8th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

Molecular Determinants of T-Cell Immunity

June 7-10, 2008

Štrbské Pleso, Slovakia

PROGRAMME

8th EFIS-EJI TATRA IMMUNOLOGY CONFERENCE

Molecular Determinants of T-Cell Immunity

CONFERENCE VENUE

Hotel Patria, Štrbské Pleso, High Tatra Mountains, Slovakia

June 7-10, 2008

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Saturday, 7th June

Arrival of participants

Sunday, 8th June

8:15 - 8:30 **Opening the Conference**

8:30 -12:10 ***Session 1:***

Chairperson: **H. Stockinger** (Vienna)

8:30 - 9:20 **F. Y. Liew** (Glasgow): *Novel cytokines in inflammatory diseases*

9:20 - 10:10 **A. Erdei** (Budapest): *Complement and adaptive responses*

10:10 - 10:30 ***Tea/Coffee break***

10:30 - 11:20 **A. Hayday** (London): *Immunosurveillance of tissues by T cells*

11:20 - 12:10 **R. Spisek** (Prague): *Immune response to cancer stem cells dictates cancer's course in monoclonal gammopathies*

12:10 - 13:00 ***Lunch***

13:00 - 16:10 **Afternoon trip**

16:30 - 19:00 ***Session 2:***

Chairperson: **J. Ivanyi** (London)

16:30 - 17:20 **G. Wick** (Innsbruck): *The immunology of fibrosis*

17:20 - 18:10 **Z. Hel** (Birmingham, AL): *Cytotoxic T cell-based vaccines against HIV and cancer*

18:10 - 19:00 **F. Nimmerjahn** (Erlangen): *Antibodies and Fc-receptors: a sweet relationship*

19:30 - 23:30 **Welcome Party**

Monday, 9th June

8:30-12:10 ***Session 3:***

Chairperson: **G. Wick** (Innsbruck)

8:30 - 9:20 **P. Garside** (Strathclyde): *Visualising immune responses in real time in vivo*

9:20 - 10:10 **A. Bensussan** (Paris): *Is killer cell Ig-like receptors on cutaneous CD4+ T cell lymphomas an advantage for their expansion?*

10:10 - 10:30 ***Tea/Coffee brea***

10:30 - 11:20 **M. Malkovsky** (Ann Arbor): *Gamma/delta T Cells: from bench to bedside*

11:20 - 12:10 **P. Peterson** (Tartu): *Autoimmune Regulator (AIRE): From disease to molecular mechanism*

12:10 - 13:00 ***Lunch***

13:00 - 16:10 **Afternoon trip**

16:30 - 18:00 ***Session 4: Selected poster presentations (each 13 min.)***

Chairperson: **A. Hayday** (London)

A. Caignard (Paris, France): *KIR2DL1 receptor induces opposite signals in human CD4$^+$ T cells*

Chan Woon Lin (London, UK): *Role of IL-17 produced by Th17 and NKT cells in aortic wall degeneration in Atherosclerotic Abdominal Aortic Aneurysm*

M. Novy (Vienna, Austria): *Analysis of human FOXP3 promoter regulation by measurement of destabilized enhanced green fluorescence protein expression in flow cytometry.*

M. Šírová (Prague, Czechia): *Anti-tumor immunity in mice treated with HPMA-based copolymer conjugates of doxorubicin*

E. Svirshchevskaya (Moscow, Russia): *Effect of ex vivo generated natural killers on Wnt-1 tumor growth in mice*

J. Tel (Nijmegen, Netherlands): *Generation and characterization of clinical grade pDCs for pDC-based anti-tumor vaccination studies*

P. Zaccone (Cambridge, UK): *S. mansoni soluble egg antigen (SEA) induces de novo generation of Foxp3$^+$ regulatory CD4$^+$ T cells that can prevent type 1 diabetes in NOD mice.*

18:30 - 19:30 ***Dinner***

20:00 - 22:00 ***Poster session (with refreshments and wine)***

Tuesday, 10th June

8:30 - 12:10 ***Session 5:***

Chairperson: **A. Erdei** (Budapest)

8:30 - 9:20 **C. Figdor** (Nijmegen): *Cancer immunotherapy; Towards the next generation of dendritic cell vaccines*

9:20 - 10:10 **H. Walczak** (London/Heidelberg): *Pro- and anti-apoptotic treatment options in cancer therapy*

10:10 - 10:30 ***Tea/Coffee break***

10:30 - 11:20 **P. Romero** (Lausanne): *Cancer vaccines based on molecularly defined tumor antigens and adjuvants*

11:20 - 12:10 **S. Nedospasov** (Moscow): *Immunological consequences of TNF blockade in vivo*

12:10 - 13:00 ***Lunch***

13:00 - 16:10 **Afternoon trip**

16:30 - 18:45 ***Session 6:***

Chairperson: **E. Liew** (Glasgow)

16:30 - 17:20 **McSorley** (Minneapolis): *CD4 responses to Salmonella*

17:20 - 18:10 **N. Minato** (Kyoto): *Rap1 signal in immune system: Lymphocyte development, autoimmunity and leukemia*

18:10 - 18:45 **Closing of the conference**

19:30 - 23:30 **Farewell Party**

Wednesday, 11th June

Departure

ABSTRACTS OF THE TALKS BY INVITED SPEAKERS

STRUCTURE AND FUNCTION OF KIRS EXPRESSED BY NORMAL AND MALIGNANT CD4$^+$ LYMPHOCYTES

Armand Bensussan and Anne Marie-Cardine

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We previously reported that malignant lymphocytes isolated from patients exhibiting a Sézary syndrome which is a subtype of cutaneous T cell lymphomas expressed KIR3DL2/CD158k at their cell surface. Importantly, the expression of this inhibitory NK receptor is highly restricted to the CD4$^+$ Sézary tumor cells present either in the blood or in the skin of the patients. Indeed, normal CD4$^+$ lymphocytes isolated from healthy individuals, or from patients with inflammatory skin diseases, did not express KIR3DL2. In addition, we recently found that in some patients other killer cell immunoglobulin-like receptors (KIR) such as CD158a/h or CD158b/j can also be expressed by the malignant lymphocytes. We first demonstrated that KIR3DL2/CD158k receptor is functional on Sézary cells, as assessed by the recruitment of the tyrosine phosphatase SHP-1 upon activation. Furthermore, we observed that KIR3DL2 inhibitory signaling results in a decrease in the CD3-induced cell proliferation and activation of c-Jun NH2-terminal (Jnk) kinase pathway. In contrast, CD158a/h or CD158b/j co-engagement with CD3 induced an increased malignant cell proliferation when compared to CD3 triggering alone. Although biochemical studies revealed that, in this case, Sézary cells express both inhibitory (CD158a or b) and activating (CD158j or h) receptors, we establish that only the activating forms are functional in the malignant cells. Indeed, no recruitment of SHP-1 by the inhibitory receptors was detected following cell activation, suggesting that this receptors-dependent signaling pathway is defective in Sézary cells. Activating receptors usually interact with adaptor molecules that promote the downstream recruitment of Syk-family protein tyrosine kinases. However, the regular adaptor proteins DAP10 and DAP12 are not expressed in Sézary cells, and we did not evidenced an association of CD158j or CD158h with CD3z in activated malignant cells. We

further developed $CD4^+$ T cell lines derived from the blood of healthy donors that only expressed KIR2DS2/CD158j. Consequently, CD158j engagement resulted in an enhanced CD3-mediated cell growth and Erk phosphorylation. Here again, we did not detect any interaction of the activating receptor with known adaptor proteins upon activation, suggesting the involvement of a non-conventional adaptor molecule within the activation cascade. Further studies will be needed to delineate the DAP12-independent Jnk pathway activation observed in malignant Sézary cells, and to understand the role of KIR in the pathophysiology of the disease.

COMPLEMENT AND ADAPTIVE RESPONSES

Anna Erdei[1], *Zsuzsa Bajtay*[1], *Eszter Csomor*[1], *Noémi Sándor*[1], *Steffen Thiel*[2], *Gerard J. Arlaud*[3]

[1]Eötvös L.University, Budapest, Hungary (anna.erdei@freemail.hu)
[2]Dept. Med. Microbiol.Immunol., University of Aarhus, Denmark
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Dendritic cells (DC) link innate and adaptive immunity by their ability to present antigens to naive T lymphocytes. Maturation of DC, a decisive step to initiate T-cell activation is known to be induced by several stimuli, including inflammatory cytokines, microbial products and immobilized IgG. Our aim is to reveal how complement, a major system of innate immunity influences maturation and function of human DC. Since immune complexes occuring *in vivo* contain two major complement proteins C1q and C3, we investigated their effect on the maturation and function of DC. We found that immobilized C1q induces maturation of monocyte-derived DC (MDC) to a similar extent as LPS and induces NFκB translocation from the cytoplasm to the nucleus. Secretion of IL-6, IL-10 and IL-12 along with the T-cell stimulatory capacity of C1q-matured DC were almost as high as in the case of LPS-stimulated cells. TNFα, IL-10 and IFNγ were measured in the supernatants of MDC-T cell cocultures, as well. Our data suggest that interaction of C1q with imMDC generates a TH1-type response. Regarding the effect of C3 and C3-derived fragments, an enhanced CD86 expression was detected, while the level of CD83 and MR did not change. We found that C3-treated DC strongly stimulated the proliferation of allogeneic T cells and the production of IL-6 and TNF-α were also increased. Modeling a possible *in vivo* situation by coculturing macrophages and DC derived from the same donor we found that activated macrophages are able to opsonize adjacent DC with C3-fragments and initiate their maturation. These data suggest that complement proteins produced locally or activated in various body-fluids may play an important modulatory role in the initiation of the adaptive responses by interacting with DC.

TOWARDS THE NEXT GENERATION OF DENDRITIC CELL VACCINES.

Carl G. Figdor Gosse Adema, Keep Punt, Erik Aarntzen & Jolanda de Vries

Department of Tumor Immunology and Medical Oncology, Nijmegen Centre for Molecular Life Sciences (NCMLS), Nijmegen, The Netherlands. (c.figdor@ncmls.ru.nl)

We exploit dendritic cells (DCs) to vaccinate melanoma patients. We recently demonstrated a statistical significant correlation between favorable clinical outcome and the presence of vaccine-related tumor antigen specific T cells in delayed type hypersensitivity (DTH) skin biopsies. While we find immunological responses in 30-50% of the patients, favorable clinical outcome is only observed in a minority of the treated patients. Therefore, it is obvious that current DC-based protocols need to be improved to increase clinical efficacy. For this reason, we study in small *proof of principle* trials the fate, interactions and effectiveness of the injected DCs.
We recently compared DC loaded with tumor antigen specific MHC class I binding peptides alone, in combination with MHC class II binding peptides or with defined tumor antigen mRNA (gp100 and tyrosinase). The results show that the presence of supplementary tumor antigen-specific MHC class II epitopes result in an T helper response that might be beneficial for the clinical outcome in these patients. Furthermore, comparing different routes of administration we observed that intranodal injection is not always successful (MRI) and that only a small proportion of the intradermally administered DCs reach the lymph nodes (scintigraphy). Our preliminary data clearly indicate that the cells that reach the lymph nodes are fully mature DCs that are able to induce an immune response *in vivo*. Currently we study whether pre-conditioning of the vaccination site results in enhanced migration. Updated results and strategies to improve DC vaccination will be presented.

CYTOTOXIC T CELL-BASED VACCINES AGAINST HIV AND CANCER

Zdenek Hel, Warren Denning, Jun Xu, Siqi Guo.

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The induction of potent, long-lasting cytotoxic CD8+ T lymphocytes (CTLs) controlling tumor growth by direct killing and activation of local anti-tumor immune responses is a paramount goal of cancer immunotherapy. Although multiple immunization strategies, such as dendritic cells (DCs) - based therapies, efficiently induce high levels of CTLs, the increase is temporary and the frequency of specific CTLs quickly returns to pre-immunization levels. We have proposed a novel approach based on the induction of low-level antigenic microchimerism resulting in continuous presentation of antigen and maintenance of functional effector T cell responses. Low numbers of transgene-expressing hematopoietic stem cells (HSCs) were transplanted into syngeneic mice pre-treated with non-myeloablative busulfan-based regimen. In contrast to DC-immunized mice in which the antigen-specific CTLs rapidly declined 3-4 weeks post-immunization, HSC recipient mice displayed detectable CTLs for at least 24 weeks following transplantation. Majority of antigen-specific $CD8^+$ T cells displayed central memory phenotype, efficiently killed target cells *in vivo*, and protected recipients against the growth of EG.7 thymoma. In an alternative approach, we explored the use of activated B cells as an alternative to DCs as cellular vaccine for cancer immunotherapy. We demonstrated that immunization with low numbers of antigen-expressing B cells resulted in an induction of functional cytotoxic T cell responses and protection against tumor growth in therapeutic setting.

Induction of protective CTL responses represents hope for the vaccine against HIV. We have explored the benefit of immunization with early regulatory proteins (Rev, Tat, Nef), as timely CTL response against these antigens may destroy infected cell prior to the budding of infectious particles. Immunization with early antigens in addition to structural proteins resulted in a delay in the onset and decrease in the extent of acute viremia following mucosal challenge exposure to highly pathogenic simian immunodeficiency virus. These data provide evidence of the importance of nonstructural HIV antigens as components of an HIV-1 vaccine.

