Annual Meeting
'Immunology and Infectious Diseases'

The Immune Response, Inflammation and Repair

PROGRAMME

7 - 9 October, 2024
LO-skolen
Konventum Konferencecenter, Helsingør

Table of Contents

Programme

Monday, 7 October

12.00 **Arrival and lunch**

13.00 **Welcome**

Virus

Session chair Henrik N Kløverpris

13.10 **Andreas Wack**, Francis Crick Institute, UK: Lung infections, interferons and macrophages: The good, the bad and the unexpected. Andreas.Wack@crick.ac.uk

13.50 **Jannick Cornelius Snel-Prentø**, University of Copenhagen: The hepatitis C virus envelope protein complex is a dimer of heterodimers. jprentoe@sund.ku.dk

14.30 **Break**

Immune Barrier

Session chair Lea Klingenberg Barfod

14.50 **Bill Agace**, University of Copenhagen: The Immune System of the Gut. william.agace@med.lu.se

15.30 **Paul Martin**, School of Biochemistry, University of Bristol: Cellular and molecular mechanisms of skin wound healing. paul.martin@bristol.ac.uk

16.10 **Break**

T cells

Session chair Mogens Holst Nissen

16.30 **Allan Randrup Thomsen**, University of Copenhagen: Role of resident memory T cells in immunity to respiratory virus infection: Implications for vaccination. athomsen@sund.ku.dk

17.10 **Break**

2 short presentations by PhD students

Session chair Mogens Holst Nissen

17.30 **Andreas Kok**: In vivo and in vitro systems for a novel Norway rat hepacivirus strain demonstrate cross-neutralization.

17.40 **Mireia Rocavert**: Development of a Nipah vaccine exploiting the Tag/Catcher Nanoparticle vaccine platform.

18.00 **Dinner**

20.00 **Poster session**

Tuesday, 8 October

Inflammatory Bowel Disease

Session chair Bill Agace

9.00 **Rahma Elmahdi**, University of Copenhagen: Inflammatory Bowel Disease: A phenome-wide pre- and post-diagnostic. rahma.elmahdi@sund.ku.dk

9.40 **Susanne Brix**, Department of Biotechnology and Biomedicine, Technical University of Denmark: Inflammatory Bowel Disease and Gut Microbiota. sbrix@dtu.dk

10.20 **Break**

Bacterial Pathogens

Session chair Lea Klingenberg Barfod

10.50 **Lianhui Zhang**, South China Agricultural University, Guangzhou: Mechanisms of Virulence Reprogramming in Bacterial Pathogens. lhzhang01@scau.edu.cn

11.20 **Tim Tolker-Nielsen**, University of Copenhagen: Challenges of bacterial biofilms – escape and eradication in disease. ttn@sund.ku.dk

12.00 **Lunch**

13.00 **Trip to Kronborg Castle**

15.30 **Return from trip**

4 short presentations by PhD students

Session chair Lea Klingenberg Barfod

15.40 **Venla Väänänen**: Assessing the Distribution and Functions of Plasmacytoid Dendritic Cells in the Human Intestine.

15.50 **Carsten Eriksen**: The gut microbiota composition links to future disease and is a stronger driver than age of low-grade systemic inflammation and metabolic dysregulation.

16.00 **Alexandra von Münchow**: Investigating the impact of Aryl Hydrocarbon Receptor signaling on the immune response in mice during enteric parasite infection.

16.10 **Lise Lotte Eriksen**: Asthma patients with airway autoimmunity have an altered composition of immune cells in the airway wall.

16.20 **Break**

5 short presentations by PhD students

Session chair Maria Bassi

16.50 **Alexander Kai Thomsen**: Complement Proteins and Complement Regulatory Proteins Are Associated with Age-Related Macular Degeneration Stage and Treatment Response.

17.00 **Tereza Alica Plchová**: Novel Interaction between MASP-3 and IGF-binding proteins.

17.10 **Rimsha Farooq**: Growth inhibitory effect of chicken egg yolk antibodies (IgY) against Escherichia coli E44.

17.20 **Emma Filtenborg Hocke**: Linkage of a novel intron variant to West African dhps resistance haplotypes in Plasmodium falciparum.

17.30 **Anne Martin Salazar**: Oncofetal-chondroitin sulfate as a regulator of cancer immunosurveillance.

18.00 **Dinner**

Wednesday, 9 October

4 short presentations by PhD students

Session chair Maria Abildgaard

9.00 **Hannes Linder**: Identification of a Potential Novel Immune Checkpoint.
9.10 **Benedetta Albieri**: Exploring Microbial Antigen Cross-Reactivity in tumour-infiltrating lymphocytes after adoptive cell transfer in metastatic melanoma.
9.20 **Mario Presti**: Transcriptional dynamics of constitutive HLA-II expression in Melanoma.
9.30 **Marta Velasco Santiago**: Elucidating the immune landscape of immune related adverse events to checkpoint inhibitors.

9.50 **Break**

Inflammation and brain

Session chair Lars Hviid

10.20 **Trevor Owens**, Department of Molecular Medicine, University of Southern Denmark: Resident Innate Immune Cells: Guardians of CNS Homeostasis. towens@health.sdu.dk

11.00 **Roosmarijn E Vandenbroucke**, Department of Biomedical Biology, Ghent University: The Impact of Systemic Inflammation on Alzheimer's Disease Pathology. Roosmarijn.Vandenbroucke@irc.VIB-UGent.be

11.40 **Closing remarks**

12.00 **Lunch**

13.15 **Departure**

Abstracts

Lung infections, interferons and macrophages: The good, the bad and the unexpected

Andreas Wack
Francis Crick Institute

The main function of the lung, that is oxygen supply to the organism through gas exchange at a permeable barrier, often clashes with the requirement to render this barrier impenetrable to harmful agents including pathogens. In addition, the need for uninterrupted oxygen supply requires timely and efficient barrier repair after damage. Therefore, degree of barrier damage and speed of repair are key determinants of severity in lung infections. Interferons, the prototypic antiviral cytokines, are induced upon viral sensing and trigger the establishment of an antiviral state in infected and neighbouring cells, thus contributing to virus elimination. They have however also proinflammatory and antiproliferative effects and can therefore, if present at too high levels or too late in infection, fuel ongoing inflammation or impede the proliferative response to epithelial damage required for recovery. We have dissected in vitro and in vivo the parameters for antiviral, proinflammatory and antiproliferative effects of members of the interferon type I and type III families, as well as mediators of these effects. Our findings have implications for timing and for the choice of interferon family members for antiviral therapy. We have also discovered that self-restricting acute infections leave a long-term imprint in the lung, well beyond recovery from infection. Highly immune-reactive, blood monocyte-derived alveolar macrophages are key contributors to this new steady state of lung immunity, and I will discuss factors the determine their initially high reactivity and gradual loss thereof over time (immunosedation).

