Mechanisms of cargo recognition by kinesin molecular motors

RNDr. Cyril Bařinka, Ph.D.

ID 235344

The project is aimed at structure-function studies of anterograde transport mediated by conventional kinesins and their interactions with cargo molecules. We will use a bottom-up approach to analyze a kinesin/cargo transport system at the molecular level. To this end, we will express and purify individual protein components to reconstitute kinesin/cargo complexes and analyze their structural and functional properties. We will apply mutagenesis, biophysical approaches (microscale thermophoresis, analytical ultracentrifugation, SPR, FRET) and structural biology techniques (hydrogen/deuterium exchange, X-ray crystallography, SAXS, cryoEM) to pinpoint motifs mediating cargo/kinesin interactions and delineate the interaction interface(s). The total internal reflection microscopy will be used to visualize the complexes and elucidate their functional properties and mechanistic up to the single molecule level in vitro. Finally, neuronal cell-based assays will be exploited to translate and validate in vitro data in a physiologically relevant environment of the axonal transport. Overall, we expect our data to contribute to our understanding of general molecular mechanisms governing kinesin activation and principles of a protein transport in (neuronal) cells.

Candidate profile:

A highly motivated PhD candidate (must hold a Master degree or to be completed in summer 2021) with a solid background in molecular biology, biochemistry, physical chemistry, or cell biology. We expect good communication skills, analytical thinking, and the ability for a teamwork. The successful candidate will participate in a PhD program at Charles University in Prague. The starting date is summer/fall 2021.

Modulation of structural and functional properties of the HSP90 chaperone machinery by reversible acetylation

RNDr. Cyril Bařinka, Ph.D.

ID 235345

The heat shock protein 90 (HSP90) is a molecular chaperone regulating proteostasis under both physiological and stress conditions in eukaryotic cells. Cellular functions of HSP90, such as conformational cycling and interactions with client proteins and co-chaperones, are modulated by a host of post-translational modifications, including lysine acetylation. Several acetylation sites of HSP90 have been identified by whole proteome mass spectrometry approaches and biological data suggest that histone deacetylase 6 (HDAC6) can be the principal deacetylase and a client protein of the HSP90 chaperone machinery. However, structural basis of HSP90 (de)acetylation by HDAC6 as well as functional consequences of such interactions have not been studied at the molecular level. We will use synthetic biology, structural and biophysical techniques as well as cell-based assays to unravel how the HSP90 structure and function are regulated by lysine acetylation. More specifically, we will use targeted mass spectrometry to identify acetylation sites in human HSP90, express and purify acetylated HSP90 variants using the genetic code expansion technology and compare their structural and functional properties to non-acetylated counterparts, identify histone deacetylases responsible for HSP90 deacetylation, and characterize in detail interactions between HSP90 and HDAC6 by biophysical and structural approaches. Taken together, our data shall provide mechanistic underpinnings of how cellular functions of HSP90 are regulated by reversible lysine acetylation with the special focus on the critical involvement of human HDAC6.

Candidate profile:

A highly motivated PhD candidate (must hold a Master degree or to be completed in summer 2021) with a solid background in molecular biology, biochemistry, physical chemistry, or cell biology. We expect good communication skills, analytical thinking, and the ability for a teamwork. The successful candidate will participate in a PhD program at Charles University in Prague. The starting date is summer/fall 2021.

Regulation of inflammation and hematopoiesis by membrane adaptor proteins

Mgr. Tomáš Brdička, Ph.D.

ID 235293

Membrane adaptor proteins are scafold proteins which recruit signaling molecules to the plasma membrane to regulate signal transduction by leukocyte surface receptors. This project will focus on two proteins from this group, WBP1L and PSTPIP2. The project will employ mouse models, cell lines and patient cells to study the mechanisms by which these proteins regulate hematopoiesis, efficiency of bone marrow transplantation, immune and inflammatory response, and how they contribute to human disease.

Receptor interactions at the nanotopography of immune cells monitored by advanced microscopy

Mgr. Marek Cebecauer, Ph.D.

