OR Imunologie

Vypsané doktorské práce pro akademický rok 2023/2024

Leukocyte signal transduction as a target for pharmacological interventions in pathological inflammation

Mgr. Tomáš Brdička, Ph.D.

ID 257903

This work will use a mouse model of autoinflammatory osteomyelitis to map in detail the defects in signal transduction of neutrophil granulocytes and monocytes leading to autoinflammation. It will also include testing inhibitors of these signaling pathways for therapeutic use.

Leukocyte signaling pathways and their dysregulation in autoinflammatory disease

Mgr. Tomáš Brdička, Ph.D.

ID 257902

The main objective of this work will be to compare the defects in neutrophil and monocyte signal transduction in patients with the autoinflammatory disease chronic multifocal osteomyelitis and in a mouse model of this disease with the aim of identifying mechanisms whose study in the mouse model is relevant for human patients and their treatment.

Environmental regulation of T cells and neutrophils

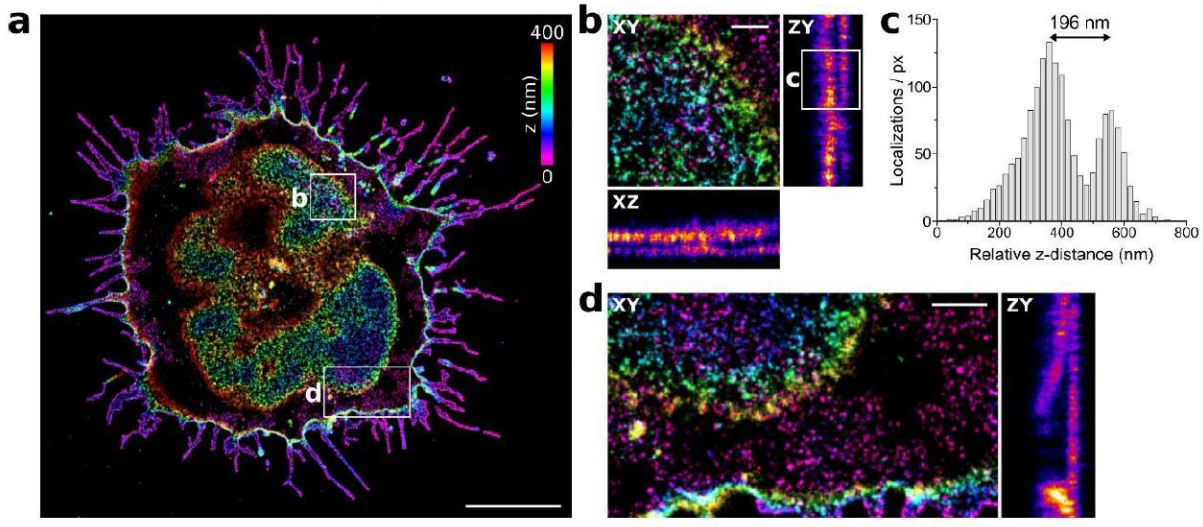
Mgr. Marek Cebecauer, Ph.D.

ID 257987

Immune cells employ diverse armoury to protect organisms from infections and other harmful conditions. A majority of these structures are associated with the plasma membrane and thus exposed to the environment. The best described protective structures in human immune cells are phagocytic sites and the immunological synapse of lymphocytes. Even though less well understood, neutrophil extracellular traps (NETs) belong to the most common tools to prevent microbial infections 1 . Similarly, small protrusions on the surface of immune cells – microvilli – are key structures regulating protective responses to pathological events. In this project, student will use two types of experimental approaches to determine how environmental factors affect human neutrophils and T cells by modifying NETs and microvilli, respectively. First, standard immunological and mol-biol techniques (standard microscopy, flow cytometry, immunoblotting – available in the Co-Supervisor's lab) will be used to find conditions modifying function of these structures. Second, high-end microscopy approaches (available in Supervisor's lab 2) will be used to learn about nanoscopic changes, which led to the altered function. Together, these studies should help to understand how environmental factors such as oxidative stress or temperature influence nanoscopic structures essential for a complex response of immune cells to the adverse conditions.

1. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 18: 134 (2019) DOI: 10.1038/nri.2017.105

2. Franke C; Chum T; Kvicalova Z; Glatzova D; Gentsch GJ; Rodriguez A; Helmerich DA.; Herdly L; Mavila H; Frank O; Brdicka T; van de Linde S and Cebecauer M. Approach to map nanotopography of cell surface receptors. Comms Biol. 5: 218 (2022); DOI: 10.1038/s42003-022-03152-y



Aire mutations mimicking APS-1 syndrome as the model of immune response against Candida albicans

Mgr. Jan Dobeš, Ph.D.

ID 257808

Roryt+ eTACs restricted expression of Aire is essential for induction of adaptive Th17 response against Candida albicans. While majority of APS-1 with muted AIRE gene suffer from C: albicans recurrent infections from early childhood, patients bearing so called "persian" mutation in AIRE are free of any candidiasis symptoms. Here we propose to utilize mouse knockouts mimicking AIRE mutations observed among APS-1 patient, including the persian mutation, as the tool to dissect molecular mechanism responsible for resistance of APS-1 patients with perisan mutation to candidiasis. In parallel, we will deepen our knowledge of molecular mechanism utilized by Aire in the immune periphery.

Coordination of antigen-presenting cells in adaptive Th17 response against C. albicans

Mgr. Jan Dobeš, Ph.D.

ID 257812

Roryt+ eTACs restricted expression of Aire is essential for induction of adaptive Th17 response against Candida albicans. However, the exact molecular mechanism how Roryt+ eTACs achieve establishment of the protective immune response against this intruder remain elusive. Based on our preliminary data we propose to test involvement of several factors in this process. Some of them might directly modulate the outcome of antigen-presentation capacity imposed by Roryt+ eTACs, others are involved in the attraction or activation of other cell types. Advanced immunology techniques utilizing transgenic and knockout mouse models, flow cytometry and transcriptomics will be used in this project.

