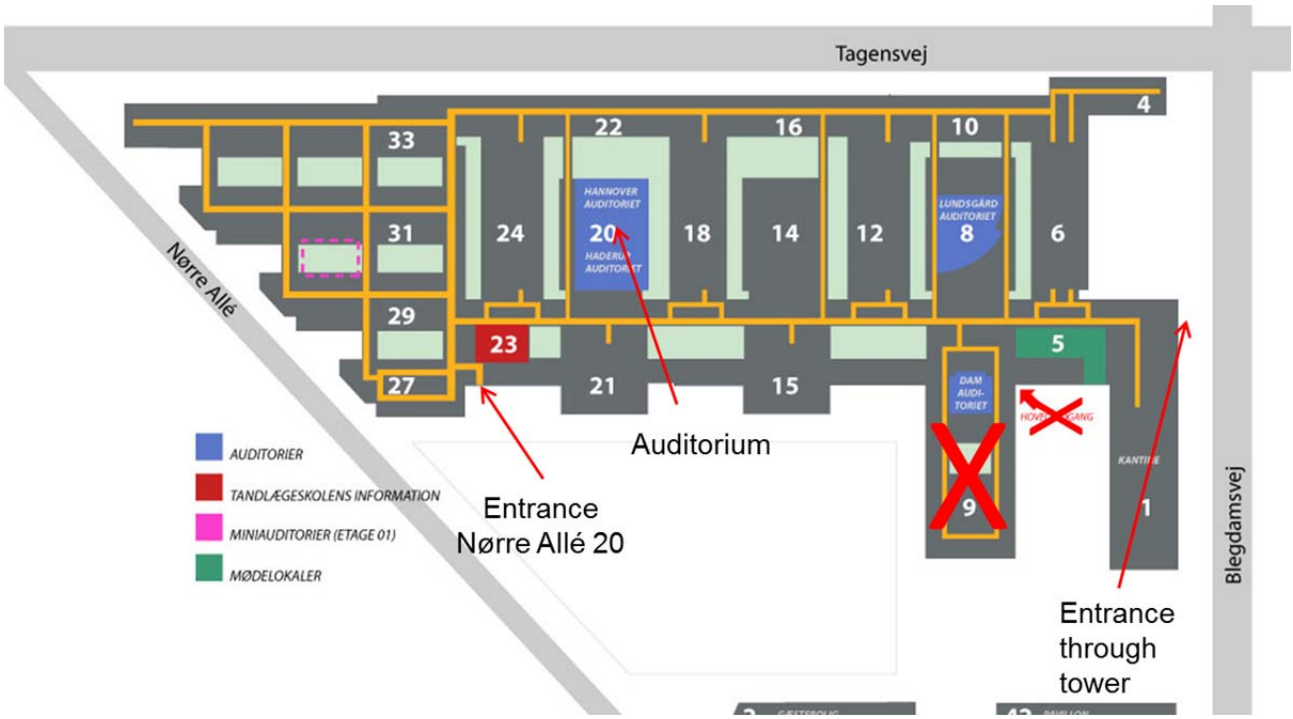
Danish Society of Immunology

Annual Meeting, April 19. 2016

University of Copenhagen

Panum

Haderup Auditoriet



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Program

12:30 Welcome

12:35-13:25 Keynote Presentation - Zika virus - coming to a town near you? By Allan R. Thomsen, Søren Buus & Jan Pravsgaard

13:25-14:45 Presentations:
13:25-13:40 Manja Idorn: *Optimizing Adoptive Cell Therapy by genetic engineering of T cells for improved homing to tumor site*
13:40-13:55 Simone Bendtsen: *Interleukin-17 Producing Vγ9Vδ2 T cells; Frequency, Phenotype and Differentiation*
13:55-14.10 Trine Hilkjær Petersen: *Increased nickel-responsiveness in filaggrin-deficient mice compared to normal mice*
14:10-14:25 Ida Uddäck: *Analyzing internal antigens to be included in a universal adenovector-based influenza A vaccine*
14:25-14:40 Rolf Billeskov: *High avidity CD4 T cells with improved antiviral efficacy are selectively induced by low-dose adjuvanted vaccination*

14:40-15:30 Coffee break/Poster session – General Assembly of IS

15:30-16:45 Presentations
15:30-15:45 Laura Lind Pedersen: *Human CD11c as a Potential Receptor for Targeted Vaccines*
15:45-16:00 Trine B Levring: *Purification of peripheral blood mononuclear cells by gradient centrifugation induces T cell priming*
16:00-16:15 Kasper Thorhauge Christensen: *Comparing the stability and activity of recombinant IFNλ3 and IFNλ4*
16:15-16:30 Rodrigo Velázquez-Moctezuma: *Culturing antibody-sensitive hypervariable region 1 deleted hepatitis C viruses with AR5A antibody reveals multiple pathways to neutralization resistance*
16:30-16:45 Troels KH Scheel: *A broad RNA virus survey reveals both miRNA dependence and functional sequestration*

16:45-17:00 Coffee break/ Poster session

17:00-18:00 Presentations
17:00-17:15 Christina Friese: *Constructing artificial antigen-presenting cells for improved T-cell function in adoptive T-cell therapy of melanoma*
17:15-17:30 Marlene Thorsen Mørch: *Antibody in cerebrospinal fluid can be directed to specific sites in brain*
17:30-17:45 Kirstine Nolling Jensen: *Role of Type I Interferon in Neuromyelitis Optica*
17:45-18:00 Peter Tougaard: *Proinflammatory cytokine, TL1A, influences the establishment of adipose tissue independent of gut microbiota*

