyrimidine resistance remained stable despite targeted treatment. Most ARGs were carried on small plasmids, with fully reconstructed plasmids ranging from 2.6 to 47.6 kb. Despite technical constraints, this work highlights the value of

plasmidome sequencing in complementing metagenomic analysis by capturing low-abundance plasmid types and providing deeper insight into plasmid-mediated ARG dissemination in the chicken gut microbiome.

Evaluating Sequence Clustering Tools in PCR Primer Design Pipelines: A Case Study of CD-HIT and MMSeqs2

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Abstract

Polymerase chain reaction (PCR) is a cornerstone of molecular diagnostics, yet the reliability of this technique depends strongly on the specificity of primers. Identifying unique gene regions suitable for primer design is therefore a critical computational task, which can be approached through different bioinformatics pipelines. This study presents and compares two workflows for the detection of unique genetic sequences from large FASTA datasets.

The first workflow integrates Prokka for genome annotation and CD-HIT-EST-2D for clustering and similarity comparison between query and reference datasets. Following annotation, gene sequences are concatenated into query and database libraries, compared with CD-HIT at a user-defined identity threshold, and subsequently filtered to extract unique genes absent from the database. The pipeline outputs include annotated gene sets, clustered sequence files, and FASTA libraries of unique genes, which form a direct basis for PCR primer development.

The second pipeline adopts the same general framework but replaces CD-HIT with MMSeqs2, a high-performance sequence search and clustering tool optimized for speed and sensitivity. MMSeqs2 employs a k-mer–based prefiltering strategy and

optimized alignment algorithms, allowing it to scale more efficiently to large datasets and to detect homologous sequences with greater sensitivity than CD-HIT. This substitution potentially reduces false negatives in unique gene detection while maintaining high computational efficiency.

By systematically comparing both pipelines, we evaluate their performance in terms of computational time, sensitivity to sequence similarity, reproducibility of outputs, and ease of integration into existing genomic workflows.

Overall, our study highlights the importance of tool selection in pipelines for PCR primer design. The comparison demonstrates that while both workflows are effective, the MMSeqs2-based pipeline provides a scalable and sensitive solution better suited to modern high-throughput genomic applications.

The Shape of Adaptation: What SARS-CoV-2 Taught Us About Protein Plasticity and Viral Evolution

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Abstract

Viruses survive by learning new molecular tricks. Within just a few years, the SARS-CoV-2 Spike protein, the molecular key that unlocks human cells, has undergone a remarkable evolution that we have been able to watch in real time. While the virus's goal is as simple as to replicate and spread, the molecular routes it takes are surprisingly diverse. Since the first variants appeared, it has been proposed that immune escape plays a major role in the virus's success. Yet the true evolutionary mechanisms, how the virus balances binding strength,
structural stability, and immune evasion, are only now being fully understood.

In my talk, I will show how, from the early days of the pandemic, we have used in vitro evolution to experimentally replay viral adaptation in the laboratory. This approach allows us to observe how SARS-CoV-2 explores and refines different molecular solutions to optimize its interaction with the human ACE2 receptor. The results not only provide a clearer picture of SARS-CoV-2's success but also offer broader insights into how proteins evolve under competing selective pressures. Ultimately, the story of the Spike–ACE2 interaction is more than a chapter in the pandemic, it is a window into how evolution of new pathogens progresses, innovates, and operates within the limits of biochemistry when numbers are high.

Effectiveness of polyphenol-based formulations in treatment of *Staphylococcus hyicus* skin infection in pigs

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Abstract

Staphylococcus hyicus is one of the causative agents of exudative epidermitis in pigs. Piglets with these skin lesions can be treated with systemic antibiotics in combination with local application of antiseptics. However, the increasing antimicrobial resistance of *S. hyicus* isolates makes treatment difficult. So, it is necessary to look for other therapeutic alternatives. Therefore, the aim of the study was to verify effectiveness of polymeric based formulations containing grape marc-derived polyphenols for a topical therapy of this skin infection. Two different concentrations of polyphenols were tested. At D0, six surface defects were created on the back of six piglets. The defects were inoculated with 3 x 10^8 CFU of *S. hyicus* field strain isolated from skin of piglet with exudative epidermitis. Every day from D4 to D13 (once per day) on each pig, the low or high polyphenol concentration was applied on two defects (3 ml on each defect), a saline solution (NaCl) in 3 ml on one defect and antibiotic (Fatroximin spray, susceptible to the strain) on one defect. Every day for the duration of the experiment (D4-D16), visual evaluation of defects (presence of erythema, scabs, oedema, exudate) was performed including monitoring of piglets' health status. On D4, D9, D14 and D16, indirect bacteriological imprints of defects were performed. Treatment with antibiotics or polyphenols successfully decreased the number of bacteria compared with non-treated defects (NaCl). Additionally, no bacteria growth was observed even after 72 hours (D16) after the last application of polyphenols formulations (independently of concentration), in contrast to the NaCl application. Defects treated with the high concentration of polyphenols were covered with a lower amount of scabs on D14 in comparison to defects treated with the low concentration of polyphenols or antibiotics. Overall, the high concentration of polyphenols is likely to be more suitable for a local treatment of exudative epidermitis. The study was supported by