The hepatitis C virus envelope protein complex is a dimer of heterodimers

Jannick Cornelius Snel-Prentø
University of Copenhagen

The Immune System of the Gut

Bill Agace
University of Copenhagen

Cellular and molecular mechanisms of skin wound healing

Paul Martin
University of Bristol

We study various aspects of tissue repair from re-epithelialisation through to inflammation in several genetically tractable model organisms from fly to mouse, and even man. We know that inflammation is both beneficial for healing in that it fights infection and drives wound angiogenesis, but has negative consequences also, in that it causes scarring and is aberrant in chronic wounds. We use Drosophila embryos and pupae and translucent zebrafish larvae, all of which are amenable to live imaging and mathematical modelling, to make movies of immune cell migration into the wound and to dissect the genetics of extravasation and inflammatory cell recruitment towards tissue damage, and its consequences, for example, how it drives wound angiogenesis and the subsequent resolution of these vessels. We have also been investigating parallels between wound inflammation and cancer inflammation. Most recently, we have used zebrafish to test drive new technologies for reprogramming macrophages, via their phagocytosis of miniature artificial protocells containing “phenotype switching” cargoes, to make them better able to guide improved wound repair and also to kill skin cancers.

Role of resident memory T cells in immunity to respiratory virus infection: Implications for vaccination

Allan Randrup Thomsen
University of Copenhagen

Tissue resident memory T (Trm) cells represent a potentially important first line of mucosal defence, and immunization aimed at eliciting mucosal T-cell memory has been suggested for improved protection against respiratory infections caused by viral variants escaping pre-existing antibodies. However, it remains unclear whether T-cell targeted vaccines not only protect the individual, but also impairs viral transmission creating herd immunity. To study this, we used a mouse model involving natural murine parainfluenza infection and an adenovirus based nucleoprotein targeting vaccine. Prior intranasal immunization inducing strong mucosal CD8+ T cell immunity provided an immediate shut-down of the incipient

infection and completely inhibited contact based viral spreading. If this first line of defense did not operate, as in parentally immunized mice, recirculating T cells effectively participated in accelerated viral control that also reduced transmission. These observations underscore the importance of developing mucosal T-cell inducing vaccines for optimal inhibition of inter-individual transmission and herd immunity. At the same time our findings explain why induction of a strong systemic T-cell response may reduce viral transmission to some degree.

In vivo and in vitro systems for a novel Norway rat hepacivirus strain demonstrate cross-neutralization

Andreas Kok
University of Copenhagen

Background & Aims: Since the development of a vaccine against HCV is severely impeded by the lack of immunocompetent animal models, researchers have explored the application of surrogate virus models. Norway rat hepacivirus 1 (NrHV) is a promising candidate, mirroring HCV genetic structure, pathogenesis, and immunity. However, NrHV experimental tools are limited to a single variant, RHV-rn1, not representing the vast genetic heterogeneity of HCV. To increase NrHV utility for HCV vaccine research, we here characterized a novel variant and developed tools to study cross-reactive neutralizing antibody responses.

Approach & Results: We sequenced the isolate, NrHV-K, and developed a molecular consensus clone, pNrHV-K, closely related to the original NrHV prototype strain NYC-C12. Intrahepatically inoculated RNA-transcripts from pNrHV-K resulted in chronic infection in Lewis rats. Passaging of NrHV-K in severe combined immunodeficiency mice led to persistent infection, although the mouse-passaged virus did not prolong infection in immunocompetent mice compared with the wild-type NrHV-K variant. Infection in naïve Lewis rats with mouse-passaged virus resulted in a subset of rats clearing the infection during the acute phase, thereby demonstrating a dichotomous infection outcome in inbred rats. We further adapted NrHV-K to efficiently infect rat hepatoma cells and demonstrated that antibodies from RHV-rn1 and NrHV-K infected rats cross-neutralized both variants.

Conclusions: The development of additional experimental systems for NrHV variants permits studies addressing the importance of strain diversity. This advancement aids in the quest for multivalent immune responses against diverse NrHV isolates, offering insights into cross-reacting immunity important for future HCV vaccine design.

Development of a Nipah vaccine exploiting the Tag/Catcher Nanoparticle vaccine platform

Mireia Rocavert
University of Copenhagen

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

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

In this study, we aim to develop a vaccine candidate against NiV using the Tag/Catcher nanoparticle nucleic acid-based platform. Here we show HEK293tt cell co-transfections with nucleic acid encoding the Catcher-nanoparticle and Tag-NivG antigen to demonstrate in vitro expression, particle and antigen conjugation as well as secretion. Lead candidates have been tested in mouse immunization studies followed by live NiV virus neutralization assays to investigate the biological efficacy of the vaccine-induced antibody response. Our results reveal the expression and secretion in mammalian cells of a variety of NiV antigens and nanoparticle combinations and elicitation of NiV specific antibodies with neutralizing capacity in mice. We anticipate this study to help effectively prevent potential future NiV epidemics and pandemics and contribute to the global vaccine field.

INFLAMMATORY BOWEL DISEASE: A PHENOME-WIDE PRE- AND POST-DIAGNOSTIC ASSOCIATION STUDY

Rahma Elmahdi
University of Copenhagen

Introduction: Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is known to be associated with various extra-intestinal manifestations, impacting organ systems beyond the gastrointestinal tract. Identifying comorbidities in IBD and the timing of their development can provide valuable insight into the mechanisms underlying IBD development.

Aims & Methods: We conducted the first population- and disease-wide phenomic association study in IBD, using >6 million ICD-10 coded healthcare contacts from 10 years before and up to 17 years after IBD diagnosis to investigate associations with 1583 diseases. To explore co-morbidities with potential aetiological significance with IBD, we additionally assessed the strength of association with all diseases in the pre-diagnosis compared with the post-diagnosis period. To correct for multiple testing, we adjust our significance threshold ($p<0.05$) with the Bonferroni correction, resulting in an adjusted p-value of $7.90\times10\text{-}6$, which we refer to as disease-wide statistical significance.

