tional ASPCR was either non-specific at all or showed specificity only in a very stringent conditions, which included low concentration of primers and magnesium chloride, high annealing temperature, and low number of PCR cycles; when any of the mentioned parameters was even slightly relaxed, non-correct genotyping occurred. However in the presence of the 3-fold excess of the depository oligonucleotide, ASPCR retained the specificity and reproducibility even if the PCR stringency was significantly reduced.

Conclusions: The deposition of allele-specific primers by complementary oligonucleotides evidently increased the reliability of ASPCR. The proposed modification may substantially facilitate SNP genotyping, either alone or in combination with other ASPCR improvements.

606 POSTER

Streptococcal preparation OK-432 is a new GMP-grade maturation factor of monocyte-derived dendritic cells

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Background: For vaccinations based on dendritic cells (DCs), maturation of DCs is important for the induction of effective T cell responses. A streptococcal preparation, OK-432, has been used as multi-cytokine inducer for management of cancer patients in Japan. We examined whether OK-432 can be a Good Manufacturing Practice (GMP)-grade maturation factor of DCs.

Material and methods: Immature monocyte-derived DCs (imDCs) generated from human peripheral blood mononuclear cells with granulocyte-macrophage colony stimulating factor and interleukin (IL)-4 were exposed to two types of common maturation factors, i.e., lipopolysaccharide and tumor necrosis factor-alpha plus prostaglandin E2, or OK-432 for another 2 days. Their surface expression of maturation-related molecules, allogeneic T cell proliferation, and cytokine secretion were analyzed with fluorescence-activated cell sorting (FACS), allogeneic mixed-lymphocyte reaction, and enzyme-linked immunosorbent assay, respectively. Activation of nuclear factor kappa B (NF- kappa B) was also examined with electrophoretic mobility shift assay.

Results: All agents examined increased both expression of maturation-related molecules such as HLA-DR, CD80, CD83, and CD86, and allogeneic T cell proliferation at a similar level in imDCs. Importantly, only OK-432 caused significant production of IL-12 p70 and interferon-gamma (IFN-gamma) at both the mRNA and protein levels. Induction of intracellular IL-12 and IFN- gamma in OK-432-stimulated DCs was also confirmed with FACS Calibur. Moreover, OK-432 induced activation of NF- kappa B in imDCs. Both cytokine secretion and NF- kappa B activation induced with OK-432 were suppressed when imDCs were pretreated with cytochalasin B, an inhibitor of endocytosis.

Conclusion: Our experimental data indicate that uptake of OK-432 by imDCs is an early critical event for secretion of both IL-12 p70 and IFN- gamma and that activation of NF- kappa B induced by OK-432 also contributes partially to these cytokine secretion. Since OK-432 is a GMP-grade agent, OK-432 may be a potential tool for vaccinations based on DCs.

607 POSTER

Induction of cytotoxic T lymphocytes that recognize a tumor-associated antigen, 90K/Mac-2 binding protein with an HLA-A2 restriction

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Background: 90K/Mac-2 binding protein (M2BP) is highly expressed in patients with various types of cancer and can influence the expression of surface molecules involved in immune responses on cultured cancer cells. We have reported that M2BP-specific immunity was observed in many lung cancer patients (Cancer 2002; 95: 1954-62). In this study, to identify HLA-A2-restricted immunogenic epitopes of M2BP, we generate cytotoxic T Lymphocytes specific for M2BP in vitro.

Materials & Methods: We selected 11 peptides (9-mer or 10-mer) derived from M2BP with an HLA-A*0201 binding motif according to peptide-motif scoring algorithms. M2BP-specific CTLs were generated from peripheral blood lymphocytes (PBLs) of HLA-A2-positive healthy donors by multiple stimulations of CD8-positive T lymphocytes with M2BP peptides. The induced CTL lines were examined for their specific responses to antigens by interferon-gamma production and standard ^{51}chromium-release assays.

