demonstrated in mice and humans. Oligodeoxynucleotides containing unmethyled CpG motifs (CpG ODN), bind to the Toll-Like Receptor 9 (TLR9) and are powerful immunostimulating agents. We investigated whether tumor antigens released after ECT could efficiently prime the immune system and induce a systemic antitumor response when associated with CpG ODN.
Materials and methods: in a first set of experiments we analysed by immunohistochemistry the nature of the cellular recruitment induced after ECT and the expression of TLR9 mRNA by quantitative RT-PCR in tumors. In a second set of experiments, we investigated the effectiveness of the association ECT-CpG-ODN in two subcutaneous mouse tumor models: a fibrosarcoma (LPB) and a melanoma (B16F10). We studied both local and systemic anti-tumoral effects of this association using a model in which two tumors were inoculated but only one was treated. The specific immune response was further studied in the subcutaneous B16OVA tumor model.
Results: ECT induced the recruitment of CD11c and Mac1 positive cells expressing TLR9 in LPB tumors up to 72 hours after ECT. Our results showed a strong local efficacy of the ECT-CpG-ODN as well as antitumor effects on the controlateral non treated tumors in the two models. In nude mice, no effect was observed on tumors, suggesting a mechanism mediated by T lymphocytes. Moreover, the combination of ECT and CpG-ODN induced a 3-fold increase of specific anti-OVA CD8 lymphocytes in the tumor-draining lymph node, compared to ECT alone.
Conclusion: the combination of ECT, allowing tumor destruction, together with a suitable immunostimulating adjuvant could be a new strategy to treat patients with subcutaneous tumor localizations.

535 POSTER

Biological, histological and clinical impact of preoperative IL-2 administration in radically operable gastric cancer patients

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Background: Surgery induced lymphocytopenia and this decrease in host defences, related to IL-2 endogenous embalance during postoperative period could promote the proliferation of possible micrometastases and the implantation of surgically disseminated tumour cells. Moreover Tumor infiltrating lymphocytes (TILs), activated by endogenous IL-2 release, is linked to prognosis in cancer patients The aim of this randomized study is to assess the biological (peripheral blood cells count, related to the grade of immunosuppression), histological (TILs) and clinical (overall and disease free survival) impact of preoperative low doses administration of IL-2 in patients with radically operable gastric cancer.
Materials and methods: This prospectic study enrolled 89 consecutive patients with histologically proven gastric adenocarcinoma who underwent radical surgery from October 1999 to December 2003 (M/F 49/40; mean age 67; range 42–82). Patient were randomized to be treated with surgery alone as controls (45 patients) or surgery plus preoperative treatment with recombinant human IL-2 (44 patients). We considered the total lymphocyte count and lymphocyte subset (CD4, CD4/CD8) during the preoperative period, before IL-2 administration, and on the 14^{th} and 50^{th} day, peritumoral stromal (fibrosis) reaction, neutrophils, lymphocytes and eosinophils infiltration in tumor histology, and morbidity disease free and overall survival were evaluated.
Results: Two groups were well-matched for type of surgery and extent of disease. All the patients underwent radical surgery plus D2 lymphadenectomy. At baseline, there were no significant differences in total lymphocyte and lymphocyte subsets between groups. The control group showed a significant decrease of total lymphocytes, CD4 cells, and CD4/CD8 ratio at the 14^{th} postoperative day relative to the baseline value. In the control group 65% of patients had a decreased of CD4 under 500 cells/mmc. Instead it has been observed in IL-2 group a significant increase over the control group values of total lymphocytes and CD4 cells (14^{th} total lymphocytes and CD4: IL-2 vs control $p < 0.05$). Moreover in this group only 15% patients had CD4 under 500 cells/mmc. This difference, in CD4 count, is significant even at the 50^{th} postoperative day ($p = 0.006$). IL-2 group showed lower postoperative complications (4/44 vs 13/45; $p < 0.05$), and higher lymphocyte/eosinophil infiltration into the tumor ($p < 0.0002$). Median follow up was 36 months (range 12–72) and median overall and disease-free survivals were longer, even if not significantly, in the IL-2 group than in the control arm ($p = 0.07$ and $p = 0.06$ respectively).
Conclusion: This randomized study would suggest that a preoperative immunotherapy with IL-2 is a well tolerated treatment able to prevent surgery induced lymphocytopenia. IL-2 seems to neutralise the immuno-suppression induced by operation and so to stimulate the host reaction against tumour tissue (lymphocytes/eosinophils infiltration). Furthermore IL-2 seems to have an impact on clinical course reducing morbidity of surgery and ameliorating overall and disease free survival

