p43/EMAP-II expression and apoptosis of tumour infiltrating lymphocytes in CRC ($p = 0.04$ by active caspase 3; $p = 0.02$ by cleaved PARP).
Conclusions: P43/EMAP-II expression is associated with hypoxia and high lymphocyte counts in colorectal cancer. Furthermore P43/EMAP-II expression is associated with apoptosis of tumour infiltrating lymphocytes.

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538 POSTER

Immunotherapy of melanoma: construction and characterization of DNA vaccines encoding mk2–23 SCFV antigen

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Anti-idiotype MK2–23 monoclonal antibody (anti-id Mk2–23mAb), structurally mimics the high molecular weight (HMW)-melanoma-associated antigen (MAA). HMW-MAA antigen is expressed in a vast majority of melanoma lesions with limited inter- and intra-lesion heterogeneity, and negligible expression in normal tissues. Because HMW-MAA is expressed on the surface of malignant cells, it represents a logical target for anti-idiotipic immunotherapy. Indeed, melanoma patients immunized with MK2–23 mAb developed anti-HMW-MAA antibodies, which associated with regression of metastases and survival prolongation in a few patients. In order to fulfill regulatory requirements for larger clinical investigations, we explored the feasibility of replacing MK2–23 antibody immunization with naked scFv DNA vaccines.
To generate scFv MK2–23 DNA expression vectors, the variables heavy (VH) and light (VL) chains of MK2–23 hybridoma were cloned in a pVAC plasmid, which allows the anchorage of the expressed antigen to the surface of mammalian cells. pVAC plasmids encoding MK2–23scFV antigen were assembled in two orientations, expressing either the VH or the VL chain at the amino terminus of the transgene product, linked together by a 10-amino acid linker (Ln). In vitro transfections of 293 cells with both (VH Ln VL) and (VL Ln VH) MK2–23 pVAC plasmids, demonstrated that the MK2–23 scFv antigen, expressed in both configurations was equally recognized by anti-HMW-MAA mAb (Cell-ELISA and FACS analysis). Next, we demonstrate that both intramuscular and gene-gun immunizations of Balb/c mice with scFv MK2–23 plasmids induced the production of antibodies against MK2–23 as well as HMW-MAA antigens.
Notably, while multiple gene-gun immunizations were required to elicit a strong immunoresponse, a single intramuscular injection of scFv MK2–23 DNA was sufficient to generate significant levels of circulating anti-HMW-MAA antibody.
The information gained from this study may be relevant for developing novel clinical vaccines for the treatment of malignant melanoma.

539 POSTER

Cell kinetic effects of chemotherapy (CT)+pegfilgrastim in circulating progenitor cells (CPCs) of breast cancer patients

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Background: Several clinical trials have been designed to evaluate the clinical effects of pegfilgrastim in dose-dense CT regimens both in lymphomas and in breast cancer. From a biological standpoint, one of the concerns of these studies is the possible cytokinetic sensitization of the CD34+ cell subsets by pegfilgrastim administration that could be detrimental when the following course of CT is started. To date, no detailed information are available on the proportion of CPCs that proliferate, remain quiescent or undergo apoptosis after in vivo treatment with CT+pegfigrastim.
Material and methods: On the basis of our previous experiences with filgrastim, we have evaluated the impact of Docetaxel (80 mg/sqm, day 1)+Epirubicin (75 mg/sqm, day 1), followed by a single dose per cycle of pegfilgrastim (6 mg s.c. on day +1) on the actual proportion of CPCs undergoing G0/G1, S and G2/M phases of the cell cycle or showing apoptotic features. The CT schedule is applied in metastatic and locally advanced breast cancer pts and it is planned every 14 days for up to 4–6 courses. Using multiparameter flow cytometry (FCM), Annexin V expression was quantitated at a single cell level and correlated with cell cycle phases (DNA content profile) in CD34+/38+ CPCs.
Results: Peripheral blood (PB) samples from 9 pts at their first course were studied. 7 days following pegfilgrastim the % of CD34+/38+ CPCs in S-phase was 12.5±5 while 4.8%±3 of this cell subset showed apoptotic features. One week later, these values were 7.9%±5 and 9.8%±3, respectively.
Conclusions: Our study is ongoing and these results show that: 1) Docetaxel/Epirubicin at standard dosages followed by pegfilgrastim exerts stimulatory effects on cell cycle status of PB-derived CD34+/38+ hematopoietic progenitors, protecting them at the same time from apoptosis; 2) this effect is particularly evident 7 days after pegfilgrastim administration and tends to decrease on one week later. These findings could be useful when dose-dense CT programs are supported with pegfilgrastim as well as when this cytokine is tested for the mobilizing capacity of CPCs for autografting.