18:00-20:00 Dinner

Abstract for Oral Presentations and Posters

Number 1:

Optimizing Adoptive Cell Therapy by genetic engineering of T cells for improved homing to tumor site

Manja Idorn[1], Gitte Holmen Olofsson[1], Maria Olsen[1], Hjalte List Larsen[2], Joest van der Berg[1], Özcan Met[1,3], Per thor Straten[1,4]

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Adoptive cell therapy (ACT) using in vitro expanded tumor infiltrating T lymphocytes (TILs) from biopsy material represents a highly promising treatment of disseminated cancer. A crucial prerequisite for successful ACT is sufficient recruitment of transferred lymphocytes to the tumor site; however, despite transfer of billions of lymphocytes, T-cell infiltration into the tumor post ACT is limited. By PCR and ELISA analyses we found that a majority of malignant melanoma (MM) cell lines expressed chemokines CXCL8/IL-8, CXCL12/SDF-1 and CCL2. Taking advantage of lentiviral transduction, successful transduction of TILs and peripheral blood T cells significantly increased receptor expression of the corresponding chemokine receptors CXCR2, CXCR4 and CCR2. All three chemokine receptors are functional *in vitro* and show ligand specific transwell migration of engineered T cells as well as increased migration towards MM conditioned medium. *In vivo* homing was assessed in a xenograft NOG mouse model. Mice with subcutaneous human melamoma were treated with ACT of MAGE-A3 specific T cells transduced with either CXCR2 or GFP. Transducing T cells with CXCR2 increased tumor infiltration. In comparison mock transfected T cells appeared to be allocated to other organ-compartments. In conclusion, our CXCR2, CXCR4 and CCR2 transduced T cells are functional *in vitro*, and transduction with CXCR2 improve *in vivo* homing of T cells to tumor site, setting the stage for mixing and matching chemokine-receptor expression to tumor microenvironments.

Number 2:

Interleukin-17 Producing Vγ9Vδ2 T cells; Frequency, Phenotype and Differentiation

Simone Bendtsen[1], Manja Idorn[1], Gitte Holmen Olofsson[1], Per thor Straten[1,2]

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Promising results of adoptive cell transfer (ATC) with tumor reactive T cells expanded from tumor infiltrating lymphocytes (TILs) are limited to cancer patients where the tumor can be surgically removed. Therefore research into other cell types like the major γδ T cell subtype in the circulation, Vγ9Vδ2, are ongoing. These cells can be isolated from a simple blood sample and expanded in vitro, and they respond to phosphoantigens, which are upregulated on cancer cells. For many different cancer cell lines, a direct correlation between the recognition of the cells by Vγ9Vδ2 T cells and accumulation of phosphoantigens have been found.

However, Vγ9Vδ2 T cells are capable of producing IL-17. This cytokine has been linked to several different pro-tumor activities like promotion of proliferation and metastatic potential of the cancer cells. Vγ9Vδ2 T cells therefore needs careful scrutiny before being used in therapy, as the tumor microenvironment contain many different stimuli.

We wish to investigate what makes in vitro expanded γδ T cells skew in an IL-17-producing direction using cytokine cocktails, co-culture with different cancer cell lines or supernatants from these. The overall amount of γδ T cells in the periphery is normally 0.5-10% in healthy donors, but it is unknown how many of these are capable of producing IL-17.

Number 3:

Increased nickel-responsiveness in filaggrin-deficient mice compared to normal mice

Trine Hilkjær Petersen, Carsten Geisler, Jonas Damgård Schmidt, Morten Milek Nielsen, Beatrice Dyring-Andersen, Steen Seier Poulsen, Christina Agerbeck, Jacob Pontoppidan Thyssen & Charlotte Menné Bonefeld

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Studies have suggested that loss-of-function mutations in the filaggrin gene are associated with increased nickel allergy in humans. However, the immune response involved in this has not yet been characterized. Thus in this study, we investigated the immune responses in filaggrin-deficient flaky tail (*ft/ft*) mice compared to wild type (WT, C57BL/6 and Balb/c) mice following epicutaneous exposure to nickel.

We used a model for contact hypersensitivity, in which *ft/ft* mice and WT mice were sensitized and challenged with nickel by repeated exposure of the ears to 10% $NiCl_2$ in petrolatum. The local immune response was analyzed by measurements of ear thickness and expression of pro-inflammatory cytokines. Furthermore, T cell responses within the draining lymph nodes were analyzed by flow cytometry.

A significant increase in ear swelling upon nickel challenge was found in nickel-sensitized *ft/ft* mice compared to WT mice. Furthermore, nickel exposure caused significantly higher levels of IL-1β in the ears of filaggrin-deficient mice as compared to WT mice. Finally, *ft/ft* mice sensitized and challenged with nickel had significantly higher numbers of Th1/Tc1 and Th17 cells within the draining lymph nodes in comparison to WT mice.

Taken together, we show that nickel elicits a stronger local and adaptive immune response in *ft/ft* mice compared to WT mice.

