1049 POSTER

LTX-315 confers long term protection in mice re-challenged with murine A20 B-cell lymphoma or murine CT26WT colon carcinoma cells after complete tumour regression following initial treatment

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Background: LTX-315 (Oncopore®) is a nonapeptide in development for local treatment of tumours. LTX-315 binds to over-expressed, negatively charged, molecules on the surface of tumour cells, where it induces lysis and cell death. LTX-315 is administered via intra-tumoural injection. The present study sought to investigate tumour growth in animals that had previously shown complete tumour regression following treatment with LTX-315
Materials and Methods: Initial studies in syngenic models of A20 B-cell lymphoma and CT26WT colon carcinoma were conducted in female Balb/c mice. Tumours were induced following injection of 5 million cells in 50 μL subcutaneously, on the abdominal surface. Once tumours had reached 20 mm^2, mice were treated with LTX-315 or vehicle (0.9% NaCl in sterile H_2O) via intra-tumoural injection, once daily for 3 days. Animals were observed for anti-tumour response and relapse after treatment.
Mice from these studies, that demonstrated complete tumour regression following treatment with LTX-315, were re-inoculated with either murine A20 B-cell lymphoma cells (n = 4) or CT26WT colon carcinoma cells (n = 9) six weeks following initial treatment with LTX-315. Tumour growth was monitored for up to 36 days following re-inoculation.
Results: Significant inhibition ($P < 0.006$) of tumour growth was observed in all 4 mice re-inoculated with A20 B-cell lymphoma compared with control animals, and while relapse was seen in 1 animal, 3 weeks later, complete tumour regression was observed in the other 3 mice. In 9 mice re-inoculated with CT26WT colon carcinoma, inhibition (P 0.01) of tumour growth was observed in comparison with control animals. Inhibition was observed in 7 mice and complete regression in 2 of the animals
Conclusions: These data suggest that complete tumour regression following initial treatment of solid murine tumours (murine A20 B-cell lymphoma or CT26WT colon carcinoma) with LTX-315 resulted in a form of endogenous long-term protection against further growth of the same tumours following re-inoculation. Inhibition of tumour growth was more pronounced in animals bearing A20 B-cell lymphoma tumours when compared with animals bearing CT26WT colon tumours.

1050 POSTER

Induction of an anti-cancer immune response following vaccination of mice with LTX-315 lysed tumour cells

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Background: LTX-315 (Oncopore®) is a nonapeptide in development for local treatment of tumours. LTX-315 binds to over-expressed, negatively charged, molecules on the surface of tumour cells, where it induces lysis and cell death. Prior work has demonstrated that treatment of solid murine tumours (murine A20 B-cell lymphoma or CT26WT colon carcinoma) with LTX-315 resulted in a form of endogenous long-term protection against further growth of the same tumours following re-inoculation. The present study sought to investigate the anti-cancer effect of prophylactic vaccination with murine A20 B-cell lymphoma cells lysed by LTX-315 in combination with LTX-315 as an adjuvant.
Materials and Methods: Murine A20 B-cell lymphoma cells were mixed with LTX-315 and left for 30 minutes to allow cell lysis. The cell-peptide mix was then injected into Balb-c mice, with or without an adjuvant injection of LTX-315. Six weeks later the treated mice, plus a control group, were injected with viable A20 cells. The animals were followed until a maximum tumour volume of 125 mm^2 was reached. Four treatment regimens were used:
Regimen 1: Single subcutaneous injection of lysate containing 5×10^6 A20 lymphoma cells and 10 mg/ml LTX-315
Regimen 2: 20 mg/ml LTX-315 subcutaneous injection followed by injection of lysate containing 5×10^6 A20 lymphoma cells
Regimen 3: Single subcutaneous injection of lysate containing 10×10^6 A20 lymphoma cells and 10 mg/ml LTX-315
Regimen 4: 20 mg/ml LTX-315 subcutaneous injection followed by injection of lysate containing 10×10^6 A20 lymphoma cells
Six weeks later all groups plus a control group were injected with 5×10^6 viable A20 lymphoma cells in a volume of 50 μl at a different abdominal site than the tumor lysate was injected.
Results: Tumour development was slower in the treated groups compared to the controls and complete regression of initially developing tumours was observed in some treated animals. Macroscopically there were morphological differences between the treated groups and the control group. The developing tumours in the treated mice were observed to be whiter and harder than the tumours observed in the control group.
Conclusions: The results indicate that an anti-cancer immune response was induced by the vaccination with LTX-315 lysed A20 B-cell lymphoma cells.