Results: Three of the 11 CTL lines produced interferon-gamma in response to T2 cells (M2BP-/HLA-A2+) pulsed with the same peptide with a dose-dependent manner. However, only one CTL line induced using M2BP216-224 could lyse both peptide pulsed-T2 cells and a breast cancer cell line, MDA-MB-231 cells (M2BP+/HLA-A2+). The cytolysis was blocked by antibodies against HLA class I but not HLA class II molecules.

Conclusion: M2BP-specific CTLs could be generated in vitro using M2BP216-224 peptide. M2BP is expected to be useful as a target antigen in cancer immunotherapy.

608 POSTER

Streptococcal preparation ok-432 induces human dendritic cells maturation via up-regulation of toll-like receptors

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Dendritic cells (DCs) are potent antigen presenting cells to promote specific anti-tumor immune response. Streptococcal preparation OK-432 is supposed to induce innate immunity and up-regulation of toll-like receptors (TLRs). In the present study, we have investigated the effect of OK-432 on expression of TLRs as well as on maturation and activation of DCs in comparison with conventional tumor necrosis factor (TNF)-alpha. Human peripheral blood mononuclear cells (PBMC) were collected from five healthy volunteers and cultured in serum-free medium (AIM-V) in the presence of interleukin-4 (IL-4; 50ng/ml) and granulocyte-macrophage stimulating factor (GM-CSF; 50ng/ml) for 6 days. Then DCs were pulsed with tumor cell lysate obtained from human gastric cancer cell line MKN-45 for 12hr and further cultured for 48hr following addition of OK-432 (0.1 KE/ml). We compared it with addition of TNF-alpha (100 ng/ml) for DCs maturation. Cell surface phenotypes of DCs (HLA-ABC, HLA-DR, CD40, CD54, CD80, CD83 and CD86) were examined by flow cytometry, and cytotoxic T cell activity was evaluated using ^{51}Cr releasing assay. Expression of toll-like receptor (TLR)-4 and TLR9 after stimulation by OK-432, TNF-alpha or lipopolysaccharide (LPS) were examined using real-time reverse transcription polymerase chain reaction (RT-PCR). Expression of cell surface phenotypes examined was increased either on the surface of TNF or OK-432 treated DCs in a time dependent manner. No significant difference of the intensity of expression was noted between the two groups. Furthermore, ^{51}Cr releasing assay showed specific cytotoxity for MKN-45 with similar killing activity between the two groups. Expression of TLR-4 and TLR-9 were highest after LPS treatment, followed by OK-432 and TNF treatment, significantly higher in OK-432 treated group than in TNF treated group. The expression of TLRs peaked at 1 hr after stimulation in LPS and TNF, while it peaked at 2 hr after stimulation in OK-432. These results suggest that OK-432 has a potential role on human DCs for generation of CTL possibly via up-regulation of TLRs, and would offer an eligible protocol for human DCs in vivo immunotherapy especially for local administration.

609 POSTER

Tumour burden and interleukin-2 dose affect the synergism between low-dose total body irradiation and interleukin-2

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Background: Low-dose total body irradiation (LTBI) is believed to initiate various immune-mediated anti-tumour effects. We have previously shown a synergistic therapeutic effects when LTBI was used in combination with Interleukin-2 (IL-2) in a murine metastatic malignant melanoma model.

Aim of the work: To optimise the use of this combination treatment this study was performed to test the effect of tumour burden and dose of both LTBI and IL-2 on the therapeutic potential of this treatment strategy.

Material and Methods: Ten-week-old female C57BL/6 mice were inoculated i.v. (Day 0) with 1 million B16F1 malignant melanoma cells. The mice received either: no treatment, single fraction of LTBI alone, IL-2 treatment alone, or a combination of LTBI and IL-2. Two dose levels of LTBI and IL-2 were tested. LTBI was given either on day +7 or on day +10. IL-2 treatment was given over 5 days starting 24 hours after LTBI. Two days after the end of treatment, the mice were sacrificed and the lungs were removed and analyzed for tumor burden. Lung sections were also tested for tumor infiltrating cells using immuno-histochemical staining.