Declaration of interest: none

Number 4:

Analyzing internal antigens to be included in a universal adenovector-based influenza A vaccine

Ida Uddbäck, Line I. Pedersen, Allan Randrup Thomsen, Jan Pravsgaard Christensen

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Current influenza vaccines are directed towards the surface proteins hemagglutinin and neuroaminidase providing an antibody-mediated immunity. High variability of these proteins results in costly annual evaluation and production of new vaccines without providing protection against heterosubtypic strains or upcoming pandemic variants. Consequently, there is a need for a new universal influenza vaccine strategy that can provide a basal protection when the conventional vaccines fail. In contrast to the surface proteins, the internal proteins of influenza A are highly conserved. Using one of these, nucleoprotein, encoded in an adenovirus construct (AdNP) we have induced a CD8 T-cell response, which provide heterosubtypic protection. Further, by vaccinating both systemically and locally mice were completely protected against challenge from influenza for at least 8 months post vaccination.

To increase the breadth of the vaccine we have investigated the potential of other conserved influenza proteins as vaccine targets with the intention of making a "vaccine cocktail" by combining the constructs in one vaccination. PB1 expressed from an adenovirus (AdPB1) elicits about the same number of antigen-specific CD8 T cells in mice as AdNP. However, the PB1 directed T cell response protects only 25% of vaccinated mice. We found that one of the underlying reasons for this lack of protection from AdPB1 vaccination relates to a significantly lower in vivo cytotoxicity of these cells compared T cells induced by AdNP. It is important to consider these results and to further evaluate why some epitopes induce a more protective response than others in future vaccine design.

Number 5:

High avidity CD4 T cells with improved antiviral efficacy are selectively induced by low-dose adjuvanted vaccination

Rolf Billeskov[1,2], Yongjun Sui[1], Else Marie Agger[2], Peter L. Andersen[2], Jay A. Berzofsky[1]

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T cells of high functional avidity sense and respond to low levels of antigen, and their superior protective efficacy was originally described using CD8 T cells cultured *in vitro* with low levels of antigen that displayed higher avidity as well as anti-tumor and anti-viral efficacy compared to low avidity T cells cultured with high antigen concentrations. Selectively priming high avidity T cells by low vaccine doses has proven very difficult.
We show for the first time that functionally high avidity CD4, but not CD8, T cells can be selectively primed *in vivo* by low concentrations of antigen in the strong crosspriming liposomal cationic adjuvant formulation (CAF09) DDA/TDB or DDA/MMG/pIC from SSI. Increased functional avidity observed after low-dose vaccinations correlated with lower surface expression of PD-1/CTLA-4/Fas and higher per cell IFN-gamma production compared to low avidity CD4 T cells, and CD4 T cell avidity was dependent on IL-15.
Importantly, high avidity CD4 T cells offered superior protection in a murine model of latent infection with *Mycobacterium tuberculosis* compared to low avidity T cells. Furthermore, adoptive transfers of HIV-specific CD4 T cells of high functional avidity in combination with TCR transgenic HIV-specific CTLs conferred superior protection against viral (recombinant vaccinia-HIV) challenge compared to TCR-Tg CTLs alone or in combination with low avidity CD4 T cells.

Number 6:

Human CD11c as a Potential Receptor for Targeted Vaccines*

Laura Lind Pedersen[1**], Jeanette Prangsgaard[1**], Mathilde Sanden[1**], Emil Kofod-Olsen[1], Ralf Agger[1]
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*The work presented is a Master's Thesis work in progress
**Has contributed equally to this work

The aim of this study is to elucidate whether the human CD11c molecule expressed on dendritic cells (DCs) can be used as a receptor for antigen uptake and presentation, and thus serve as a potential receptor for effective DC-targeted vaccination. In order to optimise antigen uptake via CD11c, new murine monoclonal antibodies against human CD11c will be generated. Therefore, a murine cell line expressing human CD11c/CD18 is being generated with the purpose of screening for novel antibodies against human CD11c.

DCs are characterised as the most potent antigen-presenting cells (APCs) and are known for initiating effective and durable T cell immunity, which renders DCs an attractive target for antibody-targeted vaccines. Vaccines directed against DCs may possibly revolutionize the field of vaccination and potentially turn vaccination into a cure for e.g. autoimmune diseases, allergies, and cancers. Antibodies targeted to the CD11c receptor induces both humoral and CTL responses without adjuvants, and CD11c has shown promising potential in murine *in vitro* and *in vivo* studies as a candidate for antibody-targeted vaccination.
The potential of CD11c as a target for antigen delivery is analysed by flow cytometry, and the fate of internalised antigens is analysed with immunofluorescence and confocal microscopy using endosome markers. Molecular cloning is carried out to develop a cell line expressing human CD11c.

Preliminary results from the ongoing experiments will be presented.

Number 7:

Purification of peripheral blood mononuclear cells by gradient centrifugation induces T cell priming

Trine B Levring[1*], Martin Kongsbak-Wismann[1], Anna O Rode[1], Niels Ødum[1], Charlotte M Bonefeld[1], Carsten Geisler[1]
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The invention of purification of peripheral blood mononuclear cells (PBMCs) by gradient centrifugation was a milestone and has been a central technique in the majority of in vitro studies of human T cells. In order to obtain reliable results, it is essential that the purification procedure does not affect the cells. However, we have found that T cells purified by gradient centrifugation downregulate the protein TXNIP, a few hours after purification despite a lack of activation. TXNIP is a proposed cell cycle inhibitor. Downregulation of a cell cycle inhibitor would suggest that T cell purification by gradient centrifugation renders the T cells "primed" and possibly easier to activate subsequently.

