pro-inflammatory M1 or the alternative anti-inflammatory/pro-tumoural M2 macrophage. The macrophage mannose receptor (MR) has been found up-regulated in the M2 phenotype and has been shown to be essential for cytokine production. MR might also interact with other canonical pattern recognition receptors in order to mediate intracellular signalling.
In an experimental model of mouse melanoma lung metastasis, we aim to describe the recruitment of myeloid cells into the lung after tumour cell challenge in a time dependent manner. Twenty-four hours after injection of B16F10 cells into the tail vein of C57BL/6 mice we observed a marked infiltration of $CD68^+CD11b^+CD11c^-$ monocytes into the lung. A fraction of these monocytes was $Gr\text{-}1^+$. The infiltration ceased within 48 h. In C57BL/6 mice lacking MR ($MR^{-/-}$), recruitment of these monocytes was abrogated. Three weeks after tumour cell injection, fewer lung colonies were scored in the $MR^{-/-}$ than in the wild type mice, suggesting a possible role for MR both in early and late stages of metastasis formation.
We aim to further characterize the monocyte and macrophage populations involved in lung colony formation with particular interest in the expression of macrophage polarization-related genes. We are using PCR arrays on monocytes and macrophages sorted from the mouse lungs following tumour cell challenge. The candidate polarization genes as potential targets in melanoma lung metastasis will be discussed.

305 Detection of circulating galectin-1 in the microvesicle fraction of serum from breast cancer patients

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Background: Galectin-1 is a β-galactose binding lectin implicated in tumour progression through its ability to regulate tumour cell migration, metastasis, and tumour-immune escape. Galectin-1 can be produced either by tumour cells or tumour stroma. High expression of galectin-1 by stromal cells in the tumour milieu has been associated with breast cancer invasiveness and progression.
Objective: To further evaluate the role of galectin-1 in breast cancer, we analyzed its expression in the microvesicle fraction of patients' serum.
Material and Methods: This study included serum samples from 12 breast cancer patients from I, II and III stages; also, 6 healthy women serum samples were analyzed as controls. Six milliliters of serum were centrifuged at $15{,}000 \times g$ for 30 minutes at 4ºC; supernatants were subjected to CL2B agarose column gel chromatography; void volume was ultracentrifuged at $105{,}000 \times g$ for 2 h at 4ºC and the pellet (microvesicles fraction, MV) was washed and resuspended in PBS. Further analysis by means of Western blot, electron microscopy and EpCAM+ magnetic isolation was performed. MV fractions were also applied to sucrose gradient centrifugation.
Results: 6 out of 12 patients showed galectin-1 expression in MV by Western blot; in these patients, galectin 1 was also detected in their EpCAM+ enriched fraction. MV electron microscopy showed the presence of a heterogeneous collection of membranous vesicles and nonmembranous particles ranging from 40 nm to 1 micron in diameter. Control samples did not show presence of microparticles in the pellets. Sucrose gradient centrifugation confirmed the presence of galectin-1 in both low and high density fractions although CD63 and Hsp70 exosome markers were not detected. Electron microscopic of low density fractions included typical MV and lipoprotein images.
Conclusions: Circulating galectin-1 in sera from breast cancer patients may be associated with tumour derived microvesicles which could control antitumour responses.

