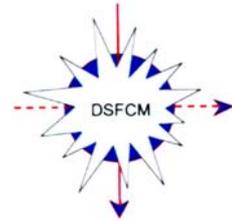




Danish Society of Immunology

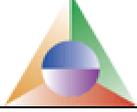
Danish Society for Flow Cytometry



**Annual meeting 2005
Danish Society of Immunology
And
Danish Society for Flow Cytometry**

**The Panum Institute,
Blegdamsvej 3,
2200 Copenhagen N
Tuesday 26/4-2005, kl. 12-18**

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Program: 12-14 in Haderup Auditorium, 14-18 in Dam Auditorium

12.00-12.05 *Welcome by Søren Buus*

12.05-13.30 *Presentations, session 1, innate immunity (Chair: Steffen Thiel)*

- Abs 1 Pernille D. Frederiksen On quantification of Mannan-binding lectin (MBL)
Abs 2 Mette Møller-Kristensen Mannan-binding lectin recognizes structures on ischemic reperfused mouse kidneys and is implicated in tissue injury
Abs 3 Christian Møller Sørensen Hormonal regulation of mannan-binding lectin synthesis in cultured hepatocytes
Abs 4 Grith L Sorensen Surfactant protein D is proatherogenic in mice
Abs 5 Lisbeth Bærentzen Assessment of immunogenicity of pharmaceuticals in vitro
Abs 6 Kajsa M. Paulsson Chaperones are not required for association and dissociation of MHC class I and tapasin

13.30-14.00 *Break w. coffee / tea (Poster session) General meeting DSFCM*

14.00-16.00 *Are Dendritic Cells the Gateway to Manipulation of Adaptive Immunity? (Chairs: F. Sallusto and H.J. Hoffmann)*

Invited main lecture Federica Sallusto Regulation of T cell immunity by dendritic cells

- Abs 7 Christian Lodberg Hvas Probiotic Bacteria Induce Regulatory Cytokine Production via Dendritic cells
Abs 8 Özcan Met Induction of immune response using dendritic cells transfected with selected tumor antigens associated with human breast carcinomas
Abs 9 Anders E. Pedersen Dendritic cell based vaccination in combination with VEGF receptor 2 and CTLA-4 blockade.
Abs 10 Claus Haase Immunomodulatory dendritic cells require autologous serum to circumvent non-specific immunosuppressive activity *in vivo*

16.00-16.30 *Break w. coffee / tea (Poster session) General meeting IS*

16.30-18.30 *Presentations, session 2, adaptive immunity (Chair: Allan R. Thomsen)*

- Abs 11 Peter J. Holst Delayed Expansion of Dysfunctional Virus Specific CD8⁺ T-Cells Following Systemic Recombinant Adenovirus Infection in mice
Abs 12 Carina de Lemos Opposing effects of CXCR3 and CCR5 deficiency on CD8⁺ T cell response to intracerebral infection with LCMV
Abs 13 Pernille Henrichsen Impaired virus control and severe CD8⁺ T cell-mediated immunopathology in chimeric mice deficient in IFN- γ receptor expression on both parenchymal and hematopoietic cells
Abs 14 Tord Labuda MEK kinase 1 is a negative regulator of virus specific CD8⁺ T cells.
Abs 15 Hans Jürgen Hoffmann Response of allergic bakers to a food challenge (DBPCFC) with flour
Abs 16 Per thor Straten Clonotype Analyses of Tumor Specific T cells; Tracking of T cells in Time and Space.

18.30-21.00 *Dinner at the Panum Kantine*



Abstract 1

On quantification of Mannan-binding lectin (MBL)

Pernille D. Frederiksen^a, Lisbeth Jensen^a, Annette G. Hansen^a, Finn Matthiesen^b, Steffen Thiel^a, Malcolm W. Turner^c, Jens Chr. Jensenius^a

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MBL is attracting considerable interest due to its role in the innate immune defence, and because the high frequency of MBL deficiency makes it feasible to evaluate clinical relevance through epidemiological investigations on fairly limited number of patients. The plasma level of MBL reflects its role as a part of the innate immune defence: it is present in newborns, and ready for action when a foreign agent needs being eliminated. The high frequency of genetically determined deficiency is a puzzle best explained by the damage it may cause in inappropriate inflammatory reactions. The exact quantification has been questioned with the re-realization of the presence of significant amounts of aberrant MBL present in plasma from individuals homozygous for allotypes determining deficiency. We find, by using TRIFMA with a variety of monoclonal antibody combinations, that B/B homozygous individuals may present signals that correspond to signals of up to 500 ng MBL per ml when using A/A wild type MBL as standard. At isotonic conditions this aberrant MBL showed a Mr of 450 kDa by GPC, but showed no binding to mannan, nor was it associated with MASP. For oligomerization-independent estimation of MBL levels we selected to quantify by applying serum samples on reduced SDS-PAGE followed by Western blotting with antibody against the CRD. This approach will presumably present the single polypeptide chains to the antibody independent of allotype differences in the collagen region. Chemiluminescent emitted photons were recorded by a CDC camera. Titrations of rMBL served as standards. Ten B/B sera showed concentrations in the range of 10 to 500 ng/ml.

No biological activity has been ascribed to the aberrant MBL. The MBL assays in widespread use are not sensitive to this apparently non-functional MBL. It seems unfortunate that a notion of MBL deficiency being an artefact is currently nurtured.

We have compared the quantification of MBL levels by several commercially available MBL assays (Immunolex A/S, Denmark, HyCult Biotechnologie b.v., The Netherlands and Dobeel Corp., Korea) with our own in-house assay and report the results.

