



Graduate Programme in Immunology and
Infectious Diseases

Abstract Book

From bench to bedside

G S I

28 - 30 SEPTEMBER

2026

KONVENTUM · HELSINGØR · DENMARK

Table of content

P01: Investigating the Immune Landscape in Atopic Dermatitis	3
P02: Germinal Center Dynamics in Crohn's Disease	4
P03: The effects of supplemental lipids on neonatal infection outcomes in preterm piglets.....	5
P04: Cell culture studies of the barrier to resistance of remdesivir for yellow fever virus	6
P05: Immunogenicity and protective efficacy of surrogate hepatitis C virus glycoprotein subunit vaccine	7
P06: Preserved frequency of innate lymphoid cell subsets in the gut and lymph-nodes in people with HIV despite CD4 T-cell depletion	8
P07: Plasma and urine profiling reveals compartment-specific complement activation in immune-mediated kidney diseases	9
P08: Pre-existing epidermal TRM cells augment the inflammatory reaction to secondary contact allergens	10
P09: The Effect of Fisetin in Aging and Diet Induced Senescence and Inflammation in Mice.....	11
P10: Germline-Encoded TCR Segments Shape allergen-specific CD4 ⁺ T Cell Responses.....	12
P11: From Fibrosis to Immunotherapy: TGFBI as a novel Immunogenic Target in Pancreatic Ductal Adenocarcinoma	13
P12: H107e/CAF-Induced Th17 Cells Orchestrate a Unique Immune Response Against Tuberculosis	14
P13: In-depth proteomic analysis of extramammary Paget's disease and associated adenocarcinomas	15
P14: The effect of mixtures of contact allergens on skin sensitization.....	16
P15: Glycoprotein A repetitions predominant (GARP): An Emerging Target for Cancer Therapeutic Immune Modulatory Vaccines	17
P16: Repurposing Thiopurines to re-sensitize Cold Tumors and expand the reach of Immunotherapy ..	18
P17: The influence of the gut microbiome and dietary-derived metabolites on type 2 immunity against T. muris infection in mice	19
P18: Butyrate is a major determinant of terminal development and function in dendritic cells.....	20
P19: Beyond complement activation: MASP-2 acts on the beta-cell surface and may influence insulin secretion.....	21
P20: Pre-clinical testing of a Nipah vaccine exploiting the Tag/Catcher Virus-like-particle vaccine platform.....	22
P21: PhIP-Seq for Serological Detection of Viral Exposure: A Herpes Virus Pilot Study Using M13 Phage Display	23
P22: Approaches in characterizing NK cell regulation: A proof-of-concept utilizing NKG2D knockout and a CD300a engager knock-in spheroid model	24
P23: AHR signalling in colonic subepithelial fibroblasts coordinates immune homeostasis, barrier integrity, and protection against colitis	25
P24: Factor D/Adipsin activation is disrupted in obesity and improved by Roux-en-Y gastric bypass.....	26
P25: Compartment-Specific Complement Dysregulation in IgA Nephropathy - Divergent Profiles of Plasma and Urinary Biomarkers	27
P26: Design of next-generation lipid nanoparticles for mRNA vaccines: Enhancing potency and safety by incorporation of adjuvants.....	28

P27: Peptide Tags to Increase Uptake and Immunogenicity of Mucosal Vaccines	29
P28: The role of fermentable fibers in <i>Trichuris muris</i> infection: host susceptibility and microbial interactions	30
P29: Screening Immunostimulators to Elicit Airway Immune Responses After Intranasal Delivery	31
P30: The role of intestinal fibroblast-derived signals in DC2 development & maturation.....	32
P31: T-cell Phenotypes and Coronary Artery Disease in People with HIV	33
P32: Clonal and Transcriptional Landscapes of Human Intestinal Plasma Cell in Homeostasis and Inflammation	34
P33: Preclinical characterization of humoral immune responses elicited by our lead inactivated hepatitis C virus vaccine candidate	35
P34: Optimization of T cell reactivity assessment and antigen-specific screening in peripheral blood of healthy donors	36
P35: Production and test of molecularly adjuvanted and multimerized recombinant vaccines against conserved protein targets representing influenza A virus.....	37
P36: A Spatially Resolved Human Tonsil Model for Studying Mucosal Immunity and Host-Pathogen Interactions	38
P37: The effect of the probiotic -ASTARTE™ on the reduction of recurrent Urinary Tract Infection in women aged 18-40 years	39
P38: A comparative study of peripheral blood-derived mast cell culturing protocols for allergy diagnosis	40
P39: Geographic heterogeneity of sulfadoxine-pyrimethamine resistance markers from 2012-2024 and <i>Plasmodium falciparum</i> genetic relatedness in the Democratic Republic of the Congo: implications for protective efficacy.....	41
P40: Multimarker profiling reveals distinct systemic inflammatory phenotypes and long-term clinical risk – a multicohort study of CRP, IL-6, and suPAR.....	42
P41: Investigating the role of NKG2D in the regulation of CD137 expression in by introducing gene knockouts in primary immune cells	43
P42: Translating Pamvac Into a Nanoparticle mRNA Vaccine Platform.....	44
P43: Development of antibody-based assays for screening of C4 isotypes	45
P44: Generation of Human Thymic Organoids to Model T Cell Selection and Tolerance Mechanisms	46
P45: Exploring adaptive immune cell crosstalk between the gut and skin in Hidradenitis Suppurativa patients with Crohn’s disease comorbidity.....	47
P46: Interactions between pathogenic CD4+ T cells and CCR7+ LAMP3+ activated dendritic cells in Crohn’s disease.....	48
P47: Biological effects of CCL5 posttranslational modifications	49
P48: Immune activation markers in severe COVID-19 and Influenza and clinical outcomes	50

P01: Investigating the Immune Landscape in Atopic Dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disease that causes intense itching and skin lesions. The pathology involves genetic, immunological, and environmental factors, leading to a compromised skin barrier and a heightened immune response.

The aim of this study was to investigate the immunological landscape in atopic dermatitis and the immunological effects of a relatively new and effective biologics treatment, dupilumab.

We analysed human skin biopsies by multiparameter flow cytometry and spatial transcriptomics. The study included lesional, non-lesional, and dupilumab treated AD skin, as well as healthy control skin. We observed that lesional AD skin had a unique immune profile compared to healthy skin, characterised by an increase in CD4 T cells producing IL-13 and IL-22, along with a decreased IFN γ and IL-17A production. Dupilumab treated skin T cells showed a similar cytokine profile, despite having complete lesion resolution. Furthermore, we saw a specific increase in IL-22 and IL-13 co-producing cells in all AD skin compared to healthy skin, with no increase in IL-22 single-producing cells.

We observed B cells, dendritic cells, and Langerhans cells in all skin types, with increased infiltration in lesional skin. Furthermore, preliminary results from spatial transcriptomics revealed localised niches of Th2 cells in proximity to classical IRF4+ type 2 dendritic cells and distinct fibroblast subsets in lesional skin.

Further work will explore the relationship between IL-22 and Th2 cells in AD, as well as investigate the effects of dupilumab on the Th2 niches in the skin.

P02: Germinal Center Dynamics in Crohn's Disease

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B cells and antibodies are essential for human health. Induction of B cells occurs in lymphoid organs, where antigen stimulation drives activated B cells into germinal centers (GCs) for affinity maturation. GC progression yields high-affinity plasma cells (PCs) and memory B cells. Intestinal lymphoid tissues, like Peyer's patch (PPs) follicles, harbor chronic GCs due to a high antigen load. Dysregulated immune responses, such as increased antimicrobial IgG, are linked to Crohn's disease (CD), but their origin remains unclear.

We aim to compare GC responses in PP follicles between CD and controls. First, we seek to confirm whether the increase in IgG production originates within PP GCs and how nearby Tfh cells may participate in regulating this increase. Second, we will examine if clonal B cell expansion occurs synchronously across follicles (suggesting trafficking) or if each GC acts independently. Lastly, we will assess clonal sharing between GC B cells and lamina propria PCs to identify antibody sources associated with CD immune dysregulation. Ultimately, we hope to unravel the antigens that these antibodies target.

We will use single-cell transcriptional and clonal profiling of GC B cells and Tfh cells in PP follicles, as well as PCs from LP samples. We will compare isotype distributions, cell-cell interactions, and clonal landscapes between CD and control gut tissues. Antibody targets will be computationally screened and confirmed by antibody expression to assess reactivity.

These insights will enhance understanding of cellular and clonal origins of antibody responses in CD, potentially highlighting inflammatory triggers and informing future therapies.

P03: The effects of supplemental lipids on neonatal infection outcomes in preterm piglets

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Approximately 200,000 premature infants die annually from infections. We have previously found that reliance on glycolysis worsens infection outcomes, while enhanced fatty acid oxidation improves outcomes. Here, we investigated the potential of fatty acid supplementation as a treatment option during neonatal infections.

Preterm piglets (90% gestation) were infected with *Staphylococcus epidermidis* (SE), a major cause of neonatal sepsis. Piglets received parenteral nutrition containing various amounts of glucose (10% or 2.5%) and fat (0 or 3.3% intralipid). In a following experiment, piglets received long chain fatty acids parenterally (intralipid), medium chain fatty acids enterally (MCT) or long- and medium chain fatty acids parenterally (SMOF). We measured sepsis biomarkers (cytokines, blood biochemistry) and gene expression (hepatic RNAseq).

We found supplementation of intralipid reduced survival, induced proinflammatory cytokine expression (TNF α , $p=0.03$), and elevated indicators of liver damage (alkaline phosphatase, $p=0.04$). Furthermore, supplementation of intralipid drove the expression of inflammation-associated pathways. In contrast, supplementation of MCT and SMOF reduced sepsis onset (factor 0.7, both) and induced anti-inflammatory cytokine expression (IL10, $p < 0.05$).

We found that administering MCT and SMOF via the same route to THP-1 cells infected with SE, led to reduced TNF α (MCT $p = 0.0713$, SMOF $p = 0.0448$) and elevated IL10 ($p < 0.0001$) gene expression levels compared to control.

Our results indicate that type of fatty acid (long or medium-chain) supplemented to nutrition affects infection outcomes in preterms. Further research is needed to develop effective nutritional strategies to ultimately reduce the number of preterms dying from neonatal infections.