The downregulation of TXNIP was not caused by blood drawing, centrifugation, or direct contact with density media. These observations suggest that the downregulation of TXNIP is induced by the gradient centrifugation itself, and that this is a general phenomenon since it was observed using 3 different kinds of density media.

Since TXNIP is a proposed cell cycle inhibitor, the fact that it is downregulated in T cells following gradient centrifugation would suggest that the method leaves the T cells primed and possibly easier to activate in subsequent experiments. It is important for researchers isolating T cells by gradient centrifugation to be aware that the cells are affected and do not accurately reflect the status of T cells in circulation.

Number 8:

Comparing the stability and activity of recombinant IFNλ3 and IFNλ4

Kasper Thorhauge Christensen[1], Hans Henrik Gad[1], Ewa Terczyńska-Dyla[1], Rune Hartmann[1].*
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Every year 3-4 million people become infected with hepatitis C virus (HCV) and of those almost 85% develop a chronic infection because they fail to clear the virus. Recently, it was shown that people with a functional *IFNL4* gene have a lower chance of clearing the virus than people with a non-functional *IFNL4* gene. The reason behind this correlation is currently not understood although a causal relationship between the activity of the IFNλ4 protein and poor HCV clearance has been demonstrated.
IFNλ4 belongs to the type III IFNs together with IFNλ1, -2, and -3. However, it differs from the others not only by its low sequence similarity but also by its impaired secretion. This impairment is not due to a weak signal peptide, as swapping the signal peptides between IFNλ3 and -4 had no effect on secretion. When we purified IFNλ3 and -4, we found that IFNλ4 is far more difficult to refold *in vitro* than IFNλ3 suggesting that the poor secretion of IFNλ4 could be due to an inherent problem of folding the protein. Because such a problem could also mean that IFNλ4 could be less stable than other type III IFNs, we decided to compare the stability of recombinant IFNλ3 and IFNλ4. Our results demonstrate that IFNλ4, like IFNλ3, is surprisingly stable once it has folded properly.

Number 9:

Culturing antibody-sensitive hypervariable region 1 deleted hepatitis C viruses with AR5A antibody reveals multiple pathways to neutralization resistance

Rodrigo Velázquez-Moctezuma[1], *Mansun Law*[2], *Jens Bukh*[1], *Jannick Prentoe*[1]

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ABSTRACT

Hepatitis C virus (HCV) is one of the leading causes of liver disease around the world [1]. Treatment accessibility is limited due to high cost [2] and development of a preventive vaccine has proven difficult, partly due to the high mutagenic rate of the virus [3–5]. Recent studies of HCV antibody resistance have revealed important information about the barrier to escape for several clinically promising human monoclonal antibodies (HMAb) [6,7]. Here, we co-cultured the novel HMAb AR5A with the neutralization sensitized HVR1-deleted viruses H77/JFH1$_{\Delta HVR1}$ and J6/JFH1$_{\Delta HVR1}$, encoding envelope protein of HCV genotypes 1 and 2, respectively. Interestingly, the path to resistance depended on the isolate. Specifically, we showed that mutation L665W increased AR5A resistance for H77/JFH1, whereas both the mutation L665S and the compensatory mutation S680T were necessary to generate resistance for J6/JFH1. We showed that the site 665 is an important site for AR5A binding and mutations in this site conferred resistance across genotypes 1-6 of HCV. Finally, we observed several cases for which L665W affected neutralization susceptibility against other clinically important HMAbs AR3A and AR4A. Thus, our results revealed important information about resistance for different genotypes, indicating that HCV can overcome the AR5A barrier to resistance in a genotype-specific manner.

Number 10:

A broad RNA virus survey reveals both miRNA dependence and functional sequestration

Troels K. H. Scheel, Joseph M. Luna, Matthias Liniger, Eiko Nishiuchi, Kathryn Rozen-Gagnon, Amir Shlomai, Gaël Auray, Markus Gerber, John Fak, Irene Keller, Rémy Bruggmann, Robert B. Darnell, Nicolas Ruggli, Charles M. Rice

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Small non-coding RNAs have emerged as key modulators of viral infection. However, with the exception of hepatitis C virus, which requires the liver-specific microRNA (miRNA)-122, the interactions of RNA viruses with host miRNAs remain poorly characterized. Here, we used crosslinking immunoprecipitation (CLIP) of the Argonaute (AGO) proteins to characterize strengths and specificities of miRNA interactions in the context of 15 different RNA virus infections, including several clinically relevant pathogens. Notably, replication of pestiviruses, a major threat to milk and meat industries, critically depended on the interaction of cellular miR-17 and let-7 with the viral 3'UTR. Unlike canonical miRNA interactions, miR-17 and let-7 binding enhanced pestivirus translation and RNA stability. miR-17 sequestration by pestiviruses conferred reduced AGO binding and functional de-repression of cellular miR-17 targets, thereby altering the host transcriptome. These findings generalize the concept of RNA virus dependence on cellular miRNAs and connect virus-induced miRNA sequestration to host transcriptome regulation.