Abstract 2

Mannan-binding lectin recognizes structures on ischemic reperfused mouse kidneys and is implicated in tissue injury

Mette Møller-Kristensen¹, Weidong Wang², Marieta Ruseva¹, Steffen Thiel¹, Søren Nielsen², Kazue Takahashi³, Lei Shi³, Alan Ezekowitz³, Jens Chr. Jensenius¹, Mihaela Gadjeva¹.

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³Developmental Immunology, Massachusetts General Hospital, Boston, USA

Organ damage as a consequence of ischemia and reperfusion (I/R) is a major clinical problem in an acute renal failure and transplantation. Ligands on surfaces of endothelial cells that are exposed due to the ischemia may be recognized by pattern recognition molecules such as mannan-binding lectin (MBL), inducing complement activation. We examined the contribution of the MBL complement pathway in a bilateral renal I/R model (45 min of ischemia followed by 24 h of reperfusion), using transgenic mice deficient in MBL-A and MBL-C (MBL double knock out, MBL DKO) and in wild type (WT) mice. Kidney damages, which were evaluated by levels of blood urea nitrogen (BUN) and creatinine, showed that MBL DKO mice were significantly protected compared with WT mice. MBL DKO mice reconstituted with recombinant human MBL showed a dose-dependent severity of kidney injury increasing to a comparable level to WT mice. Acute tubular necrosis was evident in WT mice but not in MBL DKO mice after I/R, confirming renal damages in WT mice. MBL ligands in kidneys were observed to be present after I/R but not in sham-operated mice. C3a (desArg) levels in MBL DKO mice were decreased after I/R compared with that in WT mice, indicating less complement activation that was correlated with less C3 deposition in the kidneys of MBL DKO mice. Our data implicate a role of MBL in I/R induced kidney injury.

Abstract 3

Hormonal regulation of mannan-binding lectin synthesis in cultured hepatocytes

Christian Møller Sørensen¹, Troels Krarup Hansen², Rudi Steffensen³, Jens Chr. Jensenius¹, Steffen Thiel¹



¹Department of Medical Microbiology and Immunology, Aarhus University, Aarhus, phone 89421776.

²Immunoendocrine Research Unit, Medical Department M, Aarhus University Hospital, Aarhus, ³Regional Centre for Blood Transfusion and Clinical Immunology, Aalborg Hospital, Aalborg,

Serum MBL levels are mainly genetically determined, but also influenced by growth hormone (GH) and insulin *in vivo*. Our aim was to study in more details the hormonal regulation of MBL synthesis using an *in vitro* model with cultured hepatocytes.

Cells from the human hepatocyte line HuH-7 were seeded and challenged over a 3 day period with either GH, hydrocortisone, IGF-1, insulin, IL-6, T3 or T4. The concentration of MBL and human albumin in the culture supernatants was measured with immunoassays. mRNA was measured with quantitative real time reverse PCR using β 2 microglobulin as household protein.

T3 and T4 had the strongest influence on MBL production causing an 8-fold increase ($p=0.003$), while GH augmented the production of MBL 3-fold ($p=0.028$) and IL-6 caused a weak but significant dose dependent increase in MBL production. Hydrocortisone, IGF-1 and insulin had no effect on the MBL production. None of the hormones significantly affected production of albumin. MBL mRNA levels were stable during the first 24 hours of T3 stimulation, but increased significantly between 24 and 48 hours reflecting the increased synthesis of MBL.

In conclusion, thyroid hormone and GH significantly increase MBL synthesis.

Abstract 4

Surfactant protein D is proatherogenic in mice

Grith L Sorensen¹, Jens Madsen², Karin Kejlving¹, Ida Tornoe¹, Ole Nielsen³, Paul Townsend⁴, Francis Poulain⁵, Claus H. Nielsen⁶, Kenneth B M Reid⁴, Samuel Hawgood², Erling Falk⁷, Uffe Holmskov¹

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3. Department of Pathology, Odense University Hospital, Odense, Denmark

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5. Department of Pediatrics, University of California, Davis, USA

6. Copenhagen Blood Transfusion Centre, Copenhagen University Hospital, Denmark

7. Department of Cardiology, Aarhus University Hospital (Skejby), Aarhus N, Denmark

Background Atherogenesis involves arterial inflammation and lipid deposition. We investigated the role of surfactant protein D (SP-D) in disease development because SP-D is an endogenous modulator of inflammation.

Methods and Results SP-D synthesis was localized to vascular endothelial cells. The use of a diet-induced model of atherosclerosis showed that the induced atherosclerotic lesion areas were 5.6 fold smaller in the aortic roots in SP-D deficient (*Spd*^{-/-}) mice compared to *wild-type* mice. High-density lipoprotein cholesterol (HDL-C) was significantly elevated the *Spd*^{-/-} mice and HDL-C inversely correlated to lesion areas in the study. Treatment of the *Spd*^{-/-} mice, with a recombinant fragment of human SP-D resulted in decreases of HDL-C (21%) as well as total cholesterol (TC) (26%), and low-density lipoprotein (LDL-C) (28%). Plasma tumor necrosis factor- α (TNF- α) was significantly reduced in the *Spd*^{-/-} mice (45% difference).

Conclusions SP-D is proatherogenic and retards development of atherosclerosis in the used mouse model. The effect is not likely to be due to the observed disturbances of plasma lipid metabolism. We suggest that altered progression of the inflammatory process underlies the reduced susceptibility to atherosclerosis in SP-D deficient mice.

