Graduate Programme in Immunology and Infectious Diseases

Abstract Book

Theme:
The Immune System: Friend or Foe

GSI 2025

7-9 OCTOBER

ELSINORE · HELSINGØR DENMARK

Index

Investigating Bacterial Interactions in Lung Infections by 3D Bioprinting and Air-Liquid Interface Model 3
Infection dynamyics air-way epathelial cells with macrophagers after being infected with Pseduomonas aeruginosa in 4
IL-10 Immune Modulatory Vaccine: Targeting the Tumor Microenvironment. 5
Investigating efficacy and immunologic mechanism of combination treatment with a TGFβ immunomodulatory vaccine and radiotherapy in murine models of cancer 6
Immunologic and Clinical Correlates Supporting Multi-Epitope TGFβ Vaccination in Pancreatic Cancer 7
Engineering primary CD8+ T cells with a novel PD-L1-specific TCR 8
Granzyme B dominates the tumor-specific granzyme response of expanded CD8+ tumor-infiltrating lymphocytes 9
Exercise as combination partner for cancer immunotherapy 10
Investigating Immune Profiles in Head and Neck Mucosal Melanoma: A Foundation for Personalized 11
The Transition from CHIP to Overt Myeloproliferative Neoplasms: A 13-Year Longitudinal Study of JAK2 Positive Citizens 12
Immunological and Clinical Insights into Canine Immune Thrombocytopenia in Copenhagen: Prevalence, Inflammatory Profiling, and Genetic Footprint 13
Immunological characterization of atopic dermatitis 14
Exploiting film-forming properties of poly(N-vinylcaprolactam) for cutaneous delivery of tofacitinib citrate to treat atopic dermatitis 15
Early BCG Vaccination is Potentially Beneficial for Preterm Infant Immune Systems 16
Heterogeneous generation and expansion of epidermal-resident memory T cells in patients allergic to nickel 17
The effect of mixtures of skin sensitizers and irritants on the sensitization and elicitation phase of contact allergy. 18
Developing Molecular Probes to Identify and Characterize Superantigen-Responsive (SupeR)-T cells in Atopic Dermatitis at the Single-cell Level. 19
The role of stromal aryl hydrocarbon receptor activity in controlling colonic immune responses in homeostasis and disease 20
Weaning-associated modulation of the jejunal immune barrier in piglets studied by multiplex immunohistochemistry 21
Can allergic contact dermatitis be permanently cured? 22
Efficacy of Dupilumab in Patients with Severe Asthma and Co-existing COPD 23
Polyphenol - Microbiota Interactions in Modulating Mucosal Immunity During Parasitic Infection 24
Impact of Fiber-Free Diet on Type-2 Immunity Against Helminth Infection Through Alterations of the Gut Microbiome and Metabolome in Pigs. 25
The Impact of Various Diets on Type 2 Immunity against Enteric Helminth Infection through Alterations of the Microbiome and Metabolome 26

Ongoing evolutionary patterns on the pfmdr1 hinge region and association with anti-malarial resistance mutations in P. falciparum 27
Preimmunization-Induced Trained Immunity Modulates Diet-Dependent Resistance to Intestinal Helminths in Mice 28
Sustainable saponins for next-generation vaccine adjuvants 29
Design of next-generation lipid nanoparticles for mRNA vaccines: Enhancing potency and safety by incorporation of adjuvants 30
De Novo Protein Design with RFdiffusion: A New Paradigm for Rational Vaccine Antigen and Adjuvant Development 31
Spike antibody levels and risk of SARS-CoV-2 reinfection: a two-year cohort study in previously infected adults 32
Preclinical influenza vaccine testing in mouse models 33
Optimizing mRNA-based capsid Virus-Like Particle (cVLP) vaccines: Placental Malaria. 34
Development and pre-clinical testing of a Nipah vaccine exploiting the Tag/Catcher-cVLP platform 35
A Rare HCV E2 Antibody That Defies the Classic Easy- and Hard-to-Neutralize Divide 36
Substitutions in the NS5 of tick-borne encephalitis virus confer high resistance to remdesivir 37
Preclinical characterization of humoral immune responses elicited by our lead inactivated hepatitis C virus vaccine candidate. 38
Spatial and frequency profiling of intestinal mucosal innate lymphoid cells (ILCs) in people living with HIV 39
Incidence and Clinical Outcome s of RSV and Influenza in Solid Organ Transplant Recipients 40
Human Organotypic Tonsil Slice Culture Shows Germinal Center Maturation and T-Follicular Helper Recruitment After LAIV Challenge 41
Von Willebrand Factor and Complications Related to Blood Vessel Damage Following Pediatric Hematopoietic Stem Cell Transplantation 42
Role of TSLP and mast cells among asthma patients with airway autoimmunity 43
In-depth profiling of conventional dendritic cell populations and transcriptomics in Crohn's disease . 44
Negative T cell selection and induction of regulatory T cells specific to FVIII and Factor H in Human Thymic Organoids 45
Predicting individualised treatment effects of passive immunotherapies in adults hospitalised with COVID-19 using machine learning 46
Novel approaches for treatment of community-acquired pneumonia: A presentation of a Ph.D-project 47
The role of the Met1-linked ubiquitin machinery in the immune biology of human keratinocytes and fibroblasts 48
Biological effects of CCL5 posttranslational modifications and detection of its proteoforms in biological samples 49
Characterisation of the Inflammatory and Immune-mediated Disease (IMID) Virome 50
Mapping Gene Signatures of IgA Class-Switch Recombination in Human Immune Organoids 51

P01

Investigating Bacterial Interactions in Lung Infections by 3D Bioprinting and Air-Liquid Interface Model

Fusco S[1]

[1]Rigshospitalet, [2]DTU

Theme: Bacteriology

Chronic lung infections are shaped by complex polymicrobial communities, where microscale interactions within lung niches influence bacterial virulence and strain dynamics. A major pathogen in this context is Pseudomonas aeruginosa (Pa), which undergoes intra-clonal diversification through genome-wide mutations and selective pressures unique to each micro-niche. Reproducing these microenvironments, where Pa interacts with other pathogenic strains, is a central focus of our study. This is particularly important because existing models lack spatial control, making it challenging to mimic lung infections and to address how microscale spatial arrangements affect bacterial communication, virulence, and infection progression.

To address this, we developed a 3D bioprinting method to precisely position bacteria on bronchial airway epithelial cells which are cultured at the air–liquid interface to closely mimic human airways. This technique was optimized for technical precision and bioink compatibility and enables direct visualization of bacterial interactions at defined spatial arrangements, which conventional models cannot achieve. Alongside 3D bioprinting, we are applying cell biology techniques to track infection progression, including immunofluorescence staining, measurement of IL-8 inflammatory responses, LDH-based cytotoxicity assays, and live/dead staining.

From the microbiology side, we are engineering reporter fusion strains of Pseudomonas aeruginosa to create “sender” and “receiver” systems for studying quorum sensing signals.

By combining 3D bioprinting with a physiologically relevant lung model and reporter quorum sensing strains we can be able to measure how spatial separation at the micro scale influences bacterial communication, virulence and infection progression.

P02

Infection dynamyics air-way epathelial cells with macrophagers after being infected with Pseduomonas aeruginosa in

Melanson A[1,3,4], Colque A[1,3], Martinez P[1,3], Persson J[2], Molin S[3], Johansen H[1,2]

[1]Copenhagen University, [2]Lund University, [3]Center for biosustanability, [4]Rigshospitalet

Theme: Bacteriology

Air–liquid interface (ALI) culture systems are increasingly used to model respiratory infections because they enable airway epithelial cells to differentiate into a pseudostratified epithelium resembling the in vivo lung environment. A major limitation of current ALI models, however, is the absence of immune components. To address this, we incorporated macrophages into an ALI system and examined how their presence influences the progression of Pseudomonas aeruginosa infection.

We assessed infection outcomes using confocal immunofluorescence microscopy, ELISA, transepithelial electrical resistance (TEER), cytotoxicity assays, and quantification of bacterial distribution across compartments. Cytokine secretion was not detectable until ~10 hours post-infection, after which levels increased substantially. Similarly, epithelial damage was minimal during the first 10 hours but became pronounced thereafter. Strikingly, P. aeruginosa rapidly entered epithelial cells at early time points, while delaying expansion in the apical and basolateral compartments. The intracellular compartment ultimately contained the majority of the bacterial burden. Notably, secretion of TNFα and IFNγ was not observed under these conditions.

In conclusion, inclusion of macrophages in the ALI system revealed a delayed onset of cytokine production and epithelial damage relative to the early intracellular entry of P. aeruginosa. These results underscore the value of immune-competent ALI models for dissecting host-pathogen interactions and highlight the importance of considering immune cell contributions when studying respiratory infections.

P03

IL-10 Immune Modulatory Vaccine: Targeting the Tumor Microenvironment.

Lara de la Torre L[1], Pérez Penco M[1], Lecoq Molinos I[2], Hald Andersen M[1,3]

[1]Herlev Hospital-National Center for Cancer Immunotherapy (CCIT-dk), [2]IO Biotech, [3]Department of Immunology and Microbiology, University of Copenhagen

Theme: Cancer immunology

Introduction
Cancer immunotherapy has revolutionized oncology, and Immune Modulatory Vaccines (IMVs) have emerged as a novel strategy. IMVs are peptide-based vaccines that target immunoregulatory molecules in the tumor microenvironment (TME), including indoleamine 2,3-dioxygenase (IDO), programmed death-ligand 1 (PD-L1), transforming growth factor β (TGF-β), and arginase 1 (Arg1). Clinical studies have demonstrated both safety and efficacy, with results from a phase III trial recently reported. Interleukin-10 (IL-10), a key immunosuppressive cytokine highly expressed in the TME, represents another potential target. IL-10-specific T cells have been detected in both healthy individuals and cancer patients, suggesting vaccination could expand these responses to promote antitumor immunity.

Methods
MHC-I and MHC-II IL-10-derived peptides were predicted using NetMHC 4.0. C57BL/6 mice were vaccinated subcutaneously with peptide–Montanide ISA 51VG emulsions. IFN-γ ELISpot assays evaluated peptide-specific responses. Immunogenic peptides were then tested in a syngeneic pancreatic cancer model, PanO2. Tumor growth, body weight, immune infiltrates (flow cytometry), and transcriptomic changes (Nanostring) were assessed.

Results
Vaccination with IL-$10_{87–95}$ (MHC-I) and IL-$10_{87–104}$ (MHC-II), collectively termed IL-10 IMV, significantly delayed tumor growth in Pan02-bearing mice. Tumors from vaccinated animals were smaller, with no adverse effects on body weight observed. Flow cytometry revealed increased $CD4^+$ and $CD8^+$ T-cell infiltration, while Nanostring analysis indicated upregulation of interferon signaling, cytotoxicity-related pathways, and altered myeloid cell signatures, including enhanced macrophage infiltration.

Conclusion
IL-10-derived peptide vaccination elicited functional antitumor responses, remodeled the TME, and inhibited tumor growth in vivo. These findings support IL-10 IMV as a promising immunotherapeutic approach targeting immunosuppressive pathways in cancer.

P04

Investigating efficacy and immunologic mechanism of combination treatment with a TGFβ immunomodulatory vaccine and radiotherapy in murine models of cancer

Michler Byrdal M[1], Hald Andersen M[1]

[1]National Center For Cancer Immune Therapy (ccit-dk), Herlev Hospital

Theme: Cancer immunology

Purpose
The purpose of this project is to combine an immunomodulatory vaccine, a novel cancer treatment modality, with radiotherapy, to investigate if a synergistic effect can be achieved. TGFβ is upregulated in several cancer types following radiotherapy, providing the rationale for combining radiotherapy and TGFβ-targeting treatments.

Methods
Identification of radiotherapy responsive murine cancer cell lines was done by in vitro irradia-tion of cell lines and assessment of survival and ability to form colonies. TGFβ ELISA was used to investigate upregulation of TGFβ following irradiation. In vivo vaccination of mice with subcutaneous syngeneic tumors with TGFβ epitope peptides allows assessment of anti-tumorigenic potential. ELISPOT assay was used to confirm vaccine-induced TGFβ-specific T cells. Irradiation of tumors with a linear accelerator combined with vaccination allows as-sessment of treatment synergy. Flow cytometry analysis of tumors is used to identify immu-nological changes.