306 Identification of novel cancer-testis antigens by studying humoral response against cancer

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Exploration of humoral responses to cancer may reveal diagnostic and prognostic biomarkers and may help to discover potential immunotherapeutic targets. In a previous study we identified a set of 1321 antigens eliciting humoral responses in patients with melanoma, prostate or gastric cancer by phage display-based SEREX approach and studied the frequency of autoantibody responses to these antigens by exploiting phage-displayed antigen microarrays. The goal of the current study is to identify novel cancer antigens that could be used for development of polyantigenic immunotherapy approaches.
An initial set of 49 potential therapeutic targets was selected by including antigens with cancer-associated autoantibody responses, low or absent mRNA expression in normal tissues or the presence of cancer-associated splice variants determined by *in silico* analysis and no previous knowledge of their immunogenicity. These genes were subjected to sequential analysis of their mRNA expression in 14 different normal human tissues by real-time RT-PCR, followed by cancerous and adjacent normal tissue pairs from 50 patients with melanoma, breast, colon or gastric cancer and then by immunohistochemistry.
mRNA expression analysis in normal tissues revealed that 15 out of 49 antigens were expressed preferentially in immunoprivilleged tissues such as testis. Nine of them, including SPAG8, SPAG16, CFL1 etc, were expressed at various levels and frequencies in cancerous tissues. Restricted expression of SPAG8 in normal tissues was confirmed by IHC on tissue arrays. Interestingly, 4 of them are encoded by testis-restricted splice variants of ubiquitously expressed genes. We propose that deregulation of splicing controls in cancer cells may result in the production of splice variants that are normally produced only in germ cells and if these protein isoforms are expressed in cancers they may elicit immune response in cancer patients, hence representing novel category of tumour antigens − "cancer-testis spliced" antigens.
Thus, the systematic analysis of humoral responses to cancer revealed 5 novel cancer-testis antigens and a novel category of tumour antigens – cancer-testis spliced antigens and all of them are a subject to further analysis of their immunogenicity and relevance as immunotherapeutic targets.

307 Anti-cancer immune reaction induced by cryo-ablation therapy

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Background: Cryogenic treatment sometimes stimulates the immune system by releasing intracellular antigens. We evaluated anti-tumour immune response after cryotherapy by analyzing alterations in serum cytokine levels.
Methods: Percutaneous cryosurgery was performed in 13 patients with unresectable advanced tumours. The size of the ice ball was confirmed by intraoperative ultrasound 15 minutes freezing to make a 3 cm ice ball. The therapy was performed for three freeze/thaw cycles per a tumour per a treatment and was continued eight times for once a week. Evaluation of serum factors was done before and after ablation therapy, and serum tumour markers were measured after every four treatments. Tumours were evaluated by abdominal computed tomography after eight treatments. Serum levels of interleukin (IL) -4, -6, and -10, tumour necrosis factor (TNF)-alpha, and interferon (IFN)-gamma were measured by ELISA. The Th1/Th2 ratio was estimated from the IFN-gamma/IL-4 ratio.
Results: In five cases, tumour necrosis was identified not only in the treated area but also away from the treated area, and then these cases belonged to immune reaction (IR) group. In other cases, just local effect was noted (LE), and then levels of serum factors were compared with those in IR. Serum levels of AA and CRP were increased in both the IR and LE groups after the third treatment, and that of IL-6 paralleled CRP increases. No differences in the level of serum IL-2 was observed after treatment in any of the patients. The serum level of IL-10 was low in three patients in the IR group and in one patient in the LE, but it group tended to increase with the number of treatments. In contrast, the level of TNF-alpha was increased in the IR group but showed no remarkable changes in the LE group. The Th1/Th2 ratio was increased in the IR group, compared to that in the LE group. To evaluate the clinical significance of these alterations in serum cytokines, pretreatment levels, maximum levels in response to therapy, and the number of treatments necessary to induce maximum levels were compared between the two groups. Pretreatment levels of IL-10 in the LE group were significantly greater than those in the IR group ($p = 0.0071$), and the maximum value (67.9 ± 6.3 pg/mL) was greater than that for the IR group (58.4 ± 8.1 pg/mL), but no significant difference was found between the two groups. In contrast, both pretreatment levels and maximum levels in response to treatment of TNF-alpha were significantly greater in the IR group than in the LE group. The maximum Th1/Th2 ratio was significantly greater in the IR group than in the LE group, despite the factor that pretreatment levels and treatment times to induce maximum levels were similar between the two groups.
Conclusion: It might be possible to evaluate the appearance of immune responses to cryosurgery by monitoring serum cytokine levels.