Abstract 5

Assessment of immunogenicity of pharmaceuticals in vitro

L. Bærentzen, J. Glamann

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Insulin mimetics are non-self synthetic proteins that react specifically with the insulin receptor which lead to a reduction in blood sugar levels. Due to the fact that insulin mimetics are foreign proteins to both mouse and man, it is interesting to utilize these proteins in the assessment of immunogenicity. Based on antibody Elisa assays testing antiserum from immunized Balb/c inbred and NMRI outbred mice, we showed a marked difference in immunogenicity, although the proteins included in the study only differed by one conservative substitution. In the Balb/c mice the antibody titer was high towards one of the proteins and low towards the other, whereas in the NMRI mice it was low at all times. Scrutinizing the T cell response in the immunized Balb/c mice, we performed a proliferation study where mice were



treated with the proteins in adjuvant every 2 weeks for 4 weeks total. In this immunization setting a difference in immunogenicity between the peptides was not detectable, but interestingly a marked difference was seen when we added 12-mer overlapping peptides to the wells, allowing us to discover the immunogenic epitopes of the proteins. In the Elispot assay we showed a ten-fold increase in sensitivity as compared to the proliferation assay. These methods and in particular the Elispot assay are a valuable supplement to the antibody Elisa in measuring immunogenicity in vitro.

Abstract 6

Chaperones are not required for association and dissociation of MHC class I and tapasin

Kajsa M. Paulsson^{1,2}, Catarina Betou¹, Ping Wang¹, Suling Li³.

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Tapasin is important for the quality control of MHC class I assembly. It has been discovered that the chaperones calreticulin and/or ERp57, are also associated with tapasin. However, it is unknown whether the association of tapasin with chaperones and/or association of MHC class I with chaperones are compulsory for the process of quality control. In this study, we have characterised the interaction of chaperone-free tapasin with MHC class I or chaperone-free MHC class I with tapasin. In both conditions, the interaction of tapasin and MHC class I was detected and the interaction could be dissociated with class I binding peptides. Furthermore, the interaction of tapasin and MHC class I could be reconstituted in the absence of any other cellular proteins. Thus, the interaction of tapasin with MHC class I is independent on the association of calreticulin or ERp57 with tapasin, as well as on MHC class I association with these two chaperones. In conclusion the regulation of MHC class I assembly by tapasin relies on the *de novo* interaction between tapasin and MHC class I, but not on the interaction of chaperones with either tapasin or MHC class I.

Abstract 7

Probiotic Bacteria Induce Regulatory Cytokine Production via Dendritic cells

Christian Lodberg Hvas^{*}, Jens Kelsen^{*}, Jørgen Agnholt^{*}, Per Höllsberg[†], Michael Tvede[§], and Jens F. Dahlerup^{*} from

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Probiotic bacteria, e.g. *Lactobacillus spp.*, may improve inflammatory diseases by altering the signaling between dendritic cells and T cells. Here we present an *in vitro* model system where gut-derived CD4⁺ T cells and autologous monocyte-derived dendritic cells are used in co-culture to study the effects of various probiotic strains. T cell cultures were stimulated with autologous bacterial sonicates or strains of *Lactobacillus spp.*, with and without addition of autologous dendritic cells. Cytokine levels (IFN- γ and IL-10) and phenotype (CD4, CD25, CD69) were measured on day 4.

Lactobacillus spp. induced higher IL-10 production than autologous bacteria, and dendritic cells induced an increased production of all cytokines. However, the increase of IFN- γ was more pronounced in wells with autologous bacteria than in wells with *Lactobacillus spp.* The addition of dendritic cells upregulated CD25 expression without simultaneous upregulation of CD69. The upregulation was pronounced after stimulation with *Lactobacillus rhamnosus GG* compared with autologous bacteria and other *Lactobacilli*. Future studies will address whether such IL-10 producing T cells with a CD25⁺ phenotype represent a differentiation into competent regulatory T cells. In a clinical context, such cells might be used for treatment of inflammatory diseases.

Abstract 8

Induction of immune response using dendritic cells transfected with selected tumor antigens associated with human breast carcinomas

Özcan Met¹, Jens Eriksen¹, Mogens H. Claesson², Inge Marie Svane¹

¹Department of Oncology, Herlev University Hospital, Herlev, Denmark

²Department of Medical Anatomy, University of Copenhagen, the Panum Institute, Copenhagen, Denmark

The development of protocols for the ex vivo generation of DC has provided a rationale to design and develop DC-based vaccination studies for the treatment of infectious and malignant diseases. The efficacy of antigen loading and delivery into DC is pivotal for the optimal induction of T-cell-mediated immune responses. The use of DC transfected



with RNA encoding tumor antigen offers the prospect of antigen specific immunization without requiring prior knowledge of the immunogenic epitope or restricting allele, since epitopes from the translated protein are processed by the endogenous antigen-presentation machinery.

We have previously established an anti-tumor vaccine using autologous DC pulsed with p53 peptides for treatment of metastatic breast cancer, and the object now is to improve this treatment by transfecting DCs with mRNA encoding selected tumor antigens.

We have developed a non-viral transfection method protocol that uses *in vitro* synthesized mRNA and square-wave electroporation for transient expression of antigens in DC.

Using the mRNA encoding the green fluorescence protein (EGFP), the square-wave electroporation data show high yield and viability (>90 %), and high transfection efficiency (>90 %) of DC. The flow cytometry analysis also demonstrated that the expression of EGFP peaked 48-72 h after electroporation in DC transfected either before or after maturation. However, higher levels of expression and viability were obtained when DC were electroporated after maturation. Furthermore, cryopreservation of DC before and after electroporation did not alter the level of EGFP expression. Taken together, these preliminary results show that the use of square-wave electroporation as a transfection method seems to be a useful and effective technique to charge DC with tumor antigens.