Results
In vitro radiation of 5 cell lines showed differences in radiosensitivity in both short-term cell survival and clonal expansion. TGFβ ELISA showed differential upregulation of TGFβ follow-ing irradiation. A dose-response relationship between irradiation and cellular response was observed. In vivo TGFβ vaccination induced TGFβ-specific T cells against several epitopes included in the vaccine. TGFβ vaccination of mice harboring Pan02 murine pancreatic tu-mors reduced tumor growth, showing therapeutic potential.

Conclusions
Different cell lines exhibit variation in radiosensitivity and TGFβ response to irradiation, show-ing that model choice in in vivo experiments is important for conclusions.

Immunologic and Clinical Correlates Supporting Multi-Epitope TGFβ Vaccination in Pancreatic Cancer

Ruders J[1], Ahmad S[1], Mortensen R[1], Met Ö[1], Byrdal M[1], Chen I[2], Theile S[2], Holmström M[1], Andersen M[1,3]

[1]National Center for Cancer Immune Therapy (CCIT-DK), Department of Oncology, Copenhagen University Hospital, [2]Department of Oncology, Copenhagen University Hospital, [3]Department of Immunology and Microbiology, University of Copenhagen

Theme: Cancer immunology

Transforming Growth Factor Beta (TGFβ) is a critical driver of pancreatic ductal adenocarcinoma (PDAC) progression through mechanisms involving immune suppression, fibrosis, and metastasis. Given its central role in shaping the tumor microenvironment (TME), TGFβ has emerged as an attractive target for immunotherapy. An innovative approach entails the use of TGFβ-based immune-modulatory vaccines (IMVs). In this study, we investigated the immunogenicity of various TGFβ-derived epitopes and evaluated the clinical significance of TGFβ-specific T cell responses in PDAC patients.

We observed T cell reactivity to several epitopes, namely TGFβ-15, TGFβ-33, and TGFβ-38, in both healthy donors and PDAC patients, with TGFβ-33 eliciting particularly robust responses in patients. These mainly $CD4^+$ T cells demonstrated pro-inflammatory and cytotoxic features, recognizing target cells in a TGFβ-dependent manner. Moreover, higher baseline frequencies of TGFβ-33-specific T cells were associated with improved outcomes following immune checkpoint inhibitor (ICI) therapy and radiotherapy. Additionally, patients exhibiting baseline responses to multiple TGFβ-derived epitopes experienced longer overall and progression-free survival compared to those responding to none or only a single epitope.

Employing a single mRNA construct encoding TGFβ epitopes, we further confirmed the potent activation of both $CD4^+$ and $CD8^+$ T cells specific for distinct TGFβ-derived epitopes.
Collectively, these findings emphasize the significance and potential of a multi-epitope, TGFβ-derived IMV to augment anti-tumor immunity and improve therapeutic efficacy in PDAC.

Author 9 developed a TGFβ-based vaccine, with rights initially assigned to Copenhagen University Hospital, and later licensed to IO Biotech ApS. He holds roles and shares; other authors report no competing interests.

P06

Engineering primary CD8+ T cells with a novel PD-L1-specific TCR

Jacobsen E[1], Lisle T[1], Munir S[1], Martinenaite E[1,3], Met Ö[1], Bak R[2], Andersen M[1]

[1]National Center for Cancer Immune Therapy (CCIT-DK), Department of Oncology, Copenhagen University Hospital, [2]Department of Biomedicine, Aarhus University, [3]IO Biotech ApS

Theme: Cancer immunology

The immunosuppressive tumor microenvironment limits the efficacy of cancer immunotherapy. Targeting immunosuppressive molecules, such as programmed death ligand 1 (PD-L1) and indoleamine 2,3-dioxygenase 1 (IDO1), with proinflammatory PD-L1- and IDO1-specific T cells boosted by immunomodulatory vaccination has shown promise in a recent clinical phase III trial. This study investigates whether primary CD8+ T cells can be engineered with a native PD-L1-specific TCR to effectively target immunosuppression.

A PD-L1–specific TCR (PDL101) was integrated into the TCR alpha locus of primary $CD8^+$ T cells via CRISPR-Cas9 and a PCR-generated homology-directed repair template, replacing the endogenous TCRs of HLA-A2-restricted donors. Integration was confirmed by tetramer staining, and PDL101-TCR-T cells were functionally validated by intracellular cytokine staining and ^{51}Cr release assays. PDL101-TCR-T cells were enriched by fluorescence-activated cell sorting and expanded using either a rapid expansion protocol or repeated stimulation with PDL101-loaded dendritic cells.

The HLA-A2-restricted PDL101-TCR was successfully integrated into primary CD8+ T cells, generating 3-10% PDL101-TCR-T cells that were enriched into highly specific cultures with robust antigen-specific cytokine secretion and potent cytotoxicity towards the PDL101 peptide. Repeated stimulation with PDL101-loaded dendritic cells enhanced enrichment, suggesting potential for in vivo boosting via immunomodulatory vaccines. Lastly, co-cultures with PD-L1+ cancer cell lines confirmed PD-L1-dependent recognition by the PDL101-TCR-T cells.

These results demonstrate the first generation and functional characterization of PD-L1-specific CD8+ TCR-T cells, which exhibit potent, PD-L1-dependent cytolytic activity. They show promise for targeting immunosuppressive molecules, warranting further investigation into their capacity to reprogram the tumor microenvironment.

P07

Granzyme B dominates the tumor-specific granzyme response of expanded CD8+ tumor-infiltrating lymphocytes

Dionisio E[1], Chamberlain C[1], Rasmussen A[1], Weiss A[1], Khan S[1], Lauss M[2], Schina A[1], Draghi A[1], Harbst K[2], Presti M[1], Jönsson G[2], Borch T[1], Svane I[1], Donia M[1]

[1]CCIT DK, [2]Lund University

Theme: Cancer immunology

Adoptive cell therapy using in vitro-expanded tumor-infiltrating lymphocytes (TILs) has demonstrated clinical efficacy in metastatic melanoma, yet the cytotoxic mechanisms underlying tumor elimination remain incompletely defined. We investigated granzyme (Gzm) expression and secretion profiles of expanded CD8⁺ TILs upon autologous tumor recognition to better understand effector functions relevant to TIL therapy. Using co-culture assays, ELISA, bulk mRNA sequencing, and single-cell RNA/TCR sequencing, we observed that GzmB dominated both the transcriptomic and secretory responses of tumor-reactive CD8⁺ TILs, accounting for over 60% of total Gzm secretion at early timepoints. While GzmA and GzmM were also released, their secretion occurred partly independently of tumor recognition, suggesting constitutive or inflammation-driven release. GzmH secretion was minimal and correlated weakly with GzmB, whereas GzmK was largely absent. Single-cell analyses confirmed that tumor-reactive T cells strongly co-expressed GZMB and established activation markers (e.g., TNFRSF9, IFNG), while non-tumor-reactive or interferon-responsive cells were enriched for GZMA/K/M. These findings reveal a distinct Gzm usage hierarchy in CD8⁺ TILs, highlighting GzmB as the principal effector associated with tumor-specific cytotoxicity. Our results provide mechanistic insights into TIL-mediated tumor killing and suggest potential strategies to enhance therapeutic potency by modulating GzmB expression and secretion in TIL products.

P08

Exercise as combination partner for cancer immunotherapy

Fresnillo Saló S[1], Kurzay A[1], Viet Luu T[1], Yixin Fjæstad K[1], Hargbøl Madsen D[1,2], Holmen Olofsson G[1], thor Straten P[1]

[1]National Center For Cancer Immune Therapy (CCIT-DK), [2]Department of Immunology and Microbiology, Copenhagen University

Theme: Cancer immunology

It is widely recognized that exercise reduces the risk of cancer and disease recurrence. Yet the mechanisms behind these benefits remain to be elucidated. We previously showed that voluntary wheel running in tumor-bearing mice reduced tumor incidence and growth by more than 60% across different tumor models. Moreover, this effect was mediated via exercise-associated increases in serum epinephrine levels, which in turn led to immune cell mobilization and enhanced immune cell infiltration into tumors (Pedersen, 2016). Thus, exercise may represent a simple means to improve immune-mediated tumor cell killing and, more broadly, immunotherapy.

To assess the effect of exercise on tumor growth, three different models were used (subcutaneous, spontaneous, and chemically induced). Mice were divided into an exercise group, which was provided with a running wheel, and a sedentary group. Activity and tumor growth were monitored throughout the studies and flow cytometry was used to assess immune infiltration.

Our data showed that voluntary exercise affected tumor growth differently across models. In the MC38 model, exercise enhanced survival when in combination with checkpoint inhibitor (CPI) therapy. Further analyses are underway to investigate the mechanisms underlying this improved response.

P09

Investigating Immune Profiles in Head and Neck Mucosal Melanoma: A Foundation for Personalized Treatment Approaches

Mogensen D[1,2], Eriksen P[4], Svane I[3], Lelkaitis G[2], Heegaard S[2], von Buchwald C[1]
[1]Dept. of Otorhinolaryngology & Head-and-neck Surgery, Rigshospitalet, University Hospital of Copenhagen, [2]Dept. of Pathology, Rigshospitalet, University Hospital of Copenhagen, [3]Dept. of Oncology, Herlev Hospital, University Hospital of Copenhagen, [4]Dept. of ENT, OUH, University Hospital of Odense

Theme: Cancer immunology

Purpose:
Mucosal melanoma of the head and neck (MMHN) is a rare and aggressive malignancy with poor prognosis and limited treatment options. This study aims to characterize the tumor microenvironment (TME) in MMHN to identify prognostic markers and therapeutic targets that may improve future treatment outcomes.

Methods:
We will analyze tumor samples from 183 patients diagnosed with MMHN between 2003 and 2023. Tissue microarrays (TMAs) will be constructed from formalin-fixed paraffin-embedded (FFPE) specimens, sampling intratumoral, peripheral, and adjacent non-tumor regions. Multiplex Immunofluorescence (mIF) combined with RNAscope will be used to assess immune cell populations and cytokine expression. Markers include CD45, CD14, CD68, CD20, CD4, CD8, Ki67, and selected cytokines. Spatial immune profiling will be performed to quantify immune cell clustering and activity, and findings will be correlated with clinical outcomes such as treatment response and disease progression.

Expected Results and Conclusions:
While results are pending, this study is expected to provide novel insights into the immune landscape of MMHN. By investigating the role of immune cell organization and activity, including potential Tertiary Lymphoid Structures (TLS), the project aims to inform personalized immunotherapy strategies and support the inclusion of MMHN patients in future clinical trials. Findings may also have translational relevance for other cancer types with similar immune profiles.

Conflict of Interest:
The authors declare no conflicts of interest.

The Transition from CHIP to Overt Myeloproliferative Neoplasms: A 13-Year Longitudinal Study of JAK2 Positive Citizens

Löffler L[1], Davidsen I[1], Skov V[1], Kjær L[1], Bech S[1], Eickhardt-Dalbøge C[1], Christensen S[1], Knudsen T[1], Sørensen A[1], Dahl M[2,3], Nielsen C[2,4], Larsen M[1], Ellervik C[2,3,5], Hasselbalch H[1,2]
[1]Department of Hematology, Zealand University Hospital, [2]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, [3]Department of Clinical Biochemistry, Zealand University Hospital, [4]Institute for Inflammation Research (IIR), Department of Rheumatology and Spine Diseases, Capital Region University Hospital, [5]Department of Laboratory Medicine, Boston Children's Hospital & Harvard Medical School

Theme: Cancer immunology

Purpose: The JAK2V617F mutation (JAK2) is causing clonal hematopoiesis of indeterminate potential (CHIP) in 3.1% of the population. JAK2 CHIP increases the risk of blood cancers such as myeloproliferative neoplasms (MPNs) and promotes chronic inflammation, potentially accelerating disease progression. This study aimed to investigate risk factors and characterize temporal dynamics of progression from CHIP to MPN.

Methods: 19,985 citizens were enrolled in The Danish General Suburban Population Study between the years 2010 and 2013. Retrospective analysis identified 599 citizens with JAK2 CHIP, of which 92 had a variant allele frequency (VAF) ≥1%. Among these, 39 progressed to MPN. Nineteen of the incident MPN cases were identified by screening, while 20 were diagnosed following referral by their general practitioner prior to the screening study. Data included clinical records, blood counts, lifestyle factors, and bone marrow biopsies. Statistical analyses used mixed-effects models and distributional tests.