Results: We identified 312 disease associations with disease-wide statistical significance and 125 of these diseases appear up to 10 years before diagnosis. The risk of immune-mediated diseases and extra-intestinal manifestations are among those diseases increased up to 10 years prior to IBD diagnosis (psoriasis: RRCD: 2.57, 95% CI: 2.00-3.29; RRUC: 1.54, 95% CI: 1.25-1.87; enteropathic arthropathies: RRCD: 3.57, 95% CI: 2.65-4.78; RRUC: 1.8, 95% CI: 1.38-2.32). This was also the case for gastroenterological and liver disorders (gall stones: RRCD: 1.82, 95% CI: 1.62-2.04; RRUC: 1.26, 95% CI: 1.15-1.37; acute pancreatitis: RRCD: 1.83, 95% CI: 1.30-2.53; RRUC: 2.27, 95% CI: 1.84-2.79). The risk of cardiometabolic diseases and neuropsychological disorders had increased disease wide statistical significance both pre- and post-diagnostically, whereas potential sequelae of treatment, such as osteoporosis (HRCD: 2.56, 95% CI: 2.30-2.86; HRUC: 1.92, 95% CI: 1.79-2.07) or herpes simplex infections (HRCD: 4.04, 95% CI: 2.76-5.91; HRUC: 1.69, 95% CI: 1.2-2.38) were primarily seen post-diagnostically. Of potential aetiological importance to CD, diagnosis with infectious mononucleosis (RRCD: 1.87, 95% CI: 1.37-2.52) was limited to the pre-diagnostic period.

Conclusion: Our results demonstrate IBD as an essentially multisystemic disease, particularly manifesting as gastrointestinal, metabolic, immune, and neuropsychological disorders, present up to 10 years prior to IBD diagnosis.

Inflammatory Bowel Disease and Gut Microbiota

Susanne Brix
Department of Biotechnology and Biomedicine, Technical University of Denmark

The inflammatory bowel diseases Crohn's disease and Ulcerative colitis represent gut inflammatory conditions with great heterogeneity in disease presentation and treatment responses, where individual gut microbiota dissimilarities may play a role. However, when combining microbiota data across patients and cohorts, the focus is often to identify generally disease-associated gut microbiota signatures, hence bacteria with high prevalence across patients. Zooming in on disease heterogeneity versus antibody-coating of the gut microbiota in Crohn's disease patients, we recently discovered a distinct phenotype in the subgroup of patients with severe disease. Here, specific gut pathobionts were found to escape antibody coating in the gut and to be enriched during flares. The non-coated pathobionts exhibited low prevalence, rarely coincided in the same patients and were strongly enriched during disease flares across independent and geographically distant cohorts, hence pointing to distinct gut pathobionts being involved in the disease pathology in severe Crohn's disease.

Virulence reprogramming in bacterial pathogens

Lianhui Zhang
South China Agricultural University

Bacteria are single-celled organisms that carry a comparatively small set of genetic information, typically consisting of a few thousand genes that can be selectively activated or repressed in an energy-efficient manner and transcribed to encode various biological functions in accordance with environmental changes. Research over the last few decades has uncovered various ingenious molecular mechanisms that allow bacterial pathogens to sense and respond to different environmental cues or signals to activate or suppress the expression of specific genes in order to suppress host defenses and establish infections. In addition, in the setting of infections, pathogenic bacteria have evolved various intelligent mechanisms to reprogram their virulence to adapt to environmental changes and maintain a dominant advantage over host and microbial competitors in new niches, including switching from acute to chronic infection, from local to systemic infection, and from infection to colonization. These findings may provide useful clues for the development of new strategies to combat bacterial infections.

Challenges of bacterial biofilms – escape and eradication in disease

Tim Tolker-Nielsen
University of Copenhagen

Assessing the Distribution and Functions of Plasmacytoid Dendritic Cells in the Human Intestine

Venla Väänänen
University of Copenhagen

Intestinal immune homeostasis relies on balancing between protecting from invading pathogens and tolerating commensal microbiota and food antigens. Failure in this, can lead to the development of inflammatory bowel diseases (IBD) such as Crohn's disease (CD). Intestinal immunity can be divided into immune priming sites, such as gut-associated lymphoid tissues (GALT) and the draining lymph nodes, and immune effector sites in the lamina propria (LP) and epithelial layer. Plasmacytoid dendritic cells (pDC) are central in antiviral immunity due to their rapid capacity to produce type 1 interferons. However, other roles of pDC, especially in peripheral tissues such as the intestine, are poorly understood. Here, we used our novel method (Fenton et al. Immunity 2020; Jørgensen et al. Nat Protoc. 2021) to isolate GALT and surrounding GALT-free LP from human intestinal samples to assess the location and transcriptional profile of intestinal pDC. We find pDC to be found within GALT follicles, restricted to the T cell zone, surrounded by both CD4+ and CD8+ T cells. Further, single cell RNA sequencing revealed GALT pDC to express high levels of several factors suited for interactions with T cells, especially genes indicating roles in regulating T cell responses such as GZMB and AREG, compared to blood pDC. Surprisingly, GALT pDC did not express type 1 interferon genes in homeostasis or CD, and in contrast to blood derived pDC, they did not express IFNA2 in response to in vitro CpG stimulation. To conclude, our preliminary analysis suggests that GALT pDC could have a more regulatory function compared to blood pDC.