P04: Cell culture studies of the barrier to resistance of remdesivir for yellow fever virus

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No antiviral treatment is currently approved against yellow fever virus (YFV), a mosquito-borne Orthoflavivirus responsible for an estimated 180,000 severe infections and up to 78,000 deaths annually, despite the availability of a safe and effective vaccine.

Remdesivir, a broad-spectrum nucleotide analog approved for SARS-CoV-2, has shown strong activity against YFV in multiple human cell lines, as well as efficacy in a hamster model. Here, we investigated the potential for YFV to develop resistance by serially passaging the YFV 17D strain in human hepatoma cells (Huh7.5) under increasing concentrations of remdesivir.

Next-generation sequencing revealed that after 12 passages (up to 15 μ M remdesivir), the viral population had acquired four resistance-associated substitutions (RAS) within the RNA-dependent RNA polymerase domain of the non-structural protein 5 (E460D, K462Q, G538R and K835E), which were absent in the untreated control virus. In concentration-response assays, the passage-12 treated virus exhibited a 2.5-fold increase in the effective 50% concentration (EC₅₀) of remdesivir compared to the original virus. After 32 passages (40 μ M remdesivir), the treated virus showed a 5.5-fold increase in EC₅₀ and harbored three additional NS5 RAS (N391K, A590T and A610S). The specific contribution of each RAS should be confirmed through reverse-genetics experiments.

In conclusion, YFV 17D can evolve resistance to remdesivir in cell culture. However, high resistance is likely associated with the accumulation of multiple RAS, suggesting a high barrier to resistance for remdesivir against YFV.

P05: Immunogenicity and protective efficacy of surrogate hepatitis C virus glycoprotein subunit vaccine

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Hepatitis C virus (HCV) vaccine development is hindered by the lack of immunocompetent animal models that are permissive to HCV. This necessitates the use of surrogate models like Norway rat hepatitis virus (NrHV). NrHV resembles HCV in genetic structure, pathogenesis, and immune responses. We evaluated the NrHV envelope glycoprotein, E2, as a surrogate strategy for an HCV vaccine by assessing its immunogenicity in vivo and its protection efficacy against acute infection in mice and chronic infection in rats. The induced antibody response was characterized by ELISA and neutralization assay. After NrHV infection, protection was monitored by viral RNA quantification. Escape mutations were assessed by next-generation sequencing. In vivo neutralization was assessed by passive immunization of NrHV E2-antibodies in immunodeficient mice followed by NrHV infection. Two NrHV E2 variants elicited high-titer, cross-reactive neutralizing antibodies, however, with different magnitudes. Around 62% of the E2-immunized mice cleared the virus before the control group. Surprisingly, despite high-titer in vitro neutralization, none of the E2-immunized rats prevented chronic infection. Several escape mutations were discovered in the E2-immunized rats. Moreover, the E2-antibodies showed minimal in vivo neutralization.

This shows the importance of evaluating surrogate vaccine candidates both in vitro and in vivo, given the observed discrepancies in neutralization and lack of protection against chronic infection. The NrHV surrogate platform enables the testing of different vaccine strategies, the investigation of correlates of immunity, and protection efficacy. Ultimately, this can direct analogous HCV vaccine concepts and guide the pursuit of a future HCV vaccine.

P06: Preserved frequency of innate lymphoid cell subsets in the gut and lymph-nodes in people with HIV despite CD4 T-cell depletion

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Antiretroviral therapy (ART) reduces AIDS-related morbidity and mortality in people with HIV (PWH) but fails to restore gut barrier integrity despite sustained viral suppression, contributing to chronic inflammation and multimorbidity. Innate lymphoid cells (ILCs) are critical for mucosal defense and barrier maintenance, yet their role in HIV-associated gut dysfunction remains unclear.

We examined duodenal and colon intestinal biopsies from 105 participants undergoing gastrointestinal (GI) endoscopy/colonoscopy and gut-draining lymph-node (LN) collection in 75 participants undergoing clinically indicated GI surgery within an HIV-endemic region in South Africa. Using flow cytometry, we identified intraepithelial ILC1s (ieILC1s; Lin⁻CD127⁻CD103⁺NKp44⁺) and ILC3s (Lin⁻CD127⁺CD117⁺) in gut tissue, and NK cells (Lin⁻CD94⁺CD56⁺/CD16⁺/) and ILC3s in LN. Despite CD4 T-cell depletion in the duodenum and colon, ILC3 frequencies were preserved regardless of viral suppression, with only a trend toward reduced duodenal ieILC1s. Consistently, LNs also showed stable ILC subset frequencies despite significant CD4 T-cell depletion (p<0.001). Single-cell transcriptomic analysis revealed downregulation of the NF-κB pathway and other immune regulatory pathways in LN ILC3s and NK cells, suggesting functional reprogramming. Multiplexed immunofluorescence (IF) showed that ILC3s were enriched in organized gut-associated lymphoid tissue (GALT) relative to the lamina propria (p<0.0001), but with no overall reduction by tissue area quantification in PWH, consistent with flow-cytometry. Ongoing work is applying 30-plex IF (Lunaphore) to validate these findings in LN from PWH. These findings challenge the view that tissue ILC depletion drives HIV-associated gut dysfunction and instead implicate altered ILC function as a potential mechanism contributing to persistent mucosal immune dysregulation in PWH.

P07: Plasma and urine profiling reveals compartment-specific complement activation in immune-mediated kidney diseases

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Background

Dysregulation of the complement system is implicated as a primary or secondary driver in various glomerular diseases, including focal segmental glomerulosclerosis (FSGS), IgA nephropathy (IgAN), lupus nephritis (LN), and primary membranous nephropathy (MN). Invasive kidney biopsy remains the diagnostic gold standard, highlighting the need for non-invasive biomarkers to support early diagnosis, monitor disease activity, predict relapse, and guide therapy.

Methods

Plasma and urine samples from healthy donors and patients were analyzed by sandwich-ELISA for complement activation markers (C4c, C3bc, terminal complement complex [TCC]); lectin-pathway pattern recognition molecules (mannose-binding lectin [MBL], ficolin-3 [FCN-3], collectin-11 [CL-11]); and pentraxin-3 (PTX-3). Groups were compared using Kruskal–Wallis with Dunn's multiple comparison; urine values were not adjusted for urinary creatinine.

Results

In plasma, C4c and C3bc were elevated in FSGS, IgAN, and MN versus HD, whereas TCC was elevated in FSGS, LN, and MN. In urine, all three activation markers were elevated in IgAN, LN, and MN, with C3bc additionally elevated in FSGS. FCN-3 was elevated in plasma (FSGS, LN) and in urine across most disease groups. PTX-3 distinguished FSGS, IgAN, and MN in plasma, and LN and MN in urine. In contrast, MBL and CL-11 levels were largely unchanged across all groups in both compartments.

Conclusions

Complement activation and inflammatory biomarkers were detectable in both plasma and urine across immune-mediated kidney diseases — most strikingly in LN, where complement activation in urine was substantially higher than in plasma. These findings support urine as a valuable, noninvasive compartment for capturing local intrarenal complement activity.

P08: Pre-existing epidermal TRM cells augment the inflammatory reaction to secondary contact allergens

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Purpose

Allergic contact dermatitis (ACD) is an inflammatory skin disease driven by T cells, particularly tissue-resident memory T (TRM) cells. Allergen-specific TRM cells mediate the rapid and strong inflammatory response that occurs upon re-exposure to the specific allergen. However, whether TRM cells within allergen experienced skin can influence subsequent sensitizations to other unrelated allergens is not known. The purpose of this study was to determine how sensitization to one allergen affects the immune response to a subsequent unrelated allergen, and whether un-specific TRM cells can modulate inflammation through bystander activation mechanisms.

Methods

In a mouse model of contact hypersensitivity (CHS), we investigated the immune responses to two different experimental contact allergens, 2,4-dinitrofluorobenzene (DNFB) and oxazolone (OXA). Mice were sensitized to one or both allergens in a three-week period and subsequently re-challenged with either one.

Results

Distinct T cell subsets were induced depending on the allergen, and sensitization to both allergens resulted in enhanced inflammation upon re-exposure. Interestingly, double-sensitized mice showed altered T cell subsets during allergen re-challenge compared to single-sensitized mice. This suggests that TRM cells in allergen-experienced skin can modulate the immune environment and influence the immune response during later allergen exposures.

Conclusion

Our results indicate that previous sensitizations amplify skin inflammation during subsequent allergen exposures. We hypothesize that this increase and modulation of inflammation is potentially caused through bystander activation of pre-existing, unspecific TRM cells. This response is likely mediated by cytokines such as IL-1 β , IL-18, and type I interferons, which promote local inflammation and immune cell recruitment.

P09: The Effect of Fisetin in Aging and Diet Induced Senescence and Inflammation in Mice

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Background:

Aging is associated with multimorbidity and chronic inflammation, processes to which cellular senescence contributes. High-fat diets exacerbate systemic inflammation and may increase senescent cell burden. Fisetin has been proposed as a senotherapeutic, but its in vivo effects remain unclear, particularly under metabolic stress.

Aim:

To assess whether fisetin reduces systemic and tissue-specific senescence markers in young and aged mice fed either a normal or high-fat/high-fructose diet.

Methods:

Young (3 months) and aged (18 months) male C57BL/6 mice were fed either a normal diet (ND) or high-fat/high-fructose diet (HF-HFD; 10 weeks). Mice received vehicle or fisetin (20 mg/kg) by oral gavage (2 days/week for 4 weeks; n=8/group). Blood and tissues (brain, heart, adipose, thymus, spleen, kidney, liver) were collected. Senescence markers were analyzed using ELISA, Luminex, qPCR, flow cytometry, and immunohistochemistry.

Results:

Senescence markers increased with age in plasma (e.g., GDF15, MMP-12) and tissues (p16, p21, Gdf15, Plaur), with p16 consistently elevated across organs. HF-HFD further increased plasma senescence markers. Fisetin restored Lmn1 expression in aged HF-HFD brains. Trends toward reduced p16 with fisetin were observed in some tissues of aged ND mice but not under HF-HFD. Effects on other markers were inconsistent. Additional analyses are ongoing.