Results: Progression to MPN was associated with higher baseline VAF and blood counts. The annual VAF increases were 1.2% in the group identified via screening and 4.7% in the group identified by referral, respectively. Patients identified by screening were diagnosed at lower VAF (median 13% vs 36.6%), with lower platelet and leukocyte counts, and less bone marrow fibrosis at diagnosis compared to cases diagnosed by referral.

Conclusion: Screening for JAK2 CHIP carriers allows earlier MPN diagnosis with lower fibrotic burden, offering potential benefits for both patients and healthcare systems by enabling earlier interventions, reducing complications, and improving long-term outcomes.

P11

Immunological and Clinical Insights into Canine Immune Thrombocytopenia in Copenhagen: Prevalence, Inflammatory Profiling, and Genetic Footprint

Sjödin E[1], Nikolic Nielsen L[1], Langhorn R[1], Kirstine Havnsøe Krogh A[1], Graarup-Hansen Lyngby J[1]

[1]Copenhagen Universityhospital for Companion Animals

Theme: Immune regulations

The purpose of this abstract is to present the ongoing work of the PhD project regarding immune thrombocytopenia (ITP) in dogs.

ITP is one of the most common canine acquired disorders of primary hemostasis and can be subtyped as either primary or secondary. Although the overall survival rate for ITP is 70-80%, only 50% of dogs are alive after two years, suggesting that unknown factors in the immunological process might yet be to be discovered.

The aim of this prospective observational study, is to explain the immunological processes behind ITP in dogs. The objective is to map anti-platelet antibodies (APA) as well as investigating the T-cell response. Additionally, specific microRNA profiles and their relationship with the immune system will be examined.

The study population will consist of 40 dogs with primary and secondary ITP, also including 10 dogs with thrombocytopenia of non-immune origin. Flow cytometry will be applied for the detection of APA, and CD antibodies of canine leukocyte subgroups. MicroRNA will be quantified by real-time qPCR. Level of fold-changed miRNAs of dogs with both forms of ITP will be compared to other baseline parameters such as clinical bleeding score, routine inflammatory biomarkers, time to therapeutic response, remission, and overall survival.

The overall hypothesis is that combined innate and adaptive immune reactions exist in relation to the development of ITP in dogs, and that these are measurable, with specific immunological profiles associated with the subtype of disease as well as outcome.

P12

Immunological characterization of atopic dermatitis

Lindell A[1], Heinrichsen A[2], Skov L[2], Coquet J[1]

[1]Department of Immunology and Microbiology, University of Copenhagen., [2]Department of Dermatology and Allergy, Copenhagen University Hospital – Herlev and Gentofte, Copenhagen, Denmark

Theme: Immune regulations

Atopic dermatitis (AD), is an inflammatory skin disease which affects individuals of all ages. The pathology involves genetic, immunological, and environmental factors, leading to a compromised skin barrier and an exaggerated immune response. Understanding the underlying mechanisms is crucial for developing effective treatments and management strategies to alleviate the disease burden. The aim of this study is to characterize the immunological profiles in lesional, non-lesional, and dupilumab treated skin from AD patients.

Skin biopsies were obtained from individuals diagnosed with AD and healthy controls. The skin was digested and stained for flow cytometry and cell sorting. For analysis, 2 antibody panels were used: one to analyze the cellular composition of innate and adaptive immune cells in the skin, and one to assess cellular functions through cytokine production in T cells and innate lymphoid cells.

The immunological profile of AD skin demonstrated several key differences compared to healthy skin. Specifically, lesional and treated skin showed an increased ratio of CD4+ relative to CD8+ T cells when compared to non-lesional and healthy skin. AD skin was characterized by a higher proportion of Th2/Tc2 and cells and a reduced proportion of Th1/Tc1 cells when compared to healthy controls. Finally, IL-22 production was notably increased in AD skin and associated with IL-13-producing Th2 cells but not with those producing IL-4.

This study highlights the immunological changes in AD, particularly within the T cell compartment. The increased presence of Th2/Tc2 cells and IL-22 underscores the role of these immune pathways in sustaining inflammation.

P13

Exploiting film-forming properties of poly(N-vinylcaprolactam) for cutaneous delivery of tofacitinib citrate to treat atopic dermatitis

Gregersen C[1,2], Bērziņš K[3], Heinz A[2], Nielsen H[1]

[1]Center for Biopharmaceuticals and Biobarriers in Drug Delivery (BioDelivery), Department of Pharmacy, University of Copenhagen, [2]LEO Foundation Center for Cutaneous Drug Delivery (LFCCDD), Department of Pharmacy, University of Copenhagen, [3]Center for Pharmaceutical Data Science Education (CPDSE), Department of Pharmacy, University of Copenhagen

Theme: Immune regulations

Purpose: Poly(N-vinylcaprolactam) is gaining increasing focus for its potential in biomedical applications. Poly(N-vinylcaprolactam) is a thermoresponsive polymer with proposed film-forming properties, making it suitable for cutaneous drug delivery. Janus kinase-inhibitors like tofacitinib citrate are promising in treating atopic dermatitis but rely on local delivery to minimize systemic side effects. This study aims to characterize the thermoresponsive properties of poly(N-vinylcaprolactam) and assess its potential in film-formation and cutaneous delivery of tofacitinib citrate in an atopic dermatitis-like skin model.

Methods: Thermoresponsive properties of poly(N-vinylcaprolactam) were investigated using ultraviolet-visible spectrophotometry and Raman spectroscopy. Skin studies were performed in Franz diffusion cells mounted with porcine skin, impaired by tape stripping to mimic atopic dermatitis-like skin. Poly(N-vinylcaprolactam) and tofacitinib citrate were dissolved in ethanol and applied to the skin, allowing solvent evaporation to drive film formation. Drug diffusion was monitored continuously for 24 hours, and drug retention in the skin was evaluated after 24 hours.

Results: Poly(N-vinylcaprolactam) exhibit thermoresponsive behaviour near skin temperature at certain polymer concentrations. Poly(N-vinylcaprolactam) displays excellent film-forming properties on skin and facilitates tofacitinib citrate delivery to atopic dermatitis-like skin. Tuneability between diffusion and retention can be obtained by varying poly(N-vinylcaprolactam) content in the film forming system.

Conclusions: The thermoresponsive nature and tuneable drug release profiles support further exploration of poly(N-vinylcaprolactam) for cutaneous delivery of Janus kinase-inhibitors to improve atopic dermatitis treatment.

Acknowledgements: The authors acknowledge Jesper Skovsgaard Nielsen for conducting the Franz diffusion cell studies. The authors would also like to thank the LEO Foundation for financially supporting the project.

P14

Early BCG Vaccination is Potentially Beneficial for Preterm Infant Immune Systems

Praneviciute A[1], Nguyen N[1], Bæk O[1]

[1]University of Copenhagen, Comparative Pediatrics and Nutrition

Theme: Immune regulations

Bacillus Calmette-Guérin (BCG) is a live vaccine of Mycobacterium bovis that prevents tuberculosis (TB). Since its introduction in 1921, BCG is still widely used. Currently, most BCG vaccines are administered shortly after birth to individuals in TB-prone areas. However, many preterm infants get delayed vaccinations. Health officials are concerned that live vaccines may cause disseminated infection in infants with weak, underdeveloped immune systems. Animal studies, however, demonstrate possible immune benefits from early BCG vaccination. Recent mouse studies indicate that BCG prevents non-TB-related neonatal sepsis by inducing emergency granulopoiesis. This effect may be especially beneficial for immunocompromised infants; unfortunately, few such studies exist. We therefore investigated the effect of BCG on preterm newborn pigs, an animal model physiologically similar to humans. Firstly, BCG was administered shortly after birth in C-section-delivered preterm pigs. Then, pigs were sacrificed either 1, 2, or 4 days after vaccination for examination of tissue and various immune cell types. According to our results, all animals in all experiments were clinically unaffected by BCG vaccination. After 1 day, preterm BCG-vaccinated pigs had statistically differential gene expression related to granulopoiesis, including increased expression of CD16. After 2 days, preterm BCG-vaccinated animals showed increased neutrophil and basophil counts. After 4 days, preterm BCG-vaccinated animals showed increased spleen weight and decreased monocyte counts. Our results indicate that BCG vaccination for preterm newborns is not harmful and may be beneficial, helping mature underdeveloped immune systems. Next, we intend to investigate whether BCG may improve immune response to infections.

P15

Heterogeneous generation and expansion of epidermal-resident memory T cells in patients allergic to nickel

Stegenborg-Grathwohl S[1], Yeung K[1,2], F. Weber J[1], Bischofberger N[1], Vaher H[1], Jacobsen J[3], Sørensen F[4], Khodosevich K[4], Coquet J[1], Geisler C[1], Bonefeld C[1], Skov L[2,5]

[1]The LEO Foundation Skin Immunology Research Center, Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, [2]Department of Dermatology and Allergy, Copenhagen University Hospital - Herlev and Gentofte, [3]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, [4]Biotech Research and Innovation Centre, Faculty of Health and Medical Sciences, University of Copenhagen, [5]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenha-gen

Theme: Immune regulations

Background: Nickel is the most frequent cause of allergic contact dermatitis (ACD). Recent stud-ies have shown that skin-resident memory T (TRM) cells play a central role in ACD. However, how TRM cells accumulate and differentiate in the epidermis of allergic individuals repeatedly exposed to nickel is not known.

Methods: Twelve participants with nickel allergy were patch-tested three times with nickel with a 21-day interval applying the patch tests to the same skin sites each time. 48 hours after each patch test, the skin reaction was assessed and clinically scored. 21 days after each patch test, skin biop-sies were taken and analysed by flow cytometry and single-cell RNA sequencing (scRNA-seq).

Results: The number of CD4+ and CD8+ T cells in the epidermis and the clinical score increased with each exposure to nickel. 90-95% of the T cells were CD69+ TRM cells. We found expanded Th2 and Th17-like CD4+ and cytotoxic CD8+ TRM cell clones in all participants; however, CD4+ TRM cells dominated in some participants, whereas CD8+ TRM cell dominated in others. As a com-mon feature, all participants exhibited a polyclonal Treg cell population and exhausted-liked popu-lations of CD4+ and CD8+ TRM cells and Treg cells in the epidermis.

Conclusions:
In this study we determined the complexity of epidermal T cells in allergic individuals repeatedly exposed to nickel, identifying several distinct clusters of effector and exhausted CD4+ and CD8+ TRM and Treg cells.

P16

The effect of mixtures of skin sensitizers and irritants on the sensitization and elicitation phase of contact allergy.

Seibel A[1], Lohmann R[1], Johansen J[2], Bonefeld C[1]

[1]LEO Foundation Skin Immunology Research Center, Department of Immunology and Microbiology, University of Copenhagen, [2]National Allergy Research Center, Department of Dermatology, Gentofte Hospital

Theme: Immune regulations

Allergic contact dermatitis (ACD) is an inflammatory skin disease caused by repeated exposure to contact allergens. An estimated 21 % of the adult population and 16 % of children and adolescents are sensitized to at least one contact allergen from consumer products and/or exposures in the work environment. Most consumer and occupational products contain several sensitizing chemicals as well as irritants. The combination of multiple contact allergens and/or irritants is known to influence the immune system, however not much is known about how mixtures affect the immunological mechanisms during the sensitization and elicitation phases. Furthermore, no generally accepted risk assessment model for mixtures has been established in the EU, meaning that many skin sensitizing and irritating mixtures remain uncontrolled. Therefore, we seek to investigate the impact and the mechanisms of mixtures on skin sensitization and elicitation as well as to develop predictive models and test systems. Additionally, in light of increasing interest in alternatives for animal testing of chemicals, we aim to establish the use of in vitro New Approach Methods such as H-Clat and KeratinoSens for the assessment of mixtures. We also seek to develop different 3D skin models, which will allow us to gain more insights into the mechanisms of skin sensitization and irritation by mixtures compared to current 2D cell models.

P17

Developing Molecular Probes to Identify and Characterize Superantigen-Responsive (SupeR)-T cells in Atopic Dermatitis at the Single-cell Level.

Ewing E[1], Blümel E[1], Buus T[1]

[1]LEO Foundation Skin Immunology Research Center, Department of Immunology and Microbiology, University of Copenhagen

Theme: Immune regulations

Staphylococcus aureus (S. aureus) colonization contributes to the worsening of the inflammatory skin disease, atopic dermatitis (AD), through the production of various toxins and proteases, including superantigens (SAgs). SAgs activate T cells by cross-linking MHC-II molecules on antigen-presenting cells with specific T-cell receptor (TCR) V-chains, bypassing peptide presentation and inducing widespread polyclonal T-cell expansion.