The gut microbiota composition links to future disease and is a stronger driver than age of low-grade systemic inflammation and metabolic dysregulation

Carsten Eriksen
Technical University of Denmark

Low-grade systemic inflammation is a common hallmark for non-communicable diseases (NCDs), such as type 2 diabetes and cardiovascular diseases. It increases by age, but it is not known whether age itself, the gut microbiota or both play a major role in this. Here, we investigated the influence of age and the gut microbiota composition on a broad array of plasma cytokines and biochemical and physiological variables using a sample of 1199 adults from a population-based cohort. Although age influenced levels of 67% of the variables, the gut bacterial composition was a more important contributor than age to the variation in plasma levels of inflammatory cytokines. This manifested particularly among individuals with a Bacteroides 2 microbiota enterotype (n = 198), who displayed evidence of early-onset systemic inflammation on average 37 years before individuals with other enterotypes. In addition, gut microbiota composition was a stronger driver than age of markers of metabolic dysregulation. Having a Bacteroides 2 microbiota enterotype linked to a higher risk of future hospital diagnoses in 7 out of 13 ICD-10 chapters, while individuals with a high bacterial richness displayed reduced risk in 5 out of 13. This further strengthen the link between the gut microbiota, early onset low grade-inflammation and risk of future disease. Overall, our findings show a stronger influence by the gut microbiota than age on markers of low-grade systemic inflammation and metabolic dysregulation at all ages, and that gut microbiota composition associates with future disease risk.

Investigating the impact of Aryl Hydrocarbon Receptor signaling on the immune response in mice during enteric parasite infection

Alexandra von Münchow
University of Copenhagen

Background: Immunity to parasitic helminths relies on the type-2 immune response with IL-4, IL-13, IL-25, and IL-33 as essential cytokines. The Aryl Hydrocarbon Receptor (AhR) is a ligand dependent receptor in mucosal barriers sensing ligands originating from the diet, host cells, and gut microbiota. Previous research indicates that AhR-deficient mice display resistance to

infection with the helminth Heligmosomoides polygyrus, while AhR-activation impairs the type-2 immune response by selectively promoting type-17 responses.

Aim: This study aims to dissect the impact of AhR-activation from diet-derived ligands in the type-2 immune response.

Methods: Female C57BL/6J mice (N=72) were fed either a refined semisynthetic diet (without AhR ligands), the same semisynthetic diet containing the AhR proligand indole-3-carbinol, or normal mouse chow (rich in natural ligands). Half of the mice in each group were infected with Heligmosomoides polygyrus. On day 14 or day 28 post infection the mice were sacrificed, and blood, feces, and intestinal tissue samples were taken.

Results: No significant effect of diet was evident on worm burdens at both timepoints. Total transit time and small intestinal length were influenced by diet at both time points. Paneth cells and goblet cells were influenced by infection. Both semisynthetic diets promoted transcriptomic pathways related to oxidative stress, adaptive immunity, innate immunity, and muscle contractions. Microbial beta-diversity was significantly influenced by infection status and diet. Hypoxia staining of the gut, and flow cytometry are ongoing.

Conclusion: We observed that activation of the AhR did not affect worm burdens. Diet affected physical properties of the intestines, and the microbiome diversity was influenced by infection status and diet.

Asthma patients with airway autoimmunity have an altered composition of immune cells in the airway wall

Lise Lotte Eriksen
Copenhagen University Hospital – Bispebjerg and Frederiksberg

Background: Airway autoantibodies reactive towards macrophages (MΦ) characterize some asthma patients. These individuals exhibit a distinct immunological response compared to those without airway autoimmunity. The origin of these autoantibodies against MΦ (α-MARCO) in asthma is unclear, but similar to findings in conditions such as Sjögren's syndrome with pulmonary involvement, local production may occur in tertiary lymphoid structures, which are primarily composed of B cells.

Aim: To investigate whether asthma patients showing signs of airway autoimmunity towards MΦ have an increased presence of B cells or other major immune cells in the airway wall compared to patients lacking such autoimmunity.

Methods: Cryobiopsies from mild, steroid-naïve asthma patients with (n=12) and without (n=31) elevated α-MARCO were analyzed using chromogenic immunohistochemistry to

quantify immune cells including macrophages, neutrophils, eosinophils, CD20+ B cells, CD138+ plasma cells, and tryptase+ or chymase+ mast cells. α-MARCO in sputum was measured via ELISA.

Results: Asthma patients with airway autoimmunity towards M□ had a higher degree of airway hyperresponsiveness to mannitol (p=0.0057) but were clinically similar in other aspects. Patients with elevated α-MARCO had a higher number of CD20+ B cells in the tissue (p=0.027). Additionally, autoimmunity correlated with fewer tryptase+ mast cells (p=0.007) and a trend towards fewer neutrophils (p=0.067).

Conclusions: Airway autoimmunity in asthma correlates with a buildup of tissue-resident B cells. These cells have the potential to differentiate into plasma cells, possibly acting as a local source for autoantibodies in asthma.

Complement Proteins and Complement Regulatory Proteins Are Associated with Age-Related Macular Degeneration Stage and Treatment Response

Alexander Kai Thomsen
University of Copenhagen

Background: Dysregulation of the complement system is involved in development of age-related macular degeneration (AMD). The complement cascade is regulated by membrane bound complement regulatory proteins (Cregs) on mononuclear leukocytes among others. This study aims to investigate systemic complement proteins and Cregs in AMD stages and their association with treatment response in neovascular AMD (nAMD).

Methods: In this clinical prospective study, treatment-naïve patients with nAMD, intermediate AMD (iAMD) and healthy controls were recruited and systemic complement proteins C3, C3a and C5a were investigated with electrochemiluminescence immunoassays, and Creg expression (CD35, CD46 and CD59) on T cells (CD4+ and CD8+) and monocytes (classical, intermediate and non-classical) were investigated with flow cytometry. Treatment response in nAMD patients was evaluated after loading dose and after one year, and categorized as good, partial or poor. Complement proteins and Creg expression levels were compared between healthy controls, iAMD and nAMD, as well as between good, partial and poor nAMD treatment response groups.

Results: The concentrations of systemic C3 and C3a were significantly increased in patients with nAMD compared to healthy controls ($P < 0.001$ and $P = 0.002$, respectively). Systemic C3 was also increased in iAMD compared to healthy controls ($P = 0.031$). The proportion of CD4+CD46+ T cells and CD59+ intermediate monocytes were significantly decreased in patients with nAMD compared to healthy controls ($P = 0.018$ and $P = 0.042$, respectively). The

post-loading dose partial treatment response group had significantly lower concentrations of C3a and C5a compared to the good response group (P = 0.005 and P = 0.042, respectively). The 1-year partial treatment response group had a significantly lower proportion of CD35+ monocytes compared to good responders (P = 0.039).

Conclusion: Elevated concentrations of systemic complement proteins were found in patients with iAMD and nAMD. Decreased Creg expression levels were found in patients with nAMD. Partially responding nAMD patients had a dysregulated complement system and Cregs compared to good responders.