Next, *in vitro* analysis of anti-tumor T cell-reactivity from patients operated for primary breast cancer will be initiated using square-wave electroporation for transfection of DC with the tumor antigens p53, survivin and hTERT.

Abstract 9

Dendritic cell based vaccination in combination with VEGF receptor 2 and CTLA-4 blockade.

A. E. Pedersen*, S. Buus[□], and M. H. Claesson*.

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We investigated the anti CT26 tumour effect of dendritic cell based vaccination with the MuLV gp70 envelope protein-derived peptides AH1 and p320-333. Vaccination lead to generation of AH1 specific cytotoxic lymphocytes (CTL) and some decrease in tumour growth of simultaneously inoculated CT26 cells. After combination with an antibody against VEGF receptor 2 (DC101), a significant increase in survival of the tumour cell recipients was observed. Also, monotherapy with an antibody against CTLA-4 (9H10), lead to nearly 100% survival of tumour cell recipients. However, effective treatment of mice with already established tumours was only obtained after combination of vaccination, DC101 and 9H10 treatment in which setting 80% of the mice rejected their tumours.

Abstract 10

Immunomodulatory dendritic cells require autologous serum to circumvent non-specific immunosuppressive activity *in vivo*

Claus Haase¹, Mette Ejrnaes², Amy E. Juedes², Tom Wolfe², Helle Markholst¹ and Matthias G. von Herrath²

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²La Jolla Institute for Allergy and Immunology, 10355 Science Center Drive, San Diego, CA 92121, USA

In immunotherapy, dendritic cells (DCs) can be used as powerful antigen-presenting cells to enhance or suppress antigen-specific immunity upon *in vivo* transfer in mice or humans. However, to generate sufficient numbers of DCs most protocols include an *ex vivo* culture step, wherein the cells are exposed to heterologous serum and/or antigenic stimuli. In mouse models of virus infection and virus-induced autoimmunity, we tested how heterologous serum affects the immunomodulatory capacity of immature DCs generated in the presence of IL-10, comparing FBS- or normal mouse serum (NMS)-supplemented DC-cultures. We show that immature DCs generated in FBS-supplemented cultures induced a non-antigen-specific systemic immune deviation characterized by reduction of virus-specific T cells, delayed viral clearance and enhanced systemic production of IL-4 and IL-10 to FBS-derived antigens, including BSA. By contrast, DCs generated in NMS-supplemented cultures modulated immunity and autoimmunity in an antigen-specific fashion, did not induce systemic IL-4 or IL-10 production and inhibited generation of virus-specific T-cells or autoimmunity only if pulsed with a viral antigen. These data underscore the importance of using autologous serum-derived immature DCs in preclinical animal studies to accurately assess their immunomodulatory potential in future human therapeutic settings, where application of FBS will not be feasible.

Abstract 11

Delayed Expansion of Dysfunctional Virus Specific CD8+ T-Cells Following Systemic Recombinant Adenovirus Infection in mice



Peter J. Holst¹, Cathrine Orskov², Allan R. Thomsen¹, Jan P. Christensen^{1*}

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2: Institute of Medical Anatomy, University of Copenhagen, The Panum Institute bldg.: 18.2, Blegdamsvej 3C, DK-2200

Infection with hepatotropic viruses are characterised by prolonged infection, delayed immune activation, immunopathology, and for some viruses the tendency to cause persistent infection. As systemically administered adenovirus has pronounced hepatotropism we compared the immune response and viral clearance following i.v. and peripheral infection with recombinant adenovirus. Our results demonstrate a marked qualitative and kinetic impairment of the immune response following i.v. administered adenovirus, remarkably similar to what can be seen in human viral hepatitis. These effects are demonstrated to be route and not virus specific. By infection of T-cell receptor transgenic mice targeting an adenovirus encoded epitope we demonstrate that naïve T-cells initially become activated, then dysfunctional after systemic infection with this hepatotropic model virus. Thus, systemic adenoviral infection recapitulates key features of human viral hepatitis and allows for dissection of mechanisms responsible for the induction of immunity and immune dysfunction following infection.

Abstract 12

Opposing effects of CXCR3 and CCR5 deficiency on CD8⁺ T cell response to intracerebral infection with LCMV

*Carina de Lemos, Jeanette Erbo Christensen, Annelise Nansen, Torben Moos, Bao Lu, Craig Gerard, Jan Pravsgaard Christensen and *Allan Randrup Thomsen

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To study the interplay of the chemokine receptors CXCR3 and CCR5 in regulating virus-induced CD8⁺ T-cell mediated inflammation in the brain, CXCR3/CCR5-deficient mice were generated and infected intracerebrally (i.c.) with lymphocytic choriomeningitis virus. Since these chemokine receptors were expressed by overlapping subsets of activated CD8⁺ T cells, it was expected that absence of both receptors would additively impair effector T cell invasion, and therefore protect mice against the fatal meningitis. Contrary to expectations double deficient mice were more susceptible to i.c. infection than CXCR3-deficient mice. Analysis of effector T cell generation revealed an accelerated antiviral CD8⁺ T cell response in CXCR3/CCR5-deficient mice. Furthermore, while the accumulation of CD8⁺ T cells into the neural parenchyma was delayed in both CXCR3- and CXCR3/CCR5-deficient mice the number of CD8⁺ T cells is statistically significant higher in the parenchyma of the CXCR3/CCR5-deficient mice around the time were most mice succumbed the infection. Taken together these results indicate that while CXCR3 play an important role in controlling CNS invasion, other receptors but not CCR5 also contribute significantly. Furthermore, CCR5 primarily seems to play a role as a negative regulator of the antiviral T cell response.

