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Expression of coding (mRNA) and non-coding (microRNA) RNA in lung tissue and blood isolated from pigs suffering from bacterial pleuropneumonia

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*Translational Immunology: from vaccines to
immunotherapy*

ABSTRACTS

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#1 Anne-Marie Carola Andersson

Study of the immunogenicity and protection of a universal influenza A vaccine

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There is a constant threat of a potential outbreak of a severe influenza pandemic and therefore also a great need for a universal influenza vaccine. In addition, the available vaccines against seasonal epidemics are sub-optimal with their major antigen being hemagglutinin (HA). The ectodomain of the matrix protein 2 (M2e) is highly conserved amongst human influenza A viruses and M2e specific antibodies have been shown to decrease morbidity in animals infected with influenza. Therefore, M2e is considered to have great potential as a vaccine target. The M2e-NSP4 protein consists of four M2e peptides based on the H1N1 consensus sequence fused to the NSP4₉₈₋₁₃₅ fragment of rotavirus. M2e-NSP4 formulated with CAF-01 proved to be highly immunogenic and induced M2 peptide, M2e-NSP4, and presumably native M2e IgG antibody responses quantitatively greater than those induced by M2 peptide immunization with CAF-01. The induced isotypes were primarily IgG1 and IgG2a. In addition, the sera from M2e-NSP4 immunized mice showed to be cross reactive with M2 peptides derived from avian, equine, and swine influenza A strains. The immunogenicity of the M2e-NSP4 construct was dependent on the linkage between four M2e peptides to the NSP4 fragment. Finally, two immunizations with 10 µg M2e-NSP4 protected against lethal infection with PR8. Further experiments will include studies of the M2e-NSP4 construct with M2e based on a H5N1 sequence and the immunogenicity and protection in ferrets.

#2 Åsa Andersson

Studies of genes controlling development of disease in an experimental model for multiple sclerosis

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AIM: To identify disease factors for Multiple Sclerosis (MS) by defining genes important for development of Experimental Autoimmune Encephalomyelitis (EAE), an animal model for Multiple Sclerosis.

BACKGROUND: Development of autoimmune diseases is dependent on both hereditary and environmental factors. We have defined genetic regions in the mouse linked to development of EAE. EAE develops in susceptible mice after immunization with myelin proteins or peptides and leads to a T cell dependent attack on the myelin in the CNS. To further dissect the identified quantitative trait loci (QTL) linked to disease, and to define candidate genes within the regions, we have produced mice congenic for the respective genetic regions. Disease-controlling genetic regions from the resistant mouse strain RIIS/J (H-2^I) have been bred on to the MHC congenic susceptible B10.RIII strain (H-2^K).

RESULTS: In studies of EAE development in the congenic and littermate control mice, we have confirmed that the novel *Eae* loci *Eae27* and *Eae42* on mouse chromosome 1 contain genes controlling susceptibility to EAE. By bioinformatics, DNA sequencing, and gene expression studies (with real-time PCR) we have identified specific candidate genes primarily involved in T cell activation. The candidate genes are further studied by *in vitro* functional assays and flow cytometry. Preliminary results show that polymorphisms in genes for proteins involved in T cell signalling and extra-cellular glycosylation patterns located in *Eae27* and *Eae42*, respectively, might change the T cell activation pattern in resistant (congenic) compared to susceptible (control) mice.

CONCLUSION: Two genetic loci on mouse chromosome 1 have been identified to harbour genes controlling EAE. Polymorphic genes within the respective loci controlling T cell activity could be important for disease development.

#3 Sara Marie Atkinson

Establishment and histopathological and immunological characterization of a sustained delayed type hypersensitivity model with characteristics of arthritis in mice

Sara Marie Atkinson

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The use of animal models designed to mimic important aspects of human diseases is crucial to the understanding of pathogenesis and provide valuable insight into how the immune system works in a disease setting. Moreover, animal models of disease provide an opportunity to perform pre-clinical screening and selection of new drug candidates.

Rheumatoid arthritis (RA) is a progressive, destructive systemic autoimmune human disease, characterized by chronic synovial joint inflammation, including infiltration of inflammatory cells, fibrin deposition and cytokine-mediated enzymatic erosion of cartilage and bone. Synovial hyperplasia results [1]. Signs of systemic inflammation are also present, including upregulation of acute-phase proteins [1]. Autoantibodies against type II collagen (CII) can be detected in some RA patients, but it is not well understood how antibodies against type II collagen are involved in the progression of RA [2].

Animal models of arthritis can be induced by immunizing mice with CII antigen in Freund's complete adjuvant (CFA), this model is known as collagen induced arthritis (CIA). The inflammatory arthritis induced following immunization develops primarily in the paws and is dependent on both T and B cells but also involves other inflammatory cells such as neutrophils and macrophages [2]. Another, mouse model of arthritis is the anti-type II collagen antibody-induced arthritis model (CAIA), which is induced by an injection of mixtures of anti-CII monoclonal antibodies followed by a subsequent injection of LPS [3]. The arthritis that develops in the CAIA model is highly neutrophil driven. However, T-cells are also involved [3].

The immune reaction thought to underlay many autoimmune disorders is delayed-type hypersensitivity (DTH), a reaction that is T-cell dependent and induced by antigen [4]. The organism response in DTH is essentially the same as in the response to an exogenous pathogen or antigen. DTH elicited in murine footpads peaks 24-48 hrs after the antigen challenge, and does not persist for long enough for any significant bone destruction or synovial hyperplasia to occur [5]. In the present project we would like to investigate whether injection of a mixture of anti-CII antibodies during the sensitization phase can induce a more sustained DTH reaction with characteristics of arthritis, since CII antibodies appear crucial for the development of a chronic inflammation in joint tissue [2]. Moreover, we would like to characterize the underlying histopathology and immune mechanisms. The model has been described by Tanaka et al. in Balb/c mice [5], However we would as a first choice aim at establishing the model in C57Bl/6 mice, since we already have a DTH model established in this strain. Also establishing the model in C57Bl/6 mice is practical, as many knockout mice are only available in this strain, and for control and comparison purposes it is desirable to use only one mouse strain.

DTH is a T-cell dependent immune mediated response, which develops in two phases. In the sensitization phase, T cells are sensitized and antigen-specific effector T cells are formed. Next, in the elicitation phase, recall T-cell responses are induced upon secondary challenge with antigen. These effector T cells release cytokines and chemokines, which lead to activation of endothelium and recruitment of inflammatory cells such as neutrophils and macrophages at the site of the DTH reaction. In mice, the DTH reaction is typically elicited in the footpad, and result in a transient acute footpad swelling that peaks at 24 hrs. This transient DTH model has several advantages. It is a short (7 days + 2-3 days) and highly reproducible animal model. Moreover, a DTH reaction is only seen in the antigen-challenged footpad and draining lymph node (LN), thus the contralateral PBS challenged footpad (and draining LN) serves as in intra-animal control. This very site-directed and local reaction makes the model ideally suited for mode of action studies (MoA) and efficacy studies of drugs that interfere with or block T-cell priming, cell-migration or inflammatory cytokines. However, due to the transient nature of the DTH response the model is best suited for preventive treatment studies, i.e. treatment prior to elicitation. The establishment of a more sustained and chronic DTH model would allow therapeutic intervention and MoA studies drugs which would be of great value in the pre-clinical screening and selection of new drug candidates for the treatment of autoimmune diseases and if the model shows characteristics of arthritis it would add to its value.

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#4 Maria Rosaria Bassi

Yellow fever: a proof of concept for vaccine design

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Yellow fever (YF) is a severe hemorrhagic disease caused by a virus belonging to the family of Flaviviridae that can cause up to 50% fatality among the infected individuals.

The disease is underreported and WHO estimates that YF infects as many as 200.000 people causing 20-30.000 deaths in Africa and Latin America.

There is no cure for YF; a very safe and effective vaccine, based on a live attenuated version of the virus (YF-17D), is available since 1930, and over 540 million doses of it have been administered worldwide.

Despite its success, still very little is known about how the vaccine induces protection in humans: limitations in understanding the vaccine-induced immunity are also due to the lack of a well-established small animal model.

This is why we aim to establish a mouse model for YF infection in our lab using the vaccine strain YF-17D.

This model will enable us to fully characterize the composition of the innate immune response to the virus and to identify the lymphocytes subsets involved in the virus clearance.

Once the mouse model for YF infection is established, we will adapt it to *HLA-A2.1-/HLA-DR1-transgenic H-2 class I-/class II-knockout* mice: these mice represent a unique in vivo experimental model for human immune function studies without any interference with mouse MHC response. We will use this model to validate the importance of viral epitopes targeted by human CD8+T cells, as identified by a collaborator, from the blood of human volunteers vaccinated with the YF17D-204.

#5 Simon Bendtsen

β 2 microglobulin and desLys58 β 2 microglobulin and their roles in immune system

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β 2 microglobulin (β 2m) is a part of the major histocompatibility complex class I, which presents peptides to CD8⁺ cells. The role the protein plays in the formation of this complex represents the so far only known and described function of β 2m.

In serum of patients undergoing chronic haemodialysis, a modified form of β 2m, desLy58 β 2m, is present. DesLys58 is formed by proteolytic cleavage by C1 complement and subsequent removal of Lys⁵⁸ by carboxypeptidase B-like activity. This modification of β 2m can also be done *in vitro*. The modified version of the protein has, in several studies, been shown to have an apoptotic effect on different kinds of cells derived from the immune system. We have shown that β 2m KO develop an increased severity of experimental autoimmune induced uveitis (EAU) induced by the autoantigen interphotoreceptor retinol binding protein (IRBP) compared to wt mice. Since IRBP induced EAU is considered as CD4⁺ T-cell mediated disease it might indicate a role of β 2m in regulation of the immune response as a negative regulator.

Most cells express MHC-1 and are therefore able to bind β 2m. However, the cell line K562 has no MHC-1 expression, but FACS-data show that desLys58 β 2m and β 2m are bound to the cell surface as detected by antibodies recognizing β 2m and desLys58 β 2m. In addition, binding of desLys58 β 2m cannot be displaced by excessive amounts of β 2m. This indicates the presence of an unknown receptor for β 2m and/or desLys58 β 2m on the surface of these cells. In our future efforts we will focus on identifying the presence of a possible new receptor for desLys58 β 2m and elaborate on whether this protein interaction is of importance for the increased severity of EAU observed in β 2m KO mice.

#6 Lasse Boding

Polymorphisms of the T cell receptor CD3delta and CD3epsilon chains affect anti-CD3 antibody binding and T cell activation

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T cell receptor (TCR) structure and function have been thoroughly studied for decades. Production and analyses of knock-out and knock-in mice with mutations in the CD3 chains have contributed significantly to these studies. The generation of such gene-modified mice relies on the availability of suitable embryonic stem (ES) cell lines. Traditionally, ES cell lines from the 129 mouse strains have been used followed by backcrossing to the C57BL/6 strain. In the present study, we demonstrate the existence of polymorphisms in the CD3 genes from mice of the 129 and C57BL/6 strains. These polymorphisms result in amino acid substitutions in the ectodomains of both the CD3delta and CD3epsilon chains in 129 mice compared to C57BL/6 mice. The amino acid substitutions do not change the stoichiometry or surface expression level of the TCR complex in 129 T cells but cause reduced anti-CD3 antibody binding to 129 T cells. Further, when stimulated with mitogenic anti-CD3 antibodies, T cells from the 129 strains show reduced expression of the activation marker CD69, Ca²⁺ flux, IL-2 production and proliferative responses compared to C57BL/6 T cells. These findings demonstrate that polymorphisms of the CD3delta and epsilon ectodomains exist in mice, and that some of these polymorphisms lead to amino acid substitutions which cause structural changes and affect anti-CD3 antibody binding. Thus, functional T cell studies should be interpreted with caution when anti-CD3 antibodies are used for stimulation of T cells derived from gene-modified mice originating from 129 ES cell lines.

#7 Christina Pries Bramsnæs

Analysis of antiviral T cell responses primed through adenoviral vaccines encoding multiple viral antigens

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The aim of this newly started project is to analyze the T cell response raised by adenoviral vaccines encoding multiple viral proteins under the same promoter.

T cells constitute an important part of the defense against viruses, intracellular bacteria and tumors. For that reason, vaccines that induce strong T cell responses, are in focus for treatment of several malignant diseases, as well as for infections like HIV, Hepatitis C virus and Tuberculosis, for which normal antibody-based vaccines have proven inefficient. Replication deficient adenoviral vectors encoding microbial or tumor antigens are promising vaccine candidates, as they can induce strong antigen-specific T cell responses upon vaccination. Using such vectors, it is also possible to construct multivalent vaccines that encode more than one antigen. However, T cell responses tend to be focused towards a small number of epitopes as a consequence of immunodominance occurring at the antigen and/or T cell level; a feature that not only increases the risk of selecting viral or tumor escape variants, but also challenges the concept of vaccinating against multiple antigens in the same vector. We want to study this phenomenon further and have for this purpose constructed adenoviral vectors encoding two or three antigens under the same promoter. Preliminary results with constructs encoding the glycoprotein (GP) from lymphocytic choriomeningitis virus (LCMV) followed by the second matrix protein (M2) from murine gammaherpes virus 68 (MHV68) have shown that the strengths and the breadth of the T cell response to especially the M2 gene is compromised upon vaccination, when compared to constructs encoding the M2 gene alone. We will extrapolate on the findings using other combinations of viral genes and change the order, of which they appear in the construct. Moreover, we will analyze the functional relevance of the compromised T cell response by measuring the protection level towards subsequent viral challenge with LCMV or MHV68 in mice. Finally, we will look into various factors of importance for T cell competition, including affinity to the MHC:ag complex, T cell precursor frequencies and competition at the APC level. This information is of significance relevance for design of T cell-based vaccines with broad specificities.

#8 Peter Brændstrup

Novel T-cell epitopes in the immunodominant proteins IE-1 and IE-2 in human cytomegalovirus (CMV)

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Background: Latent CMV infection in recipients of hematopoietic cell transplants and solid organ transplants is associated with increased morbidity and mortality compared to non-infected recipients despite the advent of antivirals and preemptive therapy. T-cells are of crucial importance in virus control. IE-1 and IE-2 are among the most frequently recognized CMV proteins.

Methods: In this project we used a rational approach for complete identification of CD8 and CD4 T-cell epitopes in IE-1 and IE-2. Matrices of IE-1 and IE-2 (pooled 15-mer peptides) were used to stimulate PBMC's from 16 healthy seropositive donors. T-cells were analysed after either a 6 hour stimulation period or a 24 hour stimulation period followed by 8 days of expansion. After stimulation CMV specific T-cells were detected by cytokine flow cytometry. The 15-mer peptides found to elicit T-cell responses along with the HLA-profile of the donor were then subjected to a prediction programme capable of suggesting possible restriction and the exact length of the peptide.

