(PBMC), unstimulated and stimulated to proliferate by mitogen phytohemagglutinin, using MTT cell survival test, 72 h after the continuous tea action.
Results: Tea prepared from *Artemisia annua* dry leaves exhibited selective dose-dependent cytotoxic effect against malignant cell lines and both unstimulated and stimulated PBMC. The strongest cytotoxic action was observed against K562 cells ($IC_{50} = 1.33 \pm 0.38$ mg/ml). Moreover, tea exerted pronounced cytotoxic effect on melanoma BG and Fem-x cells ($IC_{50} = 3.20 \pm 0.65$ and $IC_{50} = 3.76 \pm 1.20$ mg/ml respectively), and to HeLa cells ($IC_{50} = 3.06 \pm 0.62$ mg/ml). Cytotoxic activity was found to be weaker against MDA-MB-361 and LS174 cells ($IC_{50} = 8.86 \pm 0.42$ and $IC_{50} = 10.45 \pm 0.26$ mg/ml respectively). Cytotoxicity of tea on human healthy immunocompetent PBMC, was slightly weaker on unstimulated PBMC in comparison to stimulated PBMC ($IC_{50} = 10.38 \pm 0.49$ and $IC_{50} = 9.27 \pm 0.54$ mg/ml respectively).
Conclusions: The present research clearly demonstrates stronger and selective (Ksel > 6.95) antitumour effect of *Artemisia annua* tea to leukemia K562 cells in comparison to healthy PBMC. To melanoma BG and Fem-x cells and to HeLa cell tea was also selective in its antitumour action but to a less extent (Ksel > 2.24), pointing that some tea constituents might have choosy anticancer action.

288 Effect of curcumin on vascular endothelial growth factor levels in rat fibrosarcoma

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Background: Fibrosarcoma is a malignant tumour derived from fibrous connective tissue and characterized by immature proliferating fibroblasts. This cancer invades long or flat bones such as femur or tibia. It also involves the periosteum and the overlying muscle. Fibrosarcoma usually seen in males in ages between 30 and 40. It could not treated with surgery, radiotherapy or chemotherapy always. Vascular endothelial growth factor (VEGF) stimulates vasculogenesis (new formation of a vessel) and angiogenesis (the growth of blood vessels from pre-existing vasculature). Solid cancers that can express VEGF are able to grow and metastasize because of their dependency to blood supply. The aim of this study was to determine the VEGF levels in rat fibrosarcoma.
Materials and Methods: In this study eighteen Wistar male rats were used and were divided into three groups: I. Control group (C, n = 6), II. Fibrosarcoma group (F, n = 6) and III. Curcumine treated Fibrosarcoma group (F+C, n = 6). Fibrosarcoma was induced by 3-metilcholantren and curcumin was given in a dose of 200 mg/per rat, via oral gavaj for ten days. After the experiment, the homojenized tissues were analysed with Western blot and ELISA with anti-VEGF antibodies.
Results: VEGF levels were increased in the F group ($p \leqslant 0.05$) and decreased after curcumine (F+C group) ($p \leqslant 0.05$) by both methods.
Conclusion: We thought that curcumine have an antiangiogenic effect of fibrosarcoma and it could possible act as an alternative assistant therapy.