Conclusion:

Age and metabolic stress drive senescence across tissues, while fisetin showed limited senolytic effects under these conditions. Future studies should address dosing, exposure, and metabolic context.

P10: Germline-Encoded TCR Segments Shape allergen-specific CD4⁺ T Cell Responses

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T cells recognize antigens through highly diverse cell-surface receptors known as T cell receptors (TCRs). While TCR specificity is largely attributed to the random insertion of non-templated nucleotides within the complementarity-determining region 3 (CDR3), the contribution of genetically encoded TCR elements to antigen recognition remains poorly defined. We hypothesize that loss of a single germline allele can substantially alter immune responses to allergens and pathogens. To address this, we characterized the CD4⁺ T cell response to house dust mite (HDM) allergen extract using MHC class II I-A^b tetramers presenting the immunodominant Der p_{117–127} peptide. Single-cell RNA sequencing revealed a consistent bias in TCR usage, with selection of Trbv31-expressing T cells across multiple mice. We validated that Der p_{117–127} peptide-restricted T cells expressed Vβ14, encoded by the Trbv31 gene, across multiple HDM experiments. Moreover, the biased expansion of Vβ14⁺ tetramer⁺ CD4⁺ T cells could be recapitulated following intraperitoneal immunization with Der p_{117–127} and lipopolysaccharide (LPS); this indicates that the peptide antigen selectively induces the activation and expansion of a very limited selection of T cell clones that primarily express Vβ14. Further work on Trbv31-deficient mice will determine whether this biased response is essential or whether compensatory TCR repertoires can emerge in its absence. These findings raise the possibility that variation or loss of specific germline TCR alleles could meaningfully alter immune responsiveness to allergens and pathogens, potentially explaining inter-individual differences in disease susceptibility.

P11: From Fibrosis to Immunotherapy: TGFBI as a novel Immunogenic Target in Pancreatic Ductal Adenocarcinoma

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Background: Pancreatic ductal adenocarcinoma (PDAC) is characterized by extensive desmoplasia and an immunosuppressive tumor microenvironment that limits therapeutic efficacy. Transforming growth factor-beta-induced protein (TGFBI) is an extracellular matrix protein implicated in fibrosis and tumor progression, but its potential as an immunotherapeutic target in PDAC remains poorly understood.

Methods: Publicly available bulk- and single-cell RNA sequencing data were analyzed to evaluate TGFBI expression, prognostic significance, and cellular localization in PDAC. Gene Ontology (GO) enrichment analysis was performed on significantly upregulated genes in activated pancreatic stellate cells (aPSCs) from PDAC relative to healthy tissue. TGFBI-derived peptides were selected based on in silico epitope prediction and immunopeptidomics data and screened for immunogenicity using IFN γ ELISPOT assays. Antigen-specific rapid expansion protocol (REP) cultures and T-cell clones were subsequently generated.

Results: TGFBI expression was significantly elevated in PDAC compared with normal pancreatic tissue, and high TGFBI expression was associated with inferior overall survival. Single-cell analyses identified aPSCs, precursors to cancer-associated fibroblasts (CAFs), as a major source of TGFBI expression. Moreover, GO analysis demonstrated enrichment of pathways associated with extracellular matrix remodeling and fibrosis in PDAC-associated aPSCs. ELISPOT screening identified several immunogenic TGFBI-derived peptides that induced antigen-specific T-cell responses, enabling the generation of peptide-specific REP cultures and T-cell clones.

Conclusions: TGFBI is a clinically relevant stromal antigen associated with fibrosis and poor prognosis in PDAC. Our findings demonstrate that TGFBI-derived peptides can elicit antigen-specific T-cell responses and provide a rationale for further evaluation of TGFBI as a candidate target for immunomodulatory vaccination in PDAC.

P12: H107e/CAF-Induced Th17 Cells Orchestrate a Unique Immune Response Against Tuberculosis

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Tuberculosis (TB) is responsible for over 1.3 million deaths annually according to the World Health Organization. Despite the availability of the Bacillus Calmette-Guérin (BCG) vaccine, its protective efficacy against pulmonary TB, especially in adults, remains highly variable. Consequently, there is a pressing need to develop more effective vaccines against *Mycobacterium tuberculosis* (M.tb). H107e/CAF is a novel subunit vaccine formulation, developed at Statens Serum Institut (SSI) in Denmark, currently undergoing clinical trials. While human clinical trials can assess vaccine safety and efficacy, detailed investigation of vaccine-induced immune mechanisms at the single-cell level remains challenging due to the invasive nature of lung tissue sampling.

To address this challenge, we utilized a murine model to study vaccine-induced immune responses within the lung tissue. Mice were immunized with H107e/CAF prior to M.tb infection, then whole lung tissues were harvested. Single-cell RNA sequencing and spatial imaging revealed strong IL-17 expression in vaccinated mice, and improved intralesional positioning of CD4 T cells, which correlate with improved infection control.

To enable detailed characterization of the intended Th17-polarized immune response of H107e/CAF vaccine design, we currently investigate Th17 cells' role in orchestrating this unique immune response by leveraging an adoptive transfer murine model to compare the immune response in the presence and absence of Th17 cells in vivo.

This study provides novel insights into the cellular orchestration of immunity against TB. Our results contribute to a deeper understanding of how vaccines like H107e/CAF shape the immune landscape during M.tb infection, with implications for future vaccine design and optimization.

P13: In-depth proteomic analysis of extramammary Paget's disease and associated adenocarcinomas

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Extramammary Paget's disease (EMPD) is a rare epidermal adenocarcinoma, presenting as slow-growing lesions in genital areas. It can be primary or secondary to other adenocarcinomas, such as breast or prostate cancers. The molecular mechanisms distinguishing anatomical EMPD subtypes and their association with underlying tumors remain poorly understood. To identify pathophysiological features and potential therapeutic targets, we analysed the proteomes of microdissected tumor cells and cell islands from formalin-fixed paraffin-embedded tissue of 19 EMPD patients and nine adenocarcinomas from the same patients, using liquid chromatography tandem mass spectrometry. We detected 7977 proteins across all samples, with 20 unique to EMPD and 288 unique to the other cancers. EMPD-specific proteins were implicated in melanin synthesis and kinase binding pathways. Differential expression (DE) analysis revealed 1165 significantly DE proteins between EMPDs and other tumors (FDR<0.05, |log2FC|>1). Top EMPD-enriched proteins included CALML3, LGALS7B and TRIM29, implicating oncoproteins, tumor suppressors, calcium signaling, cell proliferation, differentiation, trafficking, and adhesion. Furthermore, RNA regulatory pathways were highly enriched. Site-specific signatures were also identified, with anal EMPD exhibiting 79 unique proteins compared to other EMPD subtypes, and 1014 proteins with |log2FC|>1 compared to non-EMPD anorectal adenocarcinomas. These included an enrichment of CD46 and GOLGA7 (log2FC=3), suggesting a role for immune evasion and protein trafficking in EMPD, independent from anorectal proteomic signatures. This study characterises EMPD-specific pathways distinct from concurrent adenocarcinomas, and provides an in-depth proteomic analysis of anatomical EMPD subtypes, contributing towards improved diagnosis and, with continued functional exploration, improved treatments, for this poorly understood cancer.

P14: The effect of mixtures of contact allergens on skin sensitization

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Most consumer and occupational products contain multiple contact allergens as well as skin irritants, however, not much is known about the effect of mixtures on skin sensitization and the immunological pathways involved. Furthermore, no generally accepted risk assessment has been established in the EU, meaning that many mixtures of allergens and irritants remain unregulated in consumer products. This study aims to investigate how mixtures impact skin sensitization as well as to develop models to identify these mixture effects and it is carried out in the framework of the European Partnership for the Assessment of Risks from Chemicals (PARC).

In line with EU regulatory requirements, New Approach Methods (NAMs) accepted by the OECD, such as the human cell line activation assay (h-CLAT), are increasingly used for the identification and potency assessment of skin sensitizers. In this study, the h-CLAT was performed, and multiple contact allergens and irritants were applied to THP-1 cells individually or in combination. CD54 and CD86 expression was measured to assess THP-1 cell activation.

For all tested combinations the allergen concentrations needed for the induction of THP-1 cell activation was lower in mixtures compared to single allergens, indicating that mixing two contact allergens can increase the ability of each one to activate immune cells and induce sensitization. In conclusion, these findings demonstrate that mixtures of contact allergens can enhance immune cell activation compared to single allergens, highlighting the need for improved understanding, test models, and regulatory approaches addressing mixture in consumer and occupational products.

P15: Glycoprotein A repetitions predominant (GARP): An Emerging Target for Cancer Therapeutic Immune Modulatory Vaccines

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Purpose:

Immunotherapy has transformed cancer treatment but has had limited success in myeloid malignancies. Immune modulatory vaccines represent a novel strategy, activating anti-regulatory T cells against antigens derived from immunosuppressive proteins in the tumor microenvironment, which has already shown clinical activity in solid tumors. We assess the preclinical potential of an immune modulatory vaccine targeting GARP, a docking protein that activates TGF- β . GARP is expressed by FOXP3+ regulatory T cells, megakaryocytes and activated B-cells. Tumor cells express GARP in many cancer diseases, and antibody blockade of GARP:TGF- β 1 has shown favorable effects in murine models of myeloid cancer.

Methods:

Sixty-five GARP-derived 20-mer peptides were generated, spanning the entire GARP sequence. Peptide immunogenicity was evaluated by IFN- γ ELISPOT after in vitro stimulation of peripheral blood mononuclear cells from healthy donors and myeloid cancer patients. Lead epitopes were further characterized for CD4 and CD8 T-cell responses by intracellular cytokine staining for IFN γ and TNF α .

Results:

Twelve immunogenic lead epitopes were identified in the initial screening. Screening in cancer patients and in an expanded healthy donor cohort narrowed these to six that remained highly immunogenic. GARP_32 emerged as the strongest candidate, eliciting robust CD4 and CD8 responses, and was used to generate highly reactive GARP-specific T-cell cultures.

Conclusion:

GARP contains immunogenic epitopes capable of inducing specific T-cell responses, with GARP_32 as the leading candidate. These findings support therapeutic vaccination against GARP to target immunosuppressive cells in myeloid malignancies and warrant further preclinical evaluation.