540 POSTER

Action of immunotherapy with Interleukin-2 on innate immunity cells in peripheral blood and in tumoral tissue of pancreatic adenocarcinoma patients

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Background: Recent evidences indicate a crucial role of innate immunity cells, like Natural Killer (NK) cells and eosinophils, in host anticancer defence. Cancer patients with high levels of NK cells and eosinophils in peripheral blood show a lower incidence of metastases and a better prognosis. Recombinant Interleukin-2 (rIL-2) immunotherapy is known to stimulate the innate immunity cells, that in pancreatic cancer patients are usually reduced and furthermore impaired by surgical operation. The purpose of this study is to evaluate the toxicity of preoperative high dose and postoperative low dose rIL-2 treatment, as well as the biological effects on innate immunity both in peripheral blood and in cancer tissue, in patients with resectable pancreatic adenocarcinoma.
Material and methods: Thirteen patients (8 males, 5 females, mean age = 65 years) received rIL-2 immunotherapy consisting in a preoperative subcutaneous administration of 12 millions IU/day for 3 consecutive days and two postoperative cycles (on 30thand 60th days) of 3 millions IU/day for 6 consecutive days. We evaluated absolute number of NK cells and eosinophils before rIL-2 administration, on 1st, 7th, 14thand 30th postoperative days and after each postoperative cycle. 11 patients underwent pancreaticoduodenectomy, 1 splenopancreatectomy and 1 total pancreatectomy. We also analized eosinophil and NK cell tumoral infiltration in pancreatic surgical specimens.
Results: Toxicity profile was moderate. In the whole early postoperative period we observed a significant increase of both NK cells and eosinophils, comparing to basal values ($p < 0.05$ in each sample). In the late postoperative period (from 30th day) innate cells count didn't further significantly improve. The histopathological and immunohistochimical analysis didn't find out any significant intratumoral infiltration of NK cells neither of eosinophils.
Conclusions: This work demonstrates that preoperative high doses rIL-2 administration is able to counteract surgery-induced deficiency of NK cells and eosinophils in peripheral blood in the early postoperative period, even if it can't overcome local mechanisms of immune tumor escape in cancer tissue. Considering the important role of innate immunity in anticancer defence, its immunotherapy induced amplification may improve the control of minimal residual disease and metastatic cells spreading in the perioperative period.

541 POSTER

Hybrid-primed lymphocytes and hybrid vaccination prevent tumor growth of Lewis Lung Carcinoma in mice

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Dendritic cell (DC)–tumor cell hybrids are currently being evaluated as a novel anti-tumor vaccination strategy. We here explored in an animal model whether administration of DCs fused with poorly immunogenic carcinoma cells could elicit an anti-tumor response.
Fusion of C57BL/6 mice bone marrow derived DCs with Lewis Lung Carcinoma (LLC1) cells resulted in around 50% fusion efficiency. Hybrid cells (HC) were used to explore three potential tumor-therapy strategies: protective immunization, vaccination and adoptive cellular therapy.

Immunization with HCs induced activation of proliferating and cytotoxic T cells and significantly retarded tumor growth, also confirmed by upregulated expression of distinct cytokines genes. The same observations accented by vaccination with HCs in the tumor bearing host. Finally, when T cells from HCs vaccinated mice were transferred into naive tumor-bearing mice, tumor growth was most strongly retarded and an efficient proliferative and cytotoxic T cell response was observed. Tumor growth was reduced by over 50%, and tumor development was significantly delayed.
Taken together, we demonstrate that HCs offer for an effective immunotherapy of poorly immunogenic carcinomas. This is independent of whether the HCs are taken for adoptive transfer or as a vaccine.

542 POSTER

Combination of hybrid-primed lymphocytes and hybrid vaccination prevent tumor growth of Lewis Lung Carcinoma in mice

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Immunotherapy with tumor cell-dendritic cell fusion hybrids has been shown to induce immune response against multiple tumor antigens including unknown tumor antigens. The aim of this study was to explore the possibility of optimizing the host protective anti tumor immunity by combined immunization strategies of tumor cell-dendritic cell fusion hybrids. Further, the effects of combined immunization strategies on tumors were evaluated by flat-panel volumetric Computer tomography (fpvCT) and immunohistochemical (IHC) analysis. As previously shown fusion of C57BL/6 mice bone marrow derived dendritic cells with Lewis Lung Carcinoma (LLC1) cells were effective against poorly immunogenic carcinomas with all three potential tumor-therapeutic strategies applied: protective immunization, vaccination and adoptive cellular therapy.
Interestingly, in this study combination of hybrid-primed lymphocytes and hybrid vaccination induced activation of proliferating and cytotoxic T cells and significantly retardation tumor growth (85%). In addition, a significant delay in tumor development, a reduction in the number of pulmonary metastases and survival times were observed. Further, the tumor bearing mice treated with hybrids displayed significant morphological changes of apoptosis compared to LLC1 and dendritic cell treated groups shown by IHC analysis and Tunel assay. An increased CD3 expression was also observed in these hybrid treated tumors, which was accompanied by strong involvement of tumor infiltrating T cells.
These findings were underlined by clearly increased spleen size compared to other treatment regimens. Thereby, these results demonstrate that the combination therapy of fusion hybrids is an effective immunotherapeutic regimen against poorly immunogenic carcinomas.