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Borrelia burgdorferi modulates immune tolerance through PD-1/PD-L1 pathway

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Abstract

During Lyme borreliosis, the spirochetes use several strategies to escape the host immune system, disrupt immune homeostasis, and establish chronic or persistent infection. Besides well-known strategies such as antigenic variation, host complement inhibition or utilization of tick salivary proteins, our findings suggest that affecting inhibitory immune checkpoints can be another mechanism of spirochetal immune evasion. According to our observation, *Borrelia burgdorferi* sensu lato induces immune tolerance by the increased expression of the inhibitory ligand PD-L1 (programmed death-ligand 1), but not PD-1 (programmed cell death protein 1), on both murine and human innate immune cells. In mice, blockade of the PD-1/PD-L1 axis by a neutralizing antibody leads to a reduced dissemination of spirochetes into target tissues and also a reduced number of regulatory T cells. Preliminary results from a study involving both Lyme disease patients and healthy volunteers show that peripheral blood mononuclear cells from all subjects upregulate PD-L1 and indoleamine 2,3-dioxygenase (IDO) expression after stimulation with *Borrelia* spirochetes. Moreover, Lyme disease patients upregulate PD-1 and FoxP3, suggesting enhanced Treg activity. Taken together, the PD-1/PD-L1 pathway plays a significant role in the course of borrelial infection, and targeting inhibitory immune checkpoints may represent a promising therapeutic strategy to improve treatment outcomes in the pathogen-host interface.

Grabbing On and Letting Go – Motility of the Lyme Disease Bacterium

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Abstract

The ability of bacterial pathogens to move within host environments is closely tied to their capacity to adhere to surfaces—a fundamental step in establishing infection. In Lyme disease-causing bacteria (*Borrelia sps*), surface-associated adhesins play a central role in both tissue attachment and motility. These adhesins are critical during the early phases of infection, allowing spirochetes to bind to various extracellular matrix (ECM) components like decorin, fibronectin, laminin, collagen, and integrins and facilitating colonization and tissue invasion. Among the most well-studied of these are the decorin binding proteins, DbpA and DbpB. Their importance in the virulence of *B. burgdorferi* has been demonstrated previously. DbpA and DbpB mutants show significant attenuation, particularly early in infection, while disruption of *dbpA* and *dbpB* genes reduces spirochete recovery from tissues distant from the inoculation site. The underlying reason for this phenomenon remains largely unclear, with explanations ranging from the effects of acquired and innate immunity to the inability of the organism to properly adhere to host ECM components. Interestingly, a straightforward hypothesis connecting these adhesins to optimal borrelial motility or propagation within the host has not been proposed. This omission is likely due, in part, to the lack of a system capable of accurately simulating the spirochete's movement through host tissues. The considerable variation among DbpA for three different genospecies was investigated and pointed at it affecting tissue tropism and disease manifestations. Recently, we demonstrated that DbpA and DbpB from *B. afzelii* significantly enhance spirochete motility within the host ECM via transient molecular interactions, thereby promoting dissemination. We have also been able to describe by NMR the strong binding association for DbpA from *B. afzelii* and similar preliminary data on DbpB. Our current results show that the DbpA/B enhance the motility and propagation capacity of Borrelia to different degrees based on genospecies. By dissecting the functional variability of these adhesins among pathogenic Borrelia genospecies, we can better understand the mechanisms of *Borrelia* invasiveness—insights that may inform the development of more effective therapeutics and preventive strategies against Lyme disease.