Sunday 27 June 2010 09:45–17:30

Poster Session
Tumour Immunology

289 Indoleamine 2,3-Dioxygenase (IDO) silencing for improved antitumour vaccination

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Background: As tumour progresses the efficacy of vaccination is tuned down by suppressive activities. The administration of adjuvants or the silencing of specific immune regulatory molecules will optimize the function antigen presenting cells (APC) and will permit the immune response elicited to be active at the tumour site. Indoleamine 2, 3-dioxygenase (IDO) the enzyme that degrades the essential amino acid tryptophan in mammals is overexpressed in both tumour cells and APCs in tumour-draining lymph nodes, where it promotes the establishment of peripheral immune tolerance to tumour antigens. IDO seems to be an ideal target to be silenced for the optimal induction of an antitumour immune response. We plan to use plasmids coding short shRNA specific for IDO to be administered together with the plasmid coding portion of Erbb-2, or plasmids containing both the shRNA module and the oncoantigen module, in vaccination-protection tests in BALB-neuT mice transgenic for the rat Erbb-2.
Material and Methods: Retroviral vectors (pLKO.1, Open Biosystem®) including five shRNA sequences targeting IDO mRNA have been used as template to amplify the interference cassettes (pU6-shRNA-IDO) that we cloned into the Eco72I site of both pVAX1 (Invitrogen®). The gene silencing efficacy of the various interference cassettes was evaluated in a kynurenine assay using N11 microglial cells (Grant et al. 2000). The most efficacious cassettes were subcloned into a pVAX vector containing the sequence of the extracellular and transmembrane domains of rat Erbb-2 (pVAX-ratECTM) and used for vaccination of BALB-neuT mice carrying different stages of mammary carcinogenesis.
Results: All the five interference cassettes were able to reduce kynurenine release from N11 cells, confirming their ability to silence IDO expression. Two cassettes were chosen to be subcloned into pVAX-ratECTM, and used to vaccinate BALB-neuT mice bearing atypical hyperplasia and in situ carcinomas (weeks 10 and 12 of age) or microscopic invasive carcinomas (weeks 16 and 18). The in vivo observation of mammary cancer progression is still ongoing.
Conclusions: We expect that this simultaneous alteration of tumour microenvironment and induction of an immune response against Erbb-2 elicits an anti-tumour response of therapeutic significance, in that it halts the progression of lesions that cannot be inhibited by Erbb-2 vaccination alone.

290 The role of tetraspanins in antigen presentation to CD4+ T cells via exosomes

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Exosomes are membrane vesicles released by various cell types. When derived from antigen-presenting cells, exosomes are MHC class II-positive and can induce CD4+ T cell responses. Tetraspanins are a family of transmembrane proteins which might play a role in MHV II delivery to the cell surface and/or exosomes.
We have prepared exosomes derived from Epstein–Barr virus (EBV)-infected human B lymphoblastoid cell lines (LCLs) and shown by Western blotting and immunoelectron microscopy that they contain MHC class II and tetraspanins including CD63, CD81 and CD82.
Such LCL-derived exosomes can mediate immunologically specific recognition by MHC class II matched EBV antigen-specific CD4+ T cell clones (1) when directly added to the T cells in the absence of antigen-presenting cells, and (2) when added to B cells lacking the EBV antigen but expressing the MHC class II matching alleles. Using shRNA, we have decreased CD63 expression in LCLs and we are studying the effect of such downregulation on LCL and LCL-derived exosome function.

291 Do regulatory T cells require cognate MHC/peptide recognition for endothelial transmigration?

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Background: The endothelium acts as a selective barrier for leukocyte migration into tissue, including tumour tissue, requiring recognition of non specific adhesion molecules, chemokine gradients and possible cognate MHC peptide. While it is accepted that higher numbers of Regulatory T cells (Tregs) can be found within tumour microenvironments there still remains uncertainty as to which conditions promote Treg recruitment into tumours. The aim of this study was to investigate the conditions which favour Treg transmigration.
Materials and Methods: Treg migration in response to tumour-associated chemokines and self MHC recognition was modelled using a murine model to mimic T cell transmigration across syngeneic (cognate MHC) and allogeneic (non-cognate MHC) murine lung endothelium. In addition the level of CXCL12 expression and T cell infiltration within tumours was examined by immunohistochemical analysis of ovarian tumour TMA samples.
Results: Our data shows that the level of CXCL12 expression by tumour cells can affect patient survival by potentially altering the balance of T cell subset infiltration into the tumour. Including a novel mechanism for Treg transmigration where cognate antigen-specific recognition of self-peptides is required for transmigration with preferential transmigration of Tregs across syngeneic endothelium, under conditions of inflammation and CXCL12.
Conclusion: Regulatory T cells recognising self antigen may preferentially accumulate within tumours where recognition of self peptides presented by self MHC allows migration of antigen-specific Tregs in response to CXCL12.

292 T-cell based identification of tissue antigens by automated two-dimensional protein fractionation

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Background: Here we describe a new method to comprehensively identify candidate tissue antigens that spontaneously cause T-cell responses in disease situations.