Host-segmented filamentous bacteria (SFB) interactions

Mgr. Jan Dobeš, Ph.D.

ID 257787

The immune research concerning host-segmented filamentous bacteria (SFB) interaction was so far focused only on the induction of Th17 response by SFB. Results from my laboratory suggest that immune reactions induced by SFB are much more complex, including Th1 response and induction of intraepithelial lymphocytes. Importantly, molecular and cellular mechanism responsible for intraepithelial lymphocytes induction are currently poorly understood and will be investigated by the proposed project. The project will utilized state-of-the-art methodology such as the transgenic and knock-out mouse models, advanced flow cytometry and transcriptomics.

The regulatory role of Aire in anti-canida albicans IgA production

Mgr. Jan Dobeš, Ph.D.

ID 257811

Autoimmune regulator (Aire) is the essential molecular factor responsible for the setup of adaptive Th17 response against Candida albicans. Preliminary data from my laboratory indicate that on top of this essential role, the Aire also regulate C-albicans directed IgA production. The proposed project will elucidate cellular and molecular mechanisms behind this process utilizing the cell-specific Aire knockouts. The project will also take advantage of the advanced flow cytometry and transcriptomics.

Immunomodulatory environment of bone marrow niche of myeloid leukemia

Mgr. Jan Frič, Ph.D.

ID 257831

In recent years, cellular immunotherapy has been an attractive new candidate to treat different types of leukemia along with new inhibitors and success of transplantation of hematopoietic cells. Despite preliminary success, there are still several limitations to these approaches. Leukemias are rather dynamic diseases, able to modulate the bone marrow niche and as such affect the immune system and the organism as a whole. Leukemic cells produce wide spectrum of modulatory molecules and communicate with other cells via direct cell-to-cell interaction. In this manner, leukemic cells are able to reshape the bone marrow niche and establish protective environment which can provide a shelter to particular leukemic clones. One type of cells possibly protected by the leukemic niche are the leukemic stem cells, which are believed to be responsible for the failure of therapy and relapses. This project is focused on describing the immunomodulatory microenvironment of myeloid leukemia bone marrow environment and its potential impact on therapeutically administered effector immune cells. The project will investigate novel approaches to shield the effector cells from negative effects of leukemic microenvironment using antagonists of major immunomodulatory cytokines and further explore the possibility of using particular subsets of cytokine-induced or cytokine-preconditioned cells in order to overcome the obstacles presented by leukemic niche. This project will address the influence of transforming growth factor β on cells within the bone marrow niche as well as incoming immune cells. Furthermore, the co-regulatory role of cytokine network including IL-2 and IL-15 will be explored, along with other major factors affecting the niche. Aim of this study is to develop a realistic in vitro model of leukemic niche using available cell lines and primary cells in order to describe the cytokine profile and general conditions resembling the situation in vivo. Extensive utilisation of more complex bone marrow model cultures (consisting of leukemic cells, mesenchymal stem cells and other cell types found in bone marrow) in combination with flow cytometry and advanced imaging methods should support our efforts in exploring and describing the leukemic niche in the most in vivo-resembling manner. These models should then be utilized to better understand the complex dynamics in leukemic niche in context of cell-based therapies. Generally, this project should deepen the knowledge of leukemic environment and possibly improve the efficiency of therapy.

Vliv bezlepkové diety (GFD) na imunitní parametry, střevní mikrobiom a metabolom u idiopatických střevních zánětů (IBD) a primární sklerotizující cholangitidy

MUDr. David Funda, Ph.D.

ID 246642

Idiopatické střevní záněty (IBD] je chronické, relapsující, imunitně mediované zánětlivé onemocneni gasteointestinalniho traktu. Primární sklerozující cholangitida (PSC) je progresivní onemocnění žlučovodů, které v pokročilém stádiu choroby představuje jednu z nejčastějších indikací k ortotopické transplantaci jater v rozvinutých zemích. Ačkoliv genetické faktory přispívají ke vzniku těchto onemocnění, faktory vnějšího prostředí (diety) a následné změny mikrobiomu mají pravděpodobně významný vliv v etiopatogenezi těchto onemocnění. PSC je přibližně u 70% nemocných asociováno s IBD. V poslední době se objevily nezávislé důkazy o úloze chronického střevního zánětu u celé řady imunitně mediovaných onemocnění. Lepek a jeho komponenty mají prozánětlivý efekt podobný lipopolysacharidu. U řady autoimunitních onemocnění (diabetes 1 typu, některé neurologické poruchy) byl již prokázán příznivý vliv bezlepkové diety (GFD).

Cílem tohoto projektu je objasnění mechanismů interakce bezlepkové diety, mikrobiomu a metabolomu, a studium vlivu GFD na slizniční a přirozenou imunitu u IBD a PSC. Projekt je založen jak na lidské randomizované klinicke studii (vliv bezlepkové diety na klinické symptomy PSC a IBD), tak na myších indukovaných modelech IBD (model DSS-indukované kolitidy u BALB/c myší) a PSC (DSS-indukovaná PSC na modelu Mdr2-/- KO myší).

Mechanistické studie zahrnují analýzu imunitních parametrů, změn metabolomů a změn bakteriomů ve vztahu k bezlepkové dietě. Použité metodiky zahrnují izolaci leukocytů periferní krve od pacientů a izolaci buněk z lymfatických tkání u myší, flowcytometrii, tkáňové kultury, ELISA, charakterizaci metabolomů (ve spolupráci s Lab. charakterizace molekulární struktury), přípravu vzorků pro sekvenaci bakteriomů, analýzu dat apod. Projekt zahrnuje mezinárodní spolupráci.

Vliv střevní mikroflóry a vybraných baktérií a jejich mechanismů na rozvoj diabetu 1. typu

MUDr. David Funda, Ph.D.