Number 11:

Title: **Constructing artificial antigen-presenting cells for improved T-cell function in adoptive T-cell therapy of melanoma**

Author(s): Friese C.[1]*, Donia M.[1,2], thor Straten P.[1], Svane I.M.[1,2], Met Ö.[1,2]

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Text: Adoptive T-cell therapy (ACT) is a cancer immunotherapy for metastatic melanoma patients based on autologous tumor-infiltrating lymphocytes (TILs). It takes advantage of naturally existing tumor-reactive T cells isolated from surgically resected tumor lesions, expanded *ex vivo* and re-infused into the patient after lymphodepleting chemotherapy and in combination with recombinant IL-2. Objective response rates of up to 50% including complete tumor regression in 10-20% of the patients have been reported from several independent centers.
Despite the great potential of TIL therapy, the technical protocol for TIL expansion is still requiring improvement. At present, a large number of peripheral blood mononuclear cells (PBMCs) from multiple blood donors is required to be used as feeders/stimulators for the rapid-expansion protocol (REP). Genetically engineered artificial antigen-presenting cells (aAPCs) that express T-cell activating and co-stimulatory molecules on the cell surface have the potential to eliminate the need to use PBMCs and could lead to improved effector-memory qualities with a longer persistence of TILs in the patients.
The aAPCs currently being established at CCIT are genetically modified with various co-stimulatory molecules and Fc receptors for antibody loading. Preliminary testing of aAPCs in REPs has shown clinical grade expansion of tumor-reactive melanoma TILs. The results also indicate a higher frequency of $CD8^+$ T cells versus $CD4^+$ T cells in the aAPC-expanded TILs in comparison to PBMC-supported TIL expansion. At present the aAPCs are optimized and their feasibility in expanding TILs for ACT of melanoma as well as renal cancer, ovarian cancer and sarcoma is tested in ongoing experiments.

Number 12:

Antibody in cerebrospinal fluid can be directed to specific sites in brain

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Elevated titers of IgG antibodies in the cerebrospinal fluid (CSF) are seen in the autoimmune diseases multiple sclerosis and neuromyelitis optica (NMO, in acute relapses). These autoantibodies come either from blood or are produced in the central nervous system. Disruption of blood- or CSF-brain barriers and/or tissue damage can result in access to parenchymal tissue and thereby to specific antigen. Encounter of autoantibodies with specific antigen can lead to hypersensitivity reactions and pathology. Localization of such pathology determines clinical outcomes.

We hypothesized that antibody in CSF can be directed to specific sites in brain by pre-established injury.

A unilateral non-specific injury was made to the brain of C57Bl/6 mice. One day post injury mice received an intrathecal injection into the CSF via cisterna magna of human IgG, either from NMO patients (NMO-IgG) or healthy donors, with/without human complement. Two days post injury brains were collected for histology.

Even though non-specific deposition of human IgG could be detected in both hemispheres, the unilateral injury site showed increased deposition of human IgG compared to the corresponding area in the contralateral hemisphere. Complement-dependent pathology was observed at the targeted site but only in mice that received NMO-IgG.

Stereotactic tissue injury specifically induces deposition of intrathecally-injected autoantibodies and pathology proximal to the injury site, and thus directs antibody trafficking in the brain.

Number 13:

Role of Type I Interferon in Neuromyelitis Optica

Kirstine Nolling Jensen, Agnieszka Wlodarczyk, Reza Khorooshi, Nasrin Asgari, Trevor Owens

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Neuromyelitis Optica (NMO) is an autoimmune, inflammatory disease of the central nervous system with unknown etiology. Serum antibodies against the water channel aquaporin 4 (AQP4) expressed by astrocytes are a biomarker for NMO. NMO is characterized by loss of astrocytes, blood-brain barrier breakdown and demyelination. Patients have reported a lack of effect or even exacerbation of symptoms when treated with interferon β (IFNβ), a commonly used treatment for the related disease Multiple Sclerosis.

The goal of this study was to examine the effect of exogenous IFNβ on the formation of NMO-like lesions.

To induce NMO-like lesions, immunoglobulin G (IgG) from NMO patients was injected with human complement to the striatum of C57Bl/6 mice. Our results show upregulation of type I interferon signature genes in the brains of mice with NMO-like lesions. Moreover, co-injection of recombinant IFNβ (rIFNβ) led to an increase of NMO-like pathology, with more pronounced loss of the astrocyte markers AQP4 and GFAP. These findings implicate type I IFN in the pathology of NMO and further support previous findings suggesting a detrimental effect of type I IFN signaling in the development of NMO-like lesions.

Number 14:

Proinflammatory cytokine, TL1A, influences the establishment of adipose tissue independent of gut microbiota

Peter Tougaard[1,2], Louise K. Martinsen[1], Jakob Riis, Søren Skov[1], Anders E. Pedersen[2], Axel K. Hansen[1], Camilla H.F. Hansen[1]

[1]Department of Veterinary Disease Biology, University of Copenhagen, Denmark

[2]Department of Department of Immunology & Microbiology, University of Copenhagen, Denmark