Results: We have so far found four new HLA class I epitopes in IE-1 and IE-2 as well as several HLA class II epitopes. Current work focuses on determining the HLA restriction of the novel epitopes and determining the binding affinity between the predicted peptide and HLA-molecule.

Conclusion: We report four novel IE-1 and IE-2 HLA class I epitopes and more importantly several new IE-1 and IE-2 HLA class II epitopes. The latter may be of importance in the development vaccine and immune therapies against CMV as little is known about CD4 T cell responses. We are currently working on validating these results.

#9 Thomas H.R. Carlsen

Development of novel JFH1-based Core-NS2 cell culture recombinants for HCV genotypes 1a, 1b and 3a using previously identified adaptive mutations

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Hepatitis C virus (HCV) chronically infects 180 million worldwide and leads to increased risk of end-stage liver diseases. No vaccine exists and current treatment cures only 40-80% of patients depending on HCV genotype. Furthermore development of specific antivirals and a vaccine has been hampered by the lack of laboratory based cell culture systems. However the recent development of complete viral life-cycle in vitro systems, depending on the genotype 2a isolate JFH1, has improved the research prospects for HCV.

We previously developed HCV cell culture systems for all seven genotypes and relevant subtypes by replacing the structural genes (Core, E1 and E2), p7 and NS2 from JFH1 by corresponding sequences of HCV prototype isolates. For most recombinants, efficient production of infectious viral particles in Huh7.5 hepatoma cells depended on adaptive mutations. These recombinants can be used for genotype-specific functional analysis of Core-NS2 and studies of susceptibility to neutralizing antibodies and antiviral drugs. However, for identification of HCV genotype-, subtype-, or isolate-specific differences, a panel of Core-NS2 recombinants for each genotype is required. In this study, we developed a panel of efficient genotype 1a, 1b, and 3a Core-NS2 recombinants using previously identified adaptive mutations. Following transfection of mutants, one novel genotype 1a and 1b recombinant exhibited immediate viral spread in culture. For other 1a, 1b and 3a isolates, introduction of mutations accelerated growth kinetics, although additional changes were required for efficient virus production. Furthermore, in two cases, for a 1a and a 1b recombinant, culture adaptation occurred only after introduction of previously identified mutations. In conclusion, through introduction of previously identified adaptive mutations we developed additional novel JFH1-based cell culture Core-NS2 recombinants for worldwide important genotypes. The novel recombinants will be important tools for future HCV research.

#10 Katja Denninger

The collagen induced arthritis (CIA) model: A purely rheumatoid model?

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Objective: To study the angiogenesis and bone remodelling of the CIA model.

Introduction: The CIA model is used as a widely accepted model of rheumatoid arthritis (RA). It is also used as a model for other arthritides like psoriatic arthritis (PsA), mostly due to lack of better disease models. However, PsA and RA are two different inflammatory arthritides differing in particular in angiogenesis and bone remodelling: While RA is a purely erosive disease, bone formation and bone erosion co-exist in PsA and new blood vessels have a different clinical appearance in PsA than RA (1). However, bone formation may not be coupled to inflammation, as treatment of patients suffering from AS with TNF α blockers do not stop ankylosing disease progression (1). Likewise, in Spondylosing ankylosis bone erosion and bone formation was seen in anatomically different positions and at different time points as judged by ultrasound scanning of enthesitis (2). On the other hand, in some studies a partly relationship between inflammation and pathologic bone formation was suggested (3; 4). The angiogenesis and bone remodelling in the CIA model has received less attention than other aspects of the disease in this model, however, bone formation can be seen with histology and mRNA of bone morphogenetic protein-1 (BMP-1) is up regulated in early disease (5).

Results: (Experiments conducted):

- Kinetic studies of H-E stained tarsal joints
- Gene expression (microarray) studies on tarsal joints with severe inflammation.

The experiments are performed, but awaiting data analysis

Future experiments: Kinetic studies of immunohistochemical staining for relevant antibodies. This will be coupled with serum analysis for biomarkers of erosive disease and in vivo imaging.

Perspectives: The final aim of the studies is to compare the CIA model to two new models of PsA under way in order to characterise, evaluate and compare the models for their usefulness as disease models of PsA.

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#11 Sanne Diemer

Stability and purity of capsaicin

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Capsaicin, the hot ingredient of chilli peppers, has pain and inflammation relieving potential by desensitizing or killing pain transmitting afferent neurons. Recently, we showed that prophylactic capsaicin treatment could prevent development of inflammation in the T cell transfer model of chronic colitis.

Problems with solubility of capsaicin led us to suspect the stability of capsaicin dissolved in standard PBS/ethanol/tween80 containing vehicle.

The stability of capsaicin was studied at different temperatures (20°C, 37°C, and 74°C) and at varying time intervals (15 min – 6 days). Capsaicin samples were subsequently analyzed by high-performance liquid chromatography (HPLC) and mass spectrometry (MS).

Batches of capsaicin from two suppliers were analyzed. A batch of capsaicin (purity of 98%, Calbiochem, Canada) was used in stability testing and shown to be stable in all the experimental settings mentioned above. However, by analyzing the purity of this batch by HPLC at 281 nm a significant diversity of the indicated 98% purity was observed, since only 61% purity was recorded. The Calbiochem batch of capsaicin contained an unknown impurity of 31% (molar mass 307 g/mol). In comparison, another batch of capsaicin (purity of 99%, Sigma-Aldrich, US) was analyzed to be 96% (molar mass 305 g/mol).

These data clearly demonstrate that dissolved capsaicin is stable under most experimental conditions including temperature and time. However, HPLC prior to use should check product purity of subsequent batches.

#12 Carsten Faber

Complement factor H lacks regulatory effects in retinal pigment epithelial cells

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Background: Age-related macular degeneration (AMD) is the most common cause of blindness in the western world, affecting more than 10% of persons above 60 years of age. Although the pathogenesis is not known in detail, it involves formation of drusen and degeneration of the retinal pigment epithelium (RPE).

Age and a common variant of complement factor H (CFH) confer increased risk of developing AMD. The level of complement activation correlates to CFH haplotype, and AMD patients have elevated plasma concentrations of activation products such as C3a. Drusen contains many complement proteins including C3a.

The objective of this study was to 1) examine the influence of age on expression of drusen-associated genes in RPE cells and 2) examine whether this expression - especially complement encoding genes - depends on CFH.

Methods: RPE/choroid was isolated from young (~50 days) and old (~500 days) WT and *Cfh*^{-/-} C57Bl/6 mice immediately after killing. RNA from 2 eyes/animal, 4 animals/group, was isolated and analysed with GeneChip Mouse Gene 1.0 ST whole-transcript microarrays from Affymetrix.

Results: Deficiency in CFH resulted in more than 2-fold change of 48 genes in young mice and 57 genes in old mice. Age resulted in 207 genes changing in WT and 217 genes in *Cfh*^{-/-} mice of which about 50% were common. Aging results in upregulation of several complement proteins including C1q, C3, factor B and Serping1. Though it is a major regulator of the alternative pathway, deficiency in CFH does not affect complement expression in RPE/choroid.

Conclusions: Age has a great influence on gene regulation in RPE/choroid. Deficiency in CFH impacts age-related transcriptional changes, but has no local effect on complement factor-encoding genes. Increased expression of complement genes in RPE/choroid is an age-related phenomenon not directly related to CFH expression.

#13 Sif Gudbrandsdottir

Effects of activated human platelets on proliferation of PBMCs in vitro

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Introduction

Platelets are abundantly present in the circulation, and activated platelets may have a role in regulating immune responses. In this study we have examined how activated platelets influence the cytokine production and CD4+ T-cell proliferation elicited by self- and non-self antigens in cultures of mononuclear cells (MNCs) from healthy donors.

Materials and methods

MNCs were isolated from 7 healthy donors and labelled with carboxyfluorescein succinimidyl ester (CFSE). Platelets were isolated by centrifugation and activated by thrombin-receptor activating protein (TRAP). The MNCs were cultured in media containing autologous serum and stimulated with tetanus toxoid (TT), thyroglobulin (Tg), autologous platelets (plts) or combinations of TT + plts or Tg + plts. Platelets were added to the cultures in concentrations ranging from 0.5 – 3.4 x10¹⁰/L. Culture supernatants were harvested at day 1 and analysed for Th1/Th2 cytokine production. CD4+ T cell proliferation was measured at day 7 by flow cytometry.

Results

Addition of activated platelets caused an increased production of IL-10 in MNC cultures stimulated with Tg (p=0.016), but not in cultures stimulated with TT (p=0.938). Trends toward inhibited production of TNF- α elicited by Tg and IFN- γ by TT were observed in the presence of activated platelets. Moreover, activated platelets inhibited both the Tg- and the TT-induced proliferation of CD4+ cells (p=0.016 and p=0.047, respectively).

Conclusion

Addition of activated autologous platelets to MNC cultures increases the IL-10 production and inhibits the proliferation of CD4+ T cells elicited by the self-antigen Tg in vitro. Inhibition of the CD4+ T-cell proliferation elicited by TT was also observed. We are currently investigating the mechanisms behind these findings.

14 Morten Hansen

Maturation of dendritic cells for cancer immunotherapy employing novel cocktails and biodegradable, clinical-grade microparticles as delivery vehicle

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Objectives

The current standard protocol for maturation of dendritic cells (DCs) *ex vivo* for cancer immunotherapy employing TNF- α , IL-1 β , IL-6 and PGE₂ does not promote the production of IL-12 and thereby does not polarize responders to a T_H1 phenotype of relevance for generation of cancer-specific cytotoxic T lymphocytes (CTLs). The aim of this project is to identify novel superior ways to induce immune competent mature DCs.

The project is based upon two hypotheses

- Microparticles, in particular poly(D,L-lactide-co-glycolide) (PLGA), are clinically relevant vehicles for the *ex-vivo* co-delivery of protein-based antigens and danger-signals during manufacturing of immunogenic DCs from patient blood cells.
- Phagocytosed antigens of non-microbial origin require co-delivery of “danger signals” (e.g. Toll-like receptor (TLR) ligands) in the same phagosome for efficient cross-presentation and generation of potent CTL responses.

State-of-the-art in maturation of DCs

IFN- α , IFN- γ , CD40 ligand, and/or TLR ligands have been included in cocktails for maturation of DCs to promote the production of IL-12. Recently it was shown that suppressed production of CCL19, preventing ligand-induced CCR7 internalization, is the key factor responsible for enhanced surface-CCR7 expression in DCs matured in the presence of PGE₂ (Muthuswamy Blood 2010). Furthermore, *in vivo* studies did not demonstrate an advantage of PGE₂-matured DCs in lymph-node homing. Microparticle-based delivery of tumor-associated antigens (TAA) and maturation-inducing molecules may potentially solve major issues of current DC-based cancer immunotherapy such as proper generation of both TAA-specific CD8⁺ CTLs and CD4⁺ T_H1 cells. Such delivery platform may also facilitate more timely control of DC maturation and IL-12 production necessary for T_H1 polarization of CD4⁺ T cells desirable for optimal anti-tumor immunity. Studies from several groups generally find that PLGA microparticles are safe, non-activating vehicles that prolong antigen activation. Elamanchili et al encapsulated the LPS-analogue MPLA and showed enhanced T_H1 promoting effects compared with soluble MPLA and Wischke et al. employed poly(I:C) coated PLGA microparticles for efficient DC maturation.

Research plan

- Identification of novel cocktails for DC maturation relevant for cancer immunotherapy
- Characterization of PLGA microparticles with antigens and TLR ligands
- Functional and phenotypic characterization of DCs
- Adaptation of a clinically relevant DC vaccination protocol

#15 Mikkel Harndahl

Real-time, high-throughput measurements of peptide-MHC-I dissociation using a scintillation proximity assay

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Efficient presentation of peptide-MHC class I complexes to immune T cells depends upon stable peptide-MHC class I interactions. Theoretically, determining the rate of dissociation of a peptide-MHC class I complexes is straightforward; in practical terms, however, generating the accurate and closely timed data needed to determine the rate of dissociation is not simple. Ideally, one should use a homogenous assay involving an inexhaustible and label-free assay principle. Here, we present a homogenous, high-throughput peptide-MHC class I dissociation assay, which by and large fulfill these ideal requirements. To avoid labeling of the highly variable peptide, we labeled the invariant β 2m and monitored its dissociation by a scintillation proximity assay, which has no separation steps and allows for real-time quantitative measurement of dissociation. Validating this work-around to create a virtually label-free assay, we showed that rates of peptide-MHC class I dissociation measured in this assay correlated well with rates of dissociation rates measured conventionally with labeled peptides. This assay can be used to measure the stability of any peptide-MHC Class I combination, it is reproducible and it is well suited for high-throughput screening. To exemplify this, we screened a panel of 384 high-affinity peptides binding to the MHC class I molecule, HLA-A*02:01, and observed rates of dissociation that ranged from 0.1 hours to 46 hours depending on the peptide used.

#16 Mikkel Harndahl

Immunogenic CTL epitopes tend to be stably bound to MHC class I molecules: Implications for “holes in the stably bound MHC-I repertoire”

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Efficient presentation of peptide-MHC-I (pMHC-I) complexes to immune T cells should benefit from a stable peptide-MHC-I interaction. However, it has been difficult to distinguish stability from other requirements for MHC-I binding. We have recently established a high-throughput assay for peptide-MHC-I stability. Here, we have used this assay to generate a large database containing stability measurements of pMHC-I complexes, and we re-examined a previously reported unbiased analysis of the relative contributions of antigen processing and presentation in defining CTL immunogenicity (Assarsson, Sidney et al. 2007). Using an affinity-balanced approach, we demonstrated that immunogenic peptides are more likely to be stably bound to MHC-I molecules than non-immunogenic. To predict pMHC-I stabilities, we here developed a bioinformatics method based on our pMHC-I stability data. This predictor suggested that 30% of the non-immunogenic binders that hitherto have been classified as “holes in the T cell repertoire” can be explained as being unstably bound to MHC-I. Finally, we suggest that non-optimal anchor residues in position 2 of the peptide are particularly prone to cause unstable interactions with MHC-I. We conclude that the availability of accurate predictors of pMHC-I stability may be helpful in the elucidation of MHC-I restricted antigen presentation, and that it may be instrumental in future search strategies for MHC-I epitopes.

