(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.