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Flagella-derived Regulation of the Type III Secretion System in *Bordetella pertussis*

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Abstract

Bordetella pertussis is a re-emerging strictly human pathogen and the causative agent of whooping cough, also known as pertussis, a highly contagious respiratory disease that is particularly severe in infants. To successfully colonize a host, *B. pertussis* employs an array of virulence factors that are either secreted into the extracellular environment or delivered directly into the cytosol of host cells using a specialized type three secretion system (T3SS) that acts more like a close-contact weapon. This so-called non-flagellar T3SS has been adopted by multiple Gram-negative pathogenic bacteria from the ancestral flagellar system, which diverged into several families. The transcriptional control of most T3SSs is controlled by AraC-like regulators, such as LcrF from *Yersinia*, a representative of the Ysc family. Interestingly, the regulation of the *Bordetella* T3SS, which also belongs to the Ysc type, closely resembles the regulation of flagella, where the alternative sigma factor FliA and the anti-sigma factor FlgM govern the transcription. In *Bordetella*, T3SS expression is controlled by the alternative sigma factor BtrS, which activity is antagonized by the anti-sigma factor BtrA. Recently, we identified BtcB, a chaperone that facilitates the secretion of BtrA and is required for the activity of the secretion system. Therefore, the BtrS/BtrA/BtcB node represents a unique regulation of the non-flagellar T3SS that closely resembles the flagellar regulation. Additionally, we have shown that similar to the T3SS of enteropathogenic *E. coli*, the secretion system of *Bordetella* is related to the regulation of metabolic pathways, biofilm formation and virulence. Collectively, this identifies the T3SS of *Bordetella* as a system that employs the flagellar type of regulation to link the host cell contact with the adaptation of multiple cellular functions.

The Adhesion Mechanism of *Bordetella pertussis*: A Critical Role of the Mature C-terminal domain of FhaB

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Abstract

Bordetella pertussis, the causative agent of whooping cough, remains a major global public health concern despite widespread vaccination. The pathogen utilizes multiple virulence factors, including filamentous hemagglutinin (FhaB), a large (~360 kDa) surface-exposed protein and an essential component of current acellular vaccines. FhaB is critical for the initial adherence of *B. pertussis* to human respiratory epithelial cells. Although FhaB is a well-established virulence factor, the specific region responsible for its adhesive function has not been clearly defined. Here, we generated a series of truncated FhaB variants and assessed their ability to interact with the apical surface of *in vitro* polarized primary human nasal epithelial cells grown and differentiated under air-liquid interface (ALI). Individual FhaB proteins were purified from *B. pertussis* culture supernatants using Cellufine Sulphate affinity chromatography and subsequently labeled on-column with Dy-647 fluorescent dye. Fluorescence microscopy of ALI-cultured cells revealed that the SphB1-processed, 'mature' 230-kDa form of FhaB bound specifically and exclusively to ciliated epithelial cells. In contrast, none of the truncated variants, each lacking the mature C-terminal domain (MCD), were able to interact with ciliated cells. Moreover, the in-frame deletion of codons encoding the MCD within the *fhaB* gene abolished attachment the ΔMCD mutant to ciliated cells as compared with the parental strain. These findings demonstrate that the MCD constitutes the critical adhesive module of FhaB and plays an important role in *B. pertussis* colonization of the human respiratory epithelium.

Extreme C-terminus of the FhaB prodomain is essential for interaction of *Bordetella pertussis* with nasal ciliated epithelial cells

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Abstract

Filamentous hemagglutinin (FHA), a major virulence factor of classical *Bordetella* species, is a rod-shaped molecule that plays a crucial role in bacterial adherence to ciliated epithelial cells of the upper respiratory tract while modulating the host innate and adaptive immune responses. FHA is translated as a 360-kDa FhaB precursor, which is exported across the outer bacterial membrane by a two-partner secretion mechanism involving the outer membrane protein FhaC. After secretion, FhaB undergoes processing by the surface-exposed SphB1 protease, releasing an N-terminal 'mature' 220-kDa FHA protein into the external environment. The remaining C-terminal 130-kDa FhaB prodomain is thought to regulate the maturation process and is rapidly degraded in the periplasm. Here, we demonstrate that the extreme C terminus (ECT) of the FhaB prodomain plays a pivotal role in *B. pertussis* virulence. NMR-based structural analysis of ECT, which consists of the highly conserved C-terminal 100 residues of FhaB, revealed that it adopts a rigid, 'pilin-like' protein fold. Deletion of the ECT sequence (ΔECT) resulted in a significant reduction in bacterial colonization of the nasal cavity in infected mice, comparable to the colonization defect observed in a *B. pertussis* strain lacking *fhaB* (ΔFhaB). Strikingly, the ΔECT strain completely lost its ability to bind cilia on human nasal epithelial cells cultured at the air-liquid interface. These findings provide novel insights into FhaB biology and underscore the indispensable role of ECT in *Bordetella* adherence to ciliated epithelial cells in the upper respiratory tract.