Materials and Methods: We used the new automated two-dimensional chromatography system *PF2D* to fractionate the proteome of tumour tissues and tested protein fractions for recognition by pre-existing tumour-specific CD4+ T-helper cells and cytotoxic T-cells.
Results: Applying this method to the Ovalbumin (OVA)-specific, TCRtg OT-I mouse model demonstrate efficient separation, processing and cross-presentation to CD8+ T-cells by dendritic cells of OVA expressed by the OVA-transfected mouse lymphoma RMA-OVA. Applying this method to human tumour tissues we identified in patients with head and neck cancer MUC-1 and EGFR as tumour-associated antigens selectively recognized by patients' T-cells. Finally, we detected on an exemplary patient with a malignant brain tumour CD4 and CD8 T-cell responses against two novel antigens, transthyretin and calgranulin B/S100A9, which were expressed on tumour and endothelial cells. Immunogenicity of these antigens could be confirmed in 4 out of 10 other brain tumour patients.
Conclusions: This fast and cheap method appears suitable to identify candidate T-cell antigens in various disease situations, such as autoimmune and malignant diseases without restriction to their expression by a certain cell type or HLA allele.

293 Cyclooxygenase 2-driven inflammation in pancreatic cancer

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Prostanoids perform important tasks in normal and diseased tissues by acting as local signals which coordinate the concerted activities of different cell types. In our work, we substantially focus on the role related to inflammation and cancer. Chronically inflamed pancreas, urinary bladder, and colon represent high-risk environments for tumourigenesis.
Epidemiological, pharmacologic, clinical, and genetic studies show the cause/effect relationship between over-expression of pro-inflammatory cyclooxygenase (COX)-2, COX-2-mediated prostaglandin (PG) signaling and the development of epithelial cancers. Depending on the cellular context, PG stimulate growth, angiogenesis, and modulate immune functions in inflammation-driven cancers.
Keratin 5 promoter-driven COX-2 (K5 COX-2) transgenic mouse lines established in our group develop pre-invasive neoplasms in various epithelial tissues including pancreas. Pancreata of the K5 COX-2 mice develop ductal neoplasms which resemble, on molecular and morphological levels, human precursor lesions of pancreatic ductal adenocarcinoma (PDAC), i.e. cystic intra-ductal papillary mucinous neoplasms (IPMN) and pancreatic intra-epithelial neoplasias (PanIN) (Gastroenterology 130, 2006).
These phenotypic changes are associated with pronounced inflammatory infiltrates in pancreas, thus representing a putative high-risk environment for tumourigenesis. Cytokine gene expression analysis depicts the presence of TH-1 (elevated IFN-gamma, TBX21), TH-17 (elevated Il-6, Il-17A), and T-regulatory (elevated FOXP-3) cells. At the cellular level, diffuse inflammatory infiltrates are observed, besides prominent inflammatory clusters, in the diseased pancreata of K5 COX-2 mice. These are comprised of B-cells, T-cells, follicular dendritic cells, macrophages, and high endothelial venules decorated with lymphocyte adhesion molecules. In addition, a panel of TLO-relevant chemokines is expressed. Altogether are known to be hallmarks of ectopic tertiary lymphoid organs (TLO) which arise in chronic inflammatory diseases but with a yet un-known function. Such an inflammatory phenotype is suppressed by inhibition of COX-2 activity whereby celebrex-fed transgenics exhibit fewer and smaller clusters, indicating the involvement of COX-2/PG signaling in the establishment of TLO; a putative novel COX-2-effect on local immunity. Follicular clusters rich in B-cells and T-cells, with vessels that express peripheral lymph node addressin (PNAd) are also observed in COX-2-positive human PDAC. This hypothesizes that the induction of TLO might be relevant in humans as well.