Results: LTBI (in the 2 tested dose levels), showed to independent therapeutic effects. IL-2 dose of (300.000 CU) that proved effective and

showed synergism with LTBI when mice were treated on day +7 has failed to show a therapeutic effect when mice were treated on day +10. High dose IL-2 (600.000 CU) on the other hand, led to a significant reduction in metastatic burden compared to control group. Combining high dose IL-2 with LTBI led to further significant reduction in tumour burden. Moreover, this combination was associated with less vascular leakage syndrome (VLS) compared to IL-2 alone. IL-2 and combination treatment was associated with an increase in the number of tumour infiltrating immune cells, but only the number of tumour infiltrating NK-cells reflected therapeutic efficacy.

Conclusion: We conclude that tumour burden at the time of treatment and IL-2 dose are 2 crucial factors affecting the synergism between LTBI and IL-2. The combination may not only be more effective than IL-2 alone but also less toxic.

610 POSTER

Dolichol as tumour marker in pancreatic cancer control

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Background: Urinary Dolichol (Dol) have been reported to be 5 to 40 times the normal values, suggesting a metabolic abnormality of N-glycoprotein synthesis in patients with cancer. Pancreas contains a highest level of Dol concentration in human tissues. With focus on a tumour marker, the present study was carried out to estimate blood and urinary levels of Dol in patients with pancreatic cancer (PCA) and chronic pancreatitis (CHP).

Materials and Methods: The samples obtained from 28 patients with PCA (male, 32-69 years old) and 40 patients with CHP (male, 36-65 yeras old). Dol in blood and urine was assayed by HPLC method (Turpeinen, 1986).

Results: Dol in healthy men's blood and urine are 125,9 + 7,8 ng/ml and 6,8 + 0,7 mg/mmol creatinine respectively. In CHP Dol content in urine was much the same, but Dol content in blood showed an increase of 18-22% Blood Dol concentration in patients with PCA increased at stage I up to 25%,at stage II up to 45%, at stage III up to 55%, making up 204,5 + 14,9 ng/ml at stage IV. There was a significant difference between urinary Dol content in patients with CHP and that of cancer patients. Urinary Dol concentration increased at stage I up to 75-90%, making up 44,9 + 6,9 mg/mmol at stage II. At stage III the level of urinary Dol was 7-10 -fold increased.

Conclusions: These findings suggest that Dol appeared in urinary excretion is one of the first manifestations of carcinogenesis in pancreas. In this way CHP therapy should be carried out under Dol excreation control. The interest drown to the employment of Dol as marker is explained by the fact that known PCA markers are glycoproteins (CA-50 and CA-19-9). Monitoring CHP patients with monthly urinary Dol determination is a reliable method to diagnose a PCA.

611 POSTER

EBV-DNA in unfractionated whole blood specimens of patients with EBV-related diseases and seropositive healthy individuals