IL-33 REDUCES THE DEVELOPMENT OF ATHEROSCLEROSIS

FY Liew

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Atherosclerosis is a chronic inflammatory disease of the vasculature commonly leading to myocardial infarction and stroke. Here, we show that IL-33, a novel IL-1-like cytokine that signals via ST2, can reduce atherosclerosis development in $ApoE^{-/-}$ mice on a high fat diet. IL-33 and ST2 are presence in the normal and atherosclerotic vasculature of mouse and humans. While control PBS-treated mice developed severe and inflamed atherosclerotic plaques in the aortic sinus, lesion development was profoundly reduced in IL-33-treated animals. IL-33 also markedly increased levels of IL-4, IL-5, and IL-13 but decreased the levels of IFNγ in serum and lymph node cells. IL-33-treatment also elevated the levels of total serum IgA, IgE and IgG_1 but decreased $IgG_{2a,}$ consistent with a Th1-to-Th2 switch. IL-33-treated mice also produced significantly elevated anti-ox-LDL antibodies. Conversely, mice treated with soluble ST2, a decoy receptor which neutralizes IL-33, developed significantly larger atherosclerotic plaques in the aortic sinus of the $ApoE^{-/-}$ mice compared to control IgG-treated mice. Together these results demonstrate that IL-33 may provide a novel therapeutic approach in the treatment or prevention of atherosclerotic vascular diseases. The potential role of IL-33 in other inflammatory diseases will be discussed.

RAP SIGNAL IN IMMUNE SYSTEM: LYMPHOCYTE DEVELOPMENT, AUTOIMMUNITY AND LEUKEMIA

Nagahiro Minato

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Rap (Ras-proximity) GTPase plays a crucial role in regulating homeostatic proliferation of hematopoietic progenitor cells (Cancer Cell, 2003, Cancer Res., 2004). In order to investigate the role of Rap in lymphocyte development, we conditionally expressed Rap1E63 (dominant active mutant), C3G-F (farnecylated Rap GEF), Rap1A17 (dominant negative mutant) or Spa-1 (Rap GAP) in T- and B-lineage cells. Abrogation of endogenous Rap signaling by lck promoter-driven expression of Spa-1 or Rap1A17 resulted in almost complete block of thymic □-selection, and it was indicated that Rap mediated pre-TCR signaling to promote Notch-dependent DN thymocyte survival and expansion. On the contrary, retroviral expression of C3G-F in hematopoietic progenitors followed by BM transplantation markedly enhanced thymic repopulation and eventually caused Notch-dependent T-ALL with characteristic *Notch-1* mutations bypassing pre-TCR (Blood, 2008). On the other hand, mb-1 promoter-driven expression Rap1A17 resulted in severe defect of B cell development at pre-B cell stage in BM, and the mice showed marked reduction in follicular B cells in the spleen with MZ B and peritoneal B1 cells being barely affected. In contrast, deregulated endogenous Rap signaling in Spa-1-/- mice caused age-dependent increase in B1 cells associated with lupus-like autoimmunity often followed by B1-CLL (Immunity, 2006). Altogether, Rap signal is crucial for both early T-cell and B-cell development, and its deregulation may lead to characteristic diseases including leukemia (Adv. Immunol, 2007).

AUTOIMMUNE REGULATOR: FROM DISEASE TO MECHANISM

Pärt Peterson

Molecular Pathology, University of Tartu, Tartu, Estonia (part.peterson@ut.ee)

AIRE (autoimmune regulator) protein is responsible for the expression of peripheral self-antigens in thymus. In humans, AIRE defect causes a monogenic autoimmune disease named APECED (autoimmune polyendocrine candidiasis ectodermal dystrophy) or APS1 (autoimmune polyendocrine syndrome type 1). The protein is expressed in medullary epithelial cells, which are central in the negative selection of self-reactive T cells. In concordance, Aire deficient mice have a defect in thymic negative selection of the self-antigens. AIRE intranuclear localization and protein domains are indicative of its function in transcriptional regulation. As a transcriptional activator, AIRE can activate reporter genes in vitro and interacts with CREB-binding protein (CBP), one of the common coactivator proteins having histone acetyltransferase activity. We have recently found that AIRE selectively interacts with histone H3 through its first PHD finger domain, which provides a new link between the status of histone modifications and the regulation of tissue-restricted antigen expression in thymus. Studies on the AIRE function will help to understand the mechanism how the tolerance is formed.

CANCER VACCINCES BASED ON MOLECULARLY DEFINED TUMOR ANTIGNES AND ADJUVANTS

Pedro Romero

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The molecular identification of T cell defined tumor antigens provided the rationale for the design of therapeutic cancer vaccines. To date, several hundreds of early phase clinical trials, testing a large variety of vaccines, have been reported. While the learning curve for both vaccine formulation and monitoring of antigen specific T cell responses has steadily progressed, clinical impact of such vaccines remains relatively low. Indeed, the efficacy of vaccination in terms of elicitation of specific T cell immunity has reached 100% for certain formulations and is generally 40-80% in the majority of recently reported trials. In contrast, metanalysis of their clinical results found an objective clinical response rate of around 3%. However, estimates of favourable clinical outcome associated to vaccination situate the proportion of patients with such outcome at around 25% of vaccinees. Moreover, induction of immunity correlates more often than not with favourable clinical course. The Melan-A/MART-1 protein is expressed in melanocytes and the majority of primary and metastatic melanomas. An immunodominant HLA-A2 restricted epitope defined by the 26-35 peptide lends itself to detailed studies in both healthy individuals and metastatic melanoma patients because of the unusually massive repertoire of T cell precursors available which are generated at high numbers in the thymus and maintained in the peripheral immune system. A series of phase I clinical studies performed in Lausanne have showed that it is possible to induce strong CD8 T cell responses in advanced melanoma patients when the antigenic peptide is injected subcutaneously as an emulsion in Montanide ISA51 together with a small amount of CpG-ODN, a class B TLR-9 agonist, at monthly intervals. Detailed analyses of the vaccine induced T cell response by combined multiparameter flow cytometry and molecular approaches to gene expression and TCR repertoire have allowed to obtain a detailed picture of the response. I will discuss our findings and their implications for the rational development of molecularly defined cancer vaccines.

IMMUNE RESPONSE TO CANCER STEM CELLS DICTATES CANCER'S COURSE IN MONOCLONAL GAMMOPATHIES

Radek Spisek

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Specific targets of cellular immunity in human premalignancy are largely unknown. Monoclonal gammopathy (MGUS) represents a preneoplastic lesion to multiple myeloma (MM). We performed a large comparative analysis of antigenic targets of specific immunity in patients with premalignancy (MGUS) versus clinical cancer (MM). We show that antigenic targets of spontaneous immunity in MGUS differ from myeloma. MGUS patients frequently mount a humoral and cellular immune response against SOX2, a gene critical for self renewal in embryonal stem cells, and expressed in the clonogenic tumor progenitors. Expression of SOX2 in MGUS is restricted to CD138neg. compartment that is enriched in clonogenic progenitors. In contrast to MM where SOX2 expression is acquired by more differentiated progeny. Cellular immunity to SOX2 inhibits clonogenic growth of tumors and predicts clinical outcome. Immunity to antigens expressed by tumor progenitor cells may be critical for prevention and therapy of human cancer.

PRO- AND ANTI-APOPTOTIC TREATMENT OPTIONS IN CANCER THERAPY

Henning Walczak

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TRAIL/Apo2L (TNF-related apoptosis-inducing ligand) is a promising anticancer agent due to its ability to selectively induce apoptosis in tumour cells but not in most non-transformed cells. To appreciate its full clinical potential it is important to understand the biochemical principles which govern sensitivity and resistance to apoptosis induction by TRAIL. In this talk the mechanisms of TRAIL sensitivity versus resistance will be addressed in cancer cell lines as well as in primary human hepatocytes and in primary human cancer cells. Then, data on the identification of a specific function of TRAIL-R in the suppression of metastasis from autochthonous murine tumours will be presented. In addition, a short overview on the current clinical developments with TRAIL receptor agonists, especially their potential in novel combinatorial treatments, will be discussed. Finally, a rather unexpected therapeutic application of a blocker of CD95 ligand in specific cancer treatment protocols will be discussed

THE IMMUNOLOGY OF FIBROSIS

Georg Wick, Christina Mayerl, Dolores Wolfram, Evelyn Rabensteiner, Alexandar Backovic

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Fibrosis, i.e. excessive extracellular matrix (ECM) formation, is a major health problem that receives insufficient attention in basic and clinical research with respect to its aetiology, pathogenesis, diagnosis and therapy.
In principle, fibrosis can occur as a consequence of many different pathologic conditions. The most important of these are:

(a) Fibrosis after tissue damage, e.g. post-operative adhesions, burns, alcoholic and post infectious liver cirrhosis, etc.

(b) Fibrosis after inflammatory diseases, e.g. infections, arteriosclerosis, connective tissue diseases such as scleroderma, etc.

(c) Fibrosis around foreign body implants, e.g. silicone mammary implants, etc.

(d) "Spontaneous" fibrosis, e.g. keloids, Dupuytren's contracture, etc.

(e) Tumors, e.g. neurofibromatosis etc.

Although the endstage of the development of fibrosis, i.e. the proliferation of fibroblasts and deposition of excessive amounts of collagenous and non-collagenous ECM proteins seems to be very stereotypic, the pathologic processes initiating and perpetuating these processes are rather diverse. However, in all cases studied in our laboratory the earliest stages of fibrotic conditions are characterized by immunologic-inflammatory hallmarks, i.e. perivascular infiltration by mononuclear cells and a subsequent dysbalance between anti- and profibrotic cytokine profiles. In all of these instances, the original antigenic stimuli triggering the lymphoid infiltrations have not yet been identified. Within the framework of

this presentation three examples of fibrotic diseases will be discussed, *viz* progressive systemic sclerosis (SSc-Scleroderma), peri-mammary silicone implant (SMI) connective tissue capsule formation and – as a representative of so called "spontaneous" fibroses – Dupuytren's contracture. Emphasis will be put on the phenotypic and functional characterization of the lymphoid cell infiltrate as well as molecular biological and proteomic analyses of the pathological ECM accumulations.

ABSTRACTS OF THE SELECTED POSTER PRESENTATIONS

1.

KIR2DL1 RECEPTOR INDUCES OPPOSITE SIGNALS IN HUMAN CD4$^+$ T CELLS

Emmanuelle Fourmentraux-Neves, Abdelali Jalil, Salem Chouaib, Georges Bismuth, Anne Caignard

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Inhibitory killer Ig-like receptors (KIR2DL1-2/3) which bind to HLA-C molecules are expressed by human Natural Killer cells and effector memory CD8$^+$ T cell subsets. These receptors suppress CD8+ T cell activation through recruitment of the Src homology 2 domain-containing protein tyronine phosphatase 1 (SHP-1). To further analyse the yet largely unclear role of inhibitory KIR receptors on CD4+T cells, KIR2DL1 transfectants were obtained from a CD4$^+$ T cell line and primary cells. The transfection of CD4$^+$ T cells with KIR2DL1 dramatically increased the T cell receptor (TCR)-induced production of IL-2 independently of ligand binding, but inhibited TCR-induced activation after ligation. KIR-mediated TCR activation requires intact ITIM motifs, involves KIR2DL1-ITIM phosphorylation, SHP-2 recruitment, ZAP-70 and PKC-□ phosphorylation. Synapses leading to activation were characterized by an increase in the recruitment of p-Tyr, SHP-2 and p-PKC-□ but not of SHP-1. In contrast, the KIR2DL1/HLA-Cw4 interaction led to a strong synaptic accumulation of KIR2DL1 and the recruitment of SHP-1/2, inhibiting TCR-induced IL-2 production. KIR2DL1 may induce two opposite signaling outputs in CD4$^+$ T cells, depending on whether the KIR receptor is bound to its ligand. These data highlight unexpected aspects of the regulation of T cells by KIR2DL1 receptors, the therapeutic manipulation of which is currently being evaluated.

2.

ROLE OF IL-17 PRODUCED BY TH17 AND NKT CELLS IN AORTIC WALL DEGENERATION IN ATHEROSCLEROTIC ABDOMINAL AORTIC ANEURYSM.

Woon Ling Chan[1], Nada Pejnovic[1] and Hamish Hamilton[2]

[1]Translational Medicine & Therapeutics, William Harvey Research Institute, Barts and The London School of Medicine, London, UK and [2]General Surgery, Barnet and Chase Farm Hospital, Enfield, UK (W.L.Chan@qmul.ac.uk)

Th17 cells are a novel subset of $CD4^+$ Th cells involved in the pathogenesis of various chronic inflammatory diseases formerly categorized as Th1-mediated disorders. Although we have demonstrated the presence of IFNγ-producing Th1 and natural killer (NK)T cells in atherosclerotic abdominal aortic aneurysm (AAA) tissues, the role of Th17 is not known. Using flow cytometry with intracellular cytokine staining of ex-vivo lymphoid cell subsets isolated from atherosclerotic AAA tissues, we show here that a high proportion of the Th and NKT cells produce both IL-17 and IFNγ or only IL-17. This is the first demonstration of ex-vivo NKT cells producing IL-17. The Th17 and NKT cells also produce low levels of vascular endothelial growth factor (VEGF), which could explain the increased endothelial cells and neo-vascularization in the media of AAA vessel walls. IL-17 induces vascular smooth muscle cells (VSMC) explanted from AAA tissue to produce matrix metalloproteinases (MMP)-2, MMP-8 and also platelet-derived growth factor (PDGF) *in vitro* and this is supported in immunohistochemistry by VSMC actin co-staining with MMP-8 in AAA tissues. Furthermore, IL-17 also stimulates VSMC to migrate and proliferate *in vitro*, and this could be via IL-17 induction of MMPs. Interestingly, IL-23 co-stains with VSMC actin in AAA tissues and cholesterol can stimulate VSMC to produce IL-23 *in vitro*. This suggests that in the presence of cholesterol in the lesion microenvironment, VSMC inversely stimulates Th17 proliferation and activation via IL-23. In conclusion, this study suggests that IL-17 has a pro-inflammatory role in the pathogenesis of atherosclerotic AAA, since the balance in VSMC migration, proliferation and survival is critical in determining atherogenesis and aneurysm disease progression and stability.

3.

ANALYSIS OF HUMAN FOXP3 PROMOTER REGULATION BY MEASUREMENT OF DESTABILIZED ENHANCED GREEN FLUORESCENCE PROTEIN EXPRESSION IN FLOW CYTOMETRY.