Previous studies have shown that AD patients exhibit an enrichment of T cells expressing TCR-V-chains associated with SAgs as compared with healthy controls. However, the precise role of these SAg-responsive (SupeR)- T cells in AD remains poorly understood due to the lack of tools to distinguish and study how these cells differ from other activated T cell subsets within the inflammatory microenvironment.

This study aims to develop and validate a novel tool to distinguish SupeR-T cells by engineering molecular probes that bind directly to the SupeR-T cells but not to other by-stander T cells. These SupeR-T cell probes will be optimized for high-resolution single-cell and spatial analyses, enabling detailed characterization of SupeR-T cell dynamics and functions in situ and ex vivo. We plan to use this tool to elucidate the role of SAgs in the development and exacerbation of AD lesions using both mouse models and human skin explants. Beyond AD, this approach has broader implications, as it can be adapted to investigate other T-cell subsets in diverse diseases where SAgs play a role.

P18

The role of stromal aryl hydrocarbon receptor activity in controlling colonic immune responses in homeostasis and disease

Ashworth C[1], Junghus F[1], Papalamprou F[1], Brown A[1], van Tilburg Bernardes E[1], Schussek S[2], Pæregaard S[2], Kotarsky K[3], Agace W[1]

[1]Københavns Universitet, [2]Danish Technical University, [3]Lunds Universitet

Theme: Immune regulations

The intestinal lamina propria (LP) harbours a substantial population of mesenchymal stromal cells (MSCs), including fibroblasts, pericytes, and smooth muscle cells. These tissue-resident, non-immune cells provide structural support and secrete growth factors that support the epithelium. Notably, they are situated in close proximity to immune cells, yet their role in modulating intestinal immunity remains incompletely defined. One candidate regulator is the ligand-dependent transcription factor aryl hydrocarbon receptor (AHR). AHR, which recognises various dietary, microbial, and endogenous metabolites, is an evolutionarily conserved environmental sensor expressed in epithelial, endothelial, and immune-cell subsets. Using single-cell RNA sequencing (scRNAseq), we found that AHR is highly expressed in distinct colonic MSC subsets, primarily in $CD9^+$ subepithelial fibroblasts (sFBs). These AHR^+CD9^+ sFBs show enrichment for genes involved in immune cell survival, maintenance, recruitment, and AHR signalling pathways. Mice lacking AHR in MSCs displayed altered MSC populations, including reduced numbers of $CD141^+$ sFBs, along with shifts in immune cells, such as increased numbers of induced regulatory T cells (iTregs), classical dendritic cells (cDC1 and cDC2), neutrophils, and P2 monocytes. Moreover, MSC-specific AHR deficiency conferred heightened susceptibility to dextran sodium sulfate (DSS)-induced colitis. Ongoing work is investigating the role of MSC-expressed AHR in supporting the epithelial stem cell niche and promoting epithelial differentiation in MSC-epithelial organoid co-cultures. Additional scRNAseq analyses of MSC-AHR-deficient mice under homeostatic and inflammatory conditions, including stromal-immune interaction inference, are underway. Together, these findings identify MSC-expressed AHR as a critical regulator of intestinal homeostasis and highlight its importance in protecting against inflammation-induced colonic injury.

P19

Weaning-associated modulation of the jejunal immune barrier in piglets studied by multiplex immunohistochemistry

Brandt Becker C[1], Goldstein S[2], Mikulski Z[2], Elvang Jensen H[1]

[1]Department for Veterinary and Animal Sciences, University Of Copenhagen, [2]La Jolla Institute for Immunology

Theme: Immune regulations

Purpose:
Post-weaning diarrhea (PWD) is a major health challenge in pig production, often leading to impaired animal welfare, economic losses, and antibiotic use. A better understanding of how weaning affects the intestinal immune barrier may help developing strategies to reduce disease risk and antibiotic use. The purpose of this study was to investigate weaning-associated changes in jejunal immune cell populations, with focus on the differences between resilient piglets and piglets with diarrhea.

Methods:
Jejunal tissue samples from 50 piglets were collected at three time points: one day before weaning (day 25), and at three- and six-weeks post-weaning, respectively, including both resilient and diarrheic animals. Samples were fixed in 10% neutral buffered formalin and processed for multiplex immunohistochemistry using the Orion RareCyte platform. A 10-plex antibody panel (Hoechst (nuclear stain), CD163, CD11c, porcineCD3e, humanCD3e, Granzyme B, FOXP3, CD79a, Ki67, and IRF4) was used for simultaneous and spatial characterization of macrophages, dendritic cells, T-cell subsets, B-cells, plasma cells, and proliferating cells.

Results:
Image acquisition is complete and quantitative analyses are ongoing. The study is expected to reveal temporal and health-status-associated differences in the jejunal immune architecture across the three age groups.

Conclusions:
This work will deliver novel insights into how weaning impacts the intestinal immune barrier and its role in susceptibility or resilience to PWD. Improved understanding of these mechanisms may guide alternatives to antibiotic use in pig production, and help mitigate the development and spread of antibiotic resistance.

Conflict of Interest:
The authors declare no conflicts of interest.

P20

Can allergic contact dermatitis be permanently cured?

Dragsbæk-Friis M[1], Menné Bonefeld C[1], Geisler C[1]

[1] LEO Foundation Skin Immunology Research Center, Department of Immunology and Microbiology (UCPH)

Theme: Immune regulations

This PhD project aims to investigate the impact of the anti-inflammatory responses induced by different contact allergens and therapeutics on the generation of tolerance to contact allergens. Contact allergens cause allergic contact dermatitis (ACD), characterized by pruritus, edema and vesicles, which often represent a severe burden for affected patients. Skin-resident memory T (TRM) cells play a central role in ACD, by contributing to the persistence of inflammation, making the condition difficult to manage. During sensitization, antigen-presenting cells capture allergens and migrate to lymph nodes, presenting modified self-peptides to naïve T cells. This triggers the formation of allergen-specific effector and memory T cells. Although epidermal CD8+ TRM cells seem to play the major pathogenic role in ACD, CD4+ T cells have been ascribed both inflammatory and anti-inflammatory roles in ACD. While many studies focus on preventing sensitization, patients with ACD are already sensitized when they develop symptoms. Therefore, this project aims to determine whether tolerance can be induced in established cases of ACD, potentially leading to a lasting cure.

P21

Efficacy of Dupilumab in Patients with Severe Asthma and Co-existing COPD

Ameri S[1], Hansen S, Sverrild A

[1]Bispebjerg Hospital

Theme: Immune regulations

Introduction
Dupilumab, a monoclonal antibody targeting IL4-Rα, has demonstrated efficacy in reducing exacerbations in both severe asthma (SA) and chronic obstructive pulmonary disease (COPD). SA is often late-onset, and many patients have coexisting COPD. These patients are, however, generally excluded from clinical trials, and hence little is known about their benefit from anti-IL4-Rα therapy.

Aim
Compare treatment responses to anti-IL4-Rα therapy in patients with combined SA+COPD, versus patients with SA only.

Methods
DSAR is a complete nationwide prospective cohort of patients with SA receiving biologics. All patients with at least one year of complete follow-up were included, and stratified according to coexisting COPD, defined as FEV1)/FVC) < 0.70, a tobacco exposure of at least 10 pack-years and age > 40 yr.

Results
A total of 390 patients were included, 24% (93 patients) had co-existing COPD. After 12 months of therapy, the mean reduction in exacerbations was comparable among SA+COPD vs SA only patients: 49.5% (45.14) vs 59.6% (51.6), p=0.10. Mean mOCS daily dose was reduced by 3.1 (3) in SA+COPD and 4.51 (8.75) in SA, p=0.46. Mean ACQ-6 score improved by 0.93 (1.25) in SA+COPD vs. 0.90 (1.25) in SA only, p=0.80. However, the proportion of patients with a complete response (no exacerbations and no mOCS after 12 months) was less among SA+COPD patients (51.2% vs 71.9%, p=0.0005).

Conclusion
Comorbid COPD in patients with SA is common and does not reduce the efficacy of dupilumab on exacerbations, mOCS dose or symptom-scores, but may decrease the chance of complete response.

P22

Polyphenol - Microbiota Interactions in Modulating Mucosal Immunity During Parasitic Infection

Zhang T[1], Williams A, Høyland-Kroghsbo N

[1]University of Copenhagen

Theme: Immune regulations

Parasitic infections pose a major challenge to livestock production globally, causing significant economic losses worldwide and placing increasing pressure on already strained agricultural systems. These infections can dysregulate the host intestinal mucosal immune system, leading to repeated infections and chronic intestinal inflammation.

Dietary polyphenols have demonstrated potential to improve gut health and enhance resistance to enteric infections. However, their biological effects are largely mediated by gut microbial metabolism. This project aims to decipher how specific gut bacteria, such as Lactobacillus plantarum, interact with polyphenols to produce immunomodulatory metabolites.

Using bacterial culturing, transcriptomics, metabolomics, and CRISPR-based gene editing, we will identify key metabolic pathways involved in polyphenol metabolism. Subsequently, in vitro assays will assess whether polyphenol bacteria combinations exhibit direct anthelmintic or antibacterial activity and how they affect intestinal and immune cells. Finally, controlled feeding trials in mice infected with intestinal parasites will be conducted to determine whether synbiotic treatments (polyphenols plus selected bacteria) can reduce parasite burden and enhance mucosal immune responses.

This interdisciplinary project combines microbiology, nutrition, and immunology, offering a novel approach to developing sustainable, diet-based interventions against parasitic infections in animals and potentially humans.

P23

Impact of Fiber-Free Diet on Type-2 Immunity Against Helminth Infection Through Alterations of the Gut Microbiome and Metabolome in Pigs.

Klein-ipsen P[1], Sharma M[1], Jessica Myhill L[1], Richard Williams A[1]

[1]University Of Copenhagen

Theme: Parasitology

Background: Parasitic worm (helminth) infections affect more than a billion people worldwide, resulting in increased malnutrition and morbidity. Immunity against helminths relies on the type-2 (Th2) immune response. However, the fundamental regulatory mechanisms modulating this response remain unanswered. Recent research focuses on the roles of diet and microbiome, as both regulate inflammatory reactions. Previous research indicates that mice fed non-starch polysaccharides (NSP)-free (i.e. fiber-free) diets have increased immunity against Trichuris muris, whereas NSP-rich diets hamper helminth expulsion. This is very different than the common perception that a fiber-rich diet should have a beneficial effect on the host immune function. We therefore found it relevant to conduct the same experiments in pigs, as their immune system and physiology is more translatable to humans. Thus, this study aims to gain a better understanding of the mechanisms modulating the immune-gut microbiome and metabolome interactions in Trichuris suis infected pigs fed fiber-free diets.

Methods: Eight-week-old female pigs (n=41) on NSP-rich or NSP-free diets were infected with T. suis and studied for 4 or 7 weeks post-infection. Analysis included flow cytometry, transcriptomics, microbiome profiling, metabolomics, histology and counting worm burdens.

Results: Data analysis shows that pigs on an NSP-free diet had significantly higher worm burdens, T-reg cell response, colonic pH, and gene expression than those on an NSP-rich diet, highlighting fiber's impact on immunity, the gut microbiome and metabolome. Further analysis is underway to explore interactions between diet, host- and diet-derived metabolites, microbial taxa, immunity, and T. suis infection.

P24

The Impact of Various Diets on Type 2 Immunity against Enteric Helminth Infection through Alterations of the Microbiome and Metabolome

Vedsted-jakobsen A[1], M. Jensen A[1], J. Myhill L[1], M. Thamsborg S[1], T. Limborg M[1], R. Williams A

[1]University Of Copenhagen

Theme: Parasitology

Background: Helminth infections affect a quarter of the global population, causing malnutrition and increased morbidity. Protective immunity against helminths depends on a robust type-2 immunity. Recent attention has turned towards the roles of the microbiome and the diet as potential regulators of the immune response. Evidence indicates that diets rich in soluble plant fibres lead to chronic infection. Host- and microbiome-derived metabolites seemingly influence the host's type-1/type-2 immune balance. Nevertheless, the fundamental mechanisms driving these effects remain unexplored.

Aim: We aim to elucidate how metabolites and microbiome influence shifts in immunity against T. muris. We seek to identify metabolites responsible for the altered immunity arising from distinct diets and their associated changes in infection.

