("late vaccination"). In conclusion, our data suggest that the combination of Treg temporary interference with ErbB2 specific DNA tumor vaccine reshuffles the T cell repertoire, and lead to both preventive and therapeutic anti-tumor immunity.

649 Poster

Methods for the isolation and identification of MHC-presented peptides from leukaemic cells

K. Kapoor[1], D. Barry[1], M. Mathieu[1], A.K. Miles[1], R.C. Rees[1], I.A. Dodi[2], C.S. Creaser[3], P.L.R. Bonner[1]
[1]Nottingham Trent University, School of Science and Technology, Nottingham, United Kingdom; [2] Royal Free Hospital, The Anthony Nolan Research Institute, London, United Kingdom; [3] Loughborough University, Department of Chemistry, Loughborough, United Kingdom

Aberrant kinase activity is implicated in the majority of malignancies. As specific immune responses may be generated to phosphopeptides presented by MHC molecules, the isolation of these peptides from the surface of cancer cells may form a basis for immunotherapy. In chronic myeloid leukaemia (CML) the majority of cases (95%) are caused by the fusion of the bcr and abl genes which results in the production of a deregulated tyrosine kinase, hypothetically resulting in aberrant MHC phosphopeptide expression.

Here we describe the development of a mild acid elution technique to selectively release peptides from MHC class I or II complexes, with minimal contamination by intracellular material or serum proteins. Unlike previous approaches, this method is compatible with immobilised metal ion affinity chromatography (IMAC), a powerful tool for fractionation of peptides and the subsequent simplification of mass spectrophotometric profiles. Fractionation of cell surface eluates from the CML cell line K562-A3 by a range of methods including IMAC and characterization by tandem mass spectrometry lead to the identification of numerous peptides and phosphopeptides, many of which bear strong links to malignancy. These include peptides from: ephrin-A4 precursor, elongation factor 1-alpha 1, MYEOV, and Myc binding protein 2; and phosphorylated peptides from nuclear receptor coactivator 2 and membrane-associated protein HEM-1.

650 Poster

Analyses of novel tumour antigens as targets for cancer immunotherapy

K. Silina[1], Z. Kalnina[1], I. Meistere[1], P. Zayakin[1], A. Abols[1], A. Rivosh[1], A. Line[1]
[1]Latvian Biomedical Research and Study Centre, Molecular Genetics of Cancer, Riga, Latvia

The efficiency of current immunotherapy approaches is still far from that expected. In order to identify immunogenic proteins of melanoma and prostate cancer that could be used as target genes for cancer therapy we screened phage-displayed cDNA libraries derived from melanoma prostate cancer, and testis with sera from 76 cancer patients. This resulted in the identification of >1000 different clones, however, only about 10% of them represented cDNAs fused in-frame to the T7 phage coat protein thus ensuring the exposition of natural products of genes on the phage surface. The rest likely represent mimotopes, albeit, there might be true tumour antigens among them that resulted from frame-shifting mutations or defective regulation of alternative splicing or translation.

As none of the identified clones contained a mutation we further looked for novel cancer-germ cell or overexpressed antigens. The criteria for selecting an antigen for expression analyses were: it (1) contains an uncharacterised splice variant, (2) represents a novel gene, (3) shows a cancer associated EST profile or (4) plays an important role in oncogenesis. The expression was tested in a panel of 15 normal tissues and in paired cancerous and adjacent normal tissues of 46 melanoma, breast, prostate and gastric cancer patients using qPCR. We have so far tested the expression of 18 antigens. We saw a testis specific expression for 2 of 3 genes with cancer associated EST profile, but no overexpression in cancerous tissues comparing to the normal counterparts was observed. 2 of 3 novel genes showed a predominant expression in testis and one so far tested also showed a cancer associated overexpression (clone 284). None of the 5 tested functionally relevant genes showed an elevated expression in cancer. 4 out of 7 novel splice variants of known genes were testis specific, and one of these tested so far (clone 29) showed also a cancer-associated overexpression.

The alternative splicing of immuno-privileged tissues like testis is very extensive, hence it is possible that due to splicing defects often observed in cancerous tissues such testis-associated isoforms could be formed and recognised by the immune system, leading us to hypothesise that analogously to cancer-germ cell expressed antigens a category of cancer-germ cell spliced antigens might exist. Clones 29 and 284 will further be subjected to T cell activation assays to test their potential to be used in cancer immunotherapy applications.