Michael Novy, Paul Eckerstorfer, Sebastian Burgstaller-Muehlbacher and Hannes Stockinger

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Regulatory T cells (Treg) maintain peripheral immune tolerance and thus play a major role in immune responses and prevention of autoimmunity. At present the best-characterized Tregs are described amid the CD4+CD25+ T cell population. However, this population is obviously heterogeneous as one can distinguish between natural occurring nTregs and induced CD4+CD25+ iTregs. While iTregs are discussed to represent an altered stage of T cell differentiation arising during the course of an adaptive immune response, natural Tregs develop during ontogeny through so far unknown mechanisms in the thymus. Forkhead box protein 3 (Foxp3) is indispensable for the development of Tregs and still denoted as the most specific marker of Tregs. However, Foxp3 is not restricted to Tregs as it became clear now that Foxp3 is also transiently upregulated in activated human T cells. Foxp3 acts as a transcription factor and binding sites were identified in approximately seven hundred genes. Although Foxp3 is involved as a key factor in gene regulation and signaling pathways, little is known about its own regulation and biochemistry. To investigate human FOXP3 gene expression we analyzed three prominent evolutionary conserved regions (ECRs) upstream of the transcription start site, which we named ECR1-3. We show that ECR2 and ECR3 fragments, which are located between 1.3 and 2.0 kb and between 5.0 and 6.0 kb upstream of transcription initiation, display basal transactivation in 293 cells. Reporter constructs derived from ECR1, with position -0.6/+0.23 kb the most proximal ECR in respect to transcription initiation, remained almost inactive. We modified a pd2-EGFP-N1 vector (= ΔCMV) to investigate FOXP3 promoter driven expression of destabilized green fluorescence protein (dEGFP) in human T-cells and T-cell lines. Screening for factors modulating the transcriptional activity of ECR1 revealed dominant transactivator and repressor candidates.

4.

ANTI-TUMOR IMMUNITY IN MICE TREATED WITH HPMA-BASED COPOLYMER CONJUGATES OF DOXORUBICIN

Milada Sirova [1], *Lubomir Kovar* [1], *Jiri Strohalm* [2], *Vladimir Subr* [2], *Daniela Plocova* [2], *Karel Ulbrich* [2], *Blanka Rihova* [1]

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Various conjugates of cytotoxic drugs based on water-soluble copolymer *N*-(2-hydroxypropyl)meth-acrylamide (HPMA) were studied for their significant anti-tumor capacity, low side-effects, and tumor-specific drug delivery. A conjugate containing doxorubicin (Dox) and human non-specific immuno-globulin (Dox-HPMA-HuIg) as the actively/passively targeting moiety was highly effective in experimental mouse tumor models. The complete regression of tumors was related to the development of specific immunity, ensuring long-lasting protection of the animals against the tumor re-challenge. We show that the mice developed a weak anti-tumor immune response already at the time of the treatment (day 11 post-transplantation in EL-4 lymphoma). Induction of the anti-tumor memory depended on the treatment regime and dose of the conjugate. Using the tumor neutralization (Winn's) assay *in vivo*, we demonstrated higher anti-tumor activity of immunocompetent cells derived from animals with the treatment-related tumor regression than in animals with the disease relapse. Both the direct toxic effect of the Dox-HPMA-HuIg conjugate on the tumor and an effective stimulation of the immune response are involved in successful treatment. Conjugate-induced immunogenic tumor cell death could be the reason for the potent immune stimulation.

5.
EFFECT OF EX VIVO GENERATED NATURAL KILLERS ON WNT-1 TUMOR GROWTH IN MICE

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For the development of anti-tumor immunotherapy it is essential to understand whether and when immune system recognizes tumor cells and what types of immune cells participate in anti-tumor response. The purpose of this work was to compare the effects of ex vivo generated subpopulations of Th1, Th2, Tc1, Tc2, and NK cells on the growth of breast carcinomas induced by hyperexpression of Wnt-1 gene in mice.
Donor splenic CD4, CD8 and NK cells purified by magnetic separation were cultivated in vitro for 2 weeks in the presence of specific cytokine cocktails biasing either Th1, Th2, Tc1 or Tc2 differentiation. All T cells were activated by CD3/CD28 coated beads. NK cells were cultivated with IL-2 only. Ex vivo generated cells were injected intravenously into mice with Wnt-1 tumors implanted into mouse fat pads 7 days before immune cell injection. We showed that injection of Th1/Tc1 cells did not affect tumor growth; injection of Th2/Tc2 accelerated it, while NK injection suppressed Wnt-1 tumor growth. The effect of NK cells was transient and 4 weeks later average sizes of tumors in NK and control groups were comparable. It is possible that single injection of NK cells was not sufficient for tumor suppression. Analysis of lymphoid subpopulations in mice with 1 g tumors in treated and control groups as well as in mice without tumors was conducted using flow cytometry. There was no significant difference between mice. Wnt-1 tumors were infiltrated by CD11b>CD4>CD8>CD19>NK1.1>CD4+CD25+ lymphoid cells. There was a high variation between mice in the numbers of tumor infiltrating lymphocytes. However there was no reliable difference between treated and control groups.

6.

Generation and characterization of clinical grade pDCs for pDC-based anti-tumor vaccination studies

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Dendritic cells (DCs) are the most professional antigen presenting cells of the immune system with the unique capability to stimulate naïve T-cells. DCs are found in blood, lymph, lymphoid organs and non-lymphoid organs. DCs mediate antigen transport and presentation in secondary lymphoid tissues, which is crucial for the initiation and maintenance of T-cell mediated immune responses. Three different natural occurring subsets of DC precursors can be defined: $CD14^+$ monocytes, $CD11c^-$ plasmacytoid preDC and $CD11c^+$ myeloid preDC. Plasmacytoid DCs (pDCs) are found to be present in the human immune system in very low amounts, approximately 0,5 % of peripheral blood cells consists of pDCs. Human pDCs are characterized as $CD4^+$ $CD45RA^+$IL-3Rα^+ (CD123) $ILT3^+ILT1^-$ BDCA-2^+ BDCA-4^+ $CD11c^-$ $lineage^-$ cells. Furthermore, pDC express Toll Like Receptor 1 (TLR1), TRL7 and TLR9 which enables them to be activated by viral or bacterial stimulation and secrete high levels of type I IFN (innate immune response). Upon encountering viral or bacterial stimuli, pDCs have a coordinated migration towards the T-cell rich areas through the high endothelial venules in the lymph nodes. Subsequently, pDC can initiate an adaptive immune response. Several groups have shown that pDC are capable of inducing strong human anti-tumor immune responses in-vitro. But also pDCs in mouse models have been proposed to induce and expand tumor-specific cytotoxic T-cells. This makes pDCs very interesting tools for application in anti-cancer adoptive immunotherapy. In our study we focused on the generation of clinical applicable mature pDCs which secrete high amounts of type I IFN, and have high migratory and T-cell immunostimulatory capabilities. In order to generate large amounts of highly purified clinical grade pDC (>95%) we made use of the Clinimacs system (Miltenyi Biotech). In addition, for the activation of pDCs we made use of clinical grade TLR ligands. In conclusion, we are able to generate clinical grade pDCs which have all the functional characteristics necessary for optimal application in in-vivo vaccination studies.

7.

S. mansoni soluble egg antigen (SEA) induces _de novo_ generation of Foxp3$^+$ regulatory CD4$^+$ T cells that can prevent type 1 diabetes in NOD mice.

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Schistosome worms and their antigens are known to be very powerful modulators of the mammalian immune system. In particular *S. mansoni* soluble egg (SEA) has been used in several *in vivo* and *in vitro* system/models to identify the cellular and molecular mechanisms of parasite immune regulation. We have previously found that SEA can prevent type 1 diabetes in NOD mice inducing a Th2 signature bias on the immune system of these mice. Here, we show that SEA can induce *de novo* generation of Foxp3$^+$ cells from naive NOD mice CD4$^+$ T cells cultured *in vitro*. Furthermore we demonstrate that the generation of T regulatory cells (Treg) by SEA is TGF-β dependent since its neutralization results in loss of Foxp3 expression. Our previous work demonstrated a decreased ability of splenocytes from SEA-treated mice to adoptively transfer diabetes to NOD.SCID. We now show that depletion of CD25$^+$ cells from splenocytes of SEA-treated mice restores the ability to transfer diabetes in to NOD SCID recipients. We have also found an increase in the numbers and activation status of CD4$^+$ Foxp3$^+$ T cells in the pancreas of NOD mice treated with SEA. These results newly identify an important role for T regulatory cells in SEA-mediated diabetes prevention.

ABSTRACTS OF THE POSTERS

8.

EXPRESSION OF CXCR4 IN BONE MARROW AND PERIPHERAL BLOOD TUMOR CELLS FROM CHILDREN WITH ACUTE LEUKAEMIA.

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The chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 (fusin, LESTR) can play an important role in the migration of hematopoietic and leukemia cells. Tumor cells from bone marrow and peripheral blood of 30 children with acute leukemia (23 – B-lineage acute lymphoblastic leukemia (B-ALL), 2 – T-lineage lymphoblastic leukemia (T-ALL), 5 – acute myeloblastic leukemia (AML)) were investigated by flow cytometry (FACScan) and Real-time PCR (iCycler iQ, Bio-Rad).

CXCR4 was expressed in all analyzed samples. Flow cytometry analysis demonstrated high expression of CXCR4 chemokine receptor on 90% lymphoid blasts in T-lineage and 70% - in B-lineage ALL. Expression of CXCR4 in AML was lower and detected just on 20 – 30% of blasts. It was found that expression of CXCR4 both at protein (detected by flow cytometry) and mRNA (Real-time PCR) levels was significantly higher (1.5-3 times) on peripheral blood cells comparing to cells from bone marrow ($p<0.001$, Kolmogorov-Smirnov test).

Expression of CXCR4 depends of lineage subtype of blast cells and increases in migration leukemia cells from bone marrow to peripheral blood in pediatric patients with acute leukemia.

9.

UNEXPECTED ANTIAPOPTOTIC EFFECT OF SOME HPMA BASED POLYMERIC CONJUGATES

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Water soluble polymer based on *N*-(2-hydroxypropyl)-methacrylamide (HPMA) bearing anticancer drug doxorubicin bound to GFLG peptidic side chains via amidic bond, known as PK1 conjugate, was developed to eliminate unwanted side effects of conventional chemotherapy. While free doxorubicin causes apoptosis in a population of cancer cells, apoptosis induction was never documented after treatment of cell culture with polymeric conjugate PK1.
Apoptosis was quantified by staining with AnexinV in Jurkat human T-cell leukemia and mouse splenocytes. Interestingly, apoptosis induced in the cell culture by TNF or after the exposition to the UV light could be significantly decreased by co-incubation of cells with conjugate PK1. The pro-apoptotic signals were not down regulated after the pretreatment with free doxorubicin or polymeric conjugate bearing doxorubicin bound to the HPMA backbone by the hydrolytically cleavable linkage.

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10.

KINETICS OF DENDRITIC CELLS RECONSTITUTION AND COSTIMULATORY MOLECULES EXPRESSION AFTER MYELOABLATIVE ALLOGENEIC HAEMATOPOETIC STEM CELL TRANSPLANTATION: IMPLICATIONS FOR THE DEVELOPMENT OF ACUTE GRAFT-VERSUS HOST DISEASE

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Background and objective: Peripheral blood dendritic cells (DCs) represent the only DCs compartment, in humans, accessible for direct studies. Allogeneic hematopoetic stem cell transplantation (HSCT) with myeloablative conditioning represents unique opportunity to monitor the kinetics of reconstitution of DCs and their dynamics in distinct pathologies.
Design and methods: In this study we analyzed kinetics and pattern of circulating DCs subsets reconstitution after myeloablative HSCT from unrelated donor. As DCs play a major role in the pathogenesis of acute graft versus host disease (GVHD), we separately analyzed patients who developed acute GVHD and compared this cohort to group with uncomplicated post-transplant course.
Results: Peripheral blood DCs were monitored from the earliest phase of hematopoetic reconstitution until day 365 after HSCT. Both myeloid DCs and plasmacytoid DCs appeared at earliest stages after engraftment and their frequency peaked between days 19-25 after HSCT. Their proportion then gradually declined and absolute numbers of both DC subsets remained lower for the whole follow-up period when compared to controls. Expression of costimulatory molecules transiently increased between days 15 and 35 and then went back to low steady state levels. Interestingly, patients who developed acute graft-versus-host disease (GVHD) had significantly lower numbers of circulating DCs. The decrease in DC counts preceded onset of clinical symptoms by at least 24h and was independent of corticosteroids administration.
Interpretation and conclusions: This study provides further insight into the biology of DCs in the settings of allogeneic HSCT and reveals quantification of plasmacytoid and myeloid DCs as a potential biomarker for the prediction of acute GVHD development.

11.

ZYMOSAN DELAYS TYPE 1 DIABETES THROUGH THE INDUCTION OF ALTERNATIVELY ACTIVATED MACROPHAGES

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Zymosan, a fungal cell wall component, has been shown to induce autoimmunity through the induction of T helper (Th) 17 cells in mouse models of arthritis and multiple sclerosis. We therefore investigated the potential for zymosan to alter the course of spontaneous autoimmune diabetes in the non-obese diabetic (NOD) mouse. We found that zymosan delays the onset of diabetes, inducing a population of PD-L1$^+$ alternatively activated macrophages in the pancreas. The altered pancreatic infiltrate from zymosan-treated mice suppresses αCD3-stimulated CD4 and CD8 T cell proliferation as compared to that of control mice. Furthermore, we were able to show that splenic macrophages secrete bioactive TGF-β following stimulation with zymosan *in vitro*. Finally, *in vivo* neutralization of TGF-β, but not the IL-6 receptor abrogates the ability of zymosan to protect against diabetes and reduces the induction of alternatively activated macrophages.