ID 257989

Náplní projektu je s pomocí bezmikrobních a monoasociovaných, ex-germ-free a SPF NOD myší studovat efekt vybraných lidských bakterií, jejichž změny byly detekovány v mikrobiomech diabetiků nebo v období sérokonverze, na incidenci diabetu. V druhé části budeme testovat vliv vybraných lidských probiotických baktérií na rozvoj diabetu u monoasociovaných NOD myší, jakož i v kontextu mikrobiomu SPF NOD myší. Tyto baktérie (často už schválené k užití u lidí) mohou představovat cestu pro rychlý přechod ke klinickým testům. Mechanistické studie se zaměří na studium změn složení metabolomů, změny v populacích Tregs a dalších buněk, jejich cytokinových profilů, a T buněčných odpovědí. Kapacita Tregs zabránit T1D bude testována na modelu adoptivního kotransferu NOD-SCID myší. Projekt je tak zaměřen na nové možnosti prevence T1D a stanovení možné etiopatogenetické role vybraných testovaných bakterií.

Projekt bude zahrnovat studium relevantní literatury, gnotobiotické metodiky, měření glykémie, izolaci buněk z tkání, flowcytometrii, tkáňové kultury, in vitro T buněčné metodiky, mikroskopické metody, ELISA metody, charakterizaci metabolomů (ve spolupráci s Lab. charakterizace molekulární struktury), metodiku adoptivního kotransferu diabetu, apod. Projekt zahrnuje mezinárodní spolupráce.

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Effect of early postnatal probiotic supplementation on maturation of neonatal immune system

doc. RNDr. Jiří Hrdý, Ph.D.

1.LF

The work will focus on the understanding of the effect of early postnatal supplementation with single strain or complex probiotic mixtures on maturation of neonatal immune system. Special focus will be paid to characterization of gut mucosal immunity and gut barrier function. The experimental mouse model will be used to study the impact of early postnatal probiotic supplementation on immune system maturation. Probiotic supplementation will start second day after the birth and impact of probiotic supplementation on changes in microbiota composition and dynamics of mutual interactions between microbiota and host immune system. To reach the proposed goals of thesis, different methods including flow cytometry, real-time PCR, cell separation, bacteria cultivation and experimental animal models including acute and chronic colitis induced by administration of trinitrobenzensulfonic acid (TNBS) and transient colitis induced by administration of pathogenic bacteria Citrobacter rodentium) will be employed.

Neutrophils as a possible triggers of autoimmune neurodegenerative diseases

doc. RNDr. Jiří Hrdý, Ph.D.

1.LF

The topic of dissertation thesis will be focused on characterization of proportional and functional parameters of neutrophils in health and disease with special focus on patients suffering from neurodegenerative autoimmune disorders. 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. We are planning to clarify the potential role of dysregulated granulopoesis in development of autoimmune disorders in collaboration with General University Hospital in Prague. The role of neutrophils in initiation and progression of autoimmune disorders will be studied using experimental mouse models with special focus on confirmation of changes in proportional and functional characteristics of neutrophils observed in patients suffering from autoimmune diseases. For the 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).

Characterization of immune mechanisms responsible for the prevention of development of allergic diseases upon administration of probiotic strain Escherichia coli O83:K24:H31 or postbiotics

doc. RNDr. Jiří Hrdý, Ph.D.

1.LF

The topic of dissertation thesis focus on identification of possible mechanisms of beneficial early postnatal supplementation with Escherichia coli O83:K24:H31 or postbiotics on maturation of neonatal immune system including setting appropriate immunoregulatory responses. Special focus will be paid to characterization of gut mucosal immunity and gut barrier function and subpopulation of innate lymphoid cells in gut and lung. The experimental mouse model of allergic asthma will be used to study the capacity of early postnatal probiotic supplementation starting 2nd day postnatally to prevent allergy disease onset induced by ovalbumin administration at the age of 8 weeks. We will study the impact of probiotic and postbiotic supplementation on dynamics of mutual interactions between microbiota and host immune system. To reach the proposed goals of thesis, different methods including flow cytometry, real-time PCR, cell separation, bacteria cultivation and experimental animal models will be employed.

Development, differentiation and (dys)function of T lymphocytes in patients with primary immunodeficiencies

MUDr. Adam Klocperk, Ph.D.

2.LF

This thesis will focus on the development, differentiation and (dys)function of T lymphocytes in patients with errors of the immunity, especially in those with primary immunodeficiencies. Patients with primary immunodeficiencies are characterized by increased susceptitbility to infections, however, these disorders are frequently accompanied by allergic, autoimmune or cancer phenomena. Even though the genetic mutations in PIDs may affect a plethora of cellular populations, T lymphocytes may also be affected not only by direct faults in their genomic data, but also through missing tissues important for their correct function (e.g. thymus), impaired interactions with other cellular populations, the impact of recurrent or persistent infectious loading and stimulation, and through other pathways. As the central players of the adaptive immune system, T lymphocytes coordinate the production of antibodies, potentiation of innate immune response, and may be directly cytotoxic. Deep and precise understanding of their subpopulations and function in inborn and acquired errors of the immunity is thus crucial for the prediction of clinical complications, and design of suitable therapy and follow-up.

As part of this project we will work with primary material from patients treated at the University Hospital in Motol, and from healthy donors. We will use flow, spectral and mass cytometry, RNA sequencing, functional in vitro stimulation assays, multiplexed cytokine production assays and then use bioinformatic pipelines to analyse the data. Through close connection to clinical departments and robust laboratory background, this work will truly be bed-to-benchside(-to bed). Thanks to our extensive international collaboration we will be able to acquire and consult new laboratory methods, and participate in lab rotation(s).

The work will result in one or more papers in international peer-reviewed journals.

Immune response to gut microbes and their components in health and disease

Mgr. Klára Kostovčíková, Ph.D.

ID 257922

Commensal microbes continuously influence host´s physiology, including immune response. This mainly happens through direct contact of cells or humorally by production of small compounds (e.g. metabolites, cell wall components). Triggered cellular pathways can influence the permeability of the gut and other barriers and migration, counts and activity of immune cells and thus modulate host´s sensitivity to auto-inflammatory and metabolic diseases, inflammation or cancer. In our laboratory, we use diets with increased or reduced content of specific macronutrients or commensal microbes or their components to modify gut microbiome and host´s immune response in mouse models of human diseases (e.g. experimental colitis, colitis-associated colorectal tumorigenesis, auto-immune encephalitis, psoriasis). To further investigate the mechanisms of microbial activity, we use various in vitro approaches.