Methods: We conducted a study involving dietary interventions with soluble fibres or polyphenols before and during T. muris infection. Comprehensive metabolomic analysis and 16SrRNA sequencing were performed to discern differences in the effects of various diets. The worm burden and serum antibody responses assessed the infection state and immune response.

Results: Preliminary findings indicate substantial influence from both diet and infection on the metabolome. Ongoing analysis aims to illuminate the interconnection between gut metabolites, microbial taxa, host immunity and infection.

P25

Ongoing evolutionary patterns on the pfmdr1 hinge region and association with anti-malarial resistance mutations in P. falciparum

Filtenborg Hocke E[1,2], Hansson H[1,2], Chopo-Pizarro A[3], Wellmann-Thomsen L[1,2], Mesia Kahunu G[4], Muhindo Mavoko H[5], Mandoko Nkoli P[6], Mukomena Sompwe E[7], Mbongi D[8], Mitashi P[5], Mbulli Ali I[9,11], Nji A[9,10], Mbacham W[9,10], Moussiliou A[12], Dandonougbo C[13], Affoukou C[13], Akogbeto M[12], Ngufor C[12], Beshir K[3], Mousa A[3], Roper C[3], J. Sutherland C[3], Alifrangis M[1,2]

[1]Centre for Translational Medicine and Parasitology, Department of Immunology and Microbiology, [2]Department of Infectious Diseases, Copenhagen University Hospital, [3](3) Department of Infection Biology, Faculty of Infectious and Tropical Diseases, [4]Department of Pharmacology and Therapeutics, University of Kinshasa, [5]Department of Tropical Medicine, University of Kinshasa, [6]National Institute of Biomedical Research, [7]University of Lubumbashi, National Malaria Control Program, Ministry of Health, [8]Centre Hospitalier Monkole, [9]The Biotechnology Centre, University of Yaounde I, [10]The Center for Health Implementation and Translational Research (CHITRES), The Fobang Institutes, [11]Department of Biochemistry, Faculty of Science, University of Dschang, [12]Centre de Recherche Entomologique de Cotonou (CREC), Laboratoire Nationale, Ministère de la Santé, [13]Programme National de Lutte contre le Paludisme (PNLP), Ministère de la Santé

Theme: Parasitology

The Plasmodium falciparum multidrug resistance-1 (pfmdr1) gene, encoding the ABC P-glycoprotein homolog PgH1, accumulates mutations that are associated with altered parasite susceptibility to multiple antimalarial drugs, namely N86Y, Y184F, and D1246Y. Among these drugs are artemisinin-based combination (ACT) therapies, which combine a fast-acting but rapidly clearing artemisinin with a longer-lasting partner drug. The in vitro resistance effect of each of these differs depending on the pfmdr1 genotype of the treated parasites.

The aim of this study was to examine high-throughput sequence diversity in the pfmdr1 hinge domain in population samples from three sub-Saharan African countries: Cameroon, the Democratic Republic of Congo, and Benin, after two decades of ACT use. Associations between hinge domain sequences and the point mutations at codons 86, 184 and 1246 were compared with similar data collected before or in the very early days of ACT use.

A total of 780 samples yielded a positive sequencing signal, and a high diversity of Asn-R repeats was observed across the samples. Several unknown hinge variants were uncovered, particularly in Cameroon and DRC. We found a significant correlation between the 7-2-9 variant and the 86Y mutation ($p<0.005$, OR = 3.54), and likewise a significant correlation of the 7-2-9 variant with 1246Y ($p<0.005$, OR=12.6). We also uncovered, when examining haplotypes, that the more mutations present, the less diversity was observed in the hinge region. The data demonstrates evolution in action, suggesting an ongoing selective sweep as a consequence of post-ACT introduction in Africa.

P26

Preimmunization-Induced Trained Immunity Modulates Diet-Dependent Resistance to Intestinal Helminths in Mice

von Münchow A[1], Wang Henriksen I[2], Sharma M[1], Maigaard A[1], Kornerup Hansen A[2], Thamsborg S[1], Williams A[1]

[1]University of Copenhagen, Section for Parasitology and Pathobiology, [2]University of Copenhagen, Section for Biomedicine

Theme: Parasitology

Specific pathogen-free (SPF) mice, commonly used in immunological, dietary, and parasitic research, exhibit a naïve immune status due to limited trained immunity. Our group has shown that a semi-synthetic, fermentable fiber-free diet enhances Th2 immune responses compared to grain-based chow, leading to increased resistance to Heligmosomoides polygyrus and Trichuris muris infections. To explore the role of trained immunity, models such as wildlings, rewilded mice, and preimmunized mice, exposed to diverse microbiota or inactivated pathogens, have been developed. This study investigates how preimmunization-induced trained immunity affects anti-helminth responses in mice fed either a Th2-promoting semi-synthetic diet (SSD) or a Th2-dampening grain-based chow, hypothesizing that trained immunity increases susceptibility to infection, particularly in chow-fed mice.

Preimmunized mice showed reduced Trichuris muris worm counts across both diets, with a greater reduction in chow-fed mice. Infection induced increased inflammation and Th2 cytokine expression, more pronounced in SSD-fed mice, alongside a decrease in Th1 cell proportions.
In Heligmosomoides polygyrus-infected preimmunized mice, IFNγ expression increased, while IL-5 was elevated in SSD-fed mice and reduced in chow-fed mice. Uninfected controls exhibited lower central memory cell proportions, which were slightly elevated in infected mice.

Immune training through preimmunization decreased susceptibility to Trichuris muris infection across both diets. This was evident by decreased worm counts, increased expression of Th2 cytokine, and decreased proportion of Th1 cells. Even so, mice fed synthetic diet exhibited more pronounced effects on immunological read outs than mice fed chow.

P27

Sustainable saponins for next-generation vaccine adjuvants

Dmowska-Pawłowska D[1,2]

[1]Croda Pharma, Vaccine Adjuvant Systems R&D, [2]Department of Health Technology, DTU

Theme: Vaccine design

Saponin-based adjuvants have emerged as critical components in the development of effective vaccines against airway infections, as demonstrated by their inclusion in formulations such as AS01 in the RSV vaccine (GSK) and Matrix-M in influenza and COVID-19 vaccines (Novavax), which utilize QS-21–a saponin mixture derived from Quillaja saponaria evergreen tree native to South America. QS-21 is a strong immunopotentiator; however, its natural abundance in the tree bark extracts is low and overexploitation of natural resources poses serious ecological and economic threats. As a result, saponins are considered both unsustainable and difficult to produce in large quantities. As respiratory infections such as RSV and influenza, continue to pose global public health challenges, the scalability and high cost of adjuvants can hinder quick and widespread vaccine development, particularly during outbreaks or pandemics. Among natural saponins, semisynthetic alternatives such as Momordica saponins (VSAs), extracted from the widely available and inexpensive seeds of gac fruit, are investigated as promising, cost-effective analogs to Quillaja saponins. Unlike QS-21, which depends on limited tree bark harvesting, VSAs benefit from its robustness and scalability, as the seeds are an abundant by-product of fruit consumption. In this study we investigated the cytotoxicity of VSA saponins compared to QS-21, and how we can quench their toxicity by incorporating them into nanoparticulate formulations. This research is particularly relevant for the development of next-generation vaccines targeting airway infections, where safe, scalable and affordable adjuvants are critically needed.

P28

Design of next-generation lipid nanoparticles for mRNA vaccines: Enhancing potency and safety by incorporation of adjuvants

Stratoudaki M[1,2,3], Münter R[2], Schmidt S[1], Pedersen G[1,3]

[1]Statens Serum Institut, [2]Croda Pharma, [3]University of Copenhagen

Theme: Vaccine design

Current lipid-based mRNA delivery systems were not originally designed to elicit the immunostimulatory responses required for strong, long-lasting immunity, nor to modulate the immune fingerprint of vaccines. A major challenge is that many classical immunomodulators activate pathways that inhibit mRNA translation. Therefore, the objective of this PhD project is to design lipid nanoparticle (LNP) formulations specifically optimized for RNA vaccine delivery. The key innovation is the incorporation of next-generation saponins, with the aim of safely inducing a potent adjuvant effect, without compromising transfection efficiency. Ultimately, this approach is expected to enhance vaccine efficacy, allowing for dose sparing and improved safety.

A series of Firefly luciferase (Fluc) mRNA-loaded LNPs carrying the immunostimulatory saponin QS-21 were successfully formulated. Formulations were based on the established Spikevax™ lipid composition (SM-102:DSPC:Cholesterol:DMG-PEG2000:DiD, 50:10:38.5:1.5:0.05 mol%). QS-21 was introduced either during formulation (in the aqueous or organic phase) or by post-insertion into fully-formed LNPs. Addition of QS-21 did not impact nanoparticle quality, as confirmed by DLS (z-average size) and Ribogreen assay (encapsulation efficiency). Initial in vitro studies demonstrated that Fluc-mRNA-LNPs containing 0.5–5 mol% QS-21 can successfully transfect C2C12 and THP-1 cell lines, without significant cytotoxicity. Notably, in THP-1 cells, LNPs containing 1.5 mol% QS-21 exhibited higher transfection levels than the benchmark Spikevax™ LNPs, as measured by luciferase assay. These promising results provide a strong basis for further investigation, before evaluating the novel adjuvanted formulations in vivo for their immunostimulatory effects.

P29

De Novo Protein Design with RFdiffusion: A New Paradigm for Rational Vaccine Antigen and Adjuvant Development

Bardino Mørch M[1], Erbs G[1], Toft Jakobsen J[1], Jungersen G[1]

[1]Statens Serum Institut

Theme: Vaccine design

The majority of licensed vaccines currently on the market are based on recombinant proteins or pathogens that are inactivated or attenuated. Despite great success, traditional antigen discovery faces many challenges such as variant escape, limited breadth of induced antibodies, and off-target epitopes. For adjuvants, few are approved for human use and the mechanism of many remains poorly understood. These limitations highlight the need to move beyond trial-and-error vaccine development and towards a rational and structure-guided design of both antigens and adjuvants.

RFdiffusion is an open-source tool for protein design that uses generative diffusion modeling to refine random noise into an atomic 3D backbone under defined constraints. ProteinMPNN then assigns sequences consistent with the structure, which are validated using AlphaFold.

1. Antigens can be designed through reverse antigen design strategies that incorporate epitope mimicry. This can involve conserving structural constraints of the original antigen or using motif scaffolding to embed a fixed sequence or structural motif from the native antigen into a new protein scaffold.
2. Targeted uptake of antigens can be increased using designed adjuvants. By modeling proteins around the structure of an uptake receptor, RFdiffusion can model binders that enhance uptake. Adjuvants can also be engineered to initiate pro-inflammatory signaling cascades, which generate danger signals and amplify the immune response.

Shifting from vaccine discovery towards vaccine design could help accelerate the development process. This approach offers a novel way of tackling common pitfalls of adjuvant formulations and antigen design.

P30

Spike antibody levels and risk of SARS-CoV-2 reinfection: a two-year cohort study in previously infected adults

Wiwe E[1,2], Jørgensen M[1], Brandt L[1], Talouzi N[1], Roed C[3], Harboe Z[3,4], Fischer T[2,5], Hansen Y[1], Hillig T[1]

[1]Department of Clinical Biochemistry, Nordsjællands Hospital, Copenhagen University Hospital, [2]Department of Public Health, Faculty of Health and Medical Science, University of Copenhagen, , Denmark, [3]Department of Pulmonary and Infectious Diseases, Nordsjællands Hospital, Copenhagen University Hospital, [4]Department of Clinical Medicine, Faculty of Health and Medical Science, University of Copenhagen, [5]Department of Clinical Research, Nordsjællands Hospital, Copenhagen University Hospital

Theme: Vaccine design

Purpose: To assess whether quantitative spike antibody concentrations predict SARS-CoV-2 reinfection risk in previously infected adults.

Methods: We conducted a two-year cohort study of 2,960 convalescent COVID-19 patients in Denmark with >9,000 plasma samples (2020–2022). Antibody levels were measured using Roche Elecsys® assays. Nonlinear mixed-effects models generated weekly antibody estimates, which were linked to reinfection risk in a competing-risk Cox regression adjusted for infection pressure, testing behaviour, comorbidity, and vaccination.