1051 POSTER

Protection against murine A20 B-cell lymphoma tumour re-growth can be passively transferred to untreated naïve mice via splenocytes from donors previously treated with LTX-315

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Background: LTX-315 (Oncopore®) is a nonapeptide in development for local treatment of tumours. LTX-315 has a lytic mode of action, binding to over-expressed, negatively charged, molecules on the surface of tumour cells, where it induces lysis and cell death. LTX-315 is administered via intra-tumoural injection. This study was undertaken to investigate whether protection against tumour re-growth observed in animals previously treated with LTX-315 could be passively transferred to naïve, irradiated recipients via spleen cells taken from LTX-315-treated donor animals.
Materials and Methods: A20 B-cell lymphoma tumours were induced in female Balb/c mice by injecting 5 million cells in 50 μL subcutaneously on the abdominal surface. Once tumours had reached 20 mm^2, mice were treated with LTX-315 (20 mg/ml) or vehicle (0.9% NaCl in sterile H_2O) via intra-tumoural injection, once daily for 3 days. Spleens from mice that demonstrated complete tumour regression were excised and freshly isolated splenocytes were injected (20×10^6 per 100 μl) into irradiated naïve recipient mice via the tail vein. Control mice received isolated splenocytes from untreated naive mice. 24 h later recipient mice were inoculated with 5 million murine A20 B-cell lymphoma cells as previously described and tumour growth was monitored for up to 26 days.
Results: Inhibition of tumour growth was observed in irradiated mice that received splenocytes isolated from animals that had shown complete tumour regression following treatment with LTX-315 when compared with control animals. A difference in the colour and texture of the tumours in these recipient mice was also noted, suggesting the occurrence of an immediate inflammatory response.
Conclusions: Inhibition of tumour growth in irradiated naive animals that received splenocytes from LTX-315-treated donors with complete regression of A20 B-cell lymphomas indicates that protection against tumour growth, conferred by LTX-315 treatment, is T-cell dependent and is passively transferrable. It is suggested that protection occurs via effective antigen presentation for T-cells and the subsequent development of a specific adaptive immune response.

1052 POSTER

Development and efficiency of lactobacillus drugs for cancer treatment

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Background: In recent years, *Lactobacillus* have been studied for the creation of immunomodulating drugs. The main immunoactive structural components of these cells (muramyl peptides) act as PAMPs and activates the corresponding receptors of innate immunity. In our previous studies low toxicity and weak antitumor and antimetastatic effects of muramyl peptides and DNA fragments complex from probiotic strain *Lactobacillus rhamnosus* V (Del-Immune V®) and N-acetylglucosaminyl-β (1−4)N-acetylmuramyl pentapeptides from probiotic strain *Lactobacillus delbrueckii* subsp. bulgaricus LB86 VCIM-B-5788 (Liasten) has been shown *in vivo*. Clinical trials of these compounds in complex treatment of stage II-III breast cancer patients showed decrease of hematological complications and higher survival rate.
Materials and Methods: We studied the effect of these compounds at 20 stage II-III Hodgkin's and stage II-IV non-Hodgkin's disease, 30 stage II-IV lung cancer (LC) and mesothelioma patients. Liasten was prescribed

for lymphoma patients with chemotherapy (CHOP, ABVD) and lung cancer patients with radio and/or chemotherapy – 1 injection followed by 1 injection every 5 days (total of 5 injections). Mesothelioma patient received two courses of 2 mg of Liasten every two weeks and 3 capsules Del-Immune b.i.d. Effect was measured on the basis of blood test results and survival rate.
Results: Striking decrease were found in incidence of neutropenia complications (60% versus 21%, p = 0.0032) in those patients who received chemotherapy with liasten. Postoperative cases acute conditions of chronical bronchitis, infectious pneumonia cases occurred in 25% of LC patients versus 40% in control group. Patients that received radiation therapy in combination with muramyl peptides preparations had substantial reduction of toxic side effects. There was no hospital mortality. Increased activity of T-cell immunity and plasma interferon was reported in the process of treatment. Tumor process development was consistent with the control group. There were no cases of malignant lymphoma progression. No relapses and 3.5-fold NK functional activity as well as improved activity of T3 and B-cells were reported by mesothelioma patient more than 3 years after the treatment.
Conclusions: It was possible to develop algorithm of using Liasten injections for prevention and treatment of chemotherapy-induced hematotoxic complications in malignant lymphoma and lung cancer. Because of the incurable natures of mesotheliomas, it thus seems warranted to further research the use of Liasten in combination with oral muramyl peptides preparation Del-Immune V® to extend the life expectancy of these patients.

1053 POSTER

Development of humanized monoclonal single chain antibodies, against the tumour suppressor interferon regulatory factor 1 (IRF-1), through phage display

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Background: Despite a potentially important role in cancer suppression, detailed knowledge of IRF-1 pathways has been hampered by a lack of biochemical tools, and in particular monoclonal antibodies, due to low immunogenicity, poor expression, and toxicity of the IRF-1 protein in *E. coli* and mammalian cells.
Materials and Methods: Preformed *in vitro*, phage display circumvents the need for immunogenicity, and was therefore particularly well suited to generating IRF-1 specific antibodies. Additionally, once antibodies capable of binding specifically to IRF-1 were selected, the phage continued to act as a genetically stable source of the antibody which could be stored over a long period. The antibody genes were extracted and moved into a variety of plasmids which allowed for higher level expression in E. coli, and *in vivo*. expression in a variety of human cancer cell lines.
Results: The single chain antibodies were raised against functional domains of IRF-1 spanning the length of the entire protein to ensure that a range of antigens were targeted. By expressing the antibodies that target functional domains *in vivo*. it may be possible to influence specific activities of IRF-1 within the cell, such as its ability to bind DNA or become transactivated.
Conclusions: In this way, single chain antibodies can be used to tease apart the IRF-1 pathway and determine the functional relevance of identified intracellular interactions.