The Ph.D. project aims on cultivation of microbes, isolation of different active compounds and use of them in vitro and in vivo. The work includes epithelial and immune cell cultures, co-cultures, mouse models and subsequent analyses by flow cytometry, ELISA, immunofluorescence, histology, PCR, real-time PCR, and sequencing.

HPMA copolymer conjugates bearing mitoxantrone for treatment of androgen-independent prostate cancer and multidrug resistant haematological malignancies

RNDr. Marek Kovář, Ph.D.

ID 257789

The project is focused on design and development of HPMA copolymer-based drug delivery systems bearing cytostatic drug mitoxantrone. Mitoxantrone derivative with introduced carbonyl group is attached to polymer carrier via hydrazone bond to facilitate its controlled release. This drug delivery systems will be tested particularly in the models of androgen-independent prostate cancer (mouse cell line RM-1, human cell lines PC-3 and DU145) and in the model of murine multidrug resistant P388/MDR monocytic leukemia/lymphoma overexpressing P-gp. Combination with HPMA copolymer conjugate bearing derivative of lopinavir effectively inhibiting P-gp activity will be employed. The primary testing of in vitro anticancer activities will include 3H-thymidine incorporation assay, MTT assay, calcein assay and determination of their activity to induce apoptosis by Annexin-V binding assay. In vivo antitumor activity of the polymer conjugates will be studied in mouse syngeneic tumor model (RM-1) and in human xenograft (PC-3, DU145) growing in either Nu/Nu or Rag1-/- immunocompromised mice after careful evaluation of their toxicities and determination of maximum tolerated dose. Next, we plan to study the immunomodulatory activity of these polymer conjugates through evaluating their effect on immune cell subsets playing a negative role in tumor progression, i.e. MDSCs, Treg cells and TAMs via flow cytometry. We will also investigate the potential of the polymer conjugate bearing mitoxantrone to induce so called immunogeneic cancer cell death and induce antitumor immune response as previously shown for free mitoxantrone. Finally, we want also to study the potential to improve the therapeutic efficacy of these polymer conjugates via combination with immune check point inhibitors (anti-CTLA-4 and anti-PD-1 mAbs) and IL-2 complexes selectively stimulating CD25+ T cells. The project is based on tight collaboration with T. Etrych´s group (Department of Biomedicinal Polymers, IMC, Prague) and it is founded by AZV (NU21-03-00273) and by National Institute for Cancer Research (LX22NPO5102).

Antitumor and immunomodulatory effects of HPMA copolymer conjugates bearing gemcitabine and docetaxel in combination with immune checkpoint inhibitors and IL-2 complexes

RNDr. Marek Kovář, Ph.D.

ID 257790

This project is focused on design and development of HPMA copolymer-based drug delivery systems bearing cytostatic drugs gemcitabine and docetaxel. These cancerostatic drugs are attached to polymer carrier via amide or hydrazone bond, respectively, to facilitate their controlled release and various spacers providing different release kinetics of the bound drugs will be employed. These drug delivery systems will be tested particularly in the models of pancreatic carcinoma (mouse cell lines Panc02, human cell lines Panc01, MiaPaca and BxPC-3) as well as in the models of mammary carcinoma (mouse cell line 4T1) and lung carcinoma (mouse cell line LL2). The primary testing of in vitro anticancer activities will include 3H-thymidine incorporation assay, MTT assay and determination of their activity to induce apoptosis by Annexin-V binding assay. In vivo antitumor activity of the polymer conjugates will be studied in mouse syngeneic tumor models and in human xenograft growing in either Nu/Nu or Rag1-/- immunocompromised mice after careful evaluation of their toxicities and determination of maximum tolerated dose. We will also study the effect of the treatment with these polymer conjugates on expression of genes related to cell cycle/proliferation, apoptosis, angiogenesis, metastatic spread and tumor-immune system interaction using Real-time PCR. Next, we plan to study the immunomodulatory activity of these polymer conjugates through evaluating their effect on immune cell subsets playing a negative role in tumor progression, i.e. MDSCs, Treg cells and TAMs via flow cytometry. Finally, we want also to study the potential to improve the therapeutic efficacy of these polymer conjugates via combination with immune check point inhibitors (anti-CTLA-4 and anti-PD-1 mAbs) and IL-2 complexes selectively stimulating CD25+ T cells. The project is based on tight collaboration with T. Etrych´s group (Department of Biomedicinal Polymers, IMC, Prague) and it is founded by GAČR (22-20548S) and by National Institute for Cancer Research (LX22NPO5102).

Immunogenetic backround of Interstitial Lung Diseases. Early detection of chronic lung diseases in a lung cancer screening progress. Immunobiology of early stages of Interstitial Lung Diseases

prof. MUDr. Martina Koziar Vašáková, Ph.D.

1.LF

Lung tissue has only limited and uniform types of reaction to different kinds of damage which may either lead to complete restitution, or to prolonged inflammation, or stable fibrosis but also to progressive fibrosis. Between the known factors influencing pathologic patterns of healing leading to different interstitial lung diseases are aging, smoking, exposure to organic and inorganic dusts and microbes and some genetic variations and mutations (mucine and Tollip gene polymorphisms, surfaktant genes mutations, telomeropathies), however number of the factors are still unrecognized. Pathologic typ of alveolar healing leading to devastating lung damage is also a main factor of morbidity and mortality for covid-19. Thus the aim of the postgradual study programme will be investigation of genetic and immunologic factors, bronchoalveolar lavage analytic studies (FACS, proteins) in correlation to clinical and radiologic presentation in the patients with fibrosing lung processes of different origin to identify risky genotype and phenotype and better predict possible progressive fibrosis as a response to the alveolar lesions of different origin. The work will also include research of characteristics of individuals with accidentally detected early IPPs detected in the lung cancer early detection program and their monitoring over time to detect the natural development of interstitial changes and prognosis.