Novel interaction between MASP-3 and IGF-binding proteins

Tereza Alica Plchová
University of Copenhagen

Background: Mannose-binding lectin-associated serine protease (MASP)-3 is a key enzyme in activating the complement system, with roles beyond immunity. It has been found to interact with non-canonical substrates like insulin-like growth factor-binding protein (IGFBP)-5, one of six regulatory molecules. 3MC syndrome, a congenital disorder, is linked to deficiencies in MASP-3 and its binding partners, collectin-10 and -11. We hypothesize that IGFBPs may connect MASP-3 to developmental disorders, like 3MC and investigated whether other IGFBP family members are also MASP-3 substrates.

Methods: Activation of full-length recombinant MASP-3 was achieved using PCSK-5 serum-free supernatant, followed by purification. Six different biotinylated IGFBPs underwent incubation with active MASP-3 at 37 °C, with time lapsed sampling. Negative controls included zymogen MASP-3 and PBS. IGFBP cleavage was visualized with western blot analysis.

Results: We could demonstrate enzymatic cleavage of IGFBP-1, 2, 4, and 5 by active MASP- 3 after 6 hours of incubation. The degradation patterns appeared to intensify up to 24 hours. IGFBP-1 forms a 15 kDa fragment, while IGFBP-2 is cleaved into two similar fragments at approximately 20 kDa. IGFBP-4 is degraded into 10 and 15 kDa. As anticipated, IGFBP-5 was also clearly a substrate, undergoing cleavage into 12 and 18 kDa fragments. The susceptibility of IGFBP-3 to MASP-3 cleavage was not conclusive. Notably, IGFBP-6 remained seemingly unaffected by MASP-3.

Conclusion: These findings reveal the existence of novel interactions between the complement and the IGF-signaling pathway. The results obtained provide a solid groundwork for further research into the involvement of MASP-3 in congenital disorder.

Growth inhibitory effect of chicken egg yolk antibodies (IgY) against Escherichia coli E44

Rimsha Farooq
University of Copenhagen

Egg yolk antibody (IgY) is an important immunoglobulin present in egg yolk and many reports have described its ability to inhibit the corresponding antigen. The current study aims to evaluate the inhibitory effect of IgY specific to an avian pathogenic Escherichia coli strain named E44. Specific IgY was produced by immunizing Ross 308 broilers by aerosol method with outer membrane vesicles obtained from a hyper-vesiculating mutant of the E.coli E44 strain. IgY were separated by the PEG 6000 method and run on SDS PAGE. ELISA confirmed that the produced IgY was specific to the antigen. The inhibitory effect of IgY was demonstrated by growth curves obtained using Bioscreen. After 15 hours of incubation with specific IgY (absorbance at 600nm) a significant ($p<0.05$) decrease in growth of bacteria was found as compared to the control. These findings indicate that eggs from hens immunized with specific antigens could be useful for providing passive immunity.

Linkage of a novel intron variant to West African dhps resistance haplotypes in Plasmodium falciparum

Emma Filtenborg Hocke
University of Copenhagen

Introduction and Objective: Sulfadoxine-pyrimethamine (SP) plays a critical role in malaria Plasmodium falciparum chemoprevention strategies across Africa. However, the protective efficacy of SP are impeded by resistance mediated by mutations in the genes encoding dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps). Recently, the 431V mutation in the dhps-coding region has emerged and is now widespread in West Africa, suggesting a selective advantage. A recent study suggests that emerging 431V haplotypes has more than one ancestor expanded across the region. Here we report a novel non-coding mutation, a548383t, positioned upstream in the first intron of dhps, and identifying a strong and significant association between the intron variant and dhps variants carrying the 431V mutation.

Methods: A number of 610 positive samples from Four African countries (Nigeria, Cameroon, Tanzania, and The Democratic Republic of Congo) were analyzed in dhps covering the first intron and a partial of exon 2 focusing on codon positions 431-436-437-540-581-613 by target amplicon Illumina sequencing.

Results: Among the four countries studied, the intron mutation was exclusively found in countries where a prevalence of 431V was observed. A total of 68 full dhps 431V-haplotype profiles from Nigeria and Cameroon and omitting mixed dhps-genotypes in one or more codon were analyzed. Three distinct 431V-containing haplotypes were observed at codons 431-436-437-540-581-613. The intron mutation a548383t was highly abundant in the VAGKGS haplotypes (93.5%, n=29), followed by the VAGKAS haplotypes (66.6%, n=4), and interestingly, the majority of VAGKAA did not harbor the a548383t mutation (58.1%, n=18). Additionally, a pattern between total intron length and 431V/intron variant-containing haplotypes was observed as well.

Conclusion: The study suggests that the 431V mutation may have arisen multiple times, leading to distinct evolutionary stages characterized by the presence of the a548383t mutation. The higher prevalence of a548383t in VAGKGS hints at a potential stronger selection when accompanied by additional 581G and 613S mutations. This phenomenon could be a neutral occurrence from genetic hitchhiking or confer a phenotypic advantage, possibly acting as a regulatory mechanism in gene expression.

Impact/Significance: The emergence of the 431V mutation and its association with specific haplotypes suggest a nuanced evolutionary process. The prevalence of the a548383t mutation in certain haplotypes implies potential selective pressure, and these findings provides an insight into how resistance variants emerge and spread. The observed patterns among distinct haplotypes emphasize the complexity of resistance dynamics, highlighting the urgency for continued genomic surveillance to inform strategic measures in prevention of P. falciparum in Sub-Saharan Africa.