ID 223862

CD4+ T cells co-ordinate immune responses against infections and cell transformations. The antigen receptors (TCRs) trigger signals to activate T cells, but a handful of surface receptors (e.g. CD2, CD4/8, CD6 and PD-1) can regulate the output of these processes. Molecular mechanisms co-ordinating the action of these receptors remain incompletely understood, especially, at the spatio-temporal level. In our laboratory, we have recently discovered that CD4 co-receptor accumulates at the tips of T cell microvilli, whereas its regulatory phosphatase, CD45, segregates to the remaining parts of the plasma membrane. This discovery indicates the importance of nanoscopic topographical organisation of receptors on T cells before and during recognition of antigens.

The project will investigate changes in the organisation of receptors with respect to each other upon stimulation of cells with diverse factors: e.g., antigen, adhesion, mechanical stress. Receptors such as TCR, CD2, CD4 and CD45 will be nanoscopically characterised in time and space to understand the impact of geometry on their function. The project will include the use of the state-of-the-art microscopy techniques (co-developed in our laboratory), functional imaging of living T cells and recombinant DNA technologies.

Single-cell analysis in systems immunology – an application of novel unsupervised tools in infectious diseases and cancer

RNDr. Karel Drbal, Ph.D.

ID 212586

The objective of this project is an application of novel analytical tools for the large transcriptomic and proteomic datasets in clinics. This is in principle unsupervised topological data analysis (TDA) based on clustering, which allows for immediate statistical data evaluation and visualization using dimensionality reduction down to 2D. We are going to optimize parameters, achieve minimal data distortion and maximal reproducibility as well. Galaxy platform is a central cloud environment, however, a deep knowledge of R/Python is essential (C++ programming is a bonus). In turn, this brings a completely new understanding of existing scientific data in general and allows for the reinterpretation and discovery of new relationships.

We will focus on clinical datasets in patients suffering from infectious or tumor diseases. An inherent part of the workflow is data collection and database maintenance. A prediction of directional causal relationships will be finally validated in available zebrafish and/or medaka models in our laboratory at the level of transcriptome and proteome.

Recently, an excessive boost in the development of analytical bioinformatic algorithms allows biologists to mine the available datasets originating from single cells in a completely unsupervised manner for the first time. The human body is composed of around 30 trillion cells and the objective of this application is the use of novel dimensionality reduction, trajectory inference, and clustering algorithms in order to decode their directional relationship in the field of systems immunology.

Our research focuses on dynamic systems of immune response monitoring in patients suffering from infectious diseases – tuberculosis or borreliosis – as well as various solid tumors – mainly bladder cancer. Under these pathological conditions, activation of immune cell subsets of both innate and adaptive systems regulate the outcome of the disease. A single-cell oriented statistical data evaluation and visualization finally stratify each patient. We are going to optimize the parameters of the non-linear computational methods in order to preserve data distribution and maximize reproducibility.

An inherent part of the workflow is the collection of genomic/transcriptomic/proteomic data and patient database maintenance. The predictions of directional causal relationships will be validated in available zebrafish and/or medaka models after multiparametric flow cytometry and cell sorting in our laboratory at the level of gene and cellular networks. A collaboration with local clinical partners (TH, HNB, Prague) and computation centers (IDA FEE CTU [1], IOCB CAS [2], Prague) are backed by recent publications. International collaboration within LifeTime consortia will be an inherent part of the project.

As stated above, deep knowledge of R and Python (C++ is a bonus) and immunology is an essential profile of a successful candidate. Optionally, the experience with cytometry and/or microscopy and the knowledge of one of the experimental models is a plus. Two major goals of this Ph.D. position are 1/ the integration of existing tools into a Galaxy pipeline or development of a standalone application and 2/ the identification of cellular biomarkers of either latent TB infection, late-stage Lyme disease or bladder cancer stem cells.

1. Dvorakova, E. et al. Bioinforma. Res. Appl. ICBRA 2019 (2019). at http://ida.felk.cvut.cz/zelezny/pubs/icbra2019.pdf

2. Kratochvíl, M. et al. bioRxiv 496869 (2019).doi:10.1101/496869

Role of AIRE in the development of gonads and gametogenesis.