Abstract 13

Impaired virus control and severe CD8⁺ T cell-mediated immunopathology in chimeric mice deficient in IFN- γ receptor expression on both parenchymal and hematopoietic cells

Pernille Henriksen, Christina Bartholdy, Jan Pravsgaard Christensen, and Allan Randrup Thomsen

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Bone marrow chimeras were used to determine the cellular target(s) for the antiviral activity of interferon- γ . By transfusing such mice with high numbers of naive virus-specific CD8⁺ T cells a system was created, in which the majority of virus-specific CD8⁺ T cells would be capable of responding to interferon- γ , but expression of the relevant receptor on non-T cells could be experimentally controlled. Only when the IFN- γ receptor is absent on both radioresistant parenchymal and bone marrow-derived cells, will chimeric mice challenged with a highly invasive, non-cytolytic virus completely lack the ability to control the infection and develop severe wasting disease. Further, the study shows that IFN- γ receptor expression on parenchymal cells in the viscera is more important for virus control than IFN- γ receptor expression on bone marrow-derived cells.



Abstract 14

MEK kinase 1 is a negative regulator of virus specific CD8⁺ T cells.

Tord Labuda, Jan Pravsgaard Christensen, Barbara Bonnesen, Michael Karin, Allan Randrup Thomsen and Niels Ødum.

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Phone +45 35327754 fax +45 35327876

Mek kinase 1 (MEKK1) is a potent JNK activating kinase, a regulator of T helper cell differentiation, cytokine production and proliferation *in vitro*. Using mice deficient for MEKK1 kinase activity (*Mekk1^{AKD}*) exclusively in their hematopoietic system, we show, that MEKK1 has a neegative regulatory role in the generation of a virus-specific immune response. *Mekk1^{AKD}* mice challanged with vesicular stomatitis virus (VSV) showed a 4-fold increase in splenic CD8⁺ T cell numbers. In contrast, splenic T cells in infected wt mice where only marginally increased. The CD8⁺ T cell expansion in *Mekk1^{AKD}* mice following VSV infection is virus-specific and the frequency of virus-specific T cells is significantly higher (> 3-fold) in *Mekk1^{AKD}* as compared to wt animals. Moreover, the hyper expansion of T cells seen in *Mekk1^{AKD}* mice after VSV infection is a result of increased proliferation, since a significantly higher percentage of virus-specific *Mekk1^{AKD}* CD8⁺ T cells incorporated BrdU as compared to virus-specific wt CD8⁺ T cells. In contrast, similar levels of apoptosis were detected in *Mekk1^{AKD}* and wt T cells following VSV infection. These results strongly suggest that MEKK1 plays a negative regulatory role in the expansion of virus specific CD8⁺ T cells *in vivo*.

Abstract 15

Response of allergic bakers to a food challenge (DBPCFC) with flour

HJ Hoffmann¹, T Skjold¹, M Raithe², K Adolf¹, O Hilberg¹ and R Dahl¹

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A number of occupational respiratory allergens are food related. Little is known about the responses these algs elicit in sensibilised persons that ingest them.

9 allergic exbakers were exposed in a double blind placebo controlled food challenge with relevant allergen. Significant increases in urinary methyl histamine (MH) and serum tryptase and decrease in blood basophils and nasal volume after ingestion of allergen compared with placebo suggest an allergic response to ingested allergen. There was no change in FEV1.

The number of blood DC2 decreased after exposure to allergen (p=0,011) and placebo (p=0,036). Dendritic cell HLA DR was reduced after both exposures (p<0,001). Expression of CXCR4 on these cells was reduced after allergen exposure (p=0,033). CD4+ memory T cell expression of CD25 was upregulated after placebo exposure (p=0,021) but reduced after allergen. The reduction of CD25 expression after allergen compared to placebo was significant (p=0,024). CD152 was downregulated on these cells after allergen exposure (p=0,039), less so after placebo.

Allergic bakers respond to relevant ingested allergen. The allergen challenge reduced plasmacytoid dendritic cell nrs and memory T cell expression of CD25 and CD152.

	placebo		wheat		p
	Base	Exp	Base	Exp	
urinary MH, g/mmol Krea	11,81	13,49	10,37	13,79	0,017
blood basophils, nr/250000	2152	2164	2146	1705	0,034
serum tryptase, mg/L	5,26	4,75	5,33	5,34	0,011
nasal volume fraction of baseline	1	1,18	1	0,93	0,045

Abstract 16

Clonotype Analyses of Tumor Specific T cells; Tracking of T cells in Time and Space.

Sine Reker*, Tania Køllgaard*, Lynn Wenandy*, Rikke Bæk Sørensen*, Anders Meier*[†], Pia Kvistborg*, Merete Jonassen*, Tina Seremet*, Jürgen C. Becker[‡], Mads Hald Andersen*, Per thor Straten*

* Tumor Immunology Group, Institute of Cancer Biology, Danish Cancer Society, [†]Department of Cancer and Immunobiology, Novo Nordisk, [‡] Department of Onco-Dermatology, University of Würzburg, Germany.