651 Poster

CD8+ cytotoxic T lymphocytes generated against a WT1 peptide analog enhance the lytic activity of leukemic cells

G. Al Qudaihi[1], C. Lehe[1], M. Negash[1], M. Al-Alwan[1], H. Ghebeh[1], S.Y. Mohamed[2], A.J.M. Saleh[2], A. Dickinson[3], M. Aljurf[2], S. Dermime[1]
[1]King Faisal Specialist Hospital, Tumor Immunology/Stem Cell Therapy Program, Riyadh, Saudi Arabia; [2] King Faisal Specialist Hospital, Adult Hematology/Oncology, Riyadh, Saudi Arabia; [3] Medical School University of Newcastle upon Tyne, Haematological Sciences/ School of Clinical & Laboratory, Newcastle upon Tyne, United Kingdom

Background: The WT1 antigen performs an oncogenic function in various types of cancer. It is overexpressed in human leukemias and therefore it has been considered as an attractive target for immunotherapy. Most WT1-specific CTLs described displayed a low avidity and exerted minimal lytic activity against cancer cells.

Materials & Methods: We used an approach to improve the immunogenicity of CTL epitopes consisting of substituting the first-amino-acid, of 2 known HLA-A0201-restricted WT1-derived peptides (Db126 and WH187), with tyrosine (Y).

Results: This modification resulted in the enhancement of the binding ability of the 126Y analog and CTL generated against this peptide exerted a significantly lytic activity against the 126Y peptide-loaded target cells and importantly cross-reacted with the 126N native peptide. Another interesting finding is the significant high lytic activity recorded for the 126Y CTL against freshly isolated HLA-A0201-matched leukemic cells expressing the WT1 antigen. This data confirms that T cells generated against the 126Y analog peptide cross-react also with the naturally processed 126N native peptide. Moreover, it seems that stimulation with the peptide analog induced CTLs with a high TCR avidity. Finally, the high lytic activity provoked by the 126Y CTL may be also attributed to the significant high number of anti-126 T cell frequencies in this T cell line as demonstrated by IFN-gamma production in the ELISPOT assay.

Conclusions: This study provide evidence that peptide modification results in a better immune response against cancer and further support the use of this strategy as a potential approach for the development of a leukemia-vaccine.

652 Poster

Study of Lewis y expression and anti Lewis y immune response through Lewis y-circulating immune complexes detection in breast cancer patients

M. Isla-Larrain[1], M. Crespo[1], E. Lacunza[1], A. Barbera[1], F. Terrier[1], A. Creton[1], A. Segal-Eiras[1], M. Croce[1]
[1]CINIBA, Faculty of Medical Sciences, University of La Plata, La Plata, Argentina

The aims of this study were: 1-to detect Lewis y (Ley) antigen in breast tumor samples; 2- to determine the role of MUC1 as a carrier of Ley and 3- to investigate the induction of humoral immune response through the detection of circulating immune complexes (CIC) carrying Ley in breast sera. Materials and methods: 137 breast tissue and serum samples: 72 malignant tumours, 30 benign diseases and 35 normals. The expression of Ley was determined by standard immunohistochemistry (IHC); percentage of stained cells, intensity and pattern of the reaction were analyzed. An immunoprecipitation was performed in order to determine if MUC1 may behave as a possible carrier for Ley. HMFG1, an anti MUC1 monoclonal antibody (MAb) was used to precipitate MUC1 from breast cancer sera. Immunoprecipitates (IP) obtained were run in SDS-PAGE and Western blot (WB) assays. Sheets were incubated with C14 (anti Ley) and HMFG1. An ELISA was developed to study the presence of Ley/CIC. Briefly, C14 was adsorbed in multiwell microplates and incubated overnight at 4ºC. After washing, 1% bovine serum albumin/PBS was added for 3 hours at 37ºC. Serum samples were incubated overnight at 4ºC and 1:2000 anti human IgM or 1:3000 IgG reacted with the complexes and revealed with ABTS and 30% H2O2 in sodium citrate buffer, pH 5.0; OD was measured at 405 nm. Results: By IHC with C14, positive results were found in 34% malignant tumors; 33% benign diseases and 35% normal samples; no statistical difference was found. The pattern of expression differed between malignant and non malignant samples: cancer specimens showed more frequently a cytoplasmic and membrane non apical reaction while non malignant samples showed an apical membrane reaction. By WB, IP displayed a band at >200KDa with both C14 and HMFG1 MAbs. By ELISA, mean OD for IgM/LeyCIC in breast cancer sera, benign and normal samples were: 0.538, 0.949 and 0.942, respectively. By ANOVA, significant statistical differences between breast cancer and normal and benign samples were found. Mean OD for IgG/LeyCIC were: 0.414, 0.438 and 0.492,

respectively. Conclusions: 1- By IHC, non statistical differences among breast cancer, benign and normal samples were found although a different pattern of expression was observed; 2- Immunoprecipitation and WB indicated that MUC1 may behave as a possible carrier for Ley in breast cancer and 3- IgM/LeyCIC showed a significant statistical difference between breast cancer and normal and benign samples.