RNDr. Dominik Filipp, CSc.

ID 235318

Fertility is defined as the natural capability of an organism to produce offspring, the utmost important attribute ensuring the continuity of all living species. Available data suggests that infertility affects about 7% of men in their reproductive age which accounts for up to 50% of couple's infertility cases. While at least 10% of male infertility is exogenous in nature (lifestyle, environment, psychological factors) and 15% is attributed to genetic abnormalities, approximately 50% of cases remain idiopathic and/or undiagnosed. Since the molecular and cellular processes as well as the structures that are involved in the production and quality of sperm appear to be conserved among rodents and humans, inevitably suggests that much of the unknown information that will aid in a better understanding of the processes underpinning infertility in humans will necessarily come from molecular studies. In particular, we focus on protein AIRE (Autoimmune regulator) previously reported to be present not only in immune organs but also in testes and ovaries. Thus, we embark on studies focused on the role of Aire in the processes underpinning fertility. AIRE-deficient mouse strains were reported to recapitulate human APECED symptoms, including male and female sterility/subfertility. Relevant to the relationship between Aire function and fertility in men and women, a recent study revealed that previously unrecognized Aire dominant point-mutations, that are characterized by milder phenotypes and reduced penetrance, some of which might affect the fertility. Main goal of this project is to uncover the role of AIRE in gonad development, gametogenesis, sexual maturation and ability to reproduce in both sexes.

Experimental aims:

1. Identification of cells expressing AIRE in ovaries and testes;

2. Uncovering the kinetic of AIRE expression profile in gonads, identification of genes regulated by AIRE

3. Mechanism of AIRE involvement in the development of gonads and gametogenesis.

Intestinal microbiota-dependent regulatory mechanisms of central tolerance

RNDr. Dominik Filipp, CSc.

ID 235726

Central tolerance represents fundamental thymic processes which control the prevention of autoimmunity. Its major mediators, medullary thymic epithelial cells (mTECs) which express microbe-sensing Toll-like receptors (TLRs), the signaling of which affects their maturation state, unidirectional cooperative antigen transfer from mTECs to dendritic cells (DCs) as well as the conversion of self-reactive T cells into T regulatory cells (Tregs). Recently, the impact of intestinal microbiota on the processes of central tolerance has been recognized. As an example, thymic DCs can carry homeostatic and/or inflammatory signals from the intestine to the thymus in a TLR signaling-dependent manner. Similarly, the origin and maintenance of thymic innate lymphoid cells (ILCs) is also highly dependent on the presence of intestinal microbiota.

Given that mTECs express a battery of TLRs which are capable of sensing microbial products, the aim of this project is to characterize the impact of intestinal microbiota on the mTEC compartment and consequently on other cell subsets and their functions which collectivelly and simultaneously underpin the outcome of thymic central tolerance.

In this project, the mTEC compartment found in the wild-type, Specific pathogen-free (SPF) as well as germ-free (GF) mice colonized, or uncolonized, with a single or mix of particular microbiotic strains will be analyzed using the single cell RNA sequencing (scRNAseq). The specific impact of microbiota will be studied using specific mouse transgenic models, advanced multicolor flow cytometry along with ex vivo spinning disc microscopy of thymic slices.

Mechanobiology of lymphocyte motility

Mgr. Miroslav Hons, Ph.D.

ID 235197

An efficient immune response requires cells of the immune system to be at the right place at the right time and depends on their migration and correct positioning in tissues. We work at the interface between cell biology and immunology and study how leukocytes establish motility, distinguish various environmental cues and interpret them in their behavior. Our primary focus are mechanical aspects – we want to understand how leukocytes recognize physical stress, adapt to obstacles and integrate mechanical and chemical signals from the environment. We concentrate on the role of cytoskeleton and signaling pathways that trigger cytoskeletal rearrangement. To this end, we use combination of artificial environments, pharmacologic/genetic interventions and various types of imaging.