12.
NON-TOXIC FRAGMENTS OF LIPOPOLYSACCHARIDES (LPS) INDUCE CYTOKINES IN PERITONEAL MACROPHAGES

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Generally, lipopolysaccharides (LPS) as the dominant surface immunogens of Gram-negative bacteria are isolated, detoxified and conjugated to a protein carrier to construct modern subcellular vaccines. The rationale is to change T-cell independent processing of saccharidic antigen to protein assisted T-dependent memory cells activated processing.

Here we prepared two detoxified LPS structures originated from *Vibrio cholerae* O1: Acid hydrolysis treatment afforded saccharidic structure (O-SP with core) without toxic lipid A part and hydrazinolysis gave non-toxic structure with no O-acetyl chains in lipid A part (deOAcLPS). Possible immunomodulatory effects of both preparations were examined by measurements of cytokines released from isolated mouse peritoneal macrophages. Unexpectedly, both detoxified saccharidic structures induced substantial release of pro-inflammatory cytokines IL-1α, IL-6 and TNF- α. Multiplex flow cytometry analysis provided quantitative relations: IL-6 >> IL-1α > TNF-α. The saccharidic structure without lipid A was even more effective inductor than deOAcLPS. The activation of the intracellular signal pathway of the macrophages resulted also in the noticeable increase of the reactive nitrogen-oxygen products (ROI, RNI) registered at the same time.

The activation of macrophages by non-toxic saccharidic structures as a new phenomenon should be considered in the design of the glycoconjugates, the components of modern subcellular vaccines.

13.

SPATIO-TEMPORAL ANALYSIS OF EARLY SIGNALING EVENTS OF T CELL ACTIVATION BY LIVE-FRET AND SINGLE MOLECULE ANALYSIS

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During the past decades, great efforts have been made to get insights into the complex process of antigen-induced T cell activation and the corresponding signal transduction pathways. The T cell antigen receptor (TCR) signaling cascade itself is initiated by phosphorylation of ITAM-tyrosine residues by the human T-lymphocyte-specific protein tyrosine kinase Lck. During T cell activation, Lck undergoes structural changes which result in an open and active conformation of Lck followed by phosphorylation of the ITAM-motif and TCR signaling. Recently, we found by single molecule analysis homophilic interaction of Lck forming monomers, dimers and higher order oligomers. We are now aiming to analyze the molecular mechanisms underlying formation of homophilic interaction of Lck as well as the role of homo-mulitmerization for T cell activation. We will use high-resolving, real-time fluorescence resonance energy transfer (FRET) technique to monitor intermolecular interactions of Lck in a Lck deficient Jurkat T cell line (JCaM 1.6) transduced by self-made biosensors, i.e. Lck fused to cyan and/or yellow fluorescent proteins. Further, we will identify the determinants responsible for homophilic interaction, modulate the interaction status of Lck during T cell activation and study the kinase activities of the Lck multimers. Together, these studies should provide a more comprehensive picture of the function of this key kinase in signaling via the T cell antigen receptor.

14.
OVERCOMING THE RESISTANCE OF HIV AGAINST COMPLEMENT-MEDIATED LYSIS

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HIV has been shown to protect itself from complement mediated lysis (CML) by regulators of complement activation (RCA). Among them is the fluid phase RCA factor H (fH), which is attach to the surface of the virus. fH is organised in 20 modules referred to as short consensus repeats (SCRs). The first 5 SCRs of fH exhibit "decay -accelerating activity" and promote C3b inactivation. SCR 7, 9, 18-20 and probably SCR 13 mediate the binding of fH to negative charged surface elements such as heparin sulphates expressed on the cell. Thus, binding of fH to negatively charged host cells contributes to the protection against damage induced by the host's own complement. As HIV acquires the envelope from the host cell, the surface of the virus is similarly charged as the host cell and fH is binding to the virus. Therefore, we aimed to block the fH-HIV interaction by recombinant SCRs which contain the motifs for the interaction with negatively charged surfaces. To harvest sufficient amounts of the respective fH-derived SCRs, the SCRs were expressed in the Pichia pastoris system and purified by affinity chromatography. In lysis experiments we were able to show that the SCRs, which are involved in the interaction of fH to the cell surface, induced a significant decrease of the viral titres. Infection assay revealed that the presence of fH-derived SCRs reduced the amount of HIV when compared to the controls. Thus, fH-derived SCRs provide an alternative means to control the infection with HIV by overcoming the resistance of HIV against complement-mediated destruction.

15.

COMPARATIVE STUDY ON INDIRECT IMMUNOFLUORESCENT AND IMMUNOPEROXIDASE ASSAYS FOR DETECTION OF ANTIMITOCHONDRIAL ANTIBODIES USING MCCOY-PLOVDIV SERUM-FREE CELL LINE

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Antimitochondrial antibodies (AMA) are the main serological criterion for diagnostics of primary biliary cirrhosis (PBC). Their detection is routinely performed by the indirect immunofluorescent assay (IFA) on cellular or tissue substrates. Our recent experience with McCoy-Plovdiv serum-free cell line showed that it is a reliable alternative to rat liver cryo-sections and HEp-2 cells in IFA for antinuclear antibody testing. Another diagnostic approach for AMA determination is the indirect immunoperoxidase assay (IPA) producing color reaction for visualization of positive AMA. Its main advantage is that it uses light microscope reading and does not require specialized equipment. The aim of this study was to compare the diagnostic sensitivity and specificity of IFA-AMA and IPA-AMA using McCoy-Plovdiv serum-free cell substrate. A total number of 138 serum samples were included in the study (20 from patients with PBC, 32 from patients with chronic viral hepatitis B and C, and 87 from healthy controls). IFA and IPA for AMA with McCoy-Plovdiv cell line showed similar diagnostic characteristics (100% sensitivity for PBC patients and 100% specificity for chronic viral hepatitis and healthy individuals).The comparison in end-point titers between IFA and IPA for AMA from PBC patients demonstrated moderate agreement. These data imply that McCoy-Plovdiv serum-free cell line is an appropriate substrate for both IFA and IPA determination of AMA from PBC patients.

16.

GENERATION OF CLINICAL-GRADE DENDRITIC CELL-BASED VACCINE FOR IMMUNOTHERAPY OF OVARIAN CANCER

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Introduction: Dendritic cells (DCs) the most potent antigen presenting. Recent technological advances allow for generation of large numbers of DCs from peripheral blood monocytes. Administration of activated DCs loaded with tumor antigens is thus an attractive approach for immunotherapy of cancer. Prerequisite for the initiation of clinical trials in cancer immunotherapy is the development of protocols for DC-based vaccine generation according to Good Manufacturing Practice (GMP) conditions.
Aim of the study: Development of protocol for the generation of clinical grade vaccine based on activated DCs loaded with killed tumor cells for use in the immunotherapy of ovarian cancer.
Materials and methods: Immature DCs were generated from peripheral blood monocytes of healthy donors. We tested Cell Gro and RPMI+5% pooled human serum (5% PHS) as clinical grade culture media. Immature DCs were then activated by three distinct stimuli (Poly I:C, LPS and cocktail of proinflammatory cytokines (TNF, IL-1 and IL-6)). Activated DCs were evaluated for their phenotypic and functional characteristics and for their capacity to activate antigen specific and/or regulatory T cells.
Results: Culture of monocytes in Cell Gro yielded highest numbers of immature DCs. Stimulation with Poly I:C, LPS and cytokines mixture induced comparable phenotypic maturation. However, only Poly I:C and LPS activated DCs produced proinflammatory cytokines. In accordance with this finding Poly I:C and LPS were more efficient in inducing antigen specific T cells. Interestingly, cytokines activated DCs induced more regulatory T cells. There was no difference between the two tested media in the induction of antigen specific T cells.
Conclusions: Generation of immature DCs in serum free Cell Gro media followed by Poly I:C activation is an optimal combination for generation of DCs with high capacity to stimulate antigen specific T cells. DCs activated by coctail of proinflammatory cytokines induce higher frequencies of regulatory T cells. This protocol was approved for clinical use by the Czech Drug Agency.
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17.

DENDRITIC CELLS' MARKERS IN IDIOPATHIC INFLAMMATORY MYOPATHIES

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Just recently, dendritic cells (DCs) have been reported to occur among the inflammatory infiltrating cells in muscle tissue, in idiopathic inflammatory myopathies (IIM). DCs indicate substantial variability of phenotypes, depending on their differentiation stage, and in addition to dendritic markers' expression, one can detect molecules, which, until not such a long time ago, were being assumed to be lymphocyte markers. Currently, several markers allowing DCs immunohistochemical visualization are being used. No reports on studies concerning langerin or fascin (markers of immature/mature dendritic cells, respectively) expression in idiopathic inflammatory myopathies have been published so far. We have performed analyses of fascin and langerin expression in muscle specimens from normal and polymyositis, as well as from dermatomyositis affected muscles. Single langerin positive cells have been detected in some patient muscles, while fascin positive ones were numerous in majority of the studied patient muscle specimens in perimysial and endomysial infiltrations. Revealing of high numbers of fascin positive DCs in muscles affected by idiopathic inflammatory myopathies may become meaningful for diagnosing and pathogenetic mechanisms' explaining, and thus result in increasing treatment efficacy.

This work has been supported by the Medical University of Łódź (grant numbers: 502-18-558 and 502-17-555).

18.

SIGNIFICANCE OF CIRCULATING SOLUBLE CTLA-4 (sCTLA-4) IN PATIENTS WITH BREAST CANCER

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In the present study, the probable interference of circulating sCTLA4 in immune suppression in breast cancer patients and also to find the explanation for gene-disease association studies from sCTLA4 point of view is investigated. sCTLA4 was evaluated in serum samples from 43 new cases of breast cancer, who had received no chemotherapy and no radiation therapy before specimen collection using an Enzyme Linked Immunosorbent Assay (ELISA). The results were compared with the data from 44 age/sex-matched healthy individuals. sCTLA4 associations with the clinicopathological characteristics of the patients as well as with the important *CTLA4* single nucleotide polymorphism (SNPs) (three in promoter region -1722 T/C, -1661 A/G, -318 C/T), one in exon 1 (+49A/G), one in intron 1 (+1822 C/T) and one in 3' untranslated region (+6230 (CT60) A/G) and the resulted haplotypes were then investigated. Statistical analyses revealed very significant higher level of sCTLA4 in patients with breast cancer than healthy controls (23.5±14.96 ng/ml versus 7.69±4.04 ng/ml, $P<0.0001$). No significant difference was found in serum sCTLA4 level between LN- and LN+ patients. (23.8±13.4 ng/ml and 24.5±16.8 ng/ml, $P=0.794$). sCTLA4 observed to be significantly higher in patients harboring +49 AG genotypes as well as +1822 CT genotypes than the other genotypes at these positions. *CTLA4* haplotype combination (T A C A C A / T A C G T G) observed also to be associated with higher sCTLA4 expression in patients in comparison to other haplotype combinations (35.4±9.6 and 21.5± 15.1 ng/ml respectively, $P<0.024$). We found no association of circulating sCTLA4 with CT60 (+6230) marker in *CTLA4* gene. Our data indicate that patients with breast cancer have increased sCTLA4 expression. Our data provided not only the explanation for association of *CTLA4* gene with cancer, but also suggest that increased circulating sCTLA4 may be one of the ways by which CTLA4 suppresses tumor immunity.

19.

IMPACT OF COMPLEMENT SPLIT PRODUCT C4D ON IMMUNE CELL FUNCTION

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Background. A cardinal feature of antibody mediated rejection (AMR) in solid organ transplantation is the deposition of complement split product C4d on vascular alloendothelia. C4d is generated through cleavage of C4 which is covalently bound to endothelial cell surfaces upon classical complement activation via antigen-antibody reaction. In kidney transplantation, the immunohistochemical detection of C4d in peritubular capillaries has been associated with graft dysfunction and decreased graft-outcome. No immunologic function could be attributed to C4d so far, so that it has often been referred to as an 'immunologic scar'. There is evidence, however, that C4d-positive AMR is associated with a predominant recruitment of monocytes to peritubular and glomerular capillaries, compared to C4d negative rejection. **Methods.** We hypothesise that human leukocytes possess C4d binding sites which confer immunologically relevant functions upon ligation. To elucidate such functions ligand binding assays (using FACS technology) were applied and an in-vitro stimulation assay has been established. C4d and C4b-like C4 were derived from human plasma C4, biotinylated and immobilized via streptavidin bound on culture plates in order to mimic in vivo AMR on endothelia. **Results.** Incubation of monocytes with C4d caused a significant increase of mean fluorescence intensity when compared to negative control. In contrast, for T cells or B cells, no surface binding of C4d could be observed. Similar results were obtained with C4b like C4, i.e. positive staining of monocytes, but no staining of T cells or B cells. Preliminary data of our in vitro stimulation model point to an immunomodulatory property of C4d on monocytes, i.e. dose-dependent modulation of TNF-α expression and of the early activation marker CD11b. **Conclusion.** Our findings demonstrate preferential binding of C4d to human monocytes. In addition we have established an in vitro culture system which allows for the study of the effect of C4d on immune cell function.

20.

CHANGES IN TUMOR MICROENVIRONMENT DURING HPMA DRUG BASED THERAPY

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Polymeric conjugates based on *N*-(2-hydroxypropyl) methacrylamide bearing doxorubicin were developed as a system designed to improve the therapeutic index of cytostatic drugs, and eliminate their side effects. Anti-cancer activity of polymeric conjugates based on HPMA does not affect natural mechanisms of anti-cancer immunity, and hence allows the development of fully functional immune responses. Systemic anti-cancer resistance was documented on mice cured with HPMA-based conjugates targeted or non-targeted, which have been found to be resistant to retransplantation with the same tumor. In contrast, the therapeutic dose of free doxorubicin increases the sensitivity of mice to neoplasis.

EL-4 T cell lymphoma was transfected with plasmid targeting GFP to the nucleus. Sensitivity of transfected cells to polymeric conjugates was comparable to parent cell line. Tumor growth and immune reaction during treatment with polymeric conjugates, was analyzed in vivo on OV100 Whole Mouse System and iCys Laser Scanning Cytometer.