P16: Repurposing Thiopurines to re-sensitize Cold Tumors and expand the reach of Immunotherapy

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Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of solid cancers but remain ineffective in immunologically "cold" tumors. We aim to repurpose 6-thioguanine (6-TG), a thiopurine that incorporates into DNA and induces mismatch repair-dependent point mutations, to increase tumor mutational burden and neoantigen formation. We hypothesized that 6-TG enhances tumor antigenicity, promotes immune activation within the tumor microenvironment (TME), and sensitizes cold tumors to Immune checkpoint inhibitors (ICIs) therapy. This strategy proved successful in murine models of low-mutation melanoma and will be further evaluated in models of soft-tissue sarcoma, pancreatic and breast cancer.

In vitro, prolonged exposure to low doses of 6-TG significantly increased MHC class I expression in all cancer models, consistent with enhanced neoantigen presentation and increased tumor immunogenicity. In vivo, oral 6-TG administration consistently delayed tumor growth in syngeneic sarcoma and breast cancer models. Furthermore, in the sarcoma model, combining 6-TG with anti-CTLA-4 and anti-PD-1 resulted in durable tumor control, with almost all mice achieving complete tumor regression, outperforming either monotherapy (6-TG alone and ICIs alone). Tumor immune microenvironment analysis following 6-TG monotherapy showed increased activation of dendritic cells (CD11c⁺CD103⁺) and expansion of CD4⁺ and CD8⁺ T cells, indicating enhanced antigen presentation and priming of adaptive antitumor immunity.

These findings support a model in which 6-TG increases tumor antigenicity through enhanced neoantigen presentation, reshaping the TME toward an inflamed, immune-responsive state. By converting immunologically cold tumors into hot tumors, 6-TG may broaden the population of patients who benefit from ICI therapy.

P17: The influence of the gut microbiome and dietary-derived metabolites on type 2 immunity against *T. muris* infection in mice

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Intestinal helminths infect over one billion people worldwide, posing a significant public health burden, particularly in low-income regions where infections contribute to malnutrition and impaired cognitive development. Protective immunity against these parasites relies on type-2 immune responses; however, the mechanisms governing their initiation and regulation in the gut remain incompletely understood. While dietary components and gut microbiota-derived metabolites are known to shape host immunity, their role in helminth infection remains poorly defined.

Here, we investigate how dietary modulation and microbiome composition influence host responses to *Trichuris muris* infection in a murine model. We show that dietary supplementation with phytochemicals or fibre profoundly alters infection outcomes. Contrary to the prevailing understanding that such dietary components are broadly beneficial, these interventions increase susceptibility to helminth infection. This effect is accompanied by distinct shifts in host metabolomic, microbial, and immune profiles.

Our data suggest that high worm burdens are associated with a metabolic shift towards increased aerobic respiration in the caecum and colon, promoting the expansion of facultative anaerobes. Notably, this bloom occurs predominantly in mice with simplified microbiomes, whereas mice with higher microbial diversity appear protected despite comparable worm burdens.

We further tested multiple interventions aimed at restoring metabolic homeostasis, increasing microbiome diversity, or modulating bile acid composition; however, these approaches failed to reverse diet-induced susceptibility.

Together, our findings highlight a critical and unexpected role for diet–microbiome interactions in shaping anti-helminth immunity and challenge the assumption that all plant-derived dietary components are uniformly beneficial in the context of intestinal parasitic infection.

P18: Butyrate is a major determinant of terminal development and function in dendritic cells

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Dairy products account for approximately 14% of energy intake of adults in Europe and North America. Lactose contained in these products can be metabolized into lactate, that is further converted into butyrate by the intestinal microbiome, which can act as an immunomodulatory compound.

The impact of butyrate on dendritic cell development was tested by addition of butyrate to murine dendritic cells (DC) either 48 hours or 30 minutes prior to stimulation and examination. Flow cytometry was used for measuring differentiation, maturation, antigen sampling and cytokine expression. Cytokines were further examined using ELISA.

Butyrate treatment altered the cytokine profiles of stimulated DCs in a time dependent manner, with 48 hours of treatment abolishing IL-12 responses while IL-10 and TGF- β were increased, and treatment just prior to stimulation resulted in a near complete inhibition of cytokines. Butyrate changed the size of the cells and altered the ratio of cells with intermediate and high expression of MHC-II. The intermediate population showed increased scavenger receptor expression and antigen sampling ability. Upon stimulation with LPS, the DCs treated with butyrate were capable of maturation, with expression of co-stimulatory receptors for T-cell activation being present prior to stimulation.

Based on these results, butyrate alters the development of DCs towards tolerance, promoting the growth of cells with increased antigen sampling, expression of T-cell co-receptors and anti-inflammatory cytokine responses. This study provides a deeper understanding of how butyrate may exert its immunomodulatory effects and the importance of the microbiome for intestinal health.

Author declares no conflicts of interest.

P19: Beyond complement activation: MASP-2 acts on the beta-cell surface and may influence insulin secretion

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Background: MASP-2 is the primary enzyme driving complement activation in the lectin pathway by initiating formation of the C3 convertase (C4bC2b). Recently, we found that MASP-2 suppresses insulin secretion in rodent and human models of type 2 diabetes. Whether this suppression is a direct or indirect effect on beta-cell function remains unclear. We hypothesize that MASP-2 directly affects beta-cell function.

Methods: Recombinant MASP-2 was produced in the ExpiCHO system, purified, and validated by immunoblotting. MIN6 mouse pancreatic beta-cells were treated with MASP-2. At 1, 8, and 24 hours after glucose stimulation, immunoblotting for insulin and MASP-2 was performed on untreated and treated beta-cells.

Results: No variation was detected 1 hour after MASP-2 treatment, but a significant, time-dependent reduction in intracellular insulin content was observed in cells treated with MASP-2 at 8 and 24 hours. Importantly, intracellular MASP-2 was not detected at any time point, suggesting that MASP-2 acts at the cell membrane without internalization.

Conclusions: Our findings suggest that MASP-2 plays a significant role in regulating insulin production and secretion in pancreatic beta-cells, likely via a surface-restricted interaction through a non-canonical pathway.

P20: Pre-clinical testing of a Nipah vaccine exploiting the Tag/Catcher Virus-like-particle vaccine platform

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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 aimed to develop a vaccine candidate against NiV using the Tag/Catcher nanoparticle nucleic acid-based platform. Protein and mRNA NiV vaccines were tested head-to-head in mouse immunisation studies followed by live NiV virus neutralisation test (VNT) in BSL4 conditions to investigate the biological efficacy of the vaccine-induced antibody response. To further characterise the immunological response, antibody receptor blocking activity was also measured.

Our results reveal that mRNA and protein NiV vaccines induce protection in mice, positive results in VNTs and receptor-blocking activity. Furthermore, the protein NiV vaccine has been tested in ferrets and hamsters, inducing positive immunological responses, and will be moved forward to toxicity studies.

We anticipate this study to help effectively prevent potential future NiV epidemics and pandemics and contribute to the global vaccine field.

P21: PhIP-Seq for Serological Detection of Viral Exposure: A Herpes Virus Pilot Study Using M13 Phage Display

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Background and purpose: Immune-mediated diseases (IMIDs) are a prevalent group of diseases which affect various organs, while sharing common inflammatory pathways. Genetic predisposition is a known risk factor for the onset of IMIDs, while viral infections just recently became implicated as a risk factor. This implication mostly derived from studies with limited scope on one or a few viruses. Phage immunoprecipitation sequencing (PhIP-Seq) allows to serologically detect past viral infections with a much wider scope by expressing thousands of viral antigens in a phage display library. Our project aims to develop and validate a PhIP-Seq pipeline based on an M13 phage display library. With a validated pipeline we hope to investigate the role of viral infections on the pathogenesis of IMIDs.

Methods: We designed a human herpes virus (HHV) antigen library encompassing ~1.200 unique sequences. We use a 3+3 expression system, expressing our HHV oligos on the M13 surface protein gIII. The resulting phage library is immunoprecipitated on serum samples, run on the Illumina MiSeq, and bioinformatically analysed.

Results: We successfully created a HHV phage display library with good representation based on the 3+3 expression system. The first Illumina MiSeq runs with this library showed biological meaningful enrichments, although overall background noise remained high and data quality low.

Conclusion: Optimisation of the 3+3 expression system is needed. Further, a 8+8 expression system-based (M13 protein gVIII) phage library has been created, will get sequenced, and will get compared to the 3+3 expression system-based phage library.

There is no conflict of interest.

P22: Approaches in characterizing NK cell regulation: A proof-of-concept utilizing NKG2D knockout and a CD300a engager knock-in spheroid model

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The purpose of this research is to generate a framework for studying novel NK regulatory mechanisms which is essential for understanding immune evasion. This study uses the NKG2D and CD300a receptor pathways to validate 2D and 3D cytotoxicity models.

NKG2D knockout and ctrl NK cells from three donors were generated through delivery of ribonucleoprotein by mechanoporation and CD300a engager molecule expressing melanoma cells were generated by lentiviral integration. A 2D cytotoxicity assay against melanoma, colorectal cancer and glioblastoma cell lines and a 3D spheroid cytotoxicity model of the CD300a engager expressing melanoma cells with naïve or cytokine activated NK cells from one donor was carried out.

Both the 2D assay and the 3D assay showed a reduction of NK cell killing capacity when knocking out NKG2D on the NK cells or stably integrating a CD300a engager in a melanoma respectively. In line with expectations, donors expressing higher level of NKG2D on the surface of NK cells showed a higher overall cytotoxicity in the 2D assay and this cytotoxicity was impaired when knocking out the receptor. The ability of NK cells to control spheroid size was reduced against melanomas expressing the CD300a engager correlating with the amount of surface expression of the engager and activated NK cells had a higher killing capacity of the spheroids.

We show a robust framework for studying NK cell regulatory mechanisms through knockout of receptors on human NK cells and knock-in of inhibitory receptor ligands on target cells in 2D and 3D immune killing assays.