Oncofetal-chondroitin sulfate as a regulator of cancer immunosurveillance

Anne Martin Salazar
University of Copenhagen

Oncofetal chondroitin sulfate (ofCS) glycosaminoglycan is a secondary modification of proteoglycans, abundantly expressed in malignant tissues with minimal presence in healthy organs, except the placenta. It can be detected in primary tumors, metastasis, and circulating tumor cells, and it is suggested to play key roles in tumorigenesis and cancer progression [1], [2], [3]. Although it is abundantly expressed in the malignant phenotype, its exact composition and function are poorly understood and warrant additional studies. To achieve this, we developed and characterized antibodies which bind two distinct ofCS epitopes as well as a panel of 4T1 and A375 knock-out (KO) cancer cell lines for ofCS biosynthetic enzymes. When allografting ofCS KO 4T1 cells into immunocompetent mice, we observed a significantly higher infiltration of CD3+ cells into the tumors compared to the wild type. Similarly, we observed an inverse relationship between ofCS presence and T-cells in human biopsies of colorectal cancer

in immunofluorescent staining. We next investigated which ofCS signature may contribute to immune cell infiltration. A375 WT and CHST11 KO cells were xenografted in immunocompromised mice followed by intravenous injection of human peripheral blood mononuclear cells (PBMCs). Flow cytometry analysis was run in tumor single cell suspensions, where CD45+ cells could only be detected in CHST11 KO tumors, while absent in WT. Moreover, combination treatment of anti-ofCS with PD-1 inhibitor in A375 WT xenografts, grants greater efficacy than the treatments alone. Altogether, our studies indicate that ofCS may play a key role in tumor immunosurveillance and we are exploring it further.

Identification of a Potential Novel Immune Checkpoint

Hannes Linder
University of Copenhagen

Introduction. Cancer immunoediting explains the emergence of detectable tumors despite immunosurveillance, with malignant cells evading the immune system through diverse immunosuppressive mechanisms. Although immune checkpoint inhibitors have enhanced cancer treatment, low objective response rates persist. Identifying new immunosuppressive molecules is important to improve our understanding of immune escape and to develop novel immunotherapies.

Materials and Methods. A potential novel immunosuppressive molecule was identified using several immunodeficient mouse models and bulk RNA sequencing. The candidate was knocked out in MC38 cells using CRISPR/Cas9. Chrome51 release assay was used to assess CD8+ T cell-mediated killing of wildtype (wt) and knockout (ko) cell lines. Effects of candidate gene knockout on tumor growth were evaluated in vivo by inoculating wt and ko cell lines into C57BL/6 mice. The tumor microenvironment (TME) composition of these tumors was analyzed using multicolor flow cytometry.

Results. We selected a candidate gene with minimal functional annotation following similar expression patterns like known immune checkpoints. Candidate gene expression was upregulated in various cancer cell lines upon IFN-y stimulation. Ko of the candidate in MC38 cells slowed down tumor growth in vivo, and the TME composition of ko tumors significantly differed from wt tumors. The delay in tumor growth could be partially reversed by depleting CD8+ T cells. Additionally, candidate gene ko increased susceptibility to CD8+ T cell-mediated killing in vitro.

Conclusions. We identified a potential novel immune checkpoint that might directly inhibit CD8+ T cell cytotoxicity. Targeting this molecule pharmacologically may lead to the development of innovative cancer immunotherapies.

Exploring Microbial Antigen Cross-Reactivity in tumour-infiltrating lymphocytes after adoptive cell transfer in metastatic melanoma

Benedetta Albieri
University of Copenhagen

Adoptive cell therapy (ACT) with tumour-infiltrating lymphocytes (TILs) involves isolating, expanding, and reinfusing autologous TILs to boost the immune system's ability to recognize and eliminate tumour cells. Tumour mutations can generate neoantigens with varying homology to microbial antigens. This study investigated whether the efficacy of cancer immunotherapy in some patients is linked to pre-existing immunological memory against previously encountered microbial antigens. We aimed to identify TILs specific to both tumour and microbial antigens using next-generation sequencing (NGS) to assess homology between tumour-specific mutations and known microbial antigens. Matched tumour cell lines, TILs, and serum samples were collected from 12 melanoma patients who responded to TIL therapy at our center. We measured TIL reactivity to autologous tumour cell lines using CD107a surface expression and TNF and IFNγ intracellular staining, observing consistent reactivity across all patients.

NGS identified minimal epitopes capable of binding to HLA, mapping mutations to a database of bacterial peptides. For each patient, we identified mutated short peptides with varying homology to microbes. Tandem Mini Genes (TMG) containing predicted minimal epitope sequences were designed for individual patients. TMG mRNA sequences were transfected into autologous expanded B cells, which served as antigen-presenting cells in ex vivo IFNγ ELISpot assays with TILs to evaluate reactivity to microbe-homologous antigens. Serum samples from patients with peptide-reactive TILs will be analyzed to confirm prior exposure to pathogens with high homology to the predicted mutated peptide.

Transcriptional dynamics of constitutive HLA-II expression in Melanoma

Mario Presti
University of Copenhagen

Constitutive expression of HLA-II genes is traditionally associated with antigen-presenting cells, but it has also been observed in human melanocytes and melanoma cells in vitro. This

acquired expression of HLA-II in tumors introduces complexity into its role in antitumor immunity. While such feature was initially expected to benefit the tumor, evidence suggests a positive impact on patient prognosis, with recent studies showing antitumoral CD4 T cell activity through HLA-II recognition.

In this project, we aim to uncover the biological mechanisms driving constitutive HLA-II expression in melanoma, and its role in the tumor-immune interactions. We analyzed transcriptomic data from melanoma tumor cell lines and demonstrated that tumoral-HLA-II expression is solely dependent on the upregulation of Class II Major Histocompatibility Complex Transactivator (CIITA), independent from a secondary genomic event. Transcriptomic analyses on public datasets confirmed the spontaneous, interferon-independent acquisition of HLA-II expression in melanoma tumors both on bulk and single-cell RNA sequencing data. Comparison between constitutively positive HLA-II and IFN-induced cells showed that no other genes linked to IFN stimulation but CIITA were upregulated in constitutively positive HLA-II cells.

These findings show the pivotal role of CIITA upregulation for the acquisition of constitutive HLA-II expression in Melanoma. The results pave the way for a deeper study of the role of HLA-II in the immunobiology of Melanoma and support further investigations on the mechanism leading to its upregulation.

Elucidating the immune landscape of immune related adverse events to checkpoint inhibitors

Marta Velasco Santiago
University of Copenhagen

Immune checkpoint inhibitors (ICIs) have transformed cancer treatment by boosting the immune system's ability to fight tumors. However, ICIs often cause immune-related adverse events (irAEs) that can disrupt treatment and reduce patients' quality of life. Current management relies on corticosteroids, which may compromise ICI efficacy, and few studies focus on better management strategies for irAEs. This study aims to uncover the mechanisms driving irAEs through flow cytometry analysis of fresh blood samples from patients undergoing ICI therapy.