Results: Among 1,600 participants with longitudinal follow-up, 133 reinfections occurred dur-ing a median of 22 risk weeks. Each tenfold (log10) increase in spike antibody level was associated with a 28% lower reinfection hazard (HR 0.72, 95% CI 0.57–0.90, p=0.0045, Fig.2). The association was robust across sensitivity analyses and unaffected by lockdowns or testing shifts. No discrete threshold was identified, indicating a continuous dose–response relationship between antibody level and protection.

Conclusions: Circulating spike antibody levels act as a continuous correlate of protection against SARS-CoV-2 reinfection. Antibody quantification may serve as a pragmatic marker of protection, particularly to identify immunosuppressed patients with low titres who could benefit from revaccination.

Competing interests: Roche Diagnostics supplied reagents free of charge but had no role in the study. Author 6 has received research funding and serves as advisor to Statens Serum Institut, the Danish Vaccination Council, EVASG, and ESCMID. Author 7 chairs the scientific advisory board of the One-Health Institute, Greifswald. Author 8 chairs the Committee on Nomenclature for Properties and Units. All other authors declare no competing interests.

P31

Preclinical influenza vaccine testing in mouse models

Zhou J[1], Steffensen M[1], Antonescu O[2], Kaselyte G[2], Nielsen I[2], Cacciapuoti M[2], Urioste S[2], Soegaard M[2], Buus S[1], Thomsen A[1], Christensen J[1]

[1]Department of Immunology and Microbiology, University of Copenhagen, [2]ExpreS2ion Biotechnologies ApS

Theme: Vaccine design

Introduction
Influenza viruses are highly contagious and contribute to significant global morbidity and mortality. Current seasonal influenza vaccines primarily target the genetically variable hemagglutinin (HA), which requires annual updates and provides limited protection against unpredictable pandemic strains. To achieve increased breadth of protection, a universal vaccine that elicits both B and T cell responses is essential for improving global protection and preventing future pandemics.

Methods
We tried to target conserved influenza antigens, including HA stem, nucleoprotein (NP), and ectodomain of matrix protein 2 (M2e) with protein-based or virus-like particle (VLP) vaccine platforms and screened vaccine constructs in mouse models. Protein design, antigens expression in Drosophila S2 insect cells, purification, VLP coupling, and formulation were done in collaboration with ExpreS2ion Biotechnologies. Immunogenicity was evaluated by measuring systemic and local immune responses, i.e. antibodies, B cells and T cells. Homologous and heterologous protection were assessed by performing influenza challenges with different viral strains.

Results
The HA stem constructs induced robust HA-stem-specific antibody responses, which were enhanced by boosting or by coupling to VLPs. Lung viral titers on day 3 post homologous challenge were significantly reduced after vaccination with HA-stem-VLP. Furthermore, an NP construct, delivered in AddaVax, elicited high level of serum antibodies specific to NP, indicating a strong humoral immune response.

These preliminary data highlight the potential of VLP-based vaccine platforms in presenting conserved viral antigens with enhanced immunogenicity. Further immunological profiling is necessary to ensure the quality of the vaccine-induced immune responses.

P32

Optimizing mRNA-based capsid Virus-Like Particle (cVLP) vaccines: Placental Malaria.

Carmona Fajardo M[1,2], Fougeroux C[2], Rocavert Barranco M[1], Sander Bertelsen A[1,2], Nielsen M[1,2]

[1]Centre for Translational Medicine and Parasitology, Department for Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, [2]AdaptVac Aps

Theme: Vaccine design

cVLPs are self-assembling nanoparticles composed of viral capsid proteins. By mimicking native viruses, they can be used to enhance immunogenicity, acting as molecular scaffolds that display antigens in a repetitive, virus-like format. Building on the success of modular protein-based cVLP-vaccines during the COVID-19 pandemic, this project establishes a versatile mRNA-cVLP vaccine platform.

The approach combines the rapid development capacity of mRNA with the potent immunostimulatory properties of cVLPs. We focus on placental malaria as a urgent global health challenge, where vaccine efficacy is hampered by high sequence variation in the VAR2CSA antigen responsible for sequestering of infected erythrocytes in the placental tissue. To improve antigen stability, immune response magnitude, and durability, a split-protein Tag/Catcher system is employed for efficient antigen-particle conjugation. The mRNA technology may enable mosaic display of sequence variants to enable higher cross-reactivity of the antibody response, which is difficult and expensive to achieve using protein-based vaccine production.

Preclinical work involves vaccine design, optimization, and production, with in vitro assays and animal experimentation. Placental malaria DNA-based vaccine candidates were expressed in HEK293-TT cells, evaluated for antigen coupling and secretion, and subsequently tested in vivo in BALB/c mice for immunogenicity. Promising candidates will undergo parasite neutralization assays, followed by in vitro mRNA production for comparison with DNA-based vaccines.

By merging two transformative vaccine technologies, this project seeks to develop next-generation immunizations with broad medical applications. Success would expand the reach of cVLP vaccines into parasitology, contributing to effective prevention strategies against diverse and urgent health threats.

P33

Development and pre-clinical testing of a Nipah vaccine exploiting the Tag/Catcher-cVLP platform

Rocavert Barranco M[1], Fougeroux C[2], Goksøyr L[2], Hagen S[2], Soegaard M[3], Kohs J[4], Diederich S[4], Balkema-Buschmann A[4], Lokras A[5], Foged C[5], Mordmüller B[6], Nielsen M[2], de Jongh W[2], Sander A[2]

[1]Centre for Translational Medicine and Parasitology, Department for Immunology and Microbiology, University of Copenhagen, [2]AdaptVac Aps, [3]Expres2ion Biotechnologies, [4]Friedrich-Loeffler-Institut, [5]Department of Pharmacy, University of Copenhagen, [6]Radboud University Medical Center

Theme: Vaccine design

The immense health and economic impacts of future epidemics and pandemics have become one of the defining public policies and health issues all over the world. A new urgency is required to understand, rapidly react to, and vaccinate against pathogens to prevent outbreaks. One such pathogen is the zoonotic Nipah virus (NiV), which has a case fatality rate estimated 40-75%. NiV has been classified as a pathogen with epidemic threat by the WHO. However, the current major challenge is the lack of vaccines targeting Nipah for humans.

The protein-based Tag/Catcher cVLP vaccine platform has previously been clinically proven to facilitate unidirectional and high-density capsid-like antigen display, resulting in very potent immune responses even when administered without an adjuvant.

In this study, we aim to develop a vaccine candidate against NiV using the Tag/Catcher nanoparticle nucleic acid-based platform. HEK293tt co-transfections with DNA encoding cVLP-Antigen pairs have been used to assess in vitro expression, conjugation and secretion. Lead candidates have been tested in mouse studies followed by live NiV virus neutralization test (VNT) in BSL4 conditions to investigate the biological efficacy of the vaccine-induced response. Our results reveal the expression and secretion of a variety of NiV antigens and nanoparticle combinations. Mice serum analysis after DNA immunization have proven elicitation of NiV specific antibodies. mRNA and protein NiV vaccines have demonstrated to induce high virus-specific responses with neutralization capacity. We anticipate this study to help effectively prevent potential future NiV epidemics and pandemics and contribute to the global vaccine field.

P34

A Rare HCV E2 Antibody That Defies the Classic Easy- and Hard-to-Neutralize Divide

Popovic F[1], Soerensen A[1], Lopez Mendez B[2], Holmboe Olesen C[1], Bukh J[1], Prentoe J[1]

[1]Copenhagen Hepatitis C Program (CO-HEP), [2]Novo Nordisk Foundation Center for Protein Research

Theme: Vaccine design

Objectives: Building on our previous findings that virus-like particles displaying oligomeric soluble E2 (sE2) elicit broader neutralizing antibody responses than monomeric sE2, we hypothesized that oligomeric sE2 may harbor novel, conformational or oligomer-dependent antibody epitopes. These may serve as promising targets for hepatitis C virus (HCV) vaccine development.

Methods: A Fab phage display library derived from a chronically infected HCV patient (genotype 1a) was screened against recombinant monomeric and oligomeric sE2 (genotype 1b). Enriched phage clones were selected through direct and epitope-masked panning strategies, converted to full-length immunoglobulin G (IgG), and characterized for binding and neutralization capacity.

Results: Antibodies from the VH1-69 germline were recovered from selections against both monomeric and oligomeric sE2, while Fabs O19 (VH3-21), O20 (VH3-49), and OC4 (VH4-38) were uniquely isolated using oligomeric sE2. As full-length IgGs, O19 and O20 demonstrated HCV neutralizing capacity in cell culture. O19 potently neutralized genotypes 1a, 3a, 4a, and 5a, but not genotype 6a or the resistant genotype 2a isolate. Notably, O20 fully neutralized S52 (genotype 3a), outperforming all other tested IgGs, and exhibited similar potency against H77 (genotype 1a) and slightly lower potency against J6 (genotype 2a). This reveals a novel neutralization profile that ignores the canonical easy- and hard-to-neutralize groupings of HCV isolates, which to our knowledge is the first report of any IgG with this ability.

Conclusions: The isolation of O20, with its unusual cross-genotype activity, underscores the potential of oligomeric E2 to better mimic native viral epitopes and guide rational vaccine design.

P35

Substitutions in the NS5 of tick-borne encephalitis virus confer high resistance to remdesivir

Ortigosa Elorz I[1], Binderup A[1], Mikkelsen L[1], Bukh J[1], Ramirez S[1]

[1]Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Copenhagen University Hospital Hvidovre and Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen

Theme: Virology

Objective
Tick-borne encephalitis virus (TBEV) causes severe disease in humans. Despite available vaccines, infections are increasing, and no antivirals exist. Remdesivir, a nucleotide analog, inhibits TBEV in cell culture, but its resistance profile remains unclear. We aimed to select and characterize remdesivir-resistant TBEV variants in cell culture.

Methods
TBEV was serially passaged under increasing remdesivir concentrations in human hepatoma (Huh7.5), medulloblastoma (Daoy), and neuroblastoma (SH-SY5Y) cells. Escape variants were sequenced using next-generation sequencing (NGS). Drug susceptibility (EC_{50}) was determined by concentration–response assays, and infectivity titers by 50% tissue culture infectious dose ($TCID_{50}$) assays in Huh7.5 cells.

Results
After 21 passages in Huh7.5 cells at a final remdesivir concentration of 20 µM (66-fold EC_{50}), the virus acquired five substitutions in NS5 (E460D, I569L, L611Y, S766N, Y767F). These substitutions remained stable without drug pressure. The escape virus reached higher infectivity titers than wild type (8.5 vs. 7.33 $\log_{10}$ $TCID_{50}$/mL), suggesting no fitness cost. It showed 22-fold resistance to remdesivir and 13-fold cross-resistance to galidesivir, but no cross-resistance to bemnifosbuvir or sofosbuvir. In SH-SY5Y cells, after 13 passages at 4.5 µM remdesivir, one substitution (L611F) in NS5 emerged. Daoy cells tolerated only 8 passages at 3 µM, with no substitutions detected.

Conclusions
TBEV can develop high remdesivir resistance in cell culture through five NS5 substitutions.
The authors declare no conflict of interest.

P36

Preclinical characterization of humoral immune responses elicited by our lead inactivated hepatitis C virus vaccine candidate.

Sacramento Nonay J[1,2], Czarnota A[1,2], Duarte Hernandez C[1,2], Duan Z[1,2], Visti M[3], Buus S[3], Bukh J[1,2], Quintana Varon M[3], Ghanizada M[3], Barfod L[3], Gottwein J[1,2]

[1]Copenhagen Hepatitis C Program (CO-HEP), Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University Of Copenhagen, [2]Copenhagen Hepatitis C Program (CO-HEP), Department of Infectious Diseases, Hvidovre Copenhagen University Hospital, [3]Department of Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen

Theme: Virology

Hepatitis C virus (HCV) is blood-borne and can cause chronic hepatitis, which can lead to liver cirrhosis and hepatocellular carcinoma. To date, no prophylactic HCV vaccine has been approved for human use, rendering HCV a major public health concern. In fact, as of 2024 approximately 50 million people live with chronic HCV infection. In this context, our research group has developed an innovative inactivated HCV vaccine candidate that induced broadly neutralizing antibodies against several HCV genotypes in immunized mice. This PhD project will focus on analysing the immune response to our vaccine antigen in a human context using human tonsil organoids (HTOs). Furthermore, it will define an optimal vaccine regimen through immunogenicity studies in mice, evaluating different adjuvants, including Sepivac SWE and Aluminum hydroxide, doses and boosting schedules. To this end, the HTOs and mice will be immunized with our vaccine antigen, and the presence of HCV binding and broadly neutralizing antibodies will be assessed with in-house developed assays. Furthermore, from HTOs, HCV epitopes targeted by vaccine-induced antibodies will be determined by the generation and characterization of monoclonal antibodies (mAbs). Generation of mAbs relies on antigen-specific B-cell enrichment and sequencing strategies and mAb expression in mammalian cells. Target epitopes of mAb will be identified in competition ELISA with known mAbs and structural analyses using cryoEM or crystallography of the variable region segments of the mAbs. Overall, these studies will offer new insights into the immunogenicity of the vaccine candidate and contribute to its advancement.

