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