308 Characteristics of NK cells isolated from regional lymph nodes of melanoma patients

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Background: Melanoma is an aggressive but also immunogenic malignant tumour. The first line of antitumour immune defense is mediated by natural killer (NK) cells that able to lyse malignantly transformed cells and also may play an important role in lymphoid organs in the control of spreading of malignant tumours. As NK cell activity against malignantly transformed cells is regulated by the balance between activating and inhibitory signals mediated by NK cell receptors, the aim of this study was to investigate the expression

of several NK cell receptors and early activation markers on NK cells in lymph nodes (LN) of melanoma patients.
Material and Methods: Regional (LN) were obtained from patients who underwent melanoma resection. LN were incised immideatelly after removal and cut into two parts: one half was paraffin embeded to perform pathohistological evaluation and the other was mechanically distrupted in order to obtain a single cell lymphocyte suspension. Lymphocytes were purified using Histopaque density gradient. The expression of CD16, NKG2D, CD158a, CD158b, CD25, CD69 and HLA-DR was analyzed on gated CD3-CD56+ NK cells by flow cytometer. Intracellular stainig of IFNg was done according to standard BD procedure.
Results: We show that NK cells isolated from histopathologicaly proven malignant LN have higher expression of CD16, the most important cytotoxic receptor, as well as the activating NKG2D NK cell receptor, compared to the benign LN. Regarding the expression of the inhibitory KIR receptors, malignant LN show the higher percentage of NK cells positive for CD158b, while the level of the other investigated KIR CD158a is similar to the bening LN. We also show that the presence of tumour cells in LN is associated with higher percentage of NK cells positive for CD69 activation marker, whereas the percentage of NK cells that express other investigated activation markers (CD25, HLA-DR) as well as he IFN-γ level, are similar to the benign LN.
Conclusions: Our results show that NK cells from malignant LN of melanoma patients, compared to benign LN, express higher percentage of NKG2D receptor that mediates antitumour activity by binding to stress-induced ligands on malignant cells, and also the higher level of CD158b inhibititory NK cell receptor whose ligands are MHC class I molecules that have been lost during malignant transformation. Invasion of malignant cells into LN may contribute to higher expression of CD16 cytotoxic receptor and CD69 activation marker.

309 Gene expression analysis of tumour markers associated to apoptosis and proliferation in head and neck cancer

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The development of oral and head and neck squamous cell carcinomas occurs in relation with multiple events including mainly: loss of cycle cell control, evasion from apoptosis, telomerase reactivation. Apoptosis represents a cellular "suicide" mechanism which helps keeping in normal limits the cell number in tissues and allows elimination of those cells presenting DNA mutations or having an abberant cell cycle, these cells being predisposed to malignant transformation. P53 phosphoprotein acts as a tumour suppressor and is involved in inhibiting cell proliferation when DNA damage occurs. Wildtype P53 plays a role as checkpoint protein for DNA damage during G1/S phase of cell cycle. Bcl-2 oncoprotein is a blocker of apoptotic cell death which resides on the cytoplasmic side of the mitochondrial outer membrane, endoplasmic reticulum and nuclear envelope. We have studied the gene expression of two proteins (p53 and bcl-2) related to apoptosis and proliferation of tumour cells, correlated with antigen expression by flow-cytometry and progression through cell cycle phases. Gene expression analysis was performed by real time RT-PCR on 24 tumours from patients with head and neck cancers. After total RNA extraction, cDNA synthesis and optimization of PCR reaction, data were analized by REST program in order to study the levels and variability of gene expression for the two proteins and correlations between them. In order to confirm the specificity of PCR reaction, the amplification products were examined by 2% agarose gel electrophoresis. The house-keeping gene used for normalization of C_T values was RPL 32. Progression through cell cycle phases was evaluated by PI technique and analysis, while percentages of apoptotic cells were detected by using Annexin V-FITC/PI coloration, followed by flow-cytometry. In addition, gene expression of studied molecules was correlated with antigen expression detected by flow-cytometry. Characterization of head and neck cancers by using modern methods of molecular biology and immunology might lead to a better understanding of the disease, orientation of the oncological treatment and patient's response to chemotherapy.