TL1A is a proinflammatory cytokine of the TNF family, with the closest sequence identity to TNFα. It is upregulated in the serum of patients with chronic inflammatory diseases, such as Crohn's disease and Rheumatoid arthritis (RA). Furthermore, neutralization of TL1A in animal models of inflammatory bowel disease (IBD) or RA ameliorates disease pathology. This empathises a pivotal role of TL1A in the pathogenesis of inflammatory diseases. Previously, we have shown that TL1A knock-out (KO) mice given low-fat diet display less visceral adipose tissue than wild-type mice and that this phenotype was associated with changes in the gut microbiota. The aim of this study is to determine how TL1A influence on the establishment of adipose tissue, especially whether TL1A's impact on adiposity is mediated through its capacity to regulate inflammation or whether the previously described changes in the gut microbiota affect the energy uptake and thereby adiposity. To assess whether TL1A impact on adiposity was mediated through gut microbiota changes, TL1A KO mice were revitalised in a germ-free environment, and the adiposity was evaluated. To evaluate TL1A's impact on inflammation two approaches were applied. Mice were either injected with biologically active TL1A to increase adiposity or TL1A KO mice were fed high-fat diet and leukocytes residing in adipose tissue were analysed by flow cytometry and histology. Interestingly, TL1A's effect on adiposity was independent of gut microbiota as germ-free TL1A KO mice had a lower adiposity than wild-type mice. Furthermore, TL1A injection resulted in increased adiposity as well as infiltration of leukocytes in the adipose tissue, thereby indicating a more direct effect of TL1A on the establishment of adipose tissue.

Number 15:

The activity of *IFNL4* promoter and expression of *IFNL4* mRNA

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The hepatitis C virus (HCV) is a global public health problem, as a great fraction of patients develop a chronic infection, which may lead to a progressive hepatic fibrosis, cirrhosis and liver cancer. Both spontaneous- and treatment-induced clearance of HCV depend on host genetic background and in particular on a genetic variation (rs368234815 (TT/ΔG)) within the recently discovered interferon lambda 4 gene (*IFNL4*). This variation, which changes the open reading frame, determines the expression of the IFNλ4 protein. Paradoxically, despite the protein's clear antiviral nature, the ability to express IFNλ4 correlates with the poor spontaneous- and treatment-induced clearance of HCV infection.

In our study we are investigating the transcription pattern of *IFNL4* in different cell lines and upon different stimuli. Its mRNA is clearly expressed at a very low level compared to the other type III IFNs and it is triggered by stimuli common for all type III IFNs. We have also observed that an epithelial cell line generates higher *IFNL4* mRNA levels compared to the hepatocyte cell line. To further investigate the mechanisms regulating the expression of *IFNL4* we have cloned the promoters of *IFNL4* and *IFNL3* to perform a luciferase-based assay. The *IFNL4* promoter appears to generate a very weak signal compared to *IFNL3* and the reasons for its low activity are currently being investigated.

Number 16:

The effect of ultraviolet B phototherapy on regulatory T cells in patients with atopic dermatitis with and without *FLG* mutations

Stine Simonsen*, Charlotte Menné Bonefeld, Jacob Pontoppidan Thyssen, Carsten Geisler, and Lone Skov.

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Background: Atopic dermatitis (AD) is associated with a higher prevalence of mutations in the filaggrin gene (*FLG)*. A small study has shown that UVB phototherapy of inflammatory skin diseases increases the proportion of circulatory regulatory T cells (Treg cells).

Objectives: To examine whether UVB phototherapy of AD increases the proportion of Treg cells and the impact of *FLG* mutations.

Methods: We will recruit 20 AD patients with and 20 AD patients without *FLG* mutations receiving UVB phototherapy. We collect samples at 0, 2, and 4 weeks of treatment. The proportion of Treg cells, and T cell cytokine production are determined in peripheral blood.

Results: Until now we have included 11 patients without and 6 patients with *FLG* mutations. Preliminary analysis shows that overall the mean proportion of $CD4^+$cells bearing $CD25^+Foxp3^+$ (Treg cells) changes from 4,9% at week 0 to 5,2% at week 2; to 4,4% at week 4. For patients without mutation the change is from 5,0%; to 4,5%; to 4,6% and for patients with mutation from 4,5%; to 6,5%; to 4,1%. No changes in cytokine production in T cells were found. Inclusion is ongoing

Conclusion: UVB phototherapy may lead to a transient increase in the number of Treg cells in AD patients with *FLG* mutations.

Number 17:

The Delivery of Double-Stranded DNA to Dendritic Cells and its Effect on Dendritic Cell Maturation*

Marlene Fyrstenberg Laursen[1], Emil Kofod-Olsen[1] and Ralf Agger[1]
[1]Laboratory of Immunology, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark
*The work presented is a Master's Thesis work in progress

The aim of this study is to investigate the effect of a 60 bp double-stranded DNA (dsDNA) on dendritic cell (DC) maturation and to elucidate how the cells sense dsDNA.

DCs are potent antigen presenting cells (APCs), which have the ability to induce a cytotoxic immune response, making them promising for the use in anti-cancer vaccination therapy. Damage-associated molecular patterns (DAMPs) are endogenous molecules, including dsDNA, which are released from damaged cells in response to cell stress or tissue injury. DAMPs are recognized by different pattern-recognition receptors and have the ability to activate APCs, making them interesting as potential adjuvant for anti-cancer vaccines.

dsDNA with 5' Cy5 labelling are used to transfect DCs, which are analyzed with flow cytometry to investigate the uptake of dsDNA. The effect of dsDNA on DC maturation is analyzed with flow cytometry by transfecting the cells with different DAMPs and comparing their effect on the expression of three maturation markers: CD83, CD86, and HLA-DR.
Confocal microscopy will be used to visualize the uptake of dsDNA by DCs and to elucidate if the intracellular adaptor protein Stimulator of Interferon Genes (STING) is involved in the sensing of dsDNA.

Preliminary results from the ongoing experiments will be presented.