#17 Chris J. Hedegaard

Interferon-beta increases systemic BAFF levels in multiple sclerosis without affecting disease activity or increasing autoantibody production

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Treatment with interferon-beta (IFN-beta) is standard therapy in multiple sclerosis (MS). To investigate whether IFN-beta therapy affects the circulating levels of B-cell activating factor of the TNF family (BAFF) and autoantibodies against myelin basic protein (MBP) we followed 23 patients with relapsing-remitting MS (RRMS) longitudinally from initiation of IFN-beta therapy. Their blood levels of BAFF correlated positively at baseline with the expanded disability status scale ($P < 0.009$) and MS severity score ($P < 0.05$), but not with disease activity as determined by the number of gadolinium-enhanced lesions. A second group of 26 patients with RRMS were followed for up to 26 months, during which the BAFF levels remained increased without being associated with increased disease activity. IFN-beta therapy caused an increase in plasma BAFF levels after both 3 and 6 months of therapy ($P < 0.002$), but an 11% decrease in IgM- and a 33% decrease in IgG anti-MBP autoantibodies ($P < 0.09$ and $P < 0.009$, respectively) after 6 months. The increased circulating BAFF levels during IFN-beta therapy do not have detrimental effects in terms of increased disease activity, and does not translate into increased levels of anti-MBP autoantibodies, in line with the known beneficial effect of IFN-beta treatment.

#18 Peter Johannes Holst

Vaccination against lymphocytic choriomeningitis virus infection in MHC class II deficient mice

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The impact of prophylactic vaccination against acute and chronic infection in a T helper deficient host has not been adequately addressed due to difficulties in generating protective immunity in the absence of CD4⁺ T cell help. In this study we demonstrate that a broad CD8⁺ T cell immune response can be elicited in MHC class II deficient mice by vaccination with adenovirus encoding LCMV³ glycoprotein⁴ tethered to MHC class II associated Invariant chain (Ad-IiGP⁵). Moreover, the Ad-IiGP induced response confers significant cytolytic CD8⁺ T-cell mediated protection against challenge with the invasive clone 13 strain of LCMV. In contrast, vaccination with adenovirus encoding unlinked LCMV GP induced marginal virus control in the absence of CD4⁺ T cells, and mice may die from increased immunopathology associated with incomplete protection. Following infection with the less invasive Traub strain, no acute mortality was observed in any vaccinated mice. However, LCMV Traub infection caused accelerated late mortality in unvaccinated MHC class II deficient mice, and in this case we observed a strong trend towards delayed mortality in vaccinated mice irrespective of the nature of the vaccine. These results indicate that optimized vaccination may lead to efficient protection against acute viral infection even in T helper deficient individuals, but that the duration of such immunity is limited.

#19 Tina Jehs

The effect on T-cell derived cytokines on astrocyte cultures

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Background: With age low-grade chronic inflammation becomes more prominent with increasing plasma concentration of proinflammatory cytokines primarily due to rearrangement of the T- cell compartment. Since astrocytes form part of the blood brain barrier and they play a central role in maintaining the homeostasis within the CNS, we want to investigate whether there may be a correlation between age-related changes in the immune system, astrocytes and the development of age-related neurodegenerative diseases such as Alzheimer´s disease (AD).

Methods and results: PBMCs were purified from healthy human donors, activated with CD3/CD28 beads, and co-cultured with human astrocytoma cell lines. This co-culture was performed by inserting a membrane between activated PBMCs and astrocytes so only soluble factors can pass. Microarray analysis of astrocytes showed induction of a great number of genes after co-cultured with activated PBMCs. Among these genes were pro-inflammatory cytokines and chemokines such as IL6, IL8, IL1 β , CXCL10 and CCL5. Additionally the up-regulation of CXCL9, CXCL10, IL8, CCL3, CCL5, IL1 β and IL6 was validated on protein level by luminex.

Conclusion: Cytokines derived from activated PBMCs affect the astrocytes transcriptome towards a more activated state including upregulation of IL6 and IL1 β . Both activated astrocytes and overexpression of IL6 and IL1 β have been associated with AD. Thus increased levels of plasma derived cytokines might have a role in AD pathogenesis.

#20 Benjamin Anderschou Holbech Jensen

The role of endogenous IL-2 expression by adenoviral vaccine vectors

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Recombinant adenoviral vectors are among the most potent and versatile antigen delivery vehicles available. By tethering the vaccine antigen to the MHC class II associated Invariant chain (Ii) we have previously increased both the potency and the versatility.

The aim of this project is to investigate the potential of endogenous IL-2 production in antigen presenting cells, presenting endogenous antigen to CD8⁺ T-cells selectively or to both CD8⁺ and CD4⁺ T-cells. For that purpose we have constructed replication deficient adenoviral vaccine vectors (Ad) expressing the Lymphocytic Choriomeningitis virus (LCMV) Glycoprotein (GP) with and without linkage to Ii in the adenoviral E1 reading frame, and with and without an IL-2 expression cassette in the E3 reading frame.

The IL-2 encoding vectors have been confirmed to secrete biologically active IL-2.

The Ad-GP construct is known to induce a rather weak, narrow and slow response, which can be dramatically augmented by the linkage of the antigen to Ii. We are testing whether additional IL-2 expression can increase it further.

The kinetics, breadth and stability of the antigen specific response will be determined by measuring IFN- γ producing CD8⁺ and CD4⁺ T-cells at different time points (7, 11, 14, 21, and 60-90 days post vaccination). The potency of the constructs will be determined by dose/response experiments, where the immune responses are measured at early and late time points.

To determine the quality of the memory response we will investigate the expression of surface markers such as KLGR-1, CD27, CD127, CD43, CXCR3 and CD62L on the antigen specific T-cells and the ability of the elicited T-cells to co-produce multiple cytokines such as IFN- γ , TNF- α and IL-2. Finally, the ability of the induced T-cells to expand in response to infection will be assessed by adoptive transfer into naive hosts which are then challenged by LCMV.

The outlined experiments should form the basis of an initial characterization of the vaccines' capabilities in wild type mice, and make the basis for further characterization in immunodeficient hosts such as CD80/CD86 and MHC-II deficient animals.

#21 Janne Koch

Investigation of the cancer resistant phenotype of the SR/CR mouse

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The SR/CR mouse (Spontaneous Regression/Complete Resistance) model of cancer resistance was discovered in 1999. It is resistant to a number of different cancer cell lines and the heritable phenotype was demonstrated on different genetic backgrounds. The cancer resistance is transferable to other strains of mice by adoptive transfer of innate immune cells. We for the first time, confirm the findings of the SR/CR phenotype of cancer resistance to the S180 sarcoma cell line in mice of two different genetic backgrounds: BALB/c and C57BL/6.

FACS profiles of peritoneal cells from SR/CR mice and wild type mice 48 hours after intraperitoneal injection of S180 cells reveal large morphological differences: In the wild type mice a large population of the large granulated S180 cells is recognised, while this population is nearly absent in the SR/CR mice. The amount of infiltrating immune cells is also markedly larger in the SR/CR mice. Interestingly the SR/CR mouse has significantly higher proportions of neutrophils, macrophages and NK cells compared to wild type mice, while the proportion of CD19 positive cells is significantly higher in wild type mice. In FACS analysis of the splenocytes of SR/CR mice after stimulation with S180 cells there are also significantly increased levels of neutrophils, macrophages and NK cells, compared to the splenocytes in wild type mice injected with S180 cells at the same time.

Adoptive transfer of immune cells from SR/CR mice to wild type mice with subcutaneous S180 cell tumours have resulted in complete regression of the tumours. In histology sections of the regressing tumours large areas with necrosis and infiltrating neutrophils are found.

The SR/CR mouse thus shows a unique ability to kill even large populations of injected cancer cells, and this resistance to cancer cells seems to be mediated by the innate immune system.

#22 Martin Kongsbak

Vitamin D uptake and activation by human T cells

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The literature describes an important role for vitamin D in the function of the immune system. The vitamin D receptor (VDR) is expressed in activated but not in naïve human T cells. Vitamin D interacts with the VDR in the cytosol of T cells, leading to the translocation of the vitamin D-VDR complex into the nucleus where it acts as a transcription factor for various genes controlling T cell function. Vitamin D circulates in the blood mainly as the inactive mono-hydroxylated precursor (25(OH)D₃). In order to become biologically active, a second hydroxylation must occur (1,25(OH)₂D₃). This hydroxylation step is catalyzed by the enzyme CYP27B1 found in the kidneys. In the present study we show that naïve human primary T cells express low levels of CYP27B1 and that CYP27B1 expression is greatly upregulated during T cell activation, an upregulation that precedes VDR upregulation. In the blood, vitamin D is bound to vitamin D binding protein (DBP). For vitamin D to be taken up by kidney cells, vitamin D-DBP binds to the receptor megalin leading to receptor-mediated endocytosis of the complex. We find that megalin is expressed by both naïve and activated T cells, and that megalin expression is up-regulated following T cell activation. Altogether, this study shows an increased capacity of activated human T cells to internalize vitamin and convert it into its metabolic active form.

#23 Michael Kongsgaard

Large scale T cell epitope discovery in yellow fever virus

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This project involves a comprehensive, large-scale discovery effort directed at identifying T cell epitopes targeted in the acute immune response following vaccination with attenuated yellow fever (YF) virus in an out bred population of vaccinees. YF is an acute viral hemorrhagic disease and is transmitted by mosquitos (the yellow fever mosquito, *Aedes aegypti*, and other species) and is found in tropical and subtropical areas in South America and Africa. YF is on the NIAID list of “Biodefense Category A, B, C Pathogens” as one of the emerging infectious diseases (i.e. Category C). The YF vaccine is a live attenuated virus. It is therefore an ideal system for T cell epitope discovery: the vaccine itself is extremely safe; vaccinees are regularly and easily accessible due to the frequent and widespread use of the YF vaccine; and the strength of the ensuing T cell immune responses allow easy ex vivo monitoring of T cell responses. Volunteers donate blood before and about 2 weeks after vaccination. For comparison both pre-and post PBMCs from the vaccinees will initially be analyzed using multicolor flowcytometry to determine the frequency of activated T cells using intracellular phenotypic markers (e.g. CD4, CD8, KI-67, Bcl-2 and CD38) and surface markers (e.g. CD4, CD8, HLA-DR, CD45RA, CCR5, CCR7, CD27 and CD57). We will screen for specific T cell responses against the entire YF proteome (an effort involving a mere ≈ 900 overlapping peptides), and attempt to identify all peptide-specific, HLA-restricted T cell responses arising in vaccinees using ELISpot analysis with a 30x30 peptide matrix. Identified peptide epitopes will be validated using multicolor flowcytometry to determine the nature of the YF specific T cell response (phenotypic markers e.g. CD4, CD8 and CD38, functional markers such as IFN γ and TNF α and specificity markers (e.g. HLA tetramers)). Sera from donors will also be analyzed for YF specific antibodies using ELISA.

#24 Katharina L. M. Kopp

COX-2-dependent PGE₂ acts as growth factor in mycosis fungoides (MF)

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Cancer often originates from sites of persistent inflammation whereas the mechanisms turning chronic inflammation into a driving force of carcinogenesis are not completely understood. Cyclooxygenase-2 (COX-2) is an inducible key modulator of inflammation that carries out the rate-limiting step in prostaglandin synthesis. Aberrant COX-2 expression as well as induction of prostaglandin E synthase 1 (PGES1) and prostaglandin E₂ (PGE₂) production have been implicated in the pathogenesis of several cancer types. Chronic inflammation is a hallmark of cutaneous T cell lymphoma (CTCL). The most common form, mycosis fungoides (MF), is a lymphoproliferative disorder which in early stages (patch stage) resembles chronic inflammatory dermatitis. In the later stages (plaque, tumor stage) skin tumors form and malignant cells spread to extracutaneous sites. We show that COX-2 is ectopically expressed in malignant, but not in non-malignant T cell lines from patients with CTCL as well as *in situ* in lymphoma cells in 11 out of 22 patients suffering from MF in plaque or tumor stage. COX-2 is not expressed in lymphocytes of patients with patch stage MF; however, COX-2 positive stroma cells are detectable in the majority of patients regardless of disease state. COX-2 expression correlates with a constitutive production of PGE₂ in malignant T cells *in vitro*. These cells express PGE₂ receptors EP3 and EP4 as well as PGES1 and spontaneous proliferation is strongly reduced by the receptor antagonist and PGES inhibitors. Furthermore, malignant cell growth is heavily decreased by COX-2 inhibitors and siRNA directed against COX-2. Our data indicate that COX-2 mediated PGE₂ acts as a tumor growth factor in MF. We propose COX-2 and downstream players of PGE₂ synthesis as novel therapeutic targets in CTCL.

#25 Birte Kristensen

Induction of T-cell responses to a self-antigen, thyroglobulin, by B cells

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In addition to being antibody-producing, B cells are highly efficient at antigen-presentation as well as cytokine production. We investigated the responses of normal mononuclear cells (MNCs) to the self-antigen thyroglobulin (TG) and observed a significant production of IL-6, TNF- α , IL-10 and IL-2, as well as CD4⁺ T-helper (T_H) cell proliferation. Depletion of monocytes from the cultures abrogated these events, while depletion of B cells caused non-significant inhibitions, showing that monocytes were the more efficient APCs. However, B cells pulsed with TG induced a significant production of TNF- α , IL-6, IL-2 and considerable amounts of IL-10 upon co-culture with purified T cells. An induced T_H-cell proliferation was also seen. B cells pulsed with the foreign recall antigen, tetanus toxoid, induced a different cytokine profile including IFN- γ and IL-2, but almost no IL-10. Moreover, the subsequent T_H-cell proliferation was higher than that induced by TG-pulsed B cells. Staining with bi-specific anti-CD45/anti-cytokine antibodies revealed that 11% of the T_H cells produced IL-10 in the presence of B cells pulsed with TG, compared with 1% in presence of unpulsed B cells ($p < 0.03$). At this time point, IL-10 producing B-/T-cell aggregates were observed. At day 7, IL-10 was produced by 4% of the T_H cells, primarily within the proliferating subset. Thus, despite the observation that monocytes are the dominant APCs in APC cultures, B cells are capable of inducing TNF- α , IL-6- and IL-10 production by, and proliferation of, T cells in the absence of monocytes, which is likely to reflect the conditions in lymph nodes.

#26 Nanna Ny Kristensen

***L. acidophilus* NCFM™ and *L. Salivarius* Ls-33 increase the effect of regulatory T cells in experimental transfer colitis**

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Background: Probiotics may alter immune regulation. Recently we showed that the probiotic bacteria *Lactobacillus acidophilus* NCFM™ influenced the activity of regulatory T cells (Tregs) *in vitro*. The aim of the present work was to demonstrate if *Lactobacillus acidophilus* NCFM™ also affects the function of Tregs *in vivo*.