Recent advances in our understanding of basic and tumor immunology has fueled the notion of specific activation of the immune system to control cancer growth. Thus, a large number of tumor associated antigens has been characterized over the past decade, and increased knowledge of natural as well as synthetic vaccination adjuvants has created new



impetus in attempt employ the immune system in anti-cancer therapy. To this end, specific therapeutic vaccinations against cancer have been ongoing for the past decade, and have produced complete and durable regressions, however, still in an unsatisfactory small fraction of patients. Strikingly - although controversial – it appears that biological responses may not coincide with clinical responses. During the past few years, several methods have become available for monitoring of cellular immunotherapy. Thus, tumor specific T cells can now be studied functionally, phenotypically as well as molecularly. We recently developed the T-cell receptor clonotype mapping methodology in which the clonal distribution of the TCR is exploited for detection and tracking of specific clonally expanded T cells based upon detection of the unique TCR. In particular when used in combination with techniques that provide data with regard to phenotype and functional capacity of clonally expanded T cells, this is a powerful technique that may help provide new insight as to the molecular and cellular events that governs the success or failure of current anti-cancer vaccination strategies. We have combined TCR clonotype mapping with EliSpot and HLA-multimer analyses in studies of spontaneous as well as treatment induced T-cell responses in cancer patients.

Abstract 17

Maternal dietary n-3 polyunsaturated fatty acid during gestation and lactation reduces the specific antibody response and TNF- α production in neonatal mice.

Kjær T¹, Porsgaard T¹, Lauritzen L², Lund P¹, Fruekilde M-B¹ and Frøkiær H.¹

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An adequate supply of n-3 polyunsaturated fatty acids (PUFA) during pregnancy and lactation is crucial for optimal fetal and postnatal development. However, the effect of differences in maternal PUFA intake on immune function in the offspring is less well established. Using a mouse model, we studied the effect of maternal dietary fatty acid composition on the immune response in mice pups. From the day of conception and throughout lactation dams were fed low-fat diets containing 4% fat of which 25% was n-3 PUFA from linseed or fish oil. A control group receiving a saturated diet with no n-3 PUFA was included. Pups were injected with ovalbumin within 24 hours of birth. Three weeks later plasma antigen-specific antibodies, spleen cells proliferation, cytokine production *ex vivo*, and fatty acid analysis of spleen cell phospholipids were measured in the pups. The fatty acid composition of pup spleens reflected maternal diet with higher levels of n-3 PUFA in the experimental groups compared to the control group. Maternal dietary long-chain n-3 PUFA gave a reduced antigen-specific antibody response and TNF- α production in their pups compared to pups of dams fed saturated fatty acids. These data show that maternal dietary long-chain n-3 PUFA influence the immune response in their pups.

Abstract 18

Characterization of Graft-versus-Tumor and Graft-versus-Host T-cell responses following Allogeneic Bone Marrow Transplantation for Malignant Hematological Disease

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Allogeneic bone marrow transplantation (BMT) has a well-documented ability to cure a number of malignant hematological diseases. Alloreactive donor T cells are known to be involved in the curative mechanism of allogeneic BMT called Graft-versus-Tumor (GVT). However, alloreactive T cells also play a role in Graft-versus-Host disease (GVHD) which is one of the major limitations of BMT leading to high patient mortality. Development of GVT is seen to be strongly correlated with induction of GVHD in patients. Thus, characterization of the cells and molecules involved in both GVHD and GVT may provide means to separate the two reactions, i.e. to induce GVT without GVHD. This project aims at identifying and characterizing clinically significant CD8+ T-cell clones in CLL patients receiving allogeneic BMT with non-myeloablative conditioning (mini-BMT). Specificity, frequency, functional capacity, and phenotype of relevant T-cell clones are analysed. Peripheral Blood Lymphocytes (PBL) isolated from donors and patients before and at different time-points after transplantation have been analysed with T-cell receptor clonotype mapping which is based on RT-PCR and denaturing gradient gel electrophoresis (DGGE). Comparative clonotype data demonstrate that clonally expanded T cells are present throughout the analysed period. During early phases after BMT clones emerge and disappear in a highly dynamic way. Eventually, this is replaced by more stable persistent clones. Recipient T cells were cytotoxic when co-cultured with autologous tumor cells, and subsequent clonotypic analyses of cytotoxic T cells demonstrated the *in vivo* clonal expansion of these cells.



Abstract 19

Crystal Structures of HLA-B*1501 in Complex with Peptides from Human Ubch6 and Epstein-Barr Virus EBNA-3A

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Based on their peptide binding specificity, human Major Histocompatibility Class I (MHC-I) molecules are currently divided into 12 supertypes. Here, we present the first crystal structures of a member of the B62 supertype, the HLA-B*1501. One structure involves an antigenic and immunogenic peptide (LEKARGSTY) from the Epstein-Barr virus protein, EBNA-3A, and the other an autologous peptide (ILGPPGSVY) from human Ubch6. The LEKARGSTY peptide is modeled into the observed electron density in a unique conformation. The Arg5 in the peptide points directly towards the usual location for the antigen recognizing CDR3 loops in the T cell receptor. Based on the electron density the ILGPPGSVY peptide is found to have two alternative conformations in the HLA-B*1501 complex. The C- and D-pockets in HLA-B*1501 are open and thus meet the spatial requirements for the two ILGPPGSVY conformations. The HLA-B*1501/ILGPPGSVY complex was studied using molecular dynamics. The results show a continuous swap between the two ILGPPGSVY conformations during the run. In contrast to LEKARGSTY, the ILGPPGSVY peptide does not extend from the peptide binding groove and thus the contact surface with a T cell receptor is severely diminished.