To expose cells to mechanical stress or defined obstacles in their migratory paths we use silicon devices with custom-made imprinted patterns. This way we apply on cells defined deformations or force them to migrate through channels with a given diameter. The role of individual genes is assed mainly by genome editing as we take advantage of the CRISPR/Cas9 system. Moreover, the basis of our work lies in broad spectrum of imaging methods. We benefit from exceptional core resources and equipment in BIOCEV and we use many modalities of live cell imaging (FLIM, FRET, TIRF) and electron microscopy

For more insight please see:

· Cellular locomotion using environmental topography. Nature. 2020 Jun;582(7813):582-585.

· Chemokines and integrins independently tune actin flow and substrate friction during intranodal migration of T cells. Nat Immunol. 2018 Jun;19(6):606-616.

Changes in immune responses during the course of SARS-CoV-2 infection

doc. RNDr. Jiří Hrdý, Ph.D.

ID 234997

The work is focused on the understanding of the effect of infectious agents on the host immune system with special focus on mechanisms contributing to inappropriate immune response leading to the cytokine storm and exhaustion of organism ending by the death. Neutrophils were considered as a homogeneous population of terminally differentiated cells. Recently, it has been shown that neutrophils consist of very heterogeneous subpopulations of cells with distinct functions including immunoregulatory capacity. During infection, emergency granulopoesis occurs leading to the changes of homeostatic interactions of host immune system. The immune response of experimental animals to vaccine and infection itself will be tested in experimental animal models (transgenic mice with ACE2, hamsters). Both humoral and cellular immune responses will be followed. To reach the proposed goals of the thesis, different methods including flow cytometry, real-time PCR, ELISA, cell separation, in vitro cultures will be employed.

The role of dysbiosis on proportional and functional characteristics of particular neutrophil subsets

doc. RNDr. Jiří Hrdý, Ph.D.

1.LF

The work will be focused on the understanding of mutual interaction between the microbiota and host immune system with special focus on neutrophils. Neutrophils were considered as a homogeneous population of terminally differentiated cells. Recently, it has been shown that neutrophils consist of very heterogeneous subpopulations of cells with distinct functions including immunoregulatory capacity. During dysbiosis (changes in microbiota composition and its functions), homeostatic interactions between microbiota and host immune system are altered. Dysbiosis will be induced by antibiotics administration and we will try to correct for dysbiosis and restoration of mutual homeostatic interactions between microbiota and host immune system by probiotic supplementation using experimental mouse model. To reach the proposed goals of thesis, different methods including flow cytometry, real-time PCR, cell separation, bacteria cultivation will be employed.

Role of neutrophils in health and disease

doc. RNDr. Jiří Hrdý, Ph.D.

1.LF

The topic of dissertation thesis will be focused on characterisation of proportional and functional parameters of neutrophils in health and disease. Neutrophils were considered as relatively homogeneous terminally differentiated population of leukocytes with relatively short survival time in periphery. Recent studies highlight the neutrophil heterogeneity with distinct functions including immunoregulatory one. The role of neutrophils in initiation and progression of tumour will be studied using experimental mouse models. Further, we will focus on the impact dysbiosis on changes of homeostatic interaction between host immune system and microbiota in the context of tumour. For successful solvent of dissertation thesis, it will be important to get familiar with following methods: flow cytometry, real-time PCR, particular cell subsets isolation and separation, handling with experimental animal models (mouse).

Identification and characterization of novel mechanisms regulating emergency granulopoiesis

Meritxell Alberich Jorda, M.Sc., Ph.D.

ID 234454

Granulocytes are innate immune cells that represent the first line of defense in the body. Upon exposure to fungal and bacterial infections, steady-state granulopoiesis is shifted towards a stress program known as emergency granulopoiesis. Emergency granulopoiesis is characterized by a rapid and massive production of granulocytes that will kill the pathogen and clear the infection. It is well reported that activation of myeloid progenitor cells is key to this process, however, how hematopoietic stem cells (HSCs) respond to the infection and contribute to the emergency granulopoiesis program is vaguely known. The present project aims to identify novel regulatory mechanisms of emergency granulopoiesis at the HSC level. We will focus on early changes that occur in HSCs upon infection, leading to the accelerated production of granulocytes. We will employ a diverse set of cell and molecular biology techniques such as single cell RNA sequencing, ATAC sequencing, in vitro culture assays, transgenic mice, and induction of emergency granulopoiesis in murine models. The results of this project will lead to the identification of novel regulatory molecules/pathways activated in HSCs during emergency granulopoiesis. Further, we will functionally validate some of the identified molecules and dissect their role in emergency granulopoiesis.