Methods: Development of colitis after transfer of CD4+CD25- T cells and protection from colitis by Tregs was studied in immunodeficient SCID mice which were simultaneously tube fed with *L. acidophilus* NCFM™ or *L. salivarius* Ls-33 or water (control) for 5 weeks. Changes in gene expression related to inflammation and autoimmunity in rectum of protected mice was compared with naïve mice.

Results: Probiotic fed SCID mice transplanted with low numbers of Tregs in addition to the disease-inducing T cells were completely protected from colitis. This was in contrast to the control group that showed intermediate levels of inflammation. In addition, feeding with probiotics lowered serum levels of inflammatory cytokines in both colitic mice and in mice protected from colitis by Tregs. Gene expression patterns of rectum samples of protected mice that receive either one of the probiotics showed a closer resemblance to naïve SCID mice than did patterns of the control group. Enteroantigen specific T cells from mesenteric lymph nodes of *L. acidophilus* NCFM™ fed protected mice showed an upregulation of Th2 cytokines compared to the other groups.

Conclusion: *L. acidophilus* NCFM™ and *L. salivarius* Ls-33 feeding of SCID mice increases the *in vivo* effect of Tregs, resulting in a phenotype resembling that of the naïve SCID mouse.

#27 Marianne B. Løvendorf

Biomarkers in psoriasis

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Psoriasis represents a chronic inflammatory skin disease with an approximate prevalence of more than 2 % of adults in the Western population. Today psoriasis is considered to be an autoimmune disease with skin manifestations. MicroRNAs (miRNAs) are a class of posttranscriptional regulators of gene expression with critical functions in health and disease. We have recently identified a specific miRNA expression-profile in psoriatic skin that differs from normal skin. Here we hypothesize, that the miRNA expression-profile in blood from patients with psoriasis may reflect chronic systemic disease with involvement of skin. Thus, the overall aim of this project is to identify specific biomarkers in blood from patients with psoriasis. We plan to identify miRNA and messenger RNA (mRNA) expression-profiles in the blood from patients with psoriasis and compare these profiles with profiles previously identified in psoriatic skin. Furthermore, we plan to examine biomarker profiles in relation to comorbidities and treatment response. The importance of relevant miRNAs and their potential target mRNAs will be examined and validated *in vitro* and *in vivo*. We hope that the identification of systemic biomarker profiles in patients with psoriasis will improve our disease understanding. Additionally, the use of miRNAs as biomarkers in this complex disease may lead to the identification of new potential targets for therapies.

#28 Kristina Maaetoft-Udsen

The immunomodulatory capacity of bacteria of marine origin

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Whole bacteria can stimulate the immune response in various ways. We live in symbiosis with an extremely high number of bacteria. Many factors determine whether bacteria can be beneficial or harmful to human health. These include timing, dosage and virulence factors of the bacterium.

In this study, we used bone marrow derived dendritic cells from mice to study the immune stimulating effects of a whole new array of microbes; bacteria of marine origin. In a time of continuous search for beneficial microorganism on human health e.g. towards chronic inflammation of the gut, we ask, if this unexploited resource of bacteria could show novel or improving functions on the immune system.

So far, it has been seen that, dependent on the strain of marine bacteria, it can induce different immune responses. We found that bacteria from both the *Ruegeria* and *Phaeobacter* genus were able to induce high IL-12 production in dendritic cells, indicating a directed T_{H1} response for the *Ruegeria* and *Phaeobacter*. Some bacteria from the *Pseudoalteromonas* genus were not able to induce an IL-12 response in dendritic cells. The cause of the low immune response induced by some *Pseudoalteromonas* is under investigation. Especially the high production of exopolysaccharides in these strains is considered to impact the immune response. Preliminary results indicate that the exopolysaccharides decrease the pro-inflammatory response otherwise induced by the lipopolysaccharides of these *Pseudoalteromonas* strains.

#29 Caroline B. Madsen

A MUC1-derived peptide elicits MUC1 specific antibodies and CD8 T cells in HLA-A2 transgenic mice

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Background: MUC1 is a glycoprotein highly overexpressed in the majority of human adenocarcinomas. MUC1 contains multiple tandem repeats carrying O-linked glycans, which are incompletely glycosylated in cancer resulting in presentation of short tumor-associated carbohydrate antigens. Several MUC1-derived peptides that map outside the tandem repeat domain and comply with the peptide-binding-motif for HLA-A*0201 is also known, one such peptide being DegMUC1. In the search for a vaccine, which induce both antibodies and cytotoxic T-cells we have investigated the humoral and cellular immune response to DegMUC1 with and without O-linked glycans.

Methods: Balb/c and HLA-A*0201 mice were immunized with peptides with and without glycosylation. Responses were monitored by flow cytometry, proliferation assay, and ELISA. The significance of glycosylation on peptide processing and MHC I presentation was monitored indirectly by SIINFEKL-DegMUC1 fusion peptide experiments.

Results: MUC1 specific IgG antibodies were elicited in Balb/c and HLA-A2 mice and DegMUC1 elicited cellular responses in both mouse strains. Glycosylation enhanced the response in Balb/c mice but blocked CD8-response in HLA-A2 mice. We assessed the significance of glycosylation on the uptake, presentation, and CD8 T-cell stimulation of MUC1-derived peptides *in vitro*, using DegMUC1- SIINFEKL fusion peptides. This demonstrated that O-glycosylation inhibits the processing and/or presentation of DegMUC1 peptide and thereby CD8 T-cell activation.

Conclusion: Glycosylation leads to enhanced cancer specific humoral immunresponse while blocking the CD8 T cell response to DegMUC1.

#30 Christian K. Mathiesen

Properties of HCVcc particles produced in serum-free medium

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Hepatitis C virus (HCV) currently infects ~180 million people worldwide. No vaccine exists, and the lack of appropriate cell culture systems, from which HCV particles could be purified has until recently prevented studies of the possibility of developing whole virus inactivated vaccine candidates. Recombinant HCV grown in cell culture (HCVcc) has been shown to consist of a heterogeneous population in regard to physiochemical properties as determined in density gradient analysis. Therefore, the purification of HCVcc from FBS derived components present in conventional DMEM has proven difficult using traditional methods such as ultracentrifugation. In an attempt to solve this problem, we harvested HCVcc from cells kept in a serum-free medium (AEM). The peak infectivity titers of the serum-free HCVcc (sf-HCVcc) particles could easily be determined and was similar to those of HCVcc. To further characterize sf-HCVcc we analyzed the density profile of the infectious particles and saw a shift from a heterogeneous HCVcc population to a single-peak homogenous sf-HCVcc population. The kinetics of this shift seemed to be genotype dependent, as HCVcc of a genotype 5a isolate shifted more rapidly than that of a genotype 2a isolate. We also tested the ability of sf-HCVcc to infect target cells with or without presence of FBS. Sf-HCVcc diluted in AEM was less infectious than HCVcc diluted in complete DMEM, at the same multiplicity of infection. Finally we tested the stability of sf-HCVcc. Storage of sf-HCVcc at 4°C induced a loss of infectivity of ~50%. This loss was not observed with HCVcc. Our results demonstrate that HCVcc associates with FBS components, and this association seems to be required for proper infectivity as well as stability of the virus particles. Understanding the physiochemical properties of sf-HCVcc may prove important for the production of a HCV antigen to be used in future vaccine candidates.

#31 Karen Nørgaard Nielsen

Perspectives on improvement of adenovectored DNA vaccines by MHC class II-associated invariant chain

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A central issue in vaccinating against viral infections is the induction of a CD8 T cell response. DNA vaccines are safe and have been shown to stimulate such responses; still the responses are inefficient and so far no clinical trial has demonstrated sufficient protection in humans. It has previously been established that linking invariant chain (Ii) to certain DNA vaccine antigens will improve adenovectored DNA vaccines in terms of duration, time of onset, breadth and CD8 T cell quality. However, this effect was not seen when Ii was linked to the highly potent NP gene of LCMV.

The first part of this study aims to investigate whether the benefits of linking Ii to the antigen is restricted to specific antigens, or if it will improve the quality of the NP vaccine under certain conditions such as reduced vaccine loads, prolonged incubation period, and lack of co stimulatory signals. Furthermore, the quality of the induced CD8 T cell response will be investigated in terms of differential cytokine production.

The second section of the project concerns vaccination with the two GP mutants, GPNoB and GPTCR, carrying a dominant epitope which cannot bind MHC class I and TCR, respectively. The aim of this part is to describe how Ii interferes with the T cell competition and cooperation by describing if Ii assists the subdominant epitopes in propagating an immune response sufficient for protection against subsequent viral infection.

Thus, this study aims to describe the possibilities and limitations of Ii-linking and will illustrate perspectives for a potential future vaccine for humans.

#32 Anja Østergren Nielsen

Interplay between monocytes and regulatory T cells in relation to interleukin-10 production

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An imbalance in cytokine homeostasis is involved in the pathogenesis of autoimmune diseases. The anti-inflammatory cytokine interleukin (IL)-10 plays a major role in down-regulating immune responses to self-antigens, and has been shown to delay or prevent the onset of autoimmune disease in experimental animal models. IL-10 is produced by inducible regulatory T cells (iTregs or Tr1) and B cells, but the major reservoir of IL-10 in the circulation is associated with monocytes.

Our previous work shows that the self-antigen thyroglobulin (TG) induces proliferation of normal CD4⁺ T-helper cells, and that the outcome of this stimulation is a relatively high production of IL-10, as compared to stimulation with foreign antigens. IL-10 was produced by a minor subset of memory CD4⁺CD4R0⁺ T cells and by the bulk of monocytes. However, depletion of T cells as well as of monocytes abrogated the IL-10 production, suggesting that it involves interplay between the two cell types. We therefore hypothesize that a positive feedback system exists, in which IL-10 production by iTregs regulates the release of larger quantities of IL-10 by monocytes. Moreover, IL-10 may activate the IL-10 receptor on monocytes in an autocrine manner, creating a positive feed-back loop.

The interaction between iTregs and monocytes in relation to IL-10 production induced by self-antigens will be studied by antibody-mediated blockade of the IL-10 receptor on either cell type, knock-down of IL-10 production by either cell type using siRNA, and by addition of exogenous IL-10 to cultures of purified monocytes or T cells. Moreover, antibodies blocking MHC II will be used to determine whether inter-cellular IL-10 signalling is associated with antigen-presentation. Production and secretion of IL-10 will be assessed by intracellular staining and Luminex technology, respectively.

Advanced understanding of the role of IL-10 in autoimmune diseases and how to regulate its production may have therapeutic implications.

#33 Kajsa M. Paulsson

Tapasin independently discriminates immunogenic from non-immunogenic peptides

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Tapasin binds and integrates the MHC-I molecule into the peptide-loading complex (PLC) where it exerts quality control of the peptide – MHC-I complex and retains it in the ER until an optimal peptide is bound to the MHC-I. The exact mechanism by which tapasin exerts this quality control is still unknown. Here, we show that the first 87 residues of tapasin, Tpn₁₋₈₇ facilitates folding of peptide – MHC-I complexes both in a peptide and HLA-I molecule dependent manner. Tpn₁₋₈₇ facilitates the folding of peptide – MHC-I complexes with HLA-A*02:01, HLA-B*08:01 and HLA-B*44:02 but not the tapasin-independent HLA-B*27:05 or the HLA-A*02:01-T134K mutant molecule. The stabilities of several peptide – HLA-A*02:01 complexes were measured and the degree of Tpn₁₋₈₇ facilitation of peptide – HLA-A*02:01 complexes inversely correlated with their intrinsic stabilities. The stability of peptide – HLA-A*02:01 complexes loaded with immunogenic peptides were higher than for those loaded with non-immunogenic peptides. Hence, Tpn₁₋₈₇ can be used to discriminate immunogenic from non-immunogenic peptides.

#34 Annika H. Rasmussen

Identification of novel minor histocompatibility antigens

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Minor histocompatibility antigens (mHags) were originally identified as antigens causing graft rejection or graft-versus-host disease in otherwise HLA-matched recipients following allogeneic hematopoietic cell transplantation (HCT) due to leukaemia or other haematological malignancies.

mHags are allelic variants of non-MHC genes that leads to protein polymorphism and can be either non-synonymous single nucleotide polymorphisms or HY-antigens which are disparities between male vs. female specific variants of homologous genes (Y/X-polymorphism).

In connection with HCT for the treatment of leukaemia, mHags could be utilized as targets for immunotherapy if T cells specific for mHags solely expressed in hematopoietic cells could be generated, expanded and adoptively transferred to the patient as a donor lymphocyte infusion leading to a highly desirable graft-versus-leukaemia effect eradicating any residual leukemic cells in the patient.

Having a large panel of mHags to choose from when selecting donors for a given recipient should allow for donor selections leading to better anti-tumor effects and reduced side-effects, however, at this time only very few minors are known.

In order to identify novel mHags approximately 200 HCT donor/recipient pairs have been analyzed with regards to their mHag disparities. MHC class I binding peptide epitopes from the differentially expressed proteins have been predicted using a predicting algorithm available at <http://www.cbs.dtu.dk/services/>.

mHags with predicted MHC class I binding have been synthesized as peptides with a length of 8-11 amino acids. The immunogenicity of these peptide epitopes will be examined in vitro in T cell cultures and the binding of the epitope to MHC class I molecules will be validated in MHC-peptide binding assays.

So far, T cells from 8 female-donor/male-recipients obtained from blood samples drawn 3 months to 8 years post transplant have been stimulated with their putative mHag epitopes in T-cell stimulation assays and analysed for T cell reactivity by flow cytometry using intracellular cytokine staining.

#35 Kirsten Reichwald

Regulation of CD4 T lymphocyte activity during autoimmune inflammation

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Background

Around 20% of people in the western part of the world suffer from diseases connected to autoimmunity. While the cause of this is still elusive, it is clear that autoimmunity is associated with loss of immunological tolerance, which is the ability of an individual to ignore 'self' and still react to 'non-self'. This breakage leads to an effective and specific immune response against self determinants.

Blocking of inflammatory cytokines, primarily TNF-alpha, has recently nearly revolutionized the treatment of e.g. rheumatoid arthritis. Moreover, recent advances in blocking of cytokine production driving differentiation of CD4 T cells into autoimmune Th17 effector cells such as IL-23/IL-12 p40 mAb have an impressive effect in the treatment of arthritis and psoriasis. Thus affecting the culprit of the autoimmune inflammation, the controlling CD4 T lymphocytes, is likely an even more effective and specific treatment rationale for autoimmune disorders.