Abstract 20

Determination of binding affinities between human leukocyte antigens (HLA) and peptides using size exclusion chromatography (SEC) on-line coupled to electrospray ionization - mass spectrometry (ESI-MS)

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A new approach in the determination of binding affinities between human leukocyte antigens (HLA) and binding peptides has been developed using size exclusion chromatography (SEC) and on-line electro spray ionization mass spectrometry (ESI-MS). Protein complexes consisting of HLA A*1101, peptide and beta-2-microglobulin (β_2m) were prepared in solution containing 10 mM ammonium acetate at pH 6.6, incubated for 24 hours at 18°C, fractionated by SEC and analyzed by ESI-MS. Mixing a reference peptide with a known affinity constant (K_D) and a test peptide with an unknown affinity constant, leads to a competition assay where the SEC column separates complex bound peptides from unbound peptides. Ion chromatograms (EIC) specific for reference- and test-peptides were extracted. From the EIC and the known K_D -value, the unknown K_D -value can be calculated. The K_D -values determined by this method were comparable to those obtained from a RIA, or an ELISA. The SEC-ESI-MS assay has several key advantages over many other methods to: primarily including the use of the MS to accurately detect and identify ligand species alleviating the need for more invasive labeling- and identification-procedures such as radiolabeling and antibody detection. Fortunately, in the current implementation, the needed titrations are reduced and the addition of an autosampler allows for assay automation.

Abstract 21

MEK kinase 1 activity is required for definitive erythropoiesis in the mouse fetal liver

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MEKK1 is a JNK activating kinase that is known to be implicated in proinflammatory responses and cell motility. Using mice deficient for MEKK1 kinase activity (*Mekk1^{AKD}*) we show a role for MEKK1 in definitive mouse erythropoiesis. Although *Mekk1^{AKD}* mice are alive and fertile on a 129 x C57/BL6 background the frequency of *Mekk1^{AKD}* embryos that develop past E14.5 is dramatically reduced when backcrossed into the C57/BL6 background. At E13.5 *Mekk1^{AKD}* embryos have normal morphology but are anemic due to failure of definitive erythropoiesis. When



Mekk1^{AKD} fetal liver cells were transferred to lethally irradiated wild-type hosts, mature red blood cells were generated from the mutant cells, suggesting that MEKK1 functions non-cell autonomously. Based on Immunohistochemical and hemoglobin-chain-transcription analysis we propose that the failure of definitive erythropoiesis is due to a deficiency in enucleation activity caused by insufficient macrophage mediated nuclear DNA destruction.

Abstract 22

Natural Killer Cells are Activated by Lactic Acid Bacteria-Matured Dendritic Cells

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Natural killer (NK) cells are lymphocytes of the non-specific immune system capable of lysing cancerous cells and cells altered by viral infection. A non-cytolytic subset of human NK cells serves a regulatory role by secreting cytokines. Gut lactic acid bacteria (LAB) are presumed to gain access to NK cell compartments, as consumption of certain strains of LAB increase *in vivo* NK cytotoxicity. Here, we investigated how human gut flora-derived LAB directly and indirectly, via their interaction with dendritic cells, activate NK cells *in vitro*.

Human blood-derived NK-cells were cultured with suspensions of UV-killed LAB (*Lactobacillus acidophilus* X37 and/or *Bifidobacterium* S13.1) for 2 or 4 days. Immature dendritic cells (iDCs, 17% of total cell number) and anti-IL-12 antibody (α IL-12, 2 μ g/ml) were added to some cultures. Interferon (IFN)- γ concentration in culture supernatants and NK-cell proliferation was measured.

Lb. acidophilus X37 induced proliferation and IFN- γ production in enriched NK cells. The IFN- γ production was mediated by IL-12, possibly derived from DCs present in the NK cell preparation. In contrast, LAB-induced proliferation was independent of IL-12. In the presence of immature DCs, *Lb. acidophilus* X37 potently increased NK-cell IFN- γ production. Production of this cytokine was partially blocked both by an anti-IL-12 antibody and by *Bifidobacterium* S13.1. Both bacteria caused the NK cells to proliferate.

Different strains of LAB thus affect NK cell responses, mainly via maturation of DCs and NK-DC cross-talk, and IFN- γ production and proliferation are induced by different mechanisms. Such activation may have implications for the regulation of the intestinal immune system.

Abstract 23

Human Isolates of Lactic Acid Bacteria Differentially Affect Maturation and Cytokine Production by Human Dendritic Cells

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The intestinal micro flora is indispensable in developing and maintaining homeostasis of the gut-associated immune system. Evidence points toward, that lactic acid bacteria (LAB), such as lactobacilli and bifidobacteria, have beneficial effects on the host. Here we evaluate the potential immunomodulating properties of a range of LAB of human origin. As dendritic cells (DCs) play a pivotal role in the balance between tolerance and immunity to commensal microorganisms; *in vitro* generated immature DCs serve as a suitable model for studying the immunomodulating effects of LAB.

Human immature DCs were generated *in vitro* from monocytes and exposed to UV-killed LAB. The effect of various species of LAB in direct contact with DCs was evaluated. Furthermore the maturation pattern of DCs separated from the bacteria by an epithelial cell layer (Caco-2 cells), which should mimic the intestinal environment, was studied. Cytokine secretion and up-regulation of maturation surface markers on DCs were measured.

LAB isolated from human gut flora differentially affect DC maturation patterns when in direct contact. When separated by an epithelial layer only *E. coli* Nissle, but none of the LAB, lead to DC maturation. Interestingly addition of LAB abolished the response induced by *E. coli* Nissle.