536 POSTER

Mechanisms of transcriptional upregulation of DR5 by chemotherapeutic drugs and sensitization to TRAIL-mediated apoptosis

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TRAIL, a member of the TNF family, has been shown to kill sensitive tumor cells with minimal toxicity to normal tissues and is a new candidate for immunotherapy in the treatment of drug-refractory tumor cells. However, many drug-resistant tumor cells are also resistant to TRAIL and such tumors require sensitization to reverse TRAIL resistance. We, and others, have reported that several sensitizing agents (ex. CDDP, ADR, chemical inhibitors, etc.) in combination with TRAIL result in significant synergistic apoptosis, however the mechanisms by which this sensitization is achieved still remain unclear. Based on the observed upregulation of Death Receptor 5 (DR5) expression, induced by the sensitizing agents (*Ng et al., Prostate, 53: 286, 2002; Huerta-Yepez et al., Oncogene, 23: 4993, 2004*), we hypothesized that many of those drugs may, directly or indirectly, interfere with a repressor factor of the DR5 transcription.
Examination of the DR5 promoter revealed the presence of one binding site for the transcription repressor Yin Yang 1 (YY1), suggesting that YY1 may negatively regulate DR5 transcription. This hypothesis was tested by examining a luciferase reporter system (pDR5 wild type) and plasmids in which the YY1-binding site was either deleted (pDR5/-605), and/or mutated (pDR5-YY1 mutant).
Using the PC-3 prostate (androgen independent) tumor cell line as a model system, we showed that PC-3 transfected with pDR5 wild type resulted in basal luciferase activity, whereas treatment with CDDP or ADR significantly augmented luciferase activity. PC-3 cells transfected with pDR5/-605 or pDR5-YY1 also resulted in significant potentiation of the basal luciferase activity. Inhibition of YY1 by siRNA revealed increased sensitization of tumor cells to TRAIL-mediated apoptosis. Reduced YY1 DNA binding properties and downregulation of the NF-kB promoter activity were also shown to be triggered by drug treatment.
These findings indicate that YY1 negatively regulates DR5 transcription inducing tumor cells' resistance to TRAIL. They also support the hypothesis that drugs-induced upregulation of DR5 expression is mediated via inhibition of the transcription repressor YY1. On a clinical aspect, the above findings suggest that tumor cells overexpressing YY1 will be resistant to TRAIL-mediated apoptosis. Therefore, inhibition of YY1 may be clinically useful in the therapeutic application of TRAIL in resistant tumor cells.

537 POSTER

P43/EMAP-II expression in colorectal cancer is associated with hypoxia, enhanced lymphocyte infiltration and apoptosis

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Aims: P43/Endothelial monocyte-activating polypeptide II (p43/EMAP II) is a proinflammatory cytosine and a chemoattractant for mononuclear phagocytes and polymorphonuclear leukocytes, found in culture supernatants of many tumour cell lines. We recently demonstrated that p43/EMAP-II induces apoptosis in mitogen-stimulated lymphocytes, and suggested that it may be a constituent of a novel immune evasion mechanism employed by tumour cells [1]. Furthermore p43/EMAP-II release is enhanced by hypoxia [2]. Our study has examined the association between p43/EMAP-II expression and hypoxia in colorectal cancer (CRC), and also the association between p43/EMAP-II and lymphocyte apoptosis.
Methods: Formalin-fixed, paraffin-embedded archival tissue samples from a well-characterised population of 72 patients diagnosed with colorectal tumours were used in immuno-histochemical studies. Antibodies against p43/EMAP-II, carbonic anhydrase (CA IX) as a surrogate marker of hypoxia, and CD3 to identify tumour-infiltrating lymphocytes (TIL) were used. Areas of p43/EMAP-II and CA IX staining were quantified using computer-aided image analysis. Antibodies against active Caspase-3 and PARP were used to identify apoptosis in TIL.
Results: P43/EMAP-II expression was correlated with CA IX expression in CRC. Patients with high p43/EMAP-II expression seemed to do better than those with low, and the reverse was true for CA IX. There was also a positive correlation between p43/EMAP-II and the lymphocyte counts in CRC ($p = 0.03$), as well as between CA IX and lymphocyte counts ($p = 0.02$). The presence of CD3+ cells was a good prognostic indicator in terms of overall survival. There was a significant association between

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.

References

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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.

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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.