Patients with rheumatoid arthritis (RA) are characterized by an unusual population of CD4+ CD28-NKG2D+ lymphocytes. Several studies have now shown that autoreactive lymphocytes bear resemblance with senescent lymphocytes, that down-regulate costimulatory CD28, concomitant with up-regulating of costimulatory molecules with a more broad reactivity range. An emerging hypothesis is that autoimmunity, which normally occurs later in life, is significantly influenced by the broad activation potential of senescent cells.

Objectives

The objective of my study is to characterize surface molecules that are critical for autoimmune activation of CD4 T lymphocytes during RA or psoriatic arthritis, including examination of the mechanism that leads their increased surface expression. Currently, the project focuses on characterizing the involvement of the TNF-receptor ligand TL1A in RA inflammation, but it is the intention to screen for new factors involved in RA inflammation. Also, I will investigate how CD4 T lymphocytes are affected by stimulation through TL1A and other molecules, in terms of production of new cytokines, surface molecules and regulation of growth and apoptosis.

Finally, I will investigate if blocking TL1A or other identified receptor / ligand interactions will affect experimental models of autoimmunity, primarily collagen-induced arthritis in DBA/1 mice. This part will be conducted in collaboration with section for animal models at Leo Pharma.

#36 Troels K. H. Scheel

Development of infectious consensus cDNA clones of HCV genotype 3A and 4A: pathogenesis studies in the chimpanzee model

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Previously, RNA transcripts of cDNA clones of hepatitis C virus (HCV) genotypes 1 and 2 were found to be infectious in chimpanzees. However, only the genotype 2a strain JFH1 was infectious in human hepatoma Huh7 cells. We performed genetic analysis of HCV genotype 3a and 4a prototype strains, and generated full-length consensus cDNA clones. Transfection of Huh7.5 cells with RNA transcripts of these clones did not yield HCV Core expressing cells. However, intrahepatic transfection of chimpanzees resulted in robust infection with peak HCV RNA titers of ~5.5 log₁₀ IU/mL. Genomic consensus sequences recovered from serum at time points of peak viral titers were identical to the parental plasmids. Both chimpanzees developed acute hepatitis with elevated liver enzymes and significant necro-inflammatory liver changes coinciding with detection of interferon- γ secreting, intrahepatic T cells. However, onset and broadness of intrahepatic T-cell responses varied greatly in the two animals, with an early (week 4) multi-specific response in the 4a infected animal (three weeks before first evidence of viral control), and a late (week 11) response with limited breadth in the 3a infected animal (without evidence of viral control). Autologous serum neutralizing antibodies were not detected during the acute infection in either animal. Both animals became persistently infected. In conclusion, we generated fully functional infectious cDNA clones of HCV genotypes 3a and 4a. Proof of functionality of all genes might further the development of recombinant cell culture systems for these important genotypes.

#37 Esben Schmidt

Enteroantigen-presenting B cells efficiently stimulate CD4⁺ T cells *in vitro*

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Background: Presentation of enterobacterial antigens by antigen presenting cells and activation of enteroantigen-specific CD4⁺ T cells are considered crucial steps in IBD pathology. The detrimental effects of such CD4⁺ T cells have been thoroughly demonstrated in models of colitis. Also, we have previously established an *in vitro* assay where murine enteroantigen-specific colitogenic CD4⁺CD25⁻ T cells are activated by splenocytes pulsed with an enterobacterial extract.

Methods: CD4⁺CD25⁻ T cells were stimulated *in vitro* with various kinds of enterobacterial extract-pulsed antigen presenting cells. T helper phenotypes were detected by flow cytometry.

Results: We found that enteroantigen-pulsed splenic B cells possess a significantly higher and more sustained T cell stimulatory capacity than similarly pulsed splenic dendritic cells (DCs) measured by the level of enteroantigen-specific CD4⁺CD25⁻ T cell proliferation. In support of this, we observed upregulation of classic maturation markers in B cells following incubation with enterobacterial antigens. Peritoneal and mesenteric lymph node-derived B cells were equally effective as enteroantigen-presenting stimulator cells. B cells greatly expanded the number of stimulated CD4⁺ T cells, which acquired a T_H2 phenotype. Interestingly, regulatory T cells were primarily activated by enteroantigen-pulsed B cells but not by similarly pulsed DCs.

Conclusions: We conclude that B cells are superior stimulators of enteroantigen-specific CD4⁺ T cells *in vitro*, favoring T_H2 polarization. Thus, enteroantigen-processing and -presentation by B cells instead of by DCs might have opposing consequences for IBD development.

#38 Maria Abildgaard Steffensen

Treatment of cancer by adenovirus based prime-boost regimes

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Treatment of cancer by vaccination is not a new idea, but we are still far from having vaccines that effectively eliminate established tumours.

At present time, our laboratory has successfully prolonged the life of tumour-bearing animals by vaccination with an adenovirus vector (Ad5) encoding an exogenous tumour-associated antigen. However, the immune response wanes over time, and eventually the tumour starts growing out again. It is therefore relevant to investigate whether the tumour-specific immune response can be sustained through repeated immunizations.

In our lab, we are using an optimized adenoviral vaccine system with a vector (Ad5) encoding the glycoprotein of LCMV tethered to the invariant chain (IiGP). Following vaccination with this vector, we initially see an inhibitory effect on the ability of the vector to re-induce an immune response upon a second immunization. However, 6 months after vaccination, the inhibition is markedly reduced, and it is again possible to induce an immune response against the antigen using the same vector.

The short term inhibition can be transferred by serum and is absent in B cell deficient mice, indicating that neutralizing antibodies are the main mediators of this effect.

This result was also supported by neutralization assays showing 50% neutralization of virus particles at a serum dilution of 1:80.

To overcome this immunity, we have inserted IiGP into the Ad35 vector, which belongs to another subgroup of adenoviruses and therefore, is not neutralized by antibodies directed towards the Ad5 vector. At present time, the Ad35 vector has been tested in mice with a positive result, and prime-boost regimes using the Ad35 and Ad5 vectors have been performed, resulting in an effective boosting of the immune response.

Homologous and heterologous prime-boost regimes are being tested in tumour-bearing animals to evaluate their ability to induce an effective and long lasting immune response against a growing tumour. To this end, we also intend to test these regimes in CD46 transgenic mice, since CD46 is a receptor for Ad35 that is expressed on all nucleated cells in humans, but not in mice. Furthermore, CD46 expression is up-regulated on human tumours making Ad35 very attractive for tumour therapy.

#39 Maria Rathmann Sørensen

Development of a one-step triplex Q-PCR method for quantification of melanoma cells in lungs as a model for metastasis detection

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Background: The B16.F10 melanoma model is a well described murine model for human cancer, which is widely used in the research of tumor immunology and in the testing of a variety of anti-cancer vaccine strategies. The B16.F10 cell line can be used in a solid tumor model, in which the B16 cells are injected subcutaneously leading to the formation of a solid tumor. The growth of the tumor can then be measured using a dial caliper. Another way to use this cell line is by intravenous administration of the B16.F10 cells, leading to the formation of lung metastases, as the B16.F10 cell line has been selected for invasion of the lungs. Metastases can then be counted after several days, depending on the initial number of B16.F10 cells injected.

The aim of this project is to develop a sensitive and exact method for detection of the B16 melanoma cells, e.g. in lungs or tumor draining lymph nodes, using quantitative PCR. Therefore, primers and probes recognizing the melanoma specific tyrosinase-related protein 2 (TRP-2), GP100 (Pmel), and the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA, were designed. The probes are attached to different dyes, allowing for detection of all the genes in one sample. To further optimize handling of the detection method, we use One-step Q-PCR, in which the cDNA synthesis step is included in the Q-PCR reaction, and therefore, each sample only has to be handled once after RNA extraction.

This method is a new, very sensitive and easy-to-handle way of measuring metastasis in various tissues after challenge with B16 cells, and hopefully, this will optimize testing of new anti-cancer drugs and vaccine candidates.

#40 Hilde Hylland Uhlving

Dendritic cells and regulatory T-cells after allogeneic stem cell transplantation

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Background

Haematopoietic stem cell transplantation (HSCT) is a treatment used for high-risk leukaemia. The biological effect is based on a T- cell mediated attack on leukaemia cells (“graft-versus-leukaemia effect (GvL)). However, the beneficial effect is limited by significant morbidity and mortality caused by the graft-versus-host disease (GvHD).

In GvHD, and presumably GvL, *donor* T-cells are activated by host-antigens presented by dendritic cells, a process antagonised by FoxP3 positive regulatory T-cells. However, it is at present unknown to what degree effective antigen presentation of allo-antigens is dependent upon residual dendritic cells of recipient origin – a subset of cells that is thought to decrease with time after HSCT.

The aim of our study is to characterize the level of donor- and recipient derived dendritic cells and FoxP3 regulatory T-cells in tissue biopsies from SCT-patients with and without GvHD.

Increased knowledge of dendritic cell chimerism in relation to development of GVHD and GvL can potentially open doors to more specific GVHD-treatment, and be a valuable tool in assessment of cellular therapy as a treatment option in patients with relapse of leukaemia after HSCT.

Methods

Biopsies from skin, gut, liver and lungs from female SCT- patients with male donors will be studied. The number of dendritic cells will be assessed by staining for CD1a, CD11c and S-100, and the fraction of donor derived cells will be assayed by hybridizing these specimens with Y chromosome probes. Reactions will be detected by fluorochrom- (FISH) or chromogen- (CISH) based detection systems.

#41 Marie Torp Vennegaard

In vivo analysis of Cdc42 functions in T cells

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Background: Rho GTPases are small GTPases that regulate cytoskeleton organization, proliferation, survival and cell polarization. They are present in an active GTP-bound form and an inactive GDP-bound form. Only in the GTP-bound conformation they can interact with a wide range of effector molecules including serine-threonine kinases, lipid kinases and cytoskeleton proteins. The Rho GTPase Cdc42 is believed to be of major importance for the polarity of T cells and for the development of the immunological synapse between T cells and antigen-presenting cells. If correct Cdc42 would be of outmost importance in the development of T cells as well as in the signalling through mature TCR complexes. However, due to the essential role of Cdc42 in gastrulation, total knockout mice are non-viable, which has limited direct studies of Cdc42 function in the hematopoietic system. Using mice with conditional targeted disruptions of the Cdc42 gene in T cells, we demonstrate that Cdc42 function in different events during T cell development.

Methods: In this study a T cell specific deletion of Cdc42 was applied using a Cre-loxP-mediated inducible system. Mice carrying the Cdc42 gene flanked by loxP sites were crossed with mice expressing the Cre recombinase under control of the Ick promoter. Sufficient knockout efficiency in T cells was verified by qPCR. Cells from thymus, spleen and lymph nodes were analysed by flow cytometry.

Results: Loss of Cdc42 resulted in reduced numbers of both CD4 and CD8 single positive cells in the thymus. A considerable decrease of CD3, TCR β , CD69 and CD5 expression was observed in the CD8 T cells in the thymus, while only a small reduction in expression of these maturation and activation markers was seen in the CD4 T cells. This suggested that loss of Cdc42 lowered TCR signals in CD8 T cells. Consistent with the hypothesis of altered TCR signalling in CD8 thymocytes a higher percentage of cells with surface characteristics of activated effector and memory T cells was observed in CD8 cells present in spleen and lymph nodes.

Conclusion: Together these findings strongly suggested a role for Cdc42 in the TCR signalling of CD8 T cells, and to a lesser extent CD4 T cells.

#42 Gudrun Weiss

***L. acidophilus* induces IFN- β production in a TLR-2 dependent manner in murine bone marrow derived dendritic cells**

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Certain lactobacilli species have been shown to trigger a strong induction of the pro-inflammatory cytokine IL-12 upon stimulation of dendritic cells (DC). We hypothesized that the strong IL-12 inducing capacity of *L. acidophilus* NCFM in murine bone marrow derived DC is, at least partly, caused by an up-regulation of IFN- β , a key inducer of a strong adaptive immune response against virus, which subsequently would stimulate the induction of IL-12 and the dsRNA binding toll like receptor (TLR)-3. Gene expression analysis in DC revealed that *L. acidophilus* NCFM induced a much stronger expression of the genes encoding IFN- β , IL-12 and IL-10 compared to the synthetic dsRNA ligand Poly I:C, whereas the levels of expressed *tlr-3* were similar. By the use of whole genome microarray gene expression, we investigated whether other genes related to the viral defence were up-regulated in DC upon stimulation with *L. acidophilus* NCFM. We found that multiple virus defence related genes, both early and late, were among the strongest up-regulated genes. The IFN- β stimulating capability was also detected in another *L. acidophilus* strain, but was not a property of other probiotic bacteria tested (*B. bifidum* and *E. coli* nissle). The IFN- β inducing capacity was markedly reduced in TLR-2^{-/-} DCs, dependent on endocytosis, and the major cause of the induction of *tlr-3*, but not *Il-12*, in *L. acidophilus* NCFM stimulated cells. Collectively, our results reveal that certain lactobacilli trigger the expression of viral defence genes in DC in a TLR-2 manner through induction of IFN- β .

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#43 Massimo Andreatta

Using peptide microarrays for antibody epitope discovery and profiling of protease specificities

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Peptide arrays allow implementing high-throughput screening assays for several biological processes involving peptide-protein or protein-protein interactions, such as enzyme/substrate, kinase/phosphosite, protease/polypeptide and antibody/antigen interactions. We have tested and analyzed the data produced by a novel peptide array technology in three selected contexts: binding of antibodies raised against the FLAG tag, and proteolytic cleavage of the model proteases trypsin and chymotrypsin.

Bioinformatics techniques were employed to recognize the sequence motifs regulating the processes under study. These included a neural network-based data driven method, which can identify motifs in quantitative peptide binding data and use the learned patterns to predict the binding affinity of new peptides; a different approach aimed at finding n-mers within peptide sequences that appeared to consistently yield high signal intensities on the array.

The binding preferences of the FLAG antibody reflected the strong affinity for the Tyr-Lys contained in the FLAG tag, and also showed weaker binding to other epitopes. In the same way, the main rules for trypsin and chymotrypsin cleavage specificities were confirmed, but also a few yet unobserved patterns emerged from the analysis.