P23: AHR signalling in colonic subepithelial fibroblasts coordinates immune homeostasis, barrier integrity, and protection against colitis

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The intestinal lamina propria (LP) harbours a large and transcriptionally diverse population of fibroblasts (FBs). In addition to providing structural support to the tissue, intestinal FB secrete growth factors that support the epithelial niche as well as chemokines and cytokines that regulate immune cell positioning, survival and differentiation. The extent to which environmental sensing by intestinal FB regulates tissue homeostasis and the potential factors involved in this process remain unclear. While screening an in-house generated scRNA-seq data set of murine intestinal FB we identified transcripts for the ligand-dependent transcription factor aryl hydrocarbon receptor (AHR), to be highly expressed by a distinct subset of colonic subepithelial fibroblasts (seFBs) that co-expressed CD9. As well as Ahr related signalling pathways Ahr⁺CD9⁺ seFBs were enriched in genes involved in immune cell survival, maintenance, and recruitment. Preliminary assessment of tamoxifen treated Gli1creERT2Ahrfl/fl mice, which lack Ahr specifically in FBs demonstrated a selective reduction in CD141⁺ seFBs, along with certain alterations in immune cells, such as, but not limited to: increased numbers of CD4⁺ T cell subsets and increased numbers of dendritic cells. These mice also showed heightened susceptibility to dextran sodium sulfate (DSS)-induced colitis. In ongoing work we are investigating the role of AHR expression by intestinal FB in epithelial support and in immune-FB interactions. Together, these findings suggest that sensing of AHR ligands by a subset of colonic seFB plays a key role in maintaining intestinal homeostasis and in protecting against inflammation-induced colonic injury.

P24: Factor D/Adipsin activation is disrupted in obesity and improved by Roux-en-Y gastric bypass

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Background

Obesity is associated with chronic inflammation and adipose tissue dysfunction. Factor D/Adipsin (FD), an adipocyte-derived enzyme of the alternative complement pathway, regulates the FD–C3a axis involved in insulin secretion and lipid metabolism. FD is secreted as inactive pro-FD and activated extracellularly by MASP-3, but previous studies have measured only total FD. We hypothesized that obesity alters FD activation and that Roux-en-Y gastric bypass (RYGB) restores this balance.

Methods

Immunoassays were developed to quantify total FD, pro-FD, active FD, and MASP-3. Serum samples from 131 patients undergoing RYGB were analyzed before surgery and 24 months postoperatively and compared with 94 healthy controls. FD activation kinetics were assessed in pre- and post-surgery samples and controls by incubation in Mg²⁺-EGTA buffer with or without the MASP-3 inhibitor TFMI-3.

Results

Before surgery (mean BMI 45 kg/m²), patients had higher total FD and pro-FD (both p<0.0001) and lower active FD (p=0.0004) than controls. 24 months after RYGB (mean BMI 30 kg/m²), total FD and pro-FD decreased (both p<0.0001), while active FD increased (p=0.0056). MASP-3 levels were reduced before surgery (p=0.0004) and declined further after RYGB. Pro-FD conversion was accelerated in obesity compared with controls (69.8 vs. 16.7 Δng/mL/h; p=0.001) and decreased after surgery (36.6 Δng/mL/h). TFMI-3 attenuated pro-FD conversion in all groups.

Conclusions

Obesity is associated with pro-FD accumulation and active-FD deficiency despite elevated total FD, indicating dysregulated FD processing not captured by conventional assays. RYGB partially normalizes FD maturation, while MASP-3 remains a key regulator of extracellular FD activation.

P25: Compartment-Specific Complement Dysregulation in IgA Nephropathy - Divergent Profiles of Plasma and Urinary Biomarkers

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Background:

IgA nephropathy (IgAN) is the most common primary glomerulonephritis, yet diagnosis still depends on invasive renal biopsy, underscoring the need for noninvasive biomarker strategies. Although complement activation is central to IgAN pathogenesis, whether systemic and intrarenal activation are mechanistically distinct remains unclear. We therefore aimed to characterize complement pathway contributions by comparing biomarker profiles across both compartments.

Methods:

We analyzed 147 plasma and 166 urine samples from IgAN patients (135 paired), alongside 100 plasma and 24 urine samples from healthy controls. Complement biomarkers were quantified targeting classical/lectin, alternative, and terminal pathway activation products (C4c, C3bc, C3bP, TCC) and pathway proteins (Factor D, properdin, FCN-3, MASP-2, MASP-3, total C3). Urine values were normalized to creatinine and data analyzed using age- and sex-adjusted linear regression.

Results:

Complement activation markers (C4c, C3bc, C3bP, TCC), Factor D, and total C3 were significantly elevated in both plasma and urine. Properdin and FCN-3 were elevated exclusively in urine, while MASP-2 was significantly decreased in urine only. Strikingly, MASP-3 showed opposing directionality: reduced in plasma yet elevated in urine, suggesting differential local versus systemic regulation. Urine biomarkers showed stronger and more coordinated inter-marker correlations than plasma, whereas cross-compartment correlations were generally weak, with the notable exception of Factor D.

Conclusion:

IgAN is characterized by both systemic and robust local renal complement activation, predominantly via the lectin and alternative pathways. Compartment-specific patterns, including divergent MASP-3 behavior, highlight mechanistic distinctions between systemic and intrarenal dysregulation, supporting urine as a noninvasive matrix for monitoring local complement activity.

P26: Design of next-generation lipid nanoparticles for mRNA vaccines: Enhancing potency and safety by incorporation of adjuvants

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Lipid nanoparticles (LNPs) are the most successful mRNA vaccine delivery systems to date. Despite their inherent adjuvanticity, next-generation LNPs with tailored immunological properties are needed to expand the RNA vaccine platform. A key challenge is that many classical immunostimulators activate pathways that inhibit RNA translation, thereby dampening vaccine immune response. Additionally, limited endosomal escape remains a major bottleneck for nucleic acid therapies. To address these challenges, we are adjuvanting mRNA-LNP vaccines with the non-classical immunomodulator QS-21, with the aim of achieving robust immune stimulation and efficient cytosolic delivery with strong antigen expression. This is expected to enhance vaccine efficacy, allowing for dose sparing and improved safety.

A series of mRNA-loaded LNPs carrying the immunostimulatory saponin QS-21 were successfully prepared. Different workflows for introducing QS-21 into the formulation were tested, and the post-insertion method was ultimately selected. Addition of QS-21 did not affect nanoparticle quality, as confirmed by DLS (z-average size and z-potential), FRET analysis, and mRNA gel electrophoresis. QS-21-functionalized LNPs showed an apparent decrease in encapsulation efficiency, as measured by Ribogreen assay; however, this only resulted in a modest decrease in transfection efficiency in THP-1 and HEK-293T cell lines. H1N1 mRNA LNPs adjuvanted with 3–15 µg/ml QS-21 were administered to mice to assess the immunogenicity and safety of the novel vaccine formulations. Study results are still pending but our hypothesis is that QS-21 can induce a potent adjuvant effect, as evidenced by higher antibody and cytokine titers and stronger T-cell responses. Ultimately, this should improve the efficacy of LNP–mRNA vaccines.

P27: Peptide Tags to Increase Uptake and Immunogenicity of Mucosal Vaccines

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Despite their widespread use, parenterally administered vaccines struggle to induce strong local responses at mucosal effector sites. Mucosal administration offers a promising strategy to increase local responses, although efficient uptake of antigens across the mucosal barrier remains a big hurdle for mucosal vaccine delivery. Targeting moieties are emerging as an effective strategy to increase antigen retention, uptake, and presentation to APCs, resulting in increased immunogenicity.

However, identifying functional moieties using a trial-and-error screening approach is labor-intensive and costly. AI-based design tools such as pipelines combining Rfdiffusion, ProteinMPNN, and AlphaFold offer new opportunities to engineer and design proteins and peptides with specific biological functions, structures, and sequence motifs. By combining in silico design with the development of a multi-species in vitro screening platform, we seek to establish a translational workflow for the discovery and validation of functional tags, before moving them into appropriate in vivo models. Assays included in this in vitro screening platform include extracellular peptide binding flow cytometry assays in multiple species, fluorescence imaging, and receptor-specificity assays.

Using nature-inspired peptides, we have identified multiple peptide tags exhibiting species-translatable extracellular binding and initial assays confirm peptides are binding specifically to the target receptor, rather than through non-specific binding. Ongoing work includes assay optimization of intracellular peptide quantification and translocation ability across Caco-2 monolayers.

Together, our results support the continued development of nature-inspired peptide tags for mucosal vaccine delivery, and demonstrate the utility of an AI-guided multi-species screening platform for accelerating the identification of functional targeting moieties.

P28: The role of fermentable fibers in *Trichuris muris* infection: host susceptibility and microbial interactions

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Parasitic worms infect over a billion people globally, contributing to malnutrition and morbidity. These infections interact with the host immune system and gut microbiota (GM), with the worm's own microbiome potentially playing a role. However, the precise interactions between the intestinal worms and the bacteria in the host gut have not been elucidated. Inulin, a fermentable fiber, is metabolized by the GM and can alter microbial composition and activity. Given these effects, we investigated how dietary fermentable fibers and antibiotic treatment influence host susceptibility to *Trichuris muris* infection and the role of the worm microbiome. Mice were fed diets containing fermentable fibers (chow or inulin) or a non-fermentable fiber diet (cellulose) for 14 days prior to infection. Inulin- and chow-fed mice exhibited significantly higher worm burdens than cellulose-fed mice, suggesting that fermentable fibers enhance host susceptibility. Host microbiome analysis revealed substantial differences between inulin- and chow-fed mice, yet the worm microbiomes remained stable across dietary groups. To assess the role of the host microbiome in infection, antibiotic treatment was administered. While antibiotics significantly reduced host GM alpha diversity, they had little impact on the worm microbiome alpha diversity and did not alter worm infectivity. Worm microbiomes were consistent between antibiotic-treated and untreated mice, demonstrating their stability even under conditions of host-microbial perturbation. These findings demonstrate that dietary fermentable fibers increase susceptibility to *T. muris* infection, though apparently not via host GM alterations. Furthermore, the stability of the worm microbiome, even under antibiotic-induced perturbation, underscores its resilience within the host environment.