653 Poster

Prognostic significance of tumor-infiltrating cytotoxic T-cells in colorectal cancer

P. Fabian[1], I. Garajova[2], M. Svoboda[2], O. Slaby[1], R. Nenutil[1]
[1]Masaryk Memorial Cancer Institute, Dept. of Oncological and Experimental Pathology, Brno, Czech Republic; [2] Masaryk Memorial Cancer Institute, Dept. of Comprehensive Cancer Care, Brno, Czech Republic

Purpose: Various immune/inflammatory cells can be found in colorectal cancer (CRC) tissue. Its potentional influence on the prognosis of CRC is controversial. We have previously demonstrated that there is no prognostic significance of regulatory T-cells (TREG, CD4+ CD25+ FOXP3+) in patients (pts) with CRC. In this study, we focused on cytotoxic tumor-infiltrating T cells (CD8+) located in the tumor epithelium which are generally considered to be prognostically favorable. Moreover, pre-operative total white cell count and individual counts of eosinophiles, neutrophiles, lymphocytes were evaluated from peripheral blood as well. Methods: Formalin-fixed, paraffin embedded tumor samples from 55 pts with CRC in clinical stage I-IV according to IUCC were evaluated by immuno-histochemistry using commercially available anti-CD8 mouse monoclonal antibody. Intraepithelial CD8+ cells were enumerated in one high power magnification field in the area with the highest CD8+ cell infiltration, so called “hot spots”. Its prognostic effect was evaluated using Kaplan-Mayer method. Results: We did not prove any statistical significance between absolute number of CD8+ cells in “hot spots” and examined clinico-pathologic parameters (clinical stage, overall survival, disease-free survival, left/right localization of tumor, “T” status, adjuvant chemotherapy, total white cell count and individual counts of eosinophiles, neutrophiles, lymphocytes with overall survival or disease-free survival). Only between the pre-operative absolute number of peripheral eosinophiles and better survival we observed positive correlation and trend to statistical significance. The median follow-up was 50 months. Conclusion: We did not prove that infiltration of intraepithelial CD8+ T-cells correlates with overall survival of colorectal cancer patients and we conclude there is no prognostic significance of intraepithelial CD8+ cells in CRC. Supported by IGA MZ CR NR/9076-4.

654 Poster

Leukocyte reprogramming mimics HIF-1a knock out

S. Hoffmann[1], P. Freitag[1], S. Frede[1], J. Fandrey[1]
[1]Universitätsklinikum Essen, Institute for Physiology, Essen, Germany

Solid tumours as well as sites of bacterial inflammation recruit leukocytes to severely hypoxic areas. One of the key regulators during hypoxia and inflammation is the transcription factor complex hypoxia inducible factor-1 (HIF-1). It has already been shown that LPS as well as inflammatory cytokines induce the regulatory subunit HIF-1α in a non hypoxic manner.

In this work, we studied the consequences of a pre-treatment with low doses of LPS (reprogramming) for both HIF-1α protein and mRNA levels in different monocytic cell lines and animal models. Furthermore, we characterised the reprogrammed cells with regard to their viability under hypoxic conditions, phagocytic activity, and invasion into extracellular matrix.

Reprogrammed monocytes show diminished levels of HIF-1α protein after hypoxic stimulation and a significantly decreased LPS-induced HIF-1α gene expression. The expression of both HIF-1 target genes ADM and GLUT-1 are significantly reduced. The viability of reprogrammed cells exposed to hypoxia gets diminished, too.

From these results we conclude that prolonged exposure of leukocytes to low doses of LPS reduces the accumulation of HIF-1α and effects the induction of HIF-1 target genes. Thus, reprogrammed monocytes may fail to function properly under hypoxic conditions.