Blood samples were collected from patients at various stages: before treatment, during treatment, at irAE onset, and during irAE management. Fresh samples were analyzed within 1- 2 hours using flow cytometry to assess immune cell populations, such as T-cells, B-cells, natural killer cells, and monocytes. Unsupervised clustering and dimensionality reduction techniques identified immunological profiles linked to irAE development.

Results showed that ICI treatment led to increased effector memory (EM) T-cell subsets, particularly CD38+ and HLA-DR+ populations. Additionally, irAE onset was linked to increased transitional and antibody-producing B-cell subsets. Comparing responders and non-responders to corticosteroid management, non-responders had higher monocytic populations 3-5 days post-treatment, while responders showed elevated antibody-producing B-cells and CD38+ or HLA-DR+ EM T-cells.

These findings provide insights into the cellular mechanisms underlying irAEs, suggesting that targeting specific immune cell subsets could improve irAE management. This research highlights the potential for personalized treatment strategies in cancer immunotherapy, aiming to enhance the efficacy and safety of ICIs in clinical practice.

Resident Innate Immune Cells: Guardians of CNS Homeostasis

Trevor Owens
University of Southern Denmark

Resident innate immune cells of the CNS include parenchymal microglia and extraparenchymal border-associated macrophages (BAMs). Microglia are uniquely yolk sac-derived and self-renewing. They exert dynamic surveillance and play key roles in neuronal development and myelination in young and adult CNS. BAMs in leptomeninges, choroid plexus and perivascular spaces display mixed origins. They are ideally located for control of parenchymal access. BAMs and microglia are phagocytic and cytokine-producing and can present antigen to T cells. Evidence for context-dependent phenotypic and functional heterogeneity will be discussed.

The Impact of Systemic Inflammation on Alzheimer's Disease Pathology

Roosmarijn E Vandenbroucke
Ghent University

List of speakers

Allan Randrup Thomsen, University of Copenhagen

Andreas Wack, Francis Crick Institute

Bill Agace, University of Copenhagen

Jannick Cornelius Snel-Prentø, University of Copenhagen

Lianhui Zhang, South China Agricultural University

Paul Martin, University of Bristol

Rahma Elmahdi, University of Copenhagen

Roosmarijn E Vandenbroucke, Ghent University

Susanne Brix, Technical University of Denmark

Tim Tolker-Nielsen, University of Copenhagen

Trevor Owens, University of Southern Denmark

List of participants

Adrian Sutta
Alexander Fredrick Melanson
Alexander Kai Thomsen
Alexandra von Münchow
Alina Brandlmayr
Andreas Kok
Anemone Møllegaard
Anna Kathrine Okholm
Anne Martin Salazar
Anne Weiss
Anne-Christine Kiel Rasmussen
Ása Didriksen Apol
Astrid Pilgård Rasmussen
Benedetta Albieri
Boglar Gal
Carsten Eriksen
Cecilie Brandt Becker
Charlotte Storck-Thy
Charlotte Malassé
Charlotte Menné Bonefeld
Christian Thomas Ashworth
Christina Tsiavou
Claus Henrik Nielsen
Cyrielle Fougeroux
Dorte Soya Hansson
Edda Pupe Blümel
Eder Extramiana Zuniga
Edoardo Dionisio
Ellen Staudinger
Emma Filtenborg Hocke
Emma Ewing
Fabian Martinez
Filip Popovic
Frederic Spatafora
Frederik Kolhorn
Hannes Linder
Helena Sachmann Larsen
Henrik Kløverpris
Ibrahim Elhassan
Ida Holmeskov Hansen

Julie Dragheim
Julie Weber
Kathrine Gotfredsen
Lars Hviid
Lea Barfod
Lise Lotte Eriksen
Lizandro Rivera
Louise Wellmann Thomsen
Lucía Cermeno Fuentes
Maja Udsen
Maria Abildgaard
Maria Bassi
Maria Ines Dos Santos
Marianne Blaszkevizc
Marina Carmona Fajardo
Mario Presti
Marta Velasco Santiago
Martin Kongsbak-Wismann
Martine Dragsbæk-Friis
Mary Lopez-Perez
Matthew James Kennedy
Mattia Dervasi
Mireia Rocavert Barranco
Mogens Holst Nissen
Mustafa Ghanizada
Nethe Thane Nielsen
Penille Jensen
Pernille Klein-Ipsen
Rimsha Farooq
Sara Fresnillo Saló
Sarah Fusco
Sebastian Rask Hamm
Sebastian Bundgaard
Signe Grue Andreassen
Simone Stegenborg-Grathwohl
Sofie Espersen Poulsen
Susan Hansen
Sven Hagen
Søren Buus
Teodora Gheorghe

Ida Wang Henriksen
Itsaso Ortigosa Elorz
Jens Bukh
Jinglin Zhou
Johan Hellstrand
Josefine Rahbek

Tereza Alica Plchová
Tiana Stanisic
Troels Scheel
Ulia Renfelia Baysi
Venla Anniina Väänänen
Wenning Zheng

General information

Key/ Badge/ Programme / Abstracts

Speakers and participants will receive a key card to their room in the afternoon on the day of arrival at Konventum, Helsingør. Name badges will be available for pickup outside the auditorium at 1 pm.

The room must be left at 9 am on the day of departure.

The programme including abstracts will be published in PDF format and all speakers and registered participants will receive a copy via e-mail prior to the meeting.

Poster Session

Please place your poster in the poster area just after dinner on the day of arrival. The poster session will be on Monday, 7 October, at 8 pm.