1054 POSTER

The existence of humoral immunity to gliadin and cow's milk proteins in patients with prostatic diseases

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Background: The goal of this study was to determine the incidence of the presence of serum IgA and IgG antibodies to gliadin and IgA, IgG and IgE antibodies to cow's milk proteins by ELISA test, in patients that are having different diagnosis of malignant or nonmalignant prostatic diseases and in control group of healthy people.
Patients and Methods: Twenty-six patients with various diagnosis of prostatic diseases (carcinoma, benign prostatic hyperplasia, adenoma) were included in this research. Nine patients had prostate-specific antigen (PSA) level less than 4 ng/ml, four of them had PSA level in range 4−10 ng/ml and thirteen patients had PSA level more than 10 ng/ml. Fifty healthy people was control group.
Two kinds of antigens were used: skimmed pasteurized cow's milk powder (ICN) and crude gliadin (Sigma). Determination of IgA and IgG serum's immuno-reactivity to gliadin, or IgA, IgG and IgE to cow's milk proteins (CMP), has been performed by home made ELISA tests. The cut off value, for each test, was evaluated as the mean +2 SD of control group.
Results: Statistical analysis of obtained data reveals that the levels of anti-gliadin IgA and anti-CMP IgE were significantly higher in patients with prostatic diseases then that of controls ($p < 0.008$ and $p < 0.02$). Anti-gliadin IgG and anti-CMP IgA immunoreactivities were not significantly higher in patients, comparing to the control group. The level of anti-CMP IgG immunoreactivities in patients with prostatic diseases comparing to the control group was on the limit of statistical significance ($p = 0.0543$).
Conclusion: Results from this study, point to the non-specific association between immunity to food proteins (gliadin and cow's milk proteins) and prostatic diseases.

1055 POSTER

Expression of cancer/testis tumor antigens MAGE-A1, MAGE-A3/4 and NY-ESO 1 in medullary breast cancer

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The medullary breast carcinomas (MBC) account for <2% of breast invasive carcinomas. Recent publications on breast cancer classifications based on gene expression profile analyses indicate that MBC may be considered part of the basal-like carcinoma spectrum. As regards this uncommon type of invasive breast cancer we have recently published an article on the clinicopathological features of MBC in 48 patients who were operated on at our two hospitals between 1999 and 2005 (Matkovic B et al. Tumori 2008).
The present study includes immunohistochemical analyses of the expression of cancer/testis (C/T) antigens MAGE-A1, MAGE-A3/4 and NY-ESO 1 in these MBC samples. C/T genes are normally expressed in germ line cells. However, they may also become activated in a wide range of cancer types. Although a study of the expression of these C/T antigens in breast cancer was in part conducted in invasive ductal carcinomas of no special type (NOS), this has not been done with respect to special and/or relatively rare histological types of breast cancers (Hofmann O et al. PNAS USA 2008; Kavalar R et al. Virchows Arch 2001).
In the present study monoclonal antibodies "77B", "57B" and "B9.8.1" (Juretic A et al. The Lancet Oncol 2003) were used to immunohistochemically determine the expressions of, respectively, MAGE-A1, MAGE-A3/4 and NY-ESO-1 C/T antigens in MBC tissues. MAGE-A1, MAGE-A3/4 and NY-ESO-1 antigens were found to be expressed in 33% (16/49), 33% (16/49) and 22% (11/49) of patients, respectively. Immunohistochemical data concerning these C/T antigen expressions were correlated with the following MBC clinicopathological features: patient's age at diagnosis, type of operation, tumor size, axillary lymph node metastasis, adjuvant therapy, ER, PR, HER-2 expression, and patient's survival. No significant correlation between the above-stated clinicopathologic parameters and the antigen expression was identified. The only exception was the patients' survival data which indicate a possible association between the expression of these C/T antigens and decreased overall survival: MAGE-A1 P = 0.07960, MAGE-A3/4 P = 0.01088, NY-ESO-1 P = 0.11742.
The results of our retrospective study suggest that the aforementioned C/T antigens may be used in MBC as tumor markers of potential prognostic relevance. Due to the relative rarity of this type of breast cancer, further tests need to be performed on additional MBC tumor samples with respect to the expression of these C/T antigens before being able to definitely confirm this possibly original observation.

1056 POSTER

Cd274/Pdc1l1 as a genetic modifier controlling aggressiveness of T-cell lymphoblastic lymphoma

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Background: The use of consomic and congenic mouse-strains has greatly facilitated the identification of tumour modifier genes. Using sub-congenic interspecific mice generated between SEG/Pas and C57BL/6J strains, we report a critical region on chromosome 19 (*Tlyr1c*) which does