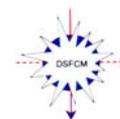
Abstract 24

Microglial Heterogeneity in Injured Central Nervous System

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Microglia are bone marrow-derived cells that constitute a facultative macrophage population when activated by trauma or pathology in the CNS. Endogenous CNS-resident microglia as well as exogenous (immigrant) bone marrow-derived cells contribute to this reactive microgliosis, raising questions about the cellular composition, kinetics and functional characteristics of the reactive microglial cell population following injury. To investigate cellular heterogeneity of reactive microglia, bone marrow chimeric mice reconstituted with green fluorescent protein expressing (GFP⁺) donor bone marrow cells were subjected to axonal lesioning of the entorhino-hippocampal perforant pathway. Microdissected lesion-reactive and contralateral whole hippocampi were dissociated and analyzed by flow cytometry 3, 5 and 7 days post-lesion. Small, but consistent, increases in the proportion of immigrant GFP⁺CD45^{dim} microglia were detected at 3 and 5 days post-lesion. A large population of bone marrow-derived microglia was evident by 7 days post-lesion, when approximately 13% of microglia in the lesion-reactive hippocampi were GFP⁺. Bone marrow-derived immigrant microglia expressed lower levels of CD11b than resident microglia, forming a distinct subpopulation on CD11b/CD45 profiles. A minor subpopulation of CD11b⁺ microglia, primarily of resident origin, expressed the haematopoietic stem cell marker CD34. Post-lesional infiltration of bone marrow-derived cells, and proliferation of bone marrow-derived cells that had taken up residence in the hippocampus prior to lesioning, are both possible sources of the increased number of exogenous cells observed in the lesion reactive hippocampi. Cells immigrating from the blood offer a potential means of therapeutic intervention.

Abstract 25

Molecular characterization of a novel human scavenger receptor cysteine-rich type I transmembrane molecule expressed by natural killer cells and T-cells

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The scavenger receptor cysteine-rich superfamily is a highly conserved group of membrane and/or secreted proteins related to the innate and adaptive immune system. The domain that defines the SRCR family of proteins consists of 100-110 amino-acid residues. Molecules with SRCR domains are divided into two groups based on the localization and number of cysteine residues. Here we report the cloning of a gene encoding human CD163c, a novel member of the group B SRCR-SF.

By searching in the NCBI genome database using murine CD163c as template we have now identified human CD163c (hCD163c). The human CD163c gene maps to chromosome 10, it spans approximately 14 kb and is composed of 13 exons and 12 introns. To obtain a full length cDNA clone of hCD163c, primers in the 3'- and 5'-end of the gene were constructed and RT-PCR was performed using human lung mRNA as template. The longest cDNA sequence of human CD163c found until now is 2200 bp encoding 5 SRCR domains and a cytoplasmic domain. We assume this possible secreted form of hCD163c lacking the transmembrane domain is an alternative spliced isoform of hCD163c and just one of many splice forms of the human gene.

RT-PCR on different human cell lines has shown hCD163c to be expressed by NK-cells, T-cells and B-cells, but not in macrophages. In addition quantitative real-time PCR analysis on human blood-fractions stimulated with pokeweed mitogen and conA has shown that hCD163c is down-regulated in activated T-cells and B-cells.

Quantitative real-time PCR on 22 different human tissues showed high expression of hCD163c in the small intestine and colon.

Abstract 26

CCR5 and CXCR3 are dispensable for liver infiltration, yet CCR5 protects against T-cell mediated virus induced hepatic steatosis.

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CCR5 and CXCR3 are important constituents of the effector T-cell chemokine receptor repertoire. Thus T-cells up-regulate CCR5 and CXCR3 upon activation and the principal chemokine ligands are expressed within inflamed tissues. Accordingly several studies have pointed to non-redundant roles of these receptors in models of allograft rejection, viral



infection and autoimmunity. In spite of this considerable controversy exists and some difficulties have emerged in the attempt to compare human epidemiological data and murine experimental data. No place more so than in the virally infected liver where murine models have pointed to requirements for CCR5 in virus induced inflammation and human studies suggest that CCR5 deficiency accelerates virus induced liver failure. In the present study we analyse CCR5, CXCR3 and CCR5/CXCR3 deficient mice for virus induced liver inflammation, generation of adaptive immunity, virus control and immunopathology. Our results indicate that CCR5 and CXCR3 are largely dispensable for tissue infiltration and virus control. In contrast adaptive immunity is somewhat accelerated in CCR5 and CCR5/CXCR3 deficient mice and the absence of CCR5 is associated with induction of a T-cell mediated immunopathology consisting of marked hepatic steatosis.

Abstract 27

Adenovirus based vaccines for Lymphocytic Choriomeningitis Virus (LCMV)

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Vaccination strategies based on the induction of protective T-cell memory holds therapeutic promise, as several infectious agents are not easily defeated by antibody responses. Of vaccination candidates capable of inducing such T-cell memory, adenovirus are among the most promising. The vectors are easy to manipulate and produce, and induce robust immune responses. The present study characterise the memory and protection induced by adenovirus vectors expressing minimal epitopes from the model virus LCMV. The vectors induce robust T-cell memory for months after a single immunisation, and protect the mice against infection with LCMV in doses that would exhaust the immune response of unvaccinated mice. In addition, vaccination with LCMV virus offers short-lived protection against a normally lethal LCMV intracerebral challenge. This protection gradually declines and it's persistence is dependent on the epitope chosen for vaccination. Our results demonstrates that T-cell memory against single epitopes is protective against lethal viral infection and suggests that adenovirus vectors can be used as tools for defining the optimal requirements for T-cell memory and protection.

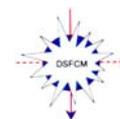


Exhibitors:

	AH Diagnostics
	BD Biosciences A/S
	Bie & Berntsen A/S
	BioTech Line
	DakoCytomation
	R&D System Europe Ltd
	Ramcon A/S
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	Soren	Buus
5	Lisbet	Bærentzen
	Jan	Christensen
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