310 Expression of cell adhesion molecules on MDR-1 positive stimuli treated breast tumour cells

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Breast cancer represents a malignancy with high incidence and mortality throughout women, its ethiology involving many genetic, immunological and biochemical factors. Proliferation of mammalian cells is tightly regulated by multiple environmental influences, adhesion to extracellular matrix (ECM), cell-cell adhesion and soluble factors. Formation and spread of tumours are closely associated with decreased dependence on adhesion for growth and survival. Co-expression of certain cell adhesion molecules (CAMs) by tumour cells, which might be involved in cellular interactions, changes in adhesivity and cellular mobility, might influence the aggressivity and metastatic potential of a certain tumour. Malignant evolution depends on the genetic profile of the tumour that dictates its reaction to the citotoxic action exerted by drugs. Those might induce modifications of gene expression (*e.g.* glycoprotein P, known also as MDR-1) that contribute to a resistant phenotype. Therefore, the use of certain natural compounds might increase the efficacy of chemotherapy and diminish tumour resistance. The present study focused on the correlation between antigen and gene expression of several CAMs (ICAM-1, MUC-1, E-cadherin, VCAM-1) and MDR-1 associated to breast cancer cell lines in the presence or absence of drugs (doxorubicin, 5-fluorouracyl) and/or natural compounds (curcumin, quercetin). Expression of membrane associated antigens was evaluated by flow cytometry, while gene expression was detected by real-time PCR. Expression analyses of antigen *vs* gene expression showed that CAMs and MDR-1 were differentially modulated by stimuli treatment. Our results bring new information regarding the co-existence of CAM and MDR-1 associated to stimuli-treated breast tumour cells that might influence the interaction between tumour cells and host immune system. Structural or gene expression alterations are responsible not only for the appearance of cancer, but also for the clinical responses of patients to chemotherapy.

311 Effect of stimuli treatment on proliferation, apoptosis and cell signalling mechanisms associated to oral cancers

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Cancer is a disease of the cell, therefore is essential the identification of evolution stages and use of this information in prediction, prevention, early diagnosis and development of new target drugs. The main obstacle against the success of therapy in many cancers seems to be the impossibility of eradication all tumour cells. Oral cancers represent some malignancies with high incidence throughout world, their ethiology involving many genetic, immunological and biochemical factors. Oral cancer can develop in any part of the oral cavity or oropharynx. Almost all oral cancers begin in the flat cells (squamous cells) that cover the surfaces of the mouth, tongue, and lips. These cancers are called squamous cell carcinomas. These are malignant and tend to spread rapidly. A new therapeutical approach could be more useful for distruction of tumour cells: renewal of the cellular pathways that lead directly to apoptosis. Traditional anti-cancer therapies have limited effects, therefore the citotoxic action exerted by drugs on tumours might be added by natural compounds and the signalling mechanisms influenced.
The present study focused on modulation of antigen expression of AMPK-alpha, -beta, LKB1, TSC2, mTOR and S6K1 by cytotoxic drugs (such as 5-fluorouracyl and cisplatinum) in the presence or absence of natural compounds such as apigenin and curcumin. Levels of expression of phosphorylated vs non-phosphorylated proteins were evaluated by immunoblotting in oral tumour cell lines derived from squamous cell carcinomas. Protein expression was detected by Western blotting using the chemiluminescence. Our results showed a differential antigen expression of the molecules under study involved in signaling mechanisms involved in kinase activities depending on the association of stimuli and drugs. Protein expression alterations, resulting frequently from gene modifications, are responsible not only for the appearance of cancer, but also for the clinical responses of patients to chemotherapy. The knowledge of the relation between the implied molecules, of the mechanisms of regulation of the gene and protein expression, as well as their functions, of the effect of therapeutic agents (oncolytic agents, natural compunds) on proliferation, induction of apoptosis and tumour cell lysis has a potential diagnostic and prognostic value of the disease evolution, but also regarding the tumour response to immunotherapy.

312 Antigenic markers, disease progression and survival in colorectal cancer (CCR) patients

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Background: Antigenic expression in colorectal (CCR) primary tumours and metastatic lymphatic nodes (N+) may be useful for early detection of disease progression. The purpose of this research was to establish the possible relationship among antigenic expression in tumour and N+ versus survival free disease (SFD) and overall survival (OS) in CCR patients.
Materials and Methods: A total of 90 CCR patients were studied: 90 primary CCR tumour samples and 64 lymphatic nodes; 22 samples from adenomas and normal colorectal mucosa specimens were employed as controls. Antigens studied were: MUC2 mucin, MUC1, MUC5AC, CEA, beta-catenin; carbohydrate antigens such as Lewis x (Lex), sialyl Lewis x (sLex), Lewis y (Ley), sialyl Lewis a (sLea) and Tn hapten. Immunohistochemistry was performed following standard procedures with antigenic retrieval. Positive