294 Successful engraftment of glioblastoma biopsy spheroids in immunocompetent rats

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Standardized implantation of biopsy spheroids generated from GBM patients into athymic nude rats has now been well established and characterized, with tumour takes close to 100%. The resulting lesions resemble human glioblastomas in their presentation, containing invasive single cells, vascular proliferations, thrombotic vessels, and necroses. Given that the brain is a site of immune privilege, and human glioblastomas evade immune surveillance, we evaluated whether human GBM spheroids would survive in fully immunocompetent rats and kill their hosts. We compared the engraftment rates of spheroids generated directly from patients or prepared after several generations of passaging in nude rats. Spheroids originally derived from four GBM patients were implanted, followed up by weekly MRI, and engraftment rates and survival data were collected.
Xenografts that were generated directly from patient biopsy tissue appeared on MRI scans only in two cases and were thereafter rejected. In contrast, xenograft tumours based on glioblastoma tissue that has been previously passaged in nude rats displayed engraftment rates of over 50% in immunocompetent rats, and once appearing on MRI scans, the lesions invariably killed their hosts. The survival time lengths for immunocompetent animals were similar to those for nude rats when implanted with the same biopsy spheroids. We sought to further characterize the mechanisms that permitted the development of human tumours in immunocompetent rats, such as the nature of immune-inflammatory host cells present and the production of immunomodulatory cytokines by the tumour and the host. The current data suggests that the elevated numbers of both CD4+ and CD8+ lymphocytes together with higher serum levels of rodent IL-1a, IL-2 and IL18 were significantly correlated with tumour xenograft rejection in immunocompetent rats. Furthermore, survival of the xenografts was associated with the inability of activated lymphocytes to penetrate the tumour bed.
In conclusion, we established that passaging of human GBM biopsy spheroids in nude rats facilitates more efficient engraftment in immunocompetent rats.

295 Cyclooxygenase-2 (COX2) gene silencing with siRNA could enhance DNA vaccination to inhibit established ErbB-2 carcinomas

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Background: Cyclooxygenase-2 (COX2) is the key enzyme in the synthesis of prostaglandin E2 from its precursor, arachidonic acid. The level of COX2 is normally very low in cells but increased amounts of COX2 are commonly detected in both premalignant and malignant tissues. In order to investigate COX2 as a potential target for the prevention and treatment of cancer, we propose a novel immunotherapeutic approach for the prevention of ErbB-2 positive breast carcinomas, based on DNA vaccination against a specific antigen (ErbB-2) in combination with the silencing of COX2.
Material and Methods: The short hairpin RNA interference cassette targeting COX2 mRNA, containing the mouse RNA promoter U6, was amplified and cloned into the Eco72I site of pVAX-ratECTM (RRT), a vector carrying the extracellular and transmembrane domains of rat ErbB-2, and into pVAXI, to obtain respectively RRT-COX2 and pVAX-COX2. The gene silencing efficacy of both plasmids was checked by transfecting COX2 over-expressing A17 cells. The plasmids were used for electroporation-based vaccination of female BALB-neuT mice of different ages, corresponding to various stages of cancer progression, from atypical hyperplasia to invasive lobular carcinoma. We are evaluating mammary cancer progression in vivo, the titer of anti-ErbB-2 antibodies in the immune sera, and the ability of spleen cells to release IFNg in response to the $H2^d$ immune dominant peptide of ErbB-2.
Results: Vaccination of 10 week-old female BALB-neuT mice with RRT plasmid significantly triggers a protective immune response toward the development of autochthonous mammary cancer in BALB-neuT mice (Quaglino et al. 2004, Cans Res). 30% of treated mice were still fully free from palpable tumours one year after vaccination, when all control animals had already died because of mammary cancer. Till now (week 46 of age) we see a similar protection using RRT-COX2. The level of anti-ErbB-2 antibodies in the sera from RRT-COX2 vaccinated mice is slightly higher than that of RRT vaccinated mice, but not significantly different. We are now collecting spleen cells from vaccinated mice to evaluate the specific CD8 response against ErbB-2. Experiments with mice vaccinated when they already have lobular carcinomas are ongoing.
Conclusions: COX2 suppression induced by shRNA might help to overcome tumour-mediated immunosuppression and generate an effective anti-tumour immunity not only in prophylactic but also in therapeutic vaccination.

296 Macrophage migration in tolerance

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Tumour Associated Macrophages (TAM) and lipopolysaccharide- (LPS)-tolerant macrophages share several characteristics, such as a massive accumulation of the p50NF-kB homodimer in the nucleus and the incapacity to express strong inflammatory programs (eg. impaired TNFa production) in response to inflammatory signals such as LPS (tolerance). Our recent study has described that both TAM and LPS-tolerant macrophages express