ACKNOWLEDGEMENTS

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21.
EFFECT OF CELLULAR AND NONCELLULAR COMPONENTS OF MILK ON REACTIVITY OF NEWBORN´S LYMPHOCYTES

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Allergic diseases belong to one of the most common diseases. Breast-feeding is the simplest prevention of development of allergy. We studied the effect of noncellular components of milk of healthy and allergic mothers on stimulation of cord blood lymphocyte of children of healthy and allergic mothers by ^{3}H thymidine incorporation and ELISPOT (immunoglobulin production). Cocultivation of milk cells with stimulated/unstimulated cord blood cells in Transwell system upregulate/downregulate cytokine mRNA expression in cord lymphocytes. As we observed, there are not larger differences between influence of milk from healthy and allergic mothers on lymphocyte functions but there is difference in lymphocytes itself. Cord blood cells of children of allergic mothers poses higher proliferation activity. Milk/colostrum in high concentration suppresses proliferation of cord blood cells after stimulation with polyclonal activators. On the other hand, we detected increased immunoglobulin production by cord blood lymphocytes after cultivation with milk/colostrum. In conclusion, cord blood cells of children of allergic mothers are more prompt to proliferation and their cytokine expression correspond to allergic phenotype.

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22.

MHC CLASS I-DEFFICIENT, HPV 16-ASSOCIATED TUMOURS: ACTIVITY AND DISTRIBUTION OF TUMOUR-INFILTRATING CELLS AFTER CHEMOTHERAPY AND SUBSEQUENT IL-12 IMMUNOTHERAPY

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Using the model of moderately immunogenic HPV 16-associated murine TC-1/A9 tumours with downregulated expression of MHC class I molecules, we examined the effects of local interleukin 12 gene therapy on the distribution and activity of tumour-infiltrating cells. The tumour-bearing animals after chemotherapy (ifosfamide derivative CBM-4A) were treated with genetically modified tumour cell vaccine producing IL-12. Histological and immunohistological examinations of CBM-4A-treated tumours showed a decrease in the infiltrated $CD4^+$ and $CD8^+$ T cells compared to untreated animals. Administration of the vaccine led to the renewal of $CD8^+$ and $CD4^+$ cells in the tumour nodules. FACS analysis of enzymatically digested tumours showed a significant increase in the number of $CD11c^+$ cells in the treated animals. On the other hand, Gr-1/CD11b-positive cells increased after chemotherapy and the amount of these cells decreased after subsequent immunotherapy. Moreover, $CD45^+$ tumour-infiltrating cells isolated from the treated animals exhibited restored cytotoxic and proliferation potential after short-term *in vitro* precultivation. These findings contribute to the relevancy of therapy of minimal residual tumour disease obtained after cytoreductive chemotherapy with genetically modified cellular vaccines.
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23.
EARTHWORMS IN HUMAN MEDICINE

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All invertebrate species including earthworms have developed a variety of defense mechanisms efficiently recognizing and responding to non-self substances. Cellular mechanisms of invertebrate innate immunity include wound repair, clotting and coagulation responses, phagocytosis and encapsulation reactions. Apart from these cellular mechanisms, invertebrates possess a broad range of antimicrobial factors such as lysozyme-like proteins, proteases, cytolytic proteins, antimicrobial peptides and components of prophenoloxidase activation cascade; humoral defense also includes pattern recognition and lectin-like molecules.

Regarding the usage of earthworms in human medicine, earthworms might be considered as a source of biologically active compounds with potential industrial or medical use. Actually, earthworm powder has been used as a traditional medicine in some South Asia countries for years to treat various diseases. Currently, the therapeutic effect of earthworm active factors is being evaluated by a modern scientific approach. Some therapeutics containing fibrinolytic enzymes from *Lumbricus rubellus* and *Eisenia fetida* earthworms are already commercially available to support coagulation and fibrinolysis balance in the body and thus prevent or treat cardiac and cerebrovascular diseases.

Here we report on sequence and functional characterization of *E. fetida* lysozyme, a bacteriolytic enzyme which catalyzes the hydrolysis of 1,4-β-D-links in the peptidoglycan of bacterial cell walls and thus efficiently protects against infections caused by Gram-positive bacteria. Moreover, our preliminary results suggest that one of the proteins participating in the fibrinolytic activity of the coelomic fluid of *Eisenia fetida* earthworms is lysozyme.

24.

VALUES OF TNF-α IN RADICULAR AND KERATOCYST OF MAXILOFACIAL REGION

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TNF-α is a pleiotropic cytokine that is considered as a primary modifier of inflammatory and immune reaction in response to various inflammatory diseases and tumour. Radicular cysts are result of inflammatory process in the periapical tissues associated with necrotic and infected pulps, while the aggressive behaviour and high recurrence rate of odontogenic keratocysts (OKC) suggests neoplastic potential and promted the WHO Working Group to classify the OKC as benign tumour with odontogenic epithelium. We compared TNF-α concentration between 43 radicular cysts and 15 odontogenic keratocysts, obtained from patients undergoing surgery, under local anaesthesia, and after aspiration of cystic fluid from non-ruptured cysts. TNF-α is elevated in both cysts fluid, but higher values were found in radicular cysts in comparison to keratocysts. The significantly higher concentration of TNF-α were associated with smaller radicular cysts, higher protein concentration, higher presence of inflammatory cells in peri cystic tissues, degree of vascularisation and cysts wall thickness (Mann-Whitney U-test, $p<0.05$). No correlation was found, based on these parameters in odontogenic keratocyst, but all cysts have detectable concentrations of TNF-α. We here for the first time present that difference in the concentration of TNF-α exist between these two cystic types.

25.

THE ROLE OF DENDRITIC CELLS IN ALLERGIC REACTION

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Introduction: Dendritic cells (DC) are professional antigen presenting cells, capable to initiate, amplify immune response and stimulate naive T lymphocytes. Different subtypes of DC polarize the specific Th immune response depending on the nature of antigen/allergen. The manner of allergen binding on DC and its engulfment are not yet elucidated. The role of DC-SIGN, specific C-lectin on the surface of DC, in this process was recently confirmed in some situations. The aim of our first study was to determine the difference in a number of DCs' subsets in peripheral blood between patients with atopic dermatitis (AD) and healthy controls. The second, we studied the modification of surface expression of DC-SIGN on DC stimulated by structurally different allergens with divers glycosylation pattern. *Patients and methods:* In the first study, we included 6 children with severe form of AD and 10 healthy age-matched donors with negative allergic history. mDC and pDC were measured by flow-cytometry as lineage negative, $CD45^{+}CD11c^{+}CD123^{lo}$, and lineage negative, $CD45^{+}CD11c^{-}CD123^{hi}$, respectively. The engulfment and presentation of allergen by DC was determined in 5 healthy donors and 5 birch allergic patients. Immature DC were obtained from the monocytes of peripheral blood and were stimulated by allergens (ovalbumine (OVA), birch allergen) and TLR ligands. Consecutively we analyzed the changes of phenotype of DC using flow-cytometry. *Results:* Total number of DC, mDC, pDC and pDC/mDC ratio did not differ between AD and controls ($p>0.999$). The expression of DC-SIGN was increased on DC of allergic patients in comparison to healthy donors. Simultaneously, its expression markedly decreased on DC stimulated by OVA, less by birch allergen. DC-SIGN appears as a candidate receptor molecule of DC in the initiation of allergic reaction. Supported by VZ MZ ČR 00064203, GAUK 7840/2007

26.

THE EFFECT OF THYMIC STROMAL LYMPHOPOIETIN ON THE SUBPOPULATIONS OF HUMAN DENDRITIC CELLS

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Thymic stromal lyphopoietin (TSLP), a IL-7 like cytokine produced mainly by epithelial cells, was described to activate human myeloid dendritic cells to induce inflammatory Th2 cells. In this study we studied effect of TSLP on the subpopulations of human dendritic cells – in vitro monocyte derived DC (MoDC), myeloid DC (mDC) and plasmacytoid DC (pDC). As dendritic cells are in a contact with epitelial cells and pathogens in mucosal tissues, we focused on interaction of TLR agonists and TSLP. Although we detected TSLPR mRNA in all of these subpopulation, their responsiveness to TSLP was profoundly different. TSLP induced strong phenotype changes (increased costimulatory molecules expression), as well as changes in T-cell proliferation and stimulation capacity of mDC. On the contrary we observed positive effect only on IL-6 and IL-12 production in MoDC. Likewise no changes in number of Tregs and Th1 were observed. Surprisingly we detected TSLP mRNA in MoDC itself, further increased by TLR stimulation. Costimulatory molecules were not upregulated by TSLP or TSLP+TLR agonists in pDC population, but in the presence of CpG ODN type A, pDC produced increased levels of INF alpha. Our observations indicate that there is a distinct responsiveness to TSLP among DC populations and under certain circumstances it has also an effect on pDC, which was so far not studied in connection with TSLP.

27.
HPMA CONJUGATES AND IMMUNOGENIC CELL DEATH

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Doxorubicin is a routinely used anthracycline antibiotic for cancer treatment and it has a broad spectrum of applicability. Polymeric carriers of drugs, copolymers of *N*-(2-hydroxypropyl) methacrylamide (HPMA), have been developed to avoid the side-effects of free drug. These conjugates consist of HPMA copolymer backbone to which the drug is bound via amide, enzymatically degradable (PK1) or pH sensitive, hydrolytically cleavable bond (HYD). Surface expression of calreticulin (CRT) is currently considered to be a key mechanism of action of free doxorubicin and is believed to determine the immunogenicity of doxorubicin-induced cell death in vivo. Incubation of four human or murine cancer cells lines with free doxorubicin enhanced the surface expression of calreticulin. Similarly the expression of CRT was enhanced after treatment with HYD conjugate, as it releases doxorubicin intracellularly. We proved that doxorubicin is not released from the PK1 conjugate and this is supported by the fact that CRT expression is not enhanced after exposure of cells to PK1 conjugate. Data obtained with six other HPMA conjugates containing doxorubicin bound either by amide or hydrazone bonds demonstrate that free doxorubicin (whether released from HPMA conjugates of free itself) is crucial for CRT expression. Both HPMA conjugates containing doxorubicin bound via amide or via hydrazone bond cure mice and induce systemic anti-cancer resistance. This means that the expression of CRT is not the only mechanism which is responsible for anti-tumor immune response induced by selected chemotherapy.

28.

IMMUNOSUPPRESSIVE AND ANTI-INFLAMMATORY ACTIVITIES OF MCS-18, A COMPOUND ISOLATED FROM A TOTAL HELLEBORUS PURPURASCENS EXTRACT

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MCS-18, a multi-anionic conjugate isolated and purified from *Helleborus purpurascens*, was classified as a New Chemical Entity (NCE). Immuno-pharmacological studies *in vitro* and *in vivo*, carried out by other research units in Romania and Germany confirmed its therapeutic efficacy by: (1) rapid and long-lasting effect in eliminating pain and increasing mobility in patients with periarthritis of the shoulder; (2) *in vitro* inhibition of dendritic cells (DC) maturation and DC-T lymphocytes cluster formation; (3) reducing paralysis associated to experimental autoimmune encephalitis (model for multiple sclerosis).
MCS -18 has been studied in our laboratory for its immunosuppressive and anti-inflammatory capacity.

We studied the effects upon: (1) modulation of production of antibodies in vivo against T-dependent antigens, the results obtained indicating that MCS-18 might be a potential antagonist of Toll-like receptors (TLRs) or an inhibitor of intracellular signaling triggered by activation of TLRs (P13K-Akt/PKB pathway); (2) modulation of production of pro-inflammatory and anti-inflammatory cytokines; (3) capacity of scavenging free oxygen radicals (FOR), using the fluorometric method ORAC (Oxygen Radicals Absorbance Capacity); (4) effect upon production and release of FOR in macrophages (Raw 264.7 cell line) activated at TLRs (TLR2, TLR4 + CD14) or Dectin 1 level (chemiluminescence); (5) inhibition of NO (Griess colorimetric method) and TNF alpha (ELISA) production; (6) capacity to reduce inflammation induced by intraplantary injection of carrageenan to CD1 mice (computer pletismometry);

The results obtained revealed that MCS-18 has immunosuppressive and anti-inflammatory capacities and pointed out new features regarding its immuno-phramacological mechanism.

29.
CD23, NO AND B CELL LYMPHOPROLIFERATIVE DISEASES

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The individual prognosis of patients with B cell chronic lymphocytic leukemia (B-CLL) is extremely variable. Although clinical stages ramain the basis for assessing prognosis in B-CLL, a number of biological markers can offer important, independent prognostic information. CD23, a type II integral membrane protein, member of type C (calcium-dependent) lectin family is constitutively and atypically expressed on malignant B cells in patients with B-CLL. High levels of the soluble CD23 (sCD23) have also been linked to adverse prognostic features such as diffuse bone marrow infiltration, rapid doubling time, and disease progression in early-stage of B-CLL therefore, sCD23 was proposed as a marker of disease activity and as an important prognostic parameter in B-CLL. B cells in patients with B-CLL spontaneusly display a low level of functional inducible nitric oxide shynthase (iNOS) and the nitric oxide (NO) produced appared to inhibit apoptosis. Our results sugest that ligation of CD23 (low affinity IgE receptor) increase iNOS expression in B-CLL cells and conversely decrease the percentage of cell undergoing apoptosis, as measured by the percentage of cells expressing annexin V. On the other hand, the anti-apoptotic role of NO was reverted by iNOS inhibitors.

30.

THE ROLE OF MESENCHYMAL STEM CELLS IN HEART TISSUE REPAIR: CARDIAC DIFFERENTIATION OR IMMUNOMODULATION?