Metabolic and neural regulation of Sertoli cell function

doc. RNDr. Magdaléna Krulová, Ph.D.

ID 257108

During the inflammatory response to a pathogen, the blood-testis barrier protecting testicular immune privilege may be disrupted, which leads to the activation of immune reactions, and, subsequently, damage to the testicular tissue and germ cells. Sertoli cells play an essential role in spermatogenesis. Besides forming and ensuring the function of the blood-testis barrier, they produce various biomolecules to support germ cell development and create an immunosuppressive environment necessary for germ cell survival. The aim of the thesis is to study neural and metabolic regulation of Sertoli cell function during testicular inflammation using both in vitro and in vivo models. The ultimate goal is to establish a tool to protect germinal cells damaged due to inflammatory reactions. Uncovering mechanisms behind the neural and metabolic dysregulation of Sertoli cells will allow us to modulate the testicular environment using targeted approaches to ensure the functional properties of Sertoli cells and thus the function of the blood-testis barrier, 2) the production of paracrine factors supporting germ cell development, 3) creating an immunosuppressive environment necessary for germ cell survival, 4) the production of lactate, which is an essential energy source for developing germ cells.

Multiparametric analysis of intracellular signalling of malignant and nonmalignant cells

Mgr. Daniela Kužílková, Ph.D.

2.LF

T buněčná akutní lymfoblastická leukemie (T-ALL) je onemocnění způsobené maligní transformací T-buněčných prekurzorů, která vede k expanzi maligních T-lymfoblastů. Představuje cca 15% všech diagnostikových případů akutní leukemií u dětí. Toto onemocnění má u dětských pacientů poměrně dobrou prognózu, nicméně u části pacientů dochází k relapsu, který má naopak velmi špatnou prognózu. Proto je potřeba vyvíjet nové přístupy vedoucí k odhalení potenciálně cílitelných molekul, popř. celých signálních drah pro léčbu. Hmotnostní cytometrie je metoda využívající značení buněčné suspenze protilátkami konjugovanými s isotopy těžkých kovů, která v současné době umožňuje detekci až 50-ti znaků současně. Vzhledem k nepřítomnosti isotopů těžkých kovů v biologických vzorcích nemají vzorky své vlastní "pozadí“ typické pro konvenční průtokovou cytometrii, což umožňuje detekci i velmi nízkých signálů charakteristických pro signální molekuly. Laboratoř CLIP (Childhood Leukemia Investigation Prague) je jediná laboratoř v České Republice, která provozuje hmotnostní cytometr CyTOF XT a má dlouholeté zkušenosti s diagnostikou dětských leukemií i monitorací průběhu léčby na základě minimální reziduální nemoci. V projektu dysregulace signálních drah v buňkách dětské T-ALL budeme pomocí hmotnostní cytometrie analyzovat signálních drah T-lymfoblastů a budeme hledat změny, ke kterým dochází v odpověď na časnou fázi léčby (diagnose, den 8 a d15). Pro tyto účely máme již vytvořený panel 42 monoklonálních protilátek, které jsou zaměřeny na detekci aktivity jednotlivých členů klíčových signálních drah T-lymfocytů, včetně apopotických a proliferačních markerů. Tento projekt vznikl ve spolupráci s Dr. Gaipou (Univerzita Milano Bicocca, Monza, Itálie), který se na single-cell analýzu dětských T-ALL dlouhodobě zaměřuje. V další fázi projektu se zaměříme na mapování povrchových znaků (n~300) buněk dětské T-ALL. Budeme mapovat všechny dostupné CD znaky. Kromě nich použijeme také nové klony protilátek proti dalším cílům, které byly poslány do workshopu HLDA11 a které byly získané v rámci konsorcia HCDM. Cílem této části projektu je získat informace o expresi daných znaků na povrchu buněk dětské T-ALL, jakožto potenciální cíle pro terapii pomocí CAR buněk a také pro detekci MRN. Metodika použitá v rámci dizertační práce bude zahrnovat práci s buněčnými liniemi (kultivace, zamražení, rozmražení, stimulace, inhibice), izolaci mononukleárních buněk, popř. leukocytů periferní krve, high-throughput průtokovou cytometrii, spektrální cytometrii, hmotnostní cytometrii, konjugaci protilátek s izotopy těžkých kovů a analýzu single-cell dat (včetně nástrojů redukce dimenzionality), základní diagnostické procesy dětských akutních leukemiích a sledování MRN.

Cíle projektu:

(i) Stanovit phospho-signature buněk dětské T-ALL při diagnose, sledovat dynamiku jednotlivých klastrů buněk v iniciální fázi léčby pacienta

(ii) stanovit, zda-li se jednotlivé klastry buněk nachází i ve vzorcích při relapsu onemocnění, vytipovat terapeuticky cílitelné signální dráhy

(iii) určit surfaceom buněk dětské T-ALL se zaměřením na získání nových markerů pro cílenou léčbu (např. pomocí CAR-T cells) a monitoraci efektu léčby z hlediska stanovení minimální reziduální nemoci (MRN)

(iv) vytvoření hmotnostně-cytometrického panelu pro detailní rozbor apoptotických drah maligních buněk rezistentních na léčbu

(v) pomocí phospho-signature buněk pacientů s vrozenými poruchami imunity stanovit aberantně aktivované signální dráhy pro nasměrování cílené léčby pomocí inhibitorů tyrosinových kinas
Laboratoř CLIP Cytometrie Kliniky dětské hematologie a onkologie se specializuje na single cell analýzy metodami cytometrie (konvenční, spektrální i hmotnostní) u hematopoetických onemocnění. V rámci Pracovní skupiny dětské hematologie České republiky (PSDH ČR) je CLIP centrální laboratoří diagnostiky dětských leukemií v ČR. Laboratoř je vybavena hmotnostním cytometrem CyTOF XT (Fluidigm corp), spektrálním cytometrem Aurora (Cytek), průtokovými cytometry (2x BD FACSLyric, BD LSR II, BD FACS Celesta) a BD FACSAria buněčným sorterem. Pracoviště je řešitelem grantů AZV MZd, GA ČR, GA UK a dalších, je zapojeno do řady mezinárodních projektů.