Nanotherapeutics delivering protease inhibitor and cytostatics for advanced treatment strategy of chemorefractory Head and Neck carcinomas

RNDr. Marek Kovář, Ph.D.

ID 234557

The general aim of the project is to investigate the therapeutic potential of novel nanotherapeutics bearing a combination of protease inhibitor derivative and conventional cytostatic drug for advanced treatment of human Head and Neck tumors (HNt). Molecules with anticancer activity will be bound to water-soluble long- circulating biocompatible and biodegradable diblock and/or three- to four-arm star polymer carriers composed of linear copolymers linked together by biodegradable spacers. These polymer carriers will enable significant accumulation in HNt via the Enhanced permeability and retention effect. Biodegradable spacers will allow the carrier degradation and elimination from the body via renal filtration. Selected protease inhibitors, i.e. nelfinavir, saquinavir, and atazanavir, will be derivatized with different oxocarboxylic acids to enable their attachment to polymer carrier via pH- sensitive hydrazone bond. These derivatives will be tested for their cytostatic and cytotoxic activities alone and in combination with docetaxel, doxorubicin, or cis-platin in vitro. The most potent derivatives will be covalently bound to polymer carriers together with selected conventional cytostatic drugs. The rate of their release from polymer carrier as well as polymer carrier degradation will be determined. Simultaneously, we will examine levels of P-gp expression and STAT3 signaling in cells of HNt resected from human patients. We will also establish the PDX model of HNt in NSG mice. We will determine the cytostatic and cytotoxic activities of synthesized nanotherapeutics in vitro. The most promising ones will be tested for their therapeutic activity in FaDu xenografts and established PDX model of HNt, preceded by a determination of the maximum tolerated dose. We presume high therapeutic activity of such nanotherapeutics due to the synergism of two biologically active molecules potentiated by enhanced accumulation in HNt due to the utilization of tailored polymer carriers.

- The project is founded by AZV it is a collaboration of Institute of Macromolecular Chemistry, Institute of Microbiology and University Hospital Motol.
- It is 3 and 1/2 year project starting 1 st May, 2021.
- The candidate will be responsible for testing the antitumor activity of above mentioned drugs and nanotherapeutics in vitro (cytostatic and cytotoxic aktivity via 3 H-thymidine incorporation and MTT assays, respectively; induction of apoptosis, inhibition of STAT3 signaling pathway, inhibition of P-gp) and in mouse and human tumor models in vivo.
- Contact: Marek Kovar, makovar@biomed.cas.cz, 241 062 362, 776 573 594.

Imunomodulační vlastnosti střevního mikrobioty a její role při vzniku zánětlivých chorob

MUDr. Miloslav Kverka, Ph.D.

1.LF

V rozvinutých zemích je jasně zřetelná vzrůstající incidence zánětlivých a autoimunitních onemocnění, jako jsou roztroušená skleróza, nespecifické střevní záněty, nebo alergie. Většina léčebných přístupů u těchto nemocí využívá farmakologické utlumení přílišné reaktivity imunitního systému, které reagují na vlastní nebo jiné neškodné antigeny. Dostupná léčba ovšem často není dostatečná, a tak pacienti trpí nejenom relapsy symptomů nemoci, ale i vedlejšími účinky terapie.

V posledních několika desetiletí se hromadí důkazy poukazující na důležitou roli mikrobiálních společenstev ve formování hostitelova imunitního systému a v regulaci mnoha patologických procesů. Složení a fungování mikrobiomu je ale také závislé na řadě zevních faktorů. Hlavním faktorem je strava, která ovlivňuje fungování slizničního i systémového imunitního systému, a to jak přímo, pomocí bioaktivních molekul, tak nepřímo, zásahem do střevní mikrobioty.