655 Poster

Salivary cytokines profile and smoking in patients with oral cancer

M.I. Nicolescu[1], L. Albulescu[2], I.F. Dragan[3], C.M. Cîrcu[3], C. Tanase[2], A.L. Bucur[4]
[1]Victor Babes National Institute of Pathology, Immunology, Bucharest, Romania; [2] Victor Babes National Institute of Pathology, Biochemistry, Bucharest, Romania; [3] Carol Davila University of Medicine and Pharmacy, Dental Faculty, Bucharest, Romania; [4] Carol Davila University of Medicine and Pharmacy, Oral and Maxillo-Facial Surgery, Bucharest, Romania

Background Cytokines play an important role in the pathology associated with chronic inflammatory diseases. Their role in cancer development is yet still underestimated. Interleukin 6 (IL-6) is the most studied mediator of the host response to tissue injury, infection and bone resorption. We also investigated several others cytokines saliva levels in our paper. Smoking is a well-documented risk factor for oral cancer, although the mechanisms of its negative influence are not yet fully understood.
Aim The current results in patients with different stages oral malignant tumors may indicate different mediator functions of salivary cytokines in response to smoking, thus leading to a milder or a more aggressive cancer phenotype.
Methods The influence of smoking on the gingival crevicular fluid (GCF) content of the pro-inflammatory cytokines was investigated in patients with oral malignant tumors and healthy controls by use of XMAP Array technology (Luminex 200). TNM calssification, histopathological data, as well as tumor localization and size were also recorded.ResultsThe expression of salivary cytokines was higher in subjects with oral tumors. The correlation with smoking was also noted, based on the differences between the results form the two groups.
Conclusion It is possible that monitoring cytokine production or its profile may allow us to early diagnose an oral malignant tumor also taking into account the smoking habits.

656 Poster

Preliminary results of a six months toxicity study in rats of the CIMAvax-EGF vaccine

A. Mancebo[1], A. Casacó[2], B. Gonzalez[1], D. Gómez[1], A. León[1], M.E. Arteaga[1], Y. González[1], A.M. Bada[1], C. González[1]
[1]CENPALAB, Center of Experimental Toxicology, Ciudad Habana, Cuba; [2] Center of Molecular Immunology, Clinical Assays, Ciudad Habana, Cuba

The CIMAvax-EGF vaccine consists of human recombinant EGF, coupled to a recombinant carrier protein, P64k from the meningitis B bacteria, and Montanide as adjuvant. The vaccine immunization induces the production of specific antibodies inhibiting the EGF/EGF-R interaction through EGF deprivation. The objective of the present study was to determine the toxicity of the CIMAvax-EGF vaccine obtained by ultra-filtration in Sprague Dawley rats after intramuscular administration of repeated doses (6 months). Rats were randomly distributed into four experimental groups: Control, Control plus adjuvant, Treated with the human total dose, and Treated with fifteen times the human total dose. The frequency of immunization was one weekly immunization for 9 weeks, plus 9 immunizations every 14 days. All rats were inspected daily for clinical signs. Body weight, food and water consumption, and rectal temperature were measured during the administration of test substances. Blood samples were collected for hematological and serum biochemical determinations at the beginning, three months later, and at the end of the study. Gross necropsy and histological examination of tissues was performed on all animals at the end of the assay. The study concluded with a survival of 95%, dying 4 animals of the Group Control + Adjuvant. The vaccine and the vehicle produced clinical signs of toxicity in the administration site, where macroscopic lesions was observed. The behaviour of the body weight gain and food and water consumption was normal for the used specie. There were not statistical differences of the behaviour of rectal temperature between groups. It could be preliminarily concluded that the intramuscular injection of CIMAvax-EGF vaccine produces only alterations limited to the administration site.

657 Poster

Comparative study of the molecular profile of the inflammatory and non-inflammatory breast cancer in Tunisia

R. Marrakchi[1], I. Khadimallah[1], K. Bougatef[1], S. Ouerhani[1], Y. Messai[1], N. Elajmi[1], A. Gammoudi[2], M. Elmay[2], K. Rahal[2], A. Ben Ammar Elgaaied[1]
[1]Faculté des sciences de Tunis, Biologie, Tunis, Tunisia; [2] Institut Salah Azaiez, Cancerology, Tunis, Tunisia

Introduction: Breast cancer is the most common type of cancer affecting women in the western world and represents approximately 13 % to 30% of new diagnosed malignancies in women in North Africa. Inflammatory cancer (IBD) is a clinical form of breast cancer characterised by a peculiar geographic distribution in incidence, particularity common in Tunisia. The aim of this study was to compare expression of two genes RhoCguanosine triphosphate and WNT-1(induced secreted protein WISP3) in IBD and non IBD tumors. Predicated on the high rate of concordance of WISP3 and RhoC changes in IBD, it has been proposed that these two genes cooperate in the development of the disease.

Methods: Our investigation is based on 45 tumors from breast cancer patients aged between 23 and 52 years diagnosed at service of Salah Azaiez Institute in Tunis: 17 tumors from patients with IBD and 28 tumors