P29: Screening Immunostimulators to Elicit Airway Immune Responses After Intranasal Delivery

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The nasal mucosa is a primary entry site for respiratory pathogens. However, conventional parenteral vaccines induce limited immune responses at this mucosal surface. Intranasal vaccines therefore represent an attractive strategy for enhancing protection against respiratory infections. A challenge in intranasal vaccine development is the identification and preclinical evaluation of effective mucosal adjuvants, as no mucosal adjuvants have yet been approved for human use. In this project, I aim to develop a pipeline for identifying novel adjuvant candidates for intranasal vaccination.

The pipeline combines in vitro and in vivo screening approaches. A central component is the incorporation of human nasal epithelial organoids (HNEOs). When fully differentiated, HNEOs contain the major epithelial cell types of the nasal mucosa and therefore better recapitulate its complexity than conventional cell lines. Candidate mucosal adjuvants will be screened for their ability to stimulate immune responses in HNEOs before in vivo validation.

Only a few studies have successfully developed HNEOs. I have isolated nasal epithelial cells from 10 donors and expanded the cells in culture for six passages to generate HNEOs. Furthermore, I have identified the HNEO's cell composition by confocal microscopy. Current efforts focus on optimizing organoid differentiation to encompass all relevant nasal epithelial cell types and on developing a flow cytometry panel for phenotypic characterization.

These findings demonstrate the feasibility of generating HNEOs from primary human cells. Incorporating HNEOs into adjuvant screening introduces a human component in the early identification of mucosal adjuvants and may improve the translation of preclinical findings to in vivo studies.

P30: The role of intestinal fibroblast-derived signals in DC2 development & maturation

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Intestinal tissues contain a substantial population of dendritic cells (DCs), which play a crucial role in orchestrating immune responses. DCs can traditionally be divided into type 1 DCs (cDC1) and type 2 DCs (cDC2), each subset contributing to different T cell responses. While cDC1 development, heterogeneity & function have been extensively studied, multiple questions remain regarding cDC2 maturation and plasticity with cDC2s appearing to be far more complex than previously appreciated. Previous studies have suggested a key role for Notch-2 signaling in DCs in the generation of the intestinal cDC2 compartment, specifically CD103⁺ SIRP α ⁺ cDC2, Isolated Lymphoid Follicle (ILF) associated cDC2 and the recently characterized cDC2A. The source and nature of the Notch-2 ligands regulating DC2 development and whether such signaling modulates additional aspects of intestinal DC biology remain unknown. Using in-house and publicly available scRNA-seq data sets we have found the Notch-2 ligand Delta like ligand 1 (DLL-1), to be expressed by human and murine intestinal LP fibroblasts (FB) but by few other cells, as well as by human & murine Peyer's Patch (PP) FB and fibroblastic reticular cells (FRCs) in the mesenteric lymph nodes. We hypothesize that fibroblast-derived DLL-1 expression is necessary for cDC2 subset development and maturation in intestinal tissues. To address that, we aim to use transgenic mouse models targeting DLL-1 expression by FBs and FRCs and assess the cDC2 compartment through multi-parameter flow cytometry, scRNA-seq and spatial transcriptomics.

P31: T-cell Phenotypes and Coronary Artery Disease in People with HIV

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Background: Living with HIV is associated with coronary artery disease (CAD), which can be accurately phenotyped using coronary computed tomography angiography (CCTA). T-cells are important in atherogenesis, but the role of circulating T-cells on CAD is poorly understood. We aimed to determine whether activated, apoptotic, or senescent CD4+ and CD8+ T-cells are associated with plaque phenotypes in people with HIV.

Methods: People with HIV (PWH) in the Copenhagen Comorbidity in HIV Infection (COCOMO) Study underwent a protocolized research CCTA and collection of blood samples for flow cytometry. We determined proportions of activated (HLA-DR+CD38+), apoptotic (CD28-CD95+), and senescent (CD28-CD57+) CD4+ and CD8+ T-cells. CCTAs were analysed for plaque volumes and its components were quantified. Multivariable regressions adjusted for age, sex, smoking status, dyslipidemia, diabetes, and current CD4+ T-cell count determined associations of T-cell proportions and plaque phenotypes.

Results: We included 547 PWH with CCTA and flow cytometry data, of whom 44% had quantifiable plaque (Table 1). Each doubling in proportions of activated CD4+ and CD8+ T-cells was associated with 52% lower total and 43% lower lipid-rich plaque volumes after adjustments, respectively (Figure 1). Neither apoptotic nor senescent CD4+ and CD8+ T cells remained significantly associated with any plaque composition in the adjusted models (Figure 1).

Conclusion: Among PWH, higher proportions of circulating activated, but not apoptotic or senescent T-cells were independently associated with less CAD and plaque burden. This highlights a role for T-cells on atherogenesis in PWH while suggesting that circulating T-cell phenotypes differ from those observed within atherogenic plaque.

P32: Clonal and Transcriptional Landscapes of Human Intestinal Plasma Cell in Homeostasis and Inflammation

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Purpose: Intestinal plasma cells (PCs) in the lamina propria (LP) are central to gut immunity, yet their heterogeneity, isotype distribution, and clonal organisation in health and disease remain incompletely understood. We characterise PC subsets, their isotype associations, and clonal dynamics across tissue sites and disease states.

Methods: Single-cell RNA and BCR sequencing was performed on PC compartments from patients with Crohn's disease (CD; paired inflamed and non-inflamed ileum) and colorectal cancer (CRC; ileum and colon, homeostatic control). Transcriptional clustering explored PC subsets, and clonal analysis assessed clone size, isotype distribution, and clonal sharing across tissue sites and inflammatory states.

Results: Preliminary analysis reveals isotype differences between disease states in ileum, with IgA2 predominating in homeostasis and IgA1 in CD, and unswitched clones appearing more abundant in the ileum than the colon in homeostasis. LP PC clonal landscape is consistently diverse across compartments, with a Gini coefficient around 0.5 and high Shannon diversity, dominated by many small and few medium-sized clones. Clonal sharing between colon and ileum LP is limited in homeostasis. In contrast, clonally related PCs span inflamed and non-inflamed ileum in CD, possibly reflecting the physical proximity of these sites.

Conclusions: These preliminary findings reveal a consistently diverse LP PC compartment whose isotype composition is shaped by both disease state and tissue site. We observe a higher IgA1/IgA2 ratio in CD relative to homeostatic ileum, and greater abundance of unswitched clones in ileum than colon. Ongoing sequencing of additional patients will determine whether these patterns hold across individuals.

P33: Preclinical characterization of humoral immune responses elicited by our lead inactivated hepatitis C virus vaccine candidate

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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

P34: Optimization of T cell reactivity assessment and antigen-specific screening in peripheral blood of healthy donors

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Purpose: Antigen-specific T cell responses have been implicated in the pathogenesis of atopic dermatitis (AD) and psoriasis (PsO). We have identified T cell clones shared between skin lesions and peripheral blood in both diseases, providing a rationale for screening antigen reactivity in blood. However, their reliable detection in PBMCs remains methodologically challenging. This study aims to establish and optimize an Activation-Induced Marker (AIM) assay for detecting antigen-specific T cells in healthy donor PBMCs.

Methods: PBMCs from healthy donors were stimulated with *Candida albicans* or viral peptide pools. T cell reactivity was measured by a multi-marker AIM assay using flow cytometry, evaluating and quantifying co-expression of CD69/CD154/CD137/CD134 on CD4⁺ and CD8⁺ T cells. Comparisons between *C. albicans* antigen preparation methods and professional antigen-presenting cell generation protocols are currently underway.

Results: Viral peptides and *C. albicans* peptides stimulation elicited detectable AIM T cell responses above unstimulated background, demonstrating assay sensitivity to prevalent immunodominant antigens. Preliminary comparisons across antigen conditions reveal differential AIM frequencies in CD4⁺ and CD8⁺ T cell compartments.

Conclusions: Multi-marker AIM assays provide a feasible and sensitive platform for detecting antigen-reactive T cells in healthy donor PBMCs. Ongoing optimization of antigen preparation and APC protocols will improve assay reproducibility and sensitivity ahead of application to AD and PsO patient cohorts.

Conflict of Interest: None declared.

P35: Production and test of molecularly adjuvanted and multimerized recombinant vaccines against conserved protein targets representing influenza A virus

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As the threat of an influenza epidemic or even pandemic continues to hang over our heads, the need for broad, preventative measures keeps growing. The current vaccination strategy is not sustainable, both in the ways that the main production uses embryonated chicken eggs, a time consuming, expensive and wasteful process, and in the fact that we are basically guessing which influenza strains we should vaccinate for in the upcoming flu season, not even covering pandemic strains.

These circumstances make for an opportunity to develop a vaccine candidate targeting universal/conserved B and T cell antigens from influenza virus. My project focuses on the production of these vaccine candidates and consider their design, expression, purification, and validation. These will be formulated with molecular adjuvants and assembled into multimeric, repetitive nanoparticles (e.g., virus-like particles). The vaccine candidates will be produced in *E. coli* assuring a fast, adaptable, inexpensive, and unlimited source. Using an established experimental animal model of influenza challenge, we will test the effect of vaccination delivered by intranasal and/or systemic application. In short, I am generating various mucosal vaccines, testing and characterizing their immunogenicity, and examining their ability to protect against influenza challenge and block transmission to naïve animals using different vaccination strategies.

P36: A Spatially Resolved Human Tonsil Model for Studying Mucosal Immunity and Host-Pathogen Interactions

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Respiratory infections such as influenza impose a major global health burden, driven by pathogens that spread efficiently across the upper airway mucosa and evade immunity through continuous antigenic variation. Yet human mucosal immunity in the upper airways remains poorly understood, largely because existing experimental models fail to capture the structural and cellular complexity of native tissue. Monolayer cultures and traditional immune organoids lack the coordinated interplay between epithelial, stromal, and immune compartments essential for mucosal immune responses.