Cílem této doktorské práce bude analyzovat vliv komenzálních a probiotických mikrobů na rozvoj zánětu ve střevě i mimo něj a možnost ovlivnit vývoj patologických změn pomocí diety, antibiotik či cílenou kolonizací vybranými mikroby. Interakce mezi mikroby a imunitním systémem hostitele bude studována jednak in vitro, s využitím buněčných linií a somatických buněk izolovaných z experimentálních zvířat a pacientů, a jednak v konkrétních aplikacích in vivo, s využitím různých zvířecích modelů. Imunitní odpověď bude analyzována základními imunologickými technikami, jako je např. ELISA a mnohobarevná průtoková cytometrie. Mikrobiální kolonizace bude stanovena technikami molekulární mikrobiologie, jako je například kvantitativní PCR a sekvenování.

Využití průtokové cytometrie v diagnostice a léčbě neurologických onemocnění

MUDr. Zuzana Libá, Ph.D.,

2.LF

Nabízené téma zahrnuje studium problematiky buněčných a solubilních markerů detekovatelných v periferní krvi, mozkomíšním moku, případně mozkové tkáni pacientů s neurologickým onemocněním. Vzhledem k zaměření školitele a pracoviště přednostně počítáme s okruhem zánětlivých onemocnění na autoimunitním podkladě u dětí, v širším pojetí také s okruhem epilepsií. Jedná se o klinický výzkum zahrnující práci s biologickým materiálem získaného převážně od pacientů Kliniky dětské neurologie 2.lékařské fakulty Univerzity Karlovy a Fakultní nemocnice Motol a korelaci klinických a laboratorních dat sledovaných pacientů. Určením jednotlivých buněčných populací a solubilních markerů zánětu (chemo/cytokinů) si klademe za cíl zjistit nové možnosti klinického využití průtokové cytometrie v diagnostice a stanovení prognózy u některých onemocnění centrálního nervového systému jakými jsou například zánětlivá demyelinizační onemocnění a autoimunitní encefalitidy nebo epilepsie. Navazujeme na naše předchozí zkušenosti s dlouhodobou péčí o tyto pacienty a výzkumného sledování různých imunologických markerů u těchto pacientů, zahrnujících mimo jiné chemo/cytokinový profil s využitím multiplexových esejí. Téma je vhodné pro studenta s lékařským vzděláním, zájmem o neurologii a imunologii a pozitivním vztahem k laboratorní práci, ideálně již s laboratorní zkušeností.

Characterization of growth and spontaneous regression of melanoma

Mgr. Helena Kupcová Skalníková, Ph.D.

ID 234632

Cutaneous melanoma is an aggressive skin tumor arising from pigmented cells – melanocytes. Melanoma diagnosed at early stages is mostly easily treatable by surgical excision. However, metastatic melanoma is mostly refractory to conventional therapies. Immune system plays a crucial role in melanoma growth control. Interaction between malignant tumor cells and immune system may lead to tumor destruction and its replacement by fibrous tissue. Currently developed immunotherapies targeting PD-1 or CTLA-4 molecules to boost the immune response significantly improved patient outcomes. In addition, adoptive cell transfer using in vitro propagated and activated T cells, originating from melanoma, promotes tumor rejection. The tumor regression may develop also spontaneously without any treatment and disappearance of the part of the tumor occurs in up to 50 percent of human melanoma cases.

Melanoma-bearing Libechov minipig (MeLiM) is a large animal model of hereditary melanoma. In the majority of the MeLiM piglets, a spontaneous regression of melanocytic loci occurs during the first year of postnatal life. The regression is among others accompanied by skin and bristle depigmentation and changes in hematological profile. Characteristic subpopulation of double positive (CD4+ CD8+) T-lymphocytes expanding during spontaneous regression was detected in MeLiM blood and tumor loci. This subpopulation carried mono-specific CDR3 region of T-cell receptor, which together with its onset during regression suggest that these cells may recognize single (melanoma?) antigen.

The aim of this project is characterization of cells and secreted factors that may participate in regulation of melanoma growth or regression. The project involves study of MeLiM animal samples as well as in vitro cultured pig and human cells. Laboratory techniques include mainly flow cytometry, cell culture, biochemical and proteomic techniques.