Meeting Location

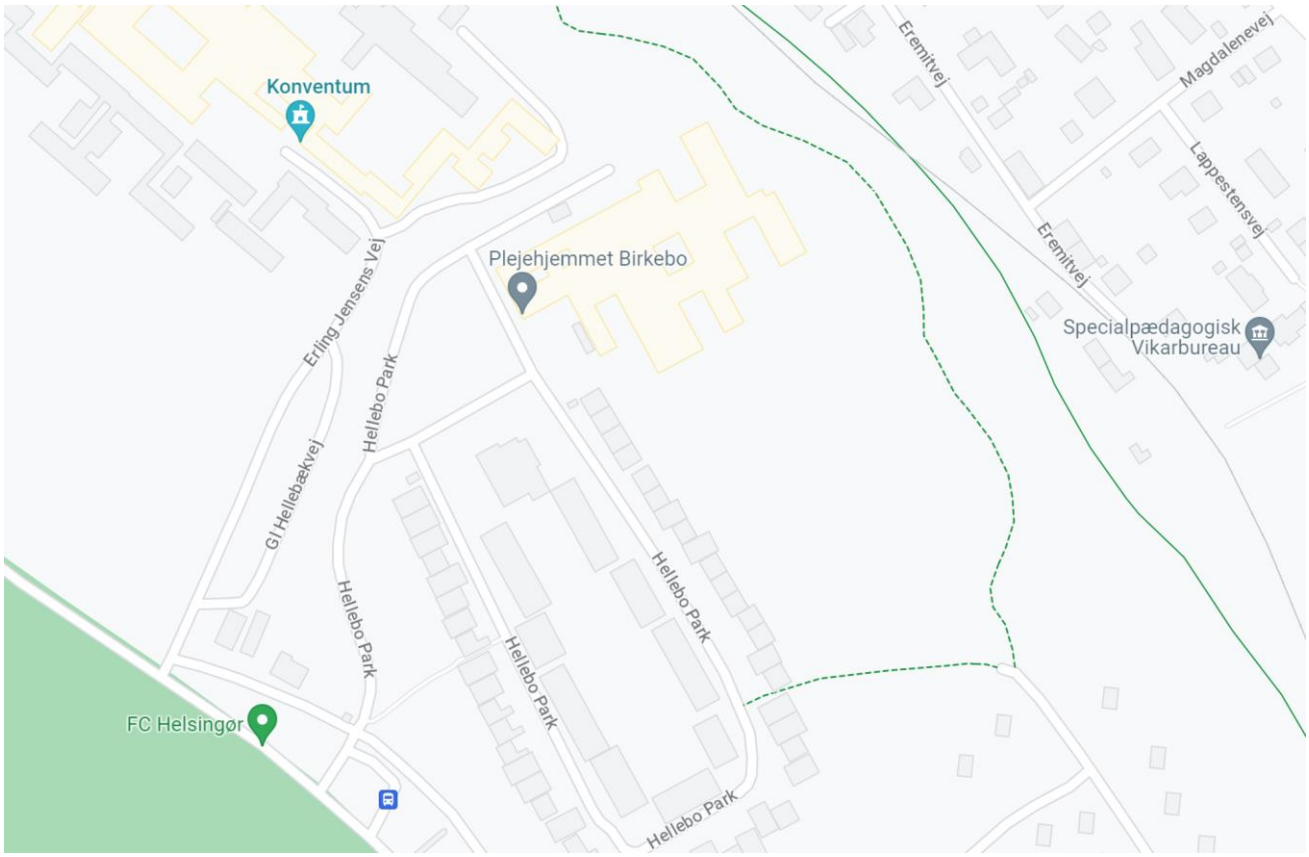
LO-skolen, Konventum Konferencecenter

Erling Jensens Vej 1

DK-3000 Helsingør

Tel: (+45) 49 28 09 00

www.konventum.dk



Transportation Details

Take the train from Copenhagen Airport to Helsingør Station. The train departs from the airport every 20 minutes and requires a change of train at Nørreport or the Copenhagen Central station.

Alternatively, if you are departing from Copenhagen, you can get on the train at Copenhagen Central Station/København H, Nørreport Station or Østerport Station.

At Helsingør Station you can take the local bus for Konventum:

Timetable – bus from Helsingør Station to Konventum

• Take **bus 353** from Helsingør station towards Hellebo Park (9 minutes)

• Get off at Hellebo Park

Hour	Minutes	
05	42	
06-07	12	42
08-17	16	46
18	16	44
19	14	44
20	14	47
21	17	47
22	06	47

<u>Timetable – bus from Konventum to Helsingør Station</u>

• Take **bus 353** from Hellebo Park towards Prøvestenscentret (9 minutes)

• Get off at Helsingør station

Hour	Minutes	
05	50	
06-07	20	50
08-17	27	57
18	27	52
19	22	52
20	22	59
21	29	59
22	15	59

Please note:

You can always plan and check up on your journey here
www.journeyplanner.dk

Organizers

Mogens Holst Nissen
Professor, Director of the Graduate School of Immunology
Department of Immunology and Microbiology, The Panum Institute, University of Copenhagen
Blegdamsvej 3
2200 Copenhagen N

Tel.: (+45) 35 32 72 68
Mobile: (+45) 28 75 72 68
Email: mhni@sund.ku.dk

Lea Klingenberg Barfod
Associate Professor
Department of Immunology and Microbiology, The Panum Institute, University of Copenhagen
Blegdamsvej 3
2200 Copenhagen N

Tel.: (+45) 35 32 64 85
Mobile: (+45) 26 74 70 19
Email: lbarfod@sund.ku.dk

Marianne Blaszkevizc
Secretary
Department of Immunology and Microbiology, The Panum Institute, University of Copenhagen
Blegdamsvej 3
2200 Copenhagen N

Tel.: (+45) 35 33 13 68
Mobile: (+45) 20 69 33 63
Email: mabl@sund.ku.dk

Anemone Chu Gui Møllegaard
Student assistant
Department of Immunology and Microbiology, The Panum Institute, University of Copenhagen
Blegdamsvej 3
2200 Copenhagen N

Email: anemone.moellegaard@sund.ku.dk

Susan Hansen
Department Secretary
Department of Immunology and Microbiology, The Panum Institute, University of Copenhagen
Blegdamsvej 3
2200 Copenhagen N

Tel.: (+45) 35 32 78 50
Mobile: (+45) 41 57 13 17
Email: sh@sund.ku.dk

Dorte Soya Hansson
Secretary
Department of Immunology and Microbiology, The Panum Institute, University of Copenhagen
Blegdamsvej 3
2200 Copenhagen N

Tel.: (+45) 35 33 45 56
Mobile: (+45) 51 30 07 39
Email: dorte.hansson@sund.ku.dk

Johan Hellstrand
Department Coordinator
Department of Immunology and Microbiology, The Panum Institute, University of Copenhagen
Blegdamsvej 3
2200 Copenhagen N

Tel.: (+45) 35 32 78 52
Mobile: (+45) 51 78 78 95
Email: johel@sund.ku.dk