Number 18:

Genetic engineering using CRISPR/Cas9: Targeting MMP23 in melanoma

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MMP23 is a metalloproteinase expressed by a subset of melanomas. It has been shown that tumors expressing high levels of MMP23 comprise fewer tumor-infiltrating lymphocytes (TIL) and that there is correlation between high levels of MMP23 and an overall worse clinical outcome. MMP23 has a ShK toxin-like protein domain that has been shown to block Kv1.3 potassium channels. Potassium channels are important for the activation and proliferation of T cells since the channels maintain the calcium homeostasis in the cell. We hypothesize that melanoma cells can suppress effector memory T cells by upregulating MMP23, which blocks Kv1.3 potassium channels on the T cells, and thereby inhibit the anti-tumor response.

Using CRISPR/Cas9, we have successfully created and validated two guide RNAs for MMP23, which target exon 5 or exon 7. By co-transfecting with plasmids expressing the guide RNAs, we have effectively knocked out exon 6, which encodes the ShK toxin-like protein domain. We have thereby created a MMP23 knock out tumor cell line, incapable of blocking the Kv1.3 potassium channel. Functional studies will be conducted to see if T cells have an improved anti-tumor response towards the knock out cell line compared to wild type. We will then move into a humanized mouse model to study the T cell response in vivo. This will hopefully result in novel insight into the immunosuppressive mechanisms of MMP23.

Number 19:

Characterization of Inhibitory Molecules on Tumor-Infiltrating Lymphocytes in Malignant Melanoma

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Adoptive T cell transfer (ACT) using in vitro expanded tumor infiltrating lymphocytes (TILs) has shown encouraging results with complete response rates of 20% and objective response rates of 50% in treatment of malignant melanoma.

Despite the tumor specificity that TILs provide, cancer cells can engage a number of inhibitory molecules on TILs and thus inhibit killing of the cancer cells. By blocking this interaction, e.g. using checkpoint inhibitors Ipilimumab and Pembrolizumab, the T cells can regain their ability to kill.

We want to characterize the expression of inhibitory molecules on TILs derived from melanoma biopsies, aiming to identify new candidates for blockade in combination with ACT.

We have isolated TILs and quantified the immune cell composition (NK-, T-, B-cells) from 10 melanoma biopsies deriving from different patients. Using multicolor flow cytometry we will analyze the expression of inhibitory molecules (including NKG2A, CD200R, TIGIT, and TIM3) on TILs from tumor digest ("ex vivo TILs"), after isolation ("Young TILs"), and after rapid expansion ("REPd TILs").

To this end we have found that NKG2A is highly expressed on natural killer (NK) cell, on a small fraction of CD8+ T cells and on γδ T cells in our TILs.

Screenings will be followed by functional studies aiming to improve the cytotoxic capacity of the TILs in ^{51}Cr-release assays through individual and combined blocking of inhibitory molecules. Based on our preliminary findings blocking studies of NKG2A on the tumor infiltrating NK cells have been initiated.

Number 20:

Characterization of the Key Chemokine-Chemokine Receptor Interactions in Ovarian Cancer

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Background: Adoptive T cell transfer (ACT) for treatment of ovarian cancer (OVC) have recently been initiated at Center for Cancer Immune Therapy, Herlev Hospital. However, a major challenge of ACT is inefficient homing of tumor infiltration lymphocytes (TILs) to the tumor site. We hypothesized that by genetic engineering of TILs to increase expression of appropriate chemokine receptors, we would be able to increase the recruitment to the tumor. We tested this by I) Mapping key ovarian tumor chemokines and measuring expression of the corresponding chemokine receptors on TILs and II) studying migration of receptor transduced T cells towards a chemokine source in vitro.

Results: We found that CCL2, CCL22, CXCL10, CXCL16 and IL-8 were pronounced chemokines of ovarian tumors. Among a panel of corresponding chemokine receptors, we found that CCR2, CCR4, and CXCR2 were only expressed by a small fraction of TILs. Hence, an inverse relationship between chemokine and chemokine receptor expression were found for the three axes CCL2/CCR2, CCL22/CCR4 and IL-8/CXCR2 in ovarian cancer. Based on these results, we showed successful enhanced migration of CCR4-transduced T cells towards its ligand, CCL22.

Conclusion and Perspective: Low expression of essential chemokine receptors may be responsible for the inadequate homing of TILs to ovarian tumors. We found that genetic engineering to increase expression of appropriate chemokine receptors is a feasible strategy for optimizing migration. For further insight into the clinical relevance we are currently exploring correlations between patient's chemokine receptor expression, chemokine concentrations in blood and ascites, and patient survival.

Number 21:

Number and affinity of IgE clones determines human mast cell activation

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Background: Allergen specific IgE consists of individual clones with unique affinity, concentration and complexity, comprising both the number of clones binding the same allergen (clonality) and their relative ratio. This study explores how these characteristics of the IgE repertoire direct mast cell activation.
Methods: Human mast cells (MC) were generated from stem cells and sensitized with combinations of well-characterised recombinant human IgE (rhIgE) clones specific for *Dermatophagoides pteronyssinus 2* (Der p 2) or *Phleum Pratense 5* (Phl p 5). Activation of mast cells was measured as upregulation of CD63 by flow cytometry. Mast cell reactivity (fraction of mast cells activated, $\%CD63^+$ MC) and sensitivity (allergen concentration triggering a half-maximal response, EC_{50}) were estimated by non-parametric curve fitting. Statistical significance was analyzed using Kruskal-Wallis test.
Results: Increasing fraction of total rhIgE specific for Der p 2 significantly increased reactivity (p=0.0006) and sensitivity (p=0.0008). Optimising the ratio of Der p 2-specific rhIgE clones from 1:99 to 1:1 increased reactivity (p=0.038) but not sensitivity (p=0.13). Linear correlations with parallel slopes were obtained when plotting total concentration of Der p 2-specific rhIgE and net concentration of the rhIgE clone expressed in the smallest fraction, respectively, against mast cell reactivity. Increasing rhIgE affinity increased reactivity (p=0.0068) and sensitivity (p=0.0005). A linear correlation exists between product of affinities (K_D) and mast cell reactivity. A 10-fold decrease in product of affinites increased reactivity with 9.5%. Increasing Der p 2-specific rhIgE clonality increased reactivity (p=0.0039) but not sensitivity (p=0.49). Increasing clonality of rhIgE antibodies specific for Phl p 5 equally only increased mast cell reactivity (p=0.0286) but not sensitivity (p=0.6571).
Conclusion: Composition of allergen specific IgE directs the human mast cell response. The number of productive IgE pairs crosslinking the allergen is more important than the total amount of one clone. When increasing affinity of the IgE pairs both reactivity and sensitivity increase in proportion to product of the affinities, and additional IgE clones binding the same allergen increase reactivity independent of IgE specificity. Knowledge of IgE epitope number, specificity and affinity may contribute significantly when predicting the development of individual patient's allergy.

Number 22:

Global gene expression profiling of six murine "AD-like" models identifies IL-23-stimulated mice to best simulate human atopic dermatitis

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Atopic dermatitis (AD) has a complex, multifactorial pathogenesis involving genetic, environmental, and immunologic factors. Therefore, identification of an appropriate mouse model for AD will help with disease understanding and drug discovery. Although several models have been proposed, none seem to capture the full human AD phenotype. We applied global gene expression profiling and qRT-PCR to characterize six inflammatory mouse models (NC/Nga, flaky-tail, Flg-mutated, ovalbumin-challenged, oxazolone-challenged, and IL-23 injected mice) and compared their gene expression profiles to those of human AD, psoriasis, and contact dermatitis. Applying criteria of fold-change ≥2 and a false discovery rate (FDR)<0.05, we showed that IL-23-injected, NC/Nga, and oxazolone-challenged mice share more differentially expressed genes (DEGs) with the human meta-analysis derived AD (MADAD) transcriptome (37%, 18%, and 17%, respectively), compared to other models. qRT-PCR analysis demonstrated robust Th1, Th2, and Th17 activation in IL-23-injected and NC/Nga mice, which is also observed in AD, and a similar, but weaker inflammatory reaction in ovalbumin-and oxazolone-challenged mice. Flaky-tail mice and Flg-mutated mice displayed down-regulation of barrier genes without significant inflammation. In conclusion, none of the mouse models captured all the features of AD in humans. Instead, each model reflects specific immune or barrier disease aspects. Among the 6 models, IL-23-injected mice seem to best simulate human AD. The translational focus, however, determines which murine model is most applicable.

Number 23:

The role of skin resident and memory T cells in a modified rat oxazolone induced contact hypersensitivity (CHS) model.

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Allergic contact dermatitis (ACD) is a type IV hypersensitivity immune reaction and common in humans. In rodents the reaction is referred to as contact hypersensitivity (CHS) and the pathophysiology of the T-cell dependent immune reaction has been widely studied in mice.
In contrast, studies of CHS in rats are more limited. The membrane potential of rat and human T cells is likewise regulated by potassium-gated channels (1), whereas this is not the case for mouse T cells, thus in mice the K+ expression pattern on repeatedly activated T cells and effector memory T cells is different from that of human and rat T cells (2).
Skin resident T cell populations are likely to play a key role in the ACD reaction (3). The aim of this project is to study skin resident T cells and memory T cells in a modified rat oxazolone induced CHS model. The allergen oxazolone is used as sensitization and elicitation chemical, since it promotes a mixed T_h1 and T_h2 immune response. Moreover, $CD8^+$ T cells play a major role in the skin reaction to oxazolone. The planned studies cover different time intervals between sensitization and elicitation, and different sites for applying oxazolone. Various methods such as measurement of ear thickness, histology, flow cytometry of epidermal and dermal cells and draining lymph nodes, and ELISAs for cytokine determination will be used in order to characterize the skin resident T cell and memory population under the different sensitization and elicitation conditions.

Number 24:

Development and experimental analyses of a novel fusion protein vaccine cassette and its interactions with dendritic cells

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This study aims to develop and confirm functionality of a novel vaccine cassette in two variants: Untargeted and targeted towards murine dendritic cells (DCs). The proposed vaccine contains an interchangeable antigen and two protein domains to promote uptake in DCs and drive them towards the wanted phenotype. One of the two protein domains allows binding to poly I:C and thus allows the vaccine itself to deliver antigen and adjuvant. The targeted variant has been developed in two editions: (a) a vaccine with a single-chain fragment variable (scFv) domain and (b) a vaccine with a Fab domain. The novel cassette is to the best of our knowledge the most advanced fusion protein vaccine to date.

The vaccine has been confirmed to bind poly I:C by electrophoretic mobility shift assay. Furthermore, binding to murine dendritic cells has also been confirmed. Currently, optimizations of the vaccine production are on-going.

Preliminary results will be presented.