A combined use of dendritic cells and T cells for cellular immunotherapy of cancer

RNDr. Daniel Smrž, Ph.D.

ID 235084

Cancer is one of the leading causes of death in developed countries. Traditional cancer treatments – surgery, chemotherapy, or radiation therapy – have shown considerable efficacy at the early stages of the disease. At very late stages of the disease, these treatments usually fail or have minimal efficacy. New treatment modalities that would allow an effective therapy even at very late stages of cancer are needed.

Adoptive cellular immunotherapy (ACI) is a modern treatment modality for cancer treatment. Ex vivo-produced T cells are used for passive ACI of late-staged cancers. Ex vivo-produced dendritic cells (DCs) for active ACI of early-staged cancers. T cells are efficient in cancer cell elimination and DCs in eliciting long-lasting anti-cancer immune immunity. The combined use of both cell types for ACI could synergize the treatment efficacy of cancer.

The Ph.D. student will investigate the efficacy of combined use of ex vivo-produced monocyte-derived DCs and T cells in the elimination of cancer cells. This efficacy will be primarily evaluated in vitro. The first part of the project will consist of ex vivo-production of both cell types from peripheral blood of healthy donors. The second part will consist of functional analyses of these cells, including their phenotype, cytotoxic activity, and maturation. As a model will be used prostate cancer cell lines LNCaP, PC-3, and DU-145. The methods used will be cell isolation and culturing, flow cytometry, immunoblotting, and microscopy.

CV and motivation letter to: daniel.smrz@lfmotol.cuni.cz

Modulation of inflammatory response with defined microbiota.

doc. Ing. Bc. Igor Šplíchal, CSc.

ID 235325

Toll-like (TLR) receptors are cell receptors recognizing various molecular structures characteristic of microorganisms.

The student will study innate immune response within his/her doctoral study, i.e. inflammatory response induced by Salmonella Typhimurium and enteropathogenic Escherichia coli.

The thesis aims to evaluate the possible modulation of over-excessive immune response (sepsis) by defined microbiota composed of commensal and probiotic bacteria. Activation of inflammatory reaction will be monitored by expression of Toll-like receptors (TLR) 2, 4, 5, and 9 and their related molecules (MyD88, TRIF, LBP, MD-2 a CD14), production of inflammatory cytokines (IL-1b, IL-6, IL-8, IL-10, IL12/23p40, and IL-18) and HMGB1, tight junction proteins, and histopathological changes in the intestine in a gnotobiotic piglet experimental model.

A student will use the following methods within his/hers doctoral study: qPCR (purification of RNA, synthesis of cDNA, Real-Time PCR), Western blot, histology, immunohistochemistry, hemocytometry, ELISA, and xMAP technology (Luminex).

Researchers of the Institute of Microbiology of the Czech Acad Sci in Nový Hrádek (closed to Náchod) have long-time experience with the study of innate immune response in the microbiologically defined (gnotobiotic) piglets. The doctoral student will be included in the grant project of Czech Science Foundation and international collaborations.

The last publication is herehttps://doi.org/10.3390/biomedicines9020183

Other publications are available on https://orcid.org/0000-0003-3665-1377

Signální dráhy T-lymfocytární kostimulace

Mgr. Ondřej Štěpánek, Ph.D.

ID 235327

V průběhu tohoto PhD projektu budou analyzovány molekulární mechanismy, jakými vybrané povrchové receptory ovlivňují aktivaci a průběh imunitních reakcí. Hlavní důraz bude kladen na objasnění, jakým způsobem receptory z rodiny TNF a B7 modulují signalizaci T lymfocytů. V první části projetku bude využita metodologie umožňující tandemovou-purifikaci těchto signálních komplexů a jejich analýzu pomocí hmotnostní spektrometrie. Cílem bude objasnit, jaké proteiny jsou součástí těchto signálních drah a jakým způsobem ovlivňují jejich aktivaci.