Minimal residual disease monitoring and composition of non malignant subsets during the therapy of acute leukemias

MUDr. Ester Mejstříková, Ph.D.

2.LF

In the laboratory we mainly use cytometric methods. We are a reference flow cytometric laboratory for the diagnosis of childhood leukaemias. The main objects of research are hematological malignancies, bone marrow failure and immunodeficiency. The project deals with the search for new markers for the detection of leukemia cells during the course of the therapy. Currently, new drugs targeting specific structures of leukemia cells, which are also found on physiological immune cells, are part of the treatment. Second part of the project is analysis of dynamics of non-malignant cell composition during the course of acute leukemia treatment.

Functional characteristics of B cells and their role in the pathogenesis of immune-mediated diseases

MUDr. Tomáš Milota, Ph.D.

2.LF

The project is focused on the characteristics of B cells and their role in the immune system dysregulation and pathogenesis of immune-mediated diseases such as primary and secondary immunodeficiencies (Common variable immunodeficiency, secondary antibody deficiencies), autoimmunities (rheumatoid arthritis, systemic lupus), lymphoproliferative and granulomatous disease (lymphomas, sarcoidosis). Within the project, we will perform deep immunophenotyping of the B cells, their subpopulation, and their properties, The analysis will be conducted using a whole spectrum of laboratory methods. Our department is fully equipped. The project will be realized within the ongoing grants providing sufficient financial resources. Moreover, will apply for other grants within the course of the project.

Thus, a Ph.D. student as a member of our team will have a unique opportunity to be a part of the research team at the department of excellence in immunology research, to meet the whole spectrum of laboratory methods, to present his/her research findings at the national and international conferences, to gain experience in project preparation and grant applications.

Methods of detection of specific immune responses in patients with inborn and aquired errors of immunity

MUDr. Tomáš Milota, Ph.D.

2.LF

The project is focused on the methods of detection of the specific immune responses of B and T cells. Reduced postvaccination responses are the hallmarks of the broad spectrum of inborn and acquired errors of immunity. While the detection of the specific postvaccination antibodies is a routine assessment, the availability of the T cell immune response analysis is very limited. Although, T cell immunity may be preserved in such patients and may play important role in the defense against viral infections particularly. Within the project, we will use various laboratory methods including flow cytometry, multiplex assays, ELISA, western blot, and PCR. Thus, a Ph.D. student as a member of our team will have a unique opportunity to be a part of the research team at the department of excellence in immunology research, to meet the whole spectrum of laboratory methods, to present his/her research findings at the national and international conferences, to gain experience in project preparation and grant applications.

Identification of novel biomarkers predicting the efficacy of donor lymphocyte infusion treatment for relapsed acute myeloid leukemia

RNDr. Jan Musil, Ph.D.

ID 257587

Acute myeloid leukemia (AML) is the most prevalent form of acute leukemia in adults. It is characterized by the accumulation of myeloid precursor cells in the bone marrow and peripheral blood. Untreated expansion of the myeloid cells leads to bone marrow failure and death.

Currently, treatment consists of two phases. Firstly, the induction phase in which chemotherapy with 7 days of cytarabine followed by 3 days of anthracyclines is used to achieve remission. The second phase, consolidation, aims to prolong remission either by using high-intensity

chemotherapy or hematopoietic stem cell transplantation (HSCT). Especially in high-risk patients, HSCT is preferred due to its capability of destroying leukemic cells by NK cells and T cells transferred in the graft during the graft-versus-leukemia effect (GvL). Despite remarkable

advances in the field of post-transplant medical care, 30-40% of patients experience disease relapse. Treatment of relapsing AML is difficult and is based on reducing post-HSCT immune suppression, donor lymphocyte infusion (DLI), and, depending on the severity, chemotherapy.

Currently, no objective markers exist that would predict the efficacy of DLI in the treatment of relapsed AML. In this thesis, we will develop a multi-omics approach centered around using flow cytometry to identify novel biomarkers of treatment efficacy in the bone marrow of relapsing

patients. Furthermore, we will compare the identified biomarkers between bone marrow and peripheral blood, with the ultimate goal of shedding light on immune-evasive mechanisms present during relapse and potentially creating a more targeted test for clinical application.

The impact of gut microbiota on the onset and the course of anorexia nervosa in patients and in an experimental animal model

RNDr. Mgr. Petra Procházková, Ph.D.

ID 257822

In our Laboratory of Cellular and Molecular Immunology, we mainly study the relationship between the gut microbiome and psychiatric or neurological diseases. We also study the role of autoantibodies against various neuroactive molecules in these diseases.

The hypothesis of the association of the microbiome with these diseases lies in the ability of microorganisms to influence the functioning of the nervous system. In the gut, humans are equipped with several nervous system cells (as large as the number of neurons in the entire spinal cord) that can receive stimuli from microorganisms and their products (e.g. serotonin) and transmit them to the brain. This is often referred to as the microbiome-gut-brain axis. Furthermore, a healthy mucosal immune system in the gut, containing 70% of the human body's immune cells, can maintain a balance between the necessary microbial stimulation and tolerance. It is believed that the reason for the frequent occurrence of autoimmune, inflammatory, and cancerous diseases is the disruption breach of the mucosal barrier (so-called "leaky gut") as a consequence of microbial dysbiosis, which leads to the penetration of some microbial components into the circulation and the activation of immune cells in various organs triggering inflammation.