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Epstein-Barr virus is related with various benign and malignant diseases. Circulating EBV-DNA has been detected in virus-related malignancies and has been shown to be valuable in the diagnosis, prognosis and monitoring of such patients. However EBV-DNA can be found in healthy individuals so caution must be used in interpreting such results. We performed a study to detect EBV-DNA in unfractionated freshly obtained whole blood in Greek patients with EBV-related diseases and healthy volunteers. Peripheral whole blood (PWB) from 95 patients with nasopharyngeal carcinoma (NPC), 34 patients with Hodgkin lymphoma (HL), 13 patients with EBV-related non Hodgkin lymphoma (NHL), 10 patients with active infectious mononucleosis (IM) and 83 seropositive healthy volunteers was collected between December 1999 and February 2003. Total genomic DNA was extracted using kit according to manufacture's protocol. Qualification and quantification of DNA were performed in all specimens using spectrophotometry. Additionally, randomly selected samples were analyzed using restriction enzymes and agarose gel electrophoresis. The extracted DNA was used as a template in PCR with specific primers to amplify region of EBNA-1 gene. The protocol used was for 45 cycles. The products of PCRs were analyzed by electrophoresis on 1.5% agarose and visualized after ethidium bromide staining. The detection rate for EBNA-1 was 83% in NPC (79/95 pts), 82% in HL (28/34 pts), 69% in (NHL) (9/13 pts), 80% in IM (8/10 pts) whereas EBV genome was found only in 11 of the 83 healthy volunteers (13%). Differences in the detection rate of EBV-DNA between patients with EBV-related diseases and healthy volunteers were statistically significant ($p<0.005$) in all cases, whereas odds ratio was 32,31 (95% CI 14.07 to 74.22) for NPC, 30.54 (95% CI 10.30 to 90.51) for HL, 14.72 (95% CI 3.86 to 56.12) for NHL and 26.18 (95% CI 4.90 to 139.69). Our results showed that detection rate was significantly higher in patients with EBV-related malignancies than in healthy controls which suggests a causative role of Epstein-Barr in human carcinogenesis. The results are similar with those reported in literature where peripheral blood cells were used to detect EBV-DNA instead of free-cell plasma or serum. It seems that whole blood could serve as the preferred clinical sample type since it is simple and reflects the total viral load in the circulation.

612 POSTER

ProteinChip® proteomics for early detection of cancer

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The emergence of new sophisticated proteomic tools has opened up a new approach to perform diagnosis, prognosis and therapy monitoring. Where previous protein based methods involving proteins have been based on single protein markers, such as PSA and CA125 with their inherent limitations, these new methods now allow for the monitoring of many proteins simultaneously, thus allowing for the use of patterns and neural networks in the evaluation of data.

Analysis of complex body fluids like serum, plasma, urine or CSF with Surface Enhanced Laser Desorption/Ionization (SELDI) (Ciphergen Biosystems Inc.) allows for direct monitoring of the proteome. This has been exploited when investigating several different cancers. When combining the information of signal intensity and mass of several hundred proteins it is possible to discover patterns of proteins that are unique for certain diseases and thereby perform diagnoses with very high specificity and sensitivity.

This approach has been applied to a range of cancers using serum samples from affected and age matched control individuals. Using as little as 20 μL of serum automated fractionation and ProteinChip® analysis on various surfaces can be performed. Including data analysis to compare profiles between groups hundreds of samples can be run in a matter of days. By submitting SELDI data to a classification software it is possible to build decision trees that yield much higher sensitivity and specificity compared to single marker assays. The same approach can be used to perform prognoses, stage determination and therapy monitoring.

613 POSTER

Effects of p53 status and wortmannin treatment on potentially lethal damage repair in vivo, with emphasis on the response of intratumor quiescent cell populations

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Purpose: To examine the effects of p53 status and wortmannin treatment on potentially lethal damage repair in vivo, referring to the response of intratumor quiescent cells.

Methods: Human head and neck squamous cell carcinoma cells transfected with mutant TP53 (SAS/mp53) or with neo vector as a control (SAS/neo) were injected subcutaneously into both the hind legs of Balb/cA nude mice. Mice bearing the tumors received 5-bromo-2'-deoxyuridine (BrdU) continuously to label all proliferating (P) cells in the tumors. The mice then received gamma-rays with or without subsequent wortmannin administration. Right after or 24 h after gamma-ray irradiation alone or 24 h after wortmannin administration following irradiation, the tumors were excised, minced and trypsinized. The tumor cell suspensions thus obtained were incubated with a cytokinesis blocker (= cytochalasin-B), and the micronucleus (MN) frequency in cells without BrdU labeling (= quiescent (Q) cells) was determined using immunofluorescence staining for BrdU. The MN frequency in total (P + Q) tumor cells was determined from the tumors that were not pretreated with BrdU.