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The multipotency of stem cells attracts the attention of scientists due to the possibility of their use for repair of tissue damage and, particularly, for treatment of heart tissue injury after infarction. Because of their low immunogenicity, feasibility of isolation and cultivation, and ability to produce mesodermal cells, the mesenchymal stem cells (MSCs) seem to be the most appropriate cell type for manipulations aimed at cellular cardiomyoplastics. Experimental and clinical trials have revealed positive effect of MSCs transplantation on structural and functional restoration of myocardium after infarction. Although cardiac-specific differentiation of human MSCs was observed in several *in vitro* studies, these results were sometimes controversial and not reproducible. In our study we tested different published protocols of cardiac-specific differentiation of human MSC *in vitro* and their modifications to develop the most effective protocol. We found that although human bone marrow derived MSCs demonstrate remarkable plasticity and undergo partial differentiation upon induction, they fail to acquire functional characteristics of mature cardiomyocytes. We suggest that mechanisms of beneficial effect of MSCs transplantation on myocardium regeneration could arise due to their anti-inflammatory properties and influence on remodeling processes and angiogenesis though secretion of cytokines and growth factors, rather then their direct impact on myocardium regeneration.

31.

KINETICS OF IL-17 AND IFN-γ EXPRESSION AND PRODUCTION IN THE CNS OF RATS WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Interferon-γ (IFN-γ) and interleukin-17 (IL-17) are involved in the pathogenesis of experimental autoimmune encephalomyelitis (EAE). Although plenty evidence suggests that IFN-γ and IL-17 are products of two distinct subpopulations of CD4+ T helper lymphocytes (Th1 and Th17, respectively) and that IL-17 production is inhibited by IFN-γ, recent data demonstrated the existence of the population of CD4+ T lymphocytes which simultaneously produce IFN-γ and IL-17 (IL-17+/IFN-γ+). Since the precise hierarchy of CNS cytokine expression still remains unclear, to test the relationship between IFN-γ and IL-17 producing cells in EAE, we immunized Dark Agouti (DA) rats with encephalitogenic emulsion and examined the kinetics of IFN-γ and IL-17-producing cellular responses during EAE. Infiltrating mononuclear cells (inMNC) isolated from CNS in different phases of EAE (onset, peak and recovery) were analyzed for cytokine mRNA expression by real time PCR and for production of proteins by ELISA and intracellular staining, measured by cytofluorimetry. Our results show that both IL-17 and IFN-γ were expressed in the CNS of DA rats in the course of EAE with the highest production at the onset of the disease. Further, number of IL-17$^+$ cells, but not of IFN-γ$^+$ cells declined among inMNC during EAE. Interestingly, among cells expressing IL-17 or IFN-γ there was a significant proportion of cells capable of expressing both cytokines, and their percentage among inMNC also decreased from the onset till the resolution of the disease. These preliminary results showing specific patterns of IFN-γ and IL-17 co-expression in DA rats suggest that IL-17 could be produced in the CNS more rapidly than IFN-γ and that IL-17 might direct the initial inflammation, whereas IFN-γ might be important in prolonging and/or resolving tissue inflammation. Therefore, it is reasonable to argue that no single dominant cytokine or effector cell population will uniquely regulate the overall process of tissue damage. Further investigations which should explain the exact roles of IFN-γ and IL-17 in the CNS autoimmunity are necessary.

32.

TECHNICAL APPROACHES OF ANTIGEN-SPECIFIC RESPONSE ESTIMATION IN EXPERIMENTAL MODEL OF AUTOIMMUNE ENCEPHALOMYELITIS

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Experimental autoimmune encephalomyelitis (EAE) is an established animal model for the human disease multiple sclerosis (MS) mediated by activated T cells specific for various myelin autoantigens. Although artificial immunization may not appropriate reproduce all the pathogenetic mechanisms most therapies of MS patients are based on EAE rodent model. Peripheral blood mononuclear cells and splenocytes were obtained from 3 groups of Wistar rats: animals with established EAE, immunized rats without clinical signs and control group. Cells were examined for their in vitro proliferative responses to ConA after 3 days and myelin antigens (syngeneic spinal cord homogenate (SCH) or myelin peptide cocktail of myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP)) after 6 days of cultivation using ^{3}H-thymidine incorporated assay in three independent experiments. The spontaneous proliferation was significantly higher in rats with EAE whereas in immunized rats it was the same as in control group. The proliferative response to ConA increased in control group ($p=0,02$) as well as in immunized rats ($p=0,05$) without any changes in rats with established EAE. The addition of autoantigens in the culture showed the increase of antigen-specific response to both SCH ($p=0,04$) and peptide cocktail ($p=0,02$) in healthy rats, the enhancement of SCH-induced proliferation in immunized rats ($p=0,04$) while in animals with established EAE the response to myelin antigens was at the same level as spontaneous proliferation. These controversial results may reflect the involvement of immunoregulatory mechanisms, e.g. anergy, by means of which the spontaneous recovery of animals with EAE may occur.

33.

NON-SPECIFIC CELLULAR IMMUNE RESPONSE AFTER SEMI SYNTHETIC OLIGOSACCHARIDE-CONJUGATE VACCINACION

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The cell surfaces of bacteria, yeast and viruses exhibit oligosaccharides that are often distinct from those of their hosts. Such cell-surface carbohydrate markers are able to be a basis for carbohydrate-based vaccines. An immune response against the carbohydrate antigens that results in long-lasting protective response to target cells is required for a carbohydrate-based vaccine.
Adaptive immunity runs in parallel with the innate immunity to control infections. Immune responses can be induced by a wide variety of antigens but only a few will be effective in the immunoprotection against infections. In this study, we used for immunization synthetically prepared dimeric and pentameric oligosaccharides conjugated to protein carrier as a model of carbohydrate-based vaccine. We monitored changes in phagocytosis, intensity of the metabolic burst and microbial killing activity of polymorphonuclear cells. We observed effective modulation of non-specific cellular immune response by these two model glycoconjugate vaccines. The investigated methods could be considered as important tools to support the modern semi synthetic glycoconjugate vaccines development.
This work was supported by the grant VEGA No. 2/7029/27 and APVV-0032-06.

34.
VACCINATION BY TATTOOING

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INTRODUCTION: The tattoo procedure non-specifically stimulates the immune system. Minor mechanical injuries are followed by hemorrhage, necrosis, inflammation and regeneration of the skin. Furthermore, tattooing has been shown to be able to deliver chemical and biological substances, e.g. tattoo-pigments, plasmid DNAs and viruses, to the skin and thus may be used for administration of vaccines. METHODS: In this work, we examined the usability of tattooing for vaccination with peptides. We compared tattooing with subcutaneous (s.c.) needle injection using peptides derived from human papillomavirus type 16 (HPV16) proteins. RESULTS: We observed that vaccination with the E7-derived peptides in combination with CpG motifs (ODN 1826) elicited higher specific cellular immune responses after delivery with a tattoo device than after s.c. inoculation. Tattooing also elicited higher level of E7-specific antibodies after vaccination with the E7 peptide containing both B-cell and Th-epitopes than s.c. injection. In the other experiment, tattooing of keyhole limpet hemocyanin-(KLH)-conjugated L2-derived peptides was as efficient in the induction of antibody production as s.c. needle-injection of KLH-conjugated peptides in mixture with Freund adjuvant (FA). CONCLUSIONS: In summary, we demonstrated that tattoo administration of peptide vaccines efficiently induces both humoral and cell-mediated immune responses.

35.
MODULATION OF HLA-G GENE EXPRESSION

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HLA-G antigen is characterized by a limited polymorphism and restricted tissue distribution. The primary transcript of HLA-G is alternatively spliced into seven mRNAs, which might be translated into seven HLA-G protein isoforms: 4 membrane-bound proteins (HLA-G1,-G2,-G3,-G4) and 3 soluble isoforms {HLA-G5,-G6,-G7). The mechanisms of *HLA-G* gene regulation differ from those of classical *HLA class I* genes because most of classical regulatory elements are in *HLA-G* disrupted. Here we studied epigenetic regulation of *HLA-G* gene expression. *HLA-G* gene activation was examined in tumor cell lines JAR (choriocarcinoma) and RAJI (lymphoblastoma) following treatment with demethylating agent 5-aza-2´- deoxycytidine (5AdC), with inhibitors of histone deacetylation (sodium butyrate and valproic acid) or by stress conditions as a heat shock (HS) or hypoxia. The highest activation of *HLA-G* transcription was achieved with 5AdC. Inhibitors of histone deacetylation were less efficient and hypoxia mimetic agents (desferrioxamine or $CoCl_2$) had no detectable effect on *HLA-G* transcription. Relatively high level of HLA-G transcripts was observed following HS treatment. Interestingly, in JAR cells HS also changed the splicing process resulting in high expression of HLA-G6 transcript. However similar HS treatment had no affect on alternative splicing of constitutively produced HLA-G mRNA in JEG3 cells. HLA-G1 protein was detected in JAR or RAJI cells only after treatment with 5AdC.

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36.

THE WAY HIV-OPSONIZATION MODULATES DENDRITIC CELLS

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Background: Already at initial phases of infection, HIV is coated with complement fragments. During the chronic phase, when HIV-specific IgGs appear, the virus circulates immune complexed with IgG and complement. Thus, we studied the interaction of dendritic cells (DCs) and DC-T cell cocultures with complement (C)-opsonized and C-IgG-opsonized HIV. **Methods:** To investigate our specific aim, we performed p24 ELISA from supernatants of DCs infected with differentially opsonized HIV preparations. In addition, we studied the expression of the distinct HIV-DNA forms by applying a real-time PCR assay. Intracellular localization of the differentially opsonized HIV preparations was assayed by confocal microscopy. **Results:** HIV infection of monocyte-derived and circulating BDCA-1^+ DCs was significantly reduced upon the presence of virus-specific but non-neutralizing IgGs. DCs exposed to C-Ig-HIV or IgG-opsonized HIV showed an impaired provirus formation and p24 production and a decreased transmission rate to autologous non-stimulated T cells upon migration along a chemokine gradient and in long-term experiments, when T cells were added delayed to IgG-HIV-exposed DCs. Similar kinetics were seen when sera from HIV-1-infected individuals before and after seroconversion were used in infection assays. Both C- and C-IgG-opsonized HIV were captured and targeted to a tetraspanin-rich endosome in DCs, but differed with respect to MHC class II colocalization. **Conclusion:** The reduced infection by IgG-opsonized HIV is possibly due to interactions of virus-bound IgG with FcγRIIb expressed on DCs. The exact mechanism has to be further elucidated. But, our data clearly indicate, that HIV exerts different modulatory effects on dendritic cells dependent on the opsonization pattern and on time.

37.

REGULATION OF TGF-BETA-INDUCED FOXP3 EXPRESSION IN ALLOANTIGEN-ACTIVATED MOUSE CD4+CD25- T CELLS BY IL-4 AND IL-12

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Transcription factor Foxp3 plays an essential role in generation and function of regulatory T cells (Treg). Foxp3 acts as a master switch controlling the development of Treg and is considered as the best marker of these cells so far. We show that the number of CD4+CD25+Foxp3+ cells was significantly increased when mouse spleen cells were stimulated with irradiated allogeneic cells in the presence of transforming growth factor-beta (TGF-beta). However, this increase in the number of Foxp3+ Treg was completely abolished when the cells were stimulated with alloantigens and TGF-beta in the presence of interleukin 4 (IL-4) or interleukin 12 (IL-12). None of the other cytokines tested had a similar effect on Foxp3 expression. Moreover, we found that the TGF-beta induced Foxp3+ cells arise exclusively from a population of CD4+CD25-Foxp3- T cells. We observed considerable proliferation of CD4+CD25-Foxp3- T cells cultivated in the presence of alloantigen and TGF-beta and simultaneously an induction of Foxp3 expression in these cells. The addition of IL-4 or IL-12 completely prevented the cell proliferation and also Foxp3 expression in this cell subset. We have not observed alloantigen-induced cell proliferation within a population of naturally occurring CD4+CD25+Foxp3+ Treg cells. Therefore, we resume that the TGF-beta-induced Foxp3+ T cells arise from the population of conventional CD4+CD25-Foxp3- T cells where the de novo induction of Foxp3 expression occurs. The results also suggest a novel role for IL-4 and IL-12, the cytokines which determine the differentiation of Th1 or Th2 subsets from Th0 precursors, in down-regulation of Foxp3 expression and in suppression of TGF-beta-induced Foxp3+ Treg proliferation.

38.

IMMUNE MECHANISMS INVOLVED IN PERI-SILICONE MAMMARY IMPLANT (SMI) FIBROSIS

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This study focused on peri-implant capsular fibrosis, the main local complication of SMI, investigating on local immune processes within the fibrous capsules and the phenotypic and functional characterization of lymphocytes present in the fibrotic tissue.
We analysed isolated intracapsular cells as well as autologous PBMCs via flow cytometry, setting a focus on the presence of T regulatory cells ($CD4^+CD25^{++}Foxp3^+$). Cytokine profiles, the TCR repertoire as well as reactivity against human heat shock protein 60 (HSP60) were determined.
The cellular composition of the fibrous capsule showed a preponderance of $CD4^+$ T helper cells and a noticeable subset of TCR γ/δ- expressing cells. IL-17, IL-6, IL-8, TGFβ and IFN-γ production prevailed, revealing a TH17/TH1 weighted immune response. Intracapsular T-cells showed a restricted TCR repertoire (monoclonal/oligoclonal pattern) as well as a preferential reaction with hHSP60. Interestingly, Tregs were more numerous in capsules with less fibrosis as compared to peripheral cells.
Taken together, our results show that silicone triggers a specific local immune response via activated TH17/TH1 cells, promoting fibrosis due to producing profibrotic cytokines. Clonal restriction of the TCR repertoire is a further evidence for a specific antigen(s) driving the immune components of capsular fibrosis. HSP60 might be a prominent candidate in this context and taking into consideration, that it is expressed all over the body, it might be the "missing link" between local and systemic side effects of SMI. Furthermore, we observed a correlation between the severity of capsular fibrosis and numbers of Tregs present in the fibrotic tissue, suggesting that in an early stage Tregs might delay capsular fibrosis.
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39.