Bordetella pertussis Toxins Drive the Emergence of a Unique CD8$^+$ T Cell Subset in the Respiratory Tract

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Abstract

Bordetella pertussis, the causative agent of whooping cough, is known for its ability to manipulate the host immune defense through the action of its enzymatically active adenylate cyclase, dermonecrotic and pertussis toxins (e.g. ACT, DNT and PT). The impact of their action on T cell populations and airway-specific mucosal immunity, however, remains poorly understood. We thus investigated the nasal cavity and lungs colonization dynamics of wild-type and toxin mutant strains of *B. pertussis* in a mouse model, focusing on the characterization of T cells in the mucosa of the upper respiratory tract (URT) and in lungs.

Our findings identified a distinct subset of CD8$^+$ T cells with an atypical phenotype that emerged both in the URT and lungs following *B. pertussis* infection. These cells exhibited an unconventional phenotype, marked by the expression of the transcription factor Eomes and checkpoint-inhibition receptors (Tigit and PD-1). Pertussis toxin (PT) activity was strongly implicated in driving this phenotype, while the effects of DNT and ACT action on T cell populations were more moderate. Additionally, we observed reduced T cell infiltration in the nose and lungs after infection with the mutant strain producing an enzymatically inactive PT-toxoid.

The emergence of the "strange" CD8$^+$ T cells following infection with PT$^+$ wild-type bacteria reveals a novel immunomodulatory mechanism by which *B. pertussis* toxin action impacts adaptive immunity. Further studies will focus on the function and antigen specificity of these atypical CD8$^+$ T cells to elucidate their role in the immune response and disease pathology.

These results provide important insights into the interplay between *B. pertussis* toxins and host immunity, with implications for understanding respiratory immune

responses and developing improved therapeutic strategies targeting pertussis and other respiratory infections.

Comparative Insights into *Bordetella pertussis* Intracellular Survival in THP-1 Cells and Primary Macrophages

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Abstract

Bordetella pertussis, the etiological agent of whooping cough, remains a re-emerging pathogen of global concern despite widespread vaccination. Recent studies suggest that intracellular survival within host immune cells may contribute to its persistence and pathogenesis. In this study, we investigated the ability of *B. pertussis* to survive inside macrophages, focusing on two distinct models: the human monocytic cell line THP-1 and primary monocyte-derived macrophages obtained from healthy donors.

Using microscopic approaches, we visualized the dynamics of bacterial survival in both systems. THP-1 cells offered a consistent and reproducible model and were therefore used for global analyses, including RNA sequencing of both host and bacterial transcripts. To validate significant transcriptional changes, we applied qPCR in both models, examining selected host and bacterial genes. This comparison revealed to what extent transcriptional trends observed in THP-1 cells were reproduced in primary macrophages, thereby strengthening confidence in the relevance of these findings. Importantly, primary macrophages, despite exhibiting high donor-to-donor variability, provided critical confirmation of expression patterns.

Our findings demonstrate that these complementary macrophage models provide valuable but distinct insights into the intracellular lifestyle of *B. pertussis*. While THP-1 cells enable standardized experiments with high reproducibility, primary macrophages better capture the heterogeneity of natural host responses. Recognition of these differences is crucial for the design and interpretation of future studies addressing *B. pertussis* persistence within host cells. Our work highlights the importance of integrating both models to obtain a more comprehensive understanding of how *B. pertussis* interacts with the innate immune system, ultimately informing strategies to counteract this re-emerging pathogen.

Environmental signals shape the T3SS tip filament structure and expression in *Bordetella*

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Abstract

Like many other bacterial pathogens, *Bordetella* species have evolved several virulence factors to invade and colonize their hosts effectively. One key mechanism is the Type III Secretion System (T3SS), a sophisticated nanomachine that injects effector proteins directly from the bacterial cytoplasm into host cells. This complex apparatus consists of a multi-ring base embedded in the bacterial membrane, which connects to cytoplasmic components and supports a needle-like structure that extends from the bacterial surface. The extracellular portion of *Bordetella* T3SS is primarily composed of a polymeric structure formed by the tip filament protein Bsp22, which represents a novel clade of tip filament proteins in Gram-negative bacteria. Bsp22 is essential for persistent colonization in mice.

We investigated the behavior of the needle tip protein Bsp22 in *B. bronchiseptica* and *B. pertussis* using super-resolution imaging and quantitative PCR. Bsp22 was found to form flexible filaments of variable lengths - up to several micrometers - protruding from the bacterial surface. These filaments grew steadily at their tips on abiotic surfaces and under conditions promoting T3SS activity. However, filament growth was restricted in DMEM, the medium used for infecting host cells. During infection of HeLa cells, the filaments reached lengths comparable to those observed on abiotic surfaces under similar conditions, and in some cases, formed short bridges between the bacteria and the host cell membrane. Despite these structural variations, T3SS activity remained functional, as demonstrated by cytotoxicity assays.