P37

Spatial and frequency profiling of intestinal mucosal innate lymphoid cells (ILCs) in people living with HIV

Aamand K[1]

[1]NIVI-R, ISIM

Theme: Virology

The introduction of antiretroviral therapy (ART) has significantly reduced AIDS-related morbidity and mortality worldwide. However, people living with HIV (PWH) remain at increased risk of developing inflammation-driven comorbidities that impair quality of life and contribute to multisystemic failure. Although the mechanisms underlying chronic immune activation are not fully understood, disruption of gastrointestinal (GI) barrier integrity and microbial translocation are considered central contributors. Innate lymphoid cells (ILCs) play a crucial role in mucosal defence and barrier maintenance, yet their role within the human gut during HIV infection remains poorly defined. Previous studies have demonstrated irreversible depletion of ILCs in the blood following HIV infection, but whether similar depletion occurs in gut mucosal tissue has remained unclear, with conflicting findings. To address this, biopsies from the small and large intestines of 49 participants without known GI disorders from a high-prevalence region in South Africa were analysed. Flow cytometry identified ILC subsets, including ILC3s, intraepithelial ILC-like cells, ILC1-like cells, and NK cells, while multiplex immunofluorescence provided spatial context on ILC3s. Despite confirmed CD4 T cell depletion, no significant loss of ILC subsets was observed in PLWH, irrespective of viral suppression status. Spatial analysis further revealed that ILC3s were significantly enriched within organised gut-associated lymphoid tissue compared with the lamina propria across both gut compartments. To extend these findings, xenium spatial transcriptomics will be applied to gut tissue samples, offering high-resolution insights into the organisation and activation states of ILCs in HIV infection and expanding the understanding of their role in gut barrier immunity.

P38

Incidence and Clinical Outcome s of RSV and Influenza in Solid Organ Transplant Recipients

Bui Le V[1], H. Thygesen H[1], Perch M[2,7], Schwartz Sørensen S[3], Rossing K[4], Schultz N[5], Lundgren J[1,6], Helleberg M[1,6]

[1]Centre of Excellence for Health, Immunity and Infections (CHIP), Copenhagen University Hospital, Rigshospitalet, Denmark, [2]Section for Lung Transplantation, Department of C ardiology, Copenhagen University Hospital Rigshospitalet, Denmark, [3]Department of Nephrology, Copenhagen University Hospital, Rigshospitalet, Denmark, [4]Department of Cardiology, Copenhagen University Hospital, Rigshospitalet, Denmark, [5]Department of Gastric Surgery, Copenhagen University Hospital, Rigshospitalet, Denmark,, [6]Department of Infectious Disease, Copenhagen University Hospital , Rigshospitalet, Denmark, [7]Department of clinical medicine, University of Copenhagen, Copenhagen, Denmark

Theme: Virology

Viral respiratory infections are common among solid organ transplant (SOT) recipients and represents a clinical concern due to their association with increased morbidity and mortality. The impact of respiratory syncytial virus (RSV) in SOT recipients has not been thoroughly studied, and its incidence may be underestimated due to low testing rates. This study aimed to compare the incidence, severity, and outcomes of influenza and RSV among SOT recipients. We had a cohort of 1862 adult who were transplanted for the first time at Rigshospitalet, Copenhagen, between 2010 to 2021. We estimated mortality rates (MR) per 100 person-years of follow-up (PYFU) according to infections status (no infection, 0 – 180 days after infection, and > 180 days after infection) by dividing number of events by the accumulated person-time at risk. Poisson regression model was used to analyze adjusted mortality rate ratios (aMRR). All analyses were adjusted for time-updated covariates. 693 (37.2%) and 202 (10.8%) were tested for influenza and RSV, respectively. Test positive rates were 17.7% for influenza and 15.8% for RSV. MR/100 PYFU for influenza was 11.6 (95% CI: 5.5 – 24.4) and 25.5 (9.6 – 60.0) for RSV. aMRR was 2.8 (1.2 - 5.5) for influenza and 4.7 (1.5 – 11.1) for RSV. Testing was more frequent for influenza than RSV, but test positive rates were similar suggesting underdiagnosis of RSV (Figure 1). Both viruses were associated with an approximately 3-5-fold increased mortality the first 6 months following infection.

P39

Human Organotypic Tonsil Slice Culture Shows Germinal Center Maturation and T-Follicular Helper Recruitment After LAIV Challenge

Dragheim J[1]

[1]University Of Copenhagen

Theme: Other

Upper airway human mucosal immunity remains poorly understood due to the lack of experimental models that preserve the structural and cellular complexity of native tissue. Existing systems, particularly conventional host–pathogen models, rely on simplified monolayer cultures or simple organoids lacking the coordinated interaction between epithelial, stromal, and immune compartments, essential for mounting a mucosal immune response.

We present a precision-cut, organotypic tonsil-slice culture system that preserves the spatial organization and cellular heterogeneity of human airway tissue. Cultured at an air–liquid interface and embedded in a collagen matrix, the model retains epithelial integrity, stromal structure, and resident immune cells for extended periods ex vivo. Six days after apical exposure to live-attenuated influenza virus (LAIV), the system recapitulates key features of mucosal immune activation by flow cytometry (n = 4), including $CD4^+$ ($CD38^+$, $log_2FC = 0.465$) and $CD8^+$ ($CD38^+$, $log_2FC = 0.565$) T cell activation, germinal center (GC) B cell differentiation ($CD27^+CD38^+$, $log_2FC = 0.806$), and plasmablast differentiation ($CD27^{++}CD38^{++}$, $log_2FC = 0.366$), compared to PBS-treated controls. LAIV-specific antibody production was also detected ($log_2FC = 0.544$). Multiplex immunofluorescence shows recruitment of $CXCR5^+CD4^+$ T follicular helper cells (TFH) to GCs at day 6 (2.7-fold, $log_2FC = 2.69$), further supporting GC development.

This platform offers a tractable system for dissecting human airway immune responses, with applications in vaccine research and host–pathogen interactions. Ongoing efforts aim to optimize culture conditions to better mimic the in vivo environment.

P40

Von Willebrand Factor and Complications Related to Blood Vessel Damage Following Pediatric Hematopoietic Stem Cell Transplantation

Horan D[1], Ifversen M[1], Nielsen C[1], Johansson P[1], Enevold C[1], Kielsen M[1], Müller K[1]

[1]Rigshospitalet

Theme: Other

Background
Chemotherapy before hematopoietic stem cell transplantation (HSCT) can damage blood vessels, causing liver damage (sinusoidal obstruction syndrome, SOS), inflammation (engraftment syndrome, ES), fluid leakage (capillary leak syndrome, CLS), and immune complications (acute graft-versus-host disease, aGvHD). Symptoms often overlap, making diagnosis difficult. Von Willebrand factor (vWF), released upon blood vessels activation, mediates platelet adhesion and thrombosis.
We investigated vWF and its association with complications in children undergoing HSCT.

Methods:
Seventy-eight children (median age 9.2) receiving HSCT for malignant (n=48) or benign (n=30) diseases were included. Donors were HLA-matched siblings (n=26) or unrelated (n=52), with bone marrow as primary graft (n=74). Chemotherapy was busulfan-based(n=50), total body irradiation-based (n=15), or other (n=13). vWF was measured before HSCT and at multiple time points up to one year.

Results:
vWF doubled from baseline (1.2 ng/mL) to peak on day +30 (2.4 ng/mL, p<0.0001) and remained elevated. Higher vWF on day +14 was associated with busulfan-based chemotherapy (2.2 vs. 1.6 ng/mL, p=0.005) and age >10 years (2.1 vs. 1.7 ng/mL, p=0.05). Twenty-seven children developed severe SOS, correlating with higher vWF on day +14 (2.4 vs. 1.6 ng/mL, p=0.007, figure 1A), though not significant after adjustment. Severe aGvHD (n=5) showed a fivefold increase in vWF (figure 1B); each twofold increase raised risk (OR=6.3 on day 0, p=0.02). No associations were seen in CLS (n=14) or ES (n=12).

Conclusion:
vWF increased and was associated with busulfan-based chemotherapy, severe SOS and aGvHD, supporting its use as a potential biomarker to guide prevention and treatment.

Conflict of interest: none

P41

Role of TSLP and mast cells among asthma patients with airway autoimmunity

Eriksen L[1], Bornesund D[2], Hvidtfeldt M[1], Sverrild A[1], Erjefält J[2], Porsbjerg C[1]

[1]Respiratory Research Unit, Copenhagen University Hospital - Bispebjerg and Frederiksberg, [2]Airway Inflammation and Immunology, Lund University

Theme: Other

Asthma is characterized by an overreactive airway epithelium. In a subset of patients with airway autoimmunity, viral encounters trigger exaggerated epithelial immune responses, notably by heightened IL-13 release. Intraepithelial mast cells (MCs), activated by epithelial TSLP, have been proposed as a potential IL-13 source.

We aimed to assess the relationship between MCs and TSLP in asthma patients with and without airway autoimmunity.

Cryobiopsies from mild, steroid-naïve asthmatics were examined: 12 with elevated anti-MARCO antibodies (autoimmune) and 33 without. MCs were identified by tryptase and chymase chromogenic immunohistochemistry and quantified in whole tissue, smooth muscle, and epithelium. Sputum and bronchoalveolar lavage (BAL) TSLP concentrations were measured by MSD, while anti-MARCO was quantified in sputum via ELISA. Logistic regression evaluated associations, adjusting for age and sex.

Autoimmune patients exhibited significantly fewer tryptase$^+$ MCs in whole tissue ($p = 0.006$) and epithelium ($p = 0.006$), with no difference in smooth muscle. Sputum TSLP was markedly elevated in the autoimmune group ($p < 0.001$); BAL TSLP showed no disparity. Each doubling in sputum TSLP raised the odds of airway autoimmunity by 2.9 (OR = 2.89, 95 % CI = 1.30–6.43, $p = 0.009$). MC counts were not independently linked to autoimmunity, and adjustment for MCs did not alter the TSLP autoimmunity relationship.

In conclusion, patients with asthma and airway autoimmunity show reduced intraepithelial MCs but elevated sputum TSLP. Sputum TSLP, but not mast cells, was strongly associated with airway autoimmunity suggesting TSLP may contribute independently and serve as a potential biomarker.

P42

In-depth profiling of conventional dendritic cell populations and transcriptomics in Crohn's disease

Väänänen V[1], Wulff L[2], Lemvigh C[1], Mörbe U[1], Agace W[1,3]

[1]SIC, ISIM, University of Copenhagen, [2]Department of Health Technology, DTU, [3]Lund University

Theme: Other

Intestinal immunity relies on a balance of tolerating food antigens and commensal microbiota but being able to mount a protective immune response towards invading pathogens. A breakage in this balance can lead to the development of inflammatory bowel diseases such as Crohn's disease (CD). Conventional dendritic cells (cDC) are key for maintaining intestinal immune homeostasis by recognizing various danger signals and priming adaptive immune responses. Here, we studied cDC populations in CD by comparing paired inflamed and uninflamed small intestinal lamina propria samples from six patients using single cell RNA sequencing. Our analysis identified cDC1, cDC2, cDC3 and cDC2-cDC3 mixed ambiguous cell populations across all samples. We found a significant decrease in the proportion of cDC2 and ambiguous cells in inflamed areas. In addition, we saw a trend of increased proportion of CCR7+ activated dendritic cells (CCR7+ DC) in inflamed areas. Strikingly, cDC subtypes were transcriptionally highly similar between inflamed and uninflamed samples apart from increased expression of interferon-induced transmembrane (IFITM) genes in the inflamed samples. Based on trajectory analysis, we hypothesize that interferon signaling drives activation of cDC2s, cDC3s and ambiguous cells into CCR7+ DCs in CD inflammation. Immunohistochemical analysis found CCR7+ DCs to be located in T cell aggregates, including proliferating T cells, in the inflamed intestine. Our results suggest that CCR7+ DCs play a role in regulating local T cell responses in CD.