#44 Edita Bartaseviciute

***In silico* prediction of T cell receptor specificity**

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A key role in the cellular immunity is dedicated to T cells, which by using their unique T cell receptors (TCRs) recognise epitopes bound to major histocompatibility complex (MHC) molecules. The immune response is triggered by TCR binding to the peptide/MHC complex and depends on strength and duration of this interaction. Understanding of molecular basis of TCR/peptide/MHC interactions is very important for immune therapy and vaccine design. It is a highly labour and cost intensive procedure to perform direct experimental screening of TCR/peptide/MHC interactions and the development of computational methods capable of guiding this procedure is hence essential. *In silico* analysis of T cell receptor binding properties was made and contributed in development of prediction method for T cell epitopes. The method is based on predicting binding energy between TCRs and peptides, using contact information obtained from known 3D structures of TCR/peptide/MHC complexes, and predicting peptide binding affinity to MHC molecules using advanced bioinformatics methods. Benchmarking revealed that the method performs significantly better than random predictions and is capable of ranking native peptides in the top 1.13% of other natural peptides. Having a list of peptides and a TCR protein sequence, the presented approach can be used to select TCR binders as potential epitopes worth checking experimentally.

#45 Stina Rikke Jensen

Quantification of specific *E. coli* bacteria adhering to gut epithelium in Crohn's disease

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Adherent-invasive *Escherichia coli* (AIEC) comprise a new group of *E. coli* species named from their distinctive ability to adhere to and invade the intestinal epithelium. The AIEC strains have been associated with the ileal mucosa in Crohn's disease (CD), and the impact of AIEC in the pathogenesis of CD has been further strengthened from the evidence that the ileum in CD harbors an abnormally high number of *E. coli* species.

The aim of this study was to implement a quantitative method based on qRT-PCR to determine the number of intestinal tissue-adhering LF82, the prototype strain of AIEC, in a sample matrix of prokaryotic and eukaryotic DNA.

Ileal tissue from two CD patients was used to study the effect of eukaryotic DNA on assay stability. Standard curves were prepared from tissue cut into 2 mg, 4 mg and 8 mg pieces and known quantities of LF82 ranging from 10^2 to 10^7 CFU. Two different TaqMan primer-probe sets were designed (GFP and pMT1), and evaluated for their specificity to GFP-transformed LF82. A third primer set was evaluated for targeting total *E. coli*.

The GFP and pMT1 primer-probe sets showed equal specificity for LF82, resulting in a detection range from $10^3/10^4$ to 10^7 CFU, and the ability to discern between 2-fold differences in bacterial counts. The coefficients of variations (%CV) between and within runs were below 2%. Interference from eukaryotic tissue DNA on assay performance was negligible. Weighing of biopsies was found appropriate to adjust for variations in tissue sizes as compared to 18S rDNA quantification or nucleic acid content.

In conclusion, a high assay robustness and specificity for detection of LF82 with low variations was demonstrated. Thus, quantification of LF82 in intestinal biopsies is feasible using a standard curve generated from known quantities of LF82, and adjustment for differences in tissue sizes by weight measurement. This method may be of importance for future studies aiming to quantify specific microbes in a complex matrix of eukaryotic DNA.

#46 Leon Jessen

Identifying immunogenic motifs in the DBL5 ϵ domain of the pregnancy associated malaria protein VAR2CSA by *in silico* pheno-genotype correlation

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Each year 10,000 women and 200,000 infants die from pregnancy-associated malaria (PAM). PAM immunity is antibody mediated and gradually obtained with multiple pregnancies. The PAM associated protein VAR2CSA is surface expressed on infected erythrocytes (IE) and mediates infection by placental adhesion. VAR2CSA thus plays a key-role in PAM pathogenesis and has been shown to induce protective antibodies. VAR2CSA is therefore considered a potential candidate for vaccine development and it is thus imperative to identify immunogenic regions. The overall aim of this study is to supply further knowledge, by single domain immunogenicity analysis. Thereby aiding the development of a broadly protecting vaccine.

The data consisted of 70 DBL5 ϵ -subdomain sequences from placental isolates from 39 *Plasmodium falciparum* infected pregnant Senegalese women. Each sequence was associated with an array of experimentally determined numerical parameters. The approach was to analyse sequence variation by correlating the genotype of each analysed sequence, with the phenotype, i.e. the associated numerical parameters. This was done by applying statistical evaluations of the distribution of each residue at each position in a protein multiple alignment of sequences ranked by each numerical parameter.

Using this approach, primi-/multigravidae motifs were indeed identified (TFKNI/deletion respectively). As the motif is parity dependent and immunity is gradually acquired with parity, the motif could be directly or indirectly involved in immunogenicity. Further experimental evaluation is proposed.

The overall aim of my PhD programme is to develop and apply *in silico* methods and tools for analysing and predicting the correlation between pheno- and genotype and its involvement in immunogenicity.

#47 Marie Kragh

Exploring the bacterial airway colonization in Danish infants of mothers with atopy

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Asthma and wheezy disorders are very common chronic diseases in children in the Western world. The diseases are heterogeneous with little to differentiate their clinical presentation, causing inadequate management and prevention. Still, our understanding of specific asthma endophenotypes is limited. In order to improve prevention, diagnosis and treatment, an increased understanding of both environmental and genetic etiologies, their interaction, and the host mechanisms involved is needed.

Our collaborators on this PhD study found in their cohort study of 411 children of asthmatic mothers (the Copenhagen Prospective Study on Asthma in Childhood (COPSAC₂₀₀₀)), that the presence of certain bacteria in the upper respiratory tract may increase the risk of asthma development. Consequently, we propose that colonization of the upper airway with certain pathogenic bacteria early in life may result in a bacteria-induced asthma endophenotype. Still, however, little is known about the diversity of bacteria in the upper airway of young children, and how certain bacteria may prime to the deregulated lung immune response leading to asthma development.

The aim of this PhD study is to investigate the nasopharyngeal microbiota from neonates enrolled in COPSAC₂₀₀₀. This is accomplished by analyzing bacterial DNA fingerprints using explorative data analysis. The obtained results are correlated with available clinical, environmental, genetic and immunological data from the children in order to reveal specific factors that influence the diversity and composition of the bacterial community of airways and promote or protect against development of atopic diseases in early childhood.

#48 Jeppe Madura Larsen

Asthma pathogenesis: Do bacteria play a role?

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Asthma is a highly complex disease that may arise from different etiology. Recent studies have indicated that bacteria may play a role in the pathogenesis of at least one particular asthma endotype. Colonization with certain pathogenic bacteria in pre-symptomatic airways of children has been associated with later asthma development. Furthermore, the airways of asthma patients have been found to be associated with a particular bacteria colonization pattern compared to lungs of healthy individuals. Contrarily, healthy lungs were found to be associated with the colonization of non-pathogenic commensal bacteria found less frequently in asthmatics. These findings suggest a divergent role of different bacteria in the development of asthma. Our studies address the physiological role of asthma-associated and lung commensal bacteria in pulmonary immune responses.

#49 Bent Petersen

NetTurnP – neural network prediction of beta-turns by use of evolutionary information and predicted protein sequence features

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β -turns are the most common type of non-repetitive structures, and constitute on average 25% of the amino acids in a protein. The formation of β -turns plays an important role in protein folding, protein stability and molecular recognition processes.

β -turns are often accessible and generally hydrophilic, two characteristics of antigenic regions. For this reason they are suitable candidates for being involved in molecular recognition processes. Pellequer et al. found that 50% of the linear B-cell epitopes in a small dataset of 11 proteins were located in turn regions. Thus prediction of β -turns could improve the prediction of epitopes. Krchnak et al. found that the parts of a protein, which can induce protein-reactive anti-peptide anti-bodies, mostly reside in regions that have a high tendency to form β -turns. A more recent article by the same authors showed that peptide sequences including a β -turn conformation tended to induce antibodies that were able to cross-react with the parent protein. β -turn and coil conformations have also previously been used to predict linear epitopes. Furthermore, β -turn types I and II, are important for binding between phospho-peptides and SH2-domains.

In the present work, neural network methods have been trained to predict β -turn or not and individual β -turn types from the primary amino acid sequence, by use of evolutionary information and predicted protein sequence features. The individual β -turn types I, I', II, II', VIII, VIa1, VIa2, VIba and IV have been predicted based on classifications by PROMOTIF, and the two-class prediction of β -turn or not is a superset comprised of all β -turn types. The performance is evaluated using a golden set of non-homologous sequences known as BT426. Our two-class prediction method achieves a performance of: MCC = 0.50, $Q_{\text{total}} = 82.1\%$, sensitivity = 75.6%, PPV = 68.8% and AUC = 0.864. We have compared our performance to eleven other prediction methods, that obtain Matthews correlation coefficients in the range of 0.17 – 0.47. For the type specific β -turn predictions, only type I and II can be predicted with reasonable Matthews correlation coefficients, where we obtain performance values of 0.36 and 0.31, respectively. NetTurnP achieves the highest reported Matthews correlation coefficient of 0.50, and furthermore shows improved performance on some of the specific β -turn types.

Conclusion: A method for the general and specific prediction of β -turns has been developed and implemented as a webserver, which is freely available at <http://www.cbs.dtu.dk/services/NetTurnP/>. NetTurnP is the only available webserver that allows submission of multiple sequences. It combines predictions from relative surface accessibility, secondary structure, β -turn/non- β -turn and position-specific β -turns into an ensemble of the best performing neural networks. The presented method shows the highest reported correlation coefficient of 0.50 for the general prediction of β -turns. For the type specific β -turn predictions, we show some improvement compared to other methods, but in general these are still of poor quality even for the best machine learning methods. We obtain the highest correlation coefficient of 0.36 for type I β -turn predictions.

#50 Kerstin Skovgaard

Expression of coding (mRNA) and non-coding (microRNA) RNA in lung tissue and blood isolated from pigs suffering from bacterial pleuropneumonia

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MicroRNAs are small non-coding RNA molecules (18-23 nt), that regulate the activity of other genes at the post-transcriptional level. Recently it has become evident that microRNA plays an important role in modulating and fine tuning innate and adaptive immune responses. Still, little is known about the impact of microRNAs in the development and pathogenesis of lung infections.

Expression of microRNA known to be induced by bacterial (i.e., LPS) ligands and thus supposed to play a role in the regulation of antimicrobial defence, were studied in lung tissue and in blood from pigs experimentally infected with *Actinobacillus pleuropneumoniae* (AP). Expression differences of mRNA and microRNA were quantified at different time points (6h, 12h, 24h, 48h PI) using reverse transcription quantitative real-time PCR (Rotor-Gene and Fluidigm). Expression profiles of miRNA in blood of seven animals were further studied using miRCURY™ LNA arrays (Exiqon, Denmark).

All AP infected animals had significantly higher levels of mRNA coding for inflammatory mediators as IL-6 and IL-8 as well as the acute phase protein SAA, in the lung compared to the control group.

MiR-223 was found to be highly up regulated, followed by miR-146a and to a lesser degree miR-155 in lung tissue of the AP infected animals. MiR-233 was also found to be up regulated in blood based on both microarray and real-time PCR. Mir-233 has been found to be a negative regulator of neutrophil proliferation and activation, and might act to limit the potentially harmful consequences of the accumulation of infiltrating neutrophils in AP infected lungs.

#51 Thomas Stranzl

***NetCTLpan*: pan-specific MHC class I pathway epitope predictions**

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Reliable predictions of immunogenic peptides are essential in rational vaccine design and can minimize the experimental effort needed to identify epitopes. In this work, we describe a pan-specific major histocompatibility complex (MHC) class I epitope predictor, *NetCTLpan*. The method integrates predictions of proteasomal cleavage, transporter associated with antigen processing (TAP) transport efficiency, and MHC class I binding affinity into a MHC class I pathway likelihood score and is an improved and extended version of *NetCTL*. The *NetCTLpan* method performs predictions for all MHC class I molecules with known protein sequence and allows predictions for 8-, 9-, 10-, and 11-mer peptides. In order to meet the need for a low false positive rate, the method is optimized to achieve high specificity. The method was trained and validated on large datasets of experimentally identified MHC class I ligands and cytotoxic T lymphocyte (CTL) epitopes. It has been reported that MHC molecules are differentially dependent on TAP transport and proteasomal cleavage. Here, we did not find any consistent signs of such MHC dependencies, and the *NetCTLpan* method is implemented with fixed weights for proteasomal cleavage and TAP transport for all MHC molecules. The predictive performance of the *NetCTLpan* method was shown to outperform other state-of-the-art CTL epitope prediction methods. Our results further confirm the importance of using full-type human leukocyte antigen restriction information when identifying MHC class I epitopes. Using the *NetCTLpan* method, the experimental effort to identify 90% of new epitopes can be reduced by 15% and 40%, respectively, when compared to the *NetMHCpan* and *NetCTL* methods. The method and benchmark datasets are available at <http://www.cbs.dtu.dk/services/NetCTLpan/>.

#52 Jonas N. Søndergaard

HIV-induced TLR-CLR crosstalk in human plasmacytoid dendritic cells

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Plasmacytoid dendritic cells (pDCs) have been associated with both beneficial and detrimental effects during an HIV-infection. Little is presently known about the intracellular pathways in pDCs that mediate these processes, but evidence suggest that C-type lectin receptor (CLR) crosstalk with Toll-like receptors (TLR) may play an important role. The present objective was to study the CLR-TLR crosstalk that is induced in human pDCs upon challenge with HIV-derived TLR and CLR ligands. We tested three different recombinant HIV-1 gp120s: IIIB (CHO-derived), 96ZM651 (Sf9-derived) and BaL (HEK-293-derived) which mediated diverse modulation of TLR7-induced IFN-alpha-2a and CD86 expression in pDCs. These differences may possibly be explained by different posttranslational glycosylations in the different gp120-expression systems. The three gp120s all lead to inhibition of TLR7-mediated CD40-upregulation and increased CCR7-upregulation. The modulating effects of gp120 were concentration-dependent and no significant modulations were obtained below concentrations of 100 µg/mL. Finally we have found that TLR7-activated pDCs express the mannose receptor (CD206), which may play an important role in the observed CLR-TLR crosstalk.

#53 Rikke Brandt Sørensen

Viable *S. typhimurium* distinctly modulate the phenotype of human dendritic cells as compared to LPS and non-replicating bacteria

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Salmonella typhimurium (ST) is a facultative intracellular anaerobe which upon colonization of the intestine is able to penetrate the intestinal epithelia and replicate both in phagocytes and non-professional antigen-presenting cells. Dendritic cells (DCs) play an important role in innate immunity against *Salmonella*, but are also believed to play an important role in *Salmonella* invasion and dissemination by acting as vehicles for the bacteria. As the bacteria-induced DC phenotype is of great importance for the ensuing T cell response, we studied the effect of viable *S. typhimurium* (ST) on various phenotypic markers in human monocyte-derived dendritic cells (moDC), and compared to the response induced by UV-inactivated, and heat-inactivated ST, as well as ST LPS. We found that the phenotype induced by viable bacteria differed significantly from that induced by inactivated bacteria, the latter resembling a LPS-induced response pattern. Compared to moDCs stimulated with ST LPS and inactivated ST, viable ST down-regulated the expression of CD40 and the mannose receptor, but did not alter the display of DC-SIGN, HLA-DR, CD86, TLR2 or TLR5. Even though secretion of both IL-10 and IL-12p70 was induced by viable ST, the IL-10/IL-12p70 ratio was highly skewed towards IL-10 production (IL-10/IL-12p70-ratio = 70), suggesting that the microenvironment favor pathogenic dissemination and not pathogenic clearance. Furthermore, when studying the effect of different bacteria-to-DC ratios (multiplicity of infection; MOI), we found that the ST-induced modulation in moDCs was dynamic and varying with MOI. Conclusively, the present results emphasize that viable *Salmonella* induces a distinct phenotypic change in moDCs that depend on the number of infecting bacteria, and diverges from that induced by non-replicating *Salmonella* bacteria.