Upon infection of differentiated human nasal epithelial cultures, *B. bronchiseptica* preferentially adhered to ciliated cells, where only sparse and short Bsp22 filaments were observed. These remaining filaments were aligned

with the direction of the cilia, oriented toward the apical surface of the epithelial cells. Notably, *bsp22* expression was significantly downregulated, unlike *bscD*, which encodes a T3SS inner-membrane ring protein and whose mRNA levels remained stable.

Our results demonstrate that environmental cues finely regulate Bsp22 production and reveal how the structural features of the *Bordetella* T3SS tip filament dynamically adapt to changing conditions.

Identification of residues involved in fatty acylation of *Bordetella pertussis* adenylate cyclase toxin

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Abstract

Bordetella pertussis adenylate cyclase toxin (CyaA) is a key virulence factor that disrupts host immune responses. CyaA translocates its N-terminal adenylyl cyclase domain into the cytosol of phagocytes, where it converts ATP to cAMP and thereby impairs their bactericidal functions. In addition, CyaA permeabilizes eukaryotic cell membranes by forming cation-selective pores. While the toxin preferentially binds to complement receptor 3 (CR3)-expressing cells, it can also enter cells lacking CR3 on their surface.

CyaA is synthesized as a protoxin (proCyaA) and requires acylation for activation, a process catalyzed by the acyltransferase CyaC. This modification involves the covalent attachment of acyl groups to Lys860 and Lys983. Using CyaA/HlyA chimeras (where HlyA is the α-hemolysin of Escherichia coli), we identified the sequence essential for CyaC-mediated acylation. Site-directed mutagenesis further revealed that Arg991 of CyaA and Trp36 of CyaC are critical for efficient acylation of Lys983. Mutations of Arg991 and Trp36 reduce CyaA acylation and decrease its cytotoxic and cytolytic activity toward sheep erythrocytes and THP-1 macrophages.

Importantly, we succeeded in defining the minimal sequence required for acylation. When this sequence was transferred into FrpC, a non-acylated RTX protein, it became acylated, demonstrating that this short segment is both necessary and sufficient for CyaC recognition. Taken together, our study defines residues involved in CyaC–proCyaA interaction using site-directed mutagenesis, functional assays, a two-hybrid system, structural modeling and mass spectrometry.

The dermonecrotic toxin of *Bordetella*: A highly toxic protein with an unknown role in pertussis infection

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Abstract

Lysing pathogenic *Bordetella* bacteria release a neurotropic dermonecrotic toxin (DNT), which is endocytosed into host cells and permanently activates RhoA family GTPases by polyamination or deamidation of glutamine residues in their switch II regions (e.g., Gln63 of RhoA).

In *B. bronchiseptica*, DNT facilitates bacterial colonization in the nasal cavity of pigs and inhibits the differentiation of nasal turbinate bone osteoblasts, contributing to atrophic rhinitis. However, the role of DNT in virulence of *B. pertussis* and pathogenesis of whooping cough remains unclear.

Recent studies identified T-type voltage-gated calcium channels (Cav3.1 and Cav3.2) as receptors for DNT. Our findings confirm that DNT interacts with these channels, facilitating calcium entry into cells and enhancing its RhoA polyaminase and deamidase activity. However, we did not observe a direct binding of DNT to cells via these channels, suggesting that additional receptor(s) may be involved.

We developed a method to purify large quantities of lipopolysaccharide (LPS)-free recombinant DNT with high biological activity on sensitive cells, including its fragments and detoxified variants. Contrary to earlier reports, we show that the C-terminal enzymatically active domain specifically binds to sensitive cells, while the N-terminal "binding" domain does not. Our results reveal that even extremely low concentrations of DNT (femtomolar) disrupt the function of primary rat

neurons cultured *in vitro*. DNT damages astrocyte protrusions, halting their support for neurons, leading to progressive neuronal death and loss of action potential transmission. Additionally, intravenous administration of as little as 3 ng (18 fmol) of DNT in mice causes weight loss and severe neurological symptoms, ultimately resulting in death. We report significant progress in understanding of the molecular mechanisms underlying DNT's effects at such low concentrations and mapping of its cell-binding domains, shedding light on its potential role in *B. pertussis* pathogenesis.

Despite these findings, unfortunately, we have so far been unable to identify a clear role for DNT in *in vivo* infections using the mouse model. We have attempted to analyze the composition of cellular populations following infection with wild-type bacteria and bacteria with a mutation of the DNT gene, but no significant differences attributable to DNT were observed.

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