Specifically, we study the microbiome and autoimmune mechanisms in patients with eating disorders such as anorexia nervosa (AN) or psychogenic binge eating. We are looking for differences in microbial composition in patients with acute or chronic forms of AN. We use an "activity-based anorexia" mouse model, which is established for both conventional and germ-free mice. Furthermore, these mice serve as recipients of stool from these patients and healthy controls (fecal microbial transplantation). Similarly, mice are administered specific probiotic bacteria, butyrate-producing bacteria, and bacteria overrepresented in the gut microbiome of AN patients. In addition to changes in the microbiome, changes in metabolites in the serum and stool of both patients and mice are monitored, and intestinal permeability and expression of genes involved in appetite regulation and genes related to dopamine neurotransmission in the hypothalamus of mice are monitored.

The influence of microbiota on the pathogenesis of skin and intestinal diseases

Mgr. Zuzana Reiss, Ph.D.

1.LF

Current epidemiology studies clearly show a relationship between changes in microbiota composition and skin and intestinal diseases. Our laboratory focuses on the influence of microbiota on diverse inflammatory diseases such as inflammatory bowel disease, psoriasis or atopic dermatitis for many years. Apart from identification of microbiota we currently aim to investigate its effect on the gut-skin axis. Dysbalanced composition of intestinal microbiota is a risk factor for the impairment of the intestinal barrier. Therefore, we hypothesize that the alteration in the composition of intestinal microbiota could affect the gut-skin axis communication and lead to skin manifestation of various diseases (psoriasis, atopic dermatitis, vitiligo, inflammatory bowel disease). During the Ph.D. study, student will focus on how the composition of the skin and gut microbiota changes in patients with skin manifestations of disease. Moreover, he will look into how microbiota of a diseased/healthy person may influence the barrier function of the gut and skin. Using biological material obtained directly from patients and healthy humans, the influence of the microbiota will be investigated under in vitro conditions. Among other things, the Ph.D. student will also address changes in the microbiota and the integrity of the gut/skin barrier in psoriasis patients following administration of anti-IL-17 biologics, which can lead to the development of non-specific intestinal inflammation in some psoriatic patients. In his/her work, the student is going to use state-of-the-art techniques such as next-generation sequencing (MiSeq, 3'mRNA Seq), co-culture of gut microbiota with human organoids, liquid chromatography with mass spectrometry (LC-qTOF-MS), ELISA, flow cytometry and immunofluorescence methods.

Vaccination with engineered antigens and antibody delivery therapy for Sars-Cov2

doc. RNDr. Radislav Sedláček, Ph.D.

ID 246652

PhD work will be focused on in-vivo preclinical testing of novel therapeutic delivery systems for RNA and proteins to mouse model sensitized for Sars-Cov2 infection and development of the immune responces. The work will rely on assembly of viral and non-viral particles, analysis of monitoring of RNA delivery efficacy and safety. The effect of delivered components for vaccination will be evaluated by detail characterization of immune system response. The efficacy of vaccination will be tested on multiple Sars2 variants of concern. The engineered antibodies prepared in collaboration with Luca Varani (Institute for Research in Biomedicine (Bellinzona, CH)) will be tested on multiple Sars2 mouse models and they protective efficiency will be evaluated. The methods used in PhD study will encompass broad variety of molecular biology methods (molecular cloning, PCR, WB, qPCR). Single cell proteomic and transcriptomic approaches will be implemented to reveal the immune system reaction to infection and reinfection in context of vaccination or antibody treatment.

Epitopes of HLA antigens and clinical significance for the organ transplantation program

MUDr. Antonij Slavčev, CSc.

ID 257740

Transplantation is currently the best treatment for end-stage kidney failure. However, the prognosis of long-term graft survival is often uncertain in risky (sensitized) patients due to possible immunological complications after transplantation. Patients with high levels of anti-HLA antibodies have limited access to transplantation due to positive crossmatch tests with the vast majority of potential organ donors. Determination of HLA antigen epitopes is a new approach for evaluating HLA compatibility, which can significantly aid in kidney allocation of patients with high levels of HLA-specific antibodies before transplantation (especially in patients before living-donor kidney transplantation). The main goal of the proposed project is to introduce the analysis and definition of HLA antigen epitopes in immunologically high-risk patients and their donors at the Department of immunogenetics in IKEM using next generation sequencing (NGS). Systematic analysis of HLA antigen epitopes can increase the likelihood of finding a compatible donor and successful transplantation. Another application of this analysis is in post-transplant monitoring of the specificity of antibodies produced de novo after transplantation. In addition, the proposed project can also help clarify the underlying mechanisms of antibody binding to HLA antigens, thereby contributing to improved diagnosis of post-transplant rejection.

The mechanism of action of the intestinal microbiota components in experimental models of food allergy and intestinal inflammation.

Mgr. Martin Schwarzer, Ph.D.

ID 257116

Allergies and inflammatory bowel disease (IBD) are widespread pathological immune reactions of the intestine which has dramatically increased during past decades. Their global prevalence have been increasing especially in industrialized countries, suggesting environmental factors playing a key role in the susceptibility and etiology of this disorder. The hygiene hypothesis postulates that behind the increased susceptibility to allergic diseases is the lack of exposure to microbial stimuli or altered microbial stimulation, which leads to aberrant immune system maturation. Along these lines, dysbiotic microbiota leading to allergy or IBD development or associated with allergic diseases has been reported to have decreased or lack of certain groups of commensal bacteria including lactobacilli and bifidobacteria.

Within the framework of the Ph.D. study you will master the handling of germ-free and conventional mice and work with mouse models of food allergies and ulcerative colitis. Specifically, you will investigate the impact of selected probiotic and commensal bacteria (such as lactobacilli, bifidobacteria or non-pathogenic E. coli) and defined isolated bacterial components on the development of naive immune system and allergic sensitization/intestinal inflammation in mouse model.

During your PhD. study you will use classical and molecular-biological methods for bacterial detection and cultivation. You will master different methods such as ELISA, Western-blot, PCR and real-time PCR, flow cytometry, in vitro cell and cell line cultivation, histology and immunohistochemistry.