#54 Aneesh Thakur

Possible effect of age at immunization and quality of immune response following MAP subunit vaccination in calves

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Mycobacterium avium subsp. *paratuberculosis* (MAP), the agent of Johne's disease, causes systemic infection and chronic intestinal inflammation in many animal species. Neonates are more susceptible to the infection due to high degree of exposure from their dams and possibly less developed immune system. The only vaccines available against MAP have been limited to veterinary use and comprised attenuated or killed organisms. However, none of these vaccines are able to completely prevent infection or shedding of bacteria. Considering the fact that MAP infection occurs at a young age and the animal remains in the subclinical phase for 2-3 years, an effective vaccine should not only elicit strong immune response in young animals, but also a quality of the T-cell response that correlates with long term protection. Here we report the possible effect of age at immunization and quality of immune response following immunization of calves with recombinant MAP proteins formulated with DDA/TDB (CAF01) adjuvant. Vaccine comprised of one heat shock protein, two ESAT-6 family members and two secreted mycobacterial components fused with CAF01 adjuvant. A total of 27 male jersey calves were divided into 3 groups of 9 calves each with first vaccination at 2, 8 and 16 weeks of age, respectively. Vaccine induced immune response, mainly the Th1 type cytokine secretion, was evaluated in different age groups following booster doses at equal time intervals. Preliminary results show higher antigen specific IFN- γ levels in response to heat shock protein and ESAT-6 family member protein antigens. However, it was observed that there was no effect of age on the IFN- γ producing capacity of the animals in the different age groups after stimulation of whole blood with SEB. As the vaccine trial has recently completed results will be compiled and presented. Findings from this work could be interesting to determine the appropriate age of vaccination so as to generate the memory T cell pool and for MAP vaccine challenge experiments.

#55 Anna Hammerich Thysen

Immunological mediators of disease initiation and progression in novel childhood asthma endotypes: Mechanistic basis for early preventive strategies

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Asthma, wheezy disorders, and other atopic diseases are the most common chronic diseases among children and represent a heterogeneous group with little to differentiate clinical presentation into defined disease endotypes. Atopic disorders arise from an aberrant immune system likely triggered by the complex interaction between individual genetic alterations and environmental factors encountered during pregnancy and early life. Thus, in depth understanding of the involved genetic, environmental, and immunological mechanisms is needed in order to improve prevention, diagnosis, and treatment.

The Copenhagen Prospective Studies on Asthma in Childhood (COPSAC2010) birth cohort is founded on an extensive clinical monitoring of children with focus on atopic diseases, environmental exposures and collection of samples for biological analysis. The purpose of the cohort is to investigate interactions between mother's diet during pregnancy, child genetics, and bacterial colonization of the airways relating to development of atopic diseases.

In the present PhD project, we aim to characterize immunological and lung mucosa-associated mechanisms within the cohort to elucidate environmental and genetic factors that shape aberrant immune responses in atopy. Innate immune responses in 18th month old children from the COPSAC2010 cohort study will be evaluated and correlated to the diet of the pregnant mother, the genetic profile of the child, and bacterial airway colonization in early life.

UNIVERSITY OF SOUTHERN DENMARK

#56 Lalit Kumar Dubey

Alkali insoluble cell wall fragments of *Aspergillus fumigatus* which induced allergic response in lungs of C57BL/6 mice is recognized by chitin binding type II receptor FIBCD1

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Purpose: *Aspergillus fumigatus* is a common opportunistic pathogen responsible for allergy and infection. The cell wall of fungus is a physically rigid layer, which protects fungal cells from environment. The major cell wall polysaccharide of *Aspergillus fumigatus* contains chitin covalently linked to β -1-3 glucan and galactomannan, which can initiate an innate immune response. The well-defined link of initial molecular recognition and immune response towards fungal chitin polysaccharides remain however unclear. Fungal cell wall polymers are classically divided in 2 groups depending on their solubility in alkali. The alkali insoluble fraction of *Aspergillus fumigatus* mainly contains the glucan-chitin complex.

Aim: To investigate the mucosal epithelial type II receptor FIBCD1 binding to chitin present on *Aspergillus fumigatus* and identify the possible role of FIBCD1 in modulation of the allergic immune response induced by fungal polysaccharides *in vivo* and *in vitro*.

Materials, Methods and Results: Using fluorescence microscopy we investigated the interaction of FIBCD1 to *Aspergillus fumigatus* (*afu*) and found that rFIBCD1 co-localized with chitin in the live organism. Later on the instillation of alkali insoluble fractions (AIF) mainly containing β -glucan and chitin was evaluated. Instillation of the AIF in mice lungs resulted in significantly increased granulocyte counts and total cell counts, increased chitinase activity and increased eosinophil peroxidase level in lung lavage when compared to mice receiving PBS. These results indicate a polysaccharide-induced recruitment of cells and production of chitinase, which may leads to Th2 immune response. We also stimulated Monocytes differentiating towards macrophages for 16 hr with AIF, and *afu* fragments and found that stimulation leads to increased expression of IL1- β , TNF- α and IL-8 measured by realtime-PCR.

Future perspective: Epithelial cells in the airways or the gut are often exposed to fungal polysaccharides by inhalation or ingestion and respond by increasing the expression of chitinase and chitin binding proteins. An immune response to chitin can develop in to Th1 or Th2 response depending upon the presence of other microbial components. FIBCD1 is a type 2 trans membrane protein preferentially present on epithelial lining which binds to *Aspergillus* chitin. The exact mechanism for recognition of chitin however remains unclear. We will now analyze the role of FIBCD1 in modulation of allergic immune responses induced by chitin, Alkali insoluble extract and by *afu in-vivo* by establishing an allergic model in wild type and FIBCD1 deficient mice. The identification of mechanisms involved in chitin induced modulation of innate and adaptive immune responses may provide novel insight into prevention of allergic airway inflammation.

#57 Mark A. Hammond

FIBCD1: A novel mammalian pattern recognition receptor for chitin

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Background: Fibrinogen C domain-containing protein 1 (FIBCD1) is a type II transmembrane protein located on the luminal side of intestinal epithelial cells. FIBCD1 binds specifically to acetylated compounds, such as chitin, through the C-terminal fibrinogen-related domain (FReD). Chitin is a highly acetylated homopolymeric β -1,4-N-acetylglucosamine carbohydrate found in organisms fungi and parasitic nematodes. We have shown that FIBCD1 binds chitin and internalises the model ligand acetylated BSA and directs it for endosomal degradation.

Aim: In this project we aim to characterise the functional aspects of the binding between FIBCD1 and chitin. We have constructed FIBCD1-expressing epithelial cell lines in which we seek to identify the intracellular signalling upon binding to chitin or model ligand. Initially this will be investigated by microarray-based methods and subsequent verification by quantitative real-time PCR. The mechanisms of internalisation of acetylated structures mediated by FIBCD1 will be investigated by performing a range of strategic point mutations in the cytoplasmic tail of FIBCD1.

Results: The colon epithelial cell line HCT-116 has been stably transfected with full length FIBCD1 in order to use for stimulatory studies and the cell line HEK293 has been stably transfected with FIBCD1 containing point mutations in the cytoplasmic tail in use for internalisation studies.

Conclusion: We have identified FIBCD1 as the first membrane bound FReD molecule in mammals and the first pattern recognition receptor that binds chitin and directs acetylated structures for degradation in the endosome. The localisation of FIBCD1 on the luminal surface of the intestinal epithelia points to a role in innate immunity and/or gut homeostasis.

#58 Anne Trommelholt Jepsen

The molecular interaction between M- and L-ficolin and chitin

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L-ficolin is a serum protein, while M-ficolin is mainly located in secretory granules and on the surface of monocytes and neutrophils. M- and L-ficolin both recognize acetylated structures, including *N*-acetylglucosamine (GlcNAc), through different sites in the fibrinogen-related domain (FReD), and functions as PRMs capable of initiating the lectin pathway.

Chitin is a linear homopolymer of GlcNAc residues, which next to cellulose is the most abundant known biopolymer. Vertebrates are exposed to chitin both through food ingestion and when infected with parasites and fungi. Chitin has recently been identified as a PAMP that modulates the allergic response in mice, and the purpose of the present study was to investigate the hypothesis that chitin would serve as a ligand for ficolins.

Recombinant M- and L-ficolin were produced and their binding properties were investigated through binding experiments using acetylated BSA, acetylated beads, chitin from crab shells and chitin beads.

Our initial observations have demonstrated that M-ficolin bound to acetylated BSA and acetylated beads in a calcium-dependent manner and the binding could be abolished by the addition of acetate, indicating that the binding is mediated through the calcium-dependent acetyl-binding S1 site. L-ficolin was likewise found to interact with acetylated BSA and acetylated beads, but in a calcium-independent manner, however only the binding to the acetylated beads could be inhibited by the addition of acetate. Both M- and L-ficolin bound chitin, but only the binding of M-ficolin to chitin was calcium-dependent. This suggests that M-ficolin binds to chitin through the S1 site, while the L-ficolin binds chitin in the S2 or S3 site.

These findings indicate that M- and L-ficolin have the potential to modulate the immune defence against inhaled fungi through chitin interaction.

#59 Jyothi Thyagabhavan Mony

CNS infiltrating CD8+ T cells in EAE express CXCR3 but not CCR6

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Introduction: T cell mediated autoimmune responses are implicated in the pathogenesis of multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE). EAE can be induced by CNS antigen specific CD4+ T cells producing IFN- γ (Th1 subtype), IL-17 (Th17 subtype), or CD8+ T cells. In contrast to CD8+ dominated MS, most EAE models are CD4+ dominated.

T cell migration is regulated by chemokines. Th17 cells express CCR6, a receptor for CCL20 and Th1 cells express CXCR3, a receptor for CXCL9, CXCL10 and CXCL11. CD8+T cells infiltrating the CNS in response to viral infection express CXCR3. Like CD4+ T cells, CD8+ T cells can differentiate into IFN- γ producing and IL-17 producing types. In EAE, CD8+ T cells are a good source of IFN- γ but poor source of IL-17. Very little is known about chemokine receptor expression by CD8+ T cells in EAE.

Aim: To study localization and chemokine receptor expression profile of CD8+ and CD4+ T cells infiltrating the CNS.

Study description: Flow cytometry was used to determine CXCR3 and CCR6 expression on CD8+ and CD4+ T cells infiltrating brain and spinal cord. EAE was induced by immunizing mice with recombinant myelin oligodendrocyte glycoprotein (MOG, residues 1-125) or with peptide p35-55.

Results and discussion: CD4+T cells constituted the majority in both EAE models. CCR6 was expressed by a small fraction of CD4+T cells. The majority of infiltrating CD8+ T cells expressed CXCR3. However, CD8+ T cells expressing CCR6 were rarely detected. This concurs with the observation that CD8+ T cells do not produce IL17 during EAE. Both models are biased towards immune response dominated by CD4+ T cells. Future studies will focus on autoimmune response dominated by CD8+ T cells.

#60 Susanne Gjørup Sækmose

Normal variation and heritability of serum microfibril associated protein 4

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Microfibril-associated protein 4 (MFAP4) was recently identified as a candidate marker of hepatic cirrhosis by the use of a proteomic approach followed by measurements of serum variation. Knowledge on normal variation and heritability of serum MFAP4 however remains to be determined in order to apply the utilization of systemic variation or possibly genetic variation in MFAP4 in fibrotic disease.

We have developed an MFAP4 ELISA assay and here we investigate the serum variation in a population of Danish adult twins (GEMINAKAR). Serum sMFAP4 was measured in 1422 self-reported healthy twins. The study population was divided in 291 pairs of monozygotic (MZ) twins, 291 pairs of dizygotic same-sex (DZ-SS) twins and 129 pairs of dizygotic opposite-sex (DZ-OS) twins. Mean sMFAP4 in the study population was 19.0 IU/ml (3.41–35.5 IU/ml), which is in accordance with previous results. The sMFAP4 distribution was right-skewed and in order to approximate a bivariate normal distribution the data were transformed to the logarithmic scale. Without adjusting for confounding effects an intraclass correlation coefficient of 0,5597 for MZ and 0.4680 for DZ-SS was calculated, indicating that the systemic level of sMFAP4 is influenced by additive genetic factors ($a=0.18$), shared genetic factors ($c=0.38$) and environmental factors ($e=0.44$). The data will be controlled for possible confounding factors, such as age, gender, and current smoking status, in the final heritability analysis.

Further knowledge of sMFAP4 variation in normal, healthy adults will be achieved by investigation of the serum variation in relation to physical activity. Together these studies will provide the background for conducting studies on sMFAP4 variation in case control studies of relevant disorders characterized by tissue remodelling and fibrosis.

UNIVERSITY OF AARHUS

#61 Christel Rothe Brinkmann

A complex of oleic acid and α -lactalbumin induces cell death resembling either necrosis or apoptosis dependent on the type of cancer cell line

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A complex called HAMLET formed from two naturally occurring constituents of human breast milk, oleic acid and α -lactalbumin, has been reported to kill tumor cells. Using an alternative preparation method and substituting the human protein for the readily available bovine α -lactalbumin, we have prepared a new variant of the α -lactalbumin/oleic acid complex. The cytotoxicity of the α -lactalbumin/oleic acid complex was comparable to that of HAMLET. Oleic acid in the absence of α -lactalbumin was also toxic to the cell lines. However, when compared on the basis of the oleic acid content, the cytotoxicity of the α -lactalbumin/oleic acid complex was 20-fold higher than of oleic acid alone. The sensitivity to treatment with oleic acid or the α -lactalbumin/oleic acid complex differed between the tested cell types. Depending on the cell type, the mode of dose-dependent cell death resembled either necrosis or apoptosis, as measured by flowcytometry using AnnexinV and DNA-dye 7AAD. The cytotoxic effect was attenuated by the presence of serum and serum albumin.