Here we present a precision-cut tonsil slice culture system that preserves the spatial organization and cellular heterogeneity of human upper airway tissue. Slices cultured at an air-liquid interface with a collagen overlay retain epithelial integrity, stromal architecture, and resident immune populations over extended periods *ex vivo*. Following apical exposure to live influenza virus, the model recapitulates key features of mucosal immune activation relative to controls, including expansion of CD27⁺⁺CD38⁺⁺ plasmablasts and antigen-specific antibody production in culture supernatants. Multiplex immunofluorescence reveals recruitment of CXCR5⁺CD4⁺ T follicular helper cells to germinal centers one week post-stimulation, consistent with functional germinal center activity. This model enables mechanistic investigation of mucosal host-pathogen interactions within a human context.

P37: The effect of the probiotic -ASTARTE™ on the reduction of recurrent Urinary Tract Infection in women aged 18-40 years

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Background:

Urinary tract infection (UTI) is among the most common bacterial infections and a major driver of antibiotic use. Probiotics may reduce infection-related risk factors by modulating the gut and vaginal microbiome. This trial investigates the effect of the orally administered probiotic combination ASTARTE™ on vaginal and gut microbial composition and the incidence of recurrent UTIs (rUTIs) in women.

Methods:

In this randomized, double-blind, placebo-controlled trial, participants were randomized 1:1 to receive either a daily probiotic capsule (5×10^9 CFU) containing *L. crispatus*, *L. rhamnosus*, *L. jensenii*, and *L. gasseri*, or placebo, for six months. Assessments included symptom questionnaires, urine culture, and shotgun-based microbiome profiling of vaginal and rectal swabs at baseline, 2, 4, and 6 months. Follow-up visits were conducted at 8, 10, and 12 months. The primary outcome was the incidence of symptomatic, bacteriologically confirmed UTIs during the intervention. Secondary outcomes included changes in vaginal and gut microbiome composition.

Results:

A total of 290 participants completed the 6-month intervention, while 46 discontinued early. Among completers, 232 (80%) experienced ≤ 1 UTI, whereas 58 (20%) had rUTI (≥ 2 UTIs). As analyses remain blinded, comparisons between the probiotic and placebo groups are not yet possible. Recruitment began in August.2022, and current projections indicate that the target sample size of 667 participants will be reached within the planned study period.

Conclusion:

Preliminary blinded results show an 80/20% distribution between non-rUTI and rUTI among completers. Final analyses will evaluate treatment effects on rUTI incidence and microbiome composition after completion of the trial.

P38: A comparative study of peripheral blood-derived mast cell culturing protocols for allergy diagnosis

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Purpose: There is no standardised protocol to culture primary mast cells (MCs) in vitro. Existing protocols differ in medium composition, cytokine concentrations, culture duration, and progenitor population (CD34+ or CD117+), resulting in variable MC phenotype and functionality. This complicates cross-study comparisons and clinical translation. We compared three peripheral blood-derived MC (PBdMC) protocols to identify the optimal model for allergy research and diagnostics.

Methods: Protocol 1 (P1): CD34+ progenitors in 2D liquid culture for 6 weeks (n=6). Protocol 2 (P2): CD117+ progenitors in 2D liquid culture for 8 weeks (n=7). Protocol 3 (P3): CD34+ progenitors in 3D semisolid culture for 4 weeks (n=8). Phenotypic characterization was performed by flow cytometry (CD117, FcεRI, MRGPRX2, tryptase, and chymase) and toluidine blue staining. Functional assessment included CD63 upregulation by flow cytometry and a Mast Cell Activation Test (MAT) using pooled peanut-sensitised patient serum.

Results: P2 and P3 yielded significantly more cells than P1 ($28.5 \pm 13.2 \times 10^6$ and $27.8 \pm 21.9 \times 10^6$ vs. $1.9 \pm 0.2 \times 10^6$; $p < 0.01$). All protocols generated CD117+FcεRI+ PBdMCs expressing tryptase and chymase. MRGPRX2 was functionally expressed in P2 ($56.3 \pm 14.3\%$) and P3 ($47.6 \pm 21.8\%$) but absent in P1. IgE-mediated degranulation was highest in P2 ($37.3 \pm 10.6\%$). In MAT, P2 showed the highest CD63 upregulation at 100 ng/mL peanut extract ($55.4 \pm 19.1\%$), followed by P1 ($49.2 \pm 13.5\%$), while P3 showed an attenuated response ($18.8 \pm 6.6\%$).

Conclusion: Culture protocol significantly impacts PBdMC phenotype and functionality. P2 outperformed others in yield, MRGPRX2 expression, and IgE-mediated degranulation, and was selected as optimal. Future work will focus on P2 optimisation for MAT-based diagnosis of food, drug, and other allergies.

P39: Geographic heterogeneity of sulfadoxine-pyrimethamine resistance markers from 2012-2024 and Plasmodium falciparum genetic relatedness in the Democratic Republic of the Congo: implications for protective efficacy

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Aims: Malaria transmission intensity and Plasmodium falciparum resistance to sulfadoxine-pyrimethamine (SP) is heterogeneous across the Democratic Republic of the Congo. This study aimed to characterize spatial and temporal variation resistance markers, estimate regional sulfadoxine-pyrimethamine protective efficacy and perennial malaria chemoprevention, and assess parasite relatedness and gene flow.

Methods: Sequencing data for dhfr (n = 7,554) and dhps (n = 7,162) from samples collected nationwide between 2012-2025 were analyzed, stratified into 2012–2017 and ≥2018 periods. Resistance haplotype frequencies, predicted SP protective efficacy, and phylogenetic relationships were assessed across provinces.

Results: The dhfr triple-mutant IRNI increased from a mean frequency of 71.8% (2012–2017) to 87.7% after 2017. Highly resistant dhps haplotypes were more prevalent in eastern DRC, with rising frequencies of I_GEA_ and I_GEG_. Predicted SP protective efficacy was lowest in Nord- and Sud-Kivu (median ~13.7 days) compared with western provinces (e.g. 29.4 days in Kinshasa), indicating post-2017 reductions in efficacy. Phylogenetic analyses revealed geographically structured parasite populations with pronounced east–west differentiation.

Conclusion: Increasing SP resistance, particularly in eastern DRC, is associated with reduced SP efficacy jeopardizing prophylactic treatment strategies. Genetic surveillance is essential to guide regional chemoprevention and cross-border malaria control strategies.

Keywords: malaria, Plasmodium, sulfadoxine–pyrimethamine, chemoprevention, Plasmodium falciparum, drug resistance, antimalaria drug resistance

P40: Multimarker profiling reveals distinct systemic inflammatory phenotypes and long-term clinical risk – a multicohort study of CRP, IL-6, and suPAR

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Soluble urokinase plasminogen activator receptor (suPAR) has emerged as a strong prognostic biomarker across clinical settings. However, it remains unclear whether suPAR reflects a chronic low-grade inflammatory state distinct from acute inflammatory processes captured by conventional biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6).

We analyzed data from four population-based Danish cohorts (n=17,804; 45% female; age range 18–72 years). Participants were classified into inflammation profiles using unsupervised K-means clustering based on plasma suPAR, CRP, and IL-6. Associations between inflammation profiles and chronic disease incidence and all-cause mortality were examined using Cox proportional hazards models.

We evaluated solutions with K=1-6 and selected a four-cluster solution as the most interpretable and well-separated, identifying (i) low inflammation (low suPAR/CRP/IL-6), (ii) acute inflammation (moderately elevated suPAR, high CRP/IL-6), (iii) chronic inflammation (high suPAR, moderately elevated CRP/IL-6); and high inflammation (high suPAR/CRP/IL-6). Profiles with elevated inflammatory biomarkers, particularly those with high suPAR (chronic and high) were associated with less favorable baseline risk-factors and with progressively higher risks of mortality and incident chronic disease, with the strongest associations observed for the high inflammation profile.

The results replicate previous findings of three distinct inflammation profiles in the independent E-Risk cohort, including a suPAR-driven profile consistent with chronic inflammation and an acute inflammation profile driven by CRP and IL-6. These findings support distinct inflammatory profiles in the general population, with suPAR capturing a chronic inflammatory dimension distinct from CRP and IL-6 with additional inflammatory vulnerability. Using multiple biomarkers may improve characterization of inflammatory risk.

P41: Investigating the role of NKG2D in the regulation of CD137 expression in by introducing gene knockouts in primary immune cells

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Investigating the regulation of co-stimulatory molecules on effector immune cells, including NK cells and T cells, is important for the understanding of immune cell activation and identification of novel immunotherapy targets. This research aims to investigate whether the co-stimulatory molecule NKG2D affects the expression of CD137, an additional co-stimulatory molecule expressed on NK and T cells in following activation.

NKG2D gene knockout (KO) was performed in primary NK cells and T cells by introducing sgRNA-Cas9 complexes to cells using mechanoporation. Subsequently, surface expression of CD137 was assessed by flow cytometry following different activating stimuli. NK cells were stimulated with monoclonal anti-CD16 antibody or a combination of IL-12/ IL-18, while T cells were stimulated with anti-CD3/CD28 beads.

NKG2D-KO NK cells showed reduced surface expression of CD137 compared to control-transfected NK cells following CD16 stimulation. A similar, though less pronounced, reduction was observed for NKG2D-KO NK cells stimulated with IL-12/IL-18. In contrast, expression of CD137 in NKG2D-KO T cells was unaltered compared to control-transfected T cells following anti-CD3/CD28 stimulation, indicating that the involvement of NKG2D in regulation of CD137 expression is specific for NK cells. These findings demonstrate that introduction of gene knockouts through mechanoporation enables studying of receptor dynamics in primary immune cells. Our data suggests that CD16-induced CD137 expression in NK cells is at least partly dependent on the presence of NKG2D. Further studies are required to elucidate the exact mechanism behind the involvement of NKG2D in the regulation of CD137 expression following NK cell activation.