INFLUENCE OF *SALMONELLA* INFECTION BY DIALYSABLE LEUKOCYTE EXTRACT IN CHICKS

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Protective effect of two dialyzable leukocyte extract (DLE) - salmonella-specific (ssDLE) and salmonella non-specific (snDLE) on the *Salmonella* Enteritidis PT4 (SE) infection in chicks was studied. Two days after intraperitoneal application of ss and snDLE to one-day-old chicks ISA BROWN breed, were chicks challenged with a single per oral dose (5.10^8) of salmonella bacteria. 120 chicks were divided into 6 groups: control, SE, ssDLE, snDLE, SE+ssDLE, and SE+snDLE. Samplings of peripheral blood were done on 3 and 14 days post infection for evaluation of white blood cell counts, lymphocyte subpopulations, phagocytic activity of phagocytes, and lymphoproliferative assay. Microbiological examination and PCR were used for proofing of SE and number of CFU in organs and faeces. The decrease of total counts of leukocytes and actual number of lymphocytes in SE group, and increase in both, ssDLE and ssDLE groups was observed. The values of phagocytic activity showed the highest peaks in SE group. Microbicidal activity was higher in SE, SE+ssDLE, and SE+nsDLE groups. Examination of lymphocyte proliferation demonstrated higher values in ssDLE and SE+ssDLE groups. An improve of CD3+, CD4+, and TCR+ lymphocytes was observed at 14 days p.i. in all experimental groups, however, the values of CD8+ were decrease, but with the highest values in SE+ssDLE group. The lower bacteria numbers were find in liver, spleen and caeca of ssDLE group, in comparison to SE and SE+snDLE groups. Our study showed protective effect of salmonella-specific DLE to experimental *Salmonella* infection in chicks.

40.

INFLUENCE OF TOLL-LIKE RECEPTOR AGONISTS ON CELLS OF B-CHRONIC LYMPHOCYTIC LEUKEMIA

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B-chronic lymphocytic leukemia (B-CLL) is most common leukemic disease in adult age. B-CLL tumour cells are weakly immunogenic which may contribute to disease progression and inhibit effective immunotherapy. Agents that enhance the immunogenicity of B-CLL cells may be useful in immunotherapeutic approaches for treatment this disease. As non-malignant counterparts of B-CLL cells express broad spectrum of Toll-like receptors and their agonists allow increase of their antigen-presentation functions we studied their expression and effect of triggering in B-CLL cells. TLR mRNA expression found in B-CLL cells was similar to the expression observed rather in memory than in naïve B-cells of healthy donors. The responsiveness to the agonists corresponded to observed expression of their respective receptors. Significant increase ($p<0.01$) of expression of CD80, CD86, HLA-DR and CD40 was observed after stimulation with TLR1/2, TLR2/6, TLR7 and TLR9 agonists. We detected production of cytokines TNF, IL-6 and chemokines IL-8 and IP-10 after stimulation with these agonists. Production of IL-6 was significantly decreased in B-CLL in comparison to healthy B cells. Similarly we found lower intensity of proliferation induced by TLR in B-CLL and on the contrary markedly enhanced process of apoptosis. Phenotype of B-CLL that correlates to the increased susceptibility to apoptosis induced by TLR agonists exposure remains unknown and will be further studied. In summary, our findings indicate that several TLR agonists are effective in stimulating B-CLL cells.

41.

ANTIBODIES SPECIFIC FOR PB1-F2 INTERFERE WITH THE COURSE OF INFLUENZA VIRUS INFECTION

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PB1-F2 is an unusual protein identified in an alternative (+1) reading frame of PB1gene of influenza A virus (IAV). PB1-F2 may function to accelerate apoptosis of infected and/or host immune cells recruited to the site of infection. We have shown that anti PB1-F2 antibodies (Abs) were induced during infection with IAV. Therefore, we decided to investigate a protective ability of antibodies specific for PB1-F2 protein of A/PR/8/34(H1N1) virus. BALB/c mice were immunized with C-terminal (3-13 aa), middle (42-53 aa), and N-terminal (71-83 aa) peptide of PB1-F2 protein conjugated with keyhole limpet hemocyanin (KLH). Each group of mice produced Abs recognizing only immunizing peptide. Ten days after the immunization, the mice were challenged with a lethal dose (1 LD50) of IAV. The protective ability of Abs was evaluated by survival rate, virus titer in the lungs, level of specific Abs in the serum, and change of body weight. We found that immunization by C-terminal peptide led to the 35% increase of the protection, when compared with the control mice immunized only with KLH. The protective effect of immunization with C-terminal peptide was accompanied by the earlier clearance of infectious virus from the lungs in comparison with the mice immunized with other peptides. Our results indicate that Abs specific for C-terminal peptide of PB1-F2 protein could confer the protection against IAV challenge.

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42.

INDUCTION OF T_H-2 CELLS IN THE PATHOGENESIS OF AUTOIMMUNE SKIN DISEASE - PEMPHIGUS VULGARIS

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BACKGROUND: Pemphigus vulgaris (PV) is classical example of antigen driven severe autoimmune bullous skin disorder. It is characterized by the presence of autoantibodies that target distinct adhesion molecules {desmoglein-3(Dsg-3)} of the epidermis and dermoepidermal basement membrane zone, leading to a loss of adhesive function of the target antigen(s) and, clinically, to the appearance of blisters and erosions. Auto reactive T cells are critical for the induction and regulation of antibody production. With regard to cytokine production profiles, it has been reported that qualitative as well as quantitative alterations in cytokine production can result in activation of inefficacious effector mechanisms and therefore, complex and severe impairment in immune functions. T-cell recognition of epitopes of Dsg 3 may be crucial for the initiation and perpetuation of the production of Dsg 3-specific autoantibodies by B-lymphocytes in Pemphigus vulgaris.

AIM: The purpose of this study was to observe the alterations in the levels of T_H1 [Interleukin-2 (IL-2), Interferon-gamma (IFN-γ)] and T_H2 (IL-4 and IL-10) cytokines in the sera from patients affected with PV and compared with Pemphigus foliaceus and healthy subjects. This work is aimed to comprehend the involvement of T_H1 and T_H2 cells as inflammatory infiltrate in the modulation of acantholysis and production of pemphigus lesions.

METHODS: Sixty PV, 12 PF and 50 healthy, age matched individuals without any generalized skin diseases like erythroderma,

Stevenson and Johnson syndrome, toxic-epidemic necrolysis were included in this study. PV and PF patients were diagnosed by Tzanck smear examination, histopathology (by hematoxylin and eosin), direct immunofluorescence and Dsg (1 & 3) ELISA. The levels of Th 1 cytokines (IL-2 & IFN-γ) and Th 2 cytokine markers (IL-4 & IL-10) were estimated by commercially available high sensitivity ELISA kits.

RESULTS: All patients with PV and PF showed significantly ($p<0.000$) elevated levels of T_H-2 cytokines IL-10 and IL-4 as compared with healthy controls. However, mean concentration of IL-2 was significantly ($p<0.000$) decreased in patients as compared to healthy individuals. No significant change was observed for IFN-γ in all groups. Both T_H1 and T_H2 cytokines did not show any significant difference between PV and PF cases.

CONCLUSIONS: Current concepts support the idea that PV, induced by autoantibodies against Dsg3, is the consequence of an imbalance between Dsg3-reactive Th2 and Th1 cells that may be critical for the maintenance of tolerance against Dsg3. Cytokine profile for confirmed PV cases showed direct evidences for involvement of T cell responses. Increase in IL-4 and IL-10 shows induction of T_H2 cells in the pathogenesis of autoimmune disorders Pemphigus vulgaris. The decreased levels of IL-2 might demonstrate the inhibitory effects by IL-4 and IL-10, which suppress the expansion of T_H1 population.

43.

M6P/IGF2R-DEPENDENT CLEAVAGE OF UPAR PROVIDES NEGATIVE FEEDBACK TO CONTROL PLASMINOGEN ACTIVATION

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The enzymatically inactive zymogen plasminogen (Plg) is activated by the urokinase plasminogen activator (uPA), bound to its receptor on cells (uPAR), to the serine protease plasmin (Plm). Plm is one of the major proteases needed for the initiation of tumor invasion, angiogenesis and the infiltration of inflamed tissues by neutrophils. Further, uPAR is also upregulated on activated T cells. Plm was shown to activate latent transforming growth factor beta (LTGFb), which can bind to the mannose 6-phosphate / insulin-like growth factor 2 receptor (M6P/IGF2R) on cell surfaces. Interestingly, we could show that M6P/IGF2R interacts with uPAR on human leukocytes, but also on various other cell types such as endothelial cells, thereby regulating uPAR functions and potentially LTGFb activation (Godar, Horejsi et al. 1999; Leksa, Godar et al. 2002; Leksa, Godar et al. 2005)

We report here that the loss of M6P/IGF2R expression by RNA interference results in accumulation of urokinase and plasminogen on the cell surface of human cancer and endothelial cells, and also in enhanced surface expression of alpha(V)beta(3) integrin on cancer cells. The silenced cells display increased plasminogen activation and higher invasive potential due to a direct effect on uPAR, as evidenced by genetic rescue experiments and inhibitor studies. In control cells, M6P/IGF2R interacts with the full-length uPAR facilitating cleavage of uPAR by urokinase/plasmin, whereas

M6P/IGF2R silenced cells express predominantly full-length uPAR due to reduced cleavage of uPAR. Equally, co-expression of human M6P/IGF2R and uPAR in mouse fibroblasts enhances cleavage of uPAR. This M6P/IGF2R-dependent proteolytic processing of uPAR results in loss of the uPA binding site on cells and therefore boosts a negative feedback loop upon onset of pericellular Plg activation.

Since both, uPAR and M6P/IGF2R, are upregulated on activated leukocytes we propose that the M6P/IGF2R dependent mechanism described here might also be relevant for inflammatory processes.

44.

VITAMIN D RECEPTOR ACTIVATORS CALCITRIOL AND PARICALCITOL MODULATE DENDRITIC CELL PHENOTYPE AND FUNCTION – IN VITRO STUDY

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Vitamin D is known as an important immunomodulatory agent. Its potential to suppress immune response in autoimmunity is intensivelly studied. Use of active form of vit. D3, calcitriol, in vivo, however, is limited due to its hypercalcemic effect. Paricalcitol, the synthetic analogue of calcitriol, is characterized by reduced hypercalcemic effect, but its immunosupressive properties have not been yet been investigated. In this study we compared the effect of calcitriol and paricalcitol on morphological and functional characterististics of dendritic cells (DC) in vitro.

DC were differentiated from monocytes with IL4 and GM-CSF for 5 days and then activated with LPS. Calcitriol or paricalcitol were added during the period of differentiation or maturation. DC differentiated in the presence of calcitriol or paricalcitol remained at immature state after maturation. They had low expression of CD80, CD83, CD86 and HLA-DR, high expression of CD14, high endocytic activity, low T-cell stimulatory capacity and produced no IL-12. DC activated in the presence of both drugs were also impaired, but to a lesser extent.

The immunosuppressive effect of both calcitriol and paricalcitol on DC was comparable. Paricalcitol thus seems to be a perspective drug for modulating immune response without significant hypercalcemia.

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45.

INVOLVEMENT OF ENDOCYTOSIS IN TRAIL-R1/DR4 AND TRAIL-R2/DR5 TRAFFICKING AND SIGNALING

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TRAIL (TNFα Related Apoptosis Inducing Ligand) is as other ligands of the TNFα family expressed mainly on surface of hematopoietic cells (T lymphocytes, monocytes, dendritic and NK cells, neutrophils). Binding to its pro-apoptotic receptors TRAIL–R1/DR4 or TRAIL-R2/DR5 triggers apoptosis preferentially of transformed cells and plays important role in the immune surveillance. Receptor clustering and their intracellular associations with adaptor protein FADD and pro-caspase-8 represent essential first steps of TRAIL-induced apoptotic signaling. Also ligand-triggered internalization of death-receptors such as TNFR1 or Fas/CD95 is apparently crucial for the induction of receptor-mediated apoptosis, but for TRAIL receptors the published data are not as unambiguous. Thus our project is aimed on clarifying significance of endocytosis and death receptor trafficking for TRAIL-induced cell death. In order to distinguish the involvement of clathrin-dependent and -independent endocytosis, we performed set of experiments with biochemical inhibitors like dansylcadaverine, methyl-β-cyclodextrin, 5-(N-ethyl-N-isopropyl)amiloride and chlorpromazine on TRAIL-sensitive colon cancer cell lines, and we compared this approach with siRNA-mediated downregulation of the gene expression of the selected members of the endocytic machinery (AP2, clathrin heavy chain, caveolin-1 or cdc42). We analyzed rate of TRAIL receptors endocytosis, efficacy of DISC formation and kinetics of TRAIL-induced apoptosis. The relevant results pointing to a role of the internalization of TRAIL-death receptors will be shown and discussed.

46.

IL-2/ANTI-IL-2 MAB IMMUNOCOMPLEXES IN COMBINATION WITH POLYMER-BOUND DRUG-BASED CHEMOTHERAPY AS A NOVEL APPROACH FOR CANCER TREATMENT

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It has been clearly shown (Boyman, 2006) that application of anti-IL-2 mAb is not always functioning as a depleting process of IL-2. Moreover, such approach augments proliferation of $CD8^+$ cells simply by increasing the biological activity of preexisting IL-2 through formation of immune complexes. According to mAb used, complexes of recombinant IL-2 and mAb show variable effects on certain cell populations. By usage of these immunocomplexes, boosting or inhibiting immune response could be achieved. Thus, we explored the possibility of combining treatment with polymeric HPMA-doxorubicin conjugates (Říhová, 2005), which have been shown previously to possess strong anti-tumor activity and immune system-preserving capability, and immunocomplexes of IL-2 and anti-IL-2 mAb, which are able to expand main anti-tumor population, $CD8^+$ T-lymphocytes. The idea behind this study is that chemotherapy is able to eradicate most of the tumor cells while immunotherapy enhances the immune system, so it can eliminate small proportion of remaining tumor cells, i.e. to treat minimal residual disease (MRD).