Location: Laboratory of Gnotobiology, Nový Hrádek, 54922

A role of clostridia in colonization resistance against enteric infections

doc. Ing. Bc. Igor Šplíchal, CSc.

ID 246727

Toll-like (TLR) receptors are cellular receptors recognizing various molecular structures typical for microorganisms and tissue damage.

During the doctoral study, the student will deal with the importance of clostridia for colonization resistance against enteric infections with Salmonella Typhimurium and enteropathogenic E. coli. The intestinal barrier state (damage/protection) will be characterized by histopathological changes in the intestine and the expression of villin, claudin-1, and occludin proteins in an experimental gnotobiotic piglet model. Activation of the inflammatory response will be assessed by the expression of TLR2 and TLR4, MD-2 and CD14 co-receptors, and MyD88 and TRIF adapter molecules. The inflammatory response will also be characterized by local and systemic levels of the inflammatory cytokines IL-1β, IL-6, IL-8, IL-10, IL12, IL-18, IFN-γ, and TNF-α.

The student will mainly use RT-qPCR (RNA isolation, cDNA synthesis, PCR), Western blot, histology, immunohistochemistry, hemocytometry, and xMAP technology (Luminex).

The doctoral thesis will be prepared at the Institute of Microbiology of the Czech Acad Sci in Nový Hrádek and the doctoral student will collaborate on a grant from the Czech Science Foundation.

Syndrom krátkých telomér u kandidátů plicní transplantace

doc. MUDr. Martina Šterclová, Ph.D.

1.LF

Úvod: Progredující fibrotizující intersticiální plicní procesy (IPP) se v čase stávají dominantní diagnózou, pro kterou jsou pacient referováni ke zvážení zařazení do programu transplantace plic. Typicky se jedná o pacienty s idiopatickou plicní fibrózou, ale i o nemocné s exogenními alergickými alveolitidami nebo systémovými chorobami pojiva s dominantně plicním postižením. Jedním z rizikových faktorů rozvoje fibrotizujícího IPP v mladším věku (4.-6. dekáda) jsou genetické predispozice, a není překvapivým zjištěním, že minimálně mezi transplantovanými pro IPF jsou významně zastoupeni pacienti s telomeropatiemi (1). Mechanismus, jak genový polymorfismus ovlivňuje patogenezi onemocnění, není vždy zřejmý, nejvíce otázek ohledně patogeneze se týká nemocných s polymorfismem genů uplatňujících se v údržbě telomér, kteří mají normální délku telomér.

Cílem předkládaného projektu je fenotypizovat kandidáty plicní transplantace s klinickou suspekcí na syndrom krátkých telomér, pacienty geneticky vyšetřit (2), stanovit délku telomér buněk periferní krve u potenciálních kandidátů plicní transplantace (pro progredující fibrotizující intersticiální plicní proces) a monitorovat vývoj délky telomér v čase (3). Pokud bude u nemocných zahrnutých do projektu indikována ošetřujícím pneumologem bronchoskopie s bronchoalveolární laváží, bude stanovena délka telomér v buňkách lavážní tekutiny (4).

Metodika: Prospektivní zařazování – všichni potenciální kandidáti plicní transplantace referovaní pro progredující fibrotizující IPP (20-30 osob ročně). Při prvním kontaktu s pacientem – sběr anamnestických údajů dle definovaného protokolu (viz příloha) + odběr krve k genetickému vyšetření a stanovení délky telomér metodou qPCR, odběr kontrolního krevního vzorku á 6 měsíců ke stanovení délky telomér metodou qPCR. Pokud ošetřující lékař indikuje z medicínských důvodů v době sledování pacienta bronchoskopii s bronchoalveolární laváží, stanovení délky telomér metodou qPCR u buněk získaných laváží. Podrobně viz literatura 2-4.

Pipeline: sběr dat a vzorků v roce 1-2, zpracování rok 2-3, práce na publikacích rok 2-3.

Význam: Dynamika zkracování telomér u kandidátů plicní transplantace, porovnání délky telomér v periferní krvi a nejvíce postiženém orgánu (plíce), fenotyp a genotyp nemocných se syndromem krátkých telomér.

Potenciální navazující projekt: dynamika délky telomér u transplantovaných pacientů (periferní krev), vztah k posttransplantačnímu průběhu a přežití pacientů.

Literatura:

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2. Revy P, Kannengiesser C, Bertuch AA. Genetics of human telomere biology disorders. Nat Rev Genet. 2023 Feb;24(2):86-108. doi: 10.1038/s41576-022-00527-z.

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Uncovering the link between autoimmunity and infections

Mgr. Ondřej Štěpánek, Ph.D.

ID 258583

The rise in autoimmune diseases necessitates identifying the causes, likely linked to lifestyle changes. The hygiene hypothesis suggests a link between a lack of infection events and autoimmune disease prevalence, but specific pathogens are also known to cause autoimmunity. Our hypothesis is that increased pathogen burden due to high population density and frequent personal contacts weakens the immune tolerance, contributing to autoimmune disease development. The student will test this hypothesis in a series of experiments by co-housing genetically uniform pathogen-free mice with feral or pet store mice to mimic traditional and modern lifestyles, respectively. We will investigate whether the transmission of specific sets of pathogens and commensals found in feral or pet store mice induce autoimmune tissue pathology and susceptibility to autoimmune encephalitis. These large-scale experiments aim to shed light on the complex interplay between microbes and autoimmunity.

Metabolically active molecules in the pathogenesis of rheumatic diseases with muscle impairment

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 physical activities, 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.

Genová imunoterapie karcinomů prostaty, plic a prsu

Mgr. David Větvička, Ph.D.

ID 257992

Studium DNA delivery systému pro experimentální imunoterapie nádorů plic, prsu a prostaty. Restart protinádorové imunitní reakce.

Studium nových TLR7/8 agonistů v kombinaci s cytostatikem a polymerním nosičem obsahujícím STING agonisty

Mgr. David Větvička, Ph.D.

ID 257991

Studium protinádorové imunoterapie kombinující STING a TLR7/8 agonisty s chemoterapií