543 POSTER

Expression of survivin, a novel inhibitor of apoptosis, in advanced rectal cancer with preoperative chemoradiotherapy

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Background: Survivin is a recently described member of the family of inhibitor of apoptosis protein. We investigated the association of survivin expression with prognosis and other apoptosis-related biological factors in advanced rectal cancer with preoperative chemoradiotherapy.
Material and methods: We examined 16 patients with rectal cancer, who were preoperatively staged as at least T3 or T4 (determined by MRI). Enrolled patients were given by 5-FU 425 mg/m^2/day and leucovorin 20 mg/m^2/day intravenously for 3 days during weeks 1 and 5 of pelvic radiotherapy (45 Gy). Surgical resection was performed 4–6 weeks after completion of the scheduled treatment and the patients were followed for up to 55 months after operation. Tumor response was divided as CR (complete response), PR (partial response; over 50% diminution of tumor volume) and NR (no/minimal response). Immunohistochemical staining of paraffin sections using monoclonal antibodies for survivin, bcl-2, p53 and ki-67 was performed on pretreatment biopsy and surgically resected tissues.
Results: No CR was achieved. PR was obtained in 10 patients (62.5%) and NR in 6 patients (37.5%). Survivin expression was found in cytoplasm or nucleus of tumor cells but not in nonneoplastic cells on pretreatment biopsy. After preoperative treatment, survivin expression tended to be decreased in tumor cells (62.5% to 31.3%) and slightly increased in adjacent normal mucosa. The NR cases showed high survivin expression on pretreatment biopsy (5/6). Survivin positivity on pretreatment biopsy showed the tendency of low apoptotic index and low median time to progression. But we failed to find any significant relationship between survivin expression and any of the parameters examined.
Conclusions: In this study, the immunohistochemical assessment of survivin status does not seem to be helpful in the prognostic characterization of rectal cancer. Further studies including more cases with sufficient follow-up period are needed in order to provide survivin as a prognostic and therapeutic target in rectal cancer.

Publication
Cytokines/immunobiology/immunotherapy

544 PUBLICATION

Tumor-associated antigens in rheumatoid arthritis

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Background: There have been scattered reports, that some tumor-associated antigens (TAA) may, apart from cancer cells, become expressed ont he surface of inflammatory cells. Carcinoembryonic antigen (CEA) is present mostly on colorectal and gastric carcinomas, CA 15–3 on breast carcinoma, CA 19–9 on pancreatic carcinoma, CA 125 on ovarian carcinoma, CA 72–4 on gastric and mucinous ovarian carcinoma and neuron-specific enolase (NSE) on small-cell lung carcinoma and neuroblastoma. However, recent studies revealed that soluble carcinoembryonic antigen (CEA), as well as CA 19–9, CA 125 and CA 15–3 TAAs may be detected in the sera or on synovial cells of patients with rheumatoid arthritis (RA), as well as in the sera of patients with scleroderma, lupus and Sjögren's syndrome.
Objectives: In this study, we assessed levels of various TAAs in the sera of RA patients and healthy subjects. Serum TAA levels were correlated with markers of disease activity.
Methods: TAAs including CEA, CA 15–3, CA 72–4, CA 125, CA 19–9 and NSE were assessed by ELISA in the sera of 78 patients with established, treated RA (disease duration >2 years) and 50 age- and sex-matched healthy controls. Normal upper limits for these TAAs were 3.4 μg/l, 25 kU/l, 6.9 kU/l, 35 kU/l, 34 kU/l and 16.3 μg/l, respectively. TAA concentrations were correlated with serum rheumatoid factor (RF; <50 U/ml), anti-CCP (<25 U/ml) and CRP (<5 mg/l). DAS28 indicating clinical disease activity was also assessed.
Results: There were more RA patients showing abnormally high levels of TAAs in comparison to controls (CEA: 12.8% vs 6%; CA 125: 11.5% vs 4%; CA 19–9: 7.7% vs 6%; CA 15–3: 15.4% vs 4%; CA 72–4: 3.8% vs 0%; NSE: 20.1% vs 8%). Significant differences were found in the case of CEA, CA 125, CA 15–3, CA 72–4 and NSE ($p < 0.05$). Among RA patients, serum NSE levels showed significant correlation with CRP ($r = 0.42$, $p < 0.05$), as well as anti-CCP levels ($r = 0.62$, $p < 0.05$). None of the assessed TAAs showed any correlation with DAS28.
Conclusion: The concentration of some TAAs may be elevated in the sera of patients with established RA in comparison to healthy subjects. Furthermore, some TAAs, such as NSE, may also correlate with laboratory markers of RA.

545 PUBLICATION

Investigation of TNF-alpha activity on new cell line from patients with myelodisplastic syndroma

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TNF-alpa is a pleiotropic cytokine which can induce apoptosis is sensitive cells, but also regulated cell proliferation, cellular activation and differentiation. To be better estimated TNF-alpha effects on new establisched cell line, entitled PC, originally developed from patients with myelodisplastic syndrome at Institute of Oncology Sremska, Kamenica, Novi Sad. In this research we monitored the kinetics of changes after in