P43

Negative T cell selection and induction of regulatory T cells specific to FVIII and Factor H in Human Thymic Organoids

Bundgaard S[1]

[1]University Of Copenhagen

Theme: Other

Purpose:
This study aims to develop a human thymic organoid (HTO) model to investigate T cell selection and tolerance mechanisms relevant to Factor VIII (FVIII) and Factor H, which are implicated in hemophilia A and atypical hemolytic uremic syndrome (aHUS), respectively.

Methods:
HTOs are generated from pediatric thymic tissue obtained during cardiothoracic surgery. Organoids are cultured using optimized media and matrix conditions to support thymic epithelial cell (TEC) growth. Flow cytometry is used to investigate HTO cell populations. Lentiviral vectors engineered for TEC-specific transduction will be used to knock out endogenous FVIII and Factor H and introduce model antigens. Peptide-HLA tetramers are developed to isolate antigen-specific T cells. T cell selection and regulatory T cell (Treg) induction are assessed via flow cytometry and bulk TCR sequencing.

Results:
Initial results demonstrate successful organoid formation from single cell suspensions of thymic tissue. Both fresh and frozen thymic cells yield organoids which can be cultured for >2 months. The organoids grow rapidly and are passaged every 10-20 days, allowing for the generation of large amounts of cells from small amounts of tissue.

Conclusions:
HTOs showing TEC specific markers can be grown from thymic tissue. Future efforts will be made to initiate differentiation of HTOs, to capture the diversity of the thymic epithelium. Once the proper culturing methods are established, differentiated HTOs and immature thymocytes will be co-cultured to investigate thymopoiesis.

Conflict of Interest:
The authors declare no conflicts of interest.

P44

Predicting individualised treatment effects of passive immunotherapies in adults hospitalised with COVID-19 using machine learning

Kjaergaard V[1], Zargari Marandi R[1], Chang W[2], Churpek M[3], Danboise B[4], Gardner E[5], Hejblum B[6], Holodniy M[7], Jensen C[1], Knowlton K[8], Kwaghe V[9], Moores R[10], Reilly C[11], Semler M[12,13], Sherman B[2], Spicer A[14], Siegel L[11], Tidswell M[15], Vock D[11], Lundgren J[1,16,17], Reekie J[1], Milojevic M[1], Jensen T[1]

[1]Centre of Excellence for Health, Immunity and Infections (CHIP), Rigshospitalet, , [2]Frederick National Laboratory for Cancer Research , [3]University of Wiscosin-Madison, [4]Christus Health-Texas A&M Spohn Emergency Medicine Residency, [5]Denver Health and Hospital Authority, [6]Univ. Bordeaux, INSERM, INRIA, BPH, U1219, F-33000, [7]Stanford University, [8]Intermountain Medical Center Heart Institute, [9]University of Abuja Teaching Hospital, [10]Department of Infectious Diseases, Royal Free London NHS Foundation Trust, [11]Division of Biostatistics and Health Data Science, University of Minnesota, [12]Division of Allergy, Pulmonary, and Critical Care Medicine, Vanderbilt University Medical Center, [13]Center for Learning Healthcare, Vanderbilt Institute for Clinical and Translational Research, [14]Division of Allergy, Pulmonary, and Critical Care Division, [15]UMASS Chan Medical School, Baystate Medical Center, Division of Pulmonary and Critical Care Medicine, [16]Department of Infectious Diseases, Copenhagen University Hospital – Rigshospitalet, , [17]Department of Clinical Medicine, University of Copenhagen

Theme: Other

Overall treatment effect of passive immunotherapies in adults hospitalised with Coronavirus disease 2019 (COVID-19) has not been shown in clinical studies. One study observed increased survival in patients seronegative at randomisation, indicating some treatment heterogeneity. Here, we explore treatment heterogeneity using machine learning models to estimate individualised treatment effects (ITEs) in a population of adults hospitalised with COVID-19.

We included adults hospitalised with COVID-19 randomised to receive passive immunotherapy or placebo. The primary outcome was 28-day mortality. The population was split into 80% training (model development) and 20% internal validation. ITEs were estimated using machine learning algorithm RBoost. Model performance was assessed using c-for-benefit, where 1·0 is perfect prediction. An independent trial was used for external validation.

Of 2,622 participants, 1,722 (82.2%) of the training, 410 (78.1%) of the internal validation sets and 467 (80.7%) of 579 participants from the external validation set had predicted reduced mortality with passive immunotherapy treatment, while the rest had a predicted harm of treatment. Patients with higher interleukin-6 and SARS-CoV-2 nucleocapsid antigen levels appeared to have a higher predicted treatment benefit. For internal and external validation, the c-for-benefit was 0.61 (95%CI: 0.53-0.71) and 0.54 (95%CI: 0.45-0.60), respectively.

We found heterogeneity in predicted ITEs, particularly driven by markers of inflammation and viral burden. While the model predicting treatment effects was internally validated, the external validation was not significantly more accurate than chance. However, the most important covariates for ITE prediction selected by all models were almost identical, suggesting some generalisability.
No known conflicts of interest.

P45

Novel approaches for treatment of community-acquired pneumonia: A presentation of a Ph.D-project

Gundersen B[1], Itenov T[2], Sivapalan P[1], Jensen J[1]

[1]Herlev & Gentofte Hospital, [2]Bispebjerg & Frederiksberg Hospital

Theme: Other

Purpose
The present project's aims are:
i) assessing the airway-tolerability of inhaled levofloxacin in patients suffering from obstructive pulmonary disease presenting with community-acquired pneumonia and
ii) assessing the effect of treatment with daily low-dose acetylsalicylic acid (ASA) towards the 1-year prognosis of community-acquired pneumonia.

Methods
The airway-tolerability will be assessed in a double-blinded randomized controlled trial (LANDCAP-1) including 36 patients admitted to a hospital with a diagnosis of community-acquired pneumonia. At inclusion they will be allocated as shown in the supplemental flow chart. The patients will receive either inhalations of hypertonic sodium-chloride or 240mg Levofloxacin. Before and after the first inhaled dose a spirometry will be performed. The proportion of patients with a drop in forced expiratory volume in one second (FEV1) of more than 300mL or 20% relative to baseline is the primary endpoint.

The effect of ASA will be assessed retrospectively in a register-based cohort study. This cohort consists of individuals with a microbiological sample being positive of bacterial infection with a pathogen suggesting community-acquired pneumonia. The endpoint is risk of death or a major adverse event within the observation period of 365 days.

Results
The LANDCAP-1 study is still actively recruiting, and no preliminary data are available. The data for the cohort study is not yet available; all permissions have been granted.

Conclusions
The results of LANDCAP-1 will be the first RCT-results in patients with obstructive pulmonary disease and inhaled antibiotics. However, no data are available, therefore, no conclusions can be made.

Conflict of Interest
None declared.

P46

The role of the Met1-linked ubiquitin machinery in the immune biology of human keratinocytes and fibroblasts

Beichler M[1], Gyrd-Hansen M[1]

[1]LEO Foundation Skin Immunology Research Center, Department of Immunology and Microbiology, University of Copenhagen

Theme: Other

The regulation of inflammation and cell death signalling in keratinocytes has emerged as a key mechanism controlling skin immune homoeostasis but can, when deregulated, contribute to inflammatory skin diseases and chronic inflammatory conditions that affect millions world-wide. The Met1-Ubiquitin (Ub) has been shown to regulate skin homeostasis and keratinocyte cell death in mice. Met1-Ub is generated by the Linear Ubiquitin chain Assembly Complex (LUBAC) which exists in complexes with the deubiquitinases (DUBs) OTULIN and CYLD that can disassemble Met1-Ub – collectively termed the Met1-Ub machinery. To investigate whether the regulation of skin inflammation and cell death by the Met1-Ub machinery is translatable to human skin, we are using the human immortalized keratinocyte cell line N/TERT-1. These cells can be differentiated into human epidermal 3D equivalent cultures. Using CRPISR-Cas9 gene editing we are creating loss-of-function (KO) mutations in components of the Met1-Ub machinery with the aim to elucidate how the Met1-Ub machinery regulates immune and cell death signalling processes in these cells. So far, we have seen that the deletion of HOIP leads to cell death signalling in N/TERT-1 cells in response to TNF treatment, indicating a regulatory role of LUBAC in skin homeostasis and cell death signalling in human keratinocytes.

P47

Biological effects of CCL5 posttranslational modifications and detection of its proteoforms in biological samples

Nielsen V[1,2], Drzazga A[2,3], Kledal T[3], Rosenkilde M[2], Proost P[1]

[1]Laboratory of Molecular Immunology, Department of Microbiology, Immunology and Transplantation, Rega Institute, Katholieke Universiteit Leuven, [2]Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, [3]Synklino

Theme: Other

Recent research has shown that a range of chemokines become posttranslationally modified. One example is CCL5 which has been observed to be N- and/or (potentially) C-terminally truncated in natural material and patient samples. Nonetheless, it is only partially known how, e.g., CCL5 truncations affect receptor interaction and downstream signaling, both in pathological as well as physiological settings. Using solid phase peptide synthesis, we have synthesized full-length CCL5 as well as the forms, CCL5[3-68] and CCL5[4-68], which have previously been identified from cell cultures. This synthetic material will allow in-depth investigation into how the variants affect receptor usage and cellular signaling using BRET-based setups and flow cytometry. Aside from these well-known CCL5 isoforms, eight to ten variants have been published. From a variety of cell lines, stimulated with an array of inflammatory conditions, we are working on verifying the presence of these and potentially other CCL5 variants using a nano-LC tandem mass spectrometry setup. Preliminary data suggest that platelets may produce CCL5 proteoforms which have not previously been described. Based on the detected natural proteoforms, new potential variants will be synthesized and tested for cellular effects in a similar fashion to the well-known variants. Finally, we will also quantify the variants in samples from patients suffering from joint inflammation, e.g., rheumatoid arthritis, as well as investigate which enzymes are responsible for the proteolytic processing of CCL5. Ultimately, this will support future drug development, highlighting which active proteoforms, receptors, and/or enzymes may be targeted for disease treatment.

P48

Characterisation of the Inflammatory and Immune-mediated Disease (IMID) Virome

Schwoon M[1]

[1]University of Copenhagen

Theme: Other

The human immune system distinguishes between harmless antigens, such as commensals or self-antigens, which it tolerates, and harmful antigens, like pathogens or toxins, which trigger defensive responses to limit damage. However, the immune system itself can induce damage to the human body, if this discrimination between harmless and harmful antigens fails. Immune-mediated diseases (IMIDs) are an undesired result of such a dysregulated immune response against otherwise harmless antigens. Genetic susceptibility is a key factor in the development of IMIDs which, however, often only manifest in disease following environmental triggers.

One such trigger could be viral infections, with multiple recent publications linking particularly herpesviruses with IMIDs like multiple sclerosis, inflammatory bowel disease (IBD), or rheumatoid arthritis.

In our project we want to investigate the role of viral infections in the onset of IMIDs, particularly IBD. For this, we will develop and utilize a high-throughput phage immunoprecipitation sequencing (PhIP-seq) platform using an M13 phage display library designed by us which presents 56-mer peptide tiles from viral antigens which react with the respective antibodies in serum samples.
To test the feasibility of viral PhIP-seq using M13 phages, we created a pilot library limited to herpesvirus antigens. Sanger sequencing confirmed successful cloning in bacteria, although the success rate is still too low with ~30%. Also, we could not show that emerging M13 phages carried the inserts or expressed viral peptides.

There is no conflict of interest of any kind.

P49

Mapping Gene Signatures of IgA Class-Switch Recombination in Human Immune Organoids

Ghanizada M[1]

[1]Department of Immunology and Microbiology, University of Copenhagen

Theme: Other

Class-switch recombination (CSR) during B-cell responses to pathogens and vaccines is essential for durable protection. Yet the cues that direct B cells toward specific antibody isotypes remain poorly defined. Here, we leverage a Human Immune Organoid model generated from human tonsil tissue and integrate systems immunology, including single-cell transcriptomics and paired VDJ sequencing, to map the gene signatures that drive isotype-specific CSR. By resolving transcriptional states and receptor clonotypes associated with switching, we aim to identify the pathways that bias B cells toward distinct outcomes. This human-focused mechanistic insight will inform the design of adjuvants and vaccine formulations tailored to elicit desired antibody isotypes and improve long-lasting protective immunity