Conclusion: A complex of α -lactalbumin and oleic acid induced cell death of cancer cells through different mechanisms depending on the cell line and concentration. The cytotoxic activity of the α -lactalbumin/oleic acid complex was inhibited by five percent serum.

#62 Søren Degn

Investigations on MASP-3 and MAp44, components of the lectin pathway of complement

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The pattern-recognition molecules of the lectin pathway, mannan-binding lectin (MBL) and the three ficolins (H-, L-, and M-ficolin), circulate in complexes with three MBL-associated serine proteases: MASP-1, -2, and -3. In addition, a non-enzymatic splice product of the *MASP2* gene, MAp19, has been described. We recently presented the first report of a fifth associated protein, MAp44, an alternative splice product of the *MASP1* gene. Three proteins are thus produced from the *MASP1* gene: MASP-1 and MASP-3, which share five domains constituting the A-chain, but have unique protease domains; and MAp44, which shares the first four domains with MASP-1 and -3, and additionally has 17 unique C-terminal residues. We have previously shown that MAp44 and MASP-3 may inhibit MASP-2 mediated C4 activation. Here, we present sandwich assays for MASP-3 and MAp44 and the results obtained using these. MASP-3 and MAp44 are found in serum in complexes eluting corresponding to 600-800 kDa and 500-700 kDa, respectively. The level of MASP-3 in donor sera (n=200) is log-normally distributed with a median value of 5.0 µg/ml (range: 1.8-10.6 µg/ml), and the corresponding value for MAp44, likewise log-normally distributed, is 1.7 µg/ml (range: 0.8-3.2 µg/ml). MASP-3 levels are low at birth and reach normal levels within the first 6 months, whereas MAp44 drops slightly during the first 6 months. Concomitant with the acute phase response in patients undergoing colon cancer surgery, levels of both proteins drop slightly within 1-2 days, but whereas MASP-3 recovers to baseline within another 2 days, MAp44 overshoots, only to reach baseline at around day 30.

STATENS SERUM INSTITUT

#63 Berit Grevstad

NGIN: Next generation HIV-1 immunogens inducing broadly reactive neutralising antibodies

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The elicitation of broadly neutralising antibodies (Nab) remains the primary and most challenging goal in HIV-1 vaccine development. Although a few anti-HIV-1 monoclonal antibodies with broadly neutralising capability have been isolated from infected individuals, none of the immunisation strategies thus far explored has proven effective in inducing similar antibodies. Objective of the project is the development of a variety of 'next-generation' HIV-1 envelope-based immunogens that in combination with new adjuvant formulations are capable of eliciting high-titer broadly Nab responses. The strategy is based on one side on the identification and cloning of envelopes that have successfully elicited broad Nabs in their natural hosts, focusing on HIV-1 strains derived from patients with high-titered broad Nabs in their sera; on the other side, to introduce rational modifications into these HIV-env based immunogens, with the aim of exposing cryptic conserved neutralization epitopes and permitting their efficient presentation to the immune system. HIV-1 envelopes will be expressed in viral vectors or as trimeric (gp150) soluble proteins and screened for their immunogenicity and antigenicity in rabbits. New immunogens will be evaluated in prime-boost regimens in rabbits using novel effective adjuvant formulations. Immunogen/adjuvant combinations that prove most effective in eliciting broadly Nabs both systemically and at the mucosal level will be evaluated in non-human primates for their immunogenicity and efficacy upon challenge with live heterologous virus. Finally, formulations that will elicit protective immunity in non-human primates will be forwarded for proof-of-principle testing in humans.

#64 Sheila T. Tang

Genome-based *in silico* identification of new *M. tuberculosis* antigens, activating polyfunctional CD8 T-cells in human tuberculosis

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Although CD8⁺ T-cells help controlling *Mycobacterium tuberculosis* (*Mtb*) infection, their *Mtb*-antigen repertoire, *in vivo* frequency and functionality in human tuberculosis (TB) remain largely undefined. We have performed genome-based bioinformatics searches to identify new *Mtb*-epitopes presented by major HLA-class I supertypes, A2, A3 and B7 (covering 80% of the human population).

432 *Mtb*-peptides predicted to bind to HLA-A*0201, HLA-A*0301 and HLA-B*0702 (representing the above supertypes) were synthesized and HLA-binding affinities determined. Peptide-specific CD8⁺ T-cell proliferation-assays (CFSE-dilution) in 44 *Mtb* responsive donors identified 70 new *Mtb*-epitopes. Using HLA/peptide tetramers for the 18 most prominently recognized HLA-A*0201-binding *Mtb*-peptides, recognition by TB-patients' CD8⁺ T-cells was validated for all 18 epitopes. ICS for IFN- γ , IL-2 and TNF- α revealed mono-, dual- as well as triple-positive CD8⁺ T-cells, indicating these *Mtb* peptide-specific CD8⁺ T-cells were functional. Moreover, these T-cells were primed during natural infection, since they were absent from *Mtb*-noninfected individuals. Control CMV-peptide/HLA-A*0201 tetramers stained CD8⁺ T-cells in *Mtb*-infected and non-infected individuals equally, whereas Ebola-peptide/HLA-A*0201 tetramers were negative.

In conclusion, the *Mtb*-epitope/antigen repertoire for human CD8⁺ T-cells is much broader than hitherto suspected; and the newly identified *Mtb*-antigens are recognized by (poly)functional CD8⁺ T-cells during control of infection. These results impact on TB-vaccine design and biomarker identification.

LUND UNIVERSITY

#65 Madelene Dahlgren

Dendritic cells induce CXCR5 surface expression on naïve CD4⁺ T cells

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T follicular helper (Tfh) cells are a subset of CD4⁺ T cells with an enhanced ability to provide B cell help and support germinal center (GC) responses, processes important for the generation of long-lived and affinity matured antibody responses. Expression of the chemokine receptor CXCR5 characterizes Tfh cells and enables their follicular localization, and thereby also facilitates interaction with cognate B cells. The transcriptional repressor Bcl-6 was recently demonstrated to be both sufficient and necessary to drive *in vivo* Tfh development, indicating that Bcl-6 acts as a master regulator in Tfh cell differentiation. However, the molecular cues that induce Bcl-6 expression and promote the Tfh phenotype is not yet defined. To address this, we have activated naïve CD4⁺ T cells *in vitro*, either in co-cultures with dendritic cells (DC) or with agonistic antibodies against CD3 and CD28. From these experiments we have seen that both the DC and antibody activated CD4⁺ T cells express comparable levels of CXCR5 mRNA, but this is not synonymous to surface expression of the protein as only the DC activated CD4⁺ T cells display CXCR5 that can be detected by flow cytometry. Further, the *in vitro* activated CXCR5⁺CD4⁺ T cells do not express elevated mRNA levels of Bcl-6 and IL-21, markers strongly associated to the Tfh phenotype. Collectively, our results indicate that DCs provide signals to the T cells necessary for surface expression of CXCR5, but are not sufficient to complete the process of Tfh development. With this observation, we are also interested in evaluating the ability of different DC subsets to induce CXCR5 expression on CD4⁺ T cells. Currently, we are looking at the CD103⁺ and CD103⁻ DCs, which previously have been shown to hold different capacities to induce gut-homing effector T cell subsets, to determine if they could also play a role in Tfh development.

#66 Thorsten Joeris

Targeting the proteasome: Deletion of the immunoproteasome-subunit LMP7 attenuates experimental colitis

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Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, is characterized by chronic relapsing inflammation of the gut. Increased proteasome activity, associated with high expression of immunoproteasomes has been shown to enhance proinflammatory signalling in the inflamed mucosa of IBD patients. Especially, immunoproteasome subunit LMP7 was highly abundant in both groups of patients. Thus, we tested whether LMP7 is a suitable target for the treatment of inflammation in experimental colitis. To assess this, wild type (WT) and *Imp7*^{-/-} mice were treated with Dextran sodium sulfate (DSS) to induce colitis. We found that *Imp7*^{-/-} compared to WT mice develop significantly attenuated colitis as judged by reduced weight loss and tissue damage. Further, we show that reduced proinflammatory signalling via NF- κ B limits the secretion of proinflammatory cytokines and chemokines, consequently reducing the infiltration of the colon by neutrophils as well as blocking the development of inflammatory Th1 and Th17 T cell responses. In summary, our experiments demonstrate that modulation of the proteasome activity by targeting LMP7 is effective in attenuating experimental colitis, and confirm that this immunoproteasome-subunit is a promising target for the therapy of IBD.

#67 Miljana Radenkovic

Studies of development of memory T cells compartment in children during the first year of life following immunization

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Objectives: To test the feasibility of investigating the frequency and phenotype of antigen-specific memory T cells post-immunization in infants using small amounts of frozen PBMC.

Methods: Frozen PBMC from children at 6 and 9 months which were immunized against pertussis, diphtheria, polio and tetanus following the same vaccine schedule were stimulated with TETRAVAC *in vitro* for 20 hrs. The cytokine secretion assay (CSA) was used in combination with multicolor flow cytometry using the following antibodies: anti-CD3, anti-CD4, anti-CD8, anti-CD45RO, anti-INF- γ and anti-CD27.

Results: We have observed a clear increase of the effector memory, central memory and naive T cells in the CD4⁺ INF γ ⁺ T cell compartment at 6 and 9 months of age in the same individual, but a decrease of the terminal effector T cells in the 9th month.

CD8⁺ T cell response showed an increase of the central memory and naive T cells at 9 months compared to the 6th month, while a decrease of the effector memory and terminal effector T cells was observed at 9 months compared to 6 months of age. The memory compartment of the non responders didn't show any differences between 6 and 9 months in either CD4⁺ or CD8⁺ T cells.

Perspectives: Our results must be confirmed with a larger number of samples. Improved understanding of the generation of functional memory T cells in children may provide new strategies to identify individuals at high-risk of developing autoimmune diseases and to follow disease progression.

DANDRIT

#68 Berit Schultz

In vitro analysis of dendritic cells pulsed with allogeneic tumor lysate

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Dendritic cells (DCs) are bone marrow-derived professional antigen presenting cells that play a key role in bridging the innate and adaptive immune defense. Their primary task is the uptake and presentation of antigen to T cells in order to initiate an adaptive immune response. Dendritic cells have been widely used in cancer therapy being loaded with different types of antigen in order to elicit an immune response against the cancer. DanDrit Biotech A/S produces a dendritic cell based vaccine loaded with allogeneic tumor lysate. The vaccine is produced from monocytes isolated from the patient's own blood which is cultured for 8 days *in vitro* to generate mature dendritic cells which is then injected into the patient. The melanoma cell lysate providing the antigen is produced from a cell line rich in the expression of cancer/testis antigens. We studied the DCs during their normal 8 days of culture and for two additional days in order to characterize their surface marker expression, cytokine secretion and antigen uptake. This verified that using our culture method does produce DCs with a mature phenotype with surface marker expression and secretion of IL-12 and IL-23 comparable to those of other studies. Furthermore, antigen uptake both via receptor-mediated endocytosis as well as macropinocytosis takes place while DCs display an immature phenotype, but not after inducing maturation, which is also consistent with previous observations.

NOVO NORDISK

#69 Kristine Holgersen

Development and validation of the interleukin-10 knockout model of Crohn's disease

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Background

The two major clinically defined forms of inflammatory bowel disease (IBD), Crohn's Disease (CD) and Ulcerative Colitis (UC) are chronic remittent or progressive inflammatory conditions. CD may affect the entire gastrointestinal tract and is associated with an increased risk of colon cancer. CD has long been appreciated to have a genetic basis and involves a response of the immune system to the natural intestinal flora and some environmental agents. There is currently no cure for CD.

Advances in understanding pathogenesis and developing therapies are hastened by the use of effective animal models of inflammatory diseases. In Crohn's disease, a variety of models have been used, including the IL-10 knockout mouse. However, in order to be truly valuable, the disease activity in the model and its response to existing therapy needs to resemble the human disease.

Objective

In the light of recent development of new biologics therapies this project is set out to

- 1) Develop assays for careful evaluation of disease activity in the IL-10 knockout model of colitis.
- 2) Compare spontaneous colitis in the IL-10 knockout mouse with a piroxicam induced colitis.
- 3) Validate the IL-10 knockout model by examining its response to various biologic therapies e.g. (anti-TNF- α , anti-IL-6, anti-IL12p40 and CTLA-4-Ig).
- 4) Increase our knowledge concerning key drivers and pathways in the IL-10 knockout model for the most accurate evaluation of novel targets.

Methods

Standard immunological assays incl. ELISA, FACS, Medonic, MACS cell purification and in vitro cell culture techniques.

Standard histopathological analysis and endoscopic analysis.

#70 Henrik Søndergaard

Humanized mouse model of graft versus host disease (GvHD) – a new tool in translational inflammation research

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Development of targeted human therapies often requires surrogate drugs or targets for preclinical evaluation *in vivo*. The aim of this study is to develop a mouse model where human immune cells mediate an inflammatory disease, allowing the investigation of novel anti-inflammatory therapies targeting molecules unique to human immune cells.

Non-obese diabetic (NOD)/severe combined immunodeficient (SCID) interleukin-2 receptor gamma-chain (IL-2R γ)^{null} or NOD/recombination activation gene-1 (Rag-1)^{null} IL-2R γ ^{null} mice were reconstituted with 20x10⁶ human peripheral blood mononuclear cells (PBMCs) from healthy donors and monitored for human immune cell engraftment and development of GvHD.

In both strains, human CD45+ cells expand *in vivo* reaching ~50% of all white cells in the blood within 3-4 weeks post injection. Engraftment mainly consists of T cells (CD4+ and CD8+), which show polyclonal expansion and acquire an activated (CD62L-CD45RO+) phenotype capable of secreting cytokines i.e. TNF α , IFN γ , IL-10 and IL-2. After ~4 weeks, mice develop a lethal GvHD measured as >20% weight loss and histologically presents with heavy mononuclear infiltration in the liver, kidney and lung. Injection of CD8+ T cell-depleted PBMCs attenuates GvHD, whereas CD4+ T cell-depleted PBMCs shows sustained GvHD activity. Treatment with Enbrel (soluble TNF α R2-Ig) significantly postpones time to GvHD, whereas Orencia (CTLA-4-Ig) can completely inhibit GvHD, validating that the model can predict responses of relevant targeted anti-inflammatory treatments.

In conclusion, we have established a mouse model supporting significant human immune cell engraftment and expansion, coinciding with the development of GvHD, which responds to anti-inflammatory biologics. We predict that this model will be a valuable new tool for preclinical evaluation of novel targeted anti-inflammatory therapies.