P42: Translating Pamvac Into a Nanoparticle mRNA Vaccine Platform

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Rational vaccine design requires precise control of antigen presentation to elicit robust, durable, and functional immune responses. This project aims to develop a modular vaccine platform that combines capsid Virus-Like Particles (cVLPs) with nucleic acid-based vaccine technologies to enhance humoral immunity and enable adaptable vaccine design against highly variable pathogens. The platform is being evaluated using the clinical-stage PAMVAC antigen against placental malaria caused by *Plasmodium falciparum*, where vaccine efficacy is challenged by extensive antigenic diversity. cVLPs are self-assembling nanoparticles that mimic viral architecture and promote strong B-cell activation through repetitive antigen display, while nucleic acid vaccines offer rapid and flexible antigen expression. To improve antigen stability, immunogenicity, and response durability, a split-protein Tag/Catcher system is employed for efficient antigen-particle conjugation. Preclinical work involves vaccine design, optimization, and evaluation through in vitro and animal studies. Placental malaria DNA vaccine candidates were expressed in HEK293-TT cells and assessed for antigen coupling, particle assembly, and secretion before immunogenicity testing in BALB/c mice. Serum samples from immunized mice were subsequently evaluated in parasite neutralization assays, and in vitro mRNA production was initiated for comparison with DNA-based vaccine approaches. Initial studies demonstrate successful antigen display on assembled particles and support their suitability for in vivo evaluation. Furthermore, controlled disassembly and reassembly of cVLPs highlight the potential for nucleic acid encapsulation, expanding platform versatility and enabling future multivalent and rapidly adaptable vaccine formulations. By integrating cVLP and nucleic acid vaccine technologies, this work seeks to establish a versatile platform for protection against antigenically diverse pathogens.

P43: Development of antibody-based assays for screening of C4 isotypes

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Complement factor 4 (C4) is a key component of the classical and lectin pathways of the complement cascade and plays dual roles in innate immunity and neurodevelopment. C4 exists as two isoforms, C4A and C4B, encoded by the C4A and C4B genes, respectively. Despite more than 99% sequence homology, these isoforms exhibit distinct functional properties. The C4 genes also show high copy-number variation (CNVs). Differences in C4A and C4B CNVs have been associated with autoimmune, infectious, and neuropsychiatric disorders, notably schizophrenia. Of particular interest is the emergence of C4A CNVs as a key regulator of synaptic pruning. Additionally, C4A and C4B define the Rodgers/Chido blood group antigens, yet clinical reagents to distinguish and quantify these isoforms at the protein level remain scarce. To address this gap, we aim to develop quantitative sandwich ELISAs to measure C4A and C4B in plasma.

We are developing monoclonal antibodies against the C4A and C4B isotypes using hybridoma technology. For immunization, we combine recombinant C4A and C4B proteins, designed, produced, and validated in our laboratory, with specific peptides as boosters. Once we have established and validated hybridoma lines that express antibodies specific to each isotype, we will develop ELISAs to assess whether CNV expression correlates with plasma C4A and C4B concentrations in relevant patient groups and to determine their potential for diagnostics and disease monitoring

P44: Generation of Human Thymic Organoids to Model T Cell Selection and Tolerance Mechanisms

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The thymus is responsible for the development of naïve T cells from bone marrow-derived progenitors through the process of thymopoiesis. The stromal microenvironment of the thymus facilitates the thymic selection process, with thymic epithelial cells (TECs) being the key cell types for T cell development. TECs present a wide variety of self-antigens, leading to reactive T cells being eliminated or becoming regulatory T cells. Understanding thymic selection mechanisms can help us to understand T cell fate decisions, and what drives self- and non-self-recognition.

Much of our current knowledge regarding thymic selection and functionality is based on animal models such as mice, which are limited in their translational value. To overcome this hurdle, we are working on creating a human thymic organoid (HTO) model, which can be used as an in vitro platform for modelling and manipulating human thymic selection processes. By utilizing specific extracellular matrix components and growth factors, TECs derived from dissociated thymic tissue grow into epithelial organoids, exhibiting rapid growth in a stem cell-like manner. Changing the growth media initiates differentiation in the organoids, causing the cells to mimic in vivo TECs. Future co-culture experiments will be conducted with epithelial organoids and thymocytes derived from the same donor to form HTOs, allowing for in vitro T cell selection. Furthermore, genetic manipulation of the HTOs will enable the investigation of the impact of genetic mutations in the thymic environment, such as the impact of gene deletions in diseases such as hemophilia A.

The authors declare no conflicts of interest.

P45: Exploring adaptive immune cell crosstalk between the gut and skin in Hidradenitis Suppurativa patients with Crohn's disease comorbidity

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Crohn's disease (CD) is a chronic, relapsing condition of the intestine characterized by an aberrant immune response. While primarily affecting the gastrointestinal tract, CD also manifests in systemic comorbidities. A major comorbidity associated with CD is hidradenitis suppurativa (HS), a chronic dermatological condition marked by recurrent nodules, abscesses, and sinus tracts in intertriginous areas, and driven by dysregulated immune responses involving excessive inflammation and cytokine production. Their co-occurrence and immune dysregulation suggest a linkage between these diseases. Biologic therapies, particularly anti-TNF- α agents, have shown some efficacy in managing both conditions, however, the exact cellular and clonal linkages driving this comorbidity remain poorly understood.

I will analyze paired biopsies (skin and gut) from patients with CD-HS comorbidity, alongside CD control patients without HS. I will perform single-cell RNA-sequencing (scRNA-seq) combined with T-cell and B-cell receptor sequencing (TCR/BCR-seq) to profile CD4⁺ T cells and plasma cells. CD4⁺ T cell phenotypes and functions will be validated via flow cytometry, while spatial transcriptomics will map the tissue immune landscape.

I anticipate identifying clonally expanded CD4⁺ T cells and plasma cells that are shared between inflamed intestinal and skin lesions. Furthermore, spatial mapping is expected to reveal distinct immune cell niches and disease promoting pathways unique to comorbidity patients.

This study will provide key insights into the shared pathogenesis of CD and HS. Identifying overlapping, pathogenic clonal populations and their spatial tissue niches will highlight novel therapeutic targets, enabling the development of more specific treatments.

The authors declare no conflicts of interest.

P46: Interactions between pathogenic CD4+ T cells and CCR7+ LAMP3+ activated dendritic cells in Crohn's disease

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Intestinal immunity relies on the balance of tolerating food antigens and commensal microbials and protection towards invading pathogens. Failure in this may lead to the development of inflammatory bowel diseases, such as Crohn's disease (CD). While CD4+ T cell responses and in particular Th1/17 cells have been linked to CD pathology, intestinal CD4+ T cell diversity and clonality, how this changes in CD remains poorly understood. Here we used scRNA-seq, scTCRseq and immunohistochemical analysis to assess the mucosal CD4+ T compartment of CD patients.

We found proliferating CD4+ T cells to be highly enriched in more- versus less-inflamed mucosa of CD patients and clonal analysis demonstrated these cells to be related to Th1/Th17 cells; cells that expressed canonical markers for both Th1 and Th17 lineages and markers of cytotoxicity. Th1/Th17 cells exhibited low clonal diversity compared to other CD4+ T cell subsets in the mucosa, indicating local clonal expansion. Immunohistochemical analysis demonstrated that proliferating CD4+ T cells lay in direct proximity with CCR7+ LAMP3+ DCs, a DC population we recently demonstrated to be expanded within the inflamed mucosa of CD patients. We further analyzed interactions between these cells using CellChat, a prediction algorithm for cell-cell interactions from scRNA-seq data. Among the predicted interactions were various costimulatory molecules as well as CXCR3 and CXCR6 signaling. Collectively these findings suggest that clonal expansion of pathogenic Th1/17 cells occurs within discrete CCR7+ LAMP3+ DC containing niches in the inflamed mucosa of CD patients.

P47: Biological effects of CCL5 posttranslational modifications

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The activity of chemokines – important regulators of inflammation – may vary through posttranslational modifications (PTMs). CCL5(3-68) and CCL5(4-68) were identified as N-terminally truncated natural CCL5 variants. Nonetheless, it is only partially known how this modification affects receptor interaction and downstream signaling, both in pathological and physiological settings. We have investigated effects of PTMs on CCR1- and CCR5-mediated G protein dissociation as well as CCR1-, CCR5-, and ACKR2-mediated β -arrestin recruitment. Deletion of the third N-terminal residue, a tyrosine, decreased signaling in efficacy and/or potency of CCL5 through CCR5. For CCR1, the first two residues, a serine and proline, seem to hold significance for promoting dissociation and recruitment of the effectors. Removal of these residues significantly reduced potency and/or efficacy of G protein activation and β -arrestin recruitment at physiological concentrations. The effects on G protein activation have been further corroborated by calcium mobilization studies. Removal of the third residue, as in CCL5(4-68), further decreased the potency or efficacy in CCR1-mediated G protein dissociation and β -arrestin recruitment, respectively. For the atypical chemokine receptor, ACKR2, only the potency was significantly reduced in β -arrestin recruitment assays when comparing CCL5(4-68) to CCL5(1-68), showcasing how the proposed chemokine scavenging function of this receptor may not be affected substantially by N-terminal truncation. Thus far, our results highlight how N-terminal truncation of CCL5 fine-tunes receptor specificity and signaling. Furthermore, it shows which residues appear to hold importance in promoting receptor-specific intracellular signaling on CCR1 and CCR5. Ultimately, this information will guide deliberate drug design to better treat inflammation.

P48: Immune activation markers in severe COVID-19 and Influenza and clinical outcomes

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Background: COVID-19 and influenza are major causes of hospitalisation and mortality, but whether specific aberrant immune responses can drive clinical events remains unclear. We hypothesized that disruption of innate immunity pathways is associated with disease progression.

Methods: Plasma collected from patients hospitalised with influenza or COVID-19 within 2 days of admission was processed for analysis of 15 markers of immunity and lung damage. Cases were those who experienced disease progression defined as increased oxygen demand, need for intensive care or death within 30 days.

Results: We find distinct biomarker profiles that reflect virus-specific immune activation patterns; severe COVID-19 infection is associated with IL-6 and IP-10, while several markers make up the severe influenza profile. When modelling we find that high levels of markers of universal inflammation (IL-6: OR 2.39, 95% CI 1.18–4.90; IL-18: OR 2.89, 95% CI 1.43–5.97), activation of antigen presenting cells (IL-8: OR 4.00, 95% CI 1.93–8.57; IP-10 and MIF) and activation of T-cells (IL2Ra: OR 3.85, 95% CI 1.77–8.70), as well as endothelial damage (Syndecan 1) are all significantly associated with disease progression.

Conclusion: Our study finds pathways of aberrant immune responses associated with severe outcomes in patients hospitalised with COVID-19 or influenza highlighting various biomarker as potential targets for therapies.