Druhá část projektu bude zaměřena na objasnění role proteinu Abin1 v regulaci imunitních reakcí. Tento protein byl v naší laboratoři identifikován jako negativní regulátor inhibující signalizaci některých T-buněčných kostimulačních receptorů. K tomuto účelu bude použit myší model, ve kterém je Abin1 deletován. Tyto modely umožní určit, jakou roli hraje Abin1 ve vývoji a funkci T buněk a v průběhu některých modelových onemocněních.

Role LCK v periferních T-buněčných odpovědích

Mgr. Ondřej Štěpánek, Ph.D.

ID 235329

Student/ka bude studovat roli LCK v periferních odpovědích T-lymfocytů v různých imunologických myších modelech. Bude studovat roli interakce LCK s CD8 a CD4 koreceptory v těchto modelech. Dále se zaměří na roli samotné LCK v imunitních odpovědích cytotoxických CD8+ a regulačních CD4+ T lymfocytů.

Mechanisms of T-cell memory

Mgr. Ondřej Štěpánek, Ph.D.

ID 235330

The student will elucidate differences between naïve and memory CD8+ T cells on per cell basis using bacterial and viral infection models established in our group. Moreover, the student will study the functional diversity of long-term memory T cells in mouse models.

Myokines and metabolically active molecules in the pathogenesis of idiopathic inflammatory myopathies

prof. MUDr. Jiří Vencovský, DrSc

ID 231950

Patients with idiopathic inflammatory myopathies (IIM) suffer from muscle weakness and muscle wasting, a condition in which increased pro-atrophic and decreased anabolic factors, such as myokines myostatin and follistatin, would be expected. However, our recent data showed reduced circulating myostatin accompanied by attenuation of its signalling in chronic IIM patients compared to healthy individuals. IIM patients struggle with the reduced ability to carry out daily physicalactivities, including walking. Thus, the complex IIM pathogenesis is further potentiated by limited contractile activity and muscle disuse, which promote muscle atrophy, lipid accumulation and mitochondrial dysfunction. The aim of the presented project is to investigate, whether the observed myostatin and metabolic dysregulation drives the pathogenesis of IIM or it is a consequence of autoimmunity-related process. To answer that, newly diagnosed patients at the onset of the disease and 6 months after pharmacotherapy will be analysed. In addition, the results between patients well- and weakly- responding to the therapy will be compared. Myokines, muscle atrophy- and muscle growth-related proteins will be analysed in sera as well as in muscle tissue of IIM patients and related to disease progression, response to therapy and daily physical activity. PhD student will be involved in all aspects of the research work such as patient recruitment, laboratory work (serological analyses, primary muscle cell cultures, gene and protein expression, fluorescent microscopy), data analysis and manuscript drafting. The project refers to a submitted grant application MZČR NU21-05-00322.

Cancer immunotherapy based on drug delivery systems: modulation of the tumor microenvironment

Mgr. David Větvička, Ph.D.

ID 235292

Imunoterapie nádorů různého původů bude rozdělena do několika přístupů: 1. Imunitní checkpoint inhibitory Indoleamine 2,3-dioxygenasy (IDO) ; 2. agonisty Toll-like receptorů (TLR); 3. terapeutické ovlivnění intratumorálních makrofágů (M2/M1); 4. imunomodulace nádorového mikroprostředí pomocí cílené DNA/RNA terapie

Vývoj a testování nových inhibitorů (IDO) a nových TLR agonistů včetně testování jejich drug delivery systémů. Vývoj drug delivery systémů aktivně cílených na makrofágy. Dále se v projektu zaměřujeme, ve spolupráci s University of Nebraska a Tampere University, na DNA/RNA delivery systémy a protinádorovou imunomodulaci nádorového mikroprostředí. Projekt je zaměřen primárně na imunoterapii karcinomu mammy, pankreatu, plic a prostaty.

Metodický přístup

In vitro: tkáňové kultury, ovlivnění aktivity rekombinantního cílového enzymu, fluorescenční mikroskopie, vícebarevná cytometrie, Western Blott, ELISA, transfekce.

In vivo: orthotopická aplikace, biodistribuce a farmakokinetika testovaných látek, intravitální zobrazování, in vivo transfekce, toxicita, imunohistochemie, terapeutické experimenty.