47.

FUNCTIONAL CONSEQUENCES OF KV1.3 ION CHANNEL REARRANGEMENT INTO THE IMMUNOLOGICAL SYNAPSE

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Formation of immunological synapse (IS), the interface between the T cell and the antigen presenting cell, is a crucial step in T cell activation. This conjugation formation results in the rearrangement and segregation of a set of membrane bound and cytosolic proteins, including that of the T cell receptor, into membrane domains. We showed previously that Kv1.3, the dominant voltage-gated potassium channels of allogen-activated human cytotoxic T cells redistribute into the IS on interaction with their specific target cells. In the present experiments we investigated the translocation of Kv1.3 channels into the IS formed between mouse helper T (Th2) and B cells. Furthermore, we characterized the functional consequences of this translocation.

Kv1.3 channels were labeled using indirect immunofluorescent method. Biophysical characteristics of whole-cell Kv1.3 current in standalone cells (C) or ones in IS (IS) were determined using voltage-clamp configuration of standard whole-cell patch-clamp technique.

Confocal microscopic images demonstrated that Kv1.3 channels of T cells quickly rearrange into the IS, within 10 minutes after the functional IS formation. Furthermore, patch-clamp recordings showed that activation of Kv1.3 current slowed ($\tau_{a,IS}$=2.3 ± 0.1 ms (n=8); $\tau_{a,C}$=1.35 ± 0.06 ms (n=18)) whereas the inactivation rate increased ($\tau_{i,IS}$=263 ± 29 ms (n=7); $\tau_{i,C}$=364 ± 26 ms (n=17)) in cells being in IS compared to the standalone cells. The equilibrium distribution between the open and the closed states of Kv1.3 (voltage-dependence of steady-state activation) was not different between control and conjugated cells. Thus, segregation of Kv1.3 channels into the IS modifies the kinetic properties of the channels, which might be explained by phosphorylation of the ion channel in the IS.

48.

GENE EXPRESSION AND EPIGENETIC CHANGES IN DENDRITIC CELL DIFFERENTIATION

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Dendritic cells are important cells of immune system because they are the main antigen presenters to T cells and act as a bridge between innate and adaptive immune responses. During the differentiation of dendritic cells, specific changes on the level of gene expression have been described, however, the epigenetic role in the regulation of dendritic cell differentiation has been poorly studied. The regulation of gene expression, through chromatin epigenetic modifications, has an important role in cell development and differentiation and, often histone modifications, such as methylation, acetylation and phosphorylation, determine whether a gene is activated or silenced. In this work monocytes were isolated from the healthy volunteers and differentiated in vitro to macrophages or dendritic cells by GM-CSF or GM-CSF + IL-4, respectively. We carried out a gene expression analysis using RT-qPCR, demonstrating an upregulation of macrophage-specific genes CD14 and FCγR1A, and dendritic cell-specific genes CCL17, CCL22, CD1A, MAOA and WNT5A. Using this model system and chromatin immunoprecipitation method we also found significant changes of active (H3K4met3, AcH3) or inactive (H3K27met3) chromatin marks in these genes during the differentiation process.

49.

SOLUBLE RECOMBINANT HUMAN CD69 RECEPTOR AND ITS LIGANDS AS EFFECTIVE TOOL IN ANIMAL TUMOR THERAPIES

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CD69, an earliest activation antigen of lymphocytes, proved important both in activation and effector functions of lymphocytes involved in anticancer immunity. However, recent studies revealed that CD69-deficient mice are more resistant to experimentally induced tumors, and thus supported the role of CD69 in downregulation of the immune response. Though physiological ligand for CD69 has not been found yet, several compounds (GlcNAc, sialylTn antigen, mycobacterial peptides and calixarenes carrying carboxy groups) appear to be high-affinity ligands in *in vitro* binding assays. When in multivalent form, these structures are able to crosslink CD69 both in solution and on the cell surface with subsequent cell activation and apoptosis, as assessed by ligand-induced receptor precipitation, Ca^{2+}/IP_3 measurement and flow cytometry. This effect can be inhibited by monovalent compound or soluble receptor, protecting CD69+ lymphocytes and rendering them available for tumor killing, providing a possible explanation for results obtained *in vivo* with sialylTn dendrimers and their monovalent components. Here we report that the same effect was observed in B16 melanoma treated with recombinant human CD69. Protein administered during the critical stage of tumor progression significantly decreased the size of tumor and prolonged the survival of treated animals. Manipulation of CD69-ligand interaction may thus modulate the immune response in tumor bearing animals.

50.

ADCC MEDIATED BENEFICIAL IN VIVO EFFECTS OF EARLY TRASTUZUMAB TEATMENT ON IN VITRO TRASTUZUMAB-RESISTANT BREAST CANCER AND CIRCULATING TUMOR CELLS

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Trastuzumab is a recombinant antibody widely used for treating breast cancer. Despite clinical benefits, some cancers are primarily resistant to trastuzumab, and a majority of those initially responding become resistant during prolonged treatment. Unexpectedly, in experiments mimicking adjuvant therapy of submacroscopic disease in vivo, where we have treated SCID mice after xenografting with the ErbB2 positive, *in vitro* trastuzumab resistant JIMT-1 cells, trastuzumab was able to inhibit the outgrowth of macroscopically detectable tumors for up to 5-7 weeks. The effect was likely mediated via ADCC, since trastuzumab-F(ab')2 was ineffective in this model. Moreover, in vitro ADCC reaction of human leukocytes was equally strong against breast cancer cells intrinsically sensitive (SKBR-3) or resistant (JIMT-1) to trastuzumab, or even against a subline of JIMT-1 that was established from xenograft tumors growing despite trastuzumab treatment. We have also shown that trastuzumab significantly reduced the number of circulating and disseminated tumor cells (CTCs and DTCs) at a time when the primary tumor was already unresponsive to trastuzumab. This observation suggests that ErbB2 positive CTCs and DTCs might be sensitive to trastuzumab-mediated ADCC even when the primary tumor is already non-responsive. Thus, measuring the ADCC activity of patient's leukocytes against the tumor cells may be a relevant predictor of clinical trastuzumab responsiveness in vivo, and trastuzumab treatment might also be beneficial in the case of patients with primary breast cancer that is already trastuzumab resistant.

51.

CHEMOTHERAPY BASED ON POLYMERIC MICELLAR DRUG DELIVERY SYSTEM WITH pH-CONTROLLED RELEASE OF DOXORUBICIN INDUCES SYSTEMIC ANTI-TUMOUR IMMUNITY IN MICE

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Amphiphilic diblock copolymer poly(ethylene oxide)-*block*-poly(allyl glycidyl ether) represents a novel class of promissing drug delivery systems for anti-cancer therapeutics. This system is characterised by unique core-shell architecture, where hydrophilic blocks form a shell, which segregate the core of hydrophobic polymer chains from the aqueous exterior. The hydrophobic core there serves as the pleasant loading space for doxorubicin (Dox). Linkage of hydrophobic Dox to a hydrophobic poly(allyl glycidyl ether) enable the increase of usefull therapeutic dose (over 30 fold). Moreover, in comparison with free drug, the micellar conjugate eliminates most of acute as well as delayed side-toxicity. The linkage of Dox via a pH sensitive bond provide release of Dox within the tumour intersticium. In EL-4 lymphoma-bearing C57BL/6 mice, a complete regression of pre-established tumours has been achieved upon treatment with micellar Dox. The treatment was effective, ranging from 20 to 70% cured mice treated with a single dose of 75-150 mg of Dox eq./kg, respectively. 85% of mice that are cured by intravenous injection of 150 mg of micellar Dox eq./kg develop a long-lasting memory and systemic antitumour resistance. It is suggested that the main activity of the polymeric drug, directly after application is - due to the high level of the drug - of cytotoxic and cytostatic nature. Thereafter, long-term conjugates persist at low concentration in the circulation, which are capable of mobilizing the defence mechanisms of the host.

52.

ANTIGENICITY OF CMV PP65 $_{495\text{-}503}$ PEPTIDE AFTER OXIDATIVE MODIFICATION

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Oxidative modifications may dramatically affect cell function during senescence and limited information is available whether oxidative modifications influence the presentation of antigenic peptides. We therefore decided to address this issue and started out by asking whether oxidative modification of CMV pp65 $_{495\text{-}503}$ (NLVPMVATV) peptide changes its antigenicity. We first oxidized the CMV pp65 (NLVPM[ox]VATV) and tested its capacity to stimulate CD8+ T cells. Using labelled peptide MHC multimers we found that the proliferation of antigen specific T cells was significantly reduced when the oxidized peptide was used instead of the native one. IFNγ secretion was also affected by the oxidative modification of the CMV peptide. Using binding and dissociation assays we could demonstrate that the decrease in proliferation and cytokine production was due to a strikingly decreased binding affinity of the oxidized peptide to the T cell receptor whereas binding to the MHC class I complex was not impaired. Taken together our results suggest that oxidation of antigens may severely affect T cell responses, which may lead to a decreased immune response against infectious agents, as well as tumor- or autoantigens.

53.

ANTIGEN-SPECIFIC RESPONSE OF SPLENOCYTES IN CO-CULTIVATION WITH AMSC FROM EAE RATS

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Current theories on the induction and progression of multiple sclerosis (MS) place autoreactive T cells in the centre of autoimmune pathogenesis. Our previous data showed that autoreactive response in MS is driven by both $CD4^+$ and $CD8^+$ memory T cells. Experimental autoimmune encephalomyelitis (EAE) is an autoimmune inflammatory disease of the CNS and represents the paradigmic model for MS.

In MS, therapeutic approaches targeting T cells have been successfully used, leading to immunosupression or tolerance. Mesenchymal stem cells (MSCs) are multipotent progenitor cells with great promise for pathogenic therapy of MS following immunomodulatory and neuroprotective properties.

In present study adipose tissue-derived MSCs (aMSCs) were co-cultured with splenocytes to compare immunosuppressive properties of aMSCs from rats with established EAE and aMSCs from healthy ones. The majority of these cells were able to differentiate into adipocytes and osteoblast-like cells. We showed that aMSCs of 1^{st} and 2^{nd} passages derived from EAE rats, as well as aMSCs from healthy one, inhibit ConA- and myelin-antigen stimulated splenocytes proliferation. Moreover, supernatants from aMSC cultures showed inhibitory effect on mitogen- and antigen-specific response of splenocytes comparable with immunosuppressive effect of direct co-culture of aMSCs with stimulated splenocytes.

Our data demonstrate that aMSC from EAE and healthy rodents exert their immunosuppressive effects through both soluble factors and cell-cell contact, these effects were similarly and don't depend from initial status of rats.

54.

IN VIVO AND IN VITRO CHANGES OF CELL-COUNTS IN PORCINE PERIPHERAL BLOOD AFTER GAMMA-IRRADIATION

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Absolute counts of porcine peripheral blood cells were quantified by flow cytometry as a marker of radiosensitivity. Irradiated human organism responds to irradiation among others by declining haemopoiesis and inducing an apoptosis of radiosensitive cells, especially lymphocytes. However can be changes of human cell-counts well examinated in peripheral blood in vitro, in vivo model is not obtainable in adequate range of doses. The experimental model of one month old large white pig was used to compare effects in these two systems. Young piglets were whole-body-irradiated and assessed in dependence on time upon irradiation. For the dose-response analyses doses 0-2-4-6 and 10 Gy were applied at 0,4 Gy/min (60Co gamma-rays). Beside that heparin-treated peripheral blood was irradiated apart and analysed at the same time frequency. Absolute counts of leukocyte populations were analysed by CytoCountTM technique (DakoCytomation) using the flow cytometry through gating within forward scatter versus side scatter. Counts of cells were expressed as a ratio of individual population with regard to nonirradiated negative control sample. The estimated decrease of lymphocytes manifested more intensive within in vivo system than in vitro. The maximum downtrend of both was marked during eight hours after irradiation. Decrease of granulocytes was very low with no distinct response to irradiation within system in vitro whereas analyses in vivo documented intensive release of granulocytes from body reserves into peripheral blood as a typical inflammation reaction. Populations of monocytes were hardly detectable and were not included in our study. Nevertheless variance in absolute counts in system in vivo versus in vitro after irradiation show irretrievability of using in vivo system within radiosensitivity studies.
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55.
DETECTION AND CHARACTERIZATION OF DENDRITIC CELLS SUBSETS BY EIGHT COLOUR FACS ANALYSES

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Dendritic cells (DCs) are specific antigen-presenting cells that play critical roles in the initiation and direction of immune responses. DCs are usually defined on the basis of expressing markers CD45, HLA-DR, their subpopulations then as cells expressing CD11c (mDC) and CD123 (pDC). However, recently were dendritic cells subdivided into newly defined four distinctive groups: CD16+, CD1c (BDCA-1) +, BDCA-3+, CD123+ (BDCA-2+). In this study, we characterised these DC subsets in peripheral blood and in BAL (bronchoalveolar lavage) by 8 colour FACS analysis, with the aim to characterize a distribution of these subpopulations and their expression of PRRs (pathogen recognition receptors) in different compartments. We created a specific panel of mAbs that identify three human dendritic cell subsets simultaneously. These subpopulations we characterized by common markers CD45, Lin- (CD3,CD19,CD20,CD56), HLA-Dr, CD14, CD16, BDCA-1 and BDCA-3. In these groups of cells we then detected PRRs by selected antibodies (DC-SIGN, MR, DEC-205, TLR2, 4).Preliminary data show that all four subsets of DCs are present in both microenvironments. However, while the most represented population of mDC (CD11c+) in blood are CD11c+CD16+, in lung play prominent role CD11cBDCA-1+ cells. Interestingly, CD11cBDCA-3+, that form the smallest population in blood, represent the second most dominant subset in BAL. As we supposed, DC subsets in bronchoalveolar lavage show stronger expression of PRRs receptors than in the blood. Data confirm successful development of novel eight colours FACS analysis for reliable monitoring of novel dendritic cells subsets that could